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PRELIMINARY RESULTS ON THE PARASITIC NEMATODE *DELADENUS SIRICIDICOLA* ASSOCIATED TO *MONOCHAMUS* SPP. IN PINE WOODS OF CAMPANIA REGION, ITALY

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Troccoli A., Garonna A.P., Fantini M., Troisi D., Barra E., Somma S., d'Errico G. - Preliminary results on the parasitic nematode *Deladenus siricidicola* associated to *Monochamus* spp. in pine woods of Campania region, Italy.

During phytosanitary monitoring activities carried out in Campania (southern Italy) in the years 2018-2021, focused on pine sawyer beetles of the genus *Monochamus* spp. considered potential insect vectors of *Bursaphelenchus xylophilus*, the facultative parasitic nematode *Deladenus siricidicola* (Nematoda Neotylenchidae) was collected from adult beetles of *Monochamus galloprovincialis*. *Deladenus siricidicola* is the primary biological control agent largely applied against the wood wasp *Sirex noctilio* (Hymenoptera, Siricidae), an invasive pest of Southern Hemisphere and North America, where it heavily damages pine wood plantations allowing the spread of its symbiotic organism, the white-rot agent *Amylostereum areolatum*. The interesting record of this beneficial nematode associated with a pine sawyer beetle of the genus *Monochamus* represents an opportunity to investigate the relationships of *D. siricidicola* with other organisms in forest ecosystems and could lead to a new step in the control of the threat posed by the harmful pine wood nematode *B. xylophilus*.

Key Words: *Deladenus siricidicola,* pine sawyer beetles*, Pinus* spp.*,* pine wilt nematode

INTRODUCTION

Nematode communities of forest ecosystems, still poorly investigated, are characterized, besides some plant parasitic species, by a large number of other species present in all trophic levels establishing complex relationships, from commensalism to saprophagy and from predatory behaviour to parasitism towards arthropods, mainly insects.

Among plant parasitic nematodes a few species belonging to the genus *Bursaphelenchus* have to be considered. This genus includes more than 130 species, mostly fungal feeders, distributed in all biogeographic regions (Kanzaki *et al*., 2023) and may be vectored by several xylophagous beetles (Penas *et al*., 2006; Robertson *et al*., 2008). An update on the genus *Bursaphelenchus* and its distribution in the Mediterranean area has been recently published (d'Errico *et al*., 2015).

In 1972, Mamiya and Kiyohara described *Bursaphelenchus lignicolus*, a forestry pest responsible of the pine wilt disease observed in Japan. This name was later synonymized with *Aphelenchoides xylophilus* (Steiner & Buhrer, 1934) before it assumed the valid name B. *xylophilus* (Nickle, 1970), species known worldwide as the Pine Wilt Nematode (PWN), native to North America, currently a pest of paramount importance worldwide.

During the '80s this species had a huge impact on pine forest ecosystems in the Far East, mainly in Japan, China and Korea causing mass mortality of pine trees (Mamiya, 2004; Yang, 2004). The spread of the PWN in Europe is considered a real threat due to the presence of susceptible host species (*Pinus pinaster*, *P. nigra*, *P. sylvestris*) and native insect vectors (Naves *et al*., 2016). Moreover the establishment could be favored by climatic factors (extreme drought and temperature increase) due to increasing global warming recorded in last decades (Mamiya, 1984; Hirata *et al*., 2017; An *et al*., 2019; Estorninho *et al*., 2022).

Unfortunately *B. xylophilus* have been introduced in Europe at the end of the XX century spreading in the Iberian peninsula (Mota *et al*., 1999). Today the species is established in Portugal (Firmino *et al*., 2017; de la Fuente *et al*., 2018) whereas strict measures are applied to eradicate the quarantine nematode from Spain (EPPO, 2022).

PWN has been included in the first list of priority quarantine pests in EU (EU, 2019). As other priority pests, PWN annual surveys are mandatory to ensure early detection of this pest by identifying pathways and monitoring wooden commodities (log, squared timber, wood packaging, dunnage, etc.) and the possible presence of alien insect vectors, pine sawyer beetles belonging to the genus *Monochamus* (Coleoptera: Cerambycidae), transported with the commodities at entry points. Mandatory monitoring is carried out also on territories of all Member States in EU searching signs attributable to the presence of PWN in host plants or in native pine sawyer beetles catched with pheromone traps (Abelleira *et al*., 2020).

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In 2018-2021 in Campania Region (Italy), during the annual monitoring activities focused on native pine sawyer species, the presence of *Deladenus siricidicola* Bedding (Nematoda: Neotylenchidae) in several dissected adult insects infesting newly dead or dying pine species was recorded. This beneficial nematode is largely used as a biocontrol agent of *Sirex noctilio* Fabricius (Hymenoptera: Siricidae) in countries of the Southern Hemisphere (Australia, Chile, New Zealand, South Africa) (Bedding, 1972; Bedding & Akhurst, 1978; Tribe & Cillie, 2004; Bedding, 2009; Fitza *et al*., 2019) and North America (Canada, USA) (Williams *et al*., 2012; Williams & Hajek, 2017; Liebhold & Hajek, 2021) invaded by the Eurasian woodwasp (Lombardero *et al*., 2016). In the non-native regions the siricid wasp may cause mass tree mortality in exotic pine plantations with the help of a symbiotic, tree-pathogenic fungus *Amylostereum areolatum* essential for larval development (Morris *et al*., 2012; Wermelinger & Thomsen, 2012; Castillo *et al*., 2018).

The present study reports for the first time the association of facultative parasitic nematode *D. siricidicola* with a pine sawyer beetle of the genus *Monochamus*.

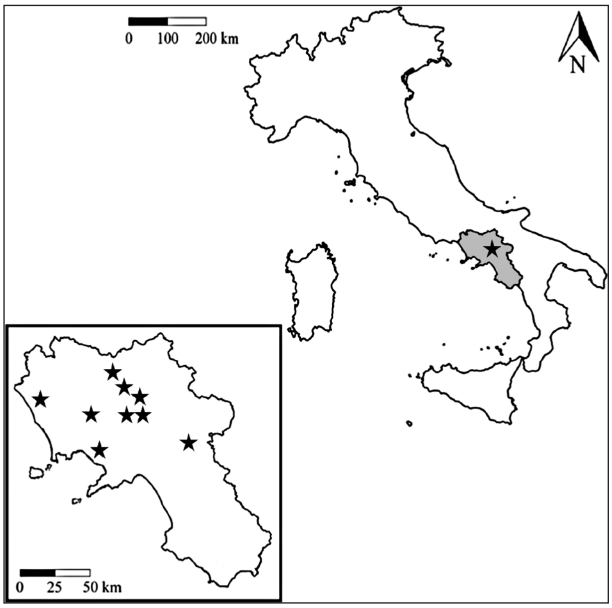

Fig. I – Map of Italy and monitored areas of Campania Region with the positions of *Deladenus* positive trapped beetles.

MATERIAL AND METHODS

Trapping of *Monochamus* spp.

Phytosanitary monitoring was carried out in Campania region (southern Italy) (Fig. I). To monitor the native pine sawyer beetles, mainly *Monochamus galloprovincialis*, trapping devices with pheromonal and kairomonal attractants were used. Apart from pinewoods, the Regional survey program also considered as risky sites to be monitored nature reserves, urban parks, entry points, sawmills and lumberyards, wood storage and wooden waste disposal sites. The traps were checked for catches every 10-12 days.

At least 50 trapping devices were installed each year during 2018-2021. CROSSTRAP® (Teflon-coated cross-vane trap) with dry collection cup for living catches, produced by SANIDAD AGRICOLA ECONEX SL (Spain) were used. On average the survey activity lasted 6 months (period May-October).

The traps were suspended with a rope in-between two trees or from one of the tree branches hanging at ca. 2 m above ground. As attractant kits GALLOPROTECT PACK (SEDQ HEALTHY CROPS SL, Spain) and MONOCHAMUS ATRAYENTE 60 DIAS (SANIDAD AGRICOLA ECONEX SL, Spain) were used. GALLOPROTECT PACK contains a first dispenser with the male aggregation pheromone of *M. galloprovincialis* (2-undecyloxy-1-ethanol), a second dispenser with ipsedienol and 2-methyl-3-buten-1-ol, and other two dispensers with α-pinene as kairomonal attractant (Fig. II). The renewal of the whole kit was made every 45-50 days, as suggested by the company.

The simplified attractant kit MONOCHAMUS ATRAYENTE 60 DIAS contains only ipsenol, methyl butenol and α-pinene.

Fig. II – Trapping device used during the monitoring survey.

The trapped adults were identified using available taxonomic keys (Bense, 1995; Wallin *et al*., 2013). All dying and living specimens were delivered to the Phytopathological Laboratory, Plant Protection Organization (PPO) of the Campania Region to assess the presence of the pine wood nematode.

Laboratory analyses and microscopic observations

Monochamus specimens were dissected in shallow water within Petri dishes, under a stereo microscope. Emerged nematodes were sieved, then handpicked and transferred in a clean water suspension. Live specimens were immobilized by gentle heating and then mounted in water temporary mounts for morphological observations and photographs (Fig. III).

Measurements and photographs of specimens were taken with a Leica DM compound microscope provided with a Leica DFC camera and LAS (Leica Microsystem®) software for computing and image digital analysis.

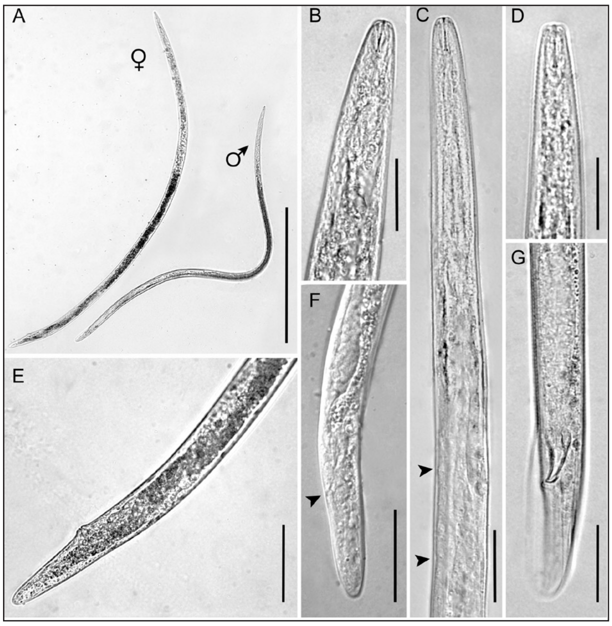

Fig. III – Light micrographs of temporary mounts of mycetophagus adults of *Deladenus siricidicola*. A: Female and male entire body; B: Female anterior end; C: Female pharyngeal region showing excretory pore position (top arrow) and hemizonid (bottom arrow); D: Male anterior end; E: Female posterior region with protruding vulval lips; F: Female tail (the arrow indicates the anus); G: Male posterior region in subventral view. (Scale bars: A = 0.5 mm; B-G = 20 µm).

Morphological identification was based on the main diagnostic characters and identification keys (Chitambar, 1991).

RESULTS

Trapping of *Monochamus* spp.

During the insect monitoring survey, we noticed that the GALLOPROTECT PACK kit always allowed higher trap catches during the whole period. Eighty-one trapped adults of *M. galloprovincialis* collected in 9 municipalities and 17 monitoring sites (Tab. 1) resulted infested by *D. siricidicola*. Nematodes (juveniles and adults) of this species recovered from dissected beetles were generally low in number (about 10 per insect), although in few cases, and particularly in beetle samples collected in autumn, a larger number of nematodes (> 50/insect) could be detected.

Laboratory analyses and observations

Among the nematodes recovered from insect dissection, a large part were juveniles. Morphology and morphometrics of a few adult specimens extracted from pine sawyer beetles are presented in Table 2. Morphometric comparison with type specimens revealed they corresponded to the free-living mycetophagus form of nematode's life cycle.

The identity of the few nematodes examined was assessed by comparing the most reliable diagnostic characters, according to Chitambar (1991), such as the vulva position, the relative position of the excretory pore to hemizonid, the distance between hemizonid and the excretory pore, and the female tail tip shape. *Deladenus siricidicola* was considered most similar to *D. wilsoni* Bedding, 1968, differing from it by the greater distance between the hemizonid and the excretory pore (more than 20 µm posterior to excretory pore *vs* just anterior to it) (Bedding, 1968). Specimens of the present study fit with type population of *D. siricidicola* (see Table 2 and Fig. III C), despite a shorter body length in female and male mycetophagous forms of our population, and a smaller 'a' value. Chitambar (1991) proposed the rank of superspecies for *D. siricidicola* and the other morphologically similar species *D. canii* Bedding, 1974*, D. imperialis* Bedding, 1974 and *D. rudyi* Bedding, 1974. More recently, Kanzaki *et al*. (2016) described the species *D. nitobei* Kanzaki, Tanaka, Fitza, Kosaka, Slippers, Kimura, Tsuchiya & Tabata, 2016*,* as not clearly distinguishable from *D. siricidicola* and *D. canii*, and considered the three species as a cryptic species complex. However, *D. nitobei*, in addition to molecular differences, sows little morphological differences from the mycetophagus female of *D. siricidicola* by having a slightly shorter stylet (9-9.7 *vs* 10-11 µm), slightly shorter excretory pore to hemizonid distance (18-30 *vs* 24-58 µm) and generally slender body. We consider the morphometrics of our population in agreement with that of the species *D. siricidicola*, although further detailed morpho-biological and molecular studies on the present population associated to pine sawyer beetles in Campania region will corroborate the species status of the nematode.

DISCUSSION AND CONCLUSION

Monochamus galloprovincialis is the main pine sawyer beetle present in Campania Region (Fig. I). This secondary xylophagous beetle develops on *Pinus* spp. and rarely on *Abies*, *Picea* e *Larix*, especially in pine plantations with abundant deadwood. Adults of this lon-

Table 1 - List of monitoring sites and number of trapped adults of *M. galloprovincialis*

Year	Samples	Municipalities	Geo-coordinates	Altitude (m asl)
2018	2	Moiano (BN)	41°05'40.0"N 14°33'39.4"E	570
2018	9	San Lorenzello (BN)	41°16'49.9"N 14°32'14.0"E	463
2018	6	Solopaca (BN)	41°10'39.0"N 14°31'54.7"E	516
2019	2	Bucciano (BN)	41°05'29.1"N 14°34'20.7"E	580
2019	12	Cautano (BN)	41°09'19.1"N 14°38'02.7"E	590
2019	4	San Lorenzello (BN)	41°16'49.1"N 14°31'56.2"E	473
2019	4	Solopaca (BN)	41°10'34.8"N 14°32'30.0"E	572
2019	8	Caserta (CE)	41°06'16.8"N 14°20'37.5"E	332
2019	3	Falciano del Massico (CE)	41°09'47.5"N 13°55'59.5"E	346
2019	2	Torre del Greco (NA)	40°47'25.6"N 14°25'37.5"E	330
2020	3	Lioni (AV)	40°49'35.7"N 15°08'55.8"E	1117
2020	2	Lioni (AV)	40°49'28.8"N 15°08'06.3"E	1130
2020	3	Solopaca (BN)	41°10'32.5"N 14°32'37.7"E	570
2020	3	Solopaca (BN)	41°10'39.8"N 14°31'52.1"E	518
2020	8	Falciano del Massico (CE)	41°09'45.1"N 13°55'48.3"E	355
2021	4	Falciano del Massico (CE)	41°09'47.0"N 13°55'52.6"E	350
2021	6	Lioni (AV)	40°49'25.8"N 15°09'04.8"E	1130

Table 2 – Morphometrics of mycetophagus *Deladenus siricidicola* specimens extracted from *Monochamus* spp. compared to the measurements of the type population (Bedding, 1968). Data are expressed as mean ± St. Error (range)

	Mycetophagus				Parasitic
	Females		Males		Infective females
	Present study	Type	Present study	Type	Type
n	4	50	3	50	50
Body length (mm)	1.44 ± 34.5 (1.37-1.54)	1.91± 0.04 (1.50-2.71)	1.36 ± 72.5 (1.22-1.45)	1.49± 0.02 (1.15-1.91)	1.220 ± 0.027 (0.80-1.60)
Max. body diam. (µm)	39.4 ± 3.8 (32.0-44.3)	-	31.2 ± 5.6 (23.5-42.0)	-	-
Stylet length (µm)	10.0 ± 0.0 (10.0-10.0)	10 ± 0.00 (10.0-11.0)	10 ± 0.00 (10.0-11.0)	10 ± 0.00 (10.0-11.0)	21 ± 0.23 (19.0-25.0)
Ex. pore to hemizonid (µm)	38.0 ± 1.2 (36.0-40.0)	40.7 ± 1.23 (24.0-58.0)	36.5 ± 0.8 (22.0-46.0)	36.5 ± 0.8 (22.0-46.0)	33 ± 0.85 (22.0-45.0)
Tail length (µm)	34.8 ± 3.2 (32.5-37.0)	-	-	-	
a	32.9 ± 6.7 (28.1-37.7)	50.9 ± 0.97 (33.0-69.1)	50.9 ± 0.97 (33.0-69.1)	53.4 ± 0.99 (43.2-77.5)	61.2 ± 1.41 (44.0-109.1)
b	15.1*	19 ± 0.39 (15.2-26.6)	-	15.7 ± 0.24 (12.1-22.4)	10.9 ± 0.13 (9.3-13.7)
c	42.5 ± 2.4 (39.8-47.4)	44.6 ± 0.74 (32.6-58.9)	28.9 ± 1.2 (26.5-30.2)	31.6 ± 0.33 (26.1-37.0)	35.1 ± 0.53 (27.0-43.8)
V	93.6 ± 0.3 (93.1-94.1)	94.8 ± 0.87 (93.2-96.2)		-	94.1 ± 0.06 (92.8-95.0)

* n = 1.

ghorn beetles emerge from late May and remain active until October, feeding on the bark of young twigs of healthy trees while females oviposit in dying pines.

Deladenus siricidicola was first recorded in 1962 infecting *Sirex noctilio* in New Zealand (Zondag, 1969) and described by Bedding (Bedding, 1968). The genus currently includes about 30 species, with *D. siricidicola* being one of the most widespread and the most studied within the genus, as it is recognised as the most important biocontrol agent of the pine sawyer beetle *S. noctilio* (Bedding & Iede, 2005; Bedding, 2009).

This nematode is characterized by having two separate life cycles with two morphologically different adult female types: the first is free-living, mycetophagous, and the second is parasitic (infective stage) to the xylophagus insect (Bedding, 1968). This nematode does not kill his host, but induces the sterilization of hymenopter females, which are still able to mate and oviposit. However, the eggs are not viable as they contain numerous nematodes which, depending on environmental stimuli, become mycetophagus adults or parasitic, whether in presence of an insect larva, or pre-pupa of *S. noctilio.*

Light microscopic observation and measurements of *Deladenus* specimens emerged from dissected pine sawyer beetles clearly revealed they belong to the mycetophagus stage. This finding represents a first record of the association between the neotylenchid *D. siricidicola* and a pine sawyer beetle of the genus *Monochamus*.

Kosaka & Ogura (1993) described a new bicyclic nematode associated to *Monochamus alternatus*, resembling a *Deladenus* species in its mycetophagus stage. However, morphological differences between the new nematode and *Deladenus* species led the authors to consider it as belonging to the genus *Contortylenchus* Ruhm, 1956, rather than to *Deladenus.*

The presence of *D. siricidicola* in adults of *M. galloprovincialis* can be explained by the coexistence of larval stages of the beetle with those of the woodwasp *S. noctilio* in the same tree or pinewood. In fact, few adult specimens of the wasp were trapped with the same devices in most of the reported sites, mainly in early fall of the whole sampling period.

The unexpected detection of *D. siricidicola* in *M. galloprovincialis* adults provides an opportunity to investigate the relationships of this facultative parasitic nematode established with other organism in forest ecosystems and also to consider alternative control options against potential harmful populations of pine sawyer beetles to prevent introduction and spread of the Pine wilt nematode.

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