Ente Nazionale Risi – Rice Research Centre – Italy
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PPM Point 6.6

Pest risk analysis

Aphelenchoides besseyi Christie

on rice (*Oryza sativa* L.)

PEST RISK ANALYSIS

Aphelenchoides besseyi **Christie**

<u>Stage 1</u> : Initiation

A- <u>Identify pest</u>:

1- <u>Is the organism clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank</u>?

YES

Name: *Aphelenchoides besseyi* Christie.
Synonyms: *Aphelenchoides oryzae* Yokoo; *Asteroaphelenchoides besseyi* (Christie) Drozdovski.

Taxonomic position: Class Nematoda; Order Aphelenchida; Family Aphelenchoididae.

Common names: Rice white tip nematode, strawberry crimp disease nematode, (English); nematode foliare (France), Nematoide da ponta branca do arroz (Spain).

Bayer computer code: APLOBE.
EPPO A2 list: No. 122.
EU Annex designation: II/A1 on rice and II/A2 on strawberry.

Species description (see C.I.H. Description of plant-parasitic nematodes, Vol. 1, n. 4) (**See Annex 1**).

Length of nematode life cycle: 3 to 6 days at 25-31.3 °C and 9 to 24 days at 14.7-20.6 °C, with optimum temperature for development at 28 °C and marginal temperature at 13 °C and 42 °C (Gergon. E.B. and Misra J.K., 1992). For normal development, the nematode needs an atmospheric humidity of at least 70% (Ou S.H., 1985).

Hosts
The main hosts are strawberry and rice. *Aphelenchoides besseyi* can also be found on *Allium cepa*, *Allium sativum*, *Boehmeria nivea*, *Brassica pekinensis*, *Chrysanthemum morifolium*, *Coleus blumei*, *Colocasia esculenta*, *Cyperus iria*, *Cyperus polystachyus*, *Dahlia variabilis*, *Digitaria adscendens*, *Digitaria sanguinalis*, *Ficus elastica*, *Hibiscus brachenridgii*, *Hydrangea macrophylla*, *Impatiens balsamina*, *Imperata cilindrica*, *Ipomoea batatas*, *Jasminum volobile*, *Narcissus pseudonarcissus*, *Narcissus* sp., *Panicum bisulcatum*, *Panicum sanguinale*, *Panicum maximum*, *Pennisetum typhoides*, *Polianthes tuberosa*, *Saccharum officinarum*, *Saintpaulia ionantha*, *Setaria italica*, *Setaria viridis*, *Sporobolus poirettii*, *Zea mays*, *Zinnia elegans* (Ou S.H., 1985; Merny G. et al., 1985; Tacconi R. and Ambrogioni L., 1993; CABI/EPPO, 1997).

Infected rice plants may or may not exhibit any symptom. Even if chlorotic discoloration can appear in susceptible varieties on seedling at 2-3 leaf stage, usually plants show the first symptoms at late tillering, as whitening of leaf tip for a length of up to 5 cm. At booting stage the flag leaf is the most affected: short, markedly twisted at the apical portion and crinkled. Panicle often emerges only partially or not emerges at all, its size is reduced as the number of the grains. The latter are smaller and deformed. Other symptoms include stunting of the plant and production of tillers from the upper nodes.
On the basis of the trials performed at the Rice Research Centre at Castello d'Agogna (PV - Italy), it seems possible to rank Italian rice varieties in 3 groups on the basis of the different leaf symptoms: 1. typical whitening of leaf tip, 2. chlorotic streaks or stripes on leaves observed with back light, 3. no leaf symptoms in stunted plants with panicle partially emerged and reduced in size (Giudici *et al*., 2003) (**See Annex 2**).

In infested rice seeds, quiescent *Aphelenchoides besseyi* is located between hulls and caryopsis, where it overwinters in anhydrobiosis. When seed germinates the nematode becomes active and, thanks to the film of water in the folded young leaf, reaches the growing point where it feeds ectoparasitically. During the plant growth it continues moving upwards and at the booting stage it reaches the panicle where it multiplies abundantly before anthesis. Afterwards reproduction declines with the age of the grains, this decline being correlated with the grain dehydration (Ou S.H., 1985; Gergon E.B. and Misra J.K., 1992).
The nematode survive from one season to the next in infected seeds and - to a lesser extent - on weeds and debris from the previous rice crop (Prot J.C., 1992). The number and infectivity of nematodes is reduced as seed age increases (Sivakumar C.V., 1987) however good seed storage conditions probably prolong nematode survival. ***Aphelenchoides besseyi* does not survive in soil** (McGawley E.C *et. al.*, 1984). *Aphelenchoides besseyi* can also be dispersed from infested seed by irrigation water (**See Annex 3**).

B- The P.R.A. area:

3- <u>Clearly define the P.R.A. area</u>

The PRA area considered is the European Union, in particular the Countries which grow rice: Italy, France, Greece, Portugal and Spain.

Rice is produced in very specific areas in each country, including rivers and estuarine areas with heavy clay soils and low permeability, where rice is the only possible crop. In fact rice in EU is mainly sown in **flooded fields**. The main producers are Italy (220,000 ha) and Spain (110,000 ha), which together represent 83% of the total area of about 400,000 ha.
Historically, rice produced in the Community belongs to the subspecies **japonica** (roundish grain), mainly consumed in the producing Member States. Japonica varieties are more suitable to temperate climate areas as the rice European regions, which are situated between 35° and 45° North latitude.
From 1988 to 1993 the Community encouraged producers to convert from surplus japonica to **indica** (slender grain) varieties, the indica rice now covers 130,000 ha, mainly located in the Southern rice areas (Sevilla in Spain, Greece).
In **Annex 4** a brief overview is reported of rice industry in the producing Member States.

C- Earlier analysis:

4- <u>Does a relevant earlier P.R.A. exist</u>?

YES

This is a review of the previous one completed with additional information as requested by the SCPH (see the Report of the meeting held on 29-30 April 2002, **Annex 5**).

5- <u>Is the earlier PRA still entirely valid, or only partly valid (out of date, applied in different circumstances, for a similar but distinct pest)</u>?

PARTLY VALID

Additional information are now available from experimental trials and seed testing.

6- <u>Proceed with the assessment, but compare as much as possible with the earlier assessment.</u>

***Stage 2** : Pest Risk Assessment*

***Section A** : Pest categorization (qualitative criteria of a quarantine pest)*

D- Geographical criteria:

7- Does the pest occur in the P.R.A. area?

YES

Aphelenchoides besseyi has been reported in rice plant and seed in Italy (Tacconi R., 1996; Moletti M., 1997; Giudici M.L. and Villa B., 1997) and found in Brazil in a rice seed accession coming from France (Tenente R.C.V. *et al.*, 1994).

Geographical distribution (CABI/EPPO, 1997; CABI/EPPO, 1998)
EPPO region: ~~Bulgaria,~~ ~~France,~~ Hungary, Italy, Russia~~, Slovakia~~.

Africa: Benin, Burkina Faso, Burundi, Cameroon, Central Africa Republic, Chad, Comoros, Cote d'Ivoire, Egypt, Gabon, Gambia, Ghana, Kenya, Madagascar, Malawi, Mali, Nigeria, Senegal, Sierra Leone, ~~South Africa,~~ Tanzania, Togo, Uganda, Zaire, Zambia, Zimbabwe.

Asia: ~~Afghanistan,~~ Azerbaijan, Bangladesh, Cambodia, China, India, Indonesia, Iran, ~~Israel~~, Japan, Korea Republic, Lao, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Sri Lanka, Taiwan, Tajikistan, Thailand, Uzbekistan, Viet ~~-~~Nam.

North America: Mexico, USA. ~~(Arizona, California, Florida, Hawaii, Louisiana, Texas).~~

Central America and Caribbean: Cuba, ~~Dominica,~~ Dominican Republic, El Salvador, ~~Guadeloupe,~~ Panama.

South America: Argentina, Brazil, Ecuador.

Oceania: Australia, Fiji.

8.- Is the pest of limited distribution in the PRA area?

YES

Aphelenchoides besseyi was first detected in Italy in 1997, since then only about 3% of seed lots resulted infested each year and the population density of the nematode was very low. In rice fields the presence of *Aphelenchoides besseyi* was detected less than 10 times in 5 years, in all cases untested paddy rice had been sown.
Concerning other EU rice countries, France notified the detection of *Aphelenchoides besseyi* in few lots of Italian seed, while no detection is reported from other countries. It is worth to outline that Italian seed is traded in all rice EU countries (Spain, France, Portugal and Greece) (**See Annex 6**). In all these countries flooded crop is the ordinary practice for growing rice and this practice is the least favourable to the nematode.
The amount of Italian seed traded inside and outside the Community has been almost the same before and after 1997, when *Aphelenchoides besseyi* was first detected in Italy. The Italian varieties sold abroad are indica varieties (Thaibonnet and Gladio) and some japonica varieties which are the most tolerant to the nematode (**See Annex 6**).

F- Potential economic importance:

18- <u>With specific reference to the host plant(s) which occur(s) in the P.R.A. area, and the parts of those plants which are damaged, does the pest in its present range cause significant damage or loss</u>?

NO

Seed is the principal dispersal way for *Aphelenchoides besseyi*. Since 1997 all rice seed lots produced in Italy have been tested for detecting *Aphelenchoides besseyi* (Table 1). **The population density of *Aphelenchoides besseyi* in infested lots is widely beneath the tolerance limit of 30 nematode/100 seeds** reported in literature (Yamaguchi T., 1977).

Table 1 Nematode population density in Italian rice seed lots.

N. of nematode/ 100 seeds	Seed lots 1997 n.	1997 %	1998 n.	1998 %	1999 n.	1999 %	2000 n.	2000 %	2001 n.	2001 %
0	845	90.0	953	96.7	936	97.1	932	96.5	943	96.3
1-30	81	8.6	32	3.2	28	2.9	34	3.5	35	3.6
31-300	8	0.9	1	0.1	0	0.0	0	0.0	1	0.1
>300	5	0.5	0	0.0	0	0.0	0	0.0	0	0.0
Total	939	100.0	986	100.0	964	100.0	966	100.0	979	100.0

Legenda - 30 nematode/100 seeds: tolerance limit density.
300 nematode/100 seeds: economic damage threshold density.

Three-years trials performed in Italy (Giudici *et al*., 2003. **See Annex 7**) confirmed that a **low population density of *Aphelenchoides besseyi* in rice seed (less than 30 nematode/100 seeds) does not cause significant damage or loss in rice flooded crop**, which is the ordinary cultural practice in EU countries. Moreover the population density of *Aphelenchoides besseyi* detected at harvest in the crop was lower than the population density of the seed used for sowing the crop.

19- <u>Could the pest, nevertheless, cause significant damage or loss in the PRA area, considering ecoclimatic and other factors for damage expression</u>?

NO

The conditions in the PRA area (temperate climate, flooded field, tolerant varieties) are adequate for pest survival but are not suitable for significant damage on the host plant.
The climate in the rice growing area (France, Greece, Italy, Portugal, Spain) in Europe is temperate. The rice European regions are located between 35° and 45° North latitude. This is the climatic limit for cultivation of rice, that is originally a tropical species. One crop per year is grown, from April to October.
Aphelenchoides besseyi is a parasite of warm regions, according to EPPO it is not found behind latitude 43° N on rice and 40° N on strawberry. In tropical countries second growth of the crop and irrigation water can be contaminated and may initiate infestation especially when the prevailing temperature is between 20° and 40° C. In fact the optimum temperature for *Aphelenchoides besseyi* development is 28 °C and the marginal temperature are 13 °C and 42 °C (Gergon. E.B. and Misra J.K., 1992).
Survival of the nematode in the Italian rice field has been never demonstrated: sowing healthy seed in paddy fields - where straw was left of badly infested crops grown the previous year, in experimental plots (about 1300 nematode/100 seeds) - the disease never occurred in three years trials, both in flooded and dry-seeded rice (Giudici M.L. *et al*., 2003).

In rice region with more favourable condition for *Aphelenchoides besseyi*, like Sevilla in Spain and Greece (35°N), mainly indica varieties are grown (**See Annex 4**) which seem to be tolerant to the pest (Prot J.C., 1992).
The ordinary sowing practice is broadcasting seed in flooded field, which is a condition less favourable to the nematode (**See Annex 7**). As previously mentioned, the population density of *Aphelenchoides besseyi* detected at harvest in the crop was lower than the population density of the seed used for sowing the crop (Giudici M.L. *et al.*, 2003).
Sowing untested paddy rice could be a powerful mean of spreading for the pest, however its use is low in the EU, in fact the use of certified rice seed is widespread, since its cost is low thanks to the Community aid granted to the seed growers.

20- Would the presence of the pest cause other negative economic impacts (social, environmental, loss of export markets)?

NO

Neither in Italy nor in other countries (EU and extra-EU countries) sowing Italian seed, negative economic impacts have ever been reported since 1997 or even before. Six years-long experience suggests that the presence of low populations of *Aphelenchoides besseyi* does not cause negative impacts of any kind.

22- This pest does not qualify as a quarantine pest for the PRA area and the assessment can stop

FINAL EVALUATIONS:

The information used to answer the questions were obtained from 6 years results of:

- analysis of rice seed (testing of all lots).
- experimental research concerning: crop damage, varietal vulnerability, pest survival in the field, control methods, detection methods, host symptoms.
- inspection of rice field by phytosanitary authority, extension service and seed certification authority.

The reliability of the pest risk assessment is considered very high: most of the questions were answered on the basis of actual facts rather than on estimate of a probability.

CONCLUSIONS: The conditions in the PRA area are adequate for pest survival but are not suitable for significant damage on the host plant.

- *Aphelenchoides besseyi* was first detected in Italy in 1997. Since then negative economic impacts have never been reported in Italy and other EU and extra-EU countries sowing Italian seed.
- Experimental trials confirmed that a low population density of *Aphelenchoides besseyi* in rice seed (less than 30 nematode/100 seeds) does not cause significant damage or loss in rice flooded crop, which is the ordinary cultural practice in EU countries.
- The chance is very low that the pest could spread reaching high level of infestation due to the ecoclimatic and agronomic conditions (temperate area, flooded crop, use of certified seed).
- At present there is no need of using chemicals which would cause negative environmental impacts. It is worth noting that no chemicals are registered in the European Union to treat rice seed.
- A hot water treatment could be recommended for foundation seed and for all seed lots exchanged for scientific research purposes.
- Since 1989 doubts have been indeed expressed by J.C. Prot on the benefits produced by quarantine applied for *Aphelenchoides besseyi* at the international level.
- A tolerance limit (less than 30 nematode/100 seeds) could be fixed for *Aphelenchoides besseyi* in rice seed.

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Picture of *Aphelenchoides besseyi* (Hunt D.J., 1993)

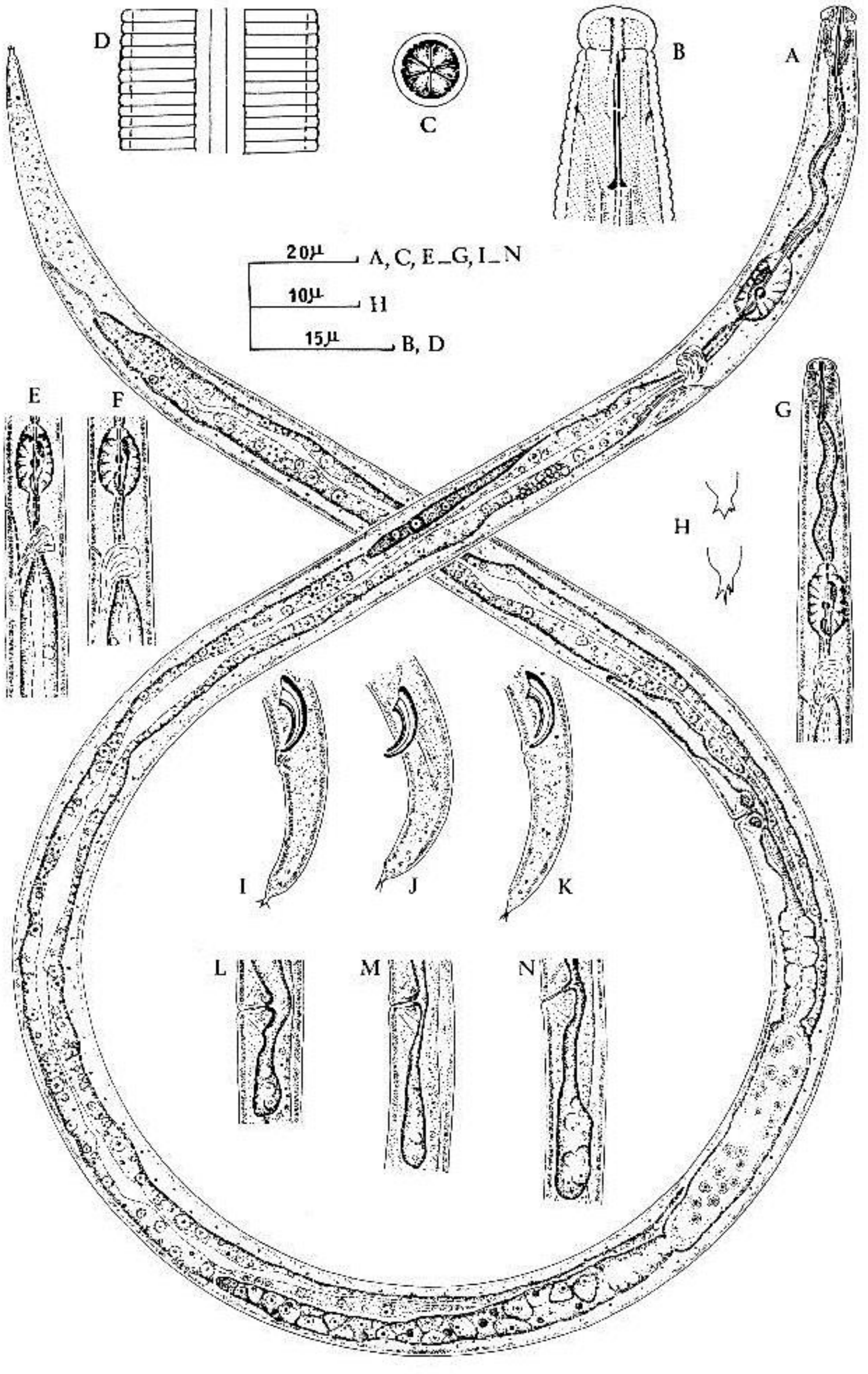

Fig. 4.6. *Aphelenchoides besseyi.* A. Female; B. Female head end; C. Female *en face* view; D. Lateral field; E, F. Variation in female median oesophageal bulb and position of excretory pore with respect to nerve ring; G. Male anterior end; H. Female tail termini showing variation in shape of mucro; I–K. Male tail ends; L–N. Variation in post uterine sac. (B, D after Siddiqi; the rest after Fortuner, 1970.)

Annex 2

Different white tip symptoms in Italian rice varieties

(Proceedings of the 3rd International Temperate Rice Conference, 10-13 March 2003, Punta de l'Este, Uruguay)

The knowledge of the different symptoms showed by a disease is a useful means to diagnosis. When a disease is new for a zone, the knowledge about it is scarce and it is difficult to arrive to a certain diagnosis. Therefore in this case it necessary to get detailed information through experimentation *ad hoc* to amplify and complete the disease syndrome allowing sure diagnosis. The seed-borne nematode *Aphelenchoides besseyi* Christie causes white tip disease of rice which is world-wide distributed in the rice growing area (Ou S.H., 1985; Gergon E.B. and Misra J.K., 1992). Its presence in the Italian rice fields was firstly reported in 1997 (Tacconi R,. 1996; Moletti M., 1997; Giudici M.L. and Villa B., 1997) and from then several studies were begun to know the performance of white tip in this country. Plants affected by this disease exhibit different symptoms well described in literature (Ou S.H., 1985; Gergon E.B. and Misra J.K., 1992; Prot J.C., 1992). In order to know the vulnerability to the attacks of *Aphelenchoides besseyi* and the symptom expression of the Italian rice varieties, a six year trial was performed.

Materials and methods

Trials tested the main Italian rice varieties in dry seeded conditions flooding at the 3-4th leaf stage of rice. At the end of April the varieties were drilled, with 2 replications, by means of a six row automatic drill-machine in 1 m x 1 m plots. As source of infestation heavily infested seed (about 1000 nematodes per 100 seeds) of a highly susceptible variety (Titanio) was used sowing it on the left and on the right of each tested variety. Therefore in each parcel rows had the following sequence: I – T – I – I – T – I, being T the tested variety an I the spreader variety. The dry seeded condition was used because it favours the primary infestation from seed to seedling. Plants born from infested seeds of cv Titanio were the source of the secondary infestation, which took place after flooding thanks to the effective spreading of nematodes from diseased to healthy plants of the tested varieties. Plants were observed along their whole life cycle taking into consideration all the symptoms reported in literature. The single rows were hand harvested and threshed. Infestation was confirmed by the nematode detection in plants or in rough rice according to the time: the former cutting young tissues into 1 cm pieces and soaking them in water (Ou S.H., 1985), the latter soaking kernels + hulls in water following the method set up by the authors (Villa B. and Giudici M.L., 1998).

Results

Only the results concerning the symptom expression are here reported, while results on the varietal vulnerability to *Aphelenchoides besseyi* will be made known in another work. The observation of rice plants during their whole life cycle evidenced that some Italian varieties do not exhibit all the white tip symptoms reported in literature. It must be pointed out that in this paper are reported only the varieties with clear and certain symptoms among those tested for six years and that sometimes symptomless but infested plants were also found. In particular the tested varieties can be ranked in three groups on the basis of the symptom expression. Usually not all the symptoms below listed for each group are present on a single plant.

The first group includes the varieties showing all the white tip symptoms: typical whitening or chlorosis of leaf tip that twists, becomes necrotic and shreds; chlorotic areas on the middle or basal parts of leaf, chlorosis of leaf edge; chlorosis of new unfolded leafs; production of tillers from the upper nodes; flag leaf shortened and twisted at the apical portion; panicle not or partially emerged; panicle noticeably reduced in length and in the number of spikelets; small spikelets with distorted glumes and deformed kernels; stunted plants. The varieties in question are: Alice, Alpe, Arco, Ares, Ariete, Asso, Baldo, Bali, Balilla, Bastia, Bravo, Castelmochi, Cervo, Chimera, Cigalon, Cobra, Dedalo, Doria, Drago, Ebro, Elba, Elvo, Europa, Flipper, Garda, Giara, Italmochi, Lago, Lido, Lieto, Loto, Mercurio, Nembo, Nettuno, Nuovo Maratelli, Padano, Perla, Pierina Marchetti, Razza 77, Redi, Ringo, Riva, Rizzotto, Roma, Roncarolo, Rosa Marchetti, Rubino, S. Andrea, S. Pietro, Santerno, Sara, Saturno, Savio, Sesiamochi, Stresa, Tea, Tejo, Titanio, Torio, Vela, Venere, Veneria, Volano, Zena, Zeus.

The second group comprehends varieties never showing chlorotic portions but only streaks or stripes chlorosis on leaves observed with back light; moreover flag leaf is never shortened but can appear crinkled. Plants stunted, panicle reduced in size and not completely emerged, small spikelets with distorted glumes and deformed kernels are also present on the varieties of this group, namely Arborio, Arborio precoce, Belgioioso, Carnaroli, Cistella, Gigante Vercelli, Giovanni Marchetti, Marengo, Molinella, Neretto, Nero, Sorriso, Viale, Vialone Nano.

The third group contains varieties showing neither chlorosis on leaves nor flag leaf reduced: the *Aphelenchoides besseyi* infestation is never manifested by any leaf symptom. Diseased plants are reduced in height and produce small panicle often not completely emerged; spikelets are reduced in number and in size. As a result not only the nematode attack is more difficult to notice in these varieties but also it is evident later than in the other two groups. The varieties under consideration are: Cripto, Diana, Elio, Raffaello, Spina.
It is easier to single out the affected plants in the first group than in the others and in the second than in the third.
In this six year trial the cv Thaibonnet did never show any symptom, while the cv Titanio was the most affected.

Conclusions
Comparing the syndrome of each group, the characteristic which more and firstly distinguishes them is the kind of leaf symptom. Therefore it seems possible to rank Italian rice varieties in 3 groups on the basis of the different leaf symptoms: 1. typical whitening of leaf tip, 2. chlorotic streaks or stripes on leaves observed with back light, 3. no leaf symptoms in stunted plants with panicle partially emerged and reduced in size. As above stated it is easier to single out the affected plants in the first group than in the others and in the second than in the third.
With regard to the varieties of the third group, it must be pointed out that among them there is a close relationship because all of them come directly or indirectly from the cv Raffaello. Also among the varieties of the second group, except for Marengo and Molinella whose genealogy is unknown, there is a relationship because they have got a common ancestor, which is the old variety Vialone nero.
The certain knowledge of the different disease symptoms allows both to do more dependable screening to evaluate the genotipic susceptibility and/or resistance to *Aphelenchoides besseyi* and to more easily notice the white tip presence in the different rice cultivation. Moreover the knowledge of the variety symptom expression can be helpful to healthy seed production and official field inspection for certified seed production.
In conclusion, this experimentation improved the knowledge on the symptom expression giving useful information to people working in our environmental conditions to arrive to more certain diagnosis.

Life cycle of *Aphelenchoides besseyi* on rice (translated from Moletti M., 1997)

Seeds infested by *Aphelenchoides besseyi*

Flooded rice: quiescent nematodes revive when immersed, begin moving about, leave the seed and are lost in the water before seed sprouts.

Dry-seeded rice: quiescent nematodes revive more slowly during germination and swim out of the hulls later, when, the seed being already sprouted, they can infest young seedling more easily than in flooded water.

In both cultural conditions the seed-borne nematodes move to seedlings and cause the primary infestation.

During the rice vegetative phase, nematodes feed ecto-parasitically on folded young leaves and on the inner surface of sheaths.

As rice grows, the nematode population increases and they can move from plant to plant by irrigation water, causing the secondary infestation. At late tillering stage the infested plants show the typical whitening of leaf tip.

During the rice reproductive phase, nematodes move to inflorescence and multiplies abundantly at late booting. Nematodes feed always ecto-parasitically, before on florets and then on embryos.

After the panicle emergence and flowering, the nematode reproduction declines and ceases in maturing grains: as the grains dehydrate, nematodes coil up, sticking on the inner surface of the hulls, and becomes quiescent. The diseased plant are stunted and their flag leaf and panicle are deformed.

At harvest only few nematodes are on stubble, most of them are in the spikelets where they over-winter.

If infested seed is sown in springtime, a new cycle starts, *Aphelenchoides besseyi* being seed-borne.

Annex 4

Rice industry in EU the producing Member States

The Member States that produce rice trade mainly within the EU, however EU also exports to a large number of third countries in the Middle East and Central and Eastern Europe. Italy is the main provider, followed by Spain: Italy sends a large part of its production to the other Member States as well as to the other extra-EU countries, Spanish production is more dedicated to internal consumption or to the rest of the EU. Importing northern countries (i.e. the Netherlands, Belgium, Germany) sell a large share of the extra-EU imported husked rice on the EU internal market.

Rice cultivation in **Italy** is mostly located in the northern regions (Po Valley) and extends at present over about 220,000 hectares, which represent only 1.3% of the total arable area (16,800,000 ha). The production is approximately 1.3-1.4 million metric tons of paddy rice and the average yield is about 6.3 tons/ha. In the last 15 years an evolution has been taking place in so far as varieties are concerned: the cultivation of indica type varieties has started and the surface grown with these genotypes reached 27% of the total rice area in 2002.

Rice was introduced in **Spain** by Arabs during the 9^{th} century, since then the cultivation has been confined to rivers and estuarine areas. At present Sevilla, Valencia, Tarragona and Estremadura are the rice areas; most of the rice area is characterised by heavy clay soils and low permeability, this makes rice the only possible crop to be grown in flooded condition. Indica varieties are grown for export and represent more than 50% of the total rice area which amounts to 110,000 ha.

Portugal has a rice growing area of about 25,000 ha; traditional japonica varieties are grown on 90% of the area, the most popular are Italian varieties i.e. Ariete. The main constraint of rice production is water shortage. Portugal has the largest rice consumption in Europe, 14 kg per capita per year.

In **Greece** rice production is concentrated in the plain of Thessaloniki. In the last 15 years Greek farmers have shifted from the cultivation of traditional to indica varieties, which are grown on about 60% of the total surface (21,000 ha). Japonica type rice is produced up to the level that domestic demand is fulfilled.

In **France** rice is almost exclusively cultivated in Camargue, around the estuarine area of Rhone river. The surface is about 20,000 ha. Japonica varieties are grown on 70% and more of the surface, the Italian variety Ariete is the most popular.

Short report of the meeting of the standing committee on plant health (SCPH) held on 9-30 April 2002

8. PRESENTATION BY ITALY OF PEST RISK ANALYSIS CARRIED OUT REGARDS *APHELENCHOIDES BESSEYI* (CHRISTIE) AND DISCUSSION ON PHYTOSANITARY MEASURES TO BE INVESTIGATED.

Italy presented the results of a Pest Risk Analysis carried out regards *Aphelenchoides besseyi* (Christie), the rice white tip nematode. Discussion took place thereon. It was concluded that Italy should complete the information presented and that additional information on the situation in the other MS is necessary in order to fully assess the situation as regards this harmful organism. MS are therefore asked to carry out a survey on the presence of this organism in their country.

Seed Export of Italian Varieties

In table 1 data are reported concerning the percentage of infested seed lots of the main Italian varieties, in the period 1997-2001. A wide difference in susceptibility is known to exist between varieties (Prot J.C. 1992). The traditional Italian varieties – grown in Italy and used to cook *risotto* (Arborio, Baldo, Carnaroli, Vialone Nano, Volano) - were the most infested, while *Aphelenchoides besseyi* was rarely recovered in the seed of indica type varieties (Gladio, Thaibonnet).

Table 1 Percentage of infested seed lots of the main Italian rice varieties.

Variety	Grain type	1997	1998	1999	2000	2001
Baldo	Long A	57.1	16.7	12.2	5.4	6.1
Carnaroli	Arborio-type	50.0	5.9	0.0	3.6	14.3
Volano		46.2	8.0	0.0	5.7	2.9
Arborio		12.2	8.9	5.9	4.4	3.3
S.Andrea		4.2	0.0	5.6	5.7	0.0
Vialone nano		3.6	4.2	0.0	17.4	33.3
Roma		0.0	0.0	0.0	3.0	0.0
Balilla	Round grain	0.0	0.0	0.0	0.0	0.0
Elio		1.8	2.9	0.0	0.0	0.0
Selenio		2.9	0.0	1.8	1.3	0.0
Ariete	Long A for	8.1	0.0	0.0	2.4	2.2
Loto	parboiling	3.6	9.4	13.5	5.6	4.3
Nembo		-	0.0	0.0	0.0	1.9
Zeus		0.0	0.0	0.0	0.0	0.0
Gladio	Long B	0.0	0.0	0.0	0.0	0.0
Thaibonnet	Indica-type	3.3	0.0	0.8	0.0	2.7

The plants of the old traditional varieties are very tall (culm length of cv Carnaroli is 120 cm) and susceptible to lodging, as a consequence they are often sown in drills and flooded afterwards. This cultural practice helps the transmission of the nematode from seed to seedling, in fact direct sowing of seeds in water rather than sowing followed by flooding is recommended to reduce the nematode population (Gergon and Misra, 1992). The results of experimental trials (see annex 7) confirm this statement.

Table 2 Rice seed export of the main Italian varieties in the last 8 years (ton).

Year Variety	1994/95	1995/96	1996/97	1997/98	1998/99	1999/00	2000/01	2001/02
Baldo	160	112	42	10	7	48	7	11
Carnaroli	-	-	-	-	-	-	-	-
Volano	-	-	-	-	19	-	-	-
Arborio	26	19	2	-	-	-	-	2
S.Andrea	-	-	-	6	-	20	-	-
Vialone Nano	-	-	-	-	-	-	-	-
Roma	8	92	196	36	27	81	35	76
Balilla	130	310	165	100	13	34	76	85
Elio	68	1,066	286	1,019	441	400	728	526
Selenio	4	11	87	174	132	179	405	73
Ariete	2,033	2,716	2,589	2,713	2,642	2,006	2,619	2,885
Loto	267	384	1,144	534	196	336	166	112
Nembo	-	-	-	-	4	1	78	34
Zeus	-	-	-	201	627	241	156	246
Gladio	-	-	-	-	422	1,021	999	1,255
Thaibonnet	787	2,025	2,276	1,967	1,756	787	959	804
Others	3,042	2,358	2,709	1,955	1,494	2,203	1770	1,817
TOTAL	**6,365**	**9,093**	**9,496**	**8,715**	**7,780**	**7,357**	**7,998**	**7,926**

In table 2 and 3 data are reported concerning Italian seed market. The Italian varieties popular abroad are the most tolerant to *Aphelenchoides besseyi*.
Besides EU growing Member States, Morocco imports significant amounts of Italian rice seed (800 ton/year).

Table 3 - Italian rice seed sold in the EU internal market in 2002 (ton).

Member State Variety	France	Spain	Portugal	Greece	Total
ALBATROS	69.2	0	144.0	0	213.2
ARIETE	1,766.0	0	1,063.7	55.0	2,884.7
ASSO	21.2	0	0	0	21.2
BALILLA	79.7	0	0	0	79.7
BRAVO	226.0	0	25	5	256
CIGALON	10.4	0	0	0	10.4
EOLO	154.7	0	77.2	0	231.9
FLIPPER	16.0	0	0	0	16.0
GANGE	0	0	12.0	1.2	13.2
GLADIO	208.3	699.0	219.0	128.6	1,254.9
KORAL	109.0	27.9	220.8	35.2	392.9
LIDO	112.2	46.5	0	0	158.6
LOTO	36.2	65.2	0	4.8	106.2
NEMBO	0.5	8.7	0	25.0	34.2
PIEMONTE	0	21.5	0	0	21.55
ROMA	0	0	0	76.1	76.1
SARA	3.6	9	0	2.2	14.8
SATURNO	111.0	0	2.2	0.5	113.7
SAVIO	90.8	0	50.0	37.0	177.8
SELENIO	69.7	0	0	0	69.7
SILLARO	52.3	0.6	0	0	52.9
TANARO	0	11.5	0	4.05	15.6
TEJO	0	0	0	90.4	90.4
THAIBONNET	113.9	147.8	38.5	219.4	519.6
ZEUS	0	0	246.0	0	246.0
OTHERS	2.7	4.8	0.4	9.1	17.0
TOTAL	3,253.5	1,042.5	2,098.8	693.6	7,088.3

Annex 7

Seed infestation and crop performance

(From "Proceedings of the 3rd International Temperate Rice Conference, 10-13 March 2003, Punta de l'Este, Uruguay")

The relation between the number of *Aphelenchoides besseyi* in the seed and the performance of the subsequent crop had been studied for three years comparing the two cultural practices used in Italy: direct sowing in water and sowing followed by flooding. On the basis of preliminary studies and of the degree of seed infestation, two susceptible varieties were used:
Baldo, a traditional Italian variety (Arborio-type), whose seed was badly infested and whose plants show typical "white tip" symptoms;
Cripto, a short grain variety, whose plants does not show any leaf symptom even if they are badly infested.

Materials and methods
The same seed was used both in flooded and in dry seeded condition. For all the trials the seed rate was 180 kg/ha and the following 4 infestation levels, expressed as number of nematodes in 100 seeds, were compared: 0 – 30 – 300 – 700/1200 (the maximum infestation level varied with variety and year). Each trial was laid in a complete randomized design with four replications.
In 1999 seed for the four infestation levels came from four different lots and the lot assumed to be healthy was hot water treated at 59-60 °C for 15' to be sure that the infestation level was "0". On the contrary in 2000 and 2001 seed came from one badly infested lot where the nematode population density corresponded with the maximum infestation level. Part of this seed was hot water treated at 55 °C for 15' to obtain the infestation level "0". This treated seed was afterwards mixed with the infested one until the desired infestation levels of "30" and "300" were got. For the nematode detection the method devised at the Rice Research Centre (Villa B. and Giudici M.L., 1998), comparing and modifying different available methods, was followed.
In flooded condition, plots, whose size was different each year ranging from 28 m^2 to 37 m^2, were separated lengthways by levees and transversely by ondolux to allow individual irrigation and draining and so preventing dissemination of the nematode through water from one plot to another. Seed was hand spread and after a week plots were drained for a short period to enhance rice seedling establishment. Before harvesting with a plot combine harvester, in each plot 20 plants at random were measured for plant height and panicle length, and 8 sub-plots of 0.25 m^2 were hand harvested in order to measure panicle density, dry matter, 1000-seed weight and number of nematodes per 100 seeds. Grain yield was evaluated on the whole plot. In 2001 ten panicles per plot were also collected to count the number of spikelets/panicle.
In dry seeded condition plots size was different each year varying from 41 m^2 to 69 m^2 and fields were drilled with a automatic drill-machine At the 3-4th leaf stage of rice plots were separately flooded. A levee surrounded each plot and individual irrigation and draining were provided to prevent movement of nematodes from plot to plot. Before harvesting with a plot combine harvester, in each plot 40 plants at random were measured and 20 sub-plots of 0.25 m^2 were hand harvested in order to get the above mentioned parameters. Also in this case grain yield was evaluated on the whole plot. In 2001 twenty panicles per plot were collected to count the number of spikelets/panicle.

Results
Results are reported in table 1-3.
Generally the higher is the number of nematodes in 100 seeds, the more serious is the yield loss. All the parameters show the same trend of the grain yield, except panicle density. The differences in the panicle density in 1999 are a consequence of the different 1000-seed weight of the seed lots used and of the reduction of germination due to hot water treatment of seed in the other two years. Taking into consideration the panicle density data it seems that the negative effect of the hot water treatment, which takes place only when rice is heavily infested (data in press), is enhanced in dry seeded rice: this aspect might be considered in future specific trials.
Comparing the two cultural conditions, it is evident a greater yield loss in drilled rice in all the years and for both the varieties. At the maximum infestation level, the greatest yield loss was reached in dry seeded crop in 2001 and in flooded rice in 2000, by cv Cripto (Table 2): the former amounts to 53.1%, the latter to 41.5%. Nevertheless it must be pointed out that in 2000 the climate was very favourable to *Aphelenchoides besseyi* and that in the other years the yield loss in flooded crop did not exceed 28.8% (Table 2). In dry seeded condition the grain yield does not show significant differences between the maximum infestation level and

level “300”, except for Cripto in 2001 (Table 2); on the contrary in flooded condition this difference is significant. The difference between the two cultural conditions is more remarkable at the infestation level “30”; in fact, while in drilled rice yield is significantly lower than yield at level “0”, in flooded rice the differences are not significant. The highest yield reduction in flooded rice, amounting to 2.5%, was got by Baldo 30 in 2000 (Table 1) when, as mentioned above, climate was very favourable to the disease. In dry seeded rice the yield reduction at the infestation level “30” ranges from 7.3% of Cripto (Table 2) to 31.8% of Baldo (Table 1), respectively in 2001 and in 2000.

Both plant height and panicle length are more reduced in drilled than in flooded rice. Also the reduction in the 1000-seed weight is greater in dry seeded crop and this is due to a reduction of the spikelet size. The number of spikelets per panicle, recorded only in 2001, is not significantly different in flooded rice crop, but differs significantly in the dry seeded one.

Table 1 Crop performance of the cv Baldo in flooded and dry-seeded crop using seed with 4 nematode infestation levels.

Treatments	Grain yield (1) t/ha	Yield reduction (2) %	Plant height (3) cm	Panicle length cm	Panicle density (4) No./m^2	Dry matter (4) g/m^2	1000-seed weight g	Spikelets/ panicle No.
BALDO - FLOODED CROP								
1999								
0	8.41 a	-	92.8 a	18.2 a	297 a	1760 a	41.0 a	-
30	8.31 a	1.2	91.9 a	18.0 a	311 a	1863 a	40.8 a	-
300	8.12 a	3.5	90.7 a	18.1 a	292 a	1749 a	40.4 a	-
1200	6.45 b	23.3	82.9 b	17.5 a	315 a	1741 a	38.2 b	-
2000								
0	7.22 a	-	95.2 a	16.4 a	386 a	1739 a	36.7 a	-
30	7.05 a	2.5	94.9 a	16.6 a	391 a	1749 a	35.4 ab	-
300	6.43 b	10.9	92.4 ab	16.7 a	381 a	1783 a	34.6 bc	-
900	5.38 c	25.6	90.0 b	15.5 b	386 a	1699 a	33.4 c	-
2001								
0	7.70 a	-	91.6 b	18.7 a	326 a	1608 a	41.8 a	104 a
30	7.85 a	0.0	95.1 a	19.1 a	321 a	1687 a	41.3 a	111 a
300	7.11 b	7.6	90.1 b	18.6 a	329 a	1543 a	39.1 b	103 a
700	6.30 c	18.1	85.8 c	17.5 b	316 a	1381 b	38.8 b	99 a
BALDO - DRY-SEEDED CROP								
2000								
0	7.61 a	-	96.5 a	16.0 a	286 c	1570 a	35.4 a	-
30	5.19 b	31.8	87.5 b	16.1 a	307 b	1510 ab	28.4 b	-
300	4.44 c	41.7	79.3 c	15.2 b	350 a	1462 bc	25.9 c	-
900	4.41 c	42.0	79.2 c	14.7 c	359 a	1408 c	25.4 c	-
2001								
0	8.30 a	-	94.8 a	18.8 a	249 b	1601 a	41.7 a	128 a
30	7.30 b	12.0	89.8 b	18.5 b	237 b	1430 b	38.1 b	133 a
300	5.73 c	31.0	81.7 c	17.0 c	281 a	1363 b	32.3 c	109 b
700	5.70 c	31.3	81.0 c	17.0 c	287 a	1323 b	32.1 c	101 b

(1) Calculated on the whole plot, 14% moisture content.
(2) Comparison with population density = 0.
(3) Measured from soil to neck node.
(4) Mean of eight 0.25 m^2 sub-plot; dry matter calculated on the whole plant without root.
Means followed by the same letter are not significantly different at $P < 0.05$ (Duncan’s test).

Table 2 Crop performance of the cv Cripto in flooded and dry-seeded crop using seed with 4 nematode infestation levels.

Infestation level	Grain yield (1)	Yield reduction (2)	Plant height (3)	Panicle length	Panicle density (4)	Dry matter (4)	1000-seed weight	Spikelets/ panicle
	t/ha	%	cm	cm	No./m^2	g/m^2	g	No.
				CRIPTO - FLOODED CROP				
1999								
0	8.95 a	-	69.2 a	15.1 a	429 a	1713 a	31.4 a	-
30	8.93 a	0.3	68.9 a	15.3 a	400 a	1761 a	31.1 a	-
300	8.59 a	4.0	67.2 a	14.2 b	423 a	1685 a	31.0 a	-
800	7.99 b	10.8	65.4 a	13.8 b	445 a	1693 a	29.7 b	-
2000								
0	7.70 a	-	72.7 a	15.2 a	464 a	1772 a	27.8 a	-
30	7.60 a	1.3	71.7 a	15.1 a	489 a	1725 a	27.9 a	-
300	6.22 b	19.2	71.4 a	15.0 ab	488 a	1781 a	26.8 b	-
900	4.50 c	41.5	70.8 a	14.7 b	485 a	1630 b	25.9 c	-
2001								
0	8.31 a	-	67.3 a	15.7 a	424 a	1544 a	31.3 a	97 a
30	8.47 a	0.0	67.1 a	15.7 a	414 a	1575 a	31.1 a	107 a
300	7.56 b	9.0	66.9 a	15.6 a	430 a	1531 a	29.7 b	101 a
900	5.92 c	28.8	62.6 b	14.2 b	428 a	1403 b	27.6 c	96 a
				CRIPTO - DRY-SEEDED CROP				
1999								
0	9.50 a	-	71.7 a	16.8 a	252 b	1560 a	32.0 a	-
30	8.27 b	13.0	68.9 b	16.0 b	262 b	1428 b	29.9 b	-
300	6.52 c	31.4	63.0 c	14.9 c	266 b	1265 d	26.5 c	-
800	6.24 c	34.4	62.4 c	14.2 d	313 a	1332 c	25.4 d	-
2000								
0	8.21 a	-	75.0 a	15.4 a	366 b	1726 a	26.7 a	-
30	5.69 b	30.7	71.8 b	14.7 b	377 b	1620 ab	24.3 b	-
300	4.90 c	40.3	66.1 c	13.5 c	385 b	1523 b	23.6 b	-
900	4.76 c	42.1	65.8 c	13.5 c	435 a	1581 b	22.5 c	-
2001								
0	8.71 a	-	67.2 a	16.8 a	307 a	1470 a	31.1 a	130 a
30	8.07 b	7.3	67.1 a	16.7 a	308 a	1448 a	31.1 a	122 ab
300	5.37 c	38.4	62.8 b	16.1 b	342 a	1287 b	26.7 b	110 bc
900	4.09 d	53.1	58.6 c	14.3 c	330 a	1158 c	24.9 c	97 c

(1) Calculated on the whole plot, 14% moisture content.
(2) Comparison with population density = 0.
(3) Measured from soil to neck node.
(4) Mean of eight 0.25 m^2 sub-plot; dry matter calculated on the whole plant without root.
Means followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test).

Taking into consideration the detection of nematodes at harvest, the number of nematodes found in 100 seeds is similar or less than the initial infestation level for all the treatments (Table 3). In particular, in flooded rice, at the infestation level "30" the population density ranges from 15 to 74, the latter was detected when the climate was very favourable to *Aphelenchoides besseyi*. The fact that some nematodes were detected also at the infestation level "0" confirms that the hot water treatment is not eradicant.

Table 3 Nematodes detected at harvest from flooded rice crop sowed using seed infested with 4 different nematode infestation levels.

BALDO		CRIPTO	
Infestation level	Nematodes at harvest (1)	Infestation level	Nematodes at harvest (1)
	1999		
0	0	0	0
30	30	30	53
300	142	300	169
1200	615	800	524
	2000		
0	2	0	1
30	42	30	74
300	422	300	395
900	673	900	668
	2001		
0	1	0	1
30	15	30	17
300	335	300	242
700	417	900	613

(1) Number of nematodes in 100 seeds, average of 5 samples each consisting of 100 seeds.