# EPPO Datasheet: Aphelenchoides besseyi

Last updated: 2020-07-24

#### **IDENTITY**

Preferred name: Aphelenchoides besseyi

**Authority:** Christie

Taxonomic position: Animalia: Nematoda: Chromadorea:

Rhabditida: Aphelenchoididae

**Other scientific names:** *Aphelenchoides oryzae* Yokoo, *Asteroaphelenchoides besseyi* (Christie) Drozdovski

Common names: rice leaf nematode, rice white-tip nematode,

strawberry crimp disease nematode, white-tip nematode

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EPPO Categorization: A2 list
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EU Categorization: RNQP (Annex IV)

**EPPO Code:** APLOBE



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# Notes on taxonomy and nomenclature

The taxonomy used in this datasheet reflects developments suggested by several recent publications, summarised in Decraemer & Hunt (2013), which place *Aphelenchoides* in the Order Rhabditida, Suborder Tylenchina. This contrasts with the taxonomy nomenclature occasionally used by some authors (such as the CABI Invasive Species Compendium CABI, 2019; Wheeler & Crow, 2020), which place *Aphelenchoides* in the Order Aphelenchida, Suborder Aphelenchina (Hunt, 1993). Whilst this makes no difference to classification from the level of Superfamily (Aphelenchoidea) to species level (*Aphelenchoides besseyi*), those studying the species might need to be aware of differences in the literature.

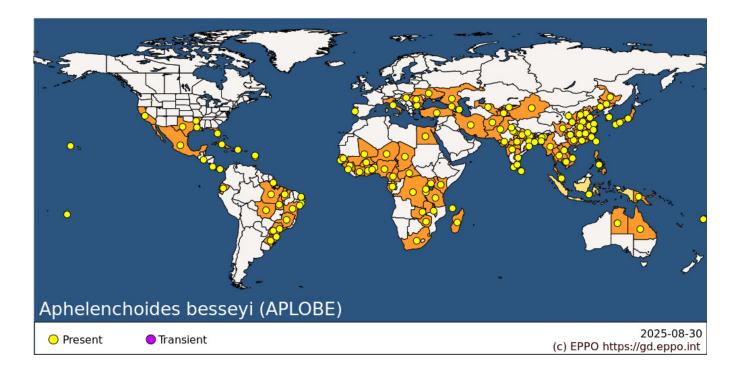
#### HOSTS

The main host plants at risk in the EPPO region are rice and strawberries. *A. besseyi* has also been found on other crop plants (including *Gossypium hirsutum*, *Capsicum annuum* var. *longum*, *Zea mays*) ornamental plants (including chrysanthemums, *Ficus elastica*, *Agave amica*), and grasses (including *Panicum*, *Setaria*). *Glycine max* appears to be a host of *A. besseyi* in laboratory tests (Oliveira *et al.*, 2019) and was reported in field infestations (Meyer *et al.*, 2017), but reports differ in the extent of the host susceptibility and subsequent damage. Extensive lists of plant groups that have been associated with *A. besseyi* in the literature are provided by Sanchez-Monge *et al.* (2015), but it should be noted that not all have necessarily been proven to be true hosts to date.

**Host list:** Abelmoschus esculentus, Agave amica, Allium cepa, Asplenium nidus, Boehmeria nivea, Capsicum annuum, Chrysanthemum indicum, Colocasia esculenta, Dioscorea cayenensis, Dioscorea trifida, Ficus elastica, Fragaria x ananassa, Glycine max, Gossypium hirsutum, Ipomoea batatas, Oryza sativa, Panicum miliaceum, Phaseolus vulgaris, Setaria italica, Sporobolus junceus, Vigna unguiculata, Zea mays

### GEOGRAPHICAL DISTRIBUTION

A. besseyi has a wide geographic distribution, mostly in tropical and subtropical countries. In the EPPO region, it is reported in a few countries of the southern and eastern part of the region.



**EPPO Region:** Azerbaijan, Bulgaria, Georgia, Hungary, Italy (mainland), Kyrgyzstan, Portugal (mainland), Romania, Russian Federation (the) (Southern Russia), Türkiye, Ukraine, Uzbekistan

Africa: Benin, Burkina Faso, Burundi, Cameroon, Central African Republic, Chad, Comoros, Congo, The Democratic Republic of the, Cote d'Ivoire, Egypt, Gabon, Gambia, Ghana, Guinea, Kenya, Madagascar, Malawi, Mali, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, South Africa, Tanzania, United Republic of, Togo, Uganda, Zambia, Zimbabwe

Asia: Afghanistan, Bangladesh, Cambodia, China (Anhui, Fujian, Guangdong, Guangxi, Guizhou, Hebei, Heilongjiang, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Jilin, Liaoning, Ningxia, Shaanxi, Shandong, Shanxi, Sichuan, Xinjiang, Zhejiang), India (Andhra Pradesh, Assam, Bihar, Delhi, Gujarat, Haryana, Jharkand, Kerala, Madhya Pradesh, Maharashtra, Meghalaya, Odisha, Punjab, Tamil Nadu, Tripura, Uttar Pradesh, West Bengal), Indonesia, Iran, Islamic Republic of, Japan (Honshu, Kyushu, Shikoku), Korea, Republic of, Kyrgyzstan, Lao People's Democratic Republic, Malaysia (West), Myanmar, Nepal, Pakistan, Philippines, Sri Lanka, Taiwan, Tajikistan, Thailand, Uzbekistan, Vietnam

North America: Mexico, United States of America (Arkansas, California, Florida, Hawaii, Louisiana, Texas) Central America and Caribbean: Costa Rica, Cuba, Dominica, Dominican Republic, El Salvador, Guadeloupe, Panama

**South America:** Brazil (Alagoas, Amapa, Bahia, Maranhao, Mato Grosso, Minas Gerais, Para, Parana, Rio Grande do Norte, Rio Grande do Sul, Santa Catarina, Sao Paulo, Sergipe, Tocantins), Ecuador

Oceania: Australia (Northern Territory, Queensland), Cook Islands, Fiji, Papua New Guinea

# **BIOLOGY**

On rice, infested seed is the primary inoculum source. When the crop is sown, the nematodes become active and move to the growing points of leaves and stems. *A. besseyi* is most active and feeds at a relative humidity greater than 70%, when symptoms are more likely to occur. At 30°C the life cycle is about 10 days and lengthens significantly at temperatures below 20°C. There is no development below 13°C (Bridge *et al.*, 2005). The optimum temperature for development is 21-25°C, the life cycle taking 10 days at 21°C and 8 days at 23°C, and there are several generations in a season. There are many publications that mention *A. besseyi* 'in the grain', but they feed mainly ectoparasitically on young tissue (Duncan & Moens, 2013). Adult females congregate in the glume axis (i.e. under the husk of the seed rather than in the seed grain itself), where they respond to the end of the growing season by living in a quiescent state until conditions become favourable to start the life cycle again, which can be up to 3 years after harvest, on dry grain (Duncan & Moens, 2013; Huang & Huang, 1972). Reproduction is by amphimixis (cross-fertilisation with male and female nematodes) but this species can also reproduce parthenogenetically (Duncan & Moens, 2013).

The nematode survives between crops by obligate quiescence and there has been research to understand the mechanisms involved but more investigations are required (Chen *et al.*, 2018). *A. besseyi* will die in 4 months on grain left in the field; the nematode is not thought to survive long periods in the soil between crops.

Mixed populations of plant-parasitic nematodes are usually found infesting plants and *A. besseyi* often occurs with another foliar nematode, *Ditylenchus angustus*, on rice. Thus, competition between the species may affect the biology of each species (Latif *et al.*, 2013).

On strawberries, the nematode is also ectoparasitic, feeding on young tissue. In Florida, nematode infestations have been uncommon since the 1950s, but in 2016 they were recorded in a few fields in Central Florida (Desaeger and Noling 2017). Oliveira *et al.* (2019) suggest that *A. besseyi* has the potential to become an emerging problem for the Florida strawberry growers, but probably not as a serious problem as it was more than 60 years ago, because the infestations of the nematode observed in the field in 2018 were less serious than those in 2017. Re-occurrence of nematode was observed in the season 2018/2019, but overall damage and yield loss appeared minimal. Details of the life cycle in strawberry crops during the season and over the winter months need to be reassessed under current cultural conditions.

Regarding other hosts, more research is required to investigate how different populations interact with their hosts. It has been thought for some time that host races exist (Bridge *et al.*, 2005). Hsieh *et al.* (2012) reported several instances where isolates from some hosts were either unable or able to multiply on other hosts, but that morphology of the species remained the same. Oliveira *et al.* (2019) suggested that *A. besseyi* may exist as a species complex as they found both morphological and molecular differences in different populations on different hosts. Other studies found different cellulases in this species, identified in different hosts (Wu & Chen, 2015; Wu *et al.*, 2016).

#### DETECTION AND IDENTIFICATION

### **Symptoms**

### On rice

Susceptible and lightly infested plants can be symptomless (*A. besseyi* was found in panicles without white tip symptoms by Tülek & Çobanog?lu, 2010), but in general yield loss only occurs in plants showing symptoms on the tillers of the affected plants, where the tips of the leaves whiten for a distance of 3 to 5 cm, and then die off and shred. The panicles are shorter and often atrophied at the tips. The fertile flowers sometimes produce misshapen grains with low germination potential and delayed date of maximum germination. Affected plants have reduced vigour and height. Infected panicles are shorter with fewer spikelets and a smaller proportion of filled grain. In severe infestations, the shortened flagleaf is twisted and can prevent the complete extrusion of the panicle from the boot. Infestations reduce seed swelling, the grain is small and distorted and the kernel may be discoloured and cracked. Infested plants mature late and have sterile panicles borne on tillers produced from high nodes (Tülek *et al.*, 2015; Bridge *et al.*, 2005).

Symptoms may be confused with calcium and magnesium deficiency.

#### On strawberries

Symptoms include leaf crinkling and distortion, and dwarfing of the plant with an associated reduction in flowering (Desaeger & Noling, 2017).

## On other hosts

A. besseyi may be endoparasitic, as in *Ficus elastica* and *Agave amica*, in which it causes leaf drop and leaf lesions, respectively. In the grass *Sporobolus poirettii*, the nematode stimulates growth, resulting in increased inflorescences (Marlatt, 1970).

Symptoms are further detailed in the EPPO Diagnostic protocol PM 7/39(2) (EPPO, 2017a).

### Morphology

Typical *A. besseyi* have a slender body 0.44-0.84 mm long and 14-22 µm wide. In females, the excretory pore is usually near the anterior edge of the nerve ring; the spermatheca is elongate-oval and usually packed with sperm; the ovary is relatively short, the post-vulval uterine sac narrow, and the terminus bears a mucro of diverse shape with three to four processes (stellate). In males, the proximal end of the spicule lacks a dorsal process. The EPPO Diagnostic Protocol PM 7/39(2) (EPPO, 2017a) sets out the morphology as described in the original description of the species, along with other previously published data up to 2017. Oliveira *et al.* (2019) have recently provided more details of the morphometrics of an *A. besseyi* population found on strawberry in Florida and compared this with other *Aphelenchoides* considered by them as having stellate tails which helps to provide further clarity to the identification process.

There is currently (June 2020) much discussion on the identity of *A. besseyi* especially when it is suspected of occurring on crops other than rice. According to de Jesus *et al.* (2016), who studied populations in infested rice and forage crops, the identity of *A. besseyi* has often been confused with a morphologically similar species, *A. fujianensis* (Zhuo *et al.*, 2010), but their molecular investigations suggested the species were so similar they were in fact the same species. Buonicontro *et al.* (2018) investigated the identities of these species as identified in forage grass seed using real-time PCR but concluded further research was required. Oliveira *et al.*, (2019), who investigated infestations of *A. besseyi* in strawberries using morphological and molecular tools (the latter not identical to those used by the previous authors), also reported that the species could be confused with other species with stellate mucros, namely *A. fujianensis* and a new species, *A. pseudogoodeyi*. They also suggested that *A. besseyi* is a complex of species. Other new species described as having stellate tails have also been described since the publication of the last edition of the EPPO Diagnostic protocol for this species (EPPO, 2017a), such as *A. medicagus* (Wang *et al.*, 2019). There is clearly more research to be done concerning the identity of *A. besseyi* and those nematodes that are morphologically similar to it, especially because, at present, only *A. besseyi* is listed as a quarantine pest. Thus identification should be done in conjunction with a laboratory quality accredited in nematological identifications, using the latest findings in research.

# **Detection and inspection methods**

Detection and identification methods are detailed in the EPPO Diagnostic protocol PM7/39(2) (EPPO, 2017a), which refers to a detection standard method, recently updated, used by the International Seed Testing Association (ISTA) method for use on rice (ISTA, 2020). Inspection of consignments of rice seed is covered in EPPO Phytosanitary Procedure PM 3/78(1) (EPPO, 2016).

Inspection of consignments of *Fragaria* plants for planting is covered in EPPO Phytosanitary procedure PM 3/73(1) (EPPO, 2008a, 2009), and inspection procedures for places of production are set out in EPPO Phytosanitary procedure PM3/83(1) (EPPO, 2017b).

The nematode may also be found in host plant detritus (EPPO, 2017a; Wheeler & Crow, 2020).

In relation to molecular methods, PCR tests and DNA barcoding can be used (EPPO, 2017a). Research on molecular methods to support the identification of *A. besseyi*, and to distinguish it from morphologically similar species, both in the laboratory and in the field, is on-going (Rybarczyk-Mydlowska *et al.*, 2012; Sanchez-Monge *et al.* 2017; Buonicontro *et al.*, 2018; Yang & Yu, 2018. Recently, Çelik & Devran (2019) and Çelik *et al.* (2020) developed a real-time PCR testbased on small subunit ribosomal DNA (SSU rDNA) of *A. besseyi*, and validated it successfully in populations collected from rice-growing fields in Turkey and subsequently developed a qualitative assay. Where molecular tools can also be used to support detection, the combined use of the 18S rRNA, 28S rRNA and COI genes appears to provide the most reliable support (Oliveira *et al.*, 2019). Such developments in research will provide support for internationally approved protocols (IPPC, 2016; EPPO, 2017a).

# PATHWAYS FOR MOVEMENT

A. besseyi is disseminated in plant material, such as infested rice seed (as it lives in a quiescent state under the hull), or associated with infested plants for planting of strawberries and other hosts. Locally, floods or irrigation water can

spread the pest from infested hosts (Duncan & Moens, 2013).

### PEST SIGNIFICANCE

# **Economic impact**

A. besseyi makes a significant contribution to the estimated 16 billion USD worth of damage caused by nematodes to rice crops (Jones et al., 2013). The economic impact of this seed-borne nematode varies between regions, countries, and localities. Yield losses are also affected by the rice cultivar used, growing year, temperature, cultural practices and other variable factors (Tulek et al., 2015). In addition, Latif et al. (2013) found infestations of either A. besseyi or D. angustus alone resulted in greater disease incidence and greater yield reductions than mixed populations of these nematodes found in some fields, probably due to the effects of competition for feeding sites.

Generally, where little information on the pest status for *A. besseyi* exists, estimates for damage prediction have used the threshold of 300 live nematodes per 100 seeds, according to Bridge *et al.* (2005). However, research has resulted in conflicting data concerning the relationship between nematode density and yield loss. Tsay *et al.* (1998) reported yield losses of 24.2, 34.7, and 44.9% when the estimated infestation rate was 18, 34 and 57% respectively. Tülek *et al.* (2015) found a significant correlation between yield loss and observation of the white tip symptoms but not nematode density.

Recent work has proposed a prediction model for losses in field crops based on a significant correlation between mean yield reduction and nematode populations (Jamali *et al.*, 2012). Where outbreaks have occurred in Japan, the economic value of infested, discoloured grain is reduced if infestation levels exceed 0.7%. In China, yield losses can be as high as 45% when plant infestation levels exceed 50%. In contrast, local cultivars in Thailand appear to be tolerant of *A. besseyi* and no symptoms have been observed despite widespread infestation (Bridge *et al.*, 2005).

On strawberries, *A. besseyi* was recorded over 60 years ago in the USA as the incitant of summer dwarf or crimp disease, and causing much reduced strawberry fruit yields. Recent field infestations in the USA have been significant but often only temporary once control measures have been put in place; it is difficult to predict the financial impact that the loss of previously available control treatments will have on the industry (Wheeler & Crow, 2020).

#### **Control**

Prophylactic methods are of utmost importance. Seed treatments and resistant cultivars have significantly reduced the economic impact of this pest in some parts of the world (Duncan & Moens, 2013; Tülek *et al.*, 2015). Cultural methods for rice include early planting if the rice season is preceded by a cooler period, and lower seedbed planting densities (Bridge *et al.*, 2005).

Contrary to that which might be expected, good seed storage conditions probably prolong nematode survival, as more nematodes survive in seeds stored with low moisture than in seeds with high moisture levels at most temperatures. Nevertheless, clean seed remains the primary means of reducing infestation levels, together with the use of resistant cultivars (Bridge *et al.*, 2005). For infested rice seeds a hot water treatment at 52-57°C for 15 min is recommended by the International Rice Research Institute to eradicate *A. besseyi* (Misra *et al.*, 1994).

Chemical treatments of rice seeds and soil have been used in the past. Similarly, nematicides, applied either as foliar sprays or as soil treatments, can reduce the nematode population sufficiently to prevent symptom expression. However, few, if any, treatments still exist. The emphasis is now on sustainable production of crops.

Strawberry runners may also be treated prior to planting by immersion in water for 10 min at 46°C (EPPO, 2012). The nematode numbers can thus be markedly reduced but not always eliminated, and symptoms can be expected to be expressed in a later growing season. However, strawberry planting material should be free from nematodes; this is best achieved within a certification scheme, which produces planting material from nematode-free nuclear stock, and is a vital component in minimising the risk of infestation. *A. besseyi* is included in the EPPO certification scheme for strawberries PM 4/11(2) (EPPO, 2008b).

### Phytosanitary risk

A. besseyi has the potential to be a significant pest outdoors in warmer climates of the EPPO region, as well as under protection in other parts of the region. It presents a risk particularly to rice, and possibly to strawberry and other hosts.

#### PHYTOSANITARY MEASURES

A. besseyi is listed on the EPPO A2 List of pests recommended for regulation as quarantine pests by EPPO. In addition, it was assessed during the EPPO RNQP Project to evaluate whether the pest was also fulfilling criteria for a RNQP, when not regulated as a quarantine pest (Picard et al., 2018). As recommended during this Project, A. besseyi is subject to a zero tolerance for rice seeds in the EU (as a RNQP) (EU, 2019). The Project suggested that the risk in strawberry plants could be managed along with the measures for other species of Aphelenchoides with tolerances for certified, basic and pre-basic material, and the absence of visual symptoms on the traded material for non-certified material. The pest is subject in the EU to a zero tolerance for plants for planting (except seeds) of strawberry (EU, 2019). Possible phytosanitary measures recommended for rice seeds in the EPPO RNQP Project (Picard et al., 2018) and in EPPO Specific quarantine requirements (EPPO, 1995) for strawberry plants for planting (except seeds) included origin from a pest-free area or treatment. In addition, the EPPO RNQP Project recommended that rice seeds could be tested. Phytosanitary procedures for rice seeds are available for inspection of consignments (EPPO, 2016), and for strawberry plants for planting for inspection (EPPO, 2008a, 2009, 2017b), hot water treatment (EPPO, 2012) and certification schemes (EPPO, 2008b).

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