

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/271839398>

EC Fifth Framework Project QLK5-1999-01554: Pest Risk Analysis for *Tilletia indica*

Technical Report · September 2006

CITATIONS

0

READS

46

19 authors, including:



Claire Sansford

My own consultancy

26 PUBLICATIONS **503** CITATIONS

[SEE PROFILE](#)



Richard Baker

Department for Environment, Food and Rural Affairs

106 PUBLICATIONS **1,770** CITATIONS

[SEE PROFILE](#)



John P. Brennan

84 PUBLICATIONS **1,451** CITATIONS

[SEE PROFILE](#)



Frank Ewert

University of Bonn

321 PUBLICATIONS **10,340** CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Exploring the Human Dynamics Affecting the Intergenerational Family Farm Transfer Process in Later Life: A Roadmap for Future Policy [View project](#)



Organics over the Ocean Modifying Particles in both Hemispheres (OOMPH) [View project](#)



EC Fifth Framework Project QLK5-1999-01554:

Risks associated with *Tilletia indica*, the newly-listed EU quarantine pathogen, the cause of Karnal bunt of wheat

Deliverable Report DL 6.1

Report on the risk of entry, establishment and socio-economic loss for *Tilletia indica* in the European Union

AND

Deliverable Report 6.5

Determination and report on the most appropriate risk management scheme for *Tilletia indica* in the EU in relation to the assessed level of risk

CONTENTS	Page
LIST OF PARTICIPANTS AND AUTHORS	4
SUMMARY	6
1. INTRODUCTION	8
2. PEST RISK ANALYSIS FOR <i>TILLETIA INDICA</i>	12
3. EXPLOITATION AND DISSEMINATION OF RESULTS	122
4. POLICY RELATED BENEFITS	122
5. REFERENCES	123
6. ACKNOWLEDGEMENTS	137
 ANNEXES	
ANNEX I: Workpackage 6 - Description of Work Plan	138
ANNEX II: Model Contingency Plan (20/07/05) – for Eradication & Containment of Karnal bunt (<i>Tilletia indica</i>)	139
ANNEX III: Draft Regulatory Impact Assessment (RIA) of the possible Management Options for Interceptions and Outbreaks of <i>Tilletia indica</i> – November 2004	151
ANNEX IV: Economic Costs of Scenario 1, ‘Large’ Karnal bunt outbreak, Year 1, managed according to an earlier draft of the model Contingency Plan.	161
ANNEX V: Economic Costs of Scenario 2, ‘Small’ Karnal bunt outbreak, Year 1, managed according to an earlier draft of the model Contingency Plan.	162
ANNEX VI: Components of Costs in Affected Region of Scenario 1, ‘Large’ Karnal bunt outbreak, Years 1 to 10) managed according to an earlier draft of the model Contingency Plan.	163
ANNEX VII: Components of Costs in Affected Region of Scenario 2, ‘Small’ Karnal bunt outbreak, Years 1 to 10) managed according to an earlier draft of the model Contingency Plan.	164

LIST OF TABLES

Table 1:	Eurostat data: Imports (tonnes) of ‘common’ and durum wheat into the former EU 15 from countries where <i>T. indica</i> is known to occur – 2000 to 2003.	23
Table 1a:	FAOSTAT data: Imports of wheat (tonnes) into the current EU 25 from countries where <i>T. indica</i> is known to occur – July 2000 to June 2001 (2001) and July 2001 to June 2002 (2002).	24
Table 2:	FAS data: Exports of unmilled wheat (tonnes) from the USA to the EU 25, 2000 - 2004	25
Table 3:	Wheat and triticale production (area harvested – hectares) in descending order of productivity by EU Member State in 2004.	36
Table 4:	Mean maximum daily temperatures (°C) recorded at the heading to flowering (GS 53–71), grain filling (GS 73–77), estimated for heading to grain filling (GS 53–77) and recorded for grain hardening (GS 83–87) stages of wheat development and levels of Karnal bunt (% CI, mean of 3 wheat cultivars) under 5 different situations after inoculation with <i>T. indica</i> (from Kumar <i>et al.</i> , 2003).	57

LIST OF FIGURES

Figure 1:	The PRA area: The European Union Member States – a subset of the EPPO Region.	13
Figure 2:	Wheat grain infected with <i>Tilletia indica</i> and free teliospores (USDA, G. Peterson).	27
Figure 3:	Unloading imported wheat at a port in northern England.	32
Figure 4:	Disease cycle of Karnal bunt (from Nagarajan <i>et al.</i> , 1997), reproduced with permission of CABI.	40
Figure 5:	Stages of development of wheat over which teliospores must germinate, sporidia infect the head and colonisation of seed begin (GS 37–75).	49
Figure 6:	Mean HTI values (1961-1990) for India in March and Great Britain in June (Baker <i>et al.</i> , 2000).	50
Figure 7:	HTI values for infection of <i>T. aestivum</i> by <i>T. indica</i> and commencement of disease development for the years 1995-2002 for three sowing dates in Europe, where the HTI was calculated to be between 2.2-3.3 during the critical phenology period of the wheat crop. The maximum number of cases (referred to as ‘years’ in the figure) is three sowings x eight years, or 24 cases.	53
Figure 8:	HTI values for infection of <i>T. durum</i> by <i>T. indica</i> and commencement of disease development for the years 1995-2002 for three sowing dates in Europe, where the HTI was calculated to be between 2.2-3.3 during the critical phenology period of the wheat crop. The maximum number of cases (referred to as ‘years’ in the figure) is three sowings x eight years, or 24 cases.	54
Figure 9:	Mean monthly air temperature (°C) in Europe in July	60
Figure 10:	Locations of four of the five named locations of Karnal bunt outbreaks in irrigated wheat crops in South Africa.	63

LIST OF PARTICIPANTS AND AUTHORS

Workpackage Manager: Dr C Sansford, CSL, York, UK					
List of Participants					
Partner no. & name	Legal status	Key contact	Postal address	Telephone no's, fax no's and email addresses	
1. CSL	Co-ordinator	Dr Claire Sansford	CSL, Sand Hutton, North Yorkshire UK, YO41 1LZ	☎ Fax. Email	+44 (0) 1904 462225 +44 (0) 1904 462250 c.sansford@csl.gov.uk
2. NSW Agriculture (Now NSW Dept. of Primary Industries)	Contractor	Dr Gordon Murray	Wagga Wagga Agricultural Institute, Private Bag, Wagga Wagga, NSW 2650, Australia	☎ Fax Email	+61 2 6938 1879 +61 2 6938 1809 gordon.murray@dpi.nsw.gov.au
	Sub-contractor to Partner 5	Dr John Brennan		☎ Fax Email	+61 2 6938 1851 +61 2 6938 1809 john.brennan@dpi.nsw.gov.au
3. DGISP	Contractor	Dr Vibeke Leth	Danish Government Institute of Seed Pathology for Developing Countries, Thorvaldsensvej 57, DK-1871, Frederiksberg C, Denmark	☎ Fax Email	+45 35 28 37 23 +45 35 28 37 01 vib@kvl.dk
4. KVL	Contractor	Prof. Dr John R. Porter	Department of Agricultural Sciences, The Royal Veterinary and Agricultural University, 10 Agrovej, Taastrup, 2630, Denmark	☎ Fax Email	+45 35 28 3377/3575 +45 35 28 3574 jrp@kvl.dk
5. TEAGASC	Contractor	Dr Paul Kelly	Teagasc, Rural Economy Research Centre, 19 Sandymount Avenue, Dublin 4, Ireland	☎ Fax Email	+353 1 6376050 +353 1 6688443 pkelly@HQ.TEAGASC.IE
7. CNR IATA (Now IBIMET)	Contractor	Dr Franco Miglietta	IBIMET, P.le delle Cascine, 18, 50144 Firenze, Italy	☎ Fax Email	+39 55 301422 +39 55 308910 migliet@sunserver.iata.fi.cnr.it
8. ISPaVe	Contractor	Dr Luca Riccioni ¹	Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero, 22 I-00156 Rome Italy	☎ Fax Email	+39 0682070329 +39 0686802296 luca.riccioni@ispave.it
9. NCRI	Contractor	Dr Håkon Magnus	The Norwegian Crop Research Institute, Plant Protection Centre, Planteforsk, Fellesbygget N-1432 AAS, Norway	☎ Fax Email	+47 64949244 +47 64949226 haakon.magnus@@bioforsk.no
10. USDA ARS	Contractor	Mr Gary Peterson	ARS/USDA, FD-WSRU, Fort Detrick, Maryland MD21702, USA	☎ Fax Email	+301 619 7313 +301 619 2880 gary.peterson@ars.usda.gov

¹ From 1 January 2004 Dr Luca Riccioni replaced Dr Porta-Puglia as the Key Contact for ISPaVe. Dr Porta-Puglia is now working as a mycologist at the Ministry for Rural Affairs and the Environment, Department of Plant Health, Agricultural Research & Development Centre, Ghammieri, Marsa CMR 01, Malta.

Authors of report:

C. Sansford (CSL), R. Baker (CSL), J. Brennan (NSW Ag.), F. Ewert (ex. KVL), B. Gioli (IBIMET), A. Inman (CSL), P. Kelly (TEAGASC), A. Kinsella (TEAGASC), V. Leth (DGISP), H. Magnus (NCRI), F. Miglietta (IBIMET), G. Murray (NSW Ag.), G. Peterson (USDA), A. Porta-Puglia (ex. ISPaVe), J. Porter (KVL), T. Rafoss (NCRI), L. Riccioni (ISPaVe), F. Thorne (TEAGASC), M. Valvassori (ISPaVe).

Project Co-ordinator:

Dr Claire Sansford, Plant Health Group, Central Science Laboratory, Department for Environment, Food and Rural Affairs (Defra), Sand Hutton, York, UK, YO41 1LZ.

SUMMARY

Tilletia indica became listed as a I/AI quarantine pest in the European Commission (EC) Plant Health Directive in 1997 following a Pest Risk Analysis produced by the UK which had been triggered by the first findings of *T. indica* in wheat crops in the USA.

Following international debate as to the risk associated with *T. indica* a revised Pest Risk Analysis (PRA) for *Tilletia indica* has been produced using a draft of the European and Mediterranean Plant Protection Organisations (EPPO) PRA scheme.

This is the culmination of a 4-year EU Fifth Framework Project ('*Karnal bunt risks*') and reflects the work of 9 Partner Organisations in 7 countries including Australia and the USA. The views expressed within this PRA do not necessarily reflect the views of the European Commission who partly-funded the work or the National Plant Protection Organisations of any of the individual countries either contributing to or cited in the PRA.

The risk of entry has been evaluated based upon trade data and data on interceptions of *T. indica* in the European Union (EU) and this shows that the pathogen has the potential to enter the EU.

The risk of establishment has been determined by a range of methods. These include:

1. Experimental work with the pathogen in European field conditions to determine the longevity of teliospores of *T. indica* (conducted under quarantine containment) as well as the likely timing of germination of teliospores in European wheat crops to examine whether or not they will produce infective spores (sporidia) at the critical infection period of the wheat crop.
2. Host susceptibility testing (*Triticum aestivum* and *Triticum durum*; bread/feed and durum wheat) to determine the critical infection period as well as the susceptibility of a range of European wheat cultivars to infection by *T. indica*.
3. Combining crop phenology models for *T. aestivum* and *T. durum* and a disease model based upon the Humid Thermal Index² of Jhorar *et al.* (1992) and outputting the findings into a series of maps using a Geographic Information System.

By combining the findings of these investigations an enhanced assessment of the risk of establishment of *T. indica* has been produced. This clearly shows that the pathogen can survive as teliospores for at least 3 years in soils in a range of locations in the EU, that some teliospores will be available to germinate to produce infective sporidia at the critical infection period of the wheat crop, and, that European wheat cultivars are susceptible to infection and disease development under European climatic conditions. Some areas will have a more frequent occurrence of favourable conditions for infection and disease development than others; this would favour a more rapid build-up of inoculum levels of *T. indica* in those locations. However, all areas have been shown to be favourable in some years within the period studied (1995-2002). It is thought that the frequency of occurrence of these conditions should not be a limiting factor in the perpetuation of the pathogen because of the longevity of teliospores present below the soil surface. The conclusion is that *T. indica* has the potential to establish in much of the wheat growing areas of the EU.

² The Humid Thermal Index covers the period from sporidial release at around the phenological growth stage of wheat known as Zadok's GS 37 through to GS 75, at which point successful infections will have occurred. We deployed the HTI in this Project from GS 37 to GS 65 (anthesis).

The likely impact that findings of the pathogen in the EU may cause has been determined. This includes a socioeconomic analysis of the costs arising from controlling any outbreaks of the pathogen in the EU.

The potential impacts are considered to be unacceptable. They justify minimal measures to reduce the risk of entry.

By evaluating the range of measures that could be undertaken to prevent entry of the pathogen or to deal with outbreaks in the EU, a model management (contingency) plan (hereafter referred to as a '*model contingency plan*') has been produced based upon outbreak scenarios in England for consideration for implementation in England and Wales. However, this plan, which formed the basis of the socio-economic impact analysis, is for illustrative purposes only. It should not be taken as indicating how any EU Member State would respond to a finding of *Tilletia indica* on its territory. Any such response will be determined by the governments concerned, in the light of consultation with stakeholders and other relevant factors at the time including the availability of resources.

Tilletia indica has been shown to have the potential to enter, establish and cause serious impacts in the EU. It should be retained as a I/AI pest and efforts made by individual EU Member States to prevent entry could be usefully applied across the EU. Contingency planning to deal with interceptions and outbreaks of *T. indica* is already underway in the UK and the Netherlands and it would be useful for other EU Member States to consider their approach should the pathogen be intercepted or gain entry to the EU.

1. INTRODUCTION

Project background

Karnal bunt of wheat, caused by the fungus *Tilletia indica*, is a floret-infecting smut pathogen of wheat (*Triticum* spp.) and triticale (\times *Triticosecale*). The fungus infects the glumes at late booting to early heading and grows via the rachilla to the florets. Seeds are infected through the germinal end of the grain and the fungus develops within the pericarp where it produces a powdery, brownish-black mass of teliospores. The fungus is restricted to the pericarp by the highly lignified external seed coat (Goates, 1988). The spore masses produce a foetid, decaying fish-like smell (trimethylamine). Infected seeds are usually only partially colonised, showing various degrees of infection. Point infections are most common, but infection may also spread down the adaxial groove and, in severe cases, the whole seed may appear bunted. Since infection occurs late in the phenological development of the host, the disease is more difficult to control than systemic smuts, which can usually be controlled effectively using seed treatments (e.g. *Tilletia tritici*).

T. indica is established in parts of Asia, including Afghanistan, India, Iraq, Nepal and Pakistan (Warham, 1986) and Iran (Torarbi *et al.*, 1996). It has also been reported in other parts of the world. It was first reported in Mexico in 1972 and detected in the USA in 1996 (Ykema *et al.*, 1996). Within the USA, it is present in certain USDA³-regulated areas in the southwest (Arizona, California and Texas). In Texas, it appears to be spreading northwards (Anon., 2001), although this may also reflect past plantings of contaminated wheat seed in combination with favourable climatic conditions rather than recent natural spread. *T. indica* was first reported from Brazil in 1993 in the southern part of the Rio Grande do Sul and some efforts were made to eradicate it (Da Luz *et al.*, 1993). However, no further publications of its current status in Brazil have been made. It was present in accessions of wheat germplasm introduced into Brazil for research purposes between 1990 and 1992 (Mendes and Ferreira, 1994). Oliveira *et al.*, (2002) also refer to the interception of *T. indica* on wheat germplasm imported into Brazil. The first report of *T. indica* in South Africa was made in 2000 in the northern Cape Province (Crous *et al.*, 2001) where it is thought to have been subject to eradication. However, between 2002 and 2004 it was found to have spread to a number of new areas (Naudé, 2002, Anon., 2004a).

The finding of the pathogen in the USA in 1996 established a significant new trade pathway for entry into Europe. Wheat had been imported into Europe from the USA before 1996, but in the absence of the pathogen in the USA, there appeared to be no risk of entry along this pathway. Since 1996, there have been several reported or suspected⁴ interceptions of *T. indica* on wheat imported to Europe:

- In Poland in 1996 in grain of *T. aestivum* from India (Klos, Plant Health and Inspection Service, Poland, *personal communication*)
- In Italy in two consignments of grain of *T. durum* from Mexico in 1998 (Anon., 1998)
- In the United Kingdom in 2003 in grain of *Triticum* sp. from India (Matthews, CSL, UK, *personal communication*)
- In the United Kingdom in 2004 in grain of *T. durum* from India (suspected; Matthews, CSL, UK, *personal communication*)

³ United States Department of Agriculture

⁴ Suspected when either there were insufficient teliospores or they failed to germinate to produce material for molecular testing

- In the United Kingdom in 2005 in two separate consignments of grain of *T. aestivum* from India (one suspected, one confirmed; Matthews, CSL, UK, *personal communication*)
- In the United Kingdom in 2006 in one consignment of grain of *T. aestivum* from India (Matthews, CSL, UK, *personal communication*)
- In Greece in 1996 on US grain (suspected on one consignment of *T. durum* and one of *T. aestivum*) (Vloutoglou, Benaki Phytopathological Institute, Greece, *personal communication*).

The establishment of the significant new trade pathway from the USA into Europe initiated a UK Pest Risk Analysis (PRA) (Sansford, 1996 *unpublished*; 1998). The PRA assessed the risk to the UK and the EU and suggested that the pathogen could establish and cause economic damage in the UK/EU. As a result, *T. indica* was added as a quarantine pest to the EC Plant Health Directive 77/93/EEC (now 2000/29/EC) in 1997 (Anon., 2000). The EC Directive applies minimal quarantine requirements to seed and grain of *Triticum*, *Secale* and \times *Triticosecale* from countries where *T. indica* is known to occur (currently listed in the EC Directive as Afghanistan, India, Iran, Iraq, Mexico, Nepal, Pakistan, South Africa and the USA).

The requirements are as follows:

- *Seeds must originate in an area where Tilletia indica Mitra is known not to occur.*
- *Grain must originate in an area where Tilletia indica Mitra is known not to occur, or no symptoms of Tilletia indica Mitra must have been observed on the plants at the place of production during the last complete cycle of vegetation and representative samples of the grain must have been taken both at the time of harvest and before shipment and have been tested and found free from Tilletia indica Mitra in these tests.*

Rye (*Secale cereale*) is to be considered for deletion from the EC Plant Health Directive because it is no longer considered to be a natural host. Iran was added to the Directive in 2004. Discussions should take place in the EC's Standing Committee on Plant Health regarding the addition of Brazil to the list of countries in which the pathogen is found. Should the Committee agree, the change will have to be notified to the World Trade Organisations Sanitary and Phytosanitary Committee (WTO/SPS) before it can be accepted.

The first UK PRA (Sansford, 1996 *unpublished*, Sansford, 1998) predicted a risk of entry based principally on trade pathways, a risk of establishment in Europe and the potential for economic damage through quality losses (downgrading of milling wheat to feed wheat) as well as losses in export markets. The risk of establishment was estimated in part by applying a published Karnal bunt disease model (Jhorar *et al.*, 1992) to the UK. The model correlates the Humid Thermal Index at 'heading' to predict disease 'severity'⁵ for that season in regions where the disease is established.

In applying the HTI, Sansford used climatic data from individual meteorological stations in the UK and showed that conditions during the 'heading' period (May and June) were favourable for infection and disease development. When applied and interpolated across the UK/Europe using European-wide meteorological data during the 'heading' period, (Baker *et al.*, 2000) the model predicted that conditions for infection and disease development were broadly favourable across most of the area.

Because data were not available on the behaviour of the pathogen in the UK/European Union (EU), such as teliospore survival, dormancy, germination and host susceptibility, the PRA (Sansford, 1996

⁵ 'Severity' is an unspecified term in Jhorar *et al.*, 1992.

unpublished; 1998) formulated conclusions from the existing biological information in the available literature. This EU Project aimed to produce a more accurate assessment of risk to the EU based upon experimentation within specific scientific objectives.

Project aims: ‘Karnal bunt risks’

The Project has eight Scientific and Technical Objectives, each of which forms a separate Workpackage. They can be summarised as follows:

1. To accurately predict the risk of *T. indica* becoming established in Europe by combining crop and pathogen models and to use these alongside new pathogen data in pest risk mapping and Pest Risk Analysis to support and underpin EU plant health policy and legislation on *T. indica*.
2. To determine the susceptibility of European wheat cultivars to *T. indica* and to estimate potential losses of yield of these cultivars if *T. indica* reaches Europe.
3. To determine the survival of *T. indica* teliospores under a range of European soil conditions.
4. To determine the abiotic factors that affect dormancy and germination of *T. indica* teliospores using controlled environments, relating the results to European climatic conditions and European wheat crop phenology.
5. To predict the likely socio-economic impact of *T. indica* should it be introduced to Europe.
6. To analyse the risk of entry, establishment and socio-economic losses for the EU and to develop harmonised risk management strategies and contingency plans to deal with actual introductions of *T. indica* to Europe, especially aspects of eradication, containment and control, whilst minimising disruption to free trade.
7. To generalise the specific findings of the Project so as to develop risk analysis techniques for other plant pathogens of potential threat to the EU, supporting EU and international plant health policy and legislation.
8. To disseminate Project outputs on: (a) ‘*The Risks to the EU from Tilletia indica*’; and (b) ‘*Advances in Pest Risk Analysis for Plant Pathogens*’. This will specifically be achieved through an internet website established for the Project and by holding a workshop at the end of the Project for representatives from EU member states, accession countries and other interested parties (including representatives from national and/or international plant health organisations).

<http://karnalpublic.pestrisk.net/>

Workpackage 6: ‘Pest Risk Analysis for Tilletia indica for the EU’

The aim of this Workpackage is ‘to analyse the risk of entry, establishment and socio-economic losses for the EU and to develop harmonised risk management strategies and contingency plans’. There are five objectives:

1. To determine the risk of entry, establishment and socio-economic loss for *T. indica* in the EU.
2. To evaluate fungicide efficacy and timing for the control of *T. indica*.
3. To evaluate published data on other control strategies.
4. To evaluate existing risk management schemes for *T. indica* outside of the EU.
5. To determine the appropriate level of risk management for the EU for *T. indica* in relation to the assessed level of risk determined by the Project.

A summary of the work plan is given in Annex I. The determination of the risk of entry, establishment and socio-economic loss for *T. indica* in the EU (objective 1) and the determination of the appropriate level of risk management for the EU for *T. indica* in relation to the assessed level of risk determined by the Project (objective 5) based on the most up-to-date FAO International Standard for Phytosanitary Measures (ISPM) for Pest Risk Analysis (FAO, 2004) is presented in this Deliverable Report. Three other Deliverable Reports have already been produced addressing objectives 2, 3 and 4 and these have already been provided to the EC.

2. PEST RISK ANALYSIS FOR *TILLETIA INDICA*

A recent draft of the European and Mediterranean Plant Protection Organisation (EPPO) standard ‘*Guidelines on Pest Risk Analysis: Decision making scheme for quarantine pests*’ (05-11845) has been used as a basis for the PRA for *Tilletia indica*. Most of the explanatory text that accompanies the draft EPPO standard has been removed for clarity; only the questions and a small part of the preamble to each of the main sections has been retained.

The EPPO PRA standard is based on FAO ISPM no. 11 (FAO, 2004). It provides detailed instructions, for the following stages of Pest Risk Analysis (PRA) for quarantine pests: initiation, pest categorisation, probability of introduction, assessment of potential economic consequences and pest risk management. It provides a simple scheme based on a sequence of questions for deciding whether an organism has the characteristics of a quarantine pest, and if appropriate to identify potential management options. The scheme is also relevant for PRAs initiated by the identification of a pathway or the review of a policy. Expert judgement may be used in interpreting the replies. In responding to the questions it has not always been possible, where required, to select a one-word answer. Where this has occurred an explanation has been provided of the difficulties encountered in responding in this way.

Phytosanitary terms used in this PRA are defined in FAO (2002).

Stage 1: Initiation

The aim of the initiation stage is to identify the pest(s) and pathways which are of phytosanitary concern and should be considered for risk analysis in relation to the identified PRA area.

1. Give the reason for performing the PRA.

This new PRA is being conducted to take into account the first ever set of experimental and economic data for the I/AI EC listed quarantine organism *Tilletia indica* that has been specifically generated for the EU/EPPO region arising from the EU Fifth Framework Project ‘*Karnal bunt risks*’. Hereafter this is referred to as the Project, or by individual unpublished Deliverable Reports by author. Summaries of each of these reports is available at: <http://karnalpublic.pestrisk.net/> under ‘*Results*’.

Go to 2

2. Specify the pest or pests of concern and follow the scheme for each individual pest in turn. For intentionally introduced plants specify the intended habitats. If no pest of concern is identified the PRA may stop at this point.

The pest of concern is:

Name: *Tilletia indica* Mitra

Synonym: *Neovossia indica* (Mitra) Mundkhur

Taxonomic position: Fungus: Basidiomycete; Ustilaginales

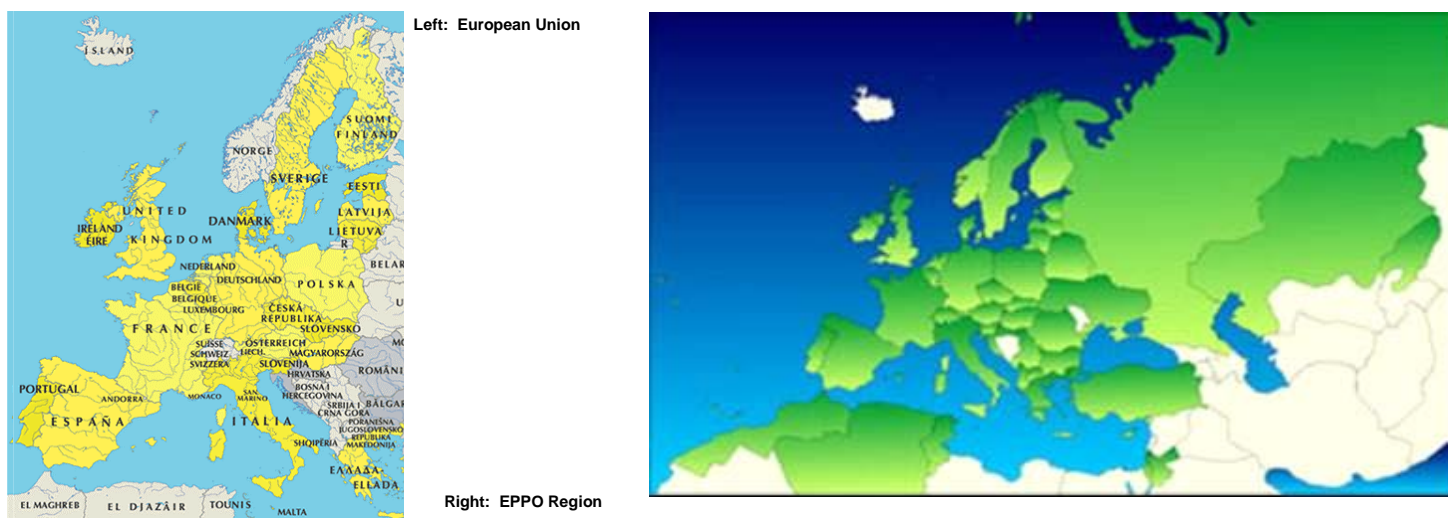
Common name(s) of the disease: Karnal bunt or partial bunt of wheat and other cereals

Go to 3

3. Clearly define the PRA area.

The PRA area is the EU. When this Project started (1st February 2000) the EU consisted of 15 Member States (EU 15) but since 1 May 2004 a further 10 countries joined, making a total of 25 Member States. The Project has concentrated on the risks to the EU 15. This forms part of the EPPO region of 46 countries. See Figure 1 below:

Figure 1. The PRA area: The European Union Member States – a subset of the EPPO Region.



Source: <http://europa.eu.int/abc/maps/images/europe.gif>

Source: http://www.eppo.org/ABOUT_EPPO/about_eppo.htm

Go to 4

4. Does a relevant earlier PRA exist?

Yes. A PRA for *Tilletia indica* was conducted in 1996 (Sansford, 1996 *unpublished*, Sansford, 1998) and revised in 2004 (Sansford, 2004). On each occasion the PRA area was principally the UK with extrapolation as appropriate to the EU/EPPO region. Subsequent to the first UK PRA in 1996, Kehlenbeck *et al.* (1997) repeated the PRA exercise for Germany based upon the UK methodology. However, the authors also conducted an in-depth study of the potential economic consequences of an outbreak of Karnal bunt which was geared towards the wheat-growing areas of Germany.

Go to 5

5. Is the earlier PRA still entirely valid, or only partly valid (out of date, applied in different circumstances, for a similar but distinct pest, for another area with similar conditions)?

The earlier UK PRAs are partly valid. A revised version has been prepared for the UK broadly incorporating the findings of the Project but with limited scientific references to make it easier to read (Sansford, 2004). This version has been used as the basis for this new PRA with a more detailed analysis of the risk of establishment and socioeconomic impact for the EU and a detailed review of the literature.

If entirely valid

End

If partly valid proceed with the PRA, but compare as much as possible with the earlier PRA
If not valid

Go to 6
Go to 6

Stage 2: Pest Risk Assessment

Section A: Pest categorisation

At the outset, it may not be clear which pest(s) identified in Stage 1 require a PRA. The categorisation process examines for each pest whether the criteria in the definition⁶ for a quarantine pest are satisfied. In the evaluation of a pathway associated with a commodity, a number of individual PRAs may be necessary for the various pests potentially associated with the pathway. The opportunity to eliminate an organism or organisms from consideration before in-depth examination is undertaken is a valuable characteristic of the categorisation process. An advantage of pest categorisation is that it can be done with relatively little information; however information should be sufficient to adequately carry out the categorisation.

Identify the pest (or potential pest)

6. Is the organism clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

Yes. As referred to at the initiation stage the pest is:

Name: *Tilletia indica* Mitra

Synonym: *Neovossia indica* (Mitra) Mundkhur

Taxonomic position: Fungus: Basidiomycete; Ustilaginales

Common name(s) of the disease: Karnal bunt or partial bunt of wheat and other cereals

If yes indicate the correct scientific name and taxonomic position

Go to 8

If no

Go to 7

7. Even if the causal agent of particular symptoms has not yet been fully identified, has it been shown to produce consistent symptoms and to be transmissible?

Not relevant.

If yes

Go to 8

If no

Go to 17

Confirm pest status (actual or potential)

8. Is the organism in its area of current distribution a known pest (or vector of a pest) of plants or plant products?

⁶ A 'quarantine pest' is defined as 'a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled'. (FAO, 2002).

Yes, it is a primary plant pathogen.

If yes, the organism is considered to be a pest

Go to 10

If no

Go to 9

9. Does the organism have intrinsic attributes that indicate that it could cause significant harm to plants?

Not relevant.

If yes or uncertain, the organism may become a pest of plants in the PRA area

Go to 10

If no

Go to 17

Presence or absence in the PRA area and regulatory status

10. Does the pest occur⁷ in the PRA area?

No.

Tilletia indica is absent from the PRA area. Cereal crops are routinely inspected by agronomists in many EU countries and certified seed crops are inspected by the relevant National Plant Protection Organisations (NPPOs); so the presence of *T. indica* may come to light depending upon the severity of any outbreak (see 1.4 for further details of the difficulties of detection). Some countries, such as the Netherlands and the UK have recently instigated domestic surveys specifically for *T. indica*. In 2004 the Netherlands inspected 103 wheat fields and submitted 8 suspect samples for laboratory testing all of which proved negative (van Leeuwen, Dutch Plant Protection Service, the Netherlands, *personal communication*, 2005). The UK has collected subsamples of wheat grain from the National Institute of Agricultural Botany (NIAB, Cambridge) and tested 25 samples from counties across the UK for harvest years 2002 and 2003 and 50 for harvest 2004, all of which also proved negative for *T. indica* using the EU/EPPO-recommended *T. indica* Diagnostic Protocol (Inman *et al.*, 2003, Anon., 2004) (Bowyer, CSL, UK, *personal communication*, 2005). The situation regarding specific surveys for *T. indica* in other EU countries is not known.

If yes

Go to 11

If no

Go to 12

11. Is the pest widely distributed in the PRA area?

Not relevant.

If not widely distributed

Go to 12

If widely distributed

Go to 17

⁷ 'Occurrence' is defined as 'the presence in an area of a pest officially reported to be indigenous or introduced and/or not officially reported to have been eradicated. This includes organisms which have been introduced intentionally and which are not subject to containment (notably cultivated plants). Organisms present for scientific purposes under adequate containment (e.g. in botanic gardens) are not included'. (FAO, 2002).

Potential for establishment and spread in the PRA area

- 12. Does at least one host-plant species (for pests directly affecting plants) or one suitable habitat (for non parasitic plants) occur in the PRA area (outdoors, in protected cultivation or both)?**

Yes. The natural host plants of *T. indica* are bread/feed and durum wheat (*T. aestivum* and *T. durum* respectively), and triticale (x *Triticosecale*). Records on triticale are rare. The pathogen was first naturally observed on this host in India in 1975 and 1976 (Agarwal *et al.*, 1977). This was later described in more detail for the years 1975-1977 (Khetarpal *et al.*, 1980).

Despite being one of the named regulated hosts in the EC Plant Health Directive (Anon., 2000), rye (*Secale cereale*) is no longer considered to be a natural host. All of the reports in the literature refer to its experimental susceptibility only and not to natural records (e.g. Aujla *et al.*, 1987; Warham, 1986, 1998). Rye is to be considered for deletion from the EC Plant Health Directive. Several grass species have been found to be experimentally susceptible (Royer and Rytter, 1988). Nagarajan *et al.* (1997) refer to the susceptibility of *Triticum boeoticum*, *Triticum ovatum*, *Triticum variabilis*, and *Triticum sharonensis* but there are no further reports of natural outbreaks on these species. Peterson and Bonde (1998) found that a cultivar of annual ryegrass (*Lolium multiflorum*) was experimentally susceptible to *T. indica*. Seven cultivars of emmer wheat (*Triticum dicoccum*), a species having an expanding niche-market in some areas of Italy, have also been shown to be experimentally susceptible to *T. indica* by boot inoculation with sporidia (Riccioni, ISPaVe, Italy, *unpublished*, 2005).

If yes **Go to 13**
If no **Go to 17**

- 13. If a vector is the only means by which the pest can spread, is a vector present in the PRA area? (If a vector is not needed or is not the only means by which the pest can spread go to 14).**

No vector is needed for spread of *T. indica*.

If yes/Not applicable **Go to 14**
If no **Go to 17**

- 14. Does the known area of current distribution of the pest include ecoclimatic conditions comparable with those of the PRA area or sufficiently similar for the pest to survive and thrive (consider also protected conditions)?**

Yes. A detailed investigation of this aspect forms part of the full assessment of the risk of establishment in Section B of this PRA.

If yes **Go to 15**
If no **Go to 17**

Potential for economic consequences in the PRA area.

- 15. With specific reference to the plant(s) or habitats which occur(s) in the PRA area, and the damage or loss caused by the pest in its area of current distribution, could the pest by itself, or acting as a vector, cause significant damage or loss to plants or other negative economic impacts (on the environment, on society, on export markets) through the effect on plant health in the PRA area?**

Yes. The pathogen normally causes minor yield and serious quality losses for the wheat crop. Because it is listed as a quarantine pest in a large number of countries, its presence can lead to a loss of export markets for the affected country. A fuller analysis of the potential economic consequences is presented in Section B of this PRA.

If yes or uncertain

Go to 16

If no

Go to 17

Conclusion of pest categorisation

- 16. This pest could present a risk to the PRA area. (Summarise the main elements leading to the conclusion that the pest presents a risk to the PRA area)**

Tilletia indica is already categorised as a quarantine pest for the EU/EPPO region. The pathogen became listed as an EC I/AI quarantine pest in 1997 as a result of the UK PRA (Sansford, 1996 *unpublished*; Sansford, 1998) and was at that time already categorised by EPPO as an A1 pest. This pathogen is absent from the PRA area, has the potential to enter, establish and cause socio-economic damage. More detail is given in Section B of this PRA.

Go to section B

- 17. The pest does not qualify as a quarantine pest for the PRA area and the assessment for this pest can stop (summarise the main reason for stopping the analysis).**

For a pathway analysis, go to 4 and proceed with the next pest. If no further pests have been identified the PRA may stop at this point.

Section B: Assessment of the probability of introduction (entry and establishment) and spread and of potential economic consequences

This part of the risk assessment process firstly estimates the probability of the pest being introduced into the PRA area (its entry and establishment) and secondly makes an assessment of the likely economic impact if that should happen. From these assessments, it should be possible to estimate the level of risk associated with the pest, which can then be used in the pest risk management phase to decide whether it is necessary to take phytosanitary measures to prevent the introduction of the pest, and if the measures chosen are appropriate for the level of risk.

The evaluation is based on the replies to a series of questions, mostly expressed in the first instance as the choice of an appropriate phrase out of a set of five alternatives (e.g. very unlikely, unlikely, moderately likely, likely, very likely). It is important to identify especially high or especially low risks. The user of the scheme should add to these replies any details which appear relevant indicating the source of information used.

Answer as many of the following questions as possible. If any question does not appear to be relevant for the pest concerned, it should be noted as 'irrelevant'. If any question appears difficult to answer no judgement should be given but the user should note whether this is because of lack of information or uncertainty.

1. Probability of introduction

'Introduction' as defined by the FAO Glossary of Phytosanitary Terms (FAO, 2002), is the entry of a pest resulting in its establishment.

Probability of Entry of a pest

Identification of pathways

'Pathway' is defined as 'any means that allows the entry or spread of a pest' (FAO, 2002).

1.1. Consider all relevant pathways and list them.

Tilletia indica is most likely to enter the PRA area through the movement of teliospores via the following pathways:

- i. Imports of seed of wheat (*T. aestivum*, *T. durum*) or triticale (x *Triticosecale*) that has been infected or contaminated with *T. indica*. The pathway for the pathogen to enter via seed is clear since planting infected or contaminated seed in arable land will allow the pathogen to enter the PRA area.
- ii. Imports of grain of wheat (*T. aestivum*, *T. durum*) or triticale (x *Triticosecale*) that has been infected or contaminated with *T. indica*. The pathway for the pathogen to enter via grain will depend upon the location of the port of entry and the route that the consignment takes post-entry, as well as its final destination and intended use. Infected or contaminated grain destined for transport through, or for processing in areas where wheat or triticale is grown, poses the highest risk, since teliospores can be released during transportation and by handling operations.
- iii. Movement as a contaminant of containers, agricultural machinery and vehicles either in soil adhering to surfaces or as sori or free teliospores.

- iv. Windborne teliospores.
- v. On feathers of birds or fur of animals or in their intestinal tract.

Other pathways may exist but are not considered further in this PRA. For example, Stansbury *et al.*, (2002), considered the risk of entry of *T. indica* to Australia and suggested that wheat, as a contaminant of herbs or other seeds for sowing, fertiliser transported in contaminated containers, straw goods or packing material, travellers personal effects, and seed brought in through mail order could all be pathways of entry.

Go to 1.2

1.2. Estimate the number of relevant pathways, of different commodities, from different origins, to different end uses.

Very few, Few, Moderate number, Many, Very many.

The number of relevant pathways is moderate.

There are six main commodities (seed and grain of wheat (*T. aestivum*, *T. durum*) and triticale (x *Triticosecale*)). The pathogen is present in ten countries (Afghanistan, Brazil, India, Iran, Iraq, Mexico, Nepal, Pakistan, South Africa and the USA). The number of pathways of import is therefore any combination of the commodities and the countries where the pathogen occurs. This will vary from month to month and year to year depending upon supply and demand in the PRA area. Data on imports into the EU of seed of all three commodities are difficult to obtain (but have been requested). Some seed import data for the UK has been obtained but the wheat seed data is generic, i.e. as with the grain data it is not broken-down into *T. aestivum* or *T. durum*. Also, for some of the countries where *T. indica* occurs no export data are available.

End uses will vary. In broad-terms grain of *T. aestivum* will mainly be used either for human or animal consumption. Grain of *T. durum* will mainly be used for pasta production but is also used to produce ethnic foods and biscuits etc. In the UK, triticale (x *Triticosecale*) is used for pig and poultry feed (Nix, 2003).

The number of each of the other pathways broadly described under 1.1 (iii, iv, and v) is not possible to enumerate. No information is available on the volume of containers, agricultural machinery and vehicles arriving in the EU from countries or areas of countries where *T. indica* occurs. These are however recognised as a pathway of entry for *T. indica* (e.g. Bonde *et al.*, 1997; Murray and Brennan, 1998; Babadoost, 2000). One example of this is the movement of teliospores of *T. indica* on rail cars moving from Mexico into California (Boratynksi *et al.*, 1985). Movement on air currents and in association with birds and animals is not enumerable. Further detail on these pathways is given under 1.3 below.

Go to 1.3

1.3. Select from the relevant pathways, using expert judgement, those which appear most important. If these pathways involve different origins and end uses, it is sufficient to consider only the realistic worst-case pathways. The following group of questions on pathways is then considered for each relevant pathway in turn, as appropriate, starting with the most important.

The most important pathways are imports of seed and grain of *T. aestivum*, *T. durum* and *x Triticosecale* originating in Afghanistan, Brazil, India, Iran, Iraq, Mexico, Nepal, Pakistan, South Africa and the USA. Of these, grain of *T. aestivum* and *T. durum* is most likely to enter and in greater quantity than seed. However, limited data on wheat seed imports (not broken-down into *T. aestivum* or *T. durum*) show that pathways of entry do exist. As much import data as are available have been summarised (see 1.6). In responding to the following questions, all of these pathways are considered together rather than in turn. This is because in practice the import pathways will vary from month to month and year to year depending upon supply and demand of each of the commodities in the PRA area.

Because of the relative isolation of the PRA area from the currently affected countries all of which are located in continents beyond Europe (North, Central and South America; Africa, Asia), movement of teliospores by wind or on/in birds or animals into the PRA area is currently considered to be very low risk. However, these modes of movement are relevant to further spread within the PRA area once the pathogen has entered the area. Post-entry spread is considered under 1.33.

Go to 1.4

Probability of the pest being associated with the individual pathway at origin.

1.4. Is the prevalence of the pest on the pathway at origin likely to be high, taking into account factors like the prevalence of the pest at origin, the life stages of the pest, the period of the year?

Note that phytosanitary measures are not considered here but are commented on for consideration under 1.10.

Very unlikely, unlikely, moderately likely, likely, very likely.

Moderately likely.

The answer to this question depends upon the country of export and the mode of inspection and testing employed by the certifying authority of the NPPO. However, grain and seed of *T. aestivum*, *T. durum* and *x Triticosecale* originating in countries where *T. indica* occurs have to be shown to be pathogen-free (rather than symptom-free) for export to the PRA area, according to current EC legislation (Anon., 2000) as amended). At low levels disease symptoms are difficult to detect in the growing crop; symptoms occur on seed within the floral bracts and are thus not clearly apparent in the crop. If crops grown in areas where the pathogen is known to occur are not tested for the pathogen itself (i.e. laboratory tests for teliospores), then it is possible that *T. indica* may be present in the harvested seed or grain and, if destined for export, the pathogen could be moved in trade.

The use of the word ‘*high*’ to describe pest prevalence for *T. indica* is subjective. The life stage that is likely to be present is the teliospore either as free spores or contained in sori on a bunted grain. Since one bunted grain has been estimated to produce up to 10⁶ teliospores (Garrett and Bowden, 2002) there is little to be gained in estimating pest prevalence in a subjective manner. This is particularly relevant as there is no minimum level of inoculum needed before infection is possible (but see 1.29 for estimates of the establishment potential based upon the inoculum load). Stansbury *et al.* (2002) support this view. The time of year at which exports occur is irrelevant to the assessment of the likelihood of prevalence; harvested grain or seed if infected or contaminated with *T. indica* will remain so irrespective of when it is exported since teliospores are very long-lived. Thus, although this question is difficult to answer, the prevalence of *T. indica* on the pathway at origin is currently unlikely to be ‘*high*’ but this is not particularly relevant to the determination of

the risk of entry since its mere presence is sufficient to ensure that it can be moved cross-continent. The USA appears to be the main country exporting to the PRA area. If grain or seed (less likely) of *T. aestivum*, *T. durum* or (least likely) x *Triticosecale* originates in areas of the USA where *T. indica* is known to occur the prevalence is 'moderately likely' to be high because of the method by which the certifying authority in the USA determines freedom from *T. indica*. Exports from other affected countries, although less in volume, could fall into the same category. Exports from countries such as India and Pakistan may be more at risk of being contaminated because of the prevalence of *T. indica* which has been there since at least 1931 (Mitra, 1931). Reported interceptions/suspect interceptions on wheat grain in the EU to date have originated in India, Mexico and possibly the USA. See 1.10 for further detail for efficacy of methods deployed for export certification.

Go to 1.5

1.5. Is the prevalence of the pest on the pathway at origin likely to be high, taking into account factors like cultivation practices, treatment of consignments?

Note: these are practices mainly in the country of origin, such as pesticide application (including herbicides for plants), removal of substandard produce, kiln-drying of wood, cultural methods, sorting and cleaning of commodities. Note that cultivation practices may change over time. Phytosanitary measures are not considered in this question (see 1.10).

Very unlikely, unlikely, moderately likely, likely, very likely.

Moderately likely.

As with the answer to 1.4, currently the prevalence of *T. indica* on the pathway at origin for consignments destined for export to the PRA area from countries where *T. indica* occurs may or may not be high (but if current phytosanitary requirements are complied with for grain or seed destined for export then the prevalence should in fact be zero in the areas from which exports arise). However, the control of *T. indica* by normal practices in any area of a country where the pathogen currently occurs is extremely difficult. A review of the literature (Sansford *et al.*, unpublished 2004a) in support of an experimental investigation into the efficacy of fungicide application, showed that foliar fungicides applied to the wheat crop from flag leaf emergence onwards can reduce the severity of the disease in wheat crops in countries where *T. indica* is established. However, fungicides will never give 100% control. Unlike many other smut fungi, *T. indica* is not systemic in the seedling and therefore the use of fungicidal seed treatments has no effect on the control of the pathogen. Non-fungicidal seed treatments are likely to damage the embryo (Sansford *et al.*, unpublished, 2004b). Warham's (1986) review of the longevity of teliospores in soil showed a range of 2 to 4 years and subsequent studies including those conducted under field conditions in Europe (Inman *et al.*, unpublished 2004), show a similar range. Bonde *et al.*, (1997) cite viability of up to 5 years in contaminated soil. This means that the use of crop rotations to reduce soil-borne populations of teliospores would have to incorporate a long host-free period of probably at least 5 years to have any impact. Even then there is no guarantee of pathogen demise. In reviewing data related to the treatments of consignments (Sansford *et al.*, unpublished 2004b) it was found that much research has been conducted on developing chemical or physical treatments for killing teliospores of other smut fungi such as *Tilletia controversa* and *T. tritici* in grain. These studies have been confined to small-scale laboratory or facilities testing with no large-scale (commercial volumes of wheat grain) research. Problems in interpreting data therefore arise. No practical treatment method has been found for killing teliospores in wheat grain while preserving milling quality. Although irradiation of grain killed teliospores of *T. controversa* and *T. tritici* and preserved milling quality, it also killed the embryo, thus causing it to break down quickly. The

dosage of methyl bromide required to kill teliospores of these species also reduced the storage life of the wheat and presented residue problems. Consequently the application of non-phytosanitary cultivation measures in the country of origin may reduce the inoculum load but consignment treatment renders consignments unfit for their intended purpose as grain or seed.

Go to 1.6

1.6. How large is the volume⁸ of the movement along the pathway?

Minimal, minor, moderate, major, massive.

Major.

Both the type of data and the actual values presented on imports of wheat (*T. aestivum*), durum wheat (*T. durum*) and triticale (x *Triticosecale*) to the PRA area vary depending upon the source of the data used. This makes detailed analysis of the trade pathways difficult but some broad conclusions can be made.

Eurostat data

Data from Eurostat on imports of ‘common wheat’ (presumed to be grain of *T. aestivum*), durum wheat, and triticale for the calendar years 2000 to 2003 to the former fifteen EU Member States (EU 15) were obtained from Defra Plant Health Division (O’Donnell, Defra, UK, *personal communication*, 2005). Data were not available from this source for the current twenty-five EU Member States (EU 25). Many countries were found to export these commodities to the EU 15. Table 1 contains a subset of the data, only showing imports originating in countries where *T. indica* occurs. During this period, India, Iraq, Mexico, Pakistan and the USA were amongst the countries exporting these commodities to nine countries of the EU 15. Italy, the Netherlands, Belgium, the UK and Spain were the main recipients. The USA was the most significant exporting country shipping high volumes of durum wheat to 9 of the EU 15 with Italy being the main recipient. A significant amount of durum wheat was also exported from Iraq to Italy in 2003. ‘Common wheat’ was only imported twice and in small amounts, once to France from the USA in 2002 and once to Greece from Mexico in 2003. According to this database, no triticale was imported into the EU 15 from countries where *T. indica* occurs over this period.

⁸ This refers to the commodity.

Table 1. Eurostat* data: Imports (tonnes) of ‘common’ and durum wheat into the former EU 15 from countries where *T. indica* is known to occur – 2000 to 2003.

Importing country**	Year, exporting country and type of wheat									
	2000	2001	2002		2003					
	USA	USA	USA	India	USA		Iraq	India	Mexico	Pakistan
	Durum	Durum	Durum	Durum	Durum	Common	Durum	Durum	Common	Durum
Italy	380541.2	590129.1	398213.5		317305.7		27789.8			
Netherlands	35188.5	40834.4	18713.1		8145.2					
Belgium	13866.0	949.5	20486.6		4568.3					
UK				14.5	16323.5			10.1		
Spain	100.0		10192.5							
Finland		1147.5								
Greece	250.0	56.0	15.0		14.0				0.1	
Sweden	305.3									
France	4.1					0.7				
Total EU 15	430255.1	63116.5	447620.7	14.5	346356.7	0.7	27789.8	10.1	0.1	***

* Source: Eurostat - Comext, Disk No S2/2003 & 12/200, extracted 24th March 2005.

** The remaining EU 15 countries of Austria, Denmark, Germany, Ireland, Luxembourg and Portugal had no recorded imports of common or durum wheat or triticale from countries where *T. indica* occurs during 2000-2003.

*** Indicates trade less than 0.1 tonnes; blank cells indicate ‘no trade’.

FAOSTAT data

Data from FAOSTAT (FAOSTAT 2005) is broken-down only into imports of ‘wheat’ (not broken-down into *T. aestivum* or *T. durum* and assumed to be grain rather than seed); no data are available for triticale. The most recent figures are for the 12 months between July 2000 and June 2001 (‘2001’) and July 2001 and June 2002 (‘2002’). For the current 25 Member States of the EU (the EU 25), noting that for some of these countries no data were available, in 2001, the only country where *T. indica* occurs that was recorded as exporting wheat to the EU 25 was the USA, shipping large amounts of wheat to at least 12 of these countries; again with Italy, Spain, Belgium-Luxembourg, the UK and the Netherlands being the recipients of the highest quantities (Table 1a). In 2002 approximately half of the 2001 total was exported from the USA to at least 9 of these countries with shipments also coming from Mexico to the Netherlands, and from Brazil and India to the UK.

Although the two recording periods used by Eurostat and FAOSTAT differ as well as the time periods currently available, discrepancies in data exist; two examples follow:

According to FAOSTAT Mexico appears to have exported a large quantity of wheat to the Netherlands between July 2001 and June 2002 (Table 1a) but no exports of any type of wheat to any EU 15 country from Mexico appear in the calendar years 2001 and 2002 for Eurostat (Table 1). Similarly, according to FAOSTAT, Brazil appears to have exported wheat to the UK between July 2001 and June 2002 (Table 1a) but Brazil is notably absent from any of the countries exporting to the EU 15 in the Eurostat dataset for 2000 to 2003.

Table 1a. FAOSTAT* data: Imports of wheat (tonnes) into the current EU 25 from countries where *T. indica* is known to occur*** – July 2000 to June 2001 (2001) and July 2001 to June 2002 (2002).

Importing country**	Year and exporting country				
	2001	2002			
	<i>USA</i>	<i>USA</i>	<i>Mexico</i>	<i>Brazil</i>	<i>India</i>
Italy	1071291	556253	-	-	-
Spain	395188	142720	-	-	-
Belgium-Luxembourg	175929	172443	-	-	-
United Kingdom	108455	109480	-	2803	26
Netherlands	99243	105704	53650	-	-
<i>Cyprus</i>	72222	62024	-	-	-
<i>Malta</i>	38157	28885	-	-	-
Germany	28893	1317	-	-	-
Portugal	17771	61664	-	-	-
Greece	15905	0	-	-	-
Sweden	2000	0	-	-	-
France	19	0	-	-	-
Finland	0	0	-	-	-
Austria	-	-	-	-	-
<i>Czech Republic</i>	-	-	-	-	-
Denmark	-	-	-	-	-
<i>Estonia</i>	-	-	-	-	-
<i>Hungary</i>	-	-	-	-	-
Ireland	-	-	-	-	-
<i>Latvia</i>	-	-	-	-	-
Total EU 25	2025073	1149941	53650	2803	26

* Source: FAOSTAT, 2005.

** Accession countries which joined the EU on May 1st 2004 are italicised.

*** All countries where *T. indica* occurs were searched for exports to the EU 25; Afghanistan and Iraq do not appear in this database as exporting countries.

- = No data were provided as a result of the search.

USDA Foreign Agricultural Service US Trade Internet System data

The USDA Foreign Agricultural Service (FAS, 2005) supplies a range of data on exports of commodities from the USA. Table 2 shows the amount of unmilled wheat (not specified into type) exported from the USA to those countries which form the EU 25 in the calendar years 2000 to 2004.

Fifteen EU Member States were shown to import wheat during this period with the top importer again being Italy.

Table 2. Exports of unmilled wheat (tonnes) from the USA to the EU 25, 2000 - 2004

<i>Importing country**</i>	<i>Year of import</i>				
	2000	2001	2002	2003	2004
Italy	776615	1022107	722928	712654	559120
Spain	76706	273841	247898	371089	184677
Belgium-Luxembourg	187005	182799	151729	145547	161854
Netherlands	125852	84727	98095	85901	37035
United Kingdom	86984	111881	124971	33358	63036
<i>Cyprus</i>	18060	41783	91701	31328	15750
Portugal	34986	0	35781	112562	13546
<i>Malta</i>	33902	38429	25082	21808	10000
Germany	3255	49179	1601	603	278
<i>Bulgaria</i>	0	0	0	23493	0
Greece	0	0	15905	0	0
Finland	9925	0	0	0	0
Sweden	0	2131	0	0	0
Ireland	287	0	0	0	0
France	48	0	19	0	73
Total	1353625	1806877	1515710	1538343	1045369

* *Source: FAS, 2005*

** Accession countries which joined the EU on May 1st 2004 are italicised.

UK Home-Grown Cereals Authority data

Details of imports to the UK from countries where *T. indica* occurs (HGCA, 2004) show that between July 2002 and June 2003, 91,708 tonnes of wheat ('wheat' not defined; i.e. not broken-down into grain or seed, or bread or durum wheat) were brought in from the USA, 2,803 tonnes from Brazil and 26 tonnes from India. Another discrepancy arises here as the values for Brazil and India appear to be the same values in the FAOSTAT database occurring between July 2001 and July 2002 (Table 1a). According to the HGCA, between July 2003 and June 2004, 24, 165 tonnes of wheat were imported into the UK from the USA and 57 tonnes from India.

Her Majesty's Customs and Excise Database for the UK – CHIEF

Data on seed imports have proven to be very difficult to obtain possibly because of the relatively small quantities involved (relative to grain) which may not always be recorded by the importing country. The Plant Health and Seeds Inspectorate for England and Wales (PHSI) have access to the CHIEF database which records import and export data for the UK for Her Majesty's Custom and Excise Database. David Butler (PHSI, UK, *personal communication*, 2005) interrogated the system and found that between October 2000 and September 2004, the UK imported seed of wheat (not broken-down into *T. aestivum* or *T. durum*) from Canada, China, Cyprus, the Czech Republic, India, the Lebanon, Poland, Russia, Switzerland, Turkey and the USA.

Seed of triticale was imported from Poland in September 2000.

The known imports of seed of wheat from India and the USA posed a potential risk of entry of *T. indica* to the PRA area.

Some EU Member States, including the UK, are sampling and testing 100% of grain and/or seed of wheat and triticale imported from countries where the pathogen is known to occur. Since 1999, the PHSI have sent samples of wheat grain for testing for *T. indica* to CSL. These have originated in the USA and Mexico as well as from some other countries, including Canada, the Ukraine and Kazakhstan because of their geographical proximity to affected countries. Not all of these imports appear in the CHIEF database.

Conclusions for 1.6

Despite the discrepancies between the data sources available to conduct this PRA it is clear that the main volume of movement of wheat grain along the pathway is from the USA to a high proportion of the EU Member States with Italy being the main recipient. Smaller volumes originate in a range of countries where *T. indica* occurs, including Brazil, India, Iraq, Pakistan and Mexico.

Data on seed imports were difficult to obtain but the UK data alone show a potential pathway of entry from the USA and from India.

Interception data complement data on imports into the PRA area from some of the affected countries and support the view that pathways for entry of *T. indica* on cereal grain from India, Mexico and the USA exist (see 1.8).

Go to 1.7

1.7. How frequent is the movement⁹ along the pathway?

Very rarely, rarely, occasionally, often, very often.

Very often.

Wheat grain (and presumably triticale) can enter the PRA area at all times of year. Between 2000 and 2004 wheat seed was found to be imported into the UK all year round. Triticale seed was imported once in September 2000. (Butler, PHSI, UK, *personal communication*, 2005).

However, the frequency of movement of the commodity is not particularly relevant to the assessment of the risk of entry because of the extreme longevity of the teliospores of *T. indica* which includes a period of dormancy in fresh teliospores.

Go to 1.8

⁹ This refers to the commodity.

Probability of survival during transport or storage

1.8. How likely is the pest to survive during transport /storage?

Very unlikely, unlikely, moderately likely, likely, very likely.

Very likely.

The pathogen is moved in trade in the form of teliospores contaminating seed or grain, or as bunted grain (see Figure 2). Generally speaking, teliospores of *T. indica* are robust structures but there is some evidence that a proportion of spores may fragment in transit. Inman *et al.*, (2003) (in Anon., 2004) found this occurred with samples despatched for ring-testing the methodology devised for the EU/EPPO-recommended *T. indica* Diagnostic Protocol (Inman *et al.*, 2003; Anon., 2004). However, if teliospores of *T. indica* are present in sori or as bunted grains they are likely to survive as intact and viable structures in transport. Teliospores survive storage for an extremely long time, years rather than months. For example, Babadoost *et al.*, (2004) citing others suggests that laboratory-stored teliospores survived 5 to 7 years; Bonde *et al.*, (1997) state that dry teliospores stored in laboratory conditions have remained viable for up to 16 years.

Figure 2. Wheat grain infected with *Tilletia indica* and free teliospores (USDA, G. Peterson).



Interception data for the PRA area support the view that the pathogen can survive transport to the PRA area as intact teliospores. Since 1996, there have been several reported or suspected¹⁰ interceptions of *T. indica* on wheat imported to Europe.

In 1996, *T. indica* was intercepted in Poland in grain of *T. aestivum* from India (Klos, Plant Health and Inspection Service, Poland, *personal communication*, 2003). Despite the existence of EC legislation and exporting country requirements since 1997, several interceptions of *T. indica* have been reported subsequently in the PRA area. The first was in Italy in 1998 on two consignments of grain of *T. durum* from Mexico (Anon., 1998). These consignments had been tested by the Mexican phytosanitary services before shipment and had been found free from bunted grains. Although testing for teliospores was common practice in Mexico at that time, grain lots found free of bunted grains were considered to be ‘*bunt-free*’ and (unfortunately) were considered to have met the EC requirement of freedom from *T. indica* (Porta-Puglia, MRAE, Malta, *personal communication*, 2005).

¹⁰ Suspected when either there were insufficient teliospores or they failed to germinate to produce material for molecular testing

All of the UK interceptions or suspect interceptions have been on wheat imported from India. These were in 2003 in grain of *Triticum* sp. from India, in 2004 in grain of *T. durum* (suspected); in 2005 in two separate consignments of grain of *T. aestivum* (one suspected, one confirmed) and in 2006 in one consignment of grain of *T. aestivum* (Matthews, CSL, UK, *personal communication*).

Greece is thought to have intercepted *T. indica* on grain of *T. durum* and also possibly on *T. aestivum* from the USA (Vloutoglou, Benaki Phytopathological Institute, Greece, *personal communication*, 2000).

Go to 1.9

1.9. How likely is the pest to multiply/increase in prevalence during transport/ storage?
Very unlikely, unlikely, moderately likely, likely, very likely.

Very unlikely. (Impossible).

The spore type that will be transported is the teliospore. This type of spore is only capable of multiplication by germinating under suitable environmental conditions to produce infective spores (sporidia). The sporidia infect the host (primarily wheat) through the ear during the heading stage. It is only at this point that more teliospores will be produced.

Go to 1.10

Probability of the pest surviving existing pest management procedures

1.10. How likely is the pest to survive or remain undetected during existing phytosanitary measures?

Very unlikely, unlikely, moderately likely, likely, very likely.

Very likely to survive; likely to remain undetected in some circumstances depending upon the exporting country.

The EC Plant Health Directive (Anon., 2000) applies minimal quarantine requirements to seed and grain of *Triticum*, *Secale* and \times *Triticosecale* exported to the EU from countries where *T. indica* is known to occur. *Secale* (rye) is no longer considered to be a host.

The requirements are as follows:

Seeds must originate in an area where Tilletia indica Mitra is known not to occur.

Grain must originate in an area where Tilletia indica Mitra is known not to occur, or no symptoms of Tilletia indica Mitra must have been observed on the plants at the place of production during the last complete cycle of vegetation and representative samples of the grain must have been taken both at the time of harvest and before shipment and have been tested and found free from Tilletia indica Mitra in these tests.

As described under 1.4, the disease is difficult to detect in the growing crop; symptoms affect the ear at heading time and can be hard to find. Therefore reliance for detection of *T. indica*, either for pre-export inspection or for determining area or place of production freedom, should not be placed upon crop inspection alone. Indeed, the EC requirements specify pathogen-freedom, not freedom

from symptoms. Although statistically-proven methods are available to determine the presence of *T. indica* in consignments destined for export to the EU (e.g. methodology of Peterson *et al.*, 2000, which has been deployed in Inman *et al.*, 2003; in Anon., 2004 for testing suspect consignments), insufficient inspection and testing, or use of less efficient methods, will result in a lack of detection of the pathogen by the exporting country. Interceptions of *T. indica* made in the PRA area suggest that, on occasion, inspection and testing have not been as thorough in the exporting countries as would be expected. (See 1.8).

The following has important implications regarding the risk of entry of *T. indica* on imports of wheat grain originating in the USA. Following the first official report of the pathogen in the USA, the USDA initiated surveys, both local and national to determine the extent of the presence of *T. indica* in the USA. Because disease levels were generally low and detection of ‘*bunted kernels*’ was considered to be ‘*difficult and unreliable*’, presence of the pathogen was based upon microscopic observation for teliospores in a 50 g seed wash. Teliospore identification was based upon morphological characteristics and, when appropriate, two different pairs of DNA PCR primers designed to be specific for *T. indica*. During the 1996 survey, teliospores that were morphologically similar to *T. indica*, but smaller, were found in seed samples from the states of Alabama, Georgia, Tennessee and Florida but with no associated bunted wheat grain. Similar spores were found in a sample of forage mix; this was traced-back to Oregon-produced ryegrass (*Lolium perenne* – perennial ryegrass, and *Lolium multiflorum* – annual ryegrass). These spores were identified as *T. indica* by four different pairs of PCR primers but again no bunted wheat grains were found (Peterson and Bonde, 1998). The pathogen was later identified as a new species known as *Tilletia walkeri* (Castlebury and Carris, 1999) a pathogen of *L. multiflorum* and *L. perenne*, in the United States and Australia, respectively. Primers were developed to differentiate *T. indica* and the (as yet unnamed) new organism and were first available in 1998 (Frederick *et al.*, 1998). Frederick *et al.* (2000) published further details on identification to species and further differential diagnostic methodology has been developed since (e.g. Levy *et al.*, 2001).

In the interim period, and due in part to the immediate difficulties of confusing *T. walkeri* with *T. indica*, the USDA Animal and Plant Health Inspection Service (APHIS) adopted an interim rule in May 1997 that there would be a requirement for a ‘*bunted kernel*’ (a wheat grain with visible disease symptoms) to be ‘*found in or associated with a field within an area, before the area be designated as regulated*’. This rule (adopted as a final rule in 1999) may allow low levels of infection to go undetected and for teliospores of the pathogen to potentially be present in grain shipments derived from infested crops originating in the USA. However, in early 1997, the USDA APHIS website for ‘*Frequently Asked Questions*’ for Karnal bunt¹¹ stated the following question and answer:

Question:

‘*Why does USDA base its testing program on the presence of spores, rather than look for bunted kernels?*’

Answer:

‘*Looking for bunted kernels is a very inefficient method of detection of Karnal bunt. Testing for spores is a much more sensitive method of detection than visual observation for bunted kernels.*’

¹¹ <http://www.aphis.usda.gov/oa/bunt/kbfaqhtm>

Very often spores are present in infected wheat without being accompanied by bunted kernels. For example, if only 1 or 2 spores are found in a 50-gram sample, in all likelihood there would only be 1 bunted kernel per 440 pounds of wheat. Because grain that contains only spores – and no bunted kernels – still presents a significant risk of spreading Karnal bunt, we must test for spores’.

The change to ‘*bunted kernels*’ as the unit of regulation could therefore result in the pathogen being spread to new areas both within and beyond the USA.

Stein *et al.* (2005) conducted soil sampling in wheat fields in Texas in June 2002 to determine the distribution of teliospores of *T. indica* in both regulated (15 fields) and non-regulated areas (1 field). Within the regulated area teliospores were found in 14 of the fields sampled including fields that had never tested positive for ‘*bunted kernels*’. Under current requirements these fields could in theory be deregulated despite the presence of teliospores.

This method of regulating areas does not meet with the EC phytosanitary requirement for freedom from the pathogen *T. indica*; all it seeks to guarantee is freedom from symptoms of Karnal bunt. In July 2003, USDA APHIS made an announcement of a proposed revision to domestic regulations in the USA for Karnal bunt (USDA, 2003); the revisions became final in 2004 (USDA, 2004). With few exceptions, the main premise of the revision is the requirement for ‘*bunted kernels*’ to be found in most circumstances before any official action is taken, rather than sampling and testing for teliospores of *T. indica*. Seed is still tested for freedom from the pathogen. Although this revision of the regulations may alleviate pressure on farmers and the officials involved in inspection, the potential result is for further spread of the pathogen in the USA. The USA exports wheat to many EU Member States (see 1.6). The potential risk of entry to the PRA area may therefore have increased. EC Plant Health officials are in correspondence with their counterparts in the United States to clarify the basis on which US exports of wheat are certified as meeting the EC’s requirements with regard to *T. indica*. Some EU Member States, including the UK, are sampling and testing 100% of grain and seed imported from countries where the pathogen is known to occur.

Go to 1.11

Probability of transfer to a suitable host or habitat

1.11. In the case of a commodity pathway, how widely is the commodity to be distributed throughout the PRA area?

Is the distribution of the commodity in the PRA area:

Very limited, limited, moderately widely, widely, very widely

Very widely.

Grain of *T. aestivum* and x *Triticosecale* will be mainly be used either for human or animal consumption. Grain of *T. durum* will be used for pasta production and production of specialist foods.

Locations of grain storage facilities, flour mills, animal feed production facilities, and processors such as bakeries and pasta producers etc will vary by country but all EU countries have these inland facilities. The movement of imported consignments from the port of entry to these facilities allows the commodity to become distributed throughout the PRA area.

As an example of the likely distribution pattern, the UK Home-Grown Cereals Authority publishes an interactive map on its website which shows the diversity and widespread locations of all of the businesses in the UK to which these commodities will be disseminated. See <http://www.hgca.com/kiwi/cerealsmap/>.

The National Association of British and Irish Millers (NABIM) states that the majority of output in most of the main flour producing EU Member States is from large mills processing more than 50,000 tonnes of wheat per year. In 2002, the UK had 68 mills producing 88% of the total flour output. In 6 of the other EU Member States the number of large flour producing mills (processing more than 50, 000 t/year) were:

France – 540; Germany – 347; Italy – 335; Spain – 221; Belgium – 54 and the Netherlands – 33. See <http://www.nabim.org.uk/images/pdf/F%20&%20F%202004.pdf> for further details.

Italy, according to Agriculture and Agrifood Canada (Anon., 2003), has the largest milling industry in the EU. The Association of Industrial Millers and Pasta Makers of Italy (ITALMOPA) estimates there are about 700 milling companies in operation throughout the country.

Of these about 190 mill durum wheat and have a total annual capacity of 7.1 Mt, wheat ‘*equivalent*’ (unspecified term). The remainder are bread wheat mills with a total annual capacity of 10.7 Mt, wheat equivalent.

As with other countries, the milling facilities in Italy are often situated in rural areas, often in close proximity to wheat fields. Workers employed in Italian mills are often resident in rural areas and some of them are part-time farmers. This adds to the potential risk of dissemination of *T. indica* into cereal-growing areas of Italy post-entry (Porta-Puglia, MRAE, Malta, *personal communication*, 2005).

Imported seed of all three commodities has the potential to be widely dispersed.

Go to 1.12

1.12. In the case of a commodity pathway, do consignments arrive at a suitable time of year for pest establishment?

Yes.

Consignments can arrive all year round. Teliospores that may be present in a consignment have extreme longevity (years rather than months). Post-entry, once introduced to agricultural land, teliospores can reside in the soil for lengthy periods and only germinate and infect the susceptible hosts at a specific period in the host’s phenology, and under specific environmental conditions. Fresh teliospores (i.e. those present in freshly-harvested crops) have a period of dormancy that delays the onset of germination under field situations until conditions are favourable for infection of the wheat host. See 1.20 for further details of these conditions.

If yes

Go to 1.13

If no

Go to 1.15

1.13. How likely is the pest to be able to transfer from the pathway to a suitable host or habitat?

Very unlikely, unlikely, moderately likely, likely, very likely.

Very likely for seed.

The pathway for the pathogen to enter via seed is clear since planting infected or contaminated seed in arable land will allow the pathogen to enter the PRA area.

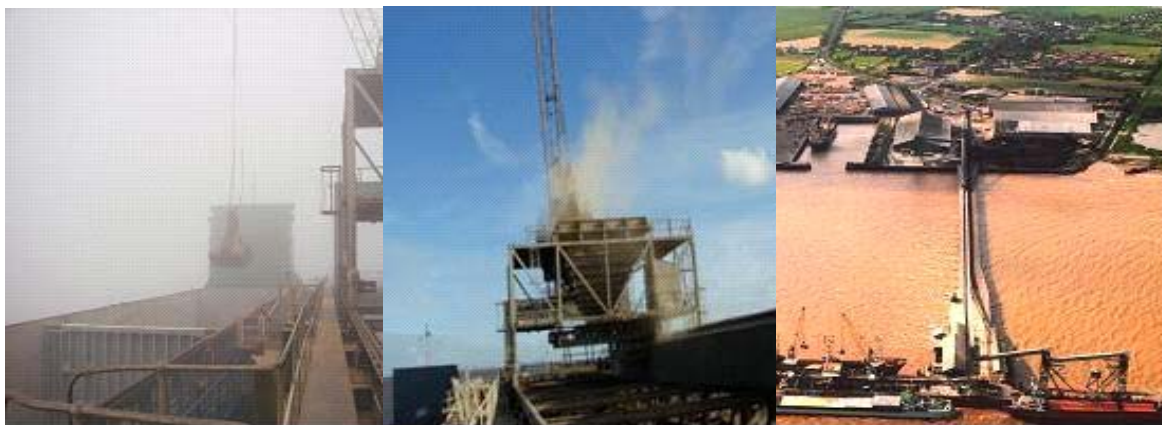
Moderately likely for grain.

The pathway for the pathogen to enter via grain will depend upon:

- The location of the port of entry in relation to agricultural land.
- The route that the consignment takes post-entry.
- The mode of transportation.
- Its final destination and intended use.

Infected or contaminated grain destined for transport through, or for processing in areas where wheat or triticale are grown, carried in vehicles that are not well-sealed, poses the highest risk, since teliospores can be released into arable production areas during transportation and by handling operations. Figure 3 illustrates some aspects of this.

Figure 3. Unloading imported wheat at a port in northern England¹².



a) Unloading the ship

b) Dust liberation – a pathway for teliospore liberation

c) Pathway from port of entry through arable production area

Go to 1.14

1.14. In the case of a commodity pathway, how likely is the intended use of the commodity (e.g. processing, consumption, planting, disposal of waste, by-products) to aid transfer to a suitable host or habitat?

Very unlikely, unlikely, moderately likely, likely, very likely.

Very likely for seed because this will be planted in arable production areas.

¹² Images a and b courtesy of Marcus Lazenby, Defra Plant Health and Seed Inspectorate

Moderately likely for grain.

At the point of unloading, during transport to storage facilities or to processing plants, and, at storage facilities and processing plants located in arable production areas, teliospores either free, or in/on bunted grain, may be liberated and transferred to fields used for growing *T. aestivum*, *T. durum* or x *Triticosecale*.

Once the infected or contaminated grain enters a facility where it is destined for processing, the risk of transfer is reduced. The risk of further spread beyond the processing facility depends upon the type of processing that is likely to be undertaken. Risks related to animal feed production and processing for flour production are considered further here.

Use as animal feed

A review of processes used to eliminate *T. indica* in known infected or contaminated wheat grain (Sansford *et al.*, unpublished 2004b) showed that processing grain into animal feed using the 'steam flake milling process' (109°C for 30 minutes) was found 100% effective at rendering teliospores non-viable, while preserving some economic value for the crop. This method was tested on a commercial scale and has been used extensively by the USDA following the first reports of *T. indica*/Karnal bunt in the USA in 1996. This is the method most commonly used in the USA for treatment of infected grain. It is the only method successfully applied on a commercial scale (Bonde *et al.*, 1997).

Pelletisation or extrusion of millfeed (the by-product of flour production) to produce livestock, pet or fish food has been shown to kill teliospores of *T. tritici* (Peterson and Kosta, unpublished data) and *T. controversa* (Bechtel *et al.*, 1999), respectively. Although not tested for efficacy against *T. indica*, milling Karnal bunt affected grain and pelleting/extruding the by-products may reduce the majority (but not all) of the risk of spread of teliospores to pathways beyond the mill.

Processing for human consumption

Sansford *et al.* (unpublished 2004b) found that although no definitive milling research has been conducted with Karnal bunt affected wheat grain, an extensive study was conducted using grain infested with teliospores of *T. controversa* (Bechtel *et al.*, 1999). Studies such as this appear to support the view that flour obtained from milling wheat grain in an urban area is not considered a high dissemination risk.

However, the milling by-products will most likely contain viable teliospores. Bechtel *et al.* (1999) showed that the greatest concentration of teliospores is likely to be in the initial cleanings, which millers commonly add back into the non-flour by-products. Bechtel *et al.* (1999) found that the cleaning and premilling processes at the mill reduced spore counts from pre-clean levels of 301 and 34 spores per 50g of wheat to 1.8 and 0.8 spores per 50g of wheat in two consecutive runs. The house filter contained the highest numbers of spores; very few spores were recovered from the mill fractions and no spores were detected in mill fractions coming from the internal parts of the grain. A few spores were found on the external parts of the grain, presumably as a contaminant.

Results from the milling study with *T. controversa* concluded that the greatest proportion of recoverable viable teliospores were present in the debris from the initial cleanings removed from the grain entering the mill, followed by a small proportion of teliospores in the non-flour by-product. It is anticipated that a study conducted with *T. indica* would lead to similar results and conclusions. If

the initial cleanings contain teliospores of *T. indica* and are disposed of or used in an unsafe manner it is possible that the teliospores could be liberated into arable production areas.

Go to 1.15

Consideration of further pathways

1.15. Do other pathways need to be considered?

Of the identified pathways (see 1.1), the pathways selected for consideration (1.3) were imports of seed and grain of *T. aestivum*, *T. durum* and x *Triticosecale* originating in Afghanistan, Brazil, India, Iran, Iraq, Mexico, Nepal, Pakistan, South Africa and the USA.

Responses have not been received to requests for information on seed imports of wheat or triticale into the EU other than the UK.

In the absence of any data no other pathways need to be or can be considered.

If yes

Go back to 1.3

If no

Go to conclusion on probability of entry and then go to 1.16

Conclusion on the probability of entry

The overall probability of entry should be described and risks presented by different pathways should be identified.

Seed

The probability of *T. indica* entering the PRA area on seed of *T. aestivum* and *T. durum* imported from countries where *T. indica* occurs is moderate. Although pathways of entry exist in theory, in practice seed should be produced in areas of these countries where the pathogen is known not to occur. Poor inspection and testing methods in areas of these countries where *T. indica* is known to occur increases the risk of undetected spread of *T. indica* to the pest-free areas. This obviously increases the risk that the pathogen will be present on seed arising in the so-called pest-free areas. Limited import data are available for seed of 'wheat' (not broken-down) and only for the UK but some of the imported material was grown in affected countries (India and the USA). Pathways of entry to other EU countries may exist. The only recent available data on seed imports for triticale is also for the UK and this material originated in Poland. As with seed of wheat, pathways of entry on seed of triticale to other EU countries may also exist where the seed was produced in affected countries.

Grain

The probability of *T. indica* entering the PRA area is moderately likely on grain of *T. aestivum* and *T. durum* originating in the countries where the pathogen occurs. In countries where *T. indica* is known to occur, grain destined for export to the EU must either be grown in an area where *T. indica* is known not to occur or no symptoms of the pathogen must have been observed in the crop. For both situations, the grain should be sampled at harvest and pre-shipment and tested for teliospores of the pathogen prior to export. If this is not done, as is the case in the USA, or is not done effectively, there is potential for *T. indica* to enter the PRA area on exports arising in these countries.

There is a lack of import data on triticale. Records of the pathogen on this host are also few and far between. It can only be assumed that imports of grain or seed of this cereal originating in countries where *T. indica* occurs pose a risk of entry.

The most likely countries from which entry is possible are the USA, India and Mexico based upon volume of trade and interception data. However, where any of the other affected countries are found to export these commodities then despite the EC requirements for pathogen freedom there may still be a risk of entry, depending upon the stringency of the methods that are used by the authority responsible for certifying exports.

Probability of Establishment

Availability of suitable hosts or suitable habitats, alternative hosts and vectors in the PRA area

1.16. Specify the host plant species (for pests directly affecting plants) or suitable habitats (for non parasitic plants) present in the PRA area?

The natural host plants of *T. indica* are bread/feed wheat (*T. aestivum*) and durum wheat (*T. durum*), as well as triticale (x *Triticosecale*).

Despite being one of the named regulated hosts in the EC Plant Health Directive (Anon., 2000), rye (*Secale cereale*) is no longer considered to be a natural host. This is because published data on susceptibility are now recognised as referring to experimental susceptibility determined when screening for sources of resistance to *T. indica* for breeding programmes (Warham, 1988). In addition, Nagarajan *et al.* (1997) refer to pathogenicity to *Triticum boeoticum*, *Triticum ovatum*, *Triticum variabilis* and *Triticum sharonensis*. Peterson and Bonde (1998) found that a cultivar of annual ryegrass (*Lolium multiflorum*) was experimentally susceptible to *T. indica*. Seven cultivars of emmer wheat (*Triticum dicoccum*), a species having an expanding niche-market in some areas of Italy, have also been shown to be experimentally susceptible to *T. indica* by boot inoculation with sporidia (Riccioni, ISPaVe, Italy, *unpublished*, 2005). However there appear to be no reports of natural outbreaks on any of these species and they are not considered further in this PRA.

1.17. How widespread are the host plants or suitable habitats in the PRA area? (specify)

Very limited, limited, moderately widely, widely, very widely

Very widely.

Wheat (not broken down into wheat type; i.e., grain or seed, or, bread/feed or durum) and triticale production (area harvested in hectares) in 2004 for the twenty-five EU Member States are presented in Table 3.

Table 3. Wheat and triticale production (area harvested – hectares) in descending order of productivity by EU Member State in 2004.

Country	Wheat area harvested (ha)	Country	Triticale area harvested (ha)
France	5231000	Poland	710000
Germany	3101000	Germany	505000
Poland	2600000	France	328000
Italy	2300000	Hungary	157000
Spain	2179000	Czech Republic	62776
United Kingdom	1991000	Lithuania	60000
Hungary	1173000	Sweden	52615
Czech Republic	863161	Denmark	40000
Greece	850000	Austria	39000
Denmark	675000	Spain	29900
Sweden	404137	Slovakia	18400
Slovakia	369400	Latvia	15000
Lithuania	360200	Portugal	15000
Austria	285000	United Kingdom	15000
Finland	235300	Belgium	8500
Belgium	220000	Estonia	6500
Portugal	200000	Netherlands	4500
Latvia	166000	Luxembourg	3800
Netherlands	135000	Slovenia	2000
Ireland	96000	Italy	0
Estonia	76200	TOTAL	2072991
Slovenia	34000		
Luxembourg	11000		
Cyprus	6000		
Malta	2300		
TOTAL	23563698		

Provisional 2004 data. Source FAOSTAT, 2004 (last updated 20 December 2004).

Details of geographic distribution by country are not readily available but clearly these crops are grown across a wide area of the EU. In 2004, all twenty-five Member States grew wheat and twenty grew triticale.

1.18. If an alternate host is needed to complete the life cycle, how widespread are alternate host plants in the PRA area (not relevant for plants).

N/A, absent, limited, moderately widely, widely, very widely

Not applicable.

1.19. If the pest requires another species for critical stages in its life cycle such as transmission, (e.g. vectors), growth (e.g. root symbionts), reproduction (e.g. pollinators) or spread (e.g. seed dispersers) how likely is the pest to become associated with such species?

Note: Is the species present in the PRA area, could it be introduced or could another species be found?

N/A, very unlikely, unlikely, moderately likely, likely, very likely

Not applicable.

Suitability of the environment

Specify the area where host plants (for pests directly affecting plants) or suitable habitats (for non-parasitic plants) are present (cf. QQ 1.16-1.19). This is the area for which the environment is to be assessed in this section. If this area is much smaller than the PRA area, this fact will be used in defining the endangered area.

1.20. How similar are the climatic conditions that would affect pest establishment, in the PRA area and in the area of current distribution?

Note: the climatic conditions in the PRA area to be considered may include those in protected cultivation. When comparing climates in a pest's current distribution with those in the PRA area, it is important to ensure that, as far as possible, the variables selected are relevant to the pest's ability to exploit conditions when these are favourable for growth and reproduction and to survive unfavourable periods, such as those of extreme cold, heat, wetness or drought.

Not similar, slightly similar, moderately similar, largely similar, completely similar.

Largely similar for the climatic conditions that affect pest establishment.

Although the areas of the world where *T. indica* successfully completes its life cycle resulting in outbreaks of Karnal bunt have pronounced rainy and dry seasons and no prolonged cold season, the key comparison is between conditions during the period when the wheat crop is grown. In arid areas, the wheat crop is often irrigated so rainfall and relative humidity data from weather stations may not always be relevant. For successful establishment in an area, not only must the pathogen life cycle be synchronised with the host but, in addition, the climatic conditions during the key vulnerable infection periods of host development must also be suitable. The timing of the vulnerable periods for host infection and the corresponding climatic conditions have been analysed in detail by the EU Project. Outside the period when wheat is grown, climate will also influence spore survival, dormancy and germination.

The similarity of climatic conditions between areas where *T. indica* occurs and the PRA area requires an in-depth consideration of the likely timing of the life cycle of the pathogen in relation to the phenology of the host crops, all of which depends upon local climatic conditions. Much is known about *T. indica* in its current geographical range. The Project has generated data that allows a better understanding of the likely behaviour of the pathogen in the PRA area than was previously possible.

An in-depth analysis of these aspects supported by experimental data on the likely timing of the life cycle of *T. indica* in the PRA area has been conducted to develop the PRA for the EU. Part of the answer to question 1.28 '*How likely is the reproductive strategy of the pest and the duration of its life cycle to aid establishment?*' cannot be dealt with separately because host phenology, pathogen life cycle including its reproductive strategy and climate are intrinsically linked. For this reason question 1.20 and **part** of 1.28 are dealt with here.

Current distribution of *T. indica*

The pathogen was first formally reported in the Karnal district of northern India in 1930 (Mitra, 1931). All countries for which there is a record of the pathogen are given below.

- North America:** USA – first reported in 1996 (Ykema *et al.*, 1996) and present since then in the states of Arizona, California and Texas.
- Central America:** Mexico - first detected in the Yaqui and Mayo Valleys of Sonora during the 1969-1970 crop season (Fuentes-Dávila, 1996a) although Durán (1972) refers to the first record being on *T. aestivum* in February 1971 in Cajeme, Sonora. According to Mexican legislation (Anon., 1996 and Anon., 2002) the disease is now established in much of Sonora and Sinaloa States as well as in Comondu, in the State of Baja California Sur. Regarding the current status in Mexico, according to the USDA (Anon., 2005) there are now 5 Mexican states that are '*Karnal bunt free*' - Chihuahua, Guanajuato, Jalisco, Michoacan and Queretaro. In addition, according to this document, the Mexicali Valley in Sonora and the State of Baja California were declared Karnal bunt free in 1998. A personal communication from G. Peterson, USDA- ARS, USA, 2005 follows: '*The Mexicali Valley [in Sonora] has been intensively surveyed for T. indica teliospores and to my knowledge has remained free of disease. Some T. indica was detected on the southern most tip of the [state of] Baja a number of years ago, but based on survey data declared T. indica free in 1998.*' It should be noted that in 1998 Italy intercepted *T. indica* on two consignments of grain of *T. durum* from Mexico (Anon., 1998).
- South America:** Brazil – present since at least 1989 in the southern part of the Rio Grande do Sul (Da Luz *et al.*, 1993). No further details.
- Caribbean:** Absent - no records.
- Europe:** Absent - no records.
- Africa:** South Africa – first found in Douglas, Northern Cape Province in December 2000 (Crous *et al.*, 2001) and since found to be present in Douglas, Koffiefontein (Naudé, 2002) and Douglas, Herbert, Hopetown and Prieska (Anon., 2004a).
- Asia:** Afghanistan - present, no details (Joshi *et al.*, 1983).
 India - present since 1930 and widespread across north-western India (Nagarajan *et al.*, 1997).
 Iran - present since 1996 (Torarbi *et al.*, 1996).
 Iraq - present, no details (Joshi *et al.*, 1983).
 Nepal –first identified in the Doti district of Nepal during 1986-87 (Singh *et al.*, 1989).
 Pakistan – present. First found in the Sind Province in 1941 (Singh, 1994).
- Australasia:** Absent – no records.

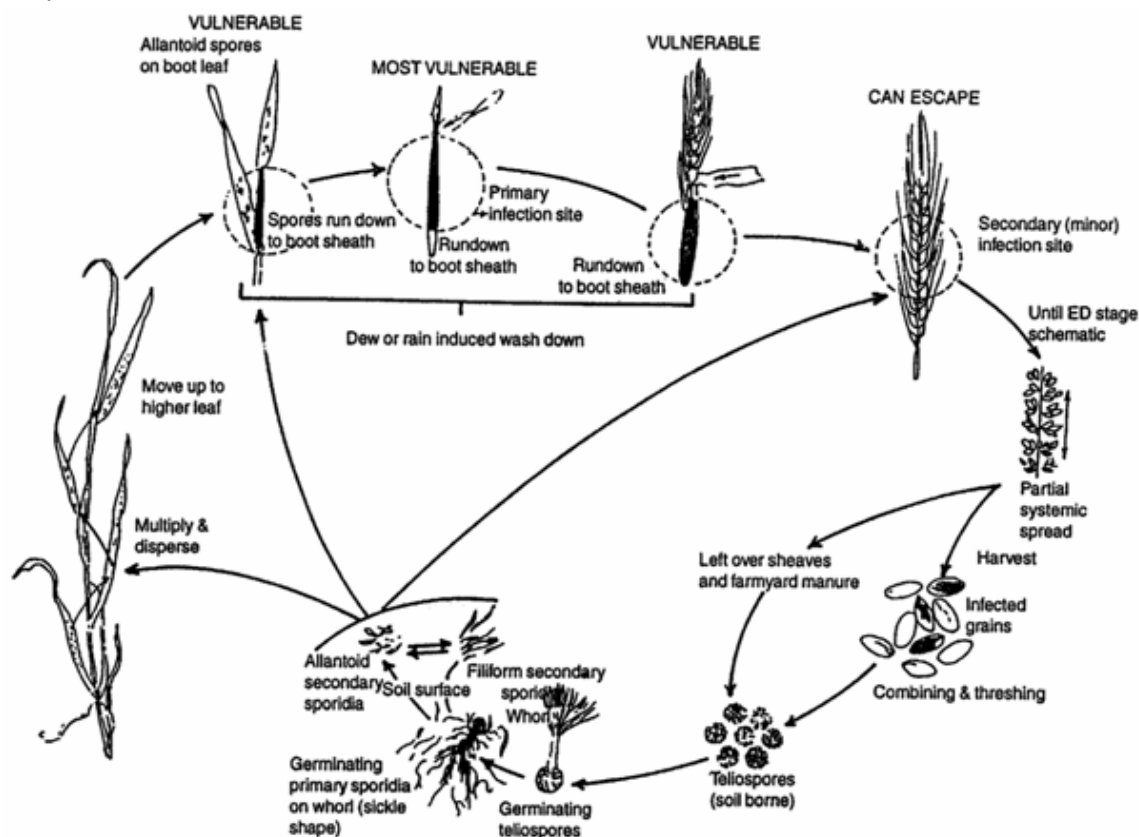
Life cycle

The full life cycle of the pathogen has been described in many publications and in earlier versions of the UK PRA (Sansford, 1996 *unpublished*, Sansford, 1998, Sansford, 2004) and therefore is only briefly described here. For the most recent published review of this pathogen see Nagarajan *et al.*, 1997. Murray (2004, *unpublished*) has reviewed the life cycle in association with published scientific data and the experimental work of this Project as well as the existing published disease models. Further comment is provided in Murray's report on the approach used in this Project for predicting the risk of establishment of *T. indica* in European wheat crops and a summary is provided below. A diagrammatic representation of the life cycle is presented in Figure 4 and more detail of specific aspects of the reproductive strategy of *T. indica* is provided under 1.28.

Karnal bunt of wheat is a monocyclic disease. The pathogen is soil-borne and seed-borne in the form of teliospores but it is not directly transmitted from the seed to the plant. The pathogen is airborne in the form of sporidia (short-distance, splash-dispersed or wind-blown spores) or teliospores (longer distance dispersal possible when liberated during harvest or during stubble-burning - where this is permitted). The life cycle commences when teliospores, present on or very near to the soil surface (Smilanick *et al.*, 1985), germinate and produce primary sporidia (basidiospores). Fresh teliospores are reported to have a period of dormancy before they will germinate (Nagarajan *et al.*, 1997). The length of this period varies in the literature. Primary sporidia germinate to produce mycelium on plant or other surfaces, which in turn produces secondary sporidia (allantoid or filiform types). Secondary sporidia are splashed by rain or blown by wind; they may germinate to produce new mycelial colonies giving rise to more secondary sporidia, this can occur in a cyclical manner. If the infective allantoid sporidia are deposited onto the flag leaf and washed into the boot cavity, or deposited directly on the emerging ear of wheat plants at the susceptible stage for infection (broadly defined as at the '*heading*' stage here for simplicity but dealt with in more detail below), under suitable climatic conditions, the pathogen can infect the developing grain through the glumes. Warham (1986) indicated that low temperatures and high humidity are necessary at '*flowering time*' for infection to occur, while dry weather, high temperatures and bright sunshine are unfavourable.

The disease typically develops in only some of the grains in the ear, and each grain usually only becomes partially bunted. At harvest, teliospores are usually dispersed locally from bunted grain by the mechanical action of harvesting; bunted grain itself will also act as a vehicle for long-distance dispersal of inoculum in trade. The teliospores or bunted grain become deposited in the soil, in the harvesting machinery, and, they can adhere to the surface of healthy grains as an external contaminant. Inoculum can be further transported in soil or in/on grain or seed on farm machinery and vehicles used for grain or seed transportation, or even by wind. The ingestion of infected or contaminated grain by livestock can also lead to the dispersal of teliospore inoculum in animal faeces/manure. Animals can act as a vehicle for surface contamination by teliospores, facilitating spread. In countries where the pathogen occurs, teliospores can survive in the soil for up to five years (Agarwal *et al.*, 1993) and it has been suggested that they are likely to be able to survive in most of the winter wheat-growing areas of the world (Zhang *et al.*, 1984). Longevity in field conditions in Europe has been studied for the first time in this Project and is described further below.

Figure 4. Disease cycle of Karnal bunt (from Nagarajan *et al.*, 1997), reproduced with permission of CABI.



The epidemiology of Karnal bunt was discussed in the first UK PRA in 1996 (Sansford, 1996 *unpublished*, Sansford, 1998) and later in the revised UK PRA (Sansford, 2004). As with the UK, prediction of the likelihood of the pathogen completing its life cycle and establishing in wheat crops in the EU depends on:

- A suitable pathway between origin and destination;
- the presence of susceptible host crops at the destination;
- the ability of the pathogen to survive between crops; and
- the ability of the over-wintering phase of the pathogen (the teliospores) to produce infective sporidia at the vulnerable growth stages for infection and under appropriate conditions to infect and cause disease, thus perpetuating the pathogen.

The first UK PRA considered each of these factors (amongst others) and concluded that there were no limiting factors preventing the establishment of the pathogen in the UK.

Host infection window and climate: early predictions for determining the risk of establishment

In predicting the risk of establishment for the UK and for the EU, because the timing of the occurrence of the susceptible phenological growth stages for infection of wheat will differ between countries, and between cultivars, a direct month-for-month match of climate between areas where the pathogen is established and the PRA area is inappropriate. Climate matching is the simplest method of predicting the suitability of a climate when information on the biology of an

organism is limited, but its geographic distribution is known (Baker, 1994). Clearly much detailed information is available on the biology of *T. indica* within its current geographic range and new data have been generated for the European context. These data have been used to their full potential in this PRA.

Because the infection window is relatively narrow, Royer (1990) found that simple climate matching using CLIMEX (Sutherst and Maywald, 1985) failed to account for the short period at the 'beginning of heading' for wheat and consequently failed to predict areas where the climate is known to be favourable i.e. where the disease is endemic.

A more refined approach is to compare mean monthly climatic data (rainfall, temperature and relative humidity) during the months when the susceptible phenological growth stages of wheat occur. This was done in the original PRA for the UK (Sansford, 1996, *unpublished*; Sansford, 1998). It was shown that during the months when wheat is in ear (broadly considered at that time to be February and March in India and May and June for the UK) there were similar climatic conditions. Comparing four UK meteorological stations (Birmingham, Cambridge, Exeter and Hull) with five Indian stations (Jammu, Dehli, Ludhiana, Alipore and Dum-Dum) it was shown that during this period, rainfall for the UK fell within the range for the Indian meteorological stations investigated. For Birmingham and Exeter in May and June the rainfall was higher than the rainfall in either February or March for any of the Indian stations. Of the Indian stations, Jammu had the highest rainfall in February and March and was the most similar to the UK stations. Rainfall in India is high (much higher than the UK) between June and September, but the wheat crop is harvested by the end of May so this falls beyond the infection window for *T. indica*. Mean daily air temperatures (mean of the minimum and maximum) were higher throughout the year in India but during the infection window all of the UK temperatures were similar to those of Jammu in the state of Jammu and Kashmir. Jammu is an area of India where Karnal bunt is known to be extremely severe. The range of mean daily % relative humidity values in the UK (mean of 15.00hrs and 09.00hrs) fell within those of the Indian stations being higher in the UK than four of the Indian stations and lower than only one of the stations (Dum-Dum).

EU Project: Research into elements of the life cycle of *T. indica* under European conditions

In support of the development of the assessment of establishment risk of *T. indica* in European wheat crops, experimental work within the Project assessed:

- The susceptibility of European wheat cultivars.
- The ability of teliospores of the pathogen to survive between crops under European conditions under quarantine containment.
- The ability of the teliospores under European conditions to be capable of germinating to produce infective sporidia during the period when European wheat cultivars have been determined by experimentation to be susceptible to infection
- The annual frequency of appropriate climatic conditions for infection of the crop and subsequent disease development. The Project also determined the maximum gap between favourable years.

The following represents a summary of the experimental results from the Project. Further details and a fuller review of the related literature are dealt with within the discussion sections of each of the Project's Deliverable Reports.

Host factors: Timing of the susceptible growth stages of wheat to infection by T. indica and European wheat cultivar susceptibility

Unpublished data from this Project (Magnus *et al.*, 2004a, *unpublished*) using European winter, spring and durum wheat cultivars and the known susceptible Indian spring wheat cultivar WL-711 as the control, as well as the existing scientific literature (e.g. Nagarajan *et al.*, 1997), suggest that the susceptible period for infection of wheat is Zadok's growth stage (GS) 43 (boot just visibly swollen) to GS 69 (anthesis complete). However, the most likely timing in the field is from GS 47/49 (flag sheath opening to first awns visible) to GS 55 (half of inflorescence emerged) depending upon environmental conditions. During this period sporidia washed down into the flag leaf sheath are most likely to find favourable conditions for infection. Once the ear is fully exposed, conditions for infection are likely to be less favourable unless conditions of high moisture predominate (rainfall and high humidity) during flowering. The potential 'window' of growth stages which are susceptible to infection may be longer for some cultivars than others and the duration of this period will vary with the season. Almost all European winter, spring and durum wheat cultivars tested were shown to be physiologically susceptible to *T. indica* by artificial inoculation of the boot cavity (Porta-Puglia *et al.*, 2002, *unpublished*). The range of susceptibilities was similar to wheat cultivars grown in countries such as India and Mexico where *T. indica* is established. Many European winter, spring and durum wheat cultivars were shown to be morphologically susceptible to infection (field susceptibility determined by spray inoculation of the ear) both in the Project (Riccioni *et al.*, 2004, *unpublished*) and for European winter wheat cultivars in other similar work in the UK and the USA (Inman, CSL, UK, *personal communication*, 2000; Peterson and Creager, 2000).

Pathogen factors: Survival of teliospores of T. indica under European conditions

Summarising the results from experimental work in the Project conducted in Italy, Norway and the UK investigating teliospore survival in Europe, teliospores of *T. indica* have been shown to survive at depths of 5, 10 and 20 cm after a range of 1-, 2- and 3- year burial periods in European soils in the field under quarantine containment. (Buried in 2000, 2001 and 2002 at approximately wheat harvest time and sampled at yearly intervals between 2001 and 2003). As the pathogen can survive for at least one year and the data suggest that survival may in fact exceed three years this supports the potential for *T. indica* to establish in cereal-growing areas of the EU. Some of the results from this part of the Project were published in 2002 (Valvassori *et al.*, 2002). Full details of the experimental work and results are reported in Inman *et al.* (2004, *unpublished*).

Experimental results from the Project also showed that a proportion of intact teliospores (*ca.* up to 50%) recovered from the containment cylinders was capable of germination under suitable conditions when brought to the soil surface after 1–3 years of burial. The timing of germination of the teliospores in the remaining 50% portion in relation to the important wheat phenological growth stages for infection is critical for the potential establishment of *T. indica* and the development of disease and is discussed below.

The results of this European study agree broadly with those reported in Indian and US studies in that teliospores can survive in soil for several years. As described in a recently-reported study under quarantine containment in Montana, USA (Babadoost *et al.*, 2004), there was evidence for a decline in teliospore numbers within the first year of burial in European soil conditions, but then very little decline over subsequent years. The Project data suggest that teliospores which survive for one year are likely to survive significantly beyond three years. In contrast, Indian studies predict a steady decline in teliospore survival over time when buried in soil, and that maximum survival is unlikely to exceed 3–4 years under Indian soil conditions (Krishna and Singh, 1983; Rattan and Aujla, 1990;

Chib *et al.*, 1990). In general, teliospore recovery (% of those buried that were retrieved intact) from European sites was usually greater after 3-years' incubation (61%, 30% and 12% mean recovery for UK, Italian and Norwegian sites) than similar studies carried out in Montana, USA (13%; Babadoost *et al.*, 2004) and Arizona, USA (23%; Bonde *et al.*, 2004a) over similar periods. Similarly, teliospore germination (percentage of recovered spores that germinated) after burial for 3 years on recovery (T_0) and after 3 months storage in the laboratory (T_3) was typically greater at the European sites (UK: $T_0 = 31\%$; $T_3 = 33\%$), (Italy: $T_0 = 36\%$; $T_3 = 29\%$), (Norway: $T_0 = 19\%$; $T_3 = 49\%$) than that reported in the Montana, USA study (17% mean germination after 32 months burial; Babadoost *et al.*, 2004) and the Arizona, USA study (4% and 14% mean germination for irrigated and non-irrigated plots respectively after 48 months burial; Bonde *et al.*, 2004a). However, it is interesting to note that mean teliospore recovery and germination was very similar between the Norwegian site and the Montana site after a 3-year incubation period, despite differences in climate and soil conditions. Zhang *et al.* (1984) suggest that teliospores of *T. indica* can probably survive in most of the winter wheat-growing area of the world.

Timing of teliospore germination relative to the susceptible growth stages of European wheat cultivars.

Detailed investigations have been undertaken in this Project to determine the timing of teliospore germination during the wheat-growing period in a range of European climates. Full details of the experimental work and results will be reported in Peterson and Leth (2005, *unpublished*). Results suggest that teliospores would be likely to germinate in most soil types and under a range of soil moistures and temperatures experienced in European countries. (See 1.21 for further detail).

Utilising five years of available soil surface and air temperature data from the time of regional autumn planting of wheat until anthesis, obtained from wheat growing regions of Sonora, a Karnal bunt-affected area in Mexico, as well as the UK, Norway, Italy and Hungary, investigations were conducted in quarantine containment growth rooms into the likely timing of teliospore germination on the soil surface under minimum, optimum and fluctuating soil moisture conditions needed for teliospore germination (moisture conditions derived from separate experiments).

Minimum/maximum temperatures were achieved via a computer controlled ramping programme to simulate a gradual rise and lowering of temperatures between minimum and maximum over the course of a 24-hour period throughout the specified period of the wheat crop phenology for each location.

Results for treatments where soil moisture was held at a constant 25% (optimum moisture for teliospore germination) showed that there was a significant drop in the number of teliospores on the soil surface available for infection at the susceptible period of infection of wheat due to early germination, except under Norwegian conditions, where sub-freezing temperatures maintained a large proportion of the teliospores that were still viable at heading time, and which were available for germination to produce infective sporidia. Results also showed however, that in all locations, despite the presence of conducive germination conditions, a small proportion of teliospores continued to germinate on the soil surface through the simulated flowering stage of wheat, demonstrating differing degrees of physiological dormancy within the population. At all locations, teliospores held at the minimum soil moisture level for germination (10%) showed that a significant number of teliospores remained ungerminated throughout the length of the profile and would be available to germinate and produce infective sporidia at the time of anthesis. Teliospores subjected to weekly periods of drying under profiles similar to UK, Hungarian and Italian climatic conditions showed a high level of teliospore recovery throughout the experiment but a significant drop in

viability over time. Those exposed to Mexican and Norwegian profiles also showed a high teliospore recovery rate throughout the experiment, but a significant level of spore viability during the entire length of the experiment. In this study, the data also indicated that, when compared to those results obtained from the Mexican profiles, Italy would be an area with the greatest establishment potential for *T. indica*.

The data obtained from these studies highlights two factors that can result in early decline of potential inoculum at the soil surface. These are either the occurrence of soil moisture conditions conducive to early teliospore germination and/or a decline in teliospore viability observed when soil moisture conditions fluctuate over the season. Results suggest that in most regions of the EU, a significant number of viable teliospores on the soil surface would be eliminated prior to the window of infection. However, some level of inoculum would remain and would be available at the susceptible period of infection for the European wheat crop, thus supporting the potential establishment of *T. indica* in the PRA area.

It should also be noted that there will always be uncertainty regarding the spread of dormancy within a population of teliospores. This will affect the prediction of the timing of teliospore germination in relation the wheat crop.

Suitability of European climates for infection of wheat by T. indica and disease development

For successful infection to occur, the teliospores of *T. indica* must germinate to produce sporidial inoculum in time for infection at the susceptible stages of the wheat crop. This stage of the life cycle of *T. indica* cannot be investigated under natural conditions in the EU because it would require the placement of teliospores of the pathogen on or near the soil surface of a wheat crop without quarantine containment, and the potential release of sporidia. While the pathogen remains a regulated quarantine pest for the EU this is not possible. Similarly, no scientist has managed to get infection of wheat to occur under quarantine containment conditions by placing teliospores on or near to the soil surface of pot-grown wheat plants. For this reason, an examination of the climatic conditions during this period in the EU was needed to aid the prediction of the risk of establishment (given that all of the other parts of the life cycle prior to this stage had been investigated under quarantine containment).

The important growth stages for infection of European wheat cultivars determined from this Project and from other literature e.g. Nagarajan *et al.*, (1997), appear to be GS (43) – 45 – 61 - (69). Teliospores must germinate on or near the soil surface at about GS 37 (commencement of flag leaf emergence) to enable production of sporidial inoculum in time for infection at the critical growth stages. Should viable infective allantoid sporidia be present at this stage then they can infect the floret tissue via the stomata. The critical stages for infection by *T. indica* in the field are from GS 45 to 55, with teliospore germination needing to occur by GS 37 and to continue, in order to provide secondary sporidia for infection, up until the latest susceptible stage of GS 69.

Secondary sporidia are thin-walled, hyaline, and are thought to require high relative humidity to survive longer than a few hours. Up until 2005, the only published data that were available on the longevity of secondary sporidia of *T. indica* suggested that they could not survive for more than 14 hours even at 95% relative humidity (Smilanick *et al.*, 1989). Subsequent to the completion of the experimental work in this Project, *in vitro* studies (Goates, 2005) suggested that secondary sporidia of *T. indica* are potentially more durable than previously thought under conditions of low humidity. Dried sporidia were found to have the ability to regenerate after 30 days at 10-20 % relative humidity and 60 days at 40-50% relative humidity. The upper limits of regeneration were not

determined. Although *T. indica* was not studied under field conditions, this preliminary work suggests that teliospores that germinate some time in advance of the host susceptibility period might produce inoculum that could regenerate during the susceptible period of the host under the favourable climatic conditions usually associated with infection and disease development. Further investigation is required into this part of the life cycle. This would include a study of the effects of ultra-violet light within the crop canopy.

With respect to the suitability of the climate in the PRA area, as stated previously, Warham (1986) (in a review of the literature) concluded that low temperatures and high humidity are necessary at wheat '*flowering time*' (anthesis) for infection to occur, whilst dry weather, high temperatures and bright sunlight are unfavourable conditions for the infection process. Rainfall is necessary but rainfall on its own at flowering is not enough to cause infection, suggesting that a specific combination of climatic factors (e.g. rainfall, high relative humidity, certain temperatures) is required. Crop irrigation during this period is an additional factor that can favour disease (see 1.25). Taking these factors into account, the first UK PRA conducted in 1996 (Sansford, 1996 *unpublished*, Sansford, 1998) showed a clear risk of establishment in the PRA area based upon this information as part of a review of the literature, a comparison of individual climatic parameters (rainfall, air temperature and humidity) between locations in India where the disease occurs and the UK, as well as the use of the Humid Thermal Index (HTI) of Jhorar *et al.*, (1992) as described below.

Jhorar *et al.*, (1992) modelled the disease, Karnal bunt, in the central Punjab, India, by an empirical method. A study of the relationships between '*plant disease intensity*' (a classification based on incidence and severity in harvested grain) of Karnal bunt in wheat and meteorological factors, using 19 years of historical meteorological data and data pertaining to disease intensity for Ludhiana district, in the central plain of the Punjab, was made for the reproductive stage of the crop. The period studied corresponded to flag leaf emergence (starting on the 12 February in Ludhiana) and subsequent stages (ending on 18 March). In this retrospective analysis, this corresponded to the most important period for the pathogen, when teliospores that germinated could lead to the production of epiphytic colonies and the generation of infective (allantoid) sporidia that survived to infect the host and for Karnal bunt to develop.

During the 6-week period that meteorological factors were analysed, the relationships between these and plant disease intensity were only found to be significant during the third, fourth and fifth week (specific growth stages of the wheat plant were not defined in this paper). Of all the factors analysed, the most significant relationship was found between disease intensity (DI) and the '*evening*' relative humidity (ERH), the maximum temperature (TMX) and the HTI.

A '*best-fit*' model was developed for forecasting the severity of Karnal bunt in the central Punjab thus:

$$\begin{aligned} (1) \text{ DI} &= -0.8 + 1.5\text{HTI} \\ (2) \text{ HTI} &= \text{ERH} \div \text{TMX} \end{aligned}$$

Where DI = Disease index rated from 1 to 4 (on the basis of Mavi *et al.*, 1992), HTI = Humid Thermal Index, ERH = '*Evening*' relative humidity recorded at 14.30 hrs (average of the third, fourth, and fifth reproductive weeks), TMX = Maximum temperature (average of third, fourth, and fifth reproductive weeks).

Jhorar *et al.*, (1992) found that the HTI during this part of the growing season varied between 1 and 5; the lowest values occurring in extremely dry and warm conditions, and the highest values representing extremely humid and cold weather, neither of which favour the disease. An HTI of 2.2 - 3.3 during the third and fourth week of the study period favoured the disease. These conditions, which occur as a result of frequent cloudiness and intermittent showers can be predicted, thus allowing a disease forecast to be made.

It is thought that the HTI covers the period from sporidial production through infection and the start of disease development. This index over the growth stages from boots just visibly swollen to medium milk in the grain ripening process (GS 43–75), has successfully predicted the extent of Karnal bunt development in the Punjab. Other models from India and Mexico show that rainfall at first awns visible to early ($\frac{1}{4}$) head emergence (GS 49–53) is particularly favourable for disease development (Nagarajan *et al.*, 1997).

The period beyond GS 75 is discussed in 1.21 under ‘*Teliosporogenesis*’.

Murray (2004, *unpublished*) assessed the applicability of four models, including the HTI, for their value in predicting the likely development of Karnal bunt in the European Union. The ideal pathogen model would estimate the sequential development of key stages of the life cycle of *T. indica*, taking into account wheat development, to simulate disease development. However, no such models exist.

In addition to the HTI (Model 1) the three other models considered by Murray were:

2. The Geophytopathology Index (Diekmann, 1993)
3. The Rainfall-Temperature Model (Smiley, 1997)
4. The Rainfall Model (Nagarajan *et al.*, 1997)

Models 1, 3 and 4 are derived from correlation relationships observed in the Indian Punjab between disease severity and weather factors. Multifactorial techniques analysing distribution data and average weather data were used to develop the second model.

Murray’s review of the models is given below (Murray, 2004 *unpublished*):

1. The Humid Thermal Index

During the period over which meteorological and disease data was gathered to develop the HTI, (which spanned 19 years), 80% of the wheat-growing area was planted with wheat cultivars susceptible to *T. indica*.

The HTI model is based on an ordinal disease index. This means that correlations calculated in support of the model need to be treated with caution because DI, the dependent variable, is ordinal rather than continuous with normal distribution. Thus, the probabilities associated with these correlations would not necessarily be those of normal data. However, Murray (2004, *unpublished*) concluded that the finding that severe Karnal bunt develops when the HTI lies between values of 2.2 and 3.3 is not affected by this.

The HTI model is used routinely in India to predict the likely levels of Karnal bunt at harvest each year in the Punjab using in-year weather data (Indu Sharma, *personal communication*).

The general success of the HTI to predict Karnal bunt levels in the Punjab suggests that conditions at heading are the most important variables controlling disease development in that environment. However, on one occasion, the HTI failed to predict Karnal bunt levels when another part of the disease cycle was not coordinated with crop development (Sharma and Nanda, 2003; described in more detail below). This suggests that a more refined model of the disease cycle is required to predict more accurately whether Karnal bunt can develop in other areas.

More detail on the applicability of the HTI to predicting the risk of establishment in the PRA area is given below.

2. The Geophytopathology Index

Diekmann (1993) used 'geophytopathology' techniques to develop a relationship between Karnal bunt presence/absence and (i) the difference between the average maximum and minimum temperature in the month of sowing; (ii) the mean daily minimum temperature in the coldest month of the year; and (iii) the mean daily maximum temperature at anthesis. Moisture-related parameters such as relative humidity, rainfall or soil moisture were not used but are critical in the life cycle of fungal plant pathogens such as *T. indica*. The method compared sites around the world where *T. indica* did and did not occur to develop the model but did not take into account whether the presence or absence of disease was because *T. indica* had or had not been introduced to the area. If the method had been applied to specific areas of India and neighbouring countries where there had been considerable time for the pathogen to reach its climate limits, the model would be more reliable. Because of these defects, it was not considered for use within this Project.

3. The Rainfall-Temperature Model

Smiley (1997) used published information to assess whether Karnal bunt could develop in the Pacific Northwest of the USA, an area where the disease is yet to be found. He developed criteria for infection to occur based on published Indian data and relationships: (i) measurable rain (>3 mm) had to occur on each of two or more successive days; (ii) at least 10 mm had to be collected within the two-day interval; and (iii) average daily relative humidity above the crop canopy must exceed 70% during both days. However, his paper does not state how these relationships were derived, which makes it difficult to show how valid it would be if used to help predict the risk of establishment of *T. indica* in the PRA area. He computed the proportion of times that these conditions were met during the heading interval for several sites in the Pacific Northwest of the USA, and concluded, '*it appears possible for T. indica to become established in selected regions*'.

The value of this model was its application to annual data to estimate the proportion of years that were favourable for Karnal bunt development. However, the model has not been validated for India or other locations where Karnal bunt is known to occur. Thus, its general applicability is unknown.

Stansbury and McKirdy (2002) compared the HTI model and their version of the Smiley model in the Western Australian wheat belt. Their '*Smiley*' model used the first two criteria (i and ii) but they were unable to obtain the relative humidity data to use Smiley's third criterion. Nevertheless, they found a close correlation between results from deploying the two models.

4. Rainfall Model

Rainfall and/or irrigation during the booting stage and ear emergence stages (GS 45–59) is necessary to allow the sporidia to develop on leaves, be washed into the leaf sheath and to infect the wheat

head (see Figure 4 and Nagarajan *et al.*, 1997). Total rainfall and number of rainy days during this two week period were highly correlated with the severity of Karnal bunt in north west India, allowing a model with R^2 of 0.89 to be developed (Nagarajan *et al.*, 1997). Rainfall and rainy days during this stage of wheat development were also highly correlated with disease severity for areas of Mexico where Karnal bunt develops, allowing a model with R^2 of 0.91 to be developed (Nagarajan *et al.*, 1997). However, the two models are location specific, containing different rainfall and rainy day parameters. In their present form they do not appear to be transferable to other locations.

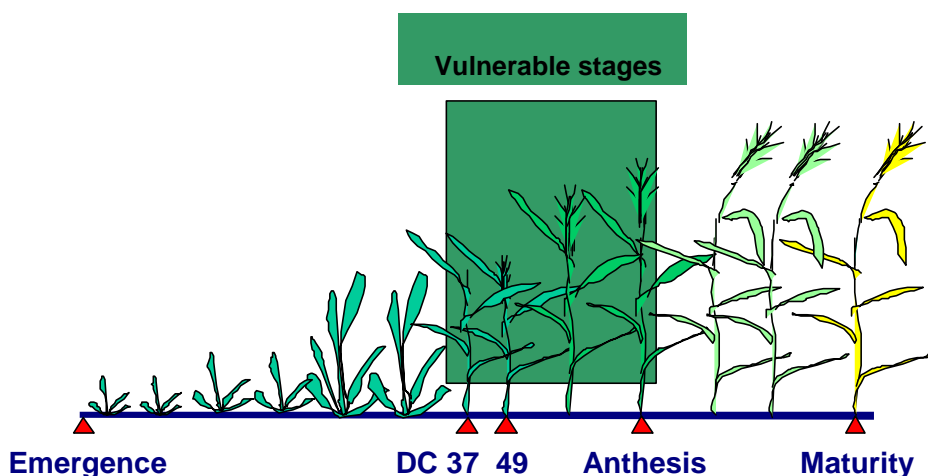
*Use of the Humid Thermal Index to aid the prediction of the risk of establishment of *T. indica* in the PRA area*

The HTI was found to be the most appropriate for helping to predict the risk of establishment of *T. indica* in the EU for the last stage of the life cycle that cannot be investigated under field conditions, i.e., covering the period from sporidial release from GS 37 to infection and the start of teliospore formation (production of sporogoneous hyphae) up until GS 75. It takes no account of other factors that may prevail in India where it was developed. However, Murray and Brennan (1998), in reviewing the use of Models 1, 2 and 3 for predicting the risk of establishment of *T. indica* in Australia had earlier concluded that the use of the HTI (Model 1) as deployed by Sansford (1996 *unpublished*; 1998) was the most appropriate for their intended purpose. The time period after GS 75 is discussed under 1.21, '*Teliosporogenesis*'.

It is recognised that various aspects of the pathogen's life cycle are not accounted for in detail by Model 1 or the other published models. However, the HTI is the best available to aid the prediction of the risk of establishment in areas where *T. indica* is not yet found. When combined with other biological data on teliospore survival, timing of teliospore germination, and host susceptibility under conditions prevailing in the area being considered as has been done within this Project a fuller picture of the likelihood of establishment in the PRA area has been obtained. As deployed within this Project, Murray (2004, *unpublished*) considered it to be the most suitable for use in estimating the potential for Karnal bunt to develop in Europe, assuming teliospores are available for germination prior to or at the susceptible period for infection. It is best used with annual data to estimate the proportion of years that are suitable for sporidial production from germinated teliospores, and for infection and the start of disease development. Stansbury and McKirdy (2002) support the view that when calculating the HTI it may not be appropriate to use long-term average data since it was originally constructed to predict disease severity by comparing disease incidence data with climatic data on an annual basis. Long-term average data are not sufficiently accurate when used to try to determine whether there is synchrony between a plant pathogen and its' host. This is why annual data has been used in this Project to help calculate the risk of establishment in the EU in individual years.

The HTI should normally be computed for the time of the year when wheat is between flag leaf emerging (GS 37) through heading/flowering until mid-milk (GS 75) (Figure 5). This time will vary with wheat maturation types and with seasonal conditions. The time will need to be estimated each year based on annual weather data.

Figure 5. Stages of development of wheat over which teliospores must germinate, sporidia infect the head and colonisation of seed begin (GS 37–75).



The HTI model assumes that teliospores germinate inside the ‘window’ required for successful infection of wheat. Data suggest that for infection to occur, teliospore germination to produce infective sporidia should coincide with the period when wheat is at the phenological stages of flag leaf emergence to heading. Models to estimate the timing of germination for teliospores are not available but it is likely that if teliospores are present on the soil surface they will germinate over a period of time (due to dormancy mechanisms intrinsic to *T. indica*) and some will germinate just prior to or during the susceptible period for infection leading to host infection. Work from the Project shows that under conditions experienced in the EU some level of teliospore inoculum will be present and capable of germinating to produce infective sporidia at this critical stage. *In vitro* work (Goates, 2005) suggests that secondary sporidia of *T. indica* are far more durable than previously thought. Dried sporidia have been shown to have the ability to regenerate after 30 days at 10-20 % relative humidity and 60 days at 40-50% relative humidity. The upper limits of regeneration were not determined. This provisional work suggests that teliospores that germinate some time in advance of the host susceptibility period might produce inoculum that could regenerate during the susceptible period of the host under the favourable climatic conditions usually associated with infection and disease development.

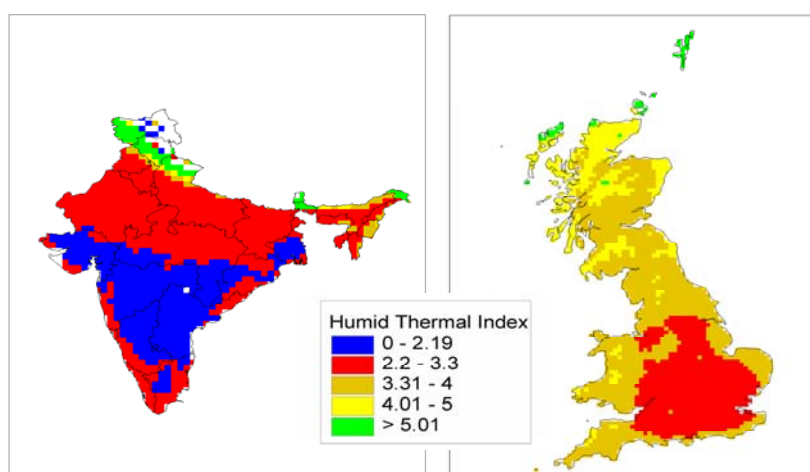
The HTI model is still used routinely in India to predict the likely levels of Karnal bunt at harvest each year in the Punjab. The model has been reliable except in one season when very little disease developed, although the model had predicted a high level. In that season, teliospores germinated during prolonged rain in December, about one month earlier than usual, and the sporidia failed to survive to infect wheat at heading in February despite conditions in February being favourable for disease development (Sharma and Nanda, 2003). An alternative theory might be that the pathogen failed to sustain itself through its epiphytic sporidial-producing colony stage.

In order to help predict the likely risk of the pathogen establishing in the PRA area, the 1996 UK PRA (Sansford, 1996 *unpublished*; Sansford, 1998) applied the HTI using long-term climatic data (1931–1960 mean monthly data) from individual meteorological stations in the UK and showed that conditions during the ‘heading’ period of wheat (broadly speaking May and June) were favourable for infection and disease development, i.e. the majority of calculated HTI values fell within the optimum 2.2 and 3.3 (i.e. favourable for infection and disease development). Kehlenbeck *et al.*, (1997) also calculated the HTI for the wheat-growing areas of Germany (mean monthly data over an unspecified period of time) and found that some of the southern areas had HTI values which also fell

within the optimum range. Murray and Brennan (1998) used this methodology (long-term mean monthly data over varying periods of time depending on location up until 1996) for Australia and found that of 122 sites tested within the Australian wheat belt, 67 had HTIs favourable for disease development. Stansbury and Pretorius (2001) used the HTI to predict that conditions at heading would be suitable for Karnal bunt to develop in some areas of South Africa.

The UK, German, Australian and South African studies were conducted using individual weather station data. To enhance the predictions for the UK, Baker *et al.*, (2000) undertook provisional climatic mapping using interpolated meteorological data (1961-90) with adjustments in the calculation to allow for the lack of mid-afternoon ('evening') relative humidity measurements. (See Figure 6). The results showed that for June, an area covering much of central and southern England had HTIs falling between 2.2 and 3.3 which was therefore considered, using this factor alone, favourable for disease development in wheat crops should they be phenologically susceptible at that time. The areas of India shown to have favourable HTI values were found to be those where Karnal bunt is known to occur. Crop phenology models were not used to enhance the predictions of risk in this study.

Figure 6. Mean HTI values (1961-1990) for India in March and Great Britain in June (Baker *et al.*, 2000).



All of these studies used long-term average monthly relative humidity and temperature data roughly approximating to the months of heading to calculate the HTI. This use differs from that in India where the HTI is applied to data within each year.

The timing of the life cycle of the pathogen will differ from country to country because the key susceptible growth stage of the wheat crop varies with time between countries. In order to conduct a more detailed assessment as to the extent to which the pathogen could establish itself in the PRA area, it was deemed necessary to compare more accurately the climatic conditions at key stages in the life cycle of the pathogen and the crop, on a country-by-country basis.

In this Project the potential infection of wheat in Europe from sporidial release at GS 37 through to infection up until GS 65 (mid-anthesis) was predicted using the HTI and two wheat phenology

models. It is not known whether by this latter stage, sorus formation would have commenced within the pericarp layer. However, sporogenic hyphal growth would be present and would be protected by the developing outer seed coat. The circumstances favouring teliospore formation are discussed under 1.21.

This work successfully combined: (a) experimental results on the developmental stages of wheat which are vulnerable to *T. indica*; (b) bread/feed and durum wheat phenology models to predict the timing of these developmental stages; (c) the potential for infection of wheat by *T. indica* and the commencement of disease development based on the HTI during this period. This was done using daily climatic data for several years as well as by evaluating the effect of sowing data and crop maturity class at low resolution across Europe and at high resolution for England, Wales, Denmark and three provinces in Italy.

Crop phenology modelling was deployed in the Project to identify whether there is overlap between the occurrences of the susceptible development phase and the meteorological conditions that have been identified as permitting *T. indica* infection and the commencement of disease development. Full details of the experimental work and results are reported in Porter *et al.* (2002, *unpublished*) and Baker *et al.* (2004, *unpublished*) and a summary of the findings is reported in Baker *et al.* (2005).

Two wheat phenology models, AFRCWHEAT (Weir *et al.*, 1984; Porter, 1993) and the IATA wheat development model (Miglietta, 1991, 1991a, 1991b), were applied to simulate the vulnerable growth stages for three cultivar maturity classes and three winter sowing dates of bread/feed and durum wheat.

Running the bread/feed wheat and durum wheat phenology models with daily meteorological data, enabled the timing of vulnerable wheat growth stages to be predicted throughout Europe annually for 1995–2002. To determine whether conditions were suitable for *T. indica* infection and disease development, the HTI was calculated for a large number of weather stations for the key crop development stages (GS 37–65) (flag leaf just visible to mid-anthesis), the earlier stage being used to account for the favourability of the climate for sporidial production from germinated teliospores (Figure 5).

By interpolating the results obtained at each between weather station, maps at low resolution for arable areas of the EU and at high resolution for selected areas of durum wheat (Tuscany, Marche, Puglia and Basilicata in Italy) and bread/feed wheat (Denmark, England and Wales) production zones were prepared, highlighting areas where HTI values fell within the critical range for teliospore germination, sporidial production, host infection and the start of disease development.

Consideration of the implication of the experimental results of the Project with the host (susceptibility studies) and the pathogen (teliospore survival and teliospore germination studies) was made to determine whether the mapping of the risk of establishment needed to be modified to reflect these.

As described earlier, European wheat cultivar susceptibility testing showed that there were no significant influences that required consideration in the deployment of the HTI. The period of European wheat morphological susceptibility determined for the Project (GS 45–69) and used in these predictions fits within and goes slightly beyond the period over which the HTI was run (GS 37–65). Because the range of susceptibilities to infection by *T. indica* for the European wheat cultivars tested had been found to be similar to those grown in Karnal bunt affected countries and as

cultivars grown in Europe would change from year to year, no modifications of the maps were required.

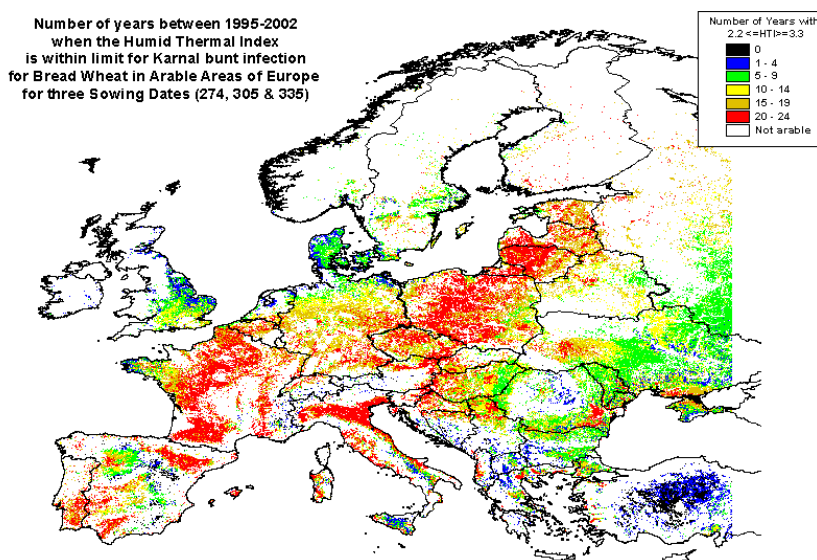
The studies of teliospore survival in EU soils in the Project showed that teliospores have the potential to survive for a minimum of three years in European soils as demonstrated at the containment sites in Italy, Norway and the UK. There will therefore be teliospores present between consecutive crops of wheat in continuous cropping situations and for periods of up to at least three years between susceptible wheat crops grown in rotation with non-wheat crops. Because of the time limitations of the Project, it has not been able to provide an upper limit to teliospore survival in the soil in the EU. However, trends in the survival data suggest that after an initial drop in the recovery of viable teliospores in the first year, a population is maintained with little decline in the next two years. The minimum survival period of 3 years for teliospores in European soils from the work of the Project has some implications for situations where the crop rotation chosen may be longer than 3 years between wheat crops. An analysis of the number of consecutive years during which the HTI was outside the critical 2.2–3.3 range showed that, in northern Europe (Denmark (82%), England and Wales (64%)), significant areas had gaps of at least four years, whereas, overall, 21% of European arable areas had gaps of over four years.

Work done to determine the timing of teliospore germination under European temperature profiles obtained from Italy, Hungary, Norway and the UK compared to Mexico, has shown that there will be some teliospores which will be capable of germination in European crops of wheat at the critical pre-infective period and thus these results have no further inferences for use in predicting establishment risk by the methods used in this Project. Indeed, Goates (2005) *in vitro* studies suggest that even if conditions are suitable for teliospores to germinate some time prior to this period there may still be some secondary sporidia capable of infecting the host.

Summary maps of the risk of infection and the commencement of disease development by *T. indica* for *T. aestivum* and *T. durum* at the European scale from Baker *et al.* (2004, *unpublished*) are shown below for agricultural land.

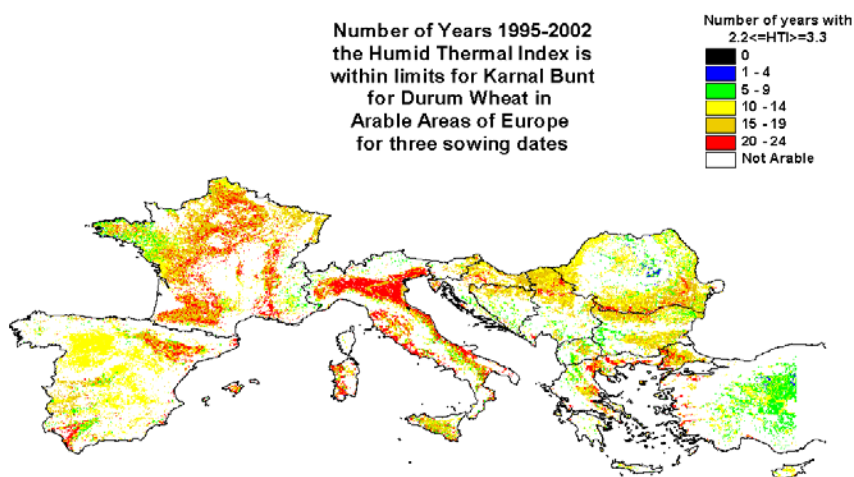
Based on predictions of the areas where the HTIs were between 2.2 and 3.3 during the critical development stages when wheat is vulnerable to infection, Figure 7 shows that in the major bread/feed wheat (*T. aestivum*) growing regions of Europe, namely western and central Europe, there is a very high probability of infection by *T. indica*. More northerly regions of Europe are predicted to have favourable HTI values in about one third of the cases studied (sowing date x years).

Figure 7. HTI values for infection of *T. aestivum* by *T. indica* and commencement of disease development for the years 1995–2002 for three sowing dates in Europe, where the HTI was calculated to be between 2.2–3.3 during the critical phenology period of the wheat crop. The maximum number of cases (referred to as ‘years’ in the figure) is three sowings x eight years, or 24 cases.



The predictions for durum wheat (*T. durum*) (Figure 8) show very high frequencies of critical HTI values for the northern Italian plain and the important pasta growing areas of Marche and Toscana. The Basilicata region in the foot of Italy seems to be less severely affected, possibly because the extremely high temperatures found in this region lower the HTI below its critical range. France and especially Spain seem less prone to critical HTIs for durum wheat; this may be related to the fact that durum wheat is generally sown later than winter wheat in these countries and thus may reach its susceptible phenological stages later, when conditions are drier and warmer, and so the HTI does not fall within the critical window in so many cases. Eastern Europe is also predicted to have a medium to high risk for infection of durum wheat, somewhat less than for northern Spain and central and eastern France.

Figure 8. HTI values for infection of *T. durum* by *T. indica* and commencement of disease development for the years 1995–2002 for three sowing dates in Europe, where the HTI was calculated to be between 2.2–3.3 during the critical phenology period of the wheat crop. The maximum number of cases (referred to as ‘years’ in the figure) is three sowings x eight years, or 24 cases.



For both bread/feed and durum wheat, sowing date and the choice of cultivars based upon their phenology (maturity class) have little effect on their potential vulnerability to infection.

Elevation was found not to have a great influence on the predictions of establishment risk.

However, climatic variation at the continental European scale is a strong determinant of the risk of establishment of *T. indica*. The risk of every year being favourable for the pathogen is lower further north and further west in Europe. Northern regions may on occasion be too cold and western regions too moist to favour infection and disease development. However, the fact that the pathogen has great longevity will most likely ensure that some level of inoculum remains present for the years when conditions are favourable for it to complete its life cycle.

The work undertaken to aid the determination of the risk of establishment concludes that there is a substantial risk of infection by *T. indica* and the commencement of disease development in most years in the EU for both durum and bread/feed wheat. This work supports the findings of the first UK PRA for *T. indica* (Sansford, 1996 *unpublished*, 1998).

Interestingly, NAPPO (2001) developed a model to attempt to predict which areas of North America were at risk using a phenology model to predict wheat anthesis. However, in deploying climatic data, they incorporated temperature regimes consistent with the work of Jhorar *et al.*, (1992) but they did not use humidity because they said the data were unavailable. This may be the reason why the model failed to predict the most northerly findings of Kernal bunt in Texas as well as failing to show the areas of Mexico where the disease occurs (Sinaloa and Sonora) as being at high risk. Relative humidity data for the USA can be obtained from sources such as the United States National Climate Data Center (Baker, CSL, UK, *personal communication*, 2005).

1.21. How similar are other abiotic factors that would affect pest establishment, in the PRA area and in the current area of distribution?

Note: the major abiotic factor to be considered is soil type; others are, for example, environmental pollution, topography/orography.

Not similar, slightly similar, moderately similar, largely similar, completely similar

Largely similar.

As demonstrated in some US and Indian studies, soil type may have an influence on the risk of establishment in as much as it can influence the survival of teliospores and teliospore germination. However, it is difficult to separate the effects of soil type from soil moisture and temperature (as well as burial depth) when reviewing the experimental results from the Project as well as those published in the scientific literature.

Teliospore survival

Results from the Project showed that teliospores survived for at least 36 months buried at 5, 10 and 20 cm in soils of different types at single locations in the field under quarantine containment in Italy (sandy clay loam), Norway (sandy loam) and the United Kingdom (clay); depth of burial did not affect survival. Thus, survival in soil does not seem to be a limiting factor for survival of *T. indica* between successive wheat crops in the range of European conditions studied.

Varying effects on teliospore survival have been reported in the literature for depth of teliospore burial, temperature, soil type and moisture content. There is some variability in the methods of reporting survival that can be measured both by the proportion of buried teliospores that remain intact after a period of burial (recovery), as well as the ability of the recovered teliospores to germinate (terms used vary). Depending upon the time period after recovery that teliospore germination is tested there may be some influence of dormancy on the results.

Babadoost *et al.* (2004) infested soils collected from 4 locations with teliospores: the soils were two silty clay loams, a loam, and a silt loam. These were placed in sealed tubes and buried in the field, in a silty clay loam soil under quarantine containment in Montana to investigate survival in a range of soil types in a Karnal bunt-free, cold, northerly area of the USA (snow cover from November to April and periods when soil temperature dipped below freezing). Initially, the mean recovery (% of those that were buried and then were retrieved intact) of teliospores declined rapidly from 90.2% on day 1 to 18.7% at month 8, but thereafter remained relatively constant with 13.3% being recovered after 32 months. The percentage of the recovered teliospores that were capable of germination on recovery (survival) similarly declined rapidly from 51.3% on day 1 to 15.1% at month 8, but remained at 16.5% after 32 months. Recovery and survival were unaffected by depth of burial. However they found that teliospore recovery was greatest from a loam soil and least from a silt loam soil. Rattan and Aujla (1990) had earlier reported a similar effect of soil type on survival, with it being higher in loamy sand soil than in clay and sandy-loam soils.

Soil moisture content can affect teliospore survival as measured by germinability. Smilanick *et al.* (1989) found that germinability of teliospores increased slightly after 7 months burial in a sandy clay loam soil. However, only the germinability of spores buried in dry soil remained high after 22 months. Recent work by Bonde *et al.* (2004) has shown that survival rates vary between soils collected from different locations: during the first 2 years, viability declined more rapidly in fields in Kansas (silt clay loam) and Maryland (clay loam) than in Georgia (sand loam) or Arizona (sand

loam) in the USA while after 2 years, viability declined nearly equally. In the laboratory over 3 years, viability decreased significantly more rapidly in dry soil from Kansas or Maryland than in dry soil from Georgia or Arizona, while the viability of pure teliospores remained unchanged (Bonde *et al.*, 2004). The results of the US study of Bonde *et al.* (2004) show that physical soil type rather than other environmental factors may influence the survival of teliospores at different locations.

Thus, teliospores can survive for at least three years in most soil types, and longer under favourable conditions. Results from several experiments show that teliospores survive better in sandier soils than in clay soils. Overall, the results show that survival in soil does not seem to be a limiting factor for the establishment of *T. indica* in the PRA area under normal cropping patterns.

Teliospore germination

Peterson and Leth (2005) have conducted investigations of the effect of soil types, moisture and temperature on teliospore germination. Qualitative tests for germination of more than one-year old teliospores mixed into four soil types (sandy loam, sandy clay loam, clay loam and silty clay) at optimum germination temperature (16°C) showed that they could germinate when soil moisture ranged from 10 % to 45% (w/w) in the first three soil types and from 15% to 45% in silty clay. Teliospore germination started after one week and they could germinate in soil at 5, 10 and 25°C, but not at 30, 36 or 60°C. Temperature and soil moisture influenced the time at which germination commenced. Low temperatures around 0°C delayed germination but did not stop it, and dry soil held the teliospores in stasis until moisture levels returned to required levels. Germination of teliospores in soil was recorded within 58 days of incubation at 5°C and 20% soil moisture and 16°C and 35% soil moisture. Results suggest that teliospores would most likely survive and germinate on the surface of most soil types and under a range of soil moistures and temperatures in European countries.

Teliosporogenesis

'*Teliosporogenesis*' is the final stage of the disease cycle of *T. indica* when the teliospores form in the pericarp of the developing grain. Thus, it occurs after the time when the HTI is deployed in India to estimate whether conditions are favourable for infection and early development of disease. When used in this way, the HTI covers the period from GS 43 (boot just visibly swollen) to GS 75 (kernel at medium milk stage) (Murray, 2004, *unpublished*). In the Project the HTI was used to help assess the risk of establishment of *T. indica* in Europe by application from GS 37–65 (flag leaf just visible to mid-anthesis), the earlier stage being used to account for favourability of sporidial production from germinated teliospores. Unlike the early stages of the disease cycle there is very little information concerning factors that influence teliosporogenesis (Gill *et al.*, 1993; Kumar *et al.*, 2003).

The time from infection at late booting/early heading to the beginning of teliosporogenesis ranges from 13 to 67 days, with shorter times associated with higher temperatures and longer times associated with lower temperatures (Gill *et al.*, 1993). Teliospore formation begins at the soft dough stage (GS 83) following growth of *T. indica* in the pericarp of the developing seed (Cashion and Luttrell, 1988; Gill *et al.*, 1993).

Kumar *et al.* (2003) hypothesised that temperatures during grain development may limit the extent of teliosporogenesis (as measured by the level of disease) and the geographic distribution of Karnal bunt. They aimed to test this hypothesis by growing inoculated plants under 5 different temperature regimes that were obtained in a mix of field and greenhouse sites. The plants were inoculated at the

‘*spikelets emerging from the boot*’ stage of growth (presumably GS 49–53) and held for 48 hours at 18–22°C and high humidity. Three susceptible wheat cultivars were used. Following this initial incubation period, the plants were moved to the 5 field or greenhouse locations. Development stages of the wheats and daily maximum and minimum temperatures were recorded. At maturity, the incidence and severity of Karnal bunt in the seeds in each head were assessed to calculate a disease index (% Coefficient of Infection; %CI – see Aujla *et al.*, 1989) for each cultivar × site treatment.

The 5 ‘*situations*’ in India used by Kumar *et al.* (2003) for post-inoculation exposure were: 1, a polyhouse with temperature and relative humidity dependent on natural conditions at Karnal; 2, a field at Karnal; 3, a polyhouse with temperature and relative humidity controlled at Karnal; 4, a glasshouse without ventilation that was heated by the sun at Karnal; and 5, a field at Shimla.

The level of Karnal bunt varied between the post-inoculation situations, with a general rise from the coldest, situation 5, to the second hottest, situation 3. No Karnal bunt developed at the hottest, situation 4. Temperature data for the 5 sites were summarised as mean daily maximum and minimum for 3 periods of wheat development: flowering (<GS 71 – starting point not stated in Kumar *et al.* 2003 but assumed from the dates supplied to commence at GS 53), grain filling (GS 73–77), and grain hardening (GS 83–87). The HTI, as deployed in India, covers the period from boot just visibly swollen (GS 43) to kernel at medium milk stage (GS 75). It is estimated that the HTI would have as its maximum temperature component a value approximately that of the mean of the first and second periods of Kumar *et al.* (2003). Part of the Kumar *et al.* (2003) data and the estimated temperature for GS 53–77 are shown in Table 4.

Table 4. Mean maximum daily temperatures (°C) recorded at the heading to flowering (GS 53–71), grain filling (GS 73–77), estimated for heading to grain filling (GS 53–77) and recorded for grain hardening (GS 83–87) stages of wheat development and levels of Karnal bunt (% CI, mean of 3 wheat cultivars) under 5 different situations after inoculation with *T. indica* (from Kumar *et al.*, 2003)

Situation	Mean daily maximum temperature				Mean %CI
	53–71	73–77	53–77	83–87	
1	20.9	23.7	22.3	27.1	16.2
2	21.4	23.4	22.4	28.9	18.2
3	22.4	25.6	24.0	33.3	40.1
4	23.7	25.4	24.6	37.7	0.0
5	12.8	13.1	13.0	16.5	1.4

Before examining the conclusions reached by Kumar *et al.* (2003) from these data, we should consider earlier meteorological studies summarised in Gill *et al.* (1993). These concluded that temperature and relative humidity from early booting to mid milk (GS 43–75) were highly correlated with Karnal bunt development. The mean daily maximum temperatures from early heading (GS 53) to end of grain fill (GS 77) recorded by Kumar *et al.* (2003) were within the favourable range for the development of Karnal bunt reported in Gill *et al.* (1993) and so probably would have had little effect on Karnal bunt development. However, Kumar *et al.* (2003) did not record relative humidity, yet the description of the five situations indicates that relative humidity would have varied between them.

Gill *et al.*, 1993 found that afternoon relative humidity from booting to mid milk was more highly correlated with the development of Karnal bunt than was mean daily maximum temperature at this time (Gill *et al.*, 1993). In the study of Kumar *et al.*, 2003, at situation 3, relative humidity was

'controlled artificially' in a polyhouse, presumably by water sprays or mist, so this was probably at a higher humidity than the polyhouse in situation 1 or in the field (situation 2). In situation 4, the unventilated glasshouse, a small rise in temperature would have reduced the relative humidity. These rises and falls in relative humidity would be predicted to increase and decrease the amount of Karnal bunt respectively from the relationships reported by Gill *et al.* (1993).

Kumar *et al.* (2003) applied an analysis of variance to the temperature and disease data to compare between sites. Such analysis is invalid because none of the conditions at the sites are the same so they are not replicated; the use of daily values of temperature and disease data for each situation is 'pseudoreplication' (Hurlbert, 1984). Nevertheless, as a result of this invalid analysis, Kumar *et al.* (2003) concluded that minimum temperatures at the three stages of wheat development were not significantly different between sites, and that the maximum temperatures did not differ significantly between the first 4 sites for the first two stages of wheat development. The analysis of variance of the level of Karnal bunt between the cultivars at each site appears to be valid, so that the conclusion that the cultivars had similar levels of disease within each site is valid. However, the conclusion that Karnal bunt levels differed significantly between sites within cultivars is invalid, again because of pseudoreplication.

Kumar *et al.* (2003) appear to have assumed that because temperatures before grain hardening in situations 1 to 4 were similar, then the differences in Karnal bunt between the sites were caused by differing temperatures during grain hardening. By referring to earlier studies (Gill *et al.*, 1993) the unrecorded but probable differences in relative humidity in situations 1 to 4 during heading, anthesis and grain filling may have accounted for the observed differences. Furthermore, the plants were inoculated and incubated at Karnal before transport to the 5 situations. Situation 5 at Shimla is about 160 km north of Karnal: Kumar *et al.* (2003) do not describe how the plants were transported so it is unknown if conditions during transport could have affected the initial infection.

The temperatures in situation 5 where the lowest levels of disease were recorded were considerably less than those in the studies reported by Gill *et al.* (1993). However, if the HTI relationship holds at these lower temperatures, depending upon the relative humidity level prevailing during the relevant growth stages, it would be considered to be outside of the favourable range for Karnal bunt development (too cold). Needless to say some level of Karnal bunt developed at this site.

It is appropriate to use regression techniques to test for relationships between the situations. The correlation matrix of the daily mean maximum and minimum temperatures that can be calculated from Table 1 in Kumar *et al.* (2003) shows that the mean temperatures over the three stages of wheat development are significantly correlated ($P < 0.05$). Thus, it is not possible to determine at what stages temperature suppressed the disease.

Kumar *et al.* (2003) excluded situation 4 (the hottest site at grain hardening) from correlation and regression analysis of the relationship between levels of Karnal bunt and temperature, because Karnal bunt failed to develop there having increased with increasing temperature at the other sites. Their Table 2 shows the following relationship:

$$Y = -39.22 + 2.239X$$

where Y = Karnal bunt (% CI) and X = mean daily maximum temperature (°C) during grain hardening (GS 83–87), with $R^2 = 0.8039$. No degrees of freedom are given. It is unclear how this relationship was calculated, since our calculations based on the data in their Table 2 yield similar but not identical slopes, intercepts and R^2 . In our calculations, the relationship based on the mean

disease incidence of the three wheat cultivars is not significant ($P > 0.05$); when data for each cultivar are included, the relationship is significant ($P < 0.05$).

Because of the significant correlation between temperatures at the three growth stages, similar relationships between disease (% CI) and temperature can be developed at each growth stage of wheat. Thus, the contention of Kumar *et al.* (2003) that temperature during grain hardening determines the development of Karnal bunt is not sustainable.

Despite a lack of evidence provided in Kumar *et al.* (2003), let us assume that temperature during grain hardening does influence teliosporogenesis. Their data would then suggest that maximum daily temperatures in excess of those in situation 3 and below those in situation 4 prevent teliospore formation. It is unclear how many days of high temperature would be required, but it would seem that these lie between the mean maximum temperature in each situation, i.e. between 33.3 and 37.7°C.

Their data and conclusions also suggest that low temperatures would restrict teliosporogenesis. 'Trace' levels of teliospores (0.02–2.1% CI) were produced in situation 5 with a mean daily maximum of 16.5°C. Thus, conditions during grain hardening somewhere between those in situation 5 (mean daily maximum 16.5°C, range 14.9–18.8°C) and situation 1 (mean daily maximum 27.1°C, range 25.3–28.3°C) would become sub-optimal for Karnal bunt development. The regression equation (Kumar *et al.*, 2003) predicts a level of Karnal bunt at 18.8°C of 2.9%CI, and at 25.3°C, 17.4%CI. Karnal bunt above 3% incidence causes wheat to be unfit for human consumption (Warham, 1986). If we assume that the average grade of infection of the affected seeds is 2, the %CI at 3% incidence is 1.5 (calculated from Aujla *et al.*, 1989): the temperature which would predict this %CI is 18.2°C from the equation in Table 2 of Kumar *et al.* (2003). The conclusion of Kumar *et al.* (2003) that the lowest maximum temperature during grain filling for development of Karnal bunt is 25.3°C seems untenable. This value is in fact not derived from the regression equation but the minimum of the maximum daily air temperature during grain hardening at situation 1.

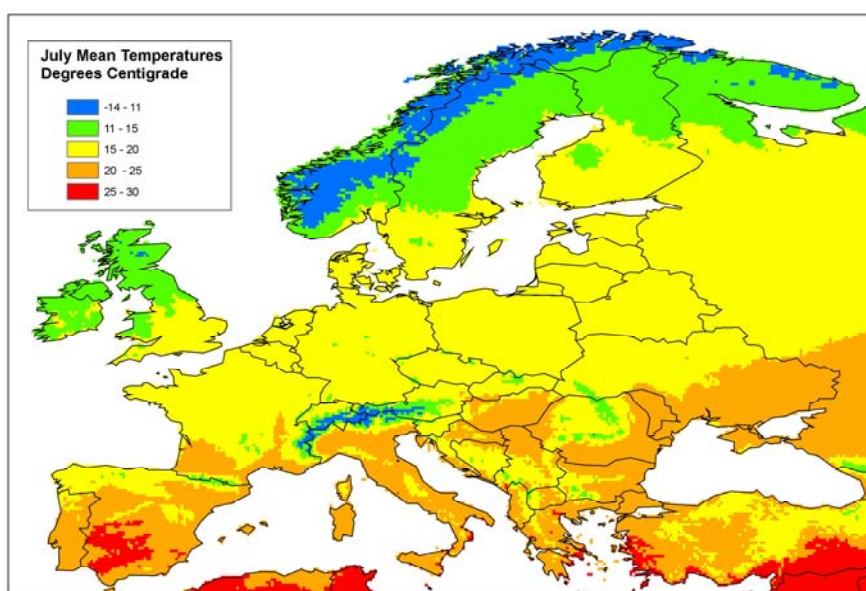
In conclusion, the data presented by Kumar *et al.* (2003) could be used with relative humidity data were it available, to present the type of conclusions that would be reached from using the HTI model for Karnal bunt development (Jhorar *et al.* 1993). This model does not utilise temperature during grain hardening for additional interpretation. This is particularly pertinent for cooler situations. It is possible that high temperatures during grain hardening will prevent Karnal bunt development. However, since the temperatures where this was observed to occur on the study of Kumar *et al.*, 2003 are well above those found in much of Europe during grain hardening, they are unlikely to influence or restrict development of Karnal bunt in Europe.

The wheat cultivar WL-711, one of three used by Kumar *et al.* (2003), was used as the susceptible control for testing the morphological susceptibility of European wheat cultivars to *T. indica* in the Project (Riccioni *et al.*, 2004, *unpublished*). Boot inoculation at GS 45 of WL-711 (to test the viability of the inoculum) and incubation at 17°C (control for winter wheat cultivars) and 20/15°C (day/night) (control for spring and durum cultivars) led to mean %CI's of 39.4 and 21.4 respectively. Despite the much lower incubation temperatures, disease still developed and at an incubation temperature of 17°C the % CI exceeded the maximum value for WL-711 found by Kumar *et al.* (2003) to occur naturally at the mean maximum air temperature during grain hardening of 33.3°C. Spray inoculation with a sporidial suspension at GS 55 of all of the European wheat cultivars under test (considered to be more representative of a natural infection situation compared to boot inoculation and at a growth stage determined as the optimum time for infection in the

Project) and incubation at 17°C (winter wheat cultivars) and 20/15°C (day/night) (spring and durum cultivars) led to maximum mean %CI's of 15.8 (winter wheat), 14.8 (spring) and 56.2 (durum). Although this work was conducted in quarantine containment growth rooms it is clear that it is not necessary for incubation temperatures during the grain hardening stage to fall between 25.3 and 34.9°C for teliosporogenesis (as demonstrated by disease development). The lower temperatures at which this occurred in the Project are more representative of the temperatures prevailing in wheat crops grown in northern Europe during this stage in the wheat crop.

Figure 9 shows the mean monthly air temperatures for Europe in July. All temperatures exceed 15°C; southern France, Spain and Italy exceed 20°C (Baker, CSL, UK, *personal communication*, 2005). All wheat growing areas have temperatures during grain hardening that lie within the range over which teliosporogenesis was found to occur in the Project.

Figure 9. Mean monthly air temperature (°C) in Europe in July.¹³



Much of the Indian wheat crop is harvested by the end of May (as determined in the UK PRA of 1996 through correspondence with CIMMYT, the International Wheat and Maize Improvement Center). The UK crop is harvested early August through to the end of September. Other European countries will have other harvest dates. In the 1996 PRA pre-harvest temperatures in India and the UK were compared, with UK values falling within the Indian range.

Seemingly therefore data generated within the Project as well as at one of the sites investigated by Kumar *et al.* (2003) shows that the range of temperatures at which teliospores are formed is much wider than that proposed by Kumar *et al.* (2003). Such temperatures are not the factor

¹³1961-1990 data, Climatic Research Unit, University of East Anglia, UK. Resolution 10 minutes longitude by 10 minutes latitude.

limiting the establishment of *T. indica* in areas where it currently is absent (or undetectable) in India; nor would they limit teliospore formation in Europe.

Regarding other abiotic factors, refer to 1.20 for comments on elevation and climatic variation.

1.22. If protected cultivation is important in the PRA area, how often has the pest been recorded on crops in protected cultivation elsewhere?

N/A, never, rarely, sometimes, often, very often

Not applicable.

Crop hosts are only grown under protection for breeding purposes. It may be possible for the pathogen to infect and cause disease under protected conditions but the pathogen should be detected under these circumstances.

1.23. How likely is establishment to be prevented by competition from existing species in the PRA area?

Very likely, likely, moderately likely, unlikely, very unlikely.

Very unlikely.

There are no other cereal-infecting smuts or bunts that would infect the wheat crop at the stage at which *T. indica* infects. However, heads already infected with common bunt (*Tilletia tritici*) would remove possible sites for infection. There are a range of other fungi, including *Fusarium* spp. that may infect the ear but there is no published information on their behaviour in the presence of *T. indica*.

1.24. How likely is establishment to be prevented by natural enemies already present in the PRA area?

Very likely, likely, moderately likely, unlikely, very unlikely.

Very unlikely.

There are no known natural enemies of *T. indica*.

Cultural practices and control measures

1.25. To what extent is the managed environment in the PRA area favourable for establishment?

Note: factors that should be considered include the time of year that the crop is grown, soil preparation, method of planting, irrigation, whether grown under protected conditions, surrounding crops, management during the growing season, time of harvest, method of harvest, soil water balance, fire regimes, disturbance etc.

Not at all favourable, slightly favourable, moderately favourable, highly favourable, very highly favourable.

Very highly favourable.

Soil cultivation practices prior to sowing the wheat crop will vary within and between countries in the PRA area. However, there has to be some tillage of soil for a wheat crop to be sown, even if direct-drilling takes place.

Where teliospores have been introduced for the first time, depending upon the time at which they are introduced to the field, some will remain on the soil surface at the point of sowing a new wheat crop. Surface-borne teliospores are the source of inoculum for the growing crop. Following on from an infected wheat crop, soil cultivation will bury some spores, some will remain on the surface and some will be brought to the surface (where burial of teliospores or bunted grain occurred through other cultivation practices prior to sowing).

The husbandry of wheat (and triticale) crops varies mainly in the timing of sowing and harvest across the PRA area as well as irrigation practice.

Baker *et al.* 2004 (*unpublished*) showed that for both *T. aestivum* and *T. durum*, sowing date and the choice of European wheat varieties based upon their phenology (maturity class) have little effect on their potential vulnerability to infection.

Irrigation during the susceptible period for infection may favour the development of Karnal bunt, (Bedi *et al.*, 1949; Fuentes-Dávila, 1996) and the effect of sowing date will have some influence where irrigation is applied. Bedi (1989) described the widespread distribution of the pathogen and the disease throughout the Indian Punjab on most of the commonly grown wheat cultivars and suggested that this was related to a range of factors. These included later sowing of wheat in a wheat and rice rotation so that favourable weather for the pathogen coincided with anthesis in the crop, use of susceptible cultivars, improved irrigation and high doses of N fertilisers, mechanical harvesting and threshing and indiscriminate movement of infested seed.

Stansbury and Pretorius (2001) used the HTI to predict the potential distribution of Karnal bunt in South Africa following the first outbreak in Douglas in the Northern Cape Province in December 2000 (Crous *et al.*, 2001). They superimposed the effects of applying conservative levels of crop irrigation on temperature, relative humidity and the HTI. They found that for early planted irrigated wheat, the HTI for the locations tested increased from below the minimum threshold of 2.2 to levels favourable for disease development. For mid- to late- planted wheat the HTI was also significantly increased but few locations tested fell within the suitable range of 2.2 to 3.3. This was borne-out for locations in South Africa known to use irrigation, with early-planted irrigated wheat being more likely to experience favourable HTI values in most years compared to mid- or late planted wheat. In discussing their findings the authors suggest that on farms where overhead irrigation is applied once every 24 hrs, the likelihood of Karnal bunt developing will be increased over those where irrigation is applied more frequently within a 24 hr period but at smaller volumes applied on each occasion within the 24 hr period (although the total volume applied in the 24 hr period may be more). This was used as a possible explanation for the first outbreak of Karnal bunt in South Africa at Douglas, where the crop was irrigated once daily. The authors report that where irrigation was applied more frequently than this, the disease was more prevalent in water-logged areas on other farms in the area. They considered that teliospores had been present in the Douglas area for some time and that alteration of irrigation schedules to less total water being applied in a 24 hr period, but applied more frequently, had favoured infection and disease development. The reason for the change in irrigation practice was because of drainage problems. Irrigation once every 24 hrs would mimic the rainfall conditions proposed by Smiley (1997) as being favourable for the development of Karnal bunt. Smiley attempted to predict the potential distribution of Karnal bunt in the USA

and suggested areas of sprinkler-irrigated wheat in the Pacific North-West appear to have a higher risk of disease than non-irrigated areas. Subsequent findings of Karnal bunt in South Africa have occurred in Douglas, Koffiefontein (Naudé, 2002) and Douglas, Herbert, Hopetown and Prieska (Anon., 2004a). All of these are irrigated areas.

Figure 10. Locations of four of the five¹⁴ named locations of Karnal bunt outbreaks in irrigated wheat crops in South Africa.



1.26. How likely are existing control or husbandry measures to prevent establishment of the pest?

Very likely, likely, moderately likely, unlikely, very unlikely.

Very unlikely.

Other crop management practices (seed treatment, fungicide application, crop rotation) have been considered under 1.5 as those which may be applied in the exporting country and as applied in the PRA area would have little influence on establishment of *T. indica* with conditions remaining favourable for the pathogen.

Cultivation practices prior to the sowing of a wheat crop will determine the level of teliospore inoculum that is present on the soil surface. It is this inoculum that will be the source of infection during that cropping year. There are no inoculum thresholds for *T. indica* so the amount of inoculum that is present is not especially relevant to the risk of establishment. (See 1.29).

No European wheat cultivars are known to be resistant to *T. indica* and in experiments, the range of susceptibilities is similar to those cultivars grown in countries where Karnal bunt occur. Thus, within the range of cultivars that are grown, some will be more susceptible than others but none will be resistant, as is the case in areas where the pathogen is established. (See 1.20).

1.27. How likely is it that the pest could be eradicated from the PRA area?

Note: Some pests can be eradicated at any time (very likely), others at an early stage (moderately likely) and others never (very unlikely). Similarly, incursions of some pests may be difficult to find and/or delimit (very unlikely). Note that intentionally imported

¹⁴ Herbert not locatable

plants, may need to be eradicated from the intended habitat as well as from the unintended habitat.

Very likely, likely, moderately likely, unlikely, very unlikely.

Very unlikely.

Although difficult to prove eradication, there has been no clear, published evidence from those countries in which the pathogen is established, or where it has been introduced, that an ‘*eradication policy*’ has succeeded based upon crop rotation, chemical control, host plant resistance, seed certification or any other quarantine measure. There are some government reports that some Mexican states are now ‘*Karnal bunt free*’ based on failure to detect *T. indica* in surveys (see 1.20). The only published report of the relatively recent introduction into Brazil (Da Luz *et al.*, 1993) referred to measures aimed at eradicating *T. indica* in Rio Grande do Sul following its first official report in the 1989 harvest. Testing of seeds collected in subsequent years (1990-91 and 1992) failed to detect teliospores in the samples examined. No further reports have been forthcoming from Brazil and so the efficacy of the measures is not clear, particularly as the teliospores of *T. indica* have the potential to be extremely long-lived. Enquiries to Brazilian scientists have not yielded any information on the current status in Brazil. The USDA attempted to eradicate the pathogen in the USA following the first official reports in 1996 when it was believed to be a localised introduction. However, after surveys determined the distribution to be more widespread a disease management scheme was adopted. Measures taken following the first report in the Douglas area of South Africa (Crous *et al.*, 2001) did not prevent it being reported later and it has since been found to be present in Douglas, Koffiefontein (Naudé, 2002) and Douglas, Herbert, Hopetown and Prieska (Anon., 2004a).

England and Wales currently has a model contingency plan¹⁵, an earlier version of which (Sansford *et al.*, 2004, *unpublished*) was incorporated into Deliverable Report 6.3 of this Project (Sansford, 2004b, *unpublished*). Options for attempting eradication presented in this plan are outlined briefly below.

In general terms, eradication of a highly localised outbreak may be possible (although probably highly unlikely), if detected in the first year of infection, through timely application of quarantine controls once trace-back and trace-forward activities to determine the extent of the problem have been completed. Measures that may be used would include decontamination of equipment, safe destruction of infected seed lots and grain, soil fumigation, long-term fallowing or grassing-down of infected land, and a prohibition on growing susceptible crops in the affected fields and in a buffer zone around the affected fields. If the pathogen was introduced over a wide area, eradication would be unlikely to be either feasible or cost-effective. From a scientific perspective, containment aimed at the eventual demise of the pathogen in the absence of its host (not guaranteed) would be the most likely option considered for managing delimited outbreaks. Options for affected fields identified by trace back and trace forward activities might be for them to be managed by keeping them under bare fallow or grassing-down. Again, based upon the

¹⁵ By evaluating the range of measures that could be undertaken to prevent entry of the pathogen or to deal with outbreaks in the EU, a model management (contingency) plan has been produced based upon outbreak scenarios in England for consideration for implementation in England and Wales. However, this plan, which formed the basis of the socio-economic impact analysis, is for illustrative purposes only. It should not be taken as indicating how any EU Member State would respond to a finding of *Tilletia indica* on its territory. Any such response will be determined by the governments concerned, in the light of consultation with stakeholders and other relevant factors at the time including the availability of resources.

model contingency plan, measures may have to be implemented for at least 5 years, if not longer, because of the potential longevity of the pathogen in the soil. The period of implementation may have to be determined by surveys and testing for the pathogen within the affected fields and in a buffer zone delimiting the area, as well as in susceptible crops grown around the outside of the buffer zone, until such time as the affected fields could be declared pathogen-free. National surveys may have to be implemented to identify pest-free areas and pest-free places of production to facilitate exports of grain and seed to countries which categorise *T. indica* as a quarantine pest. In reality, management options would be determined by the government(s) in the affected country or countries in consultation with stakeholders and by considering other relevant factors including the availability of resources.

Other characteristics of the pest affecting the probability of establishment

1.28. How likely is the reproductive strategy of the pest and the duration of its life cycle to aid establishment?

Note: consider characteristics which would enable the pest to reproduce effectively in a new environment, such as parthenogenesis/self-crossing, short life cycle, number of generations per year, resting stage, high intrinsic rate of increase, self fertility, vegetative propagation, etc.

Very unlikely, unlikely, moderately likely, likely, very likely.

Very likely.

The reproductive strategy of *T. indica*, life cycle duration and survival strategy is very likely to aid establishment. See 1.20 for a broad description of the life cycle. More detail is provided here.

T. indica is heterothallic (Durán and Cromarty, 1977). Monokaryotic diploid teliospores present on or very near to the soil surface germinate under favourable climatic conditions to produce monokaryotic haploid primary sporidia (Garrett and Bowden, 2002). For this to occur, meiosis occurs during teliospore germination and haploid nuclei migrate into a promycelium which bears primary sporidia, each of which receives one nucleus (Fuentes-Dávila and Durán, 1986). Durán and Cromarty (1977) confirmed that each haploid primary sporidium carries only one of the four mating type alleles and that a single teliospore gives rise to primary sporidia which are either one of two of the four mating types. This means that, in theory, there is no requirement for more than one teliospore to be present for sexual reproduction to occur (see 1.29). When germination of teliospores occurs within a wheat crop the pathogen is able to complete its lifecycle under the conditions described under 1.20.

Following germination, primary sporidia (basidiospores) are splash-dispersed, germinate and produce epiphytic colonies which give rise to large quantities of airborne, monokaryotic haploid secondary sporidia which are either allantoid or filiform (Nagarajan *et al.*, 1997). The filiform secondary sporidia are dispersed by rain splash but are not infective. The allantoid secondary sporidia are shot into the air and are either dispersed by wind or by rain splash. Some lodge on wheat leaves and other surfaces. There they can germinate, producing further superficial hyphal colonies from which more secondary sporidia can develop. In this way the infective allantoid sporidia reach the terminal or flag leaf of the wheat plant, where dew or rain can wash them into the boot just as the wheat head begins to emerge or becomes exposed (e.g. if the flag leaf surrounding the ear splits), or from where they can be rain-splashed or wind-dispersed onto the

emerged head. Relative humidity, water and temperature within the crop canopy influence survival and growth of the secondary sporidia (Nagarajan *et al.*, 1997).

If the secondary allantoid sporidia arrive on the wheat ear during the susceptible period for infection, they germinate under favourable environmental conditions and penetrate the glumes, lemmas, paleas and possibly the rachis. Anastomosis of germinated secondary sporidia of opposite mating types leads to dikaryotisation probably within the host tissue (the subject of some debate in a number of papers), and the formation of monokaryotic diploid teliospores.

The duration of the lifecycle from infection of the host through to the production of the long-lived teliospores synchronises with the lifecycle of the host and is completed within the cropping year.

The teliospores are the survival structures for *T. indica* and they have been shown, in the Project, to have great longevity in a range of soil types and climates found in the PRA area.

In conclusion, provided teliospores enter an area where the main host (wheat) is present and where the climatic conditions favour survival until environmental conditions are favourable for germination of teliospores on or very near to the soil surface, should this coincide with the susceptible stage for infection, under favourable conditions, completion of the lifecycle is feasible and establishment will ensue.

1.29. How likely are relatively small populations or populations of low genetic diversity to become established?

Note: if very small populations are known to survive for long periods in their current area of distribution, such evidence may be used to answer this question.

Very unlikely, unlikely, moderately likely, likely, very likely.

Very likely.

A selection of teliospores from any imported population is likely to be genetically diverse. (See 1.30).

Regarding the size of the population of teliospores, Garrett and Bowden (2002) suggest that because *T. indica* depends upon ‘*encounters on wheat spikes*’ between secondary sporidia of opposite mating types for successful infection and reproduction this results in ‘*reduced reproductive success for lower population densities*’. This so-called ‘*destabilising density dependence at low population levels*’ is generically known as an ‘*Allee*’ effect and has been described for a range of animals and plants. These authors developed a population model for *T. indica* by calculating the risk of infection for different numbers of secondary sporidia. The ‘*Allee effect*’ is described by them as being at the ‘*frontier for invasion*’ for establishment of new foci by a small teliospore population and when the environment is nonconducive for the production of secondary sporidia. The authors suggest that below a theoretical threshold population size, populations of *T. indica* may decline rather than increase; and, that seasonality, i.e. the weather dependent nature of secondary sporidia production is the key to establishment risk.

Although a single teliospore of *T. indica* will produce primary sporidia (basidiospores) which produce colonies from which many secondary sporidia of two opposite mating types are then produced, a sorus can arise from a mixed infection, so in some cases at least, teliospores from the same sorus can give rise to secondary sporidia of all four mating types. These secondary sporidia are themselves capable of multiplication prior to infection (Nagarajan *et al.*, 1997). Dhaliwal (1989) suggests the fact that generation of sporidia occurs on soil, leaves and spikes (ears) implies that the level of original inoculum in the form of soilborne teliospores plays only a ‘starting role’ in Karnal bunt epidemics. Nagarajan *et al.* (1997) confirms that the heterothallic nature of *T. indica* requires fusion between secondary sporidia of ‘compatible’ mating types but supports the view that the rapid vegetative multiplication of the secondary sporidia increases the chance of a successful fusion between opposite mating types. Whilst Garrett and Bowden (2002) suggest the ‘Allee’ effect will limit the establishment potential of *T. indica* they also state that ‘establishment of a new population of *T. indica* via teliospores is probably easier than via windblown secondary sporidia because under conducive conditions, a small number of teliospores is capable of producing a large number of secondary sporidia in a concentrated area and the frequency of different mating types is more likely to be balanced’.

Provided therefore that climatic conditions are suitable, a single teliospore can theoretically result in infection of the wheat ear and the production of many more teliospores. Garrett and Bowden (2002) suggest that a ‘teliospore threshold for establishment’ in an area will exist with or without the ‘Allee’ effect when values for a range of ‘density-independent reproductive rates’ are not too high – these ‘rates’ refer to:

- a) The number of primary sporidia (basidiospores) produced per teliospore. These have been variously estimated as having a range of 32–128 (Mundkhur (1940; in Singh, 1994), 50–140 (Durán, 1972), 60–185 (Gill *et al.*, 1981), more than 150 (Krishna and Singh, 1982) and up to 180 (Bonde *et al.*, 1997; Singh, 1998). Garrett and Bowden (2002) gave a range of 32–128 basidiospores per germinating teliospore, while Murray and Sansford (2005, see below) estimated 128 basidiospores per germinating teliospore under conditions favourable for germination.
- b) The number of secondary sporidia per primary sporidium. The literature does not provide estimates beyond ‘abundant’ or ‘many’ (e.g. Krishna and Singh, 1982; Bonde *et al.*, 1997; Nagarajan *et al.*, 1997). Garrett and Bowden (2002) assumed that the total number of secondary sporidia arising from successive generations in the crop canopy ranged from 10^2 – 10^4 per primary sporidium. Murray and Sansford (2005, see below) used the higher value for determining the risk of establishment under favourable environmental conditions.
- c) The number or proportion of secondary sporidia that produce a monokaryon infection. This refers to the proportion of the total number of secondary sporidia produced from one germinating teliospore that produce a monokaryon infection. Garrett and Bowden (2002) estimated that the proportion of secondary sporidia that remained viable and reached an infection court was 10^{-6} to 10^{-4} , and that the proportion of these that resulted in a monokaryon infection was 10^{-3} to 10^{-1} . Under favourable conditions, a high proportion of sporidia would remain viable (10^{-4}) while a high proportion of these would infect (10^{-1}), giving the proportion of secondary sporidia producing a monokaryon infection of 10^{-5} , as assumed by Murray and Sansford (2005, see below). The scientific literature including Nagarajan *et al.*, 1997 and this Project have shown that the timing of infection is most likely to be growth stage (GS) 43 (boot just visibly swollen) to GS 69 (anthesis

complete). The most likely timing in the field is likely to be from GS 47/49 (flag sheath opening to first awns visible) to GS 55 (half of inflorescence emerged) depending upon environmental conditions. Once the ear is fully exposed, conditions for infection are likely to be less favourable unless conditions of high moisture predominate (rainfall and high humidity) during flowering. The potential ‘window’ of growth stages beyond ear emergence which are susceptible to infection may be longer for some cultivars than others and the physical duration of this period will vary with the season. Individual plants in a field will vary in maturity. Individual florets within the ear of wheat come into anthesis (the end of anthesis is the latest time for infection) at different times giving a window of opportunity for primary infection to occur. Dhaliwal *et al.*, (1983) found this period to extend over 10 days.

- d) The number of dikaryon infections per monokaryon infection. After infection, the monokaryotic hypha grows within the floret tissue and if it meets a hypha of another mating type, can anastomose with it to produce the dikaryotic infection necessary to produce the bunt sorus in the developing seed. The number of dikaryotic infections per monokaryotic infection is density dependent and Garrett and Bowden (2002) use a logistic function to estimate this. They then estimated that the proportion of such infections that successfully form a sorus is 0.5–0.9. Murray and Sansford (2005) used Garrett and Bowden’s (2002) logistic function and assumed a success of 0.9 under favourable conditions to estimate the number of sorus infections.
- e) The number of new teliospores per dikaryon infection. Garrett and Bowden (2002) estimate this as 10^3 to 10^6 . This value is not recorded in the literature but once the process of infection is underway in favourable conditions this becomes irrelevant. From an initial penetration point the fungus is also capable of invading adjacent spikelets (Dhaliwal *et al.*, 1983; Goates, 1988). Bedi and Dhiman (1984) found that under favourable weather conditions the pathogen spreads from an initial infection site to adjacent spikelets on alternate sides of the rachis as well as to closely lying ovaries in the same spikelet. Presumably this spread occurs as dikaryotic hyphae after anastomosis of monokaryotic haploid hyphae of compatible mating types. They estimated that the probability of a single ovary of a spike (mean of 5 ovaries per spike) being infected by a single sporidium was 0.02 while that of simultaneous infection of 2 and 3 closely lying ovaries in a spikelet was 4×10^{-3} and 8×10^{-3} respectively. They concluded that the high frequency of simultaneous infection of ovaries in a spikelet could not be attributed to simultaneous landing of air-borne sporidia on each of the ovaries present in the same spikelet; neither could the regular occurrence of infection of adjacent spikelets, suggesting secondary and tertiary spread. Dhaliwal and Singh (1988) stated that the disease spreads to adjacent florets and spikelets, infecting as many as 31 grains around the infection site. Garrett and Bowden (2002) do not account for this secondary spread and multiplication in their model but state that it could be easily accommodated (Bowden, 2006, *personal communication*). Histological studies show masses of teliospores in each infected grain and no attempts have been made to try to count them (e.g. Fuentes-Dávila and Durán, (1986); Cashion and Luttrell, 1988). Estimates of the number of bunted grains arising from a single infection have not been calculated by Garrett and Bowden (2002) but this would seem important in determining how quickly *T. indica* can build up following entry to a new area. Murray and Sansford (2005, and *unpublished*) consider that teliospores from bunted grains that have passed through harvesting equipment will be concentrated in a small part of the field. If one bunted grain sheds half its teliospores, then about

500,000 spores will be deposited over a few square metres leading to a local concentration of 10, 000–25, 000 m⁻². ⁽¹⁶⁾.

- f) The number of teliospores that remain in the field and retain viability to the next season. Garrett and Bowden (2002) estimate this as a proportion (0.3 to 0.8). Teliospore survival studies in Europe and in countries where the pathogen occurs confirm this range of survivals and show survival for several years (see 1.20 onwards).

Garrett and Bowden's (2002) calculations are based largely upon estimates. However, there are no published inoculum thresholds for *T. indica*. The fact that once introduced to an area fresh teliospores have a dormancy period which varies in length within the same population and the fact that they can survive until conditions are suitable for germination and infection to occur, means that there appear to be no or few limiting factors in the pathogen's life cycle that would prevent establishment in the PRA area from a small population of teliospores. The main exception would be if there are any canopy conditions that might be limiting; this has not been investigated in the European context.

Despite their calculations, Garrett and Bowden (2002) acknowledge that *T. indica* 'compensates for the Allee effect' because it produces 'large numbers of primary and secondary sporidia'. So, in favourable climatic conditions the 'reproductive plasticity' of *T. indica* [may] allows it to 'maintain a population density high enough to experience the benefits of sexual reproduction without reduced reproductive potential'. They also state that 'another mechanism by which *T. indica* may counterbalance the Allee effect to some extent is through dispersal of intact bunted kernels so that teliospores tend to be distributed together and thus produce more concentrated populations of secondary sporidia'. As we have shown, different conclusions can be made if these factors are taken into account in addition to other published information.

Prior to Garrett and Bowden's paper, Murray and Brennan (1998) concluded for Australia that the entry of a small number of teliospores may give rise to a small risk of infection since there is no reported minimum threshold number of teliospores required for this to occur. Slow spread occurred in Mexico after the disease was first found in 1969-70. Murray and Brennan concluded that outbreaks could occur following the entry of a small number of teliospores which would take several years to reach detectable levels. Stansbury *et al.* (2002) estimated this time period to be between 4 and 11 years following entry to Australia, most likely to occur on bulk grain with an average of one entry per 50 years and one establishment event every 100 years.

Marshall *et al.* (2003) analysed the USDA/APHIS Port Information Network from 1984 to 2000 to determine likely pathways of introduction based upon *T. indica* interception records. All of these were made on wheat originating in Mexico and 98.8% were intercepted at land border crossings, mainly in Texas and Arizona and mainly in automobiles, trucks and railway cars. They surmise that despite having the opportunity to enter the USA since at least 1984 if not earlier, the first official report of a disease outbreak in the USA in 1996 and the recognition of known specific requirements for infection and disease development suggests a long period of 'latent survival' between initial arrival and becoming a 'thriving, established disease'.

¹⁶ Assuming one bunted grain produces 1,000,000 teliospores (Garrett and Bowden, 2002) and half of them are discharged from the header of the combine harvester with the straw over 20-50 m².

Garrett and Bowden (2002) provided estimates for the steps from teliospore germination to sorus formation as ranges from unfavourable to favourable conditions. This Project has demonstrated that large areas of Europe where wheat is grown appear to be highly favourable for Karnal bunt development. It thus seems appropriate to use the estimates for favourable conditions and the methodology of Garrett and Bowden (2002) to estimate the effect of numbers of teliospores on the establishment of *T. indica* in a new area. Using this method, Murray and Sansford (2005) found that, if the pathogen entered an area as a low number of teliospores it would result in low numbers of bunted grains which would be widely dispersed. Using assumptions based on crop yield in Australia, a localised distribution of teliospores that resulted in 15 germinating teliospores per m² would produce one bunted kernel per m². With an estimated 10⁶ teliospores being produced per bunted grain and many teliospores landing in a small area this would lead to a concentration of Karnal bunt in discrete areas. If the pathogen entered an area as a single bunted grain there would be a large local concentration of teliospores (up to 10⁶) and subsequently under favourable conditions a local concentration of Karnal bunt. Such a clumped distribution of Karnal bunt in the year after detection, as predicted by Murray and Sansford (2005) from Garrett and Bowden's (2002) method, was shown by Rush *et al.* (2005). Whether the initial introduction of *T. indica* was of teliospores distributed at relatively low density over a large area or as a bunted kernel in one spot, both cases would result in the presence of *T. indica* being undetectable and before its presence was noticed it is likely to have had the opportunity to spread to new areas.

1.30. How adaptable is the pest?

Note: is the species polymorphic, with, for example, subspecies or pathotypes? Is it known to have a high mutation rate? Does it occur in a wide range of climate and habitats? Such evidence of variability may indicate that the pest has an ability to withstand environmental fluctuations, to adapt to a wider range of habitats or hosts, to develop pesticide resistance and to overcome host resistance.

Adaptability is: Very low, low, moderate, high, very high.

Based upon the factors listed for consideration, the adaptability of *T. indica* is very high.

Genetic variability is commonly accepted as a pre-requisite for survival and evolution. Bonde *et al.*, (1997) reported that isozyme analysis of monoteliospore cultures of *T. indica* showed a high degree of genetic variation. This is believed to be because of outcrossing which occurs between secondary sporidia of differing genetic make-up. This favours sexual recombination and genetic variability. This contrasts with common bunt pathogens such as *Tilletia laevis* and *Tilletia tritici* where fusion of the basidia (sporidia) occurs mainly between basidiospores formed on the same basidium – these pathogens by contrast are considered to have 'ineffective' sexual cycles and outcrossing is rare. Nagarajan *et al.* (1997) and Bonde *et al.* (1997) discuss various aspects of genetic variability for *T. indica* including the issue of 'races' but because *T. indica* is heterothallic, genes are constantly being 'reassorted' making races unstable. In consequence a selection of teliospores from any imported population is likely to be genetically diverse.

Regarding the range of climates and types of crops in which *T. indica* occurs, Murray (2004, *unpublished*) states that until recently, *T. indica* had a limited distribution, occurring in north western India, Pakistan and some mid-eastern countries of similar latitude, and in Mexico. This suggested that the pathogen had specific environmental requirements that limited its potential

distribution. However, the recent occurrences in the south-western states of the USA, in South America (Brazil) and in South Africa show that there is potential for the pathogen to spread to new areas. Currently, the disease has been reported in areas of 24°–34° N and S latitudes, at low elevations with mild winters, hot summers and low to medium rainfall. In most cases, these areas grow spring or durum wheats that are sown in autumn and harvested in late spring or early summer. Frequently, the crops are grown under irrigation. Some winter wheat infection has been observed in Texas. The finding of *T. indica* in a survey sample from San Saba County in Texas in 1997 (Rush *et al.*, 2005) was in hard red winter bread wheat in San Saba County (Miller, undated document; Marshall *et al.*, 2003).

Host resistance is not a barrier to establishment of *T. indica* in the EU. As described under 1.20, European winter, spring and durum wheat cultivars have a range of susceptibilities that is similar to wheat cultivars grown in Karnal bunt affected countries (Riccioni *et al.*, 2004, *unpublished*). Similar findings have been made in other studies for European winter wheat cultivars (Inman, CSL, UK, *personal communication*, 2000; Peterson and Creager, 2000). European wheat cultivars with resistance to *T. indica* have not been developed by wheat breeders, as the pathogen does not occur in Europe.

In terms of the ability of the pathogen to withstand environmental fluctuations the resilient teliospore enables the pathogen to survive for long periods of time in its current range as well as in European conditions. This, along with a period of dormancy (which varies in the literature as well as with the age of the teliospore) before germination can occur, facilitates its potential presence when conditions become favourable for infection and disease development. This will result in multiplication of teliospore inoculum. The durability of secondary sporidia *in vitro* (Goates, 2005) suggests that where teliospores germinate well in advance of the critical infection window the sporidia could still be viable and capable of infection under the conditions that normally favour disease development (which includes high relative humidity).

1.31. How often has the pest been introduced into new areas outside its original area of distribution? (specify the instances , if possible)

Note: If this has happened even once before, it is important proof that the pest has the ability to pass through most of the steps in this section (i.e. association with the pathway at origin, survival in transit, transfer to the host or habitat at arrival and successful establishment). If it has occurred often, it suggests an aptitude for transfer and establishment.

Never, very rarely, occasionally, often, very often

Often.

The current distribution of *T. indica* is described under 1.20 with supporting references. The mode of entry to a number of countries is not specified and in some instances it is difficult to determine the exact point at which the pathogen entered a new area, presumably because from an initial point of entry it can take some years before the symptoms of disease are detected – this usually arises post-harvest when bunted grain is detected. Zhang *et al.*, 1984 made some attempt to explain the entry of *T. indica* to new areas and refers to the article by Joshi *et al.*, (1983) which suggests the increase in distribution of the pathogen from India (or even Pakistan, see Howard and Howard, 1909, below) to Afghanistan, Iraq, Mexico and Pakistan probably resulting from the shipment of infected seeds from India. Further elucidation for entry to all the affected areas is given below.

Asia

The borders of India and Pakistan have changed over the years and the locations given below are assigned to the country to which they belonged at the time of the record.

T. indica was first formally reported in the Karnal district of northern India in 1930. However, Warham (1986) suggests that a report of a bunt on wheat at Lyallpur, now known as Faizalabad (Pakistan) by Howard and Howard (1909) where *T. indica* was found during 1930-1934 may in fact have been the first report of the pathogen.

The first official report in Pakistan was in the Sind Province in 1941. By 1943 it was prevalent in the Punjab and North West Frontier Provinces of Pakistan. By 1969 Karnal bunt had spread to an extensive area of India, namely Jammu and Kashmir, Punjab, Haryana, Himachel Pradesh, Uttar Pradesh, Dehli and Rajasthan (Singh, 1994).

Dhaliwal *et al.* (1983) state that Karnal bunt was confined to northern India until the 1960's but had since spread to all wheat-growing states. Aggarwal *et al.* (1991) states that the disease had at first occurred in a few places in the north-western region of India but regular monitoring since 1975 showed it was much more widespread. Joshi *et al.* (1983) suggest the increased distribution (in India and elsewhere) may (in part) be due to the development and wide distribution of wheat cultivars that were more susceptible to *T. indica* than wheats grown on India prior to 1969–70. Zhang *et al.* (1984) supported this view and state that the disease was considered endemic until the 1969–70 cropping season when most of the Mexican wheat cultivars grown in India were affected by the disease.

A fuller review of Karnal bunt in India and Pakistan, including a map of distribution, was published in 1994 (Singh, 1994). The author summarises the views of many inasmuch as the disease Karnal bunt occurs sporadically but assumes epidemic proportions in certain years (see 2.1).

Records on x *Triticosecale* are few and the pathogen was first naturally observed on this host in India in 1975 and 1976 (Agarwal *et al.*, 1977).

T. indica is also present in other Asian countries (Afghanistan and Iraq – no details of dates of first findings, Joshi *et al.*, 1983; Nepal – first recorded in the Doti district between 1986 and 1987, Singh *et al.*, 1989).

The most recent record in Asia was in Iran where it was first reported in 1996 from Jiroft and Kahnooj in Kerman province. A subsequent survey in the southern provinces of Iran revealed a number of other locations were affected in the provinces of Fars and Hormozgan, some with high levels of infection. Although the mode of entry of *T. indica* into Iran is not described the authors (Torarbi *et al.*, 1996) suggest that the weather prevailing during anthesis in 1996 was favourable for disease development and that the severity and widespread distribution of the disease indicated that the pathogen must have been present there for some years.

Central America

The first record outside of Asia was in Central America, in the Yaqui and Mayo valleys of Mexico in the 1969–70 cropping season (Fuentes-Dávila, 1996a). Warham (1986) refers to the first report of *T. indica* in Mexico on wheat seed received by Chapingo college (in Durán,

(1972). Durán's paper actually refers to a specimen collected by a Chapingo scientist on *T. aestivum* in commercial wheat fields in Cajeme, Sonora in February 1971, referring to this being the first report in the New World, and suggesting it was most likely introduced from India on infected wheat seed. The International Wheat and Maize Improvement Center (CIMMYT) trades in wheat germplasm and specialises in breeding for resistance to a number of diseases including Karnal bunt. It is possible but not proven that imports of germplasm from Asia/India may have been the source of entry of *T. indica* to Mexico.

South America

The next new record outside of Asia was in South America in Brazil where it was first officially reported from the 1989 harvest in the southern part of the Rio Grande do Sul, again with no information on how it was introduced (Da Luz *et al.*, 1993). Fuentes-Dávila (1996a) refers to exports of seed from the Yaqui valley in Mexico to many countries including Brazil from 1964 to 1981 with no apparent regulation of Karnal bunt in force at that time. It is possible that the pathogen may have been introduced to Brazil from Mexico. In 1996, Fuentes-Dávila (1996a) stated that despite wheat seed exports being an unregulated activity in Mexico, the pathogen had failed to establish outside of Mexico. Clearly this is not the case. However, the situation regarding regulation of *T. indica* in Mexico is confusing as Marshall *et al.* (2003) refer to internal regulation of Karnal bunt by the Mexican Government since it was first discovered (referred to as being in 1972). *Tilletia indica* was present in accessions of wheat germplasm introduced into Brazil for research purposes between 1990 and 1992 (Mendes and Ferreira, 1994). Oliveira *et al.* (2002) also refer to the interception of *T. indica* on wheat germplasm imported into Brazil (with no further detail). Enquiries into the current status of *T. indica* in Brazil have not elicited any new information.

North America

The next continent to be affected by *T. indica* was North America. The earliest interception records of *T. indica* by the USDA were reported by Warham (1986) as being on wheat entering the USA from India in 1949 and Afghanistan in 1955.

In March 1996, Karnal bunt was first detected in a quality seed test sample from a lot of harvested seed of durum wheat in Arizona. Trace-back efforts to determine the origin of the infected seed led the USDA to fields in the vicinity of Buckeye, Arizona (Sansford and Peterson, 2004 *unpublished*). Subsequent investigations revealed that the pathogen was present in seed grown in Arizona and shipped to California, New Mexico and Texas (Podleckis *et al.*, 1996). Rush *et al.* (2005) state that reference samples of wheat maintained by the Arizona Department of Agriculture harvested in 1993 contained teliospores of *T. indica*, suggesting the pathogen had been in Arizona in 1992. Following the initial finding various theories were proposed as to how *T. indica* gained entry to the USA. Marshall *et al.* (2003) and Rush *et al.* (2005) discuss interceptions of the pathogen being made on wheat transported from Mexico by a variety of means. Mexican boxcars (rail cars) entering the USA contaminated with *T. indica* seem to have been implicated as a pathway for entry since at least 1983 when *T. indica* was confirmed on wheat kernels from a boxcar in California. In December 1984 the USDA placed restrictions on the movement of Mexican boxcars into the USA (Boratynski *et al.*, 1985). Marshall *et al.* (2003) analysed the USDA/APHIS Port Information Network (PIN) Database from 1984 to 2000 to determine pathways of entry of *T. indica* into the USA; 925 of 995 interceptions were associated with wheat seed, 70 of the 995 interceptions had no record of the type of material on which the interception was made. Of the 925 interceptions, 914 were made at land border crossings

between the USA and Mexico. Over the period analysed 36% of interceptions were made in 1986 and 1987 which was significantly greater than all other years. Marshall *et al.* (2003) conclude that the pathway for entry for *T. indica* into the USA started in Mexico crossing the border repeatedly between 1984 and 2000. They do not rule out the possibility of natural entry by wind-blown spores from infected crops in north-west Mexico to south-west areas of the USA.

Africa

Tilletia indica was found in several cultivars of *T. aestivum* in South Africa in Douglas in the Northern Cape Province in December 2000 (Crous *et al.*, 2001). The pathogen has since been found to be present in Douglas and Koffiefontein (Naudé, 2002) and Douglas, Herbert, Hopetown and Prieska (Anon., 2004a). No information as to how the pathogen entered South Africa has been published.

1.32. Even if permanent establishment of the pest is unlikely, how likely are transient populations to be maintained in the PRA area through natural migration or entry through man's activities (including intentional release into the environment)?

N/A, very unlikely, unlikely, moderately likely, likely, very likely.

Not applicable.

Probability of spread

Spread potential is an important element in determining how quickly impact is expressed and how readily a pest can be contained. In the case of intentionally imported plants, the assessment of spread concerns spread from the intended habitat or the intended use to an unintended habitat, where the pest may establish. Further spread may then occur to other unintended habitats. The nature and extent of the intended habitat and the nature and amount of the intended use in that habitat will also influence the probability of spread. Some pests may not have injurious effects on plants immediately after they establish, and in particular may only spread after a certain time. In assessing the probability of spread, this should be considered, based on evidence of such behaviour.

1.33. How likely is the pest to spread rapidly in the PRA area by natural means?

Note: consider the suitability of the natural and/or managed environment, potential vectors of the pest in the PRA area, and the presence of natural barriers. Spread depends on the capacity of a pest to be dispersed (e.g. wind dispersal) as well as on the quantity of pest that can be dispersed (e.g. volume of seeds).

Very unlikely, unlikely, moderately likely, likely, very likely.

Moderately likely.

Sporidial spread by natural means

Natural spread of *T. indica* by the release of sporidia under favourable conditions for infection and disease development will normally lead to localised spread only. Sporidia are spread by wind and water/rain splash.

Teliospore spread by natural means

Teliospores can be spread by wind to adjacent fields (Warham, 1986) during harvest or soil movement, including wind blow of dry soils.

Teliospores can be spread longer distances by wind or air currents if liberated into the upper atmosphere by activities such as stubble burning (which could be termed '*unnatural means*' or '*human assistance*') or in soil blown by wind in dry conditions.

Nelson (1996) states that at 3000 m elevation, some standard calculations show that a spore the size and mass of a *T. indica* teliospore could move in excess of 300 miles [about 500km] under appropriate wind conditions.

Schall (1988, *unpublished*) reported on the results of collecting aerial samples over burning wheat stubble in three locations in Mexico (Ciudad Obregon, Costa de Hermisillo, Tabasco) in 1988, at different times and altitudes during or after the burning process. Although the background information for the study was limited and the investigation varied between sites, Schall attempted to interpret the data. Prior to burning, only one of the locations was sampled (Tabasco) and no spores were found above the infested field. Spores were found over the field after burning (at 1500 m elevation on the same day) however, and Schall states that '*it is probable the infested air mass had travelled away*', suggesting these spores had been liberated either by natural causes or by burning, and may represent a background '*average concentration of spores*' (i.e. the absence of detectable spores prior to burning in one single flight's sampling was not proof that they would normally be absent in air currents above infested fields).

At the other two sites, the data collected in this study showed that during burning the number of spores detected above the fields on the day of burning was higher than detected the day after burning (sampled 8 km away) and they were found up to 3050 metres, the highest elevation tested. Schall concluded that burning wheat fields liberates teliospores and transports them high into the atmosphere. In this limited study, spore concentrations were highest at the highest elevation tested and on the day of burning. One day after burning the low numbers of spores detected in samples collected 8 km away from the burnt fields, was attributed either to sedimentation of spores released during burning or the movement of the spore-infested air mass away from the site(s). The updraft of air from burning fields most likely carries teliospores to high altitudes and acts as a mechanism of dispersion. Schall commented that at least 50% of the recovered teliospores were viable.

In the nearby US arid southwest, 2.5 to 3 tons of soil per acre are displaced annually by wind erosion (Northcutt, 2001) and this action may transport teliospores in a similar manner to updraughts from stubble fires.

Animals also transport teliospores as contaminants on their bodies and could in theory move them long distances in this way. Similarly they can be transported in their digestive systems after ingestion. Smilanick *et al.* (1986) fed teliospores of *T. indica* to leghorn chickens and grasshoppers (*Melanoplus sanguinipes*) and placed teliospores in the rumen of a '*rumen-fistulated*' Holstein cow, viable teliospores were collected in the faeces arising from all three. Faeces or manure derived from animals which have fed on *T. indica* teliospores are therefore two other natural means of spread.

1.34. How likely is the pest to spread rapidly in the PRA area by human assistance?

Note: consider the potential for movement with commodities or conveyances. As for 1.33 consider the capacity to be spread as well as the quantity that can be spread. For intentionally introduced plants consider spread to the unintended habitat.

Very unlikely, unlikely, moderately likely, likely, very likely.

Spread by human assistance is likely but may or may not be rapid depending upon the circumstances.

Human assistance, both direct and indirect is one of the main means of spread of teliospores of *T. indica*.

Mode of spread by human assistance from an infected crop

Teliospores first become liberated from infected grain in the ears of an infected crop of wheat at harvest. Threshing leads to teliospores being:

- Deposited in soil
- Lodging in machinery and vehicles either loose or as bunted grain
- Adhering to the surface of healthy grain as a contaminant
- Adhering to straw

From these points the pathogen can be disseminated by:

- Movement of contaminated soil either through cultivation practice or as a contaminant on any surface
- Movement of contaminated machinery and vehicles
- Movement of infected or contaminated grain by any conveyance to another arable field
- Movement of harvested infected or contaminated seed (either home-saved seed or certified seed)
- Movement of straw

Rate of spread from an infected crop

The rate of spread from an infected crop in the PRA area by any of the means outlined above would depend upon:

- The distribution of the hosts and the climate in the local area and further afield in the PRA area where long-distance movement has taken place
- How rapidly any initial or subsequent outbreak is detected and reported to the NPPO
- What action is taken as a result

Influence of detectability of T. indica on spread

Because the pathogen typically causes symptoms on only a few grains per ear and grains are usually only partially colonised this makes it difficult to detect in the first year of any outbreak, especially as infected grains are enclosed by glumes making detection by field inspection (alone) unlikely. Therefore, low levels of disease may go undetected. A slow but steady build up of the pathogen could occur in continuous wheat cropping situations if favoured by local climatic factors. Because of the longevity of teliospores, once present in the soil, these could be present for considerably longer than one cropping season and available to produce infective inoculum to infect wheat crops over a number of years during favourable climatic conditions.

In crops grown commercially for seed, following an initial outbreak, detection should be feasible post-harvest as the harvested seed will be inspected for certification purposes. In crops grown for grain, if the crop is to be managed under recognised Quality Assurance schemes, infection may be detected post-harvest since the pathogen reduces the quality of grain by the production of malodorous trimethylamine (a rotten fish smell). However, home-saved seed from infected crops may be a route by which spread occurs in the absence of any official inspection or testing.

Murray and Brennan (1998) concluded for Australia that the entry of a small number of teliospores to an area may give rise to a small risk of infection since there is no reported minimum threshold number of spores required for infection to occur. Disease outbreaks which occur at low levels may take several years to reach detectable levels, thus facilitating spread in the absence of any controls. Slow spread occurred in Mexico after the disease was first found in 1969–70.

Marshall *et al.* (2003) discussed the movement of *T. indica* in Texas following the confirmation in 1996 that some fields in Texas had been planted with infected durum wheat seed. The crops that developed from this seed were destroyed in 1996 prior to heading to prevent the potential development of Karnal bunt. In the summer of 1997 a single teliospore of *T. indica* was found in grain harvested from a ‘winter habit’ bread wheat in San Saba, Texas which is approximately 736 km east-north-east of the nearest destroyed field and was outside of the regulated area. In May 1997, the USDA introduced the ‘bunted kernel’ policy (see 1.10) which meant that fields would only be regulated if ‘bunted kernels’ (Karnal bunt) were detected. No further reports of Karnal bunt were made in the regulated areas of Texas until 2001 when it was found in grain harvested from wheat grown near San Saba and also on grain from a non-regulated area around Olney, Texas which is approximately 241 km north of San Saba.

The apparent long-distance movement of *T. indica* in 2001 within the state may have been due to one of three possible scenarios: either (i) the pathogen was spread beyond the regulated area because of the ineffective ‘bunted kernel’ inspection policy which would allow teliospores to go undetected in any survey or, (ii) the pathogen was already present in that part of the state and causing low but undetectable levels of disease for some time, or, (iii) the pathogen was already present in that part of the state and causing disease for the first time in that year. Marshall *et al.* (2003) suggest that because teliospores of *T. indica* have had ‘ample opportunity’ to enter the USA from Mexico since at least 1984 it is possible that the outbreaks in San Saba and Olney, Texas were associated with long-present populations of *T. indica*. They are of the view that low levels of undetectable infection may have occurred in these locations for several years and that weather favourable to the development of detectable levels of Karnal bunt occurred in 1997 in San Saba (and presumably in 2001 in Olney). They consider that *T. indica* has a long lag-phase following entry to a new area during which it survives until conditions are favourable before it

can infect and cause disease, remaining undetectable because of the type of symptoms it produces.

Further spread of *T. indica* may have occurred as a result of a delay in action by the USDA in response to the 2001 findings. A press report (Hegeman, 2001) suggests that the first findings in Olney, Texas were reported to the USDA on May 25th by a grain elevator operator; 7 days passed before confirmation by the USDA and 15 days before the affected counties were quarantined. By this time much of the area had been harvested and the combine harvesters had moved north into Oklahoma (the southern border of this state is 80 km north of the most northerly findings in Texas). Although borders were subsequently closed and combines entering Oklahoma had to have a USDA ‘*certificate of cleanliness*’, the delay in implementation of quarantines may have led to further spread into the wheat-growing areas of the USA; this would be facilitated by ‘*custom cutters*’ – combine harvester operators who start harvesting at the southern most end of the wheat-growing area and move north harvesting up to the border with Canada. A subsequent article published by Texas A&M University (McAlavy, 2001) states that *ca.* 400,000 acres were placed under quarantine in North Texas and confirms that the ‘*disease was not detected until after harvest was under way and some of the crop was already shipped*’.

Since the introduction of the USDA requirement for a ‘*bunted kernel*’ to be present before any field producing grain is regulated it is not possible to reliably determine the extent to which teliospores of the pathogen may have spread within Texas or beyond. The most recent publicly-available US survey is for 2003 (Anon., 2003a) and the disease is still apparently confined to Arizona, California and Texas. Whether teliospores of the pathogen have been spread further is difficult to say because the national surveys do not require the surveyors to look for them. Stein *et al.* (2005) conducted soil sampling in wheat fields in Texas in June 2002 to determine the distribution of teliospores of *T. indica* in both regulated (15 fields) and non-regulated areas (1 field). Within the regulated area teliospores were found in 14 of the fields sampled including fields that had never tested positive for ‘*bunted kernels*’. Under current requirements these fields could in theory be deregulated despite the presence of teliospores.

The USDA is progressing its strategic plan for Karnal bunt (Anon., 2003b) by making its requirements for imports of wheat, triticale and other regulated articles equivalent to its own internal domestic requirements. Recent changes to their regulations (Anon., 2005) mean that for exports of wheat and triticale arising in countries where *T. indica* occurs (referred to as Karnal bunt) the requirement for the Additional Declaration on the accompanying Phytosanitary Certificate is a statement that ‘*These articles originated in an area where Karnal bunt is not known to occur, as attested to either by survey results or by testing for bunted kernels or spores*’ This gives the exporting country several options and may increase the risk of entry of teliospores of *T. indica* to a range of locations in the USA. Because the USDA will only regulate a grain-producing area in the USA on the basis of the presence of ‘*bunted kernels*’ this may mean that many areas within the USA may be at risk of introducing *T. indica*. Where this occurs these areas will escape regulation for some considerable time.

In conclusion, because of the cryptic nature of the pathogen it is possible that following entry to the PRA area into an arable farming area, spread from an initial undetected outbreak could be slow but steady. The presence of *T. indica* may not be apparent for some considerable time post-entry.

1.35. How likely is it that the spread of the pest could be contained within the PRA area?

Note: Consider the biological characteristics of the pest that might allow it to be contained in part of the PRA area; for intentionally introduced plants consider spread to the unintended habitat.

Very likely, likely, moderately likely, unlikely, very unlikely.

The likelihood of containment depends upon how limited the first outbreak or outbreaks are, or the scenario by which the pathogen enters the PRA area.

If as happened in the state of New Mexico, USA, in 1996 it was known that a number of fields in the PRA area had been planted with seed suspected as being infected with *T. indica*, provided the crop had not reached the heading stage it could be destroyed and restrictions placed upon future planting in the contaminated fields. This should effectively contain the pathogen. In New Mexico the fields suspected of being planted with contaminated seed were put under quarantine for five years, but have since been deregulated. (Sansford and Peterson, 2004 *unpublished*). Under these circumstances, provided no undetected spread by human assistance had taken place, containment would be very likely.

In the first year of entry if the pathogen completes its lifecycle and is detected before, during or just after harvest, and, provided trace back and trace forward activities are thorough and identify areas where the pathogen may have been moved by planting, cultivation or harvesting equipment (etc), it should be possible to contain the pathogen by a series of measures; i.e. containment should be likely.

If detection does not occur in the first year then further physical spread will occur through movement of harvest grain and seed and physical movement of teliospores as contaminants on machinery, modes of transport and harvesting and handling equipment. Depending upon the findings of trace back and trace forward activities containment may be moderately likely, unlikely or very unlikely.

From a scientific perspective, containment aimed at the eventual demise of the pathogen in the absence of its host would be the most likely option needed or attempted for delimited outbreaks.

England and Wales currently has a model contingency plan¹⁷, an earlier version of which (Sansford *et al.*, 2004, *unpublished*) was incorporated into Deliverable Report 6.3 of this Project (Sansford, 2004b, *unpublished*). The most recent version of this is presented in Annex II of this report. This plan focuses on containment in the first instance. If this model plan was deployed in full (see caveat in footnote), affected fields identified by trace back and trace forward activities would be managed by keeping them under bare fallow or grassing-down. Measures

¹⁷ By evaluating the range of measures that could be undertaken to prevent entry of the pathogen or to deal with outbreaks in the EU, a model management (contingency) plan has been produced based upon outbreak scenarios in England for consideration for implementation in England and Wales. However, this plan, which formed the basis of the socio-economic impact analysis, is for illustrative purposes only. It should not be taken as indicating how any EU Member State would respond to a finding of *Tilletia indica* on its territory. Any such response will be determined by the governments concerned, in the light of consultation with stakeholders and other relevant factors at the time including the availability of resources.

would have to be implemented for at least 5 years if not longer because of the longevity of the pathogen in the soil. The period of implementation would have to be determined by surveys and testing for the pathogen within the affected fields and in a 3km buffer zone delimiting the area in which no host crops could be grown, as well as in susceptible crops grown around the outside of the buffer zone, until such time as the affected fields could be declared pathogen-free.

Conclusion on the probability of introduction and spread

‘Introduction’ is defined as the ‘*the entry of a pest resulting in its establishment*’ (FAO, 2002).

Entry

The probability of entry has already been discussed (see end of 1.15).

To recap, the most likely means of entry of *T. indica* to the PRA area would be via infected and/or contaminated wheat seed as well as through grain exported from countries where the pathogen occurs. Entry on triticale is possible but natural findings on triticale have been reported rarely. Entry into the PRA area seems possible.

Countries in the EU have made interceptions of *T. indica* on grain of bread/feed and durum wheat since 1996 (Poland, Italy, the UK, and, possibly Greece) from a variety of sources including India, Mexico and possibly the USA. The main potential route (in terms of volume alone imported from affected countries) for wheat entering the PRA area from countries where the pathogen occurs is from the USA. Italy seems to be a major importer of wheat from the USA and depending upon the area from which this wheat originates, Italy may be at significant risk of entry of *T. indica*. The Netherlands, Belgium, the UK and Spain are also countries which appear to import reasonably high quantities of wheat from the USA.

The pathogen can be detected in seed and grain, but inadequate sampling procedures or only looking for symptoms of the disease and not the pathogen itself in the country of export, will not detect low levels of the pathogen. The current USDA policy of regulating areas for wheat grain production based upon a voluntary survey and looking for the presence of a ‘*bunted kernel*’, rather than testing for the pathogen itself, does not comply with the EC phytosanitary requirement for freedom from *T. indica* in grain destined for export to EU Member States. Unless supported by other measures applied specifically for exports, such a policy may allow low levels of the pathogen to go undetected in grain harvested from infested crops and is likely to have increased the risk of entry of *T. indica* from the USA to the PRA area.

To try to prevent entry, some countries in the EU are sampling and testing 100% of consignments of grain/seed originating in countries where *T. indica* occurs at the point of entry. Transhipments of grain/seed across the EU are more difficult to pinpoint in terms of origin. In England and Wales samples are taken by the PHSI and tested by CSL; Scotland and Northern Ireland are also testing imported material (Furk, PHSI, UK, *personal communication*, 2005). Responses to recent enquiries (February 2005) show that:

- Hungary and Lithuania are inspecting 100% of grain of wheat and triticale originating in countries affected by *T. indica* (Erzsebet, Hungary, *personal communication*, 2005; Talevi *et al.*, 2004; Kerbeliene, State Plant Protection Service, Lithuania, *personal communication*, 2005).

- Italy is inspecting 100% of wheat and triticale grain and seed originating in countries affected by *T. indica* (Riccioni, ISPaVe, Italy, *personal communication*, 2005; Porta-Puglia, MRAE, Malta, *personal communication*, 2005).
- The Netherlands are not routinely sampling imported material for *T. indica* (van Leeuwen, Dutch Plant Protection Service, the Netherlands, *personal communication*, 2005).

However, no information on the deployment of any sampling and inspection regime is available for the remaining 21 Member States. The use of inspection and testing of wheat and triticale grain and seed imported from countries where the pathogen occurs would help prevent entry of *T. indica* to the PRA area.

Establishment

Following entry, establishment of *T. indica* in wheat crops across the EU seems likely. Experimental work conducted within this Project has shown the ability of the pathogen to survive for long periods (minimum of 3 years and probably longer) in field conditions as teliospores under quarantine containment, and the availability of infective sporidia at a time in the development of bread/feed wheat (*T. aestivum*) and durum wheat (*T. durum*) when infection is likely to occur. European bread/feed and durum wheat cultivars have been shown to be susceptible to infection by *T. indica* with a range of susceptibilities similar to those present in wheat cultivars grown in countries where Karnal bunt occurs. One aspect of the pathogen's life cycle that has not been investigated under field conditions in the EU is the stage from initial basidiospore production to arrival of infective allantoid sporidia on the ear of wheat – this is not possible while the pathogen is a quarantine pest for the EU and EPPO, it is also not possible to mimic this stage in containment facilities. However, climatic conditions during the period of host susceptibility are favourable for sporidial production, infection and the commencement of disease development. Recent *in vitro* studies suggest that where teliospores germinate prior to the critical infection window, the secondary sporidia produced at this stage may be extremely durable and capable of infection some time after they are first produced (Goates, 2005). Therefore these may also serve as a source of inoculum later on when the host is susceptible to infection.

In the major bread/feed wheat growing regions of Europe, namely western and central Europe, there is a very high probability of infection by *T. indica* and the commencement of disease development based on predictions of the HTI value falling between the critical values of 2.2 and 3.3 during the development stages when wheat is vulnerable. More northerly regions are predicted to have critical HTI values in about one third of the cases studied (sowing date x years). The predictions for durum wheat show very high frequencies of critical HTI values for the northern Italian plain and the important pasta growing areas of Marche and Toscana. The Basilicata region in the foot of Italy seems to be less severely affected, possibly because the extremely high temperatures found in this region raise the HTI out of its critical range. France and especially Spain seem less prone to critical HTIs for durum wheat. This may be related to durum being generally sown later than winter wheat and thus it may reach its susceptible phenological stages later when conditions are drier and warmer, so the HTI does not fall within the critical window in as many cases. Eastern Europe is also predicted to have a medium to high risk for infection of durum wheat, somewhat less than for northern Spain and central and eastern France. Although the frequency of years in which the HTI has been shown to be favourable is less in some parts of the EU than others, the ability of the pathogen to survive for long periods below the soil surface should ensure that some teliospores are present and capable of germinating when returned to or near to the surface in favourable years.

Although Kumar *et al.* (2003) suggested that high temperatures are needed during grain hardening for teliospores to form in the infected ears of wheat, this was not proven by the authors and other factors not examined by the authors could have influenced development of the teliospores. In fact, lower temperatures more akin to those experienced in the PRA area during this stage did not prevent the development of teliospores in the study reported by Kumar *et al.* (2003) and in studies within the Project.

Spread

Because of the difficulties in detecting low levels of disease, it is possible that following entry of *T. indica* to the PRA area into an arable farming area within an EU country, spread from an initial undetected outbreak could be slow but steady. Multiple entry points would facilitate more rapid spread. By the time the disease is at detectable levels at the initial site of entry, the pathogen may have spread to a number of locations.

Go to 1.36

Conclusion regarding endangered areas

1.36. Based on the answers to questions 1.16 to 1.35 identify the part of the PRA where presence of host plants or suitable habitats and ecological factors favour the establishment and spread of the pest to define the endangered area.

Within the PRA area (the EU) the endangered area is all of the wheat growing areas of all EU Member States. Although some areas of the EU will have more frequent occurrence of conditions favourable for infection and the start of disease development as demonstrated by the frequency of favourable HTI values, all areas have been shown to have some favourable years within the period studied (1995–2002 for the EU). This means that, all other factors being equal, build-up of levels of the pathogen (teliospores) would potentially be faster in those areas where the frequency of occurrence of favourable HTIs was greatest. The risk to the triticale crop has not been fully evaluated but this is also potentially at risk in areas where the HTI values are favourable.

Go to 2 Assessment of potential economic consequences

2. Assessment of potential economic consequences

The main purpose of this section is to determine whether the introduction of the pest will have unacceptable economic consequences. It may be possible to do this very simply, if sufficient evidence is already available or the risk presented by the pest is widely agreed. Start by answering Questions 2.1 - 2.9. If any of the responses to questions 2.2, 2.3, 2.4, 2.6, or 2.8 is 'major or massive' or 'likely or very likely', the evaluation of the other questions in this section may not be necessary and you can go to 2.16 unless a detailed study is required. In cases where the organism has already entered and is established in part of the PRA area, responses to questions 2.1, 2.5 and 2.7, which refer to impacts in its area of current distribution, should be based on an assessment of current impacts in the PRA area in addition to impacts elsewhere.

In any case, providing replies for all hosts (or all habitats) and all situations may be laborious, and it is desirable to focus the assessment as much as possible. The study of a single worst-case

may be sufficient. Alternatively, it may be appropriate to consider all hosts/habitats together in answering the questions once. Only in certain circumstances will it be necessary to answer the questions separately for specific hosts/habitats.

Expert judgment is used to provide an evaluation of the likely scale of impact. If precise economic evaluations are available for certain pest/crop combinations, it will be useful to provide details.

The replies should take account of both short-term and long-term effects of all aspects of agricultural, environmental and social impact.

Consider potential hosts/habitats identified in question 1.16 to answer the following questions:

Pest effects

2.1. How great a negative effect does the pest have on crop yield and/or quality to cultivated plants or on control costs within its existing area of distribution?

Note: factors to consider are types, amount and frequency of damage and crop losses in yield and quality, together with costs of treatment.

Minimal, minor, moderate, major, massive.

The question encompasses yield, quality AND control costs in the countries where *T. indica* occurs. This is difficult to answer with a single word not only because three different topics are covered but also because of the variation within and between countries where it occurs.

Yield losses are generally small but there can be local conditions that favour greater losses.

Countries where high quality wheat is produced will be affected by the negative effect on quality, especially if quality assurance schemes are in place.

Control costs will vary depending upon the spectrum of diseases that occur and whether additional costs are incurred because of *T. indica*. Also, countries where the disease is long-established and most grain is consumed locally (but with increasing export opportunities), like India, are more likely to live with the disease, compared to those where it has been more recently introduced.

Thus, the three elements (yield, quality and control costs) are considered separately for this question. The range of effects that the pathogen has in field-grown crops as well as data derived from experimental investigations are described below.

The effect of the *T. indica* on yield, quality and control costs in countries where the pathogen occurs varies from year to year and location.

The direct effect on yield and quality depends upon:

- The severity of the disease
 - The pathogen causes symptoms that affect individual grains within an infected ear. Not all the grains in an ear necessarily become infected and grains are also typically only partially colonised
- The incidence of the disease
 - Within a field the distribution of the disease can be patchy

Disease severity and incidence depend upon:

- Local climate, especially from flag leaf emergence onwards
- Degree of cultivar susceptibility
- Existing crop management practices

Information on the direct and indirect effects of the pathogen on field-grown crops are not published for a number of the affected countries. The countries which are most well-documented are those where the disease has been present the longest, namely India, Pakistan and Mexico.

Disease distribution, severity and incidence in countries where Karnal bunt occurs

Scientists in India, Pakistan and Mexico have reported on disease distribution, average severity and incidence in individual years in a number of papers. The methods used to determine incidence and severity have not been consistent and so it is difficult to make comparisons between papers.

A small selection of reported findings is given below:

India

Royer and Rytter (1985) stated that in 1965, India first imported semi-dwarf cultivars of wheat from Mexico which were widely-grown. These cultivars were considered to be more susceptible than those used previously and were considered to be the major cause of the increase in disease incidence between 1969–1975. Indian farmers apparently replanted harvested seed irrespective of whether or not it was infected thus perpetuating *T. indica* in the soil.

Agarwal *et al.* (1976) had earlier stated that Karnal bunt was considered endemic in India until the 1969–70 wheat season when most of the Mexican cultivars grown there were affected by the disease with a maximum of 7.5% seed infection in the cultivar S-331 in the foothills of the Himalayas of Uttar Pradesh. The disease did not appear in experimental plots in 1970–72 despite the pathogen being present on the seed that had been planted but when favourable conditions prevailed in 1974–75 in the Himalayan foothills, the disease was more severe and found to be present on a range of cultivars.

Joshi *et al.* (1983) described the occurrence of Karnal bunt in the ‘*plains of undivided India*’ as being at ‘*trace levels*’ in the early years. By 1969–70 it was common in Dehli, Punjab, Haryana, Rajasthan and western Uttar Pradesh. By 1974–75 it was reported as severe in many places in northern India, especially in the foothills of the Himalayas and the Tarai region of Uttar Pradesh, Punjab and Himachal Pradesh; disease severity was described as especially high in Hampur in Uttar Pradesh (15–23%). These high values refer to two other papers. In one of these (Agarwal *et al.*, 1976) data were presented on the percentage of lots of 8 wheat cultivars falling into different ranges of seed infection; the maximum being 8.3% of the lots of cultivar HD-2009 falling into the 20–50% seed infection category. However, in the second (Singh *et al.*, 1977), the field incidence at Hampur was described as ranging from 15–23% (i.e. the values appear not to refer to % severity in this particular paper).

Since 1975–76, surveys have been conducted to determine the distribution and incidence of Karnal bunt in India and the results showed that both incidence and distribution have increased.

Aujla *et al.* (1977) stated that the ‘infection % of the bunt’ during 1975–76 on different cultivars ranged between 2.2 and 52.9% in Jammu and Kashmir and 1.4 and 25.1% in the Punjab. They found that the incidence of disease was highest on late flowering cultivars which coincided with temperatures of 18–22°C and >70% relative humidity.

Joshi *et al.* (1983) viewed the change in the choice of cultivars grown in India since 1975–76 as being related to an increase in disease incidence and distribution; this includes the introduction of the cultivar WL-711 which was used as the susceptible control in the Project’s European cultivar susceptibility testing work.

A survey in 1981 revealed that the disease was present in areas where it had previously been unreported including new locations in central India (Zhang *et al.*, 1984).

Bedi (1989) described the spread of Karnal bunt in the Punjab and as with other authors, suggested there were a number of factors implicated in its spread, namely: later sowing of wheat in a wheat/rice rotation leading to anthesis coinciding with favourable conditions for infection and disease development; susceptible cultivars; improved irrigation and high doses of nitrogen fertilisers; mechanical harvesting and threshing; and ‘indiscriminate’ movement of infested seed.

Agarwal *et al.* (1993) summing-up, stated that Karnal bunt appeared in epidemic form in 1976, 1978–79, 1981–83 and 1986 in different locations in India.

Subsequently, Singh (1994) summarised the distribution, incidence, and severity of Karnal bunt in India, concentrating on Uttar Pradesh which at that time held 36% of the area of land under wheat production in India. A Karnal bunt distribution map was prepared. Surveys showed that Karnal bunt was widely distributed in various western and eastern districts of Uttar Pradesh with northern hill and southern dry areas being generally free. In reviewing the literature Singh found that during the 1974–75 cropping season the disease was severe at many places in northern India especially in the foothills of the Himalayas and the ‘tarai’ region of Uttar Pradesh, Punjab and Himachal Pradesh. Disease severity was as high as 15–50% at Hampur and Pantnagar in Uttar Pradesh. During 1975–76 maximum disease incidence in India occurred in the Punjab where 39% of grain samples were infected compared with 9% in Haryana. During 1977–78, 38% of field samples from Haryana were infected compared to 25% in Himachal Pradesh and Kashmir. The disease increased in 1978–79 when the incidence of field samples infected in Punjab, Himachal Pradesh and Kashmir were 78%, 71% and 89% respectively, compared to only 17% in Haryana. The disease was also found to be present West Bengal, Bihar, Madhya Pradesh and Gujarat covering eastern, central and western parts of India.

Singh *et al.* (1996) reported on post-harvest surveys from 1986–1994 in north-western India that showed high levels of infection in the climatically favourable years of 1986, 1987, 1990 and 1991 (34, 25, 21 and 16% of samples infected).

Beniwal *et al.* (2000a) reported that in the state of Haryana (south of the Punjab) Karnal bunt infection ranged from 0.05–9.90% and 0.05–0.30% during the 1995–96 and 1996–97 cropping seasons, respectively.

Sharma *et al.* (2004) described the level of Karnal bunt found in grain markets in the Punjab between 1994 and 2004. The highest level recorded was in 1996 when 70% of samples were infected and the mean severity was 0.6%. Considerable fluctuation in both incidence and

severity was recorded over the ten years and between the 265 grain markets sampled during the ten-year period.

Although x *Triticosecale* is not the main host under consideration in this PRA, the first records of *T. indica* on x *Triticosecale* were made in India in 1975 and 1976 (Agarwal *et al.*, 1977). This was later described in more detail for the years 1975–1977 (Khetarpal *et al.*, 1980). The level of disease in field-grown germplasm in Nainital Tarai (Pantnagar) in India between 1975 and 1977 showed higher levels of incidence of Karnal bunt in 1976 (7.3%) compared to 1977 (0.2%) (% grains affected). Disease severity was however worse in 1977 than in 1976.

Because there have only limited quarantine restrictions imposed in India the pathogen has had the opportunity to move to all of the wheat-growing areas of India. However, it remains confined to the northern cooler areas and is absent from the southern (hotter, drier) wheat growing areas.

Pakistan

Bhutta *et al.* (1999) discussed disease incidence for wheat seed samples harvested in Pakistan between 1993 and 1997. They found the highest level of % seeds infected as 3% (considered to be high) in various seed lots harvested in the Central Punjab and north-west Pakistan with southern Pakistan disease free (from the samples taken) between 1994 and 1997. Thirty-seven % and 25% of seed samples from the Central Punjab and in the North West Frontier Province contained bunted grain in 1993-1994. This was the highest level recorded. They cite earlier findings of disease severity (not defined but assumed from this study to be % of seeds infected in a sample)) declining from 4.6% in 1981-82 to 0.6% in 1983-84 with an increase to 3.2% in 1984-85 and 5.5% in 1985-86. Lower severity in the 1990s reported in this study is attributed to seed certification standards whereby seed lots above a certain level of infection are excluded from seed production. Citing other authors, Bhutta *et al.* (1999) state that southern parts of Pakistan had been free from Karnal bunt until 1987, possibly due to prevailing dry conditions in these areas.

Mexico

Fuentes-Dávila (1996a) states that levels of Karnal bunt were ‘noticeable’ in 1981–82 in the Yaqui and Mayo valleys in the state of Sonora, Mexico. Fuentes-Dávila stated that regulatory measures were introduced in 1983 and modified in later years to try to control the disease and reduce dissemination of *T. indica* to other wheat-producing areas (the time of implementation varies in the literature). The disease remained confined to north-western Mexico reaching approximately 400 km north, south and west from the first findings with disease incidence varying annually depending upon weather conditions. Disease symptoms became prevalent in years when high relative humidity and rainfall coincided with anthesis of the wheat crop. Low disease incidence occurred in the Yaqui valley in 1982, 1984, 1987, 1988, 1990, 1994 and 1996 with surveys showing 85.0 to 99.7% of samples tested to be disease-free. The highest levels of infection occurred in 1983 and 1985 with 7.5 and 11.2% of samples with >1% infected grain.

USA

The incidence and severity of the disease is not well-documented in the USA. Rush *et al.* (2005) state that the results of national surveys and samples from regulated areas indicate low disease levels citing a paper describing the effect of the pathogen on yield and quality published in 1992

as a reference source (Singh *et al.*, 1992) (i.e. providing no reference to published data on levels of disease and impacts in the USA). Tabulated data indicates the number of wheat fields designated positive for Karnal bunt in Arizona, California and Texas done by a variety of testing methods. Arizona has the greatest number of positive fields and California the least. However, disease incidence and severity are not mentioned. Rush *et al.* (2005) also cite personal communications and state one particular instance when 11% of grains in a sample of wheat were infected with Karnal bunt. However, the level of disease across the affected field was <1% (Peterson, USDA, ARS, USA, *personal communication*, 2005). This illustrates variability in findings even at the field level.

Yield

The methods used to determine the effect of *T. indica* on yield have varied and so it is difficult to make comparisons between papers. The pathogen may have different yield impacts on bread, feed and durum wheats. No precise relationship between the severity or incidence of disease and yield has been calculated. A small selection of reported findings is given below:

The effect on yield is variable because *T. indica* only invades the pericarp layer of the seed and typically only partially (i.e. the layer beneath the pericarp is not colonised). Also, *T. indica* does not normally affect every grain in an ear and the distribution of infected plants in a field is usually patchy in nature. However, the effect on individually-infected grains has been estimated. Warham (1986) stated that the weight of infected grains of wheat is directly related to disease severity – as severity increases grain weight decreases; the difference in weight between heavily and slightly infected grains can be as great as 50%. The 1000-grain weight of wheat cultivars infected by *T. indica* is reduced. Depending upon the ‘*variation and severity*’ of the disease, up to 20% loss has been recorded and grain weight can be reduced by 44–70%. Individual investigations vary in their findings. For example Singh (1980) estimated a 34% loss in 1000 grain weight in severely affected grain and Bhat *et al.* (1980) estimated a loss of up to 50%. Rai and Singh (1985) showed reductions in weight of individual wheat grains infected with *T. indica* varied with severity: low, medium and high levels of infection led to reductions of 5.9, 20.0 and 51.6% respectively. Karwasra *et al.* (1991) showed a reduction in 1000 grain weight for 6 wheat cultivars ranging from 57% for Sonalika to 66% for WL-711 under severe infection conditions. In experiments, Beniwal *et al.* (2000a) found a reduction in 1000-grain weight of 4.5 to 52.3% depending upon the severity of infection resulting from artificial inoculation of one Indian wheat cultivar [HD 2329]. The authors cite other workers’ findings giving reductions in 1000-grain weight of up to 70% in severely infected grain. Estimates of the effect of *T. indica* on the yield of European winter, spring and durum wheat cultivars in this Project were not possible in practice as only small grains were produced under controlled environment growth conditions. This was not considered representative of natural seed production (Riccioni *et al.*, 2004, *unpublished*).

At a field level, yield losses are normally low overall, but in situations where conditions favour the disease individual crops may be severely affected. The earliest reported yield loss was 20% in experimental wheat plots at Karnal, India (McRae, 1933, *in* Joshi *et al.* 1983, Warham, 1986 and Beniwal *et al.*, 2000). Munjal (1975, *in* Warham, 1986) quoted an overall incidence of 0.6% for *ca.* one-third of the wheat production area of northern India in 1969–70 and a loss in grain yield of 0.2% which equated to a loss of 40,000 metric tonnes per year. Munjal suggested that because infected grains are blown away during winnowing and cleaning, the overall yield losses may be higher. Joshi *et al.* (1983) stated that Karnal bunt occurs sporadically becoming epidemic only in certain years but may cause substantial losses; on average the total loss in India

may be *ca.* 0.3 to 0.5% of total production but in some fields infection may be as high as 89% leading to substantially greater effects. Agarwal (1993) stated that although the disease occurs sporadically in epidemic years it causes '*substantial losses*'. Singh (1994) estimated that in epidemic years in Uttar Pradesh yield and quality losses in wheat amounted to at least 1% of the total value of the crop, with a mean loss in production in epidemic years in India of 0.3–0.5%. Brennan and Warham (1990) and Brennan *et al.* (1992) evaluated the economic impact of Karnal bunt of wheat in Mexico and stated that economic losses rose sharply in the 1980s as a result of increased levels of disease. The average yield loss in north-western Mexico (southern Sonora, Sinaloa and Baja California Sur) was estimated at 0.12% per year; this was later cited by Fuentes-Dávila (1996a). Brennan *et al.* (1992) estimated yield loss to account for *ca.* 6% of the total annual cost (\$US 7,022,000) of Karnal bunt in Mexico in 1989 which amounted to \$US 452,000 at that time.

In conclusion, low incidence and low severity of disease will obviously result in low yield loss but the maximum yield loss cited in the literature investigated for this PRA was 20% (*in* Warham, 1986).

Quality including effects on seed germination

The overriding feature of this pathogen is the effect it has on the quality of infected grain, since, according to Warham (1986), when more than 3% of grains are affected the grain is no longer accepted for processing and is declared unfit for human consumption.

Joshi *et al.* (1983) discussed the effect of Karnal bunt on the quality of seed, grain, flour and products made from contaminated flour as reported in the literature. Infected wheat seeds suffer reduced germination and viability. Nutrient composition of infected grain can be affected by Karnal bunt. The main effect reported was a reduction in total lysine content of 20–25% indicating a loss in protein content. There were also effects on the content of ash (increase), thiamine (decrease) and phosphorus (increase). Infected grain emits a fishy odour due to the production of trimethylamine. Karnal bunt can cause deterioration in flour quality. Depending upon the level of infection, products made from contaminated flour may be inedible. For example, flour milled from 10% infected grain was reported to be in dark in colour; chapattis made from contaminated flour were inedible. One per cent infection of grain led to chapattis deemed '*slightly affected*' with respect to palatability; 3% infection of grain resulted in chapattis that had a disagreeable odour and were unpalatable. Beniwal *et al.* (2000) confirmed that >3% infected grains resulted in flour that was unfavourable for chapatti production.

Where infected wheat seeds had lost their embryo they were incapable of germination. However, Jatav *et al.* (2003) showed that pinpoint infected seeds were capable of germination but seedlings tillered less compared to those arising from healthy seed; they also suffered massive reductions in yield (*ca.* 50%). However, given the risk associated with infected seed (introduction of the pathogen) this is not relevant to the impact of *T. indica* since infected seed should not be used for fear of pathogen introduction.

In terms of the risk to health from consuming infected wheat, Bhat *et al.* (1980 and 1981) did not detect mycotoxins in infected wheat and no deleterious effect was observed in short-term toxicology studies on rats, chickens and monkeys fed with Karnal bunt infected seed.

In terms of economic considerations, Brennan *et al.* (1992) showed that quality losses in Mexico in 1989 amounted to 36% of the total cost of Karnal bunt equating to \$US 2,543,000.

Control costs

Few economists have worked on the costs of control of Karnal bunt. Brennan *et al.* (1992) showed that control costs for Mexico in 1989 amounted to \$US 2,927,000. Control costs will vary according to whether the aim is to attempt to eradicate or contain the pathogen in areas where it is subject to official control or whether the area concerned is content to 'live with pest' and try to manage it accordingly. In the latter case, control costs will be those associated with attempting to avoid infection of wheat and triticale crops which may be difficult because of the longevity of *T. indica* in the soil and the fact that it is not controlled by seed treatments.

2.2. How great a negative effect is the pest likely to have on crop yield and/or quality in the PRA area?

Note: the ecological conditions in the PRA area may be adequate for pest survival but may not be suitable for pest populations to build up to levels at which significant damage is caused to the host plant(s). Rates of pest growth, reproduction, longevity and mortality may all need to be taken into account to determine whether these levels are exceeded. Consider also effects on non-commercial crops, e.g. private gardens, amenity plantings.

Minimal, minor, moderate, major, massive.

Moderate for yield relative to other impacts but still a significant cost. Massive for quality.

This question is difficult to answer with a single word, not only because two different topics (yield and quality) are covered by one question, but also because of the variation within and between countries that is likely to occur in the PRA area (as happens in countries where *T. indica* currently occurs).

The first UK PRA for this pathogen in 1996 (Sansford, 1996 *unpublished*, 1998) made no specific calculations of the likely economic effect of the introduction of *T. indica* into the UK since the effect on UK-grown cultivars of wheat (which have not been bred for resistance to *T. indica*) or indeed cultivars grown in other countries in the PRA area was unknown. In addition, the scientific literature indicated that there was no consensus regarding the relationship between disease incidence, severity and yield loss. Based upon the available literature, it was assumed that if *T. indica* were to be introduced there would be some loss in yield and a greater loss in quality.

Subsequent to the first UK PRA, Kehlenbeck *et al.* (1997) repeated the PRA exercise for Germany. Again it proved difficult to estimate the true losses, given the array of data in the literature. However, considering only the direct losses in yield, given a conservative estimate of loss of 0.5% across the whole of the wheat production area of Germany, the annual losses would amount to DM 15 million.

T. indica is likely to cause similar effects on crop yield and quality in the PRA area as it does in its existing area of distribution. In other words, the effects will vary from crop to crop and year to year. European winter, spring and durum wheat cultivars have not been bred for resistance to *T. indica* and, in this Project, have been shown to exhibit a range of susceptibility to infection by the pathogen similar to that which occurs in countries where it is established. The environmental conditions in the PRA have been shown to be favourable for infection and disease development. The effect on yield will be relatively small in percentage terms except possibly in localised

‘hotspots’ of disease where conditions are highly favourable to infection and disease development.

The effect on quality will be more significant since the overriding feature of this pathogen is the effect it has on the quality of infected grain. When more than 3% of grains are affected the grain is no longer acceptable for processing for human consumption. In the UK, the National Association of British and Irish Millers (NABIM) would most likely reject any grain found to be affected by Karnal bunt irrespective of the percentage affected, since flour made from infected grain is discoloured, baking quality is impaired and palatability is reduced due to the fishy odour of trimethylamine which the fungus produces. Other countries in the PRA area where high quality wheat is produced will be affected by the negative effect of *T. indica* on quality, even more so if quality assurance schemes are in place.

A detailed analysis of the socio-economic impact that this pathogen might have if an outbreak occurred in the PRA area was undertaken within this Project (Brennan *et al.*, 2004, *unpublished*) and provisional findings (which have been subsequently updated) were published in 2004 (see Brennan *et al.*, 2004a and Thorne *et al.*, 2004). Only a broad outline of the assumptions and findings from the Project is given below (and in response to subsequent questions), more detail is available from the cited references. Because *T. indica* is already a regulated pest in the PRA area, any outbreaks would be subject to official control by the relevant NPPO. In addition to the effects on yield and quality, the strategy adopted by the NPPO will impact on the costs arising from the outbreak. For this reason, the costs arising from an outbreak are difficult to disentangle, especially the effects on quality, which encompass both direct costs including downgrading affected milling wheat to feed wheat and also include the costs arising from downgrading unaffected wheat destined for human consumption to processing for animal feed. This is an indirect cost (a reaction cost, as outlined below).

To facilitate the analysis of the impacts, an examination was made of the policies and arrangements that are in place in countries where Karnal bunt (*T. indica*) occurs and these were compared to an early draft of a model contingency plan that might (see caveat in footnote below) be adopted in parts of a country such as the UK¹⁸ (Sansford *et al.*, 2004, *unpublished*) (a later version is presented in Annex II). This formed the backbone of the analysis of the likely impact that an outbreak of Karnal bunt would have in the PRA area. A Regulatory Impact Assessment which considers the costs and benefits of implementing the model contingency plan in full in England and Wales based upon costings for outbreaks in England, is presented in Annex III.

To understand how Brennan *et al.* (2004, *unpublished*) calculated the likely impacts (costs) of an outbreak of Karnal bunt in the PRA area (which form part of the answer for this and subsequent questions), it is necessary to understand the scenarios that they envisaged in their analysis.

¹⁸ By evaluating the range of measures that could be undertaken to prevent entry of the pathogen or to deal with outbreaks in the EU, a model management (contingency) plan has been produced based upon outbreak scenarios in England for consideration for implementation in England and Wales. However, this plan, which formed the basis of the socio-economic impact analysis, is for illustrative purposes only. It should not be taken as indicating how any EU Member State would respond to a finding of *Tilletia indica* on its territory. Any such response will be determined by the governments concerned, in the light of consultation with stakeholders and other relevant factors at the time including the availability of resources.

The costs (both direct and indirect) of a Karnal bunt outbreak are likely to depend on a number of factors, including:

- The size of the outbreak
- The region in which the outbreak occurs
- The country in which the outbreak occurs
- The time of detection of the outbreak
- The mix of bread, durum and feed wheat being grown in the affected region

Because all of these factors will determine the costs involved, the costs can only be determined for specified outbreak and detection scenarios. The policy adopted by the government in the affected country will of course be the main determinant of costs arising from the initial outbreak. In the analysis of Brennan *et al.* (2004, *unpublished*), two scenarios were analysed with the measures envisaged in the model contingency plan being implemented in full:

1. A ‘*large*’ outbreak in one region of the UK (in England) affecting 50,000 ha of wheat
2. A ‘*small*’ outbreak in one region of the UK (in England) affecting 1,000 ha of wheat

In each case, the scenario envisaged is that the outbreak is detected in mid-harvest, with the pathogen being found in grain being delivered to a silo. This means that in the first year, the impact is on the harvesting, processing and storage of the existing affected grain as well as destruction of unharvested crops. In subsequent years, farmers with affected fields will, under this model contingency plan, have to either manage them as bare fallow or by grassing them down for 5 years. Official controls would be placed on the affected fields as well as a 3-km radius buffer zone around the affected fields in which non-host crops will be permitted. However, in the first year, the assumption for this analysis is that the only controls are those that would be imposed on detection at mid-harvest.

The key elements of scenario 1 (a ‘*large*’ outbreak in the UK (in England)) are taken as:

- A 0.1% yield loss in affected crops (based upon Brennan and Warham’s 1990 derived estimate for Mexico; see below);
- 20% of crops in the region are affected in year 1;
- On the basis that 50,000 ha of crop was affected in Year 1 and those crops represent 20% of the region’s wheat, the total wheat area in the affected region is 250,000 ha;
- A region in the UK (in England) with 250,000 ha of wheat would be approximated by a circle with a radius of 70 km. A buffer zone would be established around this region, extending the boundaries by a further 3 km, so the entire region including the buffer zone has a radius of 73 km from its centre;
- On initial detection, all unharvested crops identified as affected will be destroyed, amounting to 20% of the total affected crops;
- For the already harvested crops, 10% of affected grain is destroyed directly;
- All of the remaining affected grain is subjected to heat treatment to kill *T. indica* spores and it is then used as animal feed;
- No wheat from the affected region (including the buffer zone) is milled;
- An export ban is imposed on all wheat from the affected region, whether directly affected or not;

- All bread and durum wheat produced in the region is downgraded to feed wheat, even where no *T. indica* spores are detected;
- In subsequent years, fields on which Karnal bunt (*T. indica*) was detected in Year 1 must be kept as bare fallow or be grassed-down for the following five years, after which non-host crops can be grown in those fields (provided no evidence of the pathogen is found within those 5 years);
- Within the affected region including the buffer zone, no wheat can be grown for the following nine years¹⁹, even land in which no *T. indica* spores were detected, so that within the entire affected region with a radius of 73 km all land other than the fields in which Karnal bunt (*T. indica*) was detected must grow only non-host crops over that period.
- All areas in the affected region in Years 2 to 10 are subject to chemical control for volunteer cereals and other plants. Spore monitoring equipment is placed at 1 km intervals around the perimeter of the buffer zone (a total of 462) and are checked every week;
- In the UK as a whole, rail is not a significant means of transport for wheat and transport of grain/seed wheat is likely to be by road;
- The affected region has the same mix of bread, durum and feed wheat as the whole of the UK (that is 31.1% bread, 0.1% durum and 68.8% feed wheat);
- The affected region has the same average yields for wheat as the whole of the UK;
- The affected region has the same proportion of national wheat consumption as it has of national wheat production.

The price data used in the analysis were obtained from different national sources. In some cases, the appropriateness of the data was determined by the location of production, while in other cases it was dictated by the availability of the required level of detail. In all cases the average price data for the three years to 2001 were used. It was not possible to use a longer array for data for the price series due to the impact of intervention prices on the determination of national prices prior to this time period. The prices selected were averaged in the currency quoted and then converted to Euros at average exchange rates where necessary. The weighted average EU price for each wheat type was then calculated using production as weights. The final set of prices across the EU for use in the analysis was:

- Bread wheat €130 /t
- Feed wheat €118 /t
- Durum wheat €149 /t

The cost components associated with a Karnal bunt outbreak and occurrence were identified, and the were classified as:

- Direct costs.
- Reaction costs.
- Control costs.

The direct costs are the yield and quality losses in crops affected with Karnal bunt; the topics of relevance to this particular question. As with earlier attempts at quantifying this, after examining

¹⁹ This is not necessarily what would happen in practice – this is for scenario purposes only.

the literature on Karnal bunt outbreaks in other regions (Brennan and Warham, 1990; Brennan *et al.*, 1992; Murray *et al.*, 1996; Sansford, 1996 (*unpublished*), 1998; Kehlenbeck *et al.*, 1997) it became evident that it was necessary to specify, rather than estimate, a direct impact of yield losses associated with a Karnal bunt outbreak. It should also be noted that the pathogen may have different yield impacts on bread, durum and feed wheats. It was also necessary to specify the geographical disaggregation of this data for major wheat producing regions in the EU.

In order to determine the total cost associated with yield effects from a Karnal bunt outbreak it was also necessary to identify total area and production of wheat, by wheat type, and by region.

As outlined above, the yield loss in affected crops assumed for the Project's analysis of potential impacts in the EU was 0.1% and this was derived from Brennan and Warham (1990). This figure was itself a derived figure for affected areas of north-western Mexico (southern Sonora, Sinaloa and Baja California Sur) and was estimated from data on the '*quantities of grain delivered with different levels of infected grain and by assuming a 25% loss of weight in infected grains*'. It is not a definite prediction of the likely impacts of *T. indica* on yield in the PRA area.

Based upon this estimate, in the first year of the outbreak, with 50,000 ha of affected crop in the '*large outbreak*' scenario, and expected average disease-free yields of 7.63 t/ha, giving a potential total yield of 381,500 tonnes, the 0.1% yield loss would lead to a loss of production of 381 tonnes. Valued at the prices shown above, the total yield loss in the first year had an estimated value of €46, 000. Outside of the affected crops there would be no associated yield losses.

For the '*small outbreak*' scenario where 1,000 ha of crops are affected the total yield loss in the first year had a value of €1,000.

In subsequent years, the costs resulting from an outbreak varied from those in Year 1 because some other control measures and responses would be implemented. Since under this model contingency plan it is envisaged that no wheat can be grown for at least five years, there will be no wheat crops in the region affected by *T. indica* in Year 2 and beyond and therefore no yield losses, provided there is no spread of the pathogen outside of the affected area.

Direct quality losses are a substantial direct cost associated with a Karnal bunt outbreak. These occur when *infected* milling wheat is considered unsuitable for human food uses and as a result is downgraded to feed wheat. There can be a considerable economic cost associated with the loss of value of milling wheat down-graded to feed wheat and it is therefore essential that this component is captured in the analysis (Murray and Brennan, 1998).

As well as the direct quality losses mentioned above, which are associated with the downgrading of infected milling wheat, there can also be indirect quality losses associated with the downgrading of unaffected grain. As these indirect quality losses are associated with unaffected grain they are treated as reaction costs rather than direct costs.

The economic quality losses incurred by farmers when infected and unaffected bread and durum wheat are downgraded to feed wheat are a combination of the quantity of each type of wheat downgraded and the price premium for bread and durum wheats over feed wheat. For the '*large outbreak*' scenario, given the prices determined above, the 502,000 tonnes of bread wheat and

1,000 tonnes of durum wheat downgraded to feed wheat in Year 1 would result in a loss in value of €11.86 million. For the ‘*small outbreak*’ scenario the equivalent loss would be €376,000.

Finally, with respect to quality assurance costs, on the basis that all UK wheat production is subject to quality assurance, there are no specific costs associated with this, since they would be included in the market reaction to the presence of the pathogen.

As with yield losses, quality losses would not be incurred in years 2 to 10 as it is assumed under the model contingency plan that there will be no wheat grown in the affected region and under this scenario it is assumed that there is no spread of the pathogen outside of the affected area.

For the details of the financial effects of implementing the model contingency plan at Annex II, see the following Annexes:

Annex IV shows a breakdown of all the costs incurred in year 1 of a 50,000 ha outbreak in the UK (in England) (from Brennan *et al.*, 2004, *unpublished*). This also describes the actual components of the direct, reaction and control costs.

Annex V shows a breakdown of all the costs incurred in year 1 of a 1,000 ha outbreak in the UK (in England) (from Brennan *et al.*, 2004, *unpublished*).

Annex VI shows a breakdown of direct costs, reaction costs and control costs for years 1 to 10 of a 50,000 ha outbreak in the UK (in England) (from Brennan *et al.*, 2004, *unpublished*).

Annex VII shows a breakdown of direct costs, reaction costs and control costs for years 1 to 10 of a 1,000 ha outbreak in the UK (in England) (from Brennan *et al.*, 2004, *unpublished*).

In the event that *T. indica* became deregulated and countries within the PRA area had to manage outbreaks of Karnal bunt, the effect of the pathogen on yields would, on average, be less significant than that associated with a loss of quality for wheat intended for human consumption (i.e. the costs of downgrading milling wheat to feed wheat).

2.3. How great an increase in production costs (including control costs) is likely to be caused by the pest in the PRA area

Minimal, minor, moderate, major, massive.

Massive.

Whilst *T. indica* is listed as a I/AI pathogen in the PRA area, production costs in the PRA area arising from an outbreak of Karnal bunt will include the control costs associated with the efforts of the NPPO to attempt to contain and/or eradicate the pathogen. The specific control costs considered for the analysis of Brennan *et al.* (2004, *unpublished*) include the containment costs, eradication costs and surveillance and testing costs arising from implementation of the management of an outbreak in England according to an earlier version of the model contingency plan (Sansford *et al.*, 2004, *unpublished*). The most recent version of this plan is presented in Annex II of this report.

In addition to these specific control costs, *T. indica* is a seed-borne fungus and if present in purchased seed it can be particularly detrimental. Even using seed which has been certified as

free of *T. indica* teliospores but which comes from an affected area, or even a region located near an affected area, carries the risk of introducing the pathogen, as control measures may not be totally effective. This makes buyers cautious about sourcing seed and as a result they may decide to source their seed elsewhere.

The cost of production per tonne of seed is greater than that of milling or feed wheat. The inability to sell seed from an affected region can affect the producer's margin between costs and revenue to a greater extent than in the case of downgrading from milling to feed wheat. Therefore, the extra costs of production associated with seed production needs to be taken into consideration in addition to the seed premium that is foregone as a result of a Karnal bunt outbreak. This is a reaction cost.

Given the need to plant pathogen-free seed, seed producers in the affected region will lose their ability to market clean wheat seed. The estimated losses for those seed producers are €200,000 in the year of the detection of the outbreak for the '*large outbreak*' scenario. For the '*small outbreak*' scenario the loss would be €6,000. The seed normally produced in that region will need to be sourced elsewhere in the country, so that there will be equivalent gains in the rest of the affected country. As there will be no wheat planted in the affected area in years 2 to 10 of the outbreak there will no further costs.

The specific control costs associated with the '*large outbreak*' scenario in year 1 managed according to the model contingency plan at Annex II are listed in Annex IV. The total control costs (separate from the seed industry costs) for the UK amount to €17.5 million. The addition of surveillance and testing costs and administrative/compliance costs for other EU countries brings the total control costs for the EU, arising from a 50,000 ha outbreak in the UK to €17.8 million.

Over years 1 to 10 the total control costs for the '*large outbreak*' scenario in the UK amount to €428.1 million (Annex VI).

The specific control costs associated with the '*small outbreak*' scenario in year 1 managed according to the model contingency plan at Annex II are listed in Annex V. The total control costs in year 1 (separate from the seed industry costs) for the UK amount to €660,000. The addition of surveillance and testing costs and administrative/compliance costs for other EU countries brings the total control costs for the EU, arising from a 1,000 ha outbreak in the UK, to €939,000 in the first year.

Over years 1 to 10 the total control costs for the '*small outbreak*' scenario in the UK amount to €14.8 million (Annex VII).

In the event that *T. indica* became deregulated and countries within the PRA area had to manage outbreaks of Karnal bunt, the costs of control would be those associated with the management of a pathogen which is extremely long-lived in the soil. Growing alternative crops to wheat and triticale for long periods to avoid infection (and hope for a demise in the population of teliospores in the soil) is likely to impact on farm income.

2.4. How great a reduction in consumer demand is the pest likely to cause in the PRA area?

Minimal, minor, moderate, major, massive.

Major effect on (supply and) demand in the long-term²⁰. There will be no reduction in demand, particularly as wheat is a staple food for both humans and animals but sources of supply will have to change. The demand for feed wheat may even increase as outlined below.

Brennan *et al.* 2004 (*unpublished*) consider that the economic effects of downgrading bread and durum wheat to feed wheat and the effects of export bans all need to be analysed in one market framework. These components cannot be directly separated in the analysis, since the net effects of shifts in quality between market sectors and the reduction in production because of crop destruction, yield losses due to Karnal bunt, and export bans are all integrated into the one analysis. Thus it is not possible to give a definitive value to the effect of an outbreak of Karnal bunt in the PRA area on demand alone.

In the short-term, after an outbreak of Karnal bunt is detected, supply would be fixed, with no possibility of supply response, since the scenario being analysed is one in which Karnal bunt is detected at or after harvest. Only if the outbreak is detected in time for farmers to make decisions not to produce or harvest wheat will there be any supply response.

However, the impacts of an outbreak of Karnal bunt will be felt for several years afterwards. There may be planting restrictions on crops for several years after the initial outbreak which will affect supply. There may also be trade implications for several years after an outbreak, until the markets are satisfied that Karnal bunt is no longer a problem in that area.

For demand, there are few substitutes for wheat in human food production. However, for feed, there is high substitutability between animal feed ingredients (Brennan *et al.*, 2002). Consumers buying feed grain who had not previously used wheat will rethink their usage if feed wheat becomes more abundant following an outbreak of Karnal bunt (as a result of downgrading milling wheat to feed wheat).

2.5. How important is environmental damage caused by the pest within its current area of distribution?

Minimal, minor, moderate, major, massive.

Minimal.

2.6. How important is the environmental damage likely to be in the PRA area?

Minimal, minor, moderate, major, massive.

Minimal.

²⁰ NB: No other question in this PRA scheme addresses the inter-relatedness of supply and demand hence the response is not simple and cannot reflect reductions alone.

2.7. How important is social damage caused by the pest within its current area of distribution?

Minimal, minor, moderate, major, massive.

Major in some countries and minor in others.

Social damage has varied according to the plant health policies in place in countries where *T. indica* is known to occur.

No strict quarantine measures have been imposed in Asia and no analysis of the impacts arising from the presence of *T. indica* in countries in Asia has been undertaken.

In Mexico and the USA, preliminary attempts at containment or eradication of *T. indica* have had a negative social effect on farmers and those closely associated with the wheat industry. (Fuentes-Dávila, 1998; Jones, 1998; Nave, 1998). The types of social damage that have been caused by the presence of *T. indica* are those described under 2.8, below.

2.8. How important is the social damage likely to be in the PRA area?

Minimal, minor, moderate, major, massive.

Major.

Brennan *et al.* (2004, *unpublished*) made no attempt to define precisely and measure the social costs that would be associated with an outbreak of Karnal bunt in the EU. However, if an outbreak was to occur within a defined region, and some crops were destroyed, some harvested grain was destroyed, some was sent for special processing, and all grain produced in the affected region had its value reduced to that of feed wheat, there would be large financial impacts felt by farmers within the affected region. These farmers will suffer negative effects on farm income because the presence of *T. indica* will lead to long-term planting restrictions.

These impacts will lead to some social disruption within the farming community, with affected farmers having significantly reduced incomes, particularly in the short term. The extent to which those costs are borne by that group of farmers depends on the extent of any compensation payments to those farmers affected. If the affected farmers are not fully compensated, then there will be inequities in the impacts on various individuals.

In addition, others involved in the grain trade in that region will be affected, with contract harvesters, haulage contractors, grain processors, etc, either facing loss of income or additional costs associated with the control measures imposed on affected grain and unaffected grain from the region. Some of these costs will be substantial for the individuals involved. Again, the level of compensation (if any) is important in determining the extent to which the burden of an outbreak is shared equitably.

Depending on the size of the region and the extent of the outbreak, there will be multiplier effects felt in the wider regional economy if farmers and their service suppliers have reduced incomes. Thus, there are likely to be employment consequences in the local towns and cities in the affected region, the extent of which will depend on the reliance of that regional economy on agriculture. If there is a severe outbreak in a region strongly dependent on agriculture, then the

knock-on effects in terms of loss of employment and regional income could be substantial. However, if the outbreak occurred in a region only marginally reliant on farming income, then even though the impacts on the farmers and their service suppliers would be substantial, the wider regional impacts are likely to be very small. In the event of a large outbreak that leads to export bans from a region for many years, the degree of social adjustment required within the affected region could be substantial.

There will also be social effects from the disruption to daily lives associated with any quarantine policies that might be implemented to try to control and eradicate the pathogen. For example, if all vehicles leaving and entering the affected region need to be inspected and possibly cleaned and disinfected, local traffic could be substantially disrupted for some time. Similarly, restrictions on the movement of seed, machinery, equipment, etc., from the affected region could impose substantial social disruption on people who are required for other purposes to move through the region on a regular basis. Clearly, also, any imposition of such quarantine policies could restrict and damage regional tourist trade, and could lead to losses for people involved in the tourism industry in the region.

There may have to be additional inspections and surveillance activities in relation to the detection, monitoring and control of the pathogen in the affected region and its neighbouring regions. These activities are likely to lead to some additional jobs and economic activity in the region, though they are likely to be small in relation to the costs of the outbreak. In any case many of these activities will be undertaken and directed from outside the region, so that the positive impacts of that additional activity may not be felt in the affected region itself.

Given that the economic impact of a Karnal bunt outbreak on the rest of the country and the other countries in the EU is shown to be very small, there is likely to be little social cost on the regions outside the affected region. These impacts may well be positive for those other areas, given the need to obtain clean seed from outside the region, an increase in wheat prices across the EU, and a possible diversion of activities such as tourism away from the affected region.

If your answer to any of these questions is major or massive, the evaluation of the following questions may not be necessary. You may go directly to point 2.15 unless a detailed study of impacts is required.²¹

2.9. How likely is the presence of the pest in the PRA area to affect export markets?

Note: Consider the extent of any phytosanitary measures likely to be imposed by trading partners.

Impossible/very unlikely, unlikely, moderately likely, likely, very likely/certain.

Certain.

The international movement of cereal grain and seed is regulated by individual countries' plant health import regulations.

²¹ Although several answers are major/massive answers to questions 2.19 to 2.14 are given.

T. indica is listed as a quarantine pest by many countries NPPOs as well as by Regional Plant Protection Organisations (RPPOs). The individual phytosanitary requirements of the importing countries will have to be met to facilitate exports from the PRA area. In some cases imports originating in the PRA area may be banned.

Smith (2001, *unpublished*) found that 33 countries had specific restrictions on the importation of wheat from countries with *T. indica*, either through national regulation or through being a member of an RPPO.

Those listed at that time (beyond the UK) included: Argentina, Australia, Belarus, Bulgaria, Brazil, Chile, China, Croatia, Cyprus, Czech Republic, Estonia, Hungary, Latvia, Lithuania, Macedonia, Malawi, Moldova, Morocco, New Zealand, Norway, Paraguay, Romania, Russia, Saudi Arabia, Slovakia, Slovenia, Switzerland, Tunisia, Turkey, Ukraine, Uruguay, USA and Yugoslavia.

The EPPO Plant Quarantine Retrieval system (EPPO, 2005) lists some of these countries individually and several RPPOs to which some of the above-named countries belong as having some form of regulation against *T. indica*. One additional individually-named country with specific restrictions on importation of wheat from countries with *T. indica* listed by EPPO is Canada.

There may be more countries with specific import requirements regarding *T. indica* but the complexities of overseas countries import regulations makes this difficult to ascertain.

As an exporter the former EU 15 Member States exported a total of 2,136,493 tonnes of wheat between July 2003 and June 2004; the countries of destination are unavailable. The UK exported a total of 2,200,309 tonnes of wheat between July 2003 and June 2004 to a range of countries in the EU region and to 4 non-EU region countries. (HGCA, 2004).

Brennan *et al.* (2004, *unpublished*) analysed the effect of a 'small' (1,000 ha) and 'large' (50,000 ha) outbreak of *T. indica* in the UK on export markets for the EU. Information on the levels of exports from regions prior to a Karnal bunt outbreak was deemed necessary to determine the cost component associated with export bans due to Karnal bunt. The extent of the losses associated with an export ban will depend initially on the level of exports from a region prior to the Karnal bunt outbreak and also on the different types of wheat produced within a region because of possible differential responses to wheat of different types. The reaction of markets to grain from a Karnal bunt-affected region will have a significant effect on costs associated with an outbreak. Data on export quantities from the EU 15 countries were obtained from FAO (2003). The period for the export data in their analysis was the five-year average to 2001. On average, the EU was found to export 27.9 million tonnes of wheat per year, or 28% of production.

For the analysis, a distinction had to be made between exports of different wheat types.

However, although there was anecdotal information that most exports were bread wheat, data were not available on exports by wheat type, so assumptions had to be made. The basis for determining exports by wheat type was as follows:

- Durum wheat production was assumed to be consumed in the country of production (except for Italy);

- Where no other data were available, 80% of exports were taken as milling wheat, and the remainder were feed wheat;
- For Ireland, no milling wheat was exported;
- For Italy, all exports were durum wheat.

On the basis of these assumptions, a breakdown of exports into wheat types was made. From those calculations, an average of 43% of bread wheat produced in each country was envisaged as being exported, 15% of feed wheat, and, only small quantities of durum from Italy. In assessing the impacts of Karnal bunt on exports, these were the figures applied in the analysis.

Brennan *et al.* (2004, *unpublished*) consider that the economic effects of downgrading bread and durum wheat to feed wheat and the effects of export bans all have be analysed in a specified market framework. It was not possible to separate these components in the analysis, since the net effects of shifts in quality between market sectors and the reduction in production because of crop destruction, yield losses due to Karnal bunt, and export bans were all integrated into the one analysis.

Price and export losses resulting from a ‘*large outbreak*’ of 50,000 ha in the UK (in England) was calculated to be €13.47 million in the affected region in year 1 (€450,000 for the ‘*small outbreak*’ in England of 1,000 ha). Provided there is no spread of the pathogen out of the affected area and provided importing countries are content with the methods deployed by the NPPO in the affected country to comply with import requirements for consignments arising in the affected country, in years 2 to 10 there will be no losses associated specifically with loss of exports. This is because there would be no wheat produced in the affected region (the economic losses associated with the inability to grow wheat have been measured as control costs).

2.10. How easily can the pest be controlled in the PRA area?

Very easily, easily, with some difficulty, with much difficulty, impossible.

With much difficulty.

If a decision was taken to ‘*live with the pest*’ rather than to manage it as a quarantine organism, control would be difficult because of the longevity of its teliospores and the fact that it can be carried both as a surface contaminant of seed and as inoculum within the seed. Unlike other bunts and smuts of wheat it is difficult to control by seed treatments as it does not infect at the seedling stage. However, a few options for disease control are available, which, if used in an integrated manner, might reduce the incidence and severity of the disease as outlined below. (See 3.18 and 3.19).

2.11. How probable is it that natural enemies, already present in the PRA area, will suppress populations of the pest if introduced?

Very unlikely, unlikely, moderately likely, likely, very likely.

Very unlikely.

There are no reported natural enemies of *T. indica*.

2.12. How likely are control measures to disrupt existing biological or integrated systems for control of other pests or to have negative effects on the environment?

Impossible/very unlikely, unlikely, moderately likely, likely, very likely/certain.

Unlikely.

2.13. How important would other costs resulting from introduction²² be?

Note: Costs to the government, such as research, advice, publicity, certification schemes; costs (or benefits) to the crop protection industry.

Minimal, minor, moderate, major, massive

Minimal.

The majority of costs to the government are included in the control costs because *T. indica* would, while it is listed as a quarantine pest, be subject to official control. These costs are immense. (See 2.3). No further consideration is given here to this area since while *T. indica* is the subject of official control the only other likely costs would be for funding investigations into control methodologies in the PRA area.

2.14. How likely is it that genetic traits can be carried to other species, modifying their genetic nature and making them more serious plant pests?

Impossible/very unlikely, unlikely, moderately likely, likely, very likely/certain.

Very unlikely.

Within the genus *Tilletia*, although not known to occur at present, hybridisation between closely related fungal species cannot be ruled-out.

2.15. How likely is the pest to act as a vector or host for other pests?

Impossible/very unlikely, unlikely, moderately likely, likely, very likely/certain.

Impossible.

Conclusion of the assessment of economic consequences

2.16. Referring back to the conclusion on endangered area (1.36), identify the parts of the PRA area where the pest can establish and which are economically most at risk.

All of the wheat-producing areas of the PRA area are at risk from economic damage associated with the establishment of *T. indica*.

The pathogen has been shown to be able to survive in soils in several parts of the EU for at least 3 years. It can infect European wheat cultivars and lead to symptoms of Karnal bunt.

Although some areas of the EU will have more frequent occurrence of conditions favourable for infection and the start of disease development as demonstrated by the frequency of favourable

²² 'Introduction' is defined as the 'the entry of a pest resulting in its establishment' (FAO, 2002)

HTI values, all areas have been shown to have some favourable years within the period studied in this Project (1995–2002 for the EU). This means that, all other factors being equal, build-up of levels of the pathogen (teliospores) would potentially be faster in those areas where the frequency of occurrence of favourable HTIs was greatest.

In the major bread/feed wheat growing regions of Europe, namely western and central Europe, there is a very high probability of infection by *T. indica* and the commencement of disease development. (Based upon the frequency of years in which the HTI values are favourable). More northerly regions are predicted to have critical HTI values in about one third of the cases studied (sowing date × years).

The predictions for durum wheat show very high frequencies of critical HTI values for the northern Italian plain and the important pasta growing areas of Marche and Toscana. The Basilicata region in the foot of Italy seems to be less severely affected, possibly because the extremely high temperatures found in this region raise the HTI out of its critical range. France and especially Spain seem less prone to critical HTIs for durum wheat, this may be related to the fact that durum is generally sown later than winter wheat and thus may reach its susceptible phenological stages later, when conditions are drier and warmer and so the HTI does not fall within the critical window in so many cases. Eastern Europe is also predicted to have a medium to high risk for infection of durum wheat, somewhat less than for northern Spain and central and eastern France.

Although the frequency of years in which the HTI has been shown to be favourable is less in some parts of the EU than others, the ability of the pathogen to survive for long periods below the soil surface should ensure that some teliospores are present and capable of germinating when returned to or near to the surface in favourable years.

The risk to the triticale crop has not been fully evaluated but this is also potentially at risk in areas where the HTI values are favourable.

Go to degree of uncertainty

Degree of uncertainty

Estimation of the probability of introduction of a pest and of its economic consequences involves many uncertainties. In particular, this estimation is an extrapolation from the situation where the pest occurs to the hypothetical situation in the PRA area. It is important to document the areas of uncertainty and the degree of uncertainty in the assessment, and to indicate where expert judgment has been used. This is necessary for transparency and may also be useful for identifying and prioritising research needs.

For Pest Initiated Risk Assessments:	Go to conclusion of the risk assessment
For Pathway Initiated Risk Assessments:	Go to back to 1.4 to evaluate the next pest, if all pests have been evaluated go to conclusion of the risk assessment

Conclusion of the risk assessment

Entry

Evaluate the probability of entry and indicate the elements which make entry most likely or those that make it least likely. Identify the pathways in order of risk and compare their importance in practice.

Probability of entry

- Entry of *T. indica* to the PRA area is moderately likely on infected or contaminated seed and grain of *T. durum* and *T. aestivum* originating in Afghanistan, Brazil, India, Iran, Iraq, Mexico, Nepal, Pakistan, South Africa and the USA. Seed is a direct route of entry. Grain transported through the cereal producing areas of the PRA area also has the potential to disseminate teliospores of the fungus.
- Although *x Triticosecale* is recorded as a natural host of *T. indica* reports of findings are few and far between making entry on seed or grain less likely but possible.

Uncertainty and suggestions for improvement in determining the risk of entry

- There may be other countries, or parts of countries, where the pathogen occurs, but where it has not yet been discovered or reported. Some countries such as Brazil have reported initial outbreaks of *T. indica* in the literature but no later publications have been found that indicate its current status. Only the provision of survey data could improve this section of the determination of the risk of entry.
- Trade data are inconsistently reported and often do not give sufficient detail to determine whether the imported commodity is grain or seed or *T. aestivum* or *T. durum*.
- Not all affected countries appear in the various databases that have been used to determine whether or not they export the relevant commodities to the PRA area.
- More information on export trade from countries where the pathogen occurs would improve these aspects of the PRA.
- Uncertainty also arises when it comes to determining the methodology used in the exporting country to certify seed and grain of these three commodities as being free from *T. indica*. Without adequate testing the risk of entry increases. Interceptions of *T. indica* on grain of *T. aestivum* and *T. durum* in the PRA area in recent years suggests that testing methodologies in some exporting countries are not always as effective as those used in the importing countries in detecting teliospores of the pathogen.
- A study of the routes taken within the PRA area by consignments from countries where the pathogen occurs would improve the pathway analysis.

Establishment (including spread potential)

Evaluate the probability of establishment, and indicate the elements which make establishment most likely or those that make it least likely. Specify which part of the PRA area presents the greatest risk of establishment.

Probability of establishment

- The overall probability of establishment in the PRA area is high in all of the wheat producing areas. Some parts of the PRA area may be favourable for establishment post-entry more frequently than others.
- The probability of establishment in triticale is not known because it has not been investigated in this Project. Triticale is a natural host of *T. indica* but there are few records of outbreaks in the scientific literature.

Uncertainty and suggestions for improvement in determining the risk of establishment

- The data obtained from this Project on host susceptibility, the window of infection, the survival of teliospores and the availability of teliospores capable of germinating to produce infective sporidia at the timing of the infection window of wheat are experimental.
- There will always be uncertainty over the spread of dormancy within a population of teliospores. This will affect the predictions of the timing of teliospore germination in relation to the wheat crop. However, although experimental work within the Project suggests that under extremes of soil moisture (constant optimum from the beginning of the cropping season) there is a reduction in numbers of teliospores on the soil surface which would be available to germinate and infect at the period of host susceptibility, some teliospores will remain available to germinate in a number of European locations.
- There is still uncertainty with respect to sporidial production and dispersal within EU wheat crops as this could not be studied *in vivo*.
- There is always scope for further investigation. However, while the pathogen is an EC I/A1 quarantine listed organism investigation in the field without the use of quarantine containment is impossible.
- One (the HTI) of only a handful of models has been used to aid in the prediction of establishment but all of the other published models have been evaluated by Partners in this Project and found unsuitable for this purpose. In the absence of the pathogen in the PRA area the HTI cannot be validated further.
- One of the Project Partners (Australia) is attempting to develop a teliospore germination model which may assist further in the prediction of the risk of establishment.
- There is some uncertainty over the rate of spread if moved by air currents or in trade. Information obtained from countries where *T. indica* occurs is mainly anecdotal because of the difficulties of detection of the disease in the field when it occurs at low levels. All that can be assumed is that teliospores can move both in air currents and in trade. The rate of spread in the PRA area is therefore difficult to determine.

Economic importance

List the most important potential economic impacts, and estimate how likely they are to arise in the PRA area. Specify which part of the PRA area is economically most at risk.

Probable economic consequences

- Outbreaks of *T. indica* in wheat crops in the PRA area are likely to occur should the pathogen be allowed to enter the PRA area.
- This will lead to some loss of yield, a greater loss of quality and a potentially major loss of valuable export markets to countries which have a requirement for freedom from *T. indica* or Karnal bunt in imported cereals.
- Because *T. indica* is already listed as a I/A1 quarantine pest by the EU, and because countries outside of the EU have specific import requirements related to grain and seed originating in countries where *T. indica* occurs, there may, depending upon the policy adopted by government, be major costs involved in managing any outbreak which may extend over a long period of time (maybe up to at least 5 years), before it might be determined whether the management plan that has been deployed has led to the demise/eradication of the pathogen.

Uncertainty and suggestions for improvement in determining the economic impacts

- The actual loss in yield that will occur is difficult to determine as it will vary from location to location and year to year dependent upon the favourability of the climate and the cultivars for infection and disease development.
- Some of the impacts are inter-related in the analysis of the consequences in the Project and were therefore difficult to disentangle
- If no action were taken then the pathogen could enter, establish and spread and the impacts would depend both upon the potential to manage the pathogen (which because it has great longevity in the soil would rely upon growing alternative crops to wheat and triticale) and the reaction to lack of official control both within the country concerned, within the EU and by importing countries outside of the EU.
- The economic consequences of not taking action for interceptions and outbreaks in the PRA area have not been evaluated fully but could be undertaken with the methodologies designed by the economists in this Project.

Recommendation

The risk assessor should give an opinion as to whether the pest or pathway assessed is an appropriate candidate for stage 3 of the PRA: the selection of risk management options, and an estimation of the pest risk associated.

Tilletia indica is already listed as a I/A1 pest by the EC. It has the potential to enter, establish and cause serious economic impacts. For these reasons it is an appropriate candidate for the selection of risk management options.

Stage 3: Pest risk management

In all cases start with step 3.1.

*In the case of a **pest-initiated analysis**, proceed through steps 3.2-3.9, which relate to different pathways on which the pest being analysed may be carried. Thereafter continue with the questions concerned with the measures that might be applied to each pathway. Repeat the process for every major pathway.*

*In the case of a **pathway-initiated analysis**, since the precise pathway is already known, begin with question 3.10 to consider possible measures for this pathway and repeat the process as far as question 3.40 for each of the pests identified in the pest risk assessment as presenting a risk to the PRA area. When all the pests have been considered, go to 3.41 to integrate the measures for the commodity. (Note that the probabilities for entry of a particular pest with other pathways, including existing pathways, may also need to be investigated).*

In considering your responses to the following questions, please note that helpful information may be obtained from the pest risk assessment stage, particularly from the section concerning entry (1.1-1.15). References to the relevant sections of the risk assessment stage have been added.

The intentional introduction of plants that are potential pests is covered under pest initiated analysis. (The main pathway for these plants is usually the trade with ornamental plants

intended for planting.) The unintentional introduction of pest plants is covered under commodity initiated analysis (or pathway initiated analysis).

Risk associated with major pathways

Acceptability of the risk

The importing country performing the Pest Risk Analysis has to decide whether the risk from any pest/pathway combination is an acceptable risk. This decision will be based on the relationship between the level of risk identified in the pest risk assessment stage (i.e. the combination of the probability of introduction and the potential economic impact) and the importance/desirability of the trade that carries the risk of introduction of the pest.

3.1. Is the risk identified in the Pest Risk Assessment stage for all pest/pathway combinations an acceptable risk?

If yes
If no

STOP
Go to 3.2

No.

The risk is not acceptable.

Types of pathways

In most cases, the pathways to be studied will be particular commodities of plants and plant products, of stated species, moving in international trade and coming from countries where the pest is known to occur, and the questions are intended primarily for these cases. However, the pathways identified in the pest risk assessment may also include other types of pathways, e.g. natural pathway (pest spread), transport by human travellers, means of transport, packing material and traded commodities other than plants and plant products, which also need to be assessed for suitable measures. Therefore, this section explains how to cover the other types of pathways. For plants, it is particularly important to prioritize the pathways and to identify their relative importance, as some important pathways may not currently be regulated (grain, wool, hides, sand, gravel...). For intentionally introduced plants the objective is to prevent spread from the intended habitat to the unintended habitat consequently the only feasible actions are measures taken in the importing country or the prohibition of imports go directly to question 3.28. It may also be needed to consider the natural spread of the plant.

3.2. Is the pathway that is being considered a commodity of plants and plant products?

If yes
If no

Go to 3.10
Go to 3.3

Yes. See the answers to questions 1.1 to 1.3. Although this is a PRA for a specific organism it is the commodities that carry it which pose the greatest risk. Thus, although other pathways of entry exist they are not considered further in this section. However, establishment and spread including that resulting from human activities within the PRA area post-entry would have to be subject to appropriate risk management measures and this is considered further below.

3.3. Is the pathway that is being considered the natural spread²³ of the pest?

If yes **Go to 3.4**
If no **Go to 3.8**

3.4. Is the pest already entering the PRA area by natural spread or likely to enter in the immediate future? (See answer to question 1.33).

If yes **Go to 3.5**
If no **Go to 3.37**

3.5. Could entry by natural spread be reduced or eliminated by control measures applied in the area of origin?

If yes **Possible measures: control measures in the area of origin.
Go to 3.6**

3.6. Could the pest be effectively contained or eradicated after entry? (see answer to question 2.8)

If yes **Possible measures: internal containment and/or
eradication campaign.
Go to 3.7**

3.7. Was the answer ‘yes’ to either question 3.5 or question 3.6?

If yes **Go to 3.37**
If no **Go to 3.45**

3.8. Is the pathway that is being considered the entry with human travellers?

If yes **Possible measures: inspection of human travellers, their
luggage, publicity to enhance public awareness on pest
risks, fines or incentives. Treatments may also be
possible.
Go to 3.29**

If no **Go to 3.9**

3.9. Is the pathway being considered entry on contaminated machinery or means of transport?

If yes **Consider possible measures: cleaning or disinfection of
machinery/ vehicles.**

For contaminated machinery or means of transport or other types of pathways (e.g. commodities other than plants or plant products, exchange of scientific material, packing material, grain, wool, hides, sand, gravel etc), not all of the following questions may be relevant; adapt the questions to the type of pathway.

²³ Natural spread includes movement of the pest by flight (of an insect), wind dispersal, transport by vectors such as insects or birds, natural migration, rhizomial growth.

Go to 3.10

Existing phytosanitary measures

Phytosanitary measures (e.g. inspection, testing or treatments) may already be required as a protection against other (quarantine) pests (see stage B: question 1.10). The assessor should list these measures and identify their efficacy against the pest of concern. The assessor should nevertheless bear in mind that such measures could be removed in the future if the other pests are re-evaluated.

3.10. Are there any existing phytosanitary measures applied on the pathway that could prevent the introduction of the pest

Yes. *Tilletia indica* is listed as a I/A1 quarantine pest in the EC Plant Health Directive (2000/29/EC as amended) (Anon., 2000). Current EC requirements are for seeds of *Triticum*, *Secale* and x *Triticosecale* imported from countries where the pathogen occurs to originate in an area where *T. indica* is known not to occur. For grain there are two options: (a). To originate in an area where *T. indica* is known not to occur, or (b). For place of production freedom based upon inspection during the growing season and testing of the grain at harvest and before shipment for freedom from *T. indica*. This should not be interpreted as allowing exporting countries to base certification for export of grain on symptoms alone.

Go to 3.11

Identification of appropriate risk management options

This section (questions 3.11 to 3.28) examines the characteristics of the pest to determine if it can be reliably detected in consignments by inspection or testing, if it can be removed from consignments by treatment or other methods, if limitation of use of the commodity would prevent introduction, or if the pest can be prevented from infecting/infesting consignments by treatment, production methods, inspection or isolation. 'Reliably' should be understood to mean that a measure is efficient, feasible and reproducible. Measures can be reliable without being sufficient to reduce the risk to an acceptable level. In such cases their combination with other measures to reach the desired level of protection against the pest should be envisaged (see question 3.31). When a measure is considered reliable but not sufficient, the assessor should indicate this. The efficiency, feasibility and reproducibility of the measures should be evaluated by the assessor for each potential management option identified. Cost effectiveness and impact on trade are considered in the section 'evaluation of risk management options' (questions 3.33 to 3.35).

Answer all questions from 3.11 to 3.22 (but note that questions 3.11 to 3.22 are not relevant for the intentional introduction of pest plants)

Options for consignments

Detection of the pest in consignments by inspection or testing

3.11. Can the pest be reliably detected by a visual inspection of a consignment at the time of export, during transport/storage or at import?

No. Except for heavily infected/contaminated consignments, the presence of bunted grains or seed will go undetected. The presence of free teliospores would most likely go undetected except where levels were high enough to emit a detectable level of trimethylamine.

If yes **Possible measure: visual inspection.**
Go to 3.12

3.12. Can the pest be reliably detected by testing in a consignment?

Yes. Published methods to detect teliospores of *T. indica* are freely available. These include Peterson *et al.*, (2000). Inman *et al.*, (2003) (published later as Anon., 2004).

If yes **Possible measure: specified testing.**
Go to 3.13

3.13. Can the pest be reliably detected during post-entry quarantine?

Yes. As per 3.12. Post-entry quarantine would be impractical for imported grain but seed imported from countries where *T. indica* occurs could be held subject to testing if storage facilities were available at the point of entry.

If yes **Possible measure: import under special licence/permit
and post-entry quarantine.**
Go to 3.14

Removal of the pest from the consignment by treatment or other phytosanitary procedures

3.14. Can the pest be effectively destroyed in the consignment by treatment (chemical, thermal, irradiation, physical)?

No. No treatments are available for grain or seed of *T. aestivum*, *T. durum* or x *Triticosecale* that do not affect the intended use of the consignment.

Sansford *et al.*, (2004b, *unpublished*) reviewed the literature as part of this Project and no practical treatment method was found for killing teliospores in grain while preserving milling quality. Although irradiation of grain killed teliospores of *T. controversa* and *T. tritici* in wheat while preserving milling quality, it also killed the embryo, thus causing it to break down quickly. The dosage of methyl bromide required to kill teliospores of these species also reduced the storage life of the wheat and presented residue problems.

If yes **Possible measure: specified treatment.**
Go to 3.15

3.15. Does the pest occur only on certain parts of the plant or plant products (e.g. bark, flowers), which can be removed without reducing the value of the consignment? (This question is not relevant for pest plants).

No. *T. indica* affects the grain and seed of the commodities concerned and removing the affected grain or seed would be physically difficult. Such a process would not remove any free teliospores that might be present. Treatments could be applied to the consignment which would affect its intended purpose (see 1.14).

If yes **Possible measure: removal of parts of plants from the consignment.**
Go to 3.16

3.16. Can infestation of the consignment be reliably prevented by handling and packing methods?

No.

If yes **Possible measure: specific handling/packing methods**
Go to 3.17

Prevention of establishment by limiting the use of the consignment

3.17. Could consignments that may be infested be accepted without risk for certain end uses, limited distribution in the PRA area, or limited periods of entry, and can such limitations be applied in practice?

Yes. As outlined under 1.14 processing infected or contaminated grain intended for human consumption by the specific methods described for animal feed production will destroy teliospores of the pathogen. Processing infected or contaminated grain destined for animal feed by the same methods will also be effective.

If yes **Possible measure: import under special licence/permit and specified restrictions:**
Go to 3.18

Options for the prevention or reduction of infestation in the crop²⁴

Prevention of infestation of the commodity

3.18. Can infestation of the commodity be reliably prevented by treatment of the crop?

No.

Sansford *et al.*, (2004a, *unpublished*) investigated the efficacy of a range of fungicide treatments *in vitro* and *in planta* and reviewed the literature on fungicide efficacy as well as seed treatments as part of this Project. The following is a summary of the findings:

In vitro efficacy testing of fungicides against mycelial growth and sporidial germination of *T. indica* showed good activity for four out of five active ingredients tested, with azoxystrobin being the most effective. Propiconazole, epoxiconazole and tebuconazole also showed good activity. Prochloraz was the least effective of the five chemicals tested.

For the European winter wheat cultivar Kosack, *in planta* testing indicated that azoxystrobin applied at manufacturer's full-recommended rate either pre- or post-inoculation of plants at the most susceptible growth stage for infection by *T. indica* (GS 57; determined from this Project –

²⁴ This refers to the crop in the country of origin

Magnus *et al.*, 2004a) resulted in complete control of Karnal bunt at all of the timings of application (GS 39, 49, 65 or 71). The results showed that, at least for this cultivar, azoxystrobin acted as a protectant when applied at GS 39 or GS 49 and as an eradicator when applied at GS 65 or GS 71.

The same experiment was conducted on WL-711, the normally highly susceptible Indian wheat cultivar, and this was successful in part. Reasonably high levels of disease developed on the control plants for the GS 39 treatment timing and the application of azoxystrobin at this growth stage gave complete control of Karnal bunt thus showing protectant activity. Unusually for this cultivar, no disease, or low levels of disease developed on the control plants for later treatment application timings (GS 49, 65 and 71). Although azoxystrobin-treated plants had less disease the results were not significant. This could not be attributed entirely to the low infectivity of the inoculum when tested by boot inoculation because of the reasonable levels of disease that developed on the GS 39 control plants.

There are no published reports of the efficacy of azoxystrobin against *T. indica* or indeed for any other strobilurin-type fungicide.

Karnal bunt is difficult to control chemically. Teliospores resist physical and chemical treatments and this is likely to contribute to spread of the pathogen (Smilanick *et al.*, 1985, 1988; Rivera-Castaneda *et al.*, 2001). Treatments such as hot water or sodium hypochlorite can be used to disinfest seed but result in seed destruction (Smilanick, 1997). Fungicidal seed treatments can have efficacy against surface contamination by *T. indica* but teliospores within the pericarp layer may resist treatment (e.g. Aujla *et al.*, 1989a; Bryson *et al.*, 2002). Because teliospores of *T. indica* can be a contaminant of seed and soil, seed treatment alone is likely to be ineffective.

In countries where the pathogen is established, attempts at control in crops of wheat by the use of fungicides rely mainly on treatments being applied from flag leaf emergence to the end of flowering (e.g. Goel *et al.*, 2000; Salazar-Huerta *et al.*, 1997, Singh *et al.*, 2000; Singh *et al.*, 1989a).

The advent of the conazole group of fungicides in the 1980's gave promise for improved field control of *T. indica*. Fungicides of the conazole group (subgroup triazoles) seem to have good efficacy against *T. indica* when applied as a foliar spray around the boot or heading stage. Propiconazole, a well-established fungicide, was first sold commercially in 1980 (Anon., 2000a). It belongs to the triazole subgroup and seems to be one of the most effective chemicals tested in the past 16 years (Aujla *et al.*, 1989a; Aujla and Sharma, 1990; Arshad *et al.*, 1995; Bains and Dhaliwal, 1988; Chaudhry and Khan, 1990; Salazar-Huerta *et al.*, 1997; Sharma *et al.*, 1994; Sharma *et al.*, 2005; Sharma and Basandrai, 2000; Singh *et al.*, 1989a; Singh *et al.*, 1993; Singh *et al.*, 2000; Smilanick *et al.*, 1987).

Jhorar *et al.* (1993) reported efficacy of a chemical named as '*telenconazole*', (a name unknown in the literature) as being as effective as propiconazole. Sharma *et al.* (1997) reported that cyproconazole (spelt as *ciperconazole*), another triazole-type fungicide, was as effective as propiconazole as a foliar spray, one of the few chemicals to match its efficacy. Diniconazole, also a triazole-type fungicide, is reported to have efficacy against *T. indica* in the field (Singh *et al.*, 1992). Propiconazole is approved for use in the UK on a range of crops and is permitted to be used on wheat up to a maximum of 4 treatments (3 in the year of harvest) with a 35-day harvest interval (Anon., 2004).

If yes **Possible measure: specified age of plant, growth stage or time of year of harvest**
Go to 3.22

3.22. Can infestation of the commodity be reliably prevented by production in a certification scheme (i.e. official scheme for the production of healthy plants for planting)?

Current EC requirements are for seeds of *Triticum*, *Secale* and x *Triticosecale* imported from countries where the pathogen occurs to originate in an area where *T. indica* is known not to occur. This will only be effective if the pest-free area is determined by testing for freedom from the pathogen.

If yes **Possible measure: certification scheme.**
Go to 3.23

Establishment and maintenance of pest freedom of a crop, place of production or area²⁵

Note that in this set of questions pest spread capacity is considered without prejudice to any other measure that can be recommended (e.g. production in a glasshouse may provide protection against pest with high natural spread capacity). In answering questions 3.23 to 3.26 refer to the answer to question 1.33 of the risk assessment section.

3.23. Has the pest a very low capacity for natural spread?²⁶

Whilst the teliospores of *T. indica* cannot move without physical assistance and therefore could be considered to be of low natural mobility, they are difficult to detect at low levels and therefore can spread cryptically and with human or other physical assistance as described under 1.20 and summarised below:

At harvest, teliospores are usually dispersed locally from bunted grain by the mechanical action of harvesting; bunted grain itself will also act as a vehicle for long-distance dispersal of inoculum in trade. The teliospores (within or free from sori) or bunted grain become deposited in the soil, in the harvesting machinery, and, teliospores can adhere to the surface of healthy grains as an external contaminant. Inoculum can be further transported in soil or in/on grain or seed on farm machinery and vehicles used for grain or seed transportation, or even by wind. The ingestion of infected or contaminated grain by livestock can also lead to the dispersal of teliospore inoculum in animal faeces. Animals can act as a vehicle for surface contamination by teliospores, facilitating spread.

EC requirements for certifying grain for export to the PRA area offer two options: (a) To originate in an area where *T. indica* is known not to occur, or (b) For place of production freedom based upon inspection during the growing season and testing of the grain at harvest and before shipment for freedom from *T. indica*. This should not be interpreted as allowing exporting countries to base certification for export of grain on symptoms alone.

²⁵ This still refers to the country of origin of a commodity

²⁶ This should be understood to mean both the innate ability of the pest to spread and the possibility of transportation by human or other agents (e.g. vectors).

Consideration of other possible measure

3.28. Are there effective measures that could be taken in the importing country (surveillance, eradication) to prevent establishment and/or economic or other impacts?

Note: Internal measures to prevent pest establishment should be considered especially when no effective phytosanitary measures at or before import exist

Yes. Individual countries could instigate a 100% inspection and testing regime for grain and seed imported from countries where *T. indica* occurs. The UK Plant Health Service (PHS) has a target to sample and test all consignments of wheat (and triticale) grain and seed which have arisen in countries where *T. indica* occurs. This resulted in the identification of *T. indica* or suspect *T. indica* in consignments of grain from India in 2003, 2004, 2005 and 2006. These were managed post-entry according to an earlier draft of the model contingency plan (Sansford *et al.*, 2004, *unpublished*) (incorporated into Deliverable Report 6.3 of this Project, Sansford *et al.*, 2004b, *unpublished*). A later version of the model plan is presented in Annex II of this report.

The UK PHS is aware of all consignments arriving directly in UK ports from countries where *T. indica* occurs. There may be problems however in identifying consignments which have arrived in another EU Member State before being transported onwards to the UK, since these will have been cleared for movement within the EU by the official body in the country in which these first arrive. Other EU countries that are known to be actively inspecting and testing consignments of the relevant commodities originating in affected countries are Hungary (Erzsebet, Hungary, *personal communication*, 2005), Italy (Riccioni, ISPaVe, Italy, *personal communication*, 2005 and Porta-Puglia, MRAE, Malta, *personal communication*, 2005) and Lithuania (Kerbeliene, State Plant Protection Service, Lithuania, *personal communication*, 2005). Based upon interception data, other countries such as Greece and Poland appear to have (or have had) some form of sampling and testing regime in place for consignments imported from countries where *T. indica* occurs.

To show country-freedom, the UK PHS sampled and tested for *T. indica* in samples of *T. aestivum* grain harvested in 2002, 2003 and 2004 in the UK using the EU/EPPO-recommended *T. indica* Diagnostic Protocol (Inman *et al.*, 2003; Anon., 2004). The pathogen was not detected. The UK wheat crop is monitored intensively and it is likely that the presence of *T. indica* would be reported either pre-harvest, or (more likely) post-harvest. The Netherlands inspected 103 wheat fields in 2004 and submitted 8 suspect samples for laboratory testing all of which proved negative (van Leeuwen, Dutch Plant Protection Service, the Netherlands, *personal communication*, 2005).

The potential for continued exclusion is possible, but will rely on the exporting countries where *T. indica* occurs complying with the current EC legislation, and on early detection of the pathogen on imported contaminated or infected material. Increasing the number of inspections and testing of grain and seed imported from countries where *T. indica* occurs will help exclude the pathogen from the PRA area.

The EU/EPPO-recommended *T. indica* Diagnostic Protocol (Inman *et al.*, 2003; Anon., 2004) is in current use in the UK and could be deployed by other importing countries in the PRA area to help to exclude the entry of the pathogen. This protocol has already been adapted for use by Australia (Wright, Australia, *personal communication*, 2005).

Regarding the efficacy of measures that may be deployed should an outbreak of *T. indica* occur in the PRA area a model contingency plan was prepared for Defra Plant Health Division (Sansford *et al.*, 2004, *unpublished*) (incorporated into Deliverable Report 6.3 of this Project, Sansford *et al.*, 2004b, *unpublished*). A later version of the model plan is presented in Annex II of this report.

If yes **Possible measure internal surveillance and/or eradication campaign**
Go to 3.29

Evaluation of risk management options

3.29. Have any measures been identified during the present analysis that will reduce the risk of introduction of the pest?

Yes.

If yes **Go to 3.30**
If no **Go to 3.37**

3.30. Taking each of the measures identified individually, does any measure on its own reduce the risk to an acceptable level?

No.

If yes **Go to 3.33**
If no **Go to 3.31**

3.31. For those measures that do not reduce the risk to an acceptable level, can two or more measures be combined to reduce the risk to an acceptable level?

Note: The integration of different phytosanitary measures at least two of which act independently and which cumulatively achieve the Appropriate Level of Protection are known as Systems Approaches (see ISPM 14: the use of integrated measures in a systems approach for Pest Risk Management). It should be noted that Pest free places of production identified as phytosanitary measures in questions 3.23 to 3.26 may correspond to a System Approach.

Yes. Countries where *T. indica* occurs should be applying the requirements of the EC Plant Health Directive (2000/29/EC as amended) (Anon., 2000). Current EC requirements are for seeds of *Triticum*, *Secale* and x *Triticosecale* imported from countries where the pathogen occurs to originate in an area where *T. indica* is known not to occur. For grain there are two options: (a) To originate in an area where *T. indica* is known not to occur, or (b) For place of production freedom based upon inspection during the growing season and testing of the grain at harvest and before shipment for freedom from *T. indica*. This should not be interpreted as allowing exporting countries to base certification for export of grain on symptoms alone.

Imported consignments of the relevant commodities originating in the affected countries (Afghanistan, Brazil, India, Iran, Iraq, Mexico, Nepal, Pakistan, South Africa and the USA) could be sampled and tested by the NPPOs in the PRA area. This is already being undertaken in the Hungary, Italy, Lithuania and the UK. Other EU countries may also be doing this.

National surveys in the PRA area geared to detecting teliospores of *T. indica* such as the recently introduced sampling and testing of harvested UK wheat grain and wheat crop surveys in the Netherlands would help determine the presence of the pathogen (hopefully) before spread occurred.

For outbreaks in the PRA area the deployment of a contingency plan such as the model contingency plan would help prevent further spread. The details of an earlier version of this plan are presented in Annex II of this report.

If yes **Go to 3.33**
If no **Go to 3.32**

3.32. If the only measures available reduce the risk but not down to an acceptable level, such measures may still be applied, as they may at least delay the introduction of the pest. In this case, a combination of phytosanitary measures at or before export and internal measures (see question 3.29) should be considered.

Go to 3.33

3.33. Estimate to what extent the measures (or combination of measures) being considered interfere with trade.

Note: It is necessary to consider the relationship between the negative effect on trade and the importance/desirability of that trade. If this analysis concerns a pest already established in the PRA area but under official control, measures that are applied for international trade should not be more stringent than those applied domestically/internally.

None of the measures currently interferes with trade. However, some countries such as the USA have adopted a regime of testing grain for ‘*bunted kernels*’ only, both for testing consignments of grain destined for export as well as for determining pest-free areas. This is less onerous than sampling and testing for teliospores of *T. indica*. This is of concern to some of the countries in the PRA area. Although trade continues between affected countries and individual Member States within the EU, some countries NPPOs (Hungary, Italy, Lithuania and the UK) have started implementing a sampling and testing procedure for *T. indica* for any relevant consignment arising in the affected countries.

Go to 3.34

3.34. Estimate to what extent the measures (or combination of measures) being considered are cost-effective, or have undesirable social or environmental consequences.

Costs arising in affected exporting countries have not been evaluated.

Costs in the importing countries are those associated with sampling and testing imported consignments and will depend on the level of imports. Countries such as Italy, which import large quantities of wheat from the USA, will be incurring the greatest costs. However, they are at particular risk so it is beneficial to undertake this.

Brennan *et al.* (2004, *unpublished*) evaluated the costs of implementing an earlier draft of the model contingency plan (Sansford *et al.*, 2004, *unpublished*) (incorporated into Deliverable Report 6.3 of this Project, Sansford *et al.*, 2004b, *unpublished*). A later version of the plan, which is broadly similar is presented in Annex II of this report.

The costs and benefits are outlined under 2.3.

Go to 3.35

3.35. Have measures (or combination of measures) been identified that reduce the risk for this pathway, and do not unduly interfere with trade, are cost-effective and have no undesirable social or environmental consequences?

Yes.

If yes For pathway-initiated analysis, go to 3.38
For pest-initiated analysis, go to 3.37
If no go to 3.36

3.36. Envisage prohibiting the pathway

Note: Prohibition should be viewed as a measure of last resort. If prohibition of the pathway is the only measure identified for a commodity-initiated analysis, there may be no need to analyse any other pests that may be carried on the pathway. If later information shows that prohibition is not the only measure for this pest, analysis of the other pests associated with the pathway will become necessary.

For pathway-initiated analysis, go to 3.42 (or 3.38)
For pest-initiated analysis go to 3.37

This is a PRA for a specific pest, *T. indica*, but to manage the risk the pathways have had to be analysed.

Both questions 3.37 and 3.38 are therefore relevant but it is then necessary to go to 3.40.

3.37. Have all major pathways been analysed (for a pest-initiated analysis)?

Yes. The risk management options for *T. indica* relate to the trade pathways and these have been analysed.

If yes Go to 3.40
If no Go to 3.1 to analyse the next major pathway

3.38. Have all the pests been analysed (for a pathway-initiated analysis)?

Yes.

The only pest is *T. indica*.

If yes Go to 3.39
If no Go to 3.1 (to analyse next pest)

3.39. For a pathway-initiated analysis, compare the measures appropriate for all the pests identified for the pathway that would qualify as quarantine pests, and select only those that provide phytosanitary security against all the pests.

Note: the minimum effective measures against one particular pest may reduce the risk from other pests far more than necessary, but these measures would be the only ones appropriate for the pathway as a whole.

Go to 3.41

3.40. Indicate the relative importance of pathways

Note: The relative importance of the pathways is an important element to consider in formulating phytosanitary regulation

Seed of *T. aestivum*, *T. durum* and x *Triticosecale* imported from Afghanistan, Brazil, India, Iran, Iraq, Mexico, Nepal, Pakistan, South Africa and the USA is the highest risk.

Grain imported into the PRA area from these countries and transported through arable production areas and processed in mills in arable production areas is the next highest risk.

Go to 3.41

3.41. All the measures or combination of measures identified as being appropriate for each pathway or for the commodity can be considered for inclusion in phytosanitary regulations in order to offer a choice of different measures to trading partners.

Note: Only the least stringent measure (or measures) capable of performing the task should be selected. Thus, if inspection is truly reliable, it should not be necessary to consider treatment or testing. Note also that some measures may counteract each other; for example the requirement for resistant cultivars may make detection more difficult. It may be that some or all of these measures are already being applied to protect against one or more other pests, in which case such measures need only be applied if the other pest(s) is/are later withdrawn from the quarantine.

The minimum phytosanitary measure applied to any pest is the declaration in phytosanitary regulations that it is a quarantine pest. This declaration prohibits both the entry of the pest in an isolated state, and the import of consignments infested by the pest. If other phytosanitary measures are decided upon, they should accompany the declaration as a quarantine pest. Such declaration may occasionally be applied alone, especially: (1) when the pest concerned may be easily detected by phytosanitary inspection at import (i.e. general visual inspection or targeted inspection; see questions 12 and 13), (2) where the risk of the pest's introduction is low because it occurs infrequently in trade or its biological capacity for establishment is low, or (3) if it is not possible or desirable to regulate all trade on which the pest is likely to be found. The measure has the effect of providing the legal basis for the NPPO to take action on detection of the pest (or also for eradication and other internal measures), informing trading partners that the pest is not acceptable, alerting phytosanitary inspectors to its possible presence in imported consignments, and sometimes also of requiring farmers, horticulturists, foresters and the general public to report any outbreaks.

To reduce the risk of entry still further, it is suggested that a proposal be considered that EC and domestic legislation in Member States be amended, with a more precise explanation of the

existing requirements for exporting countries where the pathogen occurs to determine pest-free areas and pest-free places of production for exports of wheat and triticale. If this is considered appropriate, this amendment should be based upon an explicit explanation within the text that the requirements include testing specifically for freedom from teliospores of *T. indica* rather than just looking for visual symptoms alone. The latest version of the model contingency plan (Annex II of this report) outlines measures aimed at exclusion at the port of entry which seem economically justified. Should an outbreak occur in the PRA area long-term measures aimed at containment and eradication have significant cost implications for the farmers whose land is affected. Nevertheless, if the EU wishes to maintain its export markets then at least some of these measures are justified dependent upon the requirements of the trading partners and the policy adopted by the government(s) concerned.

Go to 3.42

3.42. In addition to the measure(s) selected to be applied by the exporting country, a phytosanitary certificate (PC) may be required for certain commodities. The PC is an attestation by the exporting country that the requirements of the importing country have been fulfilled. In certain circumstances, an additional declaration on the PC may be needed (see EPPO Standard PM 1/1(2): Use of phytosanitary certificates).

A Phytosanitary Certificate is already required.

As of 1 January 2005, implementation of a further amendment to the EC Plant Health Directive requires that specified Additional Declarations (ADs) be stated on all Phytosanitary Certificates (PCs). This will mean that ADs on all PCs accompanying seed or grain of wheat, rye or triticale from countries where *T. indica* occurs will need to be completed to show freedom from *T. indica*. Rye is no longer considered to be a host and can be deleted from UK and EC legislation.

Go to 3.43

3.43. If there are no measures that reduce the risk for a pathway, or if the only effective measures unduly interfere with trade (e.g. prohibition), are not cost-effective or have undesirable social or environmental consequences, the conclusion of the pest risk management stage may be that introduction cannot be prevented.

Irrelevant.

Conclusion of Pest Risk Management.

Summarise the conclusions of the Pest Risk Management stage. List all potential management options and indicate their effectiveness. Uncertainties should be identified.

Countries where *T. indica* occurs should be applying the current requirements of the EC Plant Health Directive (2000/29/EC as amended) (Anon., 2000). EC requirements are for seeds of *Triticum*, *Secale* and x *Triticosecale* imported from countries where the pathogen occurs to originate in an area where *T. indica* is known not to occur. For grain there are two options: (a). To originate in an area where *T. indica* is known not to occur, or (b). For place of production freedom based upon inspection during the growing season and testing of the grain at harvest and before shipment for freedom from *T. indica*. This should not be interpreted as allowing

exporting countries to base certification for export of grain on symptoms alone. The Additional Declaration on the Phytosanitary Certificate accompanying the relevant consignments now has to be completed.

It is suggested that the following proposal be considered:

That EC and domestic legislation in EU Member States be amended, with more precise requirements for exporting countries where the pathogen occurs to determine pest-free areas and pest-free places of production for exports of wheat and triticale.

As is already being undertaken by Hungary, Italy, Lithuania and the UK, imported consignments of the relevant commodities originating in the affected countries (Afghanistan, Brazil, India, Iran, Iraq, Mexico, Nepal, Pakistan, South Africa and the USA) could be sampled and tested by the NPPO in the PRA area.

National surveys in the PRA area geared to detecting teliospores of *T. indica* such as the recently introduced sampling and testing of harvested UK wheat grain and surveys of wheat crops on the Netherlands, would help determine the presence of the pathogen (hopefully) before spread occurred.

For outbreaks in the PRA area the deployment of a contingency plan such as the model plan would help prevent further spread. The most recent version of this plan is presented in Annex II of this report.

The efficacy of some of the proposed measures in the model contingency plan for controlling *T. indica* (should outbreaks occur) is not known. As part of the plan, monitoring the efficacy of the measures is suggested and this includes pathogen monitoring for at least 5 years within the affected areas and in a buffer zone and in any susceptible crops grown within a specified distance around the buffer zone, until pathogen-freedom is confirmed. A Regulatory Impact Assessment which considers the costs and benefits of implementing the model contingency plan based upon the socioeconomic impact assessment carried-out in this Project for outbreaks in England is presented in Annex III.

3. EXPLOITATION AND DISSEMINATION OF RESULTS

Results of the EU '*Karnal bunt risks*' Project will be disseminated through the Project web site. The results will be provided to the NPPOs and the EC for their consideration. Consideration of publication in a refereed journal will be undertaken

4. POLICY RELATED BENEFITS

The main policy related benefit arising from this Deliverable report is that a fully revised Pest Risk Analysis has been prepared for *Tilletia indica* and a model contingency plan has been developed for consideration by NPPOs for dealing with an outbreak of Karnal bunt (*Tilletia indica*) in Europe.

5. REFERENCES

Agarwal VK, Singh DV, Mathur S (1993). *Seed-borne diseases and seed health testing of wheat*, **3**, 31-43.

Aggarwal R, Singh DV, Srivastava KD, Bahadur P (1991). Karnal-bunt of Wheat: What it does, and how it spreads. *Indian Farming*, **40**, 9-10.

Agarwal VK, Singh A, Verma HS (1976). Outbreak of Karnal bunt of Wheat. *FAO Plant Protection Bulletin*, **24**, 99-100.

Agarwal VK, Verma HS, Kheterpal RK (1977). Occurrence of partial bunt on triticale. *FAO Plant Protection Bulletin*, **25**, 210-211.

Anon. (1996). Secretary of Agriculture, Livestock and Rural Development. Mexican Official Rule: NOM-EM-001-FITO-1995.
http://ns1.oirsa.org.sv/OIRSA/Miembros/Mexico/Decretos_Leyes_Reglamentos/NORMA01.html

Anon. (1998). EC Food and Veterinary Office Report on a Mission in Italy from 4 to 7 August 1998 on the interception of two consignments of durum wheat contaminated by *Tilletia indica*.
http://europa.eu.int/comm/food/fs/inspections/pi/reports/italia/pi_rep_ital_1440-1998_en.pdf

Anon. (2000). Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. *Official Journal of the European Communities* **43 no. L 169**, 1-112.

Anon. (2000a). *The Pesticide Manual – a World Compendium. Twelfth Edition*. British Crop Protection Council. 1250pp.

Anon. (2001). Update on the situation of *Tilletia indica* in the USA, EPPO Reporting Service, Issue 6, Item 2001/109.

Anon. (2002). Secretary of Agriculture, Livestock and Rural Development. 2002. Mexican Official Rule: NOM-001-FITO-2000.
http://www.ipfsaph.org/servlet/BinaryDownloaderServlet/FAOLEX023543_http_faolex.fao.or.doc?filename=\kopool_data\FAOLEX_0\es_mex27802.doc&refID=FAOLEX023543

Anon. (2003). Agriculture and Agrifood Canada bi-weekly Bulletin. December 19, 2003 Volume 16, Number 22. ISSN 1494-1805 AAFC No. 2081/E

Anon. (2003a). Co-operative Agricultural Pest Survey Program. USDA APHIS PPQ. Record Summary July 13th 2004. <http://ceris.purdue.edu/napis/pests/kb/table/tab03.html>

Anon. (2003b). ‘Karnal bunt strategic plan overcoming Karnal bunt as a quarantine pest while preserving export markets Fy 2004-2007’ (December 12, 2003)
(see http://www.aphis.usda.gov/ppq/ep/kb/kb_sp-2004.pdf) (earlier version was dated February 26th 2002).

Anon. (2004). EPPO Standards - Diagnostic protocols for regulated pests - PM7/29 *Tilletia indica*. *EPPO Bulletin*, **34**, 219-227.

Anon. (2004a). *Plant Health Surveillance in South Africa*. Report of 15 July 2004 from South Africa to the Meeting of the States Parties to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction. BWC/MSP/2004/MX/WP.13. Second Meeting, Geneva, 6-10 December 2004. http://www.opbw.org/new_process/mx2004/bwc_msp.2004_mx_wp13_E.pdf

Anon. (2005). USDA Karnal bunt, revision of regulations for importing wheat: Federal Register: 18 February 2005, **70**, No. 33, PP8229-8233.

Arshad KM, Ahmad S, Attaudin (no initial) (1995). Field assay of fungicides for the control of Karnal bunt of wheat. *Sarhad Journal of Agriculture* **11**, 501-504.

Aujla SS, Sharma I (1990). Evaluation of fungicides against Karnal bunt of wheat. *Journal of Research Punjab Agricultural University* **27**, 434-436.

Aujla SS, Sharma YR, Chand K, Sawney SS (1977). Influence of weather factors on the incidence and epidemiology of Karnal bunt disease of wheat in the Punjab. *Indian Journal of Ecology*, **4**, 71-74.

Aujla SS, Sharma I, Singh BB (1987). Physiologic specialization of Karnal bunt of wheat. *Indian Phytopathology*, **40**, 333-336.

Aujla SS, Sharma I, Singh BB (1989). Rating scale for identifying wheat varieties resistant to *Neovossia indica*. *Indian Phytopathology*, **42**, 161-162.

Aujla SS, Sharma I, Singh P, Singh G, Dhaliwal HS, Gill KS (1989a). Propiconazole – a promising fungicide against Karnal burnt (*sic*) of wheat. *Pesticides (Bombay)* **23**, 35-38.

Babadoost M (2000). Comments on the zero-tolerance quarantine of Karnal bunt of wheat. *Plant Disease*, **84**, 711-712.

Babadoost M, Mathre DE, Johnston RH, Bonde M (2004). Survival of teliospores of *Tilletia indica* in soil. *Plant Disease*, **88**, 56-62.

Bains SS, Dhaliwal HS (1988). Standardization of a technique for *in vivo* evaluation of fungitoxicants against *Neovossia indica*. *Indian Journal of Mycology and Plant Pathology* **18**, 47-54.

Baker RHA (1994). The potential for geographical information systems in analysing risks posed by exotic pests. Proceedings of the Brighton Crop Protection Conference, Pests and Diseases, Brighton, 1994. 3C-2, 159-166.

Baker R, Gioli B, Porter JR, Sansford CE (2004). (*Unpublished*). EU Karnal bunt risks Project. Deliverable Report 1.2. Maps of Europe and key durum and bread wheat production locations showing the area at risk from *T. indica* establishment based on existing disease models. AND Deliverable Report 1.3. Revised maps using new pathogen data obtained in Workpackages 2, 3 and 4. 36pp (plus 25pp annexes).

Baker RHA, Sansford CE, Gioli B, Miglietta F, Porter JR, Ewert F (2005). Combining a disease model with a crop phenology model to assess and map pest risk: Karnal bunt disease (*Tilletia indica*) of wheat in Europe. In Alford, DV, Backhaus, GF (Eds.). Introduction and spread of invasive species. BCPC Symposium Proceedings **81**: 89-94.

Baker RHA, Sansford CE, Jarvis CH, Cannon RJC, MacLeod A, Walters KFA (2000). The Role of Climatic Mapping in Predicting the Potential Distribution of Non-Indigenous Pests under Current and Future Climates. *Agriculture, Ecosystems and the Environment*, **82**, 57-71.

Bechtel DB, Wilson JD, Eustace WD, Behnke KC, Whitaker TB, Peterson GL, Sauer DB (1999). Fate of dwarf bunt fungus (*Tilletia controversa* Kuhn) teliospores during milling of wheat into flour. *Cereal Chemistry*, **76**, 270–275.

Bedi PS (1989). Impact of new agricultural technology on Karnal bunt of wheat. *Plant Disease Research*. **4**, 1-8.

Bedi PS, Dhiman JS (1984). Spread of *Neovossia indica* in a wheat ear. Phytopathological Notes, *Indian Phytopathology*, **37**, 335-337.

Bedi KS, Sikka MR, Mundkur BR (1949). Transmission of wheat bunt due to *Neovossia indica* (Mitra) Mundkur. *Indian Journal of Phytopathology*, **2**, 20-26.

Beniwal MS, Chawla P, Madan S, Singh R (2000). Effect of Karnal bunt on grain weight and quality of wheat. *Annals of Applied Biology*, **16**, 101-104.

Beniwal MS, Duhan JC, Singh R, Chawla P (2000a). Assessment of yield loss and seedling vigour in wheat due to Karnal bunt. *Seed Research*, **28**, 102-103.

Bhat RV, Deosthale YG, Roy DN, Vijayaraghavan M, Tulpule PG (1980). Nutritional and toxicological evaluation of Karnal bunt affected wheat. *Indian Journal of Experimental Biology*, **18**, 1333-1335.

Bhat RV, Rao B, Roy DN, Vijayaraghavan M, Tulpule PG (1981). Toxicological evaluation of Karnal bunt in monkeys – a report. Bulletin. Food and Drug Toxicological Research Centre, Jasmai-Osmania. Hyderabad, India. 5pp.

Bhutta AR, Hussain A, Ahman I (1999). Prevalence of Karnal bunt in wheat seed lots in Pakistan. RACHIS Newsletter, **18**, 38-42.

Bonde MR, Berner DK, Nester SE, Peterson GL, Olsen MW, Cunfer BM, Sim T (2004). Survival of *Tilletia indica* teliospores in different soils. *Plant Disease*, **88**, 316-324.

Bonde MR, Nester SE, Olsen MW, Berner DK (2004a). Survival of teliospores of *Tilletia indica* in Arizona field soils. *Plant Disease*, **88**, 804-810.

Bonde MR, Peterson GL, Schaad NW, Smilanick JL (1997). Karnal bunt of wheat. *Plant Disease*, **81**, 1370-1377.

Boratynski TN, Matsumoto TT, Bonde MR (1985). Interceptions of *Tilletia indica* at the California-Mexico border Mexican railroad boxcars. *Phytopathology*, **75**, 1339.

Brennan JP, Kelly PW, Thorne F (2004). (*Unpublished*). EU Karnal bunt risks Project. Deliverable Report 5.1. Report on Socio-Economic Costs of Karnal bunt in the European Union. 63pp.

Brennan JP, Singh RP, Singh IP (2002). Economic potential for improving the nutritional characteristics of feed grains. *Australian Journal of Agricultural and Resource Economics* **46** (4) 539-558.

Brennan JP, Thorne FS, Kelly PW, Murray GM (2004a). Defining the costs of an outbreak of Karnal bunt of wheat. Proceedings of the 48th Annual Conference of the Australian Agricultural and Resource Economics Society, Melbourne, Australia.

Brennan JP, Warham EJ (1990). Economic losses from Karnal bunt of wheat in Mexico. CIMMYT Economics Working Paper, 90/02. CIMMYT, Mexico, 56pp.

Brennan JP, Warham EJ, Byerlee D, Hernandez-Estrada J (1992). Evaluating the economic impact of quality-reducing, seed-borne diseases: Lessons from Karnal bunt of wheat. *Agricultural Economics* **6**, 345-352.

Bryson RJ, Froment MA, Karwasra SS (2002). Evaluation of fungicides as seed dressings against Karnal bunt (*Neovossia indica*) of wheat. *Tests of Agrochemicals and Cultivars* **23**, 6-7.

Cashion N, Luttrell ES (1988). Host-parasite relationships in Karnal bunt of wheat. *Phytopathology* **78**, 75-84.

Castlebury A, Carris LM (1999). *Tilletia walkeri*, a new species on *Lolium multiflorum* and *L. perenne*. *Mycologia* **91**, 121-131.

Chaudry MH, Khan MA (1990). Effectiveness of different spray fungicides against Karnal bunt of wheat. *Rachis* **9**, 5-6.

Chib HS, Kalha CS, Gupta BR, Tikoo ML, Gupta RS (1990). Studies on the longevity of *Neovossia indica* (the incitant of Karnal bunt of wheat) in soil. *Plant Disease Research* (special), 17-18.

Crous PW, Jaarsveld AB van, Castlebury LA, Carris LM, Frederick RD, Pretorius ZA (2001). Karnal bunt of wheat newly reported from the African continent. *Plant Disease* **85**, 561.

Da Luz MAS, Mendes MASV, Ferreira, Urben AF (1993). *Tilletia indica* on wheat in the southern part of Rio Grande do Sul and means for its eradication (in Portuguese). *Fitopatologia Brasileira*, **18** (suplemento): 329.

Dhaliwal HS (1989). Multiplication of secondary sporidia of *Tilletia indica* on soil, wheat leaves and spikes and incidence of Karnal bunt. *Canadian Journal of Botany*, **67**, 2387-2390.

Dhaliwal HS, Randhawa AS, Chand K, Singh D (1983). Primary infection and further development of Karnal bunt of wheat. *Indian Journal of Agricultural Science* **53**, 239-44.

Diekmann M (1993). *Epidemiology and geophytopathology of selected seed-borne diseases*. Aleppo, Syria: ICARDA.

Durán R (1972). Further aspects of teliospore germination in North American smut fungi II. *Canadian Journal of Botany* **50**, 2569-2573.

Durán R, Cromarty R (1977). *Tilletia indica*; a heterothallic wheat bunt fungus with multiple alleles controlling incompatibility. *Phytopathology*, **67**, 812-8115.

EPPO (2005). EPPO Plant Quarantine Information Retrieval System. Version 4.4. March 2005. CD-ROM.

FAO (2002). International Standards for Phytosanitary Measures: Glossary of Phytosanitary Terms. ISPM No. 5. Secretariat of the International Plant Protection Convention, Food and Agriculture Organisation of the United Nations.

FAO (2003). National area, yield, production and trade data from FAOSTAT. <http://apps.fao.org>

FAO (2004). International Standards for Phytosanitary Measures: Pest Risk Analysis for quarantine pests including analysis of environmental risks and living modified organisms. ISPM No. 11. (Second revision).

FAOSTAT (2004). FAOSTAT database collections: Agricultural Production –Crops Primary. Updated December 20th 2004.
<http://faostat.fao.org/faostat/form?collection=Production.Crops.Primary&Domain=Production&servlet=1&hasbulk=0&version=ext&language=EN>

FAOSTAT (2005). FAOSTAT database collections: Export of Cereals by Source and Destination. Updated data March 15th 2005.
<http://faostat.fao.org/faostat/form?collection=ExportByDestination&Domain=ExportByDestination&servlet=1&hasbulk=0&version=ext&language=EN>

FAS (2005). Foreign Agricultural Service U.S. Trade Internet System.
<http://www.fas.usda.gov/ustrade/>

Frederick RD, Tooley PW, Berthier-Schaad Y, Peterson GL, Bonde MR, Schaad NW (1998). Differentiation between *Tilletia indica*, the causal organism of Karnal bunt of wheat, and a related ryegrass smut using PCR. *Abstracts of Invited and Offered Papers, Seventh International Congress of Plant Pathology, Edinburgh, Scotland 9-16 August, 1998*.

Frederick RD, Snyder KE, Tooley PW, Berthier-Schaad Y, Peterson GL, Bonde MR, Schaad NW, Knorr DA (2000). Identification and differentiation of *Tilletia indica* and *T. walkeri* using the polymerase chain reaction. *Phytopathology*, **90**, 951-960.

Fuentes-Dávila G (1996). Karnal bunt. In: Wilcoxson RD, Saari EE, eds. *Bunt and smut diseases of wheat: concepts and methods of disease management*. CIMMYT, Mexico, 26-32.

Fuentes-Dávila G (1996a). Karnal bunt in Mexico. Transcripts of the American Phytopathological Society Karnal bunt Symposium, June 24th – August 16th, 1996. <http://www.apsnet.org/online/karnal/kbspaper/index.htm>

Fuentes-Dávila G, Durán R (1986). *Tilletia indica*: cytology and teliospore formation *in vitro* and in immature kernels. *Canadian Journal of Botany*, **64**, 1712-1719.

Fuentes-Dávila G (1998). Karnal bunt of wheat. In *Bunts and Smuts of Wheat: An International Symposium*; North Carolina, August 17-20, 1997. Eds. Malik, VS, Mathre, DE. NAPPO, Ottawa, 69-81.

Garrett KA, Bowden RL (2002). An Allee effect reduces the invasive potential of *Tilletia indica*. *Phytopathology* **92**, 1152-1159.

Gill KS, Randhawa AS, Aujla SS, Dhaliwal HS, Grewal AS, Sharma I (1981). Breeding wheat varieties resistant to Karnal bunt. *Crop Improvement*, **8**, 73-80.

Gill KS, Sharma I, Aujla SS (1993). Karnal bunt and wheat production. Punjab Agricultural University, Ludhiana, India. 153 pp.

Goates BJ (1988). Histology of infection of wheat by *Tilletia indica*, the Karnal bunt pathogen. *Phytopathology*, **78**, 1434-1441.

Goates B (2005). Durability of secondary sporidia of floret infecting *Tilletia* species: Implications for epidemiology. Abstract of poster presented at the American Phytopathological Society annual meeting. July 30th – August 3rd, 2005, Austin, Texas. <http://meeting.apsnet.org/program/pdfs/2005LatePosterAbstracts.pdf>

Goel LB, Singh DP, Sinha VC, Singh DV, Srivastava KD, Agrawal R, Aujla SS, Sharma I, Bagga PS, Singh RV, Singh AK, Singh SP (2000). Evaluation of Tilt against Karnal bunt of wheat. *Indian Phytopathology* **53**, 301-302.

Hegeman R (2001). *USDA Wheat Disease Reaction Faulted. Growers say the spread of Karnal bunt fungus could be crippling.* Associated Press Article, June 24th 2001; pA02

HGCA (2004). HGCA Market Data Centre. <http://www.hgca.com/data/>

Howard A, Howard GLC (1909). *Wheat in India I. Production, varieties and improvement.* Calcutta, India: Published by Thacker Spink and Company.

Hurlbert SH (1984). Pseudoreplication and the design of ecological field experiments. *Ecological Monographs* **54**, 187–211.

Inman AJ, Hughes KJD, Bowyer, RJ (2003). EU/EPPO Recommended Protocol for the Diagnosis of a Quarantine Organism: *Tilletia indica*. <http://www.csl.gov.uk/science/organ/ph/diagpro/tipro.pdf>

Inman A, Hughes K, Coates M, Barnes A, Barton V, Sansford C, Leach R, Porta-Puglia A, Riccioni L, Valvassori M, Magnus H, Razzaghian J, Peterson G (2004). (*Unpublished*). EU Karnal bunt risks Project. Deliverable Report 3.1. Report on the proportions of teliospores

capable of germinating after 1, 2 and 3 years in European soils in relation to soil moisture, temperature and depth. 63pp.

Jatav AL, Singh CB, Khan AA, Sachan CP (2003). Effects of Karnal bunt disease infection on the germination, tillering and yield of wheat. *Progressive Agriculture*. Society for Recent Development in Agriculture, Rawatpur, India, 2003. **3**, 145.

Jhorar OP, Mavi HS, Sharma I, Mahi GS, Mathauda SS, Singh G (1992). A biometeorological model for forecasting Karnal bunt disease of wheat. *Plant Disease Reporter* **7**, 204-209.

Jhorar OP, Sharma I, Mavi HS, Aujla SS, Nanda GS (1993). Forecasting models for effective application of fungitoxicants in the management of Karnal bunt. *Indian Journal of Mycology and Plant Pathology* **23**, 78-89.

Jones SR (1998). The effect of Karnal bunt on the Arizona wheat industry. In *Bunts and Smuts of Wheat: An International Symposium*; North Carolina, August 17-20, 1997. Eds. Malik, VS, Mathre, DE. NAPPO, Ottawa, 425-430.

Joshi LM, Singh DV, Srivastava KD, Wilcoxson RD (1983). Karnal bunt: A minor disease that is now a threat to wheat. *Botanical Review*, **49**, 309-330.

Karwasra SS, Beniwal MS, Parashar RD (1991). Effect of Karnal bunt severity on inoculum load and grain yield in different varieties of wheat. *Crop Research (Hisar)*, **4**, 327-330.

Kehlenbeck H, Motte G, Unger J-G, (1997). Analysis of the risk and consequences of *Tilletia indica* (syn. *Neovossia indica*) introduction into Germany. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes*, **49**, 65-74.

Khetarpal RK, Agarwal VK, Chauhan KPS (1980). Studies on the influence of weather conditions on the incidence of black-point and Karnal bunt of *Triticale*. *Seed Research*, **8**, 108-110.

Krishna A, Singh RA (1982). Investigations on the disease cycle of Karnal bunt of wheat. *Indian Journal of Mycology and Plant Pathology*, **12**, 124.

Krishna A, Singh RA (1983). Longevity of teliospores of *Neovossia indica* causing Karnal bunt of wheat. *Indian Journal of Mycology and Plant Pathology*, **13**, 97-98.

Kumar J, Saharan MS, Sharma AK, Nagarajan S (2003). Temperature requirement for teliosporogenesis of *Tilletia indica*, the causal agent of Karnal bunt of wheat. *Indian Phytopathology*, **56**, 439-442.

Levy L, Castlebury LA, Carris LM, Meyer RJ, Pimentel G (2001). Internal transcribed spacer sequence-based phylogeny and polymerase chain reaction-restriction fragment length polymorphism differentiation of *Tilletia walkeri* and *T. indica*. *Phytopathology*, **91**, 935-940.

Magnus H, Rafoss T, Sansford C, Murray G (2004). (*Unpublished*). EU Karnal bunt risks Project. Deliverable Report 7.1. Report on the biological features of plant pathogens suitable for Pest Risk Analysis by the methodology developed in this Project. AND Deliverable Report

7.2. Report on the development of a Pest Risk Analysis scheme for evaluating the range of pathogens identified for investigation using the ‘*Karnal bunt risks*’ Project Approach. 33pp.

Magnus H, Razzaghian J, Prince A, Porta-Puglia A, Riccioni L, Valvassori M, Inman A, Hughes K, Bowyer R, Barnes A, Coates M, Sansford C, Peterson G (2004a). (*Unpublished*). Report on the determination of the most susceptible wheat growth stage for infection by *Tilletia indica* for European cultivars. EU *Karnal bunt risks* Project. Deliverable Report 2.2. 32pp.

Marshall D, Work TT, Cavey JF (2003). Invasion pathways of *Karnal bunt* of wheat into the United States. *Plant Disease*, **87**, 999-1003.

Mavi HS, Jhorar OP, Sharma I, Singh G, Mahi GS, Mathauda SS, Aujla SS (1992). Forecasting *Karnal bunt* of wheat – a meteorological method. *Cereal Research Communications* **20**, 67–74.

McAlavy TW (2001). *Karnal bunt losses on rolling plains exceed \$27 million*. AgNews, November 12th 2001. Texas A&M University Agriculture Program.

McRae W (1933). Report of the imperial mycologist. Sci. Rep. Imper. Inst. Agric. Res. Pusa, 1932-33, 134-160.

Mendes MAS, Ferreira MASV (1994) (Abstract). Pathogenic fungi detected in vegetable germplasm introduced into Brazil from 1990 to 1992. *Fitopatologia Brasileira*, **19**, 449-454.

Miglietta F (1991). Simulation of wheat ontogenesis: I. The appearance of main stem leaves in the field. *Climate Research*, **1**, 145-150.

Miglietta F (1991a). Simulation of wheat ontogenesis: II. Predicting final main stem leaf number and dates of heading in the field. *Climate Research*, **1**, 151-160.

Miglietta F (1991b). Simulation of wheat ontogenesis: III. Effect of variety, nitrogen fertilization and water stress on leaf appearance and final leaf number in the field. *Climate Research*, **1**, 233-242.

Miller T (undated). *Karnal bunt in Texas Wheat*. Texas Agricultural Extension Service. 3pp. <http://lubbock.tamu.edu/othercrops/pdf/wheat/buntintxwheat.pdf>

Mitra M (1931). A new bunt of wheat in India. *Annals of Applied Biology*, **18**, 178-179.

Mundkhar BB (1940). A second contribution towards a knowledge of Indian Ustilaginales. *Transactions of the British Mycological Society*, **24**, 312-336.

Munjral RL (1975). Status of *Karnal bunt* (*Neovossia indica*) of wheat in northern India during 1968-1969 and 1969-1970. *Indian Journal of Mycology and Plant Pathology*, **5**, 185-187.

Murray G, Brennan J, Hare R (1996). ‘*Karnal bunt*: A concern for Australia?’ *Australian Grain*, **6** (3), (August – September), 10-12.

Murray GM (2004). (*Unpublished*). Evaluation of published data for *Tilletia indica* to compare existing disease models in relation to data obtained in Workpackages 2, 3 and 4. EU *Karnal bunt risks* Project. Deliverable Report: DL 1.4. 27pp.

Murray GM, Brennan JP (1998). The risk to Australia from *Tilletia indica*, the cause of Karnal bunt of wheat. *Australasian Plant Pathology*, **27**, 212-225.

Murray GM, Sansford CE (2005). *How Tilletia indica overcomes the Allee effect*. In Proceedings of the 15th Biennial Australasian Plant Pathology Society Conference, Geelong, Victoria, Australia. 26th – 29th September 2005.

Nagarajan S, Aujla SS, Nanda GS, Sharma I, Goel LB, Kumar J, Singh DV (1997). Karnal bunt (*Tilletia indica*) of wheat - a review. *Review of Plant Pathology*, **76**, 1207-1214.

Naudé K (2002). Karnal bunt in South Africa. Items from South Africa Small Grain Institute. *Annual Wheat Newsletter*, **49**, 137.

NAPPO (2001). An Epidemiological Approach to Assessing the Risk of Establishment of Karnal bunt, *Tilletia indica* Mitra, in North America. North American Plant Protection Organization Pest Risk Analysis Panel. 17pp plus figures. <http://www.nappo.org>

Nave RH (1998). Overview of the Karnal bunt quarantine: a U.S. perspective. In *Bunts and Smuts of Wheat: An International Symposium*; North Carolina, August 17-20, 1997. Eds. Malik, VS, Mathre, DE. NAPPO, Ottawa, 69-81.

Nelson M (1996). Karnal bunt, an Arizona perspective. American Phytopathological Society Online Symposium. <http://www.apsnet.org/online/karnal/kbspaper/artariz.htm>

Nix J (2003). Farm Management Pocketbook. Imperial College at Wye, UK. 33rd Edition. 256pp.

Northcutt G (2001). *Some downwind costs of upwind erosion*. *Erosion*. Forester Publications, Santa Barbara, CA. September 2001.

Oliveira MRV de, A Marinho VL de, Tenente RCV, Mendes MAS, Marques AS dos A, Batista M de F, Gonzaga V, Urben AF, Fonseca JNL (2002). Quarantine information and transit of plant propagation material. [Portuguese]. Documentos - Embrapa Soja. Embrapa Centro Nacional de Pesquisa de Soja, Londrina, Brazil: 2002. 180, 154-1. Anais do II Congresso Brasileiro de Soja e Mercosoja, 3-6 Junho 2002, Foz do Iguacu, PR, Brazil.

Peterson GL, Bonde, MR (1998). Results of pathogenicity tests with *T. indica* spp. associated with *Lolium* spp. In *Bunts and Smuts of Wheat: An International Symposium*; North Carolina, August 17-20, 1997. Eds. Malik, VS, Mathre, DE. NAPPO, Ottawa, 117-125.

Peterson GL, Bonde MR, Phillips, JG (2000). Size-selective sieving for detecting teliospores of *Tilletia indica* in wheat seed samples. *Plant Disease*, **84**, 999-1007.

Peterson GL, Creager RA (2000). Susceptibility of winter wheat to Karnal bunt. *Phytopathology*, **90**: S60.

Peterson GL, Leth, V (2005). (*Unpublished draft*). EU Karnal bunt risks Project. Deliverable Report 4.1. Report on teliospore dormancy and germination under a range of abiotic conditions,

interpreting the results in relation to European conditions and predicting the likely timing of teliospore germination in Europe.

Podleckis EV, Firko MJ (1996). Karnal bunt: Likelihood of spread via conveyances, harvest equipment and wheat shipments. Preliminary Probabilistic Risk Assessment. USDA-APHIS, unpublished document. 38pp.

Porta-Puglia A, Riccioni L, Valvassori M, Inman A, Hughes K, Bowyer R, Barnes A, Sansford C, Magnus H, Peterson G (2002). (*Unpublished*). EU Karnal bunt risks Project. Deliverable Report 2. 1. Report on the physiological susceptibility of 40 European wheat cultivars, including estimates of yield loss. 50pp.

Porter JR (1993). AFRCWHEAT: a model of the growth and development of wheat incorporating responses to water and nitrogen. *European Journal of Agronomy*, **2**, 69-82.

Porter JR, Ewert F, Miglietta F, Gioli B, Baker R, Murray G, Sansford C (2002). (*Unpublished*). EU Karnal bunt risks Project. Deliverable Report 1. 1. Report describing when wheat ear emergence occurs throughout Europe taking key factors into account. 55pp.

Rai RC, Singh A (1985). Estimation of loss in wheat grain weight due to Karnal bunt infection. *Indian Journal of Mycology and Plant Pathology*, **15**, 121-131.

Rajaram S, Fuentes-Dávila G (1998). Development of wheat varieties with resistance to Karnal bunt. In *Bunts and Smuts of Wheat: An International Symposium*; North Carolina, August 17-20, 1997. Eds. Malik VS, Mathre DE. NAPPO, Ottawa, 305-315.

Rattan GS, Aujla SS (1990). Survival of Karnal bunt (*Neovossia indica*) teliospores in different types of soil at different depths. *Indian Journal of Agricultural Science*, **60**, 616-618.

Riccioni L, Valvassori M, Porta-Puglia A, Inman A, Hughes K, Coates M, Bowyer R, Barnes A, Sansford C, Magnus H, Peterson G (2004). (*Unpublished*). EU Karnal bunt risks Project. Deliverable Report 2. 3. Report on the morphological resistance/susceptibility of European bread and durum wheat cultivars to *Tilletia indica*. 32pp.

Rivera-Castaneda G, Martinez-Tellez MA, Vallejo-Cohen S, Alvarez-Manilla G, Vargas-Arispuro I del C, Moya-Sanz P, Primo-Yufera E (2001). *In vitro* inhibition of mycelial growth of *Tilletia indica* by extracts of native plants from Sonora, Mexico. *Revista Mexicana de Fitopatología* **19**, 214-217.

Royer MH, Rytter J (1985). Artificial inoculation of wheat with *Tilletia indica* from Mexico and India. *Plant Disease*, **69**, 317-319.

Royer MH, Rytter J (1988). Comparison of host ranges of *Tilletia indica* and *T. barclayana*. *Plant Disease*, **72**, 133-136.

Royer MH (1990). Pros and cons of using modelling versus non-modelling approaches to predict Karnal bunt outbreaks. Proceedings of the Seventh Biennial Workshop on Smut Fungi. June 4th – 7th 1990, Frederick, Maryland, USA, page 24.

Rush C, Stein JM, Bowden RL, Riemenschneider R, Boratynski T, Royer MH (2005). Status of Karnal bunt in the United States 1996 to 2004. *Plant Disease*, **89**, 212-223.

Salazar-Huerta FJ, Figueroa-López P, Fuentes-Dávila G, Smilanick JL (1997). Evaluation of foliar fungicides for control of Karnal bunt of wheat during 1986-1989 in northwestern Mexico. *Revista Mexicana de Fitopatología* **15**, 73-80.

Sansford CE (1996). (*Unpublished*). Karnal bunt (*Tilletia indica*): Provisional Pest Risk Analysis: An assessment of the current situation in the USA and the potential risk to the European Community. CSL internal document, 21pp.

Sansford CE (1998). Karnal bunt (*Tilletia indica*): Detection of *Tilletia indica* Mitra in the US: Potential risk to the UK and the EU. *In* Bunts and Smuts of Wheat: An International Symposium; North Carolina, August 17-20, 1997. Eds. Malik, VS, Mathre, DE. NAPPO, Ottawa, 273-302.

Sansford CE (2004). Revised UK Summary Pest Risk Analysis for *Tilletia indica*. Web Revision 1, November 2004. 27pp. <http://www.defra.gov.uk/planth/pratilletia.pdf>

Sansford CE, Inman AJ, Reed P (2004). (*Unpublished*). Revised draft UK contingency plan for the eradication and containment of Karnal bunt. Internal CSL document prepared for the UK Plant Health Service.

Sansford C, Jennings P, Leach R, Inman A, Stonehouse J (2004a). (*Unpublished*). EU Karnal bunt risks Project. Deliverable Report 6.2. Report on the efficacy and timing of existing and new European fungicide sprays against *Tilletia indica*. 32pp.

Sansford C, Peterson G (2004). (*Unpublished*). EU Karnal bunt risks Project. Deliverable Report 6.4. Evaluate existing risk management schemes for the USA, Mexico and the UK. 27pp.

Sansford C, Peterson G, Murray G (2004b). (*Unpublished*). EU Karnal bunt risks Project. Deliverable Report 6.3. Evaluation of published data on the efficacy of 'other' (non-fungicide) control strategies for *Tilletia indica* (Karnal bunt) including the control of soil-borne inoculum and the treatment of infected grain or seed. 38pp.

Schall RA (1988). (*Unpublished*). Karnal bunt: The risk to the American wheat crop, *Internal document*, USDA-APHIS.

Sharma BK, Basandrai AK (2000). Effectiveness of some fungicides and biocontrol agents for the management of Karnal bunt of wheat. *Journal of Mycology and Plant Pathology* **30**, 76-78.

Sharma I, Nanda GS (2003). Effect of prolonged period of fog/rain from sowing of wheat to pre-boot stage on Karnal bunt development. *Annals of Plant Protection Sciences* **11**, 93-95.

Sharma I, Nanda GS, Kaloty PK (1997). Ciperconazole [*sic*], an effective fungicide for the control of Karnal bunt of wheat. *Plant Disease Research* **12**, 35-36.

Sharma I, Nanda GS, Singh H, Sharma RC (2004). Status of Karnal bunt disease of wheat in Punjab (1994-2004). *Indian Phytopathology* **57**, 435-439.

Sharma AK, Singh DP, Kumar J, Sharma I, Sharma BK (2005). Efficacy of some new molecules against Karnal bunt (*Tilletia indica*) of wheats (*Triticum aestivum* and *T. durum*). *Indian Journal of Agricultural Sciences*. **75**, 369-370.

Sharma KK, Singh DV, Srivastava KD, Agarwal R, Nanda SK (1994). Bio-efficacy and persistence of propiconazole against Karnal bunt of wheat. *Indian Journal of Plant Protection* **22**, 93-95.

Singh A (1994). Epidemiology and Management of Karnal bunt disease of wheat. Research Bulletin No. 127, Directorate of Experiment Station, G.B. Pant University of Agriculture and Technology, Pantnagar, India.

Singh A (1998). Epidemiology of Karnal bunt of wheat. In Bunts and Smuts of Wheat: An International Symposium; North Carolina, August 17-20, 1997. Eds. Malik, VS, Mathre, DE. NAPPO, Ottawa, 149-162.

Singh D (1980). A note on the effect of Karnal bunt infection on the vigor of wheat seed. *Seed Research*, **8**, 81-82.

Singh DV, Agarwal R, Shrestha JK, Thapa BR, Dubin HJ (1989). First report of *Tilletia indica* on wheat in Nepal (Disease note). *Plant Disease*, **73**, 273.

Singh BB, Aujla SS, Sharma I (1993). Integrated management of wheat Karnal bunt. *International Journal of Pest Management* **39**, 431-434.

Singh R, Beniwal MS, Karwasra SS (2000). Field evaluation of fungicides against Karnal bunt of wheat through foliar spray. *Tests of Agrochemicals and Cultivars* **21**, 9-10.

Singh DV, Dhaliwal HS (1989). Screening of wheat germplasm for components of resistance to Karnal bunt disease. *Indian Phytopathology*, **42**, 393-399.

Singh P, Dhaliwal HS, Gill KS (1989a). Chemical control of Karnal bunt of wheat by a single spray of fungicides at heading. *Indian Journal of Agricultural Science* **59**, 131-133.

Singh DV, Sharma KK, Nanda SK, Srivastava KD, Aggarwal R (1992). Efficacy of diniconazole against Karnal bunt of wheat. *Indian Journal of Plant Protection* **20**, 47-49.

Singh DV, Srivastava KD, Aggarwal R, Jain SK (1996). Factors associated with development and spread of Karnal bunt of wheat (*Triticum aestivum*) in north-western India. *Indian Journal of Agricultural Sciences*, **66**, 374-383.

Singh, DV, Srivastava, KD, Goel, LB, Joshi, LM, Gupta, RS (1977). Incidence of Karnal bunt in Northwestern India during 1974-1975. *Indian Phytopathology*, **30**, 431-432.

Smilanick JL (1997). Germinability of teliospores of *Tilletia indica* after hot water and sodium hypochlorite treatments. *Plant Disease* **81**, 932-935.

Smilanick JL, Dupler M, Goates BJ, Hoffmann JA, Clark D, Dobson D (1986). Germination of teliospores of Karnal, dwarf and common bunt fungi after ingestion by animals. *Plant Disease*, **70**, 242-244.

Smilanick JL, Hoffman JA, Cashion NL, Prescott JM (1987). Evaluation of seed and foliar fungicides for control of Karnal bunt of wheat. *Plant Disease* **71**, 94-96.

Smilanick JL, Hoffmann JA, Royer MH (1985). Effect of temperature, pH, light, and desiccation on teliospore germination of *Tilletia indica*. *Phytopathology* **75**, 1428-1431.

Smilanick JL, Hoffmann JA, Secret LR, Wiese K (1988). Evaluation of chemical and physical treatment to prevent germination of *Tilletia indica* teliospores. *Plant Disease* **72**, 46-51.

Smilanick JL, Prescott JM, Hoffmann JA, Secret LR, Weise K (1989). Environmental effects on survival and growth of secondary sporidia and teliospores of *Tilletia indica*. *Crop Protection*, **8**, 86-90.

Smiley RW (1997). Risk assessment for Karnal bunt occurrence in the Pacific Northwest. *Plant Disease* **81**, 689-692.

Smith, D (2001). Regulations Governing the International Movement of Cereal Seed and Grain in Relation to the Plant Pathogen, *Tilletia indica*. CSL internal document. 11pp.

Stansbury CD, McKirdy SJ (2002). Forecasting climate suitability for Karnal bunt of wheat: a comparison of two meteorological methods. *Australasian Plant Pathology* **31**, 81-92.

Stansbury CD, McKirdy SJ, Diggle AJ, Riley IT (2002). Modelling the risk of entry, establishment, spread, containment, and economic impact of *Tilletia indica*, the cause of Karnal bunt of wheat, using an Australian context. *Phytopathology* **92**, 321-331.

Stansbury CD, Pretorius ZA (2001). Modelling the potential distribution of Karnal bunt of wheat in South Africa. *South African Journal of Plant and Soil* **18**, 159-168.

Stein JM, Maples HW, Rush CM (2005). Epidemiology of *Tilletia indica* teliospores in regulated wheat fields in Texas. *Plant Disease* **89**, 828-833.

Sutherst RW, Maywald GF (1985). A computerised system for matching climates in ecology. *Agriculture, Ecosystems and Environment*, **13**, 281-299.

Talevi S, Pucci N, Nardi S, Di Giambattista G, Porta-Puglia A (2004). Analysis of the mycoflora on wheat kernels imported from USA. Micoflora presente su cariossidi di grano duro importato dagli Stati Uniti. *Informatore Fitopatologico - La difesa delle piante* **54** (10), 41-45.

Thorne FS, Brennan JP, Kelly PW, Kinsella A (2004). The potential socio-economic impact of *Tilletia indica* in Europe. Proceedings of the UK Agricultural Economics Society Conference, 2nd March, 2004, London.

Torarbi M, Mardoukhi V, Jaliani N (1996). First report on the occurrence of partial bunt on wheat in the southern parts of Iran. *Seed and Plant*, **12**, pages 8-9 (Persian) and 59-60 (English).

USDA (2003). Karnal bunt; Revision of Domestic Regulations. Proposed Rule. Federal Register, **68**, 130, 7 CFR Part 301, [Docket No. 02-056-1]. July 8th 2003.

USDA (2004). Karnal bunt; Revision of Domestic Regulations. Final Rule. Federal Register, **69**, 35, 7 CFR Part301, [Docket No. 02-056-2]. February 23rd 2004.

Valvassori M, Riccioni L, Inman A, Hughes K, Conca G, Di Giambattista G, Porta-Puglia A (2002). Survival of *Tilletia indica* teliospores in soil. *Journal of Plant Pathology*, **84**, 171-200.

Warham E (1986). Karnal bunt disease of wheat: a literature review. *Tropical Pest Management*, **32**, 229-242.

Warham EJ (1988). Screening for Karnal bunt (*Tilletia indica*) resistance in wheat, triticale, rye and barley. *Canadian Journal of Plant Pathology*. **10**, 57-60.

Warham E (1988a). Teliospore germination patterns in *Tilletia indica*. *Transactions of the British Mycological Society*, **90**, 318-320.

Weir AH, Bragg PL, Porter JR, Rayner JH (1984). A winter wheat crop simulation model without water or nutrient limitations. *Journal of Agricultural Science*, **102**, 371-382.

Ykema RE, Floyd JP, Palm ME, Peterson GL (1996). First report of Karnal bunt of wheat in the United States (Disease Note). *Plant Disease* **80**, 1207.

Zhang Z, Lange L, Mathur SB (1984). Teliospore survival and Plant Quarantine significance of *Tilletia indica* (causal agent of Karnal bunt) particularly in relation to China. *EPPO Bulletin* **14**, 119-128.

6. ACKNOWLEDGEMENTS

Partner 1: CSL

Dr Sansford thanks Defra Plant Health Division and the Plant Health and Seeds Inspectorate for their support in helping develop the PRA for *T. indica*. Thanks are also due to Mrs Sue Sainty, Plant Health Group, CSL for tidying up this manuscript.

Partner 2: NSW Ag

Dr Murray and Dr Brennan thank the Grains Research and Development Corporation, Australia, for financial support. Dr Murray also thanks the United States Department of Agriculture and Plant Health Australia for additional financial support.

Partner 8: IsPAVe

Dr Riccioni and Dr Porta-Puglia wish to thank Giovanni Conca and Giuseppe Di Giambattista for technical support at ISPaVe.

Partner 9: NCRI

Dr Magnus and Dr Rafoss wish to thank Anders Heen, Norwegian Agricultural Inspection Service for providing facilities for the quarantine work.

Workpackage 6 - Description of Work Plan

Workpackage title: Pest Risk Analysis for *Tilletia indica* for the EU

Aim: To analyse the risk of entry, establishment and socio-economic losses for the EU and to develop harmonised risk management strategies and contingency plans.

Workpackage 6. Objectives

- Determine the risk of entry, establishment and socio-economic loss for *T. indica* in the EU.
- Evaluate fungicide efficacy and timing for the control of *T. indica*.
- Evaluate published data on other control strategies.
- Evaluate existing risk management schemes for *T. indica* outside of the EU.
- Determine the appropriate level of risk management for the EU for *T. indica* in relation to the assessed level of risk determined by the Project.

Methodology (per sub-workpackage)

- 6.1** Prepare a report of the risk of entry, establishment and socio-economic losses for the EU from data obtained in Workpackages 1 and 5 using the internationally PRA standard (based on the most up-to-date FAO ISPM on ‘Guidelines for Pest Risk Analysis’) as a framework (Partner 1, with input from all other Partners)
- 6.2 Evaluate** the efficacy of propiconazole, epoxiconazole, tebuconazole, prochloraz and azoxystrobin, alone and in mixtures by in vitro screening (Partner 1). The most effective treatment will then be applied at flag leaf emergence (GS 39), awns emerging (GS 49), anthesis (GS 60-69) and post anthesis (GS 71) on plants of the most susceptible European wheat cultivar, identified in Workpackage 2, inoculated with *T. indica* at the most susceptible wheat development stage, identified in Workpackage 2. The plants will be grown in pots under quarantine in controlled environment chambers. Treatments will be replicated four times. Results will be incorporated into the risk management section of the Pest Risk Analysis (6.1).
- 6.3** Evaluate published data on the efficacy of other control strategies including the control of soil-borne inoculum and the treatment of infected grain or seed and incorporate the results into the Pest Risk Analysis (6.1). (Partners 2, 10).
- 6.4 Evaluate** existing risk management schemes for Australia, the USA and Mexico and report on the evaluation in the Pest Risk Analysis (6.1). (Partners 2, 10).
- 6.5** Determine the most appropriate risk management scheme for the EU (based on the most up-to-date FAO ISPM on ‘Guidelines for Pest Risk Analysis’) in relation to the level of risk identified in 6.1 and prepare a conclusion in the Pest Risk Analysis (6.1). (Partners 1, 2, 3, 8, 9, 10).

Deliverables

- DL 6.1 Report on the risk of entry, establishment and socio-economic loss for *T. indica* in the EU. **(Month 48)**
- DL 6.2 Report on the efficacy and timing of existing and new European fungicide sprays against *T. indica*. **(Month 34)**
- DL 6.3 Report on published data on the efficacy of other control strategies. **(Month 30)**
- DL 6.4 Report on the evaluation of existing risk management schemes outside of the EU **(Month 33)**
- DL 6.5 Determine and report on the most appropriate risk management scheme for *T. indica* in the EU in relation to the assessed level of risk using the framework of the published international standard for Pest Risk Analysis. **(Month 48)**

Milestones

- MS 6.1 The risk of entry, establishment and socio-economic loss for *T. indica* in the EU determined. **(Month 48)**
- MS 6.2 Fungicide efficacy and timing for the control of *T. indica* evaluated. **(Month 30)**
- MS 6.3 Published data on other control strategies evaluated. **(Month 30)**
- MS 6.4 Existing risk management schemes outside of the EU evaluated. **(Month 33)**
- MS 6.5 Appropriate level of risk management determined in relation to the assessed level of risk from the Project. **(Month 48)**

MODEL CONTINGENCY PLAN (Prepared by CSL in consultation with Defra) (20/07/05)

By evaluating the range of measures that could be undertaken to prevent entry of the pathogen or to deal with outbreaks in the EU, a model management (contingency) plan has been produced based upon outbreak scenarios in England for consideration for implementation in England and Wales. This plan, which formed the basis of the socio-economic impact analysis in the PRA, is for illustrative purposes only. It should not be taken as indicating how any EU Member State would respond to a finding of *Tilletia indica* on its territory. Any such response will be determined by the governments concerned, in the light of consultation with stakeholders and other relevant factors at the time including the availability of resources.

ERADICATION & CONTAINMENT OF KARNAL BUNT (*Tilletia indica*)

Contents

- 1. BACKGROUND, POLICY AND STATUTORY AUTHORITY**
 - 1.1 The pest**
 - 1.2 Scope of these instructions, policy and definitions**
 - 1.3 Statutory authority**

- 2. INSPECTION PROCEDURES**
 - 2.1 Inspection and sampling**
 - 2.2 Sampling protocol**
 - 2.3 Testing procedure**

- 3. ACTION ON SUSPICION OF *TILLETIA INDICA*/KARNAL BUNT IN IMPORTED GRAIN OR SEED**
 - 3.1 Action on finding suspect teliospores in a sample of grain or seed**

- 4. ACTION ON CONFIRMATION OF *TILLETIA INDICA*/KARNAL BUNT IN IMPORTED GRAIN OR SEED**
 - 4.1 Loading affected grain at store and transportation for processing or disposal**
 - 4.2 Handling affected grain at flour mills**
 - 4.3 Cleaning of lorries to transport and equipment to process affected consignments**
 - 4.4 Seed**

- 5. ACTION ON SUSPICION AND CONFIRMATION OF *TILLETIA INDICA*/KARNAL BUNT IN THE FIELD, OR IN GRAIN OR SEED HARVESTED IN ENGLAND OR WALES**
 - 5.1 Growing crop**
 - 5.2 Grain/seed**
 - 5.3 Straw**
 - 5.4 Milling products or by-products**
 - 5.5 Soil (associated with any article)**

- 5.6 Farm machinery, farm tools, soil moving equipment and other articles**
- 5.7 Bags, sacks**
- 5.8 Livestock**
- 5.9 Manure**
- 5.10 Subsequent actions in affected fields**
- 5.11 Removal of statutory restrictions**
- 5.12 Surveys in England and Wales**

1. BACKGROUND, POLICY AND STATUTORY AUTHORITY

1.1 The pest

Karnal bunt is a fungal disease of wheat (*Triticum aestivum*), durum wheat (*Triticum durum*) and triticale (*Triticum aestivum* X *Secale cereale*), a hybrid of wheat and rye. Karnal bunt is caused by the fungus *Tilletia indica* which is primarily a soil-borne and seed-borne pathogen. Long distance spread is primarily through the movement or planting of infected seed or grain. Local spread can occur if teliospores are liberated from grain during combining and become wind-borne, or through the movement of infested wind-blown soil. The fungus attacks the grain, replacing it with black, dusty spore masses that resemble soot. The fungus is not known to have human or animal health implications but it affects the taste and smell of flour made from infected grain. Downgrading wheat destined for human consumption to animal feed results in economic loss to the farmer. The disease may also reduce yield in the field. However, the main economic impact of the disease results from loss of exports to countries that ban imports of grain or seed grown in affected countries or areas.

The fungus is difficult to control. Seed treatments are largely ineffective because the fungus does not infect at the seedling stage. Rather, teliospores germinate on the soil surface prior to ear emergence and the fungus infects directly through the ear. Reduction in disease levels in growing crops can be achieved with timely application of effective fungicides. However, complete eradication is very difficult to achieve, as the teliospores of the fungus are extremely long-lived in the soil.

Further information on this disease can be found on the Defra website (www.defra.gov.uk/planth/pestnote/karnal.htm/) with hard copies of a poster (PB4642) and information sheet (PB4642a) also available on request.

1.2 Scope of these instructions, policy and definitions

This model contingency plan provides guidance on the measures which might be taken (*see note at the start of the plan*) following findings of *Tilletia indica* on imported grain or seed as well as outbreaks in field situations in England or Wales or in grain or seed harvested in England or Wales. These measures are based on a policy of exclusion (preventing entry) and eradication of the organism, and include long-term containment measures for field outbreak sites, as soil-borne stages of the pathogen can remain viable for 5 years (and possibly longer). Further information on general measures to be taken, in the event of an outbreak of a quarantine plant pest or disease can also be found in the Eradication & Containment-General section of the Plant Health & Seeds Inspectorate (PHSI) Handbook, which is available on request.

It is unlikely that the disease will be detected in the field in the first outbreak, and more likely that it will be detected in harvested grain or seed. However, both scenarios are dealt with in this plan. For the purposes of this plan, ‘seeds’ are defined as seeds for sowing; ‘grain’ is defined as seed for processing (e.g. milling/animal feed).

1.3 Statutory authority

Tilletia indica is listed in Annex 1A1 and Annex IVA1 of the EC Plant Health Directive 2000/29/EC and Schedule 1A1 and 4A1 of the Plant Health (Great Britain) Order 1993 (as

amended). This means that the pest is known not to occur in the EU, its introduction into and spread within the EU is banned, import requirements are imposed and its presence is notifiable. Statutory action to deal with *T. indica* is taken under the Plant Health (Great Britain) Order 1993 as amended by the 1996 (no 3) Order.

EC and UK Plant Health legislation for the Devolved Administrations requires that imports of seed of wheat and triticale must originate from an area where *T. indica* is known not to occur. Imported grain for animal feed or milling must either come from an area where *T. indica* is known not to occur, or has been found free from the fungus during both visual crop inspection and in post-harvest testing, and testing of the consignment before shipment.

2. INSPECTION PROCEDURES

In order to ensure that EC and UK Plant Health legislation requirements are being met, a proportion of grain imported from outside of the EU is currently sampled by the Defra Plant Health and Seeds Inspectorate (PHSI) and tested for the presence of *T. indica* at the Central Science Laboratory (CSL). PHSI will aim to sample all imported consignments of grain or seed originating in countries where *T. indica* is known to occur (currently USA, Mexico, parts of Asia, including Afghanistan, India, Iran, Iraq, Nepal, Pakistan, South Africa, as well as possibly Brazil). It is possible that grain or seed from these countries may be re-exported from other EU countries to the UK; where this is known to have occurred these consignments will also be sampled. Imported seed may also be liable to monitoring but, at present, seed rarely if ever originates in countries where *T. indica* occurs.

UK freedom from *T. indica* is also required to satisfy other country's' import requirements. Sampling and testing for this organism is being undertaken in the UK to demonstrate pest freedom. In addition the UK wheat crop is monitored intensively and it is likely that the presence of *T. indica* would be reported either pre-harvest, or (more likely) post-harvest.

2.1 Inspection & Sampling

The disease is not readily detected in the field as only a few grains in an ear usually become infected and they are enclosed within the glumes until harvest. *T. indica* is more likely to be found when inspecting grain after harvest, either by visual inspection of a grain sample for symptoms of bunt or by conducting a seed wash and microscopic examination for teliospores. For quarantine purposes, visual examination for symptomatic (bunted) grains is considered unreliable for detection of the pathogen, though it can be done in parallel with tests for the detection of teliospores using a seed wash.

Symptoms of Karnal bunt on wheat caused by *T. indica* can be confused with at least two diseases of the grain, namely common bunt (*Tilletia tritici*, synonym *Tilletia caries*) and blackpoint (mainly *Alternaria* species and physiological condition). Suspect symptoms must be confirmed by laboratory examinations and tests.

2.2 Sampling protocol

For seed lots, samples should be taken following ISTA (International Seed Testing Association) seed sampling rules to ensure a representative sample. Sampling equipment should be cleaned and decontaminated after use. Sampling of grain shipments is more problematic. For grain destined for human consumption, a 2 kg sub-sample is taken from the sample collected for

Customs for tariff purposes. A sample from this composite tariff sample may be taken by PHSI for testing for *T. indica*. By the time the test results are available the consignment will probably be moving inland and appropriate action will be required on finding teliospores which are suspected to be those of *T. indica*, as outlined below. Imports not sampled for tariff purposes, including grain destined for animal feed, should also be sampled by PHSI.

2.3 Testing procedure

Samples sent to CSL are tested for *T. indica* using the EU/EPPO-recommended *T. indica* Diagnostic Protocol. (See <http://www.csl.gov.uk/science/organ/ph/diagpro/tipro.pdf>). This protocol was produced by CSL and ring-tested by European laboratories and is used for teliospore detection and species identification.

Detection involves a wash test of replicate 50 g sub-samples (ca. 1000 seeds) and involves the use of size-selective sieving and centrifugation methods. One spore per 50 g sample can be detected with 99% confidence if three replicate samples are tested. Identification of suspect teliospores is then carried-out. (NB: Teliospores of other *Tilletia* species which are very similar to *T. indica* can occur as contaminants of wheat such as *T. walkeri* and *T. horrida*). In the absence of bunted kernels, morphological identification is possible with relatively small numbers of spores, but confirmation by molecular methods is recommended, especially when very low numbers are found (<10).

3. ACTION ON SUSPICION OF *TILLETIA INDICA*/KARNAL BUNT IN IMPORTED GRAIN OR SEED

Action will be required to deal with any grain or seed at a port or other premises including grain at a mill, suspected to be infected/infested with *T. indica*. Testing, notification, traceback and traceforward will be carried-out as described below.

3.1 Action on finding suspect teliospores in a sample of grain or seed

- 3.1.1. If suspect teliospores are found in a sample of grain or seed during the initial wash test, which takes ca. 24 hours, the consignment will be traced and held (if not held already) and any other known recipients of the consignment in the EU notified.
- 3.1.2. If the sample proves to be negative for *T. indica* following morphological examination of the spores (which takes a further (ca.) 24 hours) the consignment will be released.
- 3.1.3. Following morphological examination of the spores, if the sample is still suspected to be positive, importers will have the option of holding the consignment pending confirmatory tests (which could take a further 2 weeks) or taking immediate action before confirmation, which could be either processing under official control or returning the consignment to the port for re-export (see 4 for requirements for immediate action).
- 3.1.4. The final decision on options for immediate action will be made by Defra, taking into account the risk of disseminating teliospores.
- 3.1.5. At this stage a provisional notification will be sent to the exporting country, the European Commission and other Member States, and traceback and traceforward procedures will commence.

- 3.1.6. If the sample is subsequently confirmed as positive by molecular and/or morphological analysis for *T. indica* the EU notification will be updated, the exporting country will be asked for an explanation of the finding as well as assurances that measures are in place to prevent recurrence. Action on confirmation is described in section 4. Future consignments from that country will be held pending initial test results.

4. ACTION ON CONFIRMATION OF *TILLETIA INDICA*/KARNAL BUNT IN IMPORTED GRAIN OR SEED

Consignments of grain destined for human consumption confirmed as being infected with *T. indica* will require processing to destroy the pathogen or disposal of in a manner which poses no threat of dispersal of teliospores to arable land. It should be stressed that *T. indica* is not known to pose a risk to human (or animal) health. However, for grain destined for human consumption, infestation with *T. indica* may affect the taste and smell of flour made from infected grain resulting in downgrading for processing for animal feed. This processing method, as described below (4.2), is known to be sufficient to render teliospores non-viable.

4.1 Loading affected grain at store and transportation for processing or disposal

- 4.1.1 For transportation for processing or disposal, affected grain must be loaded into lorries whilst within the store.
- 4.1.2 The load must be sealed, i.e. completely covered with a suitable sheet (again while lorries are within the store) and cleaned externally (see 4.3) before it leaves the store.
- 4.1.3 Every precaution must be taken to avoid/minimise grain dust formation.
- 4.1.4 Only lorries specified as NOT to carry wheat ‘seed’ (for sowing) should be used, or once used, should not be used for transport of wheat seed or grain, until cleaned as described in 4.3.
- 4.1.5 Lorries should be driven directly to specified mills for processing or PHSI approved sites for disposal.
- 4.1.6 The route the lorry takes must be specified by the responsible PHSI.

4.2 Handling affected grain at flour mills

- 4.2.1 All sites must operate under negative pressure to suppress dust.
- 4.2.2 Affected grain and any flour fraction derived from affected grain is to be processed for animal feed according to the instructions for ‘wheat feed’ below. Alternative processing methods will need to be assessed to ensure teliospores are rendered non-viable. Full details should be provided to PHSI. N.B. In some circumstances, based on appropriate risk assessment, grain may continue to be processed for human consumption.
- 4.2.3 All waste (dust, bran, screenings etc) must be either:
- 4.2.3.1 Bagged and disposed of at a PHSI approved landfill site or

4.2.3.2 Returned to '*wheat feed*' (see below).

4.2.3.3 Sample(s) should be bagged and retained for collection by PHSI.

4.2.3.4 Closed incineration may also be an option for waste, based on an assessment of the risk e.g. for small quantities.

4.2.4 All '*wheat feed*' must be processed by a method known to render teliospores non-viable, such as the pelleting process detailed below:

4.2.4.1 Wheat feed is introduced to the pellet press at 10-30°C, 12-13% moisture.

4.2.4.2 Wheat feed is '*conditioned*' with live steam at 55-85°C, 15-17% moisture. This takes approx. 10-15 seconds.

4.2.4.3 Wheat feed is squeezed out through press (to make the pellets) at 70-90°C, 15-17% moisture (4-5 seconds).

4.2.4.4 Pellets are cooled down from press exit (45-80°C) to 15-30°C and drop back to 12-13% moisture over 15-20 minutes.

4.3 Cleaning of lorries and equipment used to process affected consignments

4.3.1 All lorries and sheeting must be cleaned down thoroughly;

4.3.2 Cleaning should be performed at the destination, following removal of all soil and plant debris, in the presence of the PHSI, by one of the following methods:

4.3.2.1 Apply steam at the critical temperature of 77°C to all surfaces to the point of runoff or

4.3.2.2 Clean with a solution of hot water and detergent (82°C minimum) applied under pressure of at least 30 pounds per square inch or

4.3.2.3 Wet all surfaces to the point of runoff with a solution of 1.5% sodium hypochlorite and let stand for 15 minutes. Thoroughly wash down after 15 minutes to minimise corrosion or

4.3.2.4 Fumigate with methyl bromide at 240g for 96 hours (CTP = 23040 gh.m3)

NOTE: The above processes may cause damage to equipment (e.g. rubber parts). Equipment should, therefore, be checked thoroughly after any of the above processes are undertaken.

4.3.3 Any waste collected during the next routine clean of the mills must be disposed of at a PHSI approved site (e.g. by closed incineration)

4.4 Seed

Seed at any premises confirmed as positive for *T. indica* as well as any known to have been contaminated should either be destroyed or be moved safely to a PHSI approved facility for processing suitable to destroy *T. indica* (see 4.2 above), or to a PHSI approved facility for closed incineration, or to a licensed landfill facility. Small amounts of breeding material can be autoclaved.

5. ACTION ON SUSPICION AND CONFIRMATION OF *TILLETIA INDICA*/KARNAL BUNT IN THE FIELD, OR IN GRAIN OR SEED HARVESTED IN ENGLAND OR WALES

On suspicion of the presence of *T. indica*/Karnal bunt in crops or (more likely) in harvested grain or seed in England and Wales, confirmation of the presence of *T. indica* will be made as described in section 2.

In the event of a confirmed finding, trace-back and trace-forward activities will have to commence immediately on a range of articles. See Table 1 below.

Articles identified as potentially contaminated by trace-back and trace-forward activities will require further sampling and testing to confirm the presence of the organism and will be subject to treatment or destruction by appropriate and effective methods as described below. Several of the actions required on confirmation in imported grain or seed (section 4) will apply.

Table 1. Articles in or related to the infested field(s) to be subjected to trace-back and trace-forward activities.

Article category	Sub-categories
Growing crop (plants)	Wheat: <i>Triticum aestivum</i> Durum wheat: <i>Triticum durum</i> Triticale: x <i>Triticosecale</i>
<i>Certified seed, home-saved seed, grain</i>	Wheat: <i>Triticum aestivum</i> Durum wheat: <i>Triticum durum</i> Triticale: x <i>Triticosecale</i>
Straw	Wheat: <i>Triticum aestivum</i> Durum wheat: <i>Triticum durum</i> Triticale: x <i>Triticosecale</i>
Milling products or by-products (including animal feed, waste and debris)	Wheat: <i>Triticum aestivum</i> Durum wheat: <i>Triticum durum</i> Triticale: x <i>Triticosecale</i>
Soil (associated with any article)	
Soil moving equipment	
Farm machinery (cultivation and harvesting equipment)	
Farm tools	
Clothes and footwear of workers	
Containers related to movement of seed, grain or straw	Includes lorries, trailers, containers, railcars, bags, sacks
Storage facilities	
Grain processing facilities	
Seed treatment machinery/seed treatment plants	
Livestock	
Manure	

5.1 Growing crop

On detection of the disease within the growing crop (most likely to occur close to harvest) or for crops at any stage, which are known to have been planted with infected seed, the crop should be destroyed and disposed of safely, to prevent further spread, in compliance with waste management legislation. It is recognised that the volume of material involved will be substantial, particularly closer to harvest, but a strategy for destruction and disposal will need to be determined. Whilst there is still green material present in the crop a non-selective herbicide should be applied *in situ* to prevent further fungal development (teliospores in maturing seeds; reduction of future volunteer wheat plants). Up until early heading the use of glyphosate will arrest grain development but later (pre-senescence) paraquat would be more appropriate.

Burning plant material from a mature crop in the field is not an option, as spores are not reliably destroyed by this process and may also be disseminated over long distances by the resulting convection. Similarly, composting is not an effective method of disposal of any infected/infested material. However, additional processing options (e.g. biomass burning) will be considered on a case-by-case basis. Full details of the processing conditions will be required to confirm suitability of the treatment as an alternative to destruction. The actions for the individual affected fields are discussed in Section 5.10.

5.2 Grain/seed

Action on confirmation of *T. indica* in grain or seed produced in England or Wales at a store or mill is outlined in section four, which details safe handling, movement, processing and destruction procedures.

Where affected seed or grain has been used for sowing the crop should be destroyed as outlined in 5.1.

5.3 Straw

No movement; safe destruction and disposal *in situ* where possible

Or

Safe movement to a PHSI approved facility for closed incineration, or to a licensed landfill facility.

5.4 Milling products or by-products

No movement; safe destruction and disposal *in situ* where possible

Or

Safe movement to a PHSI approved facility for processing suitable to destroy *T. indica* (see 4.2 above)

Or

Safe movement to a PHSI approved facility for closed incineration, or to a licensed landfill facility.

5.5 Soil (associated with any article)

Safe movement to a PHSI approved disposal site or licensed landfill facility.

5.6 Farm machinery (cultivation & harvesting equipment); farm tools; soil moving equipment; transport vehicles; worker's clothing and footwear; storage facilities; grain handling and processing machinery; seed treatment machinery and treatment plants

See 4.3 above noting risk of damage to equipment, e.g. rubber parts of mechanised equipment.

5.7 Bags, sacks

No movement; safe destruction and disposal *in situ* where possible

Or

Safe movement to a PHSI approved facility for closed incineration, or to a licensed landfill facility.

5.8 Livestock

Livestock which have grazed on infected grain, or contaminated straw or hay should be held in a non-cultivated area and fed on uninfected feed for 5 days before movement elsewhere. This is to prevent the risk of spread from manure, which may still contain viable teliospores following ingestion by livestock.

5.9 Manure

No movement; safe destruction and disposal *in situ* where possible

Or

Safe movement to a PHSI approved facility for closed incineration, or to a licensed landfill facility.

5.10 Subsequent action in affected fields

General

5.10.1 Restrict access to affected fields. All activities employing the use or potential movement of articles listed in Table 1 related to the affected field(s) will be subject to the containment actions listed under Section 5.1 to 5.9

5.10.2 Soil sterilisation may be considered as an option, subject to further discussion with Defra

5.10.3 Grow non-susceptible crops in a 3-km radius buffer zone around the affected fields for 5 years (see below).

Future Cropping

5.10.4 There are two options for subsequent action to be taken within the affected field(s)

a) Bare Fallow

Maintain affected fields as bare fallow, nil cultivation, with chemical control of volunteer cereals and other plants. This will have to be for a minimum of 5 years and the time period will have to be reviewed on the basis of ongoing monitoring activities as described below.

Or

b) Grassing Down

Grass down the affected fields for a minimum of 5 years. [NB: grassing down could prolong teliospore survival if protection from frost occurs; conversely it may reduce survival if moisture retention and microbial populations are enhanced as these could contribute to teliospore mortality]. Grazing of livestock will not be permitted, unless measures can be taken to prevent movement of soil containing teliospores. This will have to be for a minimum of 5 years, to be reviewed on the basis of ongoing monitoring activities as described below.

Monitoring

5.10.5 Conduct soil sampling in affected fields to 30cm depth at appropriate intervals and test for presence and viability of teliospores of *T. indica*.

5.10.6 Place spore traps in the affected field and at the outer edge of the buffer zone positioned in the direction of the prevailing wind. Sample for spores between GS 45 to 51 (timing to be determined based upon local observations in wheat crops outside of the buffer zone as it will vary between sites and years).

5.10.7 Conduct visual inspection of susceptible crops grown within a 1km strip around the outside of the buffer zone to ensure that containment is effective. At harvest each year, sample and test grain/seed of susceptible crops in this zone for the presence of *T. indica*. Additional sampling and testing is recommended at mills receiving grain/seed from within this 1km zone.

5.11 Removal of Statutory Restrictions

The period of pest-freedom is proposed as 5 years, as determined by non-detection of *T. indica* by the above monitoring actions during that period. If viable teliospores or sporidia are still being found, or symptoms detected in susceptible crops grown in the 1km wide area around the outside of the buffer zone, restrictions will have to be continued for a further 5 years before confirming freedom from *T. indica*.

5.12 Surveys subsequent to an outbreak in England or Wales

- 5.12.1 In addition to the actions for monitoring in the affected fields and the buffer zone as well as in susceptible crops in a 1 km radius around the buffer zone, stratified national surveys will be conducted to prove freedom from *T. indica*.
- 5.12.2 A representative number of fields throughout England and Wales will be inspected prior to harvest for visual symptoms of Karnal bunt.
- 5.12.3 A representative sample of harvested grain from each field inspected in 5.12.2 will be processed using the EU/EPPO-recommended *T. indica* Diagnostic Protocol (see Section 2) to detect teliospores of *T. indica*.

DRAFT REGULATORY IMPACT ASSESSMENT (RIA) OF THE POSSIBLE MANAGEMENT OPTIONS FOR INTERCEPTIONS AND OUTBREAKS OF *TILLETIA INDICA* – NOVEMBER 2004

1. Issue

The fungus *Tilletia indica*, the cause of Karnal (or partial) bunt of wheat (and triticale) is a potentially damaging non-indigenous plant pathogen which does not occur in Europe. When the pathogen was found in the USA for the first time in 1996, a Pest Risk Analysis (PRA) was undertaken in the UK. EC and domestic legislation have been in place since 1997; this requires exporting countries where the pathogen occurs to comply with minimal requirements for cereal seed and grain exports; this is designed to prevent the entry of the pathogen into the EU. Despite this, *T. indica* has been intercepted in the UK, Italy and (possibly) Greece in wheat grain originating in countries where the pathogen occurs (see 3.). The policy and mechanisms by which this pathogen is regulated in the USA have changed recently, focusing on looking for disease symptoms rather than the presence of the pathogen. Such a policy is not recommended by EPPO²⁸. This approach may lead to further cryptic spread of *T. indica* in the USA and a potential increase in the risk that the pathogen will be present in exports of grain arising in areas of the USA where it occurs. The European Commission is in correspondence with the USDA to clarify the basis on which US exports of wheat are certified as meeting the EU's requirements with regard to *T. indica*.

2. Objective

This RIA aims to identify the impacts of policy options that might be used to prevent or deal with future interceptions and manage potential outbreaks of the pathogen in England or Wales.

3. Risk Assessment

A UK PRA was prepared in 1996 (see 1.). The PRA predicted a risk of entry on seed and grain of wheat, rye and triticale originating in countries where the pathogen occurs. At that time the published reports suggested it occurred in Asia (Afghanistan, India, Iraq, Nepal, Pakistan), North America (USA), Central America (Mexico) and South America (Brazil). Available import data for the UK for the past 2 years suggest that of these countries Brazil, India and the USA have exported wheat to the UK. Also, *T. indica* has been detected on wheat exported from India to the UK in 2003 and 2004 (and since this RIA was written in 2005 and 2006). For other EU countries the most recent trade data (2001-2002) suggest Italy has been a major importer of wheat from the USA. Interceptions of *T. indica* in other EU Member States have been on wheat from Mexico in Italy (twice in 1998), in Poland on wheat from India (1996), and possibly in Greece, on two consignments of wheat from the USA (in 1996). The PRA concluded that the pathogen had the potential to enter and establish in the UK and cause a large socio-economic loss. To facilitate risk management, *T. indica* became listed as a I/A1 quarantine pathogen in the EC Plant Health Directive in 1997, and in domestic legislation in each EU Member State. The consequence of quarantine listing is described in 4.

In addition to the continents and countries listed above, the pathogen is now also known to occur in Africa; it was first reported in South Africa in 2000. *Tilletia indica* was also reported in Iran in 1996; another Asian country.

²⁸ The European and Mediterranean Plant Protection Organisation, the Regional Plant Protection Organisation for 45 countries including the Member States of the EU

The PRA has been updated. Findings from a 4-year EU Fifth Framework Research Project ‘*Karnal bunt risks*’ (whose key objective was to develop the PRA) have been used to develop the assessment of the risk of establishment and socio-economic impact, and to support the development of the risk management options in the form of a model contingency plan²⁹ whose aim is to prevent or deal with interceptions and manage outbreaks. The conclusion of the revised PRA is that the risk of entry, establishment and socio-economic impact remains; also that considerable effort to prevent entry and to manage any potential outbreaks of the pathogen in the UK and other EU Member States is justified. The mechanisms to prevent or deal with interceptions and manage outbreaks in England or Wales are detailed in a model contingency plan. This plan is not necessarily indicative of what management actions would be taken in the event of an outbreak. (See footnote below).

3.1 What is the pest’s potential to cause economic, environmental or social impacts in the area at risk?

T. indica presents a major economic threat to bread or feed wheat (*T. aestivum*), durum wheat (*T. durum*) and possibly triticale (x *Triticosecale*) production and trade in the PRA area which consists of the UK, other Member States of the EU and the EPPO region.

The cost components associated with an outbreak of the disease (Karnal bunt) are broadly categorised as:

- Direct costs
- Reaction costs
- Control costs

The direct costs are the yield and quality losses in crops affected with Karnal bunt. With respect to wheat, the pathogen has a relatively small effect on grain yield but a significant effect on grain quality because flour made from infected grain is discoloured, baking quality is impaired and palatability is reduced, due to the fishy odour of trimethylamine which the fungus produces.

The reaction costs arise as a result of market reactions to the fact that *T. indica* has been detected in a particular region. The pathogen is categorised as a quarantine pest by many countries and by five RPPOs. The reaction costs include indirect quality losses, loss of exports related to its categorisation as a quarantine pest in other countries, and seed industry costs.

Unlike other bunts and smuts of wheat, *T. indica* is difficult to control by seed treatments as it does not infect at the seedling stage. Control is also difficult because of the longevity of its teliospores (at least 3 years in the PRA area by experimentation, but possibly longer) and the fact that it can be carried both as a surface contaminant of seed and as inoculum within the seed. Once

²⁹ By evaluating the range of measures that could be undertaken to prevent entry of the pathogen or to deal with outbreaks in the EU, a model management (contingency) plan has been produced based upon outbreak scenarios in England for consideration for implementation in England and Wales. However, this plan, which formed the basis of the socio-economic impact analysis, is for illustrative purposes only. It should not be taken as indicating how any EU Member State would respond to a finding of *Tilletia indica* on its territory. Any such response will be determined by the governments concerned, in the light of consultation with stakeholders and other relevant factors at the time including the availability of resources.

present in an area it may be difficult to eradicate. This gives rise to the control costs that occur in an attempt to control and/or eradicate the pathogen. The specific control costs include those for containment, eradication, and surveillance and testing.

Details of these costs based upon outbreaks in England are provided in the accompanying PRA and Table 1 in this RIA.

For interceptions on imported grain or seed, there will be costs associated with storage of consignments suspected to be infected with *T. indica* pending testing, and potential costs associated with a shortage of grain for processing during that period (for example, millers producing flour for bread production have a tight schedule for buying-in grain and the storage period could upset their production schedule). If confirmed as positive for *T. indica*, there will be additional costs associated with processing to ensure death of the pathogen, or safe disposal, or re-export.

4. Main provisions (what legislation is currently in place to deal with the issue?)

Tilletia indica is listed as a I/A1 quarantine pest in the EC Plant Health Directive (2000/29/EC as amended). EC requirements are for seeds of *Triticum*, *Secale* and x *Triticosecale* imported from countries where the pathogen occurs to originate in an area where *T. indica* is known not to occur. For grain there are two options: (a). To originate in an area where *T. indica* is known not to occur, or (b). For place of production freedom based upon inspection during the growing season and testing of the grain at harvest and before shipment for freedom from *T. indica*. This should not be interpreted as allowing exporting countries to base certification for export of grain on symptoms alone.

The Plant Health (Great Britain) Order 1993 (Statutory Instrument 1993/1320; as amended) implements these controls. Defra is responsible for implementing the Order in England and Wales (on behalf of the National Assembly for Wales) and SEERAD is the responsible body in Scotland. Separate but similar arrangements are applied in Northern Ireland implemented by DARDNI.

4.1 Proposed action to be taken (pending consultation)

By evaluating the range of measures that could be undertaken to prevent entry of the pathogen or to deal with outbreaks in the EU, a model management (contingency) plan has been produced based upon outbreak scenarios in England for consideration for implementation in England and Wales. However, this plan, which formed the basis of the socio-economic impact analysis, is for illustrative purposes only. It should not be taken as indicating how any EU Member State would respond to a finding of *Tilletia indica* on its territory. Any such response will be determined by the governments concerned, in the light of consultation with stakeholders and other relevant factors at the time including the availability of resources. The proposal is for a consultation of the model contingency plan to take place.

5. Other options

In general when considering the management of the risks associated with non-indigenous plant pests, there are up to four broad options to choose from:

- (i) do nothing
- (ii) attempt exclusion
- (iii) attempt eradication, or

(iv) manage the incident to inhibit spread

These are discussed below for *T. indica*:

(i) Do nothing

T. indica is currently listed as a I/A1 pest for the EC as well as in domestic legislation in EU Member States so to meet statutory plant health obligations it seems unlikely that ‘*doing nothing*’ is a realistic option. The model contingency plan is aimed at preventing entry and managing outbreaks. By doing nothing, there is a risk of entry, establishment, and spread of *T. indica* in the UK and other EU Member States; the socio-economic impact would be significant and most likely long-term in its nature.

(ii) Attempt exclusion

This remains the main aim of the EC and domestic legislation. The potential for continued exclusion relies on the exporting countries where *T. indica* occurs complying with the current legislation, and, on early detection of the pathogen on imported contaminated or infected material in cases of non-compliance. The UK Plant Health Service (PHS) has a target to sample and test all consignments of wheat (and triticale) grain and seed which have arisen in countries where *T. indica* occurs. This resulted in the identification of *T. indica* or suspect *T. indica* in consignments of grain from India in 2003, 2004 and since this RIA was written in 2005 and 2006. These were managed post-entry according to the model contingency plan. The UK PHS is aware of all consignments arriving directly in UK ports from countries where *T. indica* occurs. There may be problems however in identifying consignments which have arrived in another EU Member State before being transported onwards to the UK, since these will have been cleared for movement within the EU by the official body in the country in which these first arrive.

(iii) Attempt eradication

There has been no clear published evidence from those countries in which the pathogen is established that an eradication policy has succeeded based upon crop rotation, chemical control, host plant resistance, seed certification or other measures. However, measures aimed at attempting eradication of the pathogen in the absence of its host, are outlined in the model contingency plan (see 4.1 above) and include grassing-down affected fields or putting them under bare fallow. If adopted in its entirety this would have to be implemented for at least 5 years if not longer, because of the longevity of the pathogen in the soil. The period of implementation would have to be determined by monitoring for *T. indica* in the affected fields as well as in a buffer zone delimiting the affected fields in which no susceptible crops would be allowed to be grown, and, in susceptible crops grown around the outside of the buffer zone, until such time as there had been no evidence of the pathogen for a period of (at least) 5 years.

(iv) Manage the incident to inhibit spread (containment)

The measures outlined in the model contingency plan (see 4.1 above) for interceptions as well as for outbreaks incorporate this option. For outbreaks, containment, aimed at preventing the spread of the pathogen would be implemented following trace back and trace forward activities to determine the extent of any outbreak. The buffer zone referred to under (iii) and monitoring for *T. indica* as described under (iii) would be implemented. Stratified national surveys would also be conducted to prove freedom from *T. indica* outside of the containment area.

The model contingency plan is aimed firstly at exclusion (option ii) but should an outbreak occur, a series of measures is anticipated aimed at containment and (hopefully) eradication (options iv and iii combined). Option (ii) is ongoing irrespective of an outbreak occurring.

6. What assumptions have been made about the risk management options identified?

It is assumed that *T. indica* is not present in the UK or any other part of the EU. It is also assumed that *T. indica* will not survive longer than 5 years in UK soil in the absence of the host and that all the management options will be effective against the pathogen. However, should a delimited outbreak occur, then statutory restrictions will be imposed for a minimum of 5 years based upon monitoring for the pathogen in the affected fields, in a buffer zone around those fields and in susceptible crops grown within a specified distance around the outside of the buffer zone. This period could be extended if the pathogen continues to be detected. This assumes that the model contingency plan is adopted in its entirety, but see 4.1 above.

7. Issues of equity and fairness from the options

UK (and EU) arable farmers and the UK cereal industry are not currently directly affected by the legislative requirements for *T. indica*. Exporting countries where *T. indica* occurs are however expected to comply.

Measures drafted in the model contingency plan, aimed firstly at excluding the pathogen from the UK, if enacted will have implications for importers and processors of grain and importers and users of seed, of wheat and triticale. (See 4.1 above).

The interceptions of *T. indica* in the UK in 2003 and 2004 (and since this RIA was written in 2005 and 2006) on wheat from India were in small consignments destined for specialist snack food production which had already been transported within the UK. Under the model contingency plan processing was therefore allowed (and in certain circumstances would be allowed in the future) under official control. Suspect interceptions on future consignments of grain or seed, which have not already been transported from the port of entry, will be held subject to official testing in order to confirm the presence of *T. indica*. During the period of testing, importers have the option of holding the consignment pending confirmatory tests (up to 2 weeks) or, if not held, they would be allowed to process the consignment under official control, or they could re-export the consignment. On official confirmation however, processing to destroy *T. indica* will be required, or the alternative would be safe disposal. Costs are involved at all stages of the process from suspicion to confirmation.

Measures aimed at containment and (hopefully) eradication of outbreaks are outlined in the model contingency plan (see 4.1 above). Farmers with directly affected fields (where trace back and trace forward activities suggest that the pathogen is present in the soil), as well as those with arable fields in a buffer zone in a 3-km radius around the affected fields will be directly affected by the measures over at least a 5-year period with the aim being the intended demise of *T. indica* in the affected areas. The affected fields will have to be maintained for the minimum period of 5 years either as bare fallow, or, they will have to be grassed-down. No wheat or triticale would be allowed to be grown in a 3-km radius buffer zone around the affected fields. These measures will have direct economic consequences for individual arable farmers, which, depending upon the extent of the outbreak may affect whole farms and could put individual farms out of business.

8. Benefits from each of the options outlined above

(i) Do nothing

By doing nothing, the UK Plant Health Service resources would not be utilised in enforcing the measures outlined in the model contingency plan. Individual businesses would not be affected by exclusion, containment and eradication measures and would not have to bear the costs. The milling wheat price may increase as a result of a shortage of milling quality wheat grain related to outbreaks of Karnal bunt.

(ii) Attempt exclusion

The benefits in attempting exclusion are to prevent entry, establishment, spread and socio-economic impacts. Export markets are maintained. There are no shortages of milling quality wheat. Individual businesses would not be affected by the containment and eradication measures and would not have to bear the costs of an outbreak.

(iii) Attempt eradication

The benefits arising from attempting eradication are (hopefully) firstly the prevention of establishment and spread. This would demonstrate to countries which have specific legislation aimed at preventing entry of *T. indica* to which the UK exports cereals, that measures are being taken. The attempted eradication of the pathogen from the affected land once completed, should ultimately lead to the ability to produce milling quality wheat on that land, commanding a premium over wheat destined for animal consumption (depending upon price differences between milling and feed wheat at the time) or, at least, the ability to market wheat for human consumption under recognised Quality Assurance Schemes. Once eradicated, seed producers would have one less pathogen to consider when producing pathogen-free seed. During the eradication process, export markets should be maintained from non-affected areas.

(iv) Inhibit spread (containment)

Arable farmers would receive benefit from protection from infestation with *T. indica*. During the containment process, the ability to demonstrate pest-free areas and pest-free places of production would reduce the risk of loss of export markets and losses of yield and quality in these areas.

9. Compliance costs for each of the options

Compliance costs for each of the options are outlined in Table 1 below. Where figures are provided, these are quoted from Brennan *et al.*, 2004³⁰ and are based upon a specific scenario affecting 50, 000 ha of wheat in a region of the UK (specifically in England) in which a containment and eradication programme based upon the model contingency plan (see 4.1 above) has been implemented. The ‘do nothing’ option was not costed in this evaluation:

³⁰ Brennan, J P, Kelly, P W, Thorne, F, (2004) (unpublished). Report on Socio-Economic Costs of Karnal bunt in the European Union. Revised Deliverable Report, 5.1, 29 July 2004. EU Fifth Framework Research, Technological and Development Project ‘Risk associated with *Tilletia indica*, the newly listed-EU quarantine pathogen, the cause of Karnal bunt of wheat’ (short title ‘Karnal bunt risks’). Contract no. QLK5-1999-01554; 2000-2004.
<http://karnalpublic.pestrisk.net/>

(i) Do nothing

While the pathogen remains categorised as a quarantine pest in many countries around the world, ‘*doing nothing*’ would increase the risk of entry, establishment and spread in the UK. The main outcome, should an outbreak occur which is not officially controlled, would most probably be a loss of export markets for UK grain and seed of wheat, triticale and possibly rye. Other negative impacts resulting from ‘*doing nothing*’ include, for example, for infected or contaminated grain intended for human consumption to be downgraded to animal feed and for seed producers to have an additional organism to inspect and test for in seed crops. The pathogen has the potential to be perpetuated in wheat production areas because it is extremely long-lived in the soil and wheat crops are grown intensively and in short rotation, or as continuous cropping for several years. The pathogen is likely to spread and therefore the costs will increase with time.

Even if *T. indica* was not categorised as a quarantine pest in third countries, it is more difficult to control than other bunts and smuts of wheat (which are normally effectively managed by the appropriate use of seed treatments). This is because it infects through the ear and not at the seedling stage. Its presence in arable land would cause ongoing and perpetual management problems for UK cereal farmers because of its longevity in the soil and its potential perpetuation and spread in wheat production areas. Although mid- to late season fungicide applications would most likely reduce the level of Karnal bunt in harvested grain there is no guarantee of 100% control; small levels of infection could lead to significant losses in quality. Even in this scenario, infected grain destined for human consumption is likely to be down-graded to animal feed with an associated loss of income for the affected farmer, the size of which depends upon the price difference between milling and feed quality wheat. Producers of UK seed would still have to ensure freedom from *T. indica*.

(ii) Attempt exclusion

The ongoing compliance costs fall on parts of the UK Plant Health Service and comprise the costs of sampling and testing grain and seed of wheat (and when it is imported, triticale). When intercepted on imports however, individual businesses will have to bear the costs. For interceptions on imported grain or seed, there will be costs associated with storage of consignments suspected to be infected with *T. indica* pending testing and possibly a shortage of grain for processing during that period. Importers may opt to incur costs of processing under official control or re-export during this period. If confirmed as positive for *T. indica*, there will be additional costs associated with processing to ensure death of the pathogen, or safe disposal.

(iii) Attempt eradication

The compliance costs fall in part on the UK Plant Health Service (surveillance and testing; administration and compliance costs) but more significantly on the affected farmers. Costs have been quantified for a specific scenario, that is, an outbreak affecting 50, 000 ha of wheat in England. These are significant and ongoing over at least a ten-year period.

(iv) Inhibit spread

The compliance costs fall in part on the UK Plant Health Service (surveillance and testing; administration and compliance costs) but more significantly on the affected farmers. Costs are significant and ongoing over at least a ten-year period

10. Business sectors affected

The business sectors affected by the model contingency plan (see 4.1 above) for *T. indica* are:

- Arable farmers
- Seed producers
- Wheat and triticale processors i.e. the millers and the animal feed producers
- Grain transport companies
- Importers and exporters of grain and seed and related businesses
- Wholesalers and retailers of milling and feed products

11. Consultation with small businesses

National bodies representing small businesses will be consulted.

12. Formal consultation (trade organisations etc)

An ‘*interested organisation*’ consultation exercise is envisaged.

13. Conclusion

Tilletia indica is a I/A1 quarantine-listed pest for the EC and is listed in domestic legislation in the UK and other EU Member States. It has been intercepted in wheat grain imported from countries where the pathogen occurs including interceptions by the UK, in 2003 and 2004 and since this RIA was written in 2005 and 2006 from India. EC and domestic legislation requires exporting countries to ensure that grain and seed of wheat, rye³¹ and triticale be free from the pathogen. The pathogen also occurs in the USA and changes in US domestic legislation may lead to an increased risk of entry on wheat grain (or seed) imported from the affected areas of the USA into the UK and other EU Member States unless exports comply with EU requirements. The European Commission is currently in correspondence with the USDA to clarify the position. There is a risk of establishment and significant socio-economic impact. The model contingency plan (see 4.1 above) outlines measures aimed at exclusion which seem economically justified. Should an outbreak occur in the UK long-term measures aimed at containment and eradication have significant cost implications (based upon costs for outbreaks in England alone) for the farmers whose land is affected.

³¹ Since the legislation was drafted, rye is no longer considered to be a host.

Table 1. A summary of the benefits and costs of pest risk management options falling on each of the sectors involved for the UK including the effect of implementing the model Contingency Plan in full. (Costs based upon outbreaks in England).

Sector	Cost or Benefit	Option:			
		(i) Do nothing	(ii) Exclusion	(iii) Eradication	(iv) Inhibit spread (Containment)
UK Plant Health Service (PHS)	Cost	Doing nothing would be contrary to UK obligations under EU law and there is a risk of infraction proceedings against the UK. The UK PHS would have to justify this to the EC as well as to UK cereal producers and the export trade and to trading partners outside of the UK.	Relatively low. Current costs are sampling and testing of grain and seed of wheat (and triticale) on imports arriving from countries where <i>T. indica</i> occurs	To implement the Eradication and Containment (E&C) Plan in the draft contingency plan there would be two identifiable component costs: A). Surveillance and testing costs B). Administrative and compliance costs These amount to a relatively small percentage of the overall E & C costs which would currently be expected to be borne by the farmer (see below). The individual farmer(s) affected would be negatively affected.	
	Benefit	PHS would not have to expend resources on the measures	High. The cereal industry view the UK PHS as protecting individual businesses as well as the UK export trade.	The main benefit for the UK PHS in implementing the E & C plan is continued facilitation of UK cereal exports.	
Public	Cost	The pathogen may enter, establish and spread. The pathogen will perpetuate itself and so the long-term costs could increase significantly over and above those cited. Milling wheat may still be downgraded to feed wheat because of quality losses and therefore there may be a shortage of milling quality wheat which could lead to a rise in the price of bread.	Not applicable.	While the E & C plan is being implemented, milling wheat harvested from affected fields may be downgraded to feed wheat or may be destroyed. A reduction in the availability of milling quality wheat may led to a rise in the price of bread. There may be restrictions in public access to rights of way across affected farmers fields.	
	Benefit	Not applicable.	Exclusion would maintain the current absence of a related grain shortage therefore no related rise in the price of bread.	None.	

Table 1 continued. A summary of the benefits and costs³² of pest risk management options falling on each of the sectors involved for the UK including the effect of implementing the model Contingency Plan in full. (Costs based upon outbreaks in England).

Sector	Cost or Benefit	Option:			
		(i) Do nothing	(ii) Exclusion	(iii) Eradication	(iv) Inhibit spread (Containment)
Business	Cost	The pathogen may enter, establish and spread. The pathogen will perpetuate itself and so the long-term costs could increase significantly over and above those cited. The consequences of doing nothing are high. For an affected area of 50,000 ha in the UK the direct costs (yield and quality losses) over a ten-year period amount to €1.7M (£1.2M); the reaction costs (downgrading milling wheat to feed wheat, price and export effects and seed industry costs) amount to €23.9M (£16.2M).	When suspected as being intercepted on imports there will be costs associated with storage of consignments pending confirmation and possibly a shortage of grain for processing during that period. Importers may opt to incur costs of processing under official control or re-export during this period. If <u>confirmed</u> as positive there will be additional costs associated with processing to ensure death of the pathogen, or safe disposal.	By implementing the E&C plan for an affected area of 50,000 ha in the UK the direct costs to the farmer (yield and quality losses) over a ten-year period amount to €1.7M (£1.2M) Individual farmers would have to bear the control costs of the E & C plan which, including the UK PHS surveillance and testing costs (breakdown not available), for an outbreak of 50, 000 ha amount to €428.1M (£291.1M) over a ten-year period. There may be surplus of feed quality wheat as a result of downgrading to feed wheat.	
	Benefit	By doing nothing, if an outbreak did occur, individual affected farmers would not have to bear the control costs associated with the E&C plan, which, including the UK PHS surveillance and testing costs (breakdown not available) for an outbreak of 50, 000 ha in the UK amount to €428.1M (£291.1M) over a ten-year period. The price of milling quality wheat may increase as a result of a shortage of supply.	The pathogen is hopefully denied entry. The benefit to the UK industry overall is avoiding a total cost of €453.7M (£308.5M) (sum of direct, reaction and control costs for a 50, 000 ha UK outbreak) Export markets will be maintained.	During implementation of the E & C plan, individual farmers who are not affected by the pathogen benefit directly because there will be a shortage of milling quality wheat and a rise in price of milling quality wheat. A significant benefit is that export markets are maintained because it will be possible to delimit pest-free area and pest-free places of production. The value of the UK wheat export market is high, with, according to the Home Grown Cereals Authority a quarter of UK wheat and barley production destined for the export trade.	

³² Where values are specified these are discounted to present values with a discount rate of 5% per annum. The exchange rate used is €1 : £0.68

Economic Costs of Scenario 1, 'Large' Karnal bunt outbreak in England, Year 1, (Brennan *et al.*, 2004, unpublished) managed according to an earlier draft of the model Contingency Plan (Sansford *et al.*, 2004, unpublished). (A later version of the plan is presented in Annex II of this report).

		<i>Region</i>	<i>Rest of UK</i>	<i>Rest of EU</i>	<i>Total UK</i>	<i>Total EU</i>
Direct Costs						
Yield losses	(€'000)	46	0	0	46	46
Downgrading affected milling wheat to feed	(€'000)	1,655	0	0	1,655	1,655
- Total Direct Costs	(€'000)	1,701	0	0	1,701	1,701
Reaction Costs						
Down-grading unaffected milling wheat to feed	(€'000)	10,206	0	0	10,206	10,206
Price and export effects	(€'000)	13,472	-279	-8,921	13,193	4,272
Seed industry costs	(€'000)	200	-200	0	0	0
Quality assurance costs	(€'000)	0	0	0	0	0
- Total Reaction Costs	(€'000)	23,878	-479	-8,921	23,399	14,478
Control Costs						
Surveillance and testing costs	(€'000)	297	112	69	409	478
Administrative - Compliance costs	(€'000)	200	60	20	260	280
Income loss from cropping restrictions	(€'000)	0	0	0	0	0
Yield reduction from tolerant variety	(€'000)	0	0	0	0	0
Additional fungicide costs	(€'000)	0	0	0	0	0
Value of standing crop destroyed	(€'000)	9,118	0	0	9,118	9,118
Costs of destroying growing crop	(€'000)	450	0	0	450	450
Value of affected grain destroyed	(€'000)	3,647	0	0	3,647	3,647
Costs of destroying affected grain	(€'000)	1,066	0	0	1,066	1,066
Treatment of mill by-products	(€'000)	0	0	0	0	0
Grain processing costs (heat treatment)	(€'000)	2,742	0	0	2,742	2,742
Livestock industry costs	(€'000)	0	0	0	0	0
Machinery cleaning costs	(€'000)	19	0	0	19	19
Facility cleaning costs	(€'000)	8	0	0	8	8
- Total Control Costs	(€'000)	17,548	172	89	17,720	17,809
Total Economic Costs	(€'000)	43,127	-307	-8,832	42,820	33,988

Economic Costs of Scenario 2, 'Small' Karnal bunt outbreak in England, Year 1, (Brennan *et al.*, 2004, unpublished) managed according to an earlier draft of the model Contingency Plan (Sansford *et al.*, 2004, unpublished). (A later version of the plan is presented in Annex II of this report).

		<i>Region</i>	<i>Rest of UK</i>	<i>Rest of EU</i>	<i>Total UK</i>	<i>Total EU</i>
Direct Costs						
Yield losses	(€'000)	1	0	0	1	1
Downgrading affected milling wheat to feed	(€'000)	33	0	0	33	33
- Total Direct Costs	(€'000)	34	0	0	34	34
Reaction Costs						
Downgrading unaffected milling wheat to feed	(€'000)	343	0	0	343	343
Price and export effects	(€'000)	450	-10	-277	440	163
Seed industry costs	(€'000)	6	-6	0	0	0
Quality assurance costs	(€'000)	0	0	0	0	0
- Total Reaction Costs	(€'000)	800	-16	-277	783	506
Control Costs						
Surveillance and testing costs	(€'000)	118	130	69	249	318
Administrative - Compliance costs	(€'000)	200	60	20	260	280
Cropping restrictions	(€'000)	0	0	0	0	0
Yield reduction from tolerant variety	(€'000)	0	0	0	0	0
Additional fungicide costs	(€'000)	0	0	0	0	0
Value of standing crop destroyed	(€'000)	182	0	0	182	182
Costs of destroying growing crop	(€'000)	9	0	0	9	9
Value of affected grain destroyed	(€'000)	73	0	0	73	73
Costs of destroying affected grain	(€'000)	21	0	0	21	21
Treatment of mill by-products	(€'000)	0	0	0	0	0
Grain processing costs (heat treatment)	(€'000)	55	0	0	55	55
Livestock industry costs	(€'000)	0	0	0	0	0
Machinery cleaning costs	(€'000)	1	0	0	1	1
Facility cleaning costs	(€'000)	0	0	0	0	0
- Total Control Costs	(€'000)	660	190	89	850	939
Total Economic Costs	(€'000)	1,493	174	-188	1,667	1,479

Components of Costs in Affected Region of Scenario 1, ‘Large’ Karnal bunt outbreak in England, Years 1 to 10, (Brennan *et al.*, 2004, *unpublished*) managed according to an earlier draft of the model Contingency Plan (Sansford *et al.*, 2004, *unpublished*). (A later version of the plan is presented in Annex II of this report).

		<i>Direct Costs</i>	<i>Reaction Costs</i>	<i>Control Costs</i>	<i>Total Costs</i>
Total Costs					
Year 1	(€ <i>m</i>)	1.70	23.88	17.55	43.13
Year 2	(€ <i>m</i>)	0.00	0.00	64.43	64.43
Year 3	(€ <i>m</i>)	0.00	0.00	64.43	64.43
Year 4	(€ <i>m</i>)	0.00	0.00	64.43	64.43
Year 5	(€ <i>m</i>)	0.00	0.00	64.43	64.43
Year 6	(€ <i>m</i>)	0.00	0.00	64.43	64.43
Year 7	(€ <i>m</i>)	0.00	0.00	47.38	47.38
Year 8	(€ <i>m</i>)	0.00	0.00	47.38	47.38
Year 9	(€ <i>m</i>)	0.00	0.00	47.38	47.38
Year 10	(€ <i>m</i>)	0.00	0.00	47.38	47.38
Present Value^a	(€ <i>m</i>)	1.70	23.88	428.12	453.70
Total Costs per Hectare					
Year 1	(€/ <i>ha</i>)	6	88	65	159
Year 2	(€/ <i>ha</i>)	0	0	237	237
Year 3	(€/ <i>ha</i>)	0	0	237	237
Year 4	(€/ <i>ha</i>)	0	0	237	237
Year 5	(€/ <i>ha</i>)	0	0	237	237
Year 6	(€/ <i>ha</i>)	0	0	237	237
Year 7	(€/ <i>ha</i>)	0	0	174	174
Year 8	(€/ <i>ha</i>)	0	0	174	174
Year 9	(€/ <i>ha</i>)	0	0	174	174
Year 10	(€/ <i>ha</i>)	0	0	174	174
Present Value^a	(€/<i>ha</i>)	6	88	1,576	1,670

^a Discounted to present values with a discount rate of 5% per annum

Components of Costs in Affected Region of Scenario 2, ‘Small’ Karnal bunt outbreak in England, Years 1 to 10, (Brennan *et al.*, 2004, *unpublished*) managed according to an earlier draft of the model Contingency Plan (Sansford *et al.*, 2004, *unpublished*). (A later version of the plan is presented in Annex II of this report).

		<i>Direct Costs</i>	<i>Reaction Costs</i>	<i>Control Costs</i>	<i>Total Costs</i>
Total Costs					
Year 1	(€ <i>m</i>)	0.03	0.80	0.66	1.49
Year 2	(€ <i>m</i>)	0.00	0.00	2.12	2.12
Year 3	(€ <i>m</i>)	0.00	0.00	2.12	2.12
Year 4	(€ <i>m</i>)	0.00	0.00	2.12	2.12
Year 5	(€ <i>m</i>)	0.00	0.00	2.12	2.12
Year 6	(€ <i>m</i>)	0.00	0.00	2.12	2.12
Year 7	(€ <i>m</i>)	0.00	0.00	1.78	1.78
Year 8	(€ <i>m</i>)	0.00	0.00	1.78	1.78
Year 9	(€ <i>m</i>)	0.00	0.00	1.78	1.78
Year 10	(€ <i>m</i>)	0.00	0.00	1.78	1.78
Present Value^a	(€ <i>m</i>)	0.03	0.80	14.77	15.61
Total Costs per Hectare					
Year 1	(€/ <i>ha</i>)	4	95	78	176
Year 2	(€/ <i>ha</i>)	0	0	250	250
Year 3	(€/ <i>ha</i>)	0	0	250	250
Year 4	(€/ <i>ha</i>)	0	0	250	250
Year 5	(€/ <i>ha</i>)	0	0	250	250
Year 6	(€/ <i>ha</i>)	0	0	250	250
Year 7	(€/ <i>ha</i>)	0	0	210	210
Year 8	(€/ <i>ha</i>)	0	0	210	210
Year 9	(€/ <i>ha</i>)	0	0	210	210
Year 10	(€/ <i>ha</i>)	0	0	210	210
Present Value^a	(€/ <i>ha</i>)	4	95	1,746	1,845

a Discounted to present values with an discount rate of 5% per annum