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PLANT PARASITIC NEMATODES ON *PAULOWNIA TOMENTOSA* IN POLAND

Short communication

Andrzej Tomasz SKWIERCZ[1], Anita ZAPAŁOWSKA*[2], Łukasz FLIS[3],
Justyna KOC-JURCZYK[2], Łukasz JURCZYK[2], Wojciech LITWIŃCZUK[2], Czesław PUCHALSKI[2]
[1]National Institute of Horticultural Research, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland
[2]University of Rzeszów, Ćwiklińskiej 1, 35-601 Rzeszów, Poland
[3]Museum and Institute of Zoology of the Polish Academy of Sciences, Wilcza 64, 00-679 Warsaw, Poland

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ABSTRACT

Short-rotation woody plants of the genus *Paulownia* are attracting more and more attention as trees that produce biomass and reduce the concentration of carbon dioxide in the atmosphere. However, plants growing in monoculture affect the properties and condition of the soil. One of the effects of changes in the soil environment is the growing population of plant parasitic nematodes (PPN). The article presents information about the PPN inhabiting the root zone of the *Paulownia tomentosa* plantation in Poland. In this study, the frequency and density of nematode populations in samples from seven plantations in Poland were determined. The extracted nematodes were identified at the species level on the basis of the male and female morphological characteristics according to several available identification resources. A total of 20 nematode species were identified, of which 9 were classified as accessory and 11 as occasional. Among them, *Trichodorus viruliferus* and *Longidorus attenuatus* belonging to the group of viral messengers were identified.

Key words: accessory and occasional nematode species, ecto- and endoparasites, virus vectors

INTRODUCTION

Fast-growing woody crops are a very important source of energy biomass. *Paulownia* spp., native to China, where they have been cultivated for more than 2000 years (Tu et al. 2017), tends to grow extremely fast. Well-established plants can grow more than 4 m in one season. With high tolerance to different soil and climate conditions, they can even grow on soils recognized as marginal (Wang & Shogren 1992). Following shifts in the Common Agricultural Policy and the expansion of the energy sector, farmers in the European Union are progressively expanding the cultivation of energy crops. *Paulownia* spp., which is considered to form a short-rotation forestry plantations, is a promising tool for reducing the atmospheric carbon dioxide concentration (Wisniewski et al. 1993; Hall 1997; Calfapietra et al. 2010; Lucas-Borja et al. 2011) due to the presence of a C4 photosynthesis pathway. Plants of *Paulownia* spp. produce as much biomass in one year as other species do in several (Kalaycioglu et al. 2005; García et al. 2011). The biomass is often used for the production of industrial wood and paper. The presence of lignin, hemicelluloses, and high content of cellulose (Yadav et al. 2013) makes their timber comparable to that of hardwoods (Ates et al. 2008), considered to be suitable as solid biofuel. As a good source of energy, it is used to produce bioethanol and biogas (Skibko et al. 2021). Species of the genus *Paulownia* are proposed for afforestation as they are highly adaptable to a wide range of climatic and soil conditions (García-Morote et al. 2014), as well as to soil contaminated with trace elements (Jiang et al. 2012). This makes this genus particularly suitable for remediation purposes (Doumett et al. 2008; Macci et al. 2012, 2013, Madejón et al. 2014). The trees are often planted along roadsides, in gardens, and in parks for decorative purposes.

Paulownia spp. is known also as a fodder plant whose aboveground parts contain high levels of useful nutrients (Barton et al. 2007). The flowers and leaves are a good source of fats, sugars, and proteins for feeding cattle. The nitrogen compounds in *Paulownia* spp. leaves can be compared with those of several leguminous family plants.

*Corresponding author
e-mail: azapalowska@ur.edu.pl

They have the same nutritious values as alfalfa and are suitable for the nutrition of sheep and goats in combination with wheat straw or silage (Woods 2008; Angelov 2010). The leaves are also utilized as green manure, particularly in China (Yadav et al. 2013).

Paulownia spp. has a range of medicinal properties (Ayan 2006). The bark, leaves, and flowers are used in traditional Chinese medicine to treat infections and inflammatory diseases (Ji et al. 2015); the antiviral effects of METPF (methanol extracts of *P. tomentosa* flowers) being especially noted. Tea and syrup extracted from the flowers of *Paulownia* spp. have a positive effect on the liver, spleen problems, and bronchitis. The flowers are used in the cosmetic industry (Angelov 2010) and also are a good source of nectar for bees (Woods 2008; Angelov 2010; Yadav et al. 2013; Bikfalvi 2014).

The most common species within the genus are *P. fortunei*, *P. kawakamii*, *P. taiwaniana*, and *P. tomentosa* but in Poland mainly the last species was planted. Presented here nematological research on *Paulownia tomentosa* Steud. (princess tree) rhizosphere was influenced by the detection of the root-knot nematode *Meloidogyne hapla*, a serious pest of many economically important plants, on the roots of this plant (Skwiercz et al. 2019). Galled roots of *Paulownia* are extremely open to other soil pathogens: bacteria, fungi, and other species of soil and foliar nematodes. *Meloidogyne incognita*, *Meloidogyne javanica*, and *Meloidogyne arenaria* are the most frequently mentioned of the dozen species of root-knot nematodes that parasitize *Paulownia* spp. (Schmelzer 1969; Wang et al. 1975; Chunram et al. 1976; Hsieh 1983; Kobayashi & de Guzman 1998; Perera et al. 2005; Kaur et al. 2007). Their possible appearance in Poland may occur as a result of the import of planting material.

The aim of the study was to obtain factual information about the composition and density of the nematode fauna in Polish plantations of the princess tree, with particular emphasis on nematodes, which are considered serious enemies in Polish agriculture.

MATERIALS AND METHODS

Soil samples with fragments of plant roots were collected from 3–6-year-old *P. tomentosa* plantations in the following locations:

- Żołynia (50.09° N, 22.15° E), south-eastern Poland;
- Rzeszów (50.01° N, 14.55° E), south-eastern Poland;
- Dębogórze (54.59° N, 18.44° E), Polish Baltic Coast;
- Kudypy (53.77° N, 20.38° E), the Warmian-Masurian region;
- Tomaszkowo (53.71° N, 20.40° E), the Warmian-Masurian region;
- Szczecin (53.43° N, 14.55° E), north-western Poland;
- Wrocław (51.10° N, 17.07° E), the region of Lower Silesia.

Based on particle size (the overall sum of fractions less than 0.02 mm amounting to 37.7%) the soil under surveyed plantations can be categorized as heavy, with its agricultural suitability classified as class IV, according to the Polish soil classification system. Soil samples were collected from the rhizosphere to a depth of 45 cm from July to August 2020, from every square of plots of 1–5 ha. Samples were taken in the number of 20 punctures per 1 ha area. After 5–6 kg of soil from each plot have been mixed, 1 kg of soil was prepared for analyzing as a pooled sample. The analytical sample consisted of 100 cm^3 of soil and 5 g of roots.

The recovery of nematodes was made in two steps. Nematodes from the soil (100 cm^3) were extracted using the centrifugation method (Szczygieł 1971). The migratory endoparasitic nematodes from roots present in the soil samples were recovered by Baermann method (1917) using 5 g of the roots (water incubation in a sieve for 4 days). Nematodes obtained from incubation and centrifugation were combined in water and fixed by adding a similar amount of 6% hot formalin.

After processing with glycerin (Baermann 1917), permanent slides of nematodes were made. Nematodes were observed in a Zeiss microscope at 1000x magnification with immersion oil and identified at the species level based on the morphological traits of male and female individuals according to different identification resources (Seinhorst 1959; Brzeski 1998; Andrássy 2007; Decraemer 1995). Frequency of occurrence (C index: $C = 100 \times Na \cdot N^{-1}$, where Na – number of samples containing a given species, N – total number of samples), the population density of the species specimens (range of minimum and maximum), and mean with standard deviations were calculated (Zapałowska & Skwiercz 2018).

The nematodes were assigned to the following trophic groups, according to the classification proposed by Yeates et al. (1993); Yeates (1999); Tytgat et al. (2000); Baldwin et al. (2004); Moens and Perry (2009), and the system approved by Fauna Europaea (Tytgat et al. 2000) as follows:

– A1: migratory endoparasites (Pratylenchidae) (Winiszewska 2008; Moens & Perry 2009);
– A2: semi-endoparasites (Hoplolaimidae) (Tytgat et al. 2000);
– A3: ectoparasites and A3 V: vectors of plant viruses (nematode species belonging to the families Longidoridae, Xiphinematidae, and Trichodoridae (Lamberti et al. 1975; Duke & Du Cellier 1993);
– A4: sedentary parasites (Baldwin et al. 2004);
– V: vectors of viral infections (Lamberti et al. 1975);
– F: hyphal and root hair feeders (Yeates et al. 1993, Yeates 1999).

RESULTS AND DISCUSSION

The nematode species identified in the 24 collective samples from the rhizosphere in plantations of the *P. tomentosa* are presented in Table 1. Altogether 20 species were detected, belonging to Trichodoridae, Longidoridae, Criconematidae, Paratylenchidae, Telotylenchidae, Hoplolaimidae, and Pratylenchidae families. Based on the frequency of occurrence, the 11 species were classified as occasional (frequency of occurrence < 25%) and 9 species (*Trichodorus viruliferus* (Fig. 1F, G), *Longidorus attenuatus* (Fig. 1A, D), *Bitylenchus dubius*, *B. maximus*, *Meloidogyne hapla*, *Helicotylenchus pseudorobustus*, *Rotylenchus buxophilus* (Fig. 1C), *Pratylenchus fallax*, and *P. neglectus*) as accessory (frequency of occurrence 29–42%). Among 20 species 12 ectoparasite species, including 6 known as virus vectors (*T. viruliferus*, and *L. attenuatus* in abundance qualified as an accessory), 1 sedentary species (*M. hapla*), 3 semi endoparasites, and 4 migratory endoparasites were detected. These are:

1. ectoparasites that migrate into the root system:
   - plant virus vectors: *Paratrichodorus pachydermus*, *T. viruliferus*, *L. attenuatus*,
   - members of the family Belonolaimidae: *B. dubius*, *B. maximus*;
2. semi-endoparasites of the Hoplolaimidae family: *H. pseudorobustus*, *R. agnetis*, *R. buxophilus* (Fig. 1C);
3. migratory internal parasites of the genus *Pratylenchus*: *P. fallax* and *P. neglectus*, which interact with a number of bacterial and fungal pathogens of plants;
4. sedentary parasites of the *M. hapla* species, which are found in 33% of *Paulownia* crops, do not pose a significant threat to yield, as this plant is not a suitable host for this species, but in light soils, *M. hapla* could be more dangerous for *Paulownia*.

The presence of *P. fallax* and *P. neglectus*, which are migratory harmful endoparasites due to phytotoxins excreted to the plant during its feeding in cortical tissues, was noted in high population densities (16–124 and 8–88, respectively). The frequency of *T. viruliferus* was 29%, and *T. sparsus* and *T. cylindricus* 8%. They are vectors of plant viruses, able to transfer several TOBRA viruses to the roots of healthy plants. *L. attenuatus* can transfer NEPO plant viruses. Nematode vectors of plant virus diseases feed as migratory ectoparasites on the roots of plant hosts, producing small galls on the root tips. In the root samples, some necrosis of cortical cells grouped linearly was observed. Also, in half of the root samples tested, elongated swellings were found, which are symptoms characteristic of colonization by nematodes belonging to the Longidoridae family, as described by Whitehead and Hooper (1970) on sugar beet. In our investigation, we found two species of *Longidorus* that should be sought in plantations, *R. buxophilus* and *R. agnetis*, semi-endoparasitic nematodes, rare species in field crops, especially in heavy soils, were detected with high frequency in the rhizosphere of *P. tomentosa*. Host testing is required to confirm the potential pathogenicity of these species to *P. tomentosa*.

Table 1. Species of plant parasitic nematodes collected from seven plantations of *Paulownia tomentosa* Steud. in Poland

Species (authors and date of the first description)	Trophic type*	**Nematodes population density		***Frequency of occurrence; C index (%)	Species dominance status
		Range	Mean ± SD		
TRICHODORIDAE					
Paratrichodorus pachydermus (Seinhorst, 1954)	A3,V	6–22	13.0±0.6	17	occasional
Trichodorus cylindricus (Hooper, 1962)	A3,V	8–12	10.0±1.1	8	occasional
T. viruliferus (Hooper, 1963)	A3,V	4–28	14.0±2.5	29	accessory
T. sparsus (Szczygieł, 1968)	A3,V	12–16	14.0±1.1	8	occasional
LONGIDORIDAE					
Longidorus attenuatus (Hooper, 1961)	A3,V	4–27	13.8±2.3	29	accessory
Longidorus spp.	A3,V	4–12	8.0±2.3	8	occasional
CRICONEMATIDAE					
Criconemoides informis (Micoletzky,1922)	A3	3–32	13.0±4.9	17	occasional
Criconema annuliferum (de Man, 1921)	A3	2–54	18.0±9.31	17	occasional
PARATYLENCHIDAE					
Paratylenchus projectus (Jenkins, 1956)	A3	12–44	24.0±5.47	17	occasional
TELOTYLENCHIDAE					
Bitylenchus dubius (Bütschli, 1873)	A3	8–87	45.0±9.56	29	accessory
B. maximus (Allen, 1955)	A3	3–42	13.0±3.6	42	accessory
Scutylenchus quadrifer (Andrássy, 1954)	A3	6–96	55.0 ±14.3	21	occasional
Meloidogyne hapla (Chitwood, 1949)	A4	6–98	49.0±11.7	33	accessory
HOPLOLAIMIDAE					
Helicotylenchus pseudorobustus (Steiner, 1914)	A2	12–90	51.0±8.47	29	accessory
Rotylenchus agnetis (Szczygieł, 1968)	A2	14–118	57.0±13.9	25	occasional
R. buxophilus (Golden, 1956)	A2	14–78	44.0±6.6	38	accessory
PRATYLENCHIDAE					
Pratylenchus fallax (Seinhorst, 1968)	A1	16–124	65.0±10.95	29	accessory
Pratylenchus neglectus (Rensch, 1924)	A1	8–88	43.5±13.07	29	accessory
Pratylenchoides laticauda (Braun & Loof, 1966)	A1	12	12.0±0.0	4	occasional
Hirschmanniella gracilis (de Man, 1880)	A1	6	6.0±0.0	4	occasional

Specimen numbers as sum of samples of 100 cm^3 of soil and 5 g of roots
*trophic type: A1 – migratory endoparasites, A2 – semi endoparasites, A3 – ectoparasites, A4 – sedentary parasites, V – vectors of plant viruses; **population density: minimum and maximum population densities counted for each species, mean and standard deviation of each species population densities; ***frequency of occurrence in relation to 24 soil samples

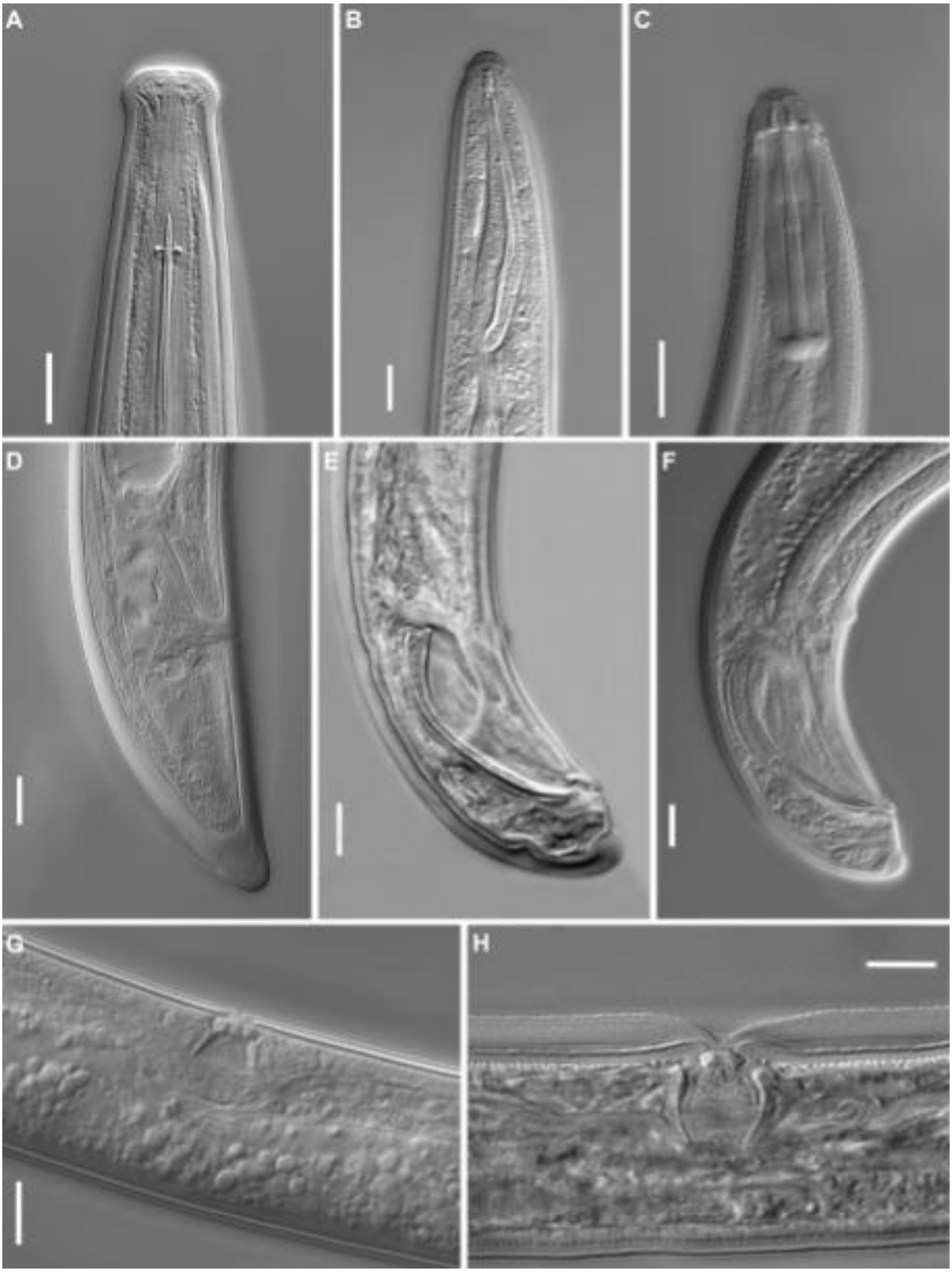

Figure 1. *Longidorus attenuatus* (A), *Trichodorus sparsus* (B), *Rotylenchus buxophilus* (C), *Longidorus attenuatus* (D), *Trichodorus sparsus* (E), *Trichodorus viruliferus* (F), *Trichodorus viruliferus* (G), *Trichodorus sparsus* (H); bars on each photograph represent 10 µM (photo. G. Winiszewska, A. Skwiercz)

Presented here nematological research on *P. tomentosa* was influenced by the detection of the root-knot nematode *M. hapla*, a serious pest of many economically important plants, on the roots of this plant (Skwiercz et al. 2019). Galled roots are extremely open to other soil pathogens: bacteria, fungi, and other species of soil and foliar nematodes. Although interest in cultivating this plant as an energy crop is increasing, there is little work done to develop an industrial method of this crop cultivation. There is also insufficient knowledge on the protection of plantations against diseases and pests. In this study, in the result of the analysis of soil samples from rhizosphere of seven *P. tomentosa* plantations, 20 nematode species were identified. Among them, *M. hapla*, which is known as a serious pest of major food crops, vegetables, fruit, and ornamental plants in temperate, tropical, and subtropical regions (Duke & Du Cellier 1993; Evans et al. 1993; Luc et al. 2005; Zapałowska & Skwiercz 2018). *Meloidogyne* spp. is difficult to control due to their high rate of reproduction. Symptoms are seen in the roots, leaves, and as a lesser growth of the infected plants. On roots, small and spherical growths called galls, situated near small roots, are formed. Defective roots can no longer transport water and nutrients properly, which results in stunted, wilted, yellowing plants and a decrease in yield. The severity of the symptoms depends on the nematode population density, host plant species, and cultivar. As the number of nematodes increases, the number and size of root galls increase as well. However, the use of a combination of control practices, known as an integrated pest management plan, has proved to be effective (Robinson 2008). *M. hapla* observed on *P. tomentosa* roots in heavy soil showed little harmfulness (Skwiercz et al. 2019), but in light soils can develop larger populations. Therefore, a search for the presence of *M. hapla* in the soil is mandatory before planting *Paulownia* in light soils.

Nematodes belonging to the families Longidoridae and Trichodoridae that were detected at high frequency in this survey are known as virus vectors. *Paulownia* plants are susceptible to cucumber mosaic virus (CMU) (Mehrotra 1997), tomato rattle virus (TRV), and elm mottle virus (EMV) (Horváth 1973). On *Paulownia* plantations in Asia, witches' broom disease was recorded that is vectored by the insect *Halyomorpha halys* Uhler (Brown marmorated stink bug) (Shiozawa et al. 1979). However, this disease can also be caused by fungi, oomycetes, mistletoes, mites, phytoplasmas, viruses, and nematodes. Such disease was also occasionally observed on Polish plantations (personal observations). The *Paulownia* witches' broom disease may lead to the premature death of the tree. It is also known that virus and virus vector nematodes can threaten *Paulownia* plantations causing deformation of the appearance of the branches of infected plants (Hsieh 1983; Burger 1989.

Even though the semi-endoparasitic nematodes *R. buxophilus* (Fig. 1C) were frequent in the root zone, a special focus should be given to the nematode species which are vectors of plant viruses. To our knowledge, this is the first record of the occurrence of plant-parasitic migratory nematode species in Poland. Also virus vector nematodes belonging to the genera *Longidoridae* and *Trichodoridae* were recorded for the first time in *Paulownia* plantations in Poland.

The presence of *M. hapla* in high population density in the rhizosphere on *P. tomentosa* plantations should draw the attention of agriculture practitioners and plant protection specialists to the succession of plants after *Paulownia* plantations in order not to transfer nematodes to other sensitive plants and vice versa from formerly cultivated crops to *Paulownia*.

In order not to destroy the existing plantations of *Paulownia* and prevent diseases on newly-established plantations, it is necessary to determine the tolerance limit of *P. tomentosa* to plant-parasitic nematodes in pot experiments under controlled conditions, especially against virus vectors and root-knot nematodes. It is necessary to analyze the nematode fauna of the soil to assess the pre-planting population of nematodes, which are potential pests of *Paulownia* plantations. *P. tomentosa* cultivation for energy and industrial purposes just started in Poland and has the expected potential for the EU-piloted Green Deal (Renewable Energy Directive, 2009/28/EC).

CONCLUSION

The detection of numerous nematodes in *P. tomentosa* plantations, including those transmitting viruses and root-knot nematodes, indicates the need to determine the pathogenicity of nematodes in relation to this plant, as well as to assess the risk of nematodes occurrence in soils intended for future plantations.

APPENDIX A

Trichodorus viruliferus (Hooper, 1963) (Figure 1F, G)
N – 10 females, L – 0.65–0.92 mm, stylet – 36–56 µm, J-shaped. Widened head, ballade with constriction and bristles, shape and length of vagina: rhomboid; > 0.3 cdb.
N – 4 males, L – 0.61–0.89 mm, stylet – 32–53 µm. Widened head, ballade with constriction and bristles, sausage-shaped nucleus, in spermathecal.
Trichodorus sparsus (Szczygieł, 1968) (Figure 1B, E, H)
N – 10 females, L – 0.80–0.95 mm, a – 24–28, b – 6.0, c – subterminal, V – 54–58%, stylet – 47–49 um. Body bends ventrally, vulval opening is pore-shaped. Posterior end of esophagus set off from intestine, end of tail oval, with paired post anal and caudal pores present. Anus ventrosubterminal.
N – 6 males, L – 0.85–0.90 mm, a – 23–28, stylet – 48–50 um, spicules – 47–49 um. Body ventrally bends, often hooked in posterior end. Two ventromedian cervical pores situated anterior to excretory pore. Three supplementary ventromedian papillae. Spicules striated and bristled on the its surface. The species occurred only at 2 localities in Żołynia and Rzeszów, Southern Poland in low population densities.
Longidorus attenuatus (Hooper, 1961) (Figure 1A, D)
N – 8 females. L – 4.65–5.20 mm, a – 105–120, c' – 1.45–1.55, V – 48–49%, odontostylet – 85–90 um, hyaline part of tail – 10–13 um. Anterior end to guide ring – 26–28 um. Lip region flattened, expanded characteristically and definitely offset from the neck contour. Symmetrically bilobed large amphids. The tail convex dorsally, conoid. Seven populations from all 7 localities were occurred. Males absent.
Rotylenchus buxophilus (Golden, 1956) (Figure 1C)
N – 24 females. L – 1.1–1.2 mm, stylet – 30–34 um, pharyngeal lobe 18–25 um, c' – 1.0–1.1, head not offset with 4 distinct annuli, cephalic framework strongly refractive, spermatheca empty, tail bearing 12–14 annuli, phasmids 5–10 annuli anterior to anus, tail with striated terminus, dorsally convex-conoid, lateral fields aerolated only anteriorly. Measurements range of 9 populations from all 7 localities. Males absent.

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