

Plant parasitic nematodes on *Paulownia tomentosa* in Poland

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Abstract

Fast growing woody crops are currently a very important source for the generation of energy biomass. As short-rotation woody crops, the genus *Paulownia* has already attracted growing attention. These trees are used to produce biomass and reduce the carbon dioxide concentration in the atmosphere. Most projects for biomass production, however, may affect soil properties and status. For this reason, it is important to know the effects of *Paulownia* plantations on the microbiological properties of the soils on agricultural areas in Poland. This article provides information on plant parasitic nematodes inhabiting the root zone of *Paulownia tomentosa* L. in Poland. The only report of *Meloidogyne hapla* Chitwood, 1949 shows the potential pathogenicity of the root-knot nematodes. Furthermore, nothing is known about plant parasitic nematodes inhabiting the root zone of *Paulownia tomentosa* L. in Poland. Determining the trophic group of plant parasitic nematodes was undertaken by a process of centrifugation. Measurements showed a decrease in the population reproduction factor (P_f/P_i) which reached a value of 0.1. *Paulownia tomentosa* L. taken from seven different locations in Poland revealed the presence of *M. hapla*.

Keywords: *Meloidogyne hapla*; *Paulownia tomentosa*; Longidorus, Trichodorus

Introduction

Fast-growing woody crops are a very important source for the generation of energy biomass. *Paulownia* spp., native to China where they have been cultivated for more than 2000 years [1], tend to grow extremely fast. Well established plants can grow more than 4 m in one season. Trees from this genus are extremely adaptive to a wide range of climate and soil factors. With vegetative propagation and tolerance to different soil and climate conditions, they even grow on soils recognised as marginal [2]. European farmers, following shifts in the Common Agricultural Policy and the expansion of the energy sector [3], are progressively expanding the cultivation of energy crops. The existing Renewable Energy Directive, adopted by a decision of 23 April 2009 (Directive 2009/28/EC), established a 20 % mandatory share of EU energy consumption from renewable energy by 2020, while the decarbonisation scenario is 30% by 2030. A key management system, like short-rotation woody crops, constitutes a renewable energy feedstock for biofuels, bioenergy, and bioproducts. *Paulownia* spp., which are considered to form short rotation forestry plantation, is a promising tool for reducing the atmospheric carbon dioxide concentration [4],[5],[6],[7]. Characterised by the presence of a C4 photosynthesis pathway involved in regulating the climate, it absorbs significant amounts of carbon dioxide and releases a large amount of oxygen. *Paulownia* spp. produce as much biomass in one year as other species do in several [8],[9] and the biomass is often used for the production of industrial wood and paper. The presence of lignin, hemicelluloses and a high cellulose concentration [10] makes their timber comparable to that of hard woods [11], considered to be suitable as solid biofuels. As a good source for energy, they are burned directly for heat, used to produce electricity and ethanol, and used to generate gas [12].

Species of the *Paulownia* genus are proposed for afforestation projects since they are highly adaptive to a wide range of climate and soil conditions [13]. They are often planted along roadsides and as park trees and used for agro-forestry plantations.

Paulownia spp. are known as fodder plants whose aboveground parts contain high levels of useful nutrients [14]. 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. 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 [12],[15].The leaves are also utilised as green manure, particularly in China [10].

Paulownia spp. have a range of medicinal properties [16]. The bark, leaves and flowers are used in traditional Chinese medicine to treat infections and inflammatory diseases [17], 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 for perfumes and cream production [15]. Moreover *Paulownia* spp. are a good nectar and honey producers [12],[10],[18].The honey has a light colour and a high quality. It is often used to treat respiratory disorders, lung problems and the digestive system [15].

Paulownia spp. are very promising, in particular for the afforestation of trace element-contaminated soils. According to many authors [19],[20],[21],[22] it could be used for remediation purposes. Recent studies have shown that some *Paulownia* species have a relatively high tolerance to the presence of a high concentrations of trace elements [23].Their ability to concentrate the radionuclides can be used to clean contaminated soil and research has been conducted by Ukrainian scientists in order to examine this ability [24]. Samples of *Paulownia* Clone 112 in Vitro, planted in the villages of Parishev, Rudnia-Illintsi, Kopachi and the town of Chernobyl are giving information on the current state of radioactive contamination and soil structure. It is known that fast growing *Paulownia* spp. absorb radionuclides much faster than any other tree, so Ukrainian scientists are going to use this characteristic to clean radioactive substances from the soil.

Nematological research on *Paulownia tomentosa* Steud in Poland was influenced by the detection on the roots of the root-knot nematode *Meloidogyne hapla*, a serious pest of many economically important plants [25]. Galled roots of *Paulownia* could be extremely susceptible to other soil pathogens: bacteria, fungi and more species of soil and foliar nematodes. Two trophic groups of plant parasitic nematodes need to be identified as inhabiting the root zone of *Paulownia* plants:

1. Migratory ectoparasites belonging to three families, Longidoridae, Xiphinematidae. Trichodoridae are known as vectors of plant viruses. The main feature noted in *Paulownia* plantations is a susceptibility to viruses and phytoplasmatic organisms (MLO), known as witches' brooms in Australia [26] and in China [27], [28].

2. Migratory endoparasites Pratylenchidae which cooperate during feeding migrations with many other pathogens, such as bacterial and fungal diseases, inside the cortical cells of the roots.

In order to obtain factual information we started with a survey and the results are shown below.

Material and methods

The aim of the survey was to gain knowledge of the parasites and pathogens which are serious enemies to *Paulownia* plantations in Poland. Soil samples with fragments of plant roots were collected from a 3 – 6 year-old plantation in:

- Żołynia (50.09° N, 22.15° E) West-Southern Poland,
- Rzeszów (50.01° N, 14.55° E) West-Southern Poland,
- Dębogórze (54.59° N, 18.44° E) Baltic Coast,
- Kudypy (53.77° N, 20.38° E) Warmia-Mazuria,
- Tomaszki (53.46° N, 20.95° E) Warmia-Mazuria,
- Szczecin (53.43° N, 14.55° E) West-Northern Poland,
- Wrocław (51.10° N, 17.07° E) Silesia.

Soil samples were collected from the rhizosphere at a depth of 45 cm. Number of samples: 24.

The recovery of nematodes was made in two steps, according to the different types of plant-parasitic nematodes. The larger nematodes (Longidoridae) and migratory endoparasitic Pratylenchidae were extracted from 50-gram samples of the roots by the Baermann method (incubation on a sieve for 4 days), while smaller sized species were extracted from 100 cm³ soil samples by the centrifugation method (Szczygieł, 1971) [29]. Nematodes obtained from incubation and centrifugation were combined in water and killed by adding a similar amount of 6% hot formalin. After processing to glycerin by the Seinhorst (1959) [30] method, permanent slides of nematodes were made. Nematode species were identified at the species level based on the morphological traits of male and female individuals following the identification keys developed by Brzeski (1998) [31] and Andrassy (2007) [32]. A species dominance index C was calculated as follows: $C = (NaN^{-1}) \times 100\%$, where: Na = number of samples containing a given species, N = total number of samples.

The nematode species analysed were classified on the basis of the calculated values of index C: (i) 0-25%: occasional species, (ii) 26-50%: accessory species, (iii) 51-75%: dominant species.

The nematodes were also divided into the following trophic groups, according to the classification proposed by other authors [33], [34], [35], [36], [37] and the system approved by Fauna Europaea [38] as follows:

- A1: migratory endoparasites (Pratylenchidae) [37]
- A2: semi-endoparasites (Hoplolaimidae),
- A3: ectoparasites and A3,V: vectors of plant viruses (nematode species belonging to the families Longidoridae, Xiphinematidae, and Trichodoridae [39], [40],
- A4: sedentary parasites,
- V: vectors of viral infections,
- F: hyphal and root hair feeders.

For every species found in each sample (consisting of specimens isolated from 100 cm³ of soil by centrifugation, and the individuals isolated on Baermann sieves), the following parameters were calculated:

- population density (number of individuals in 100 cm³ of soil or 50 g of roots),
- frequency of occurrence (number of occurrences of a species out of the total number of 24 samples), expressed in %.

Results

The nematode species identified in the 24 samples from the *Paulownia* spp. root-zone are presented in Tab.1. The population density in 100 cm³ of soil and the frequency of occurrence were determined for each species. Based on the values of the C index, from the 20 species of plant parasitic nematodes listed, there were 11 species classified as occasional (frequency of occurrence < 25%) and 9 species were classified as accessory (frequency of occurrence 29-42 %).

The species that appeared most frequently were: *T. maximus* (Allen, 1955) (Fig. 1), *R. buxopilus* (Golden, 1956) (Fig. 4) and *M. hapla*. and in addition, morphological observations revealed the presence of features typical for *M. hapla*. By equating the P_f/P_i 0.1 value obtained to the scale [41], it appears that empress tree is a poor host for *M. hapla* [25].

The presence of *P. fallax* was noted in high population densities (a migratory endoparasite which is harmful due to phytotoxins excreted to the plant during its feeding in cortical tissues).

The nematode vectors of plant viruses, which are accessory species in the *Paulownia* root zone, were from the genus *Trichodorus*: *T. viruliferus* (Hooper, 1963) and *T. sparsus* Szczygieł, 1968 (Fig.2), which were both identified at a 29% frequency. The species are able to transfer several TOBRA viruses to the roots of healthy plants. *L. attenuatus* (Hooper, 1961) (Fig.3) and *L. intermedius* Kozłowska & Seinhorst, 1979 were found from the genus *Longidorus* which transfers NEPO plant viruses. Nematode vectors of plant virus diseases feed as migratory ectoparasites on the roots of plant hosts, producing small galls on the tips of the roots. Some necrosis of cortical cells grouped in rows was observed.

Tab. 1 Species of plant feeders collected from seven plantations of *Paulownia tomentosa* in Poland.

Species	Trophic type	Nematodes/cm ³		Frequency of occurrence (%)	Species dominance status
		Range	Mean±SD		
TRICHODORIDAE					
<i>Paratrichodorus Siddigi, 1974</i>					
<i>pachydermus</i> Seinhorst, 1954	A3,V	6-22	13.0 ± 0.6	17	O
<i>Trichodorus Cobb, 1910</i>					
<i>cylindricus</i> Hooper, 1962	A3,V	8-12	10.0 ± 1,1	8	O
<i>viruliferus</i> Hooper, 1963	A3,V	4-28	14.0 ± 2.5	29	A
<i>sparsus</i> Szczygieł, 1968	A3,V	12-16	14.0 ± 1.1	8	O
LONGIDORIDAE					
<i>Longidorus Micoletzky, 1922</i>					
<i>attenuatus</i> Hooper, 1961	A3,V	4-27	13.8 ± 2.3	29	A
<i>intermedius</i> Kozłowska&Seinhorst, 1979	A3,V	4-12	8.0 ± 2.3	8	O
CRICONEMATIDAE					
<i>Criconemoides Taylor, 1936</i>					
<i>informis</i> Micoletzky, 1922	A3	3-32	13.0 ± 4.9	17	O
<i>Criconema Hofmann & Menzel, 1914</i>					
<i>annuliferum</i> de Man	A3	2-54	18.0 ± 9,31	17	O
PARATYLENCHIDAE					
<i>Paratylenchus Micoletzky, 1922</i>					
<i>projectus</i> Jenkis, 1960	A3	12-44	24.0 ± 5.47	17	O
TELOTYLENCHIDAE					
<i>Bitylenchus Filipjev, 1934</i>					
<i>dubius</i> Butschli, 1873	A3	8-87	45.00 ± 9.56	29	A
<i>maximus</i> Allen, 1955	A3	3-42	13.0± 3.6	42	A
<i>Scutylenchus Jairajpuri, 1971</i>					
<i>quadrifer</i> (Andrassy, 1974)	A3	6-96	55.0 ± 14.3	21	O
<i>Meloidogyne Goeldi, 1892</i>					

<i>Meloidogyne hapla</i> Chitwood, 1949	A4	6-98	49.0 ± 11,7	33	A
HOPLOLAIMIDAE					
Helicotylenchus Steiner, 1945					
<i>pseudorobustus</i> Steiner, 1914	A2	12-90	51.0 ± 8.47	29	A
Rotylenchus Filipjev, 1936					
<i>agnetis</i> Szczygieł	A2	14-118	57.0 ± 13,9	25	O
<i>buxophilus</i> Hooper	A2	14-78	44.0 ± 6.6	38	A
Pratylenchus Filipjev, 1936					
<i>fallax</i> Steinhorst, 1968	A1	16-124	65.0 ± 10.95	29	A
<i>neglectus</i> Rench, 1924	A1	8-88	43,50 ± 13.07	29	A
Pratylenchoides Winslow, 1958					
<i>laticauda</i> Braun&Loof, 1967	A1	12	12.0 ± 0.0	4	O
Hirschmanniella Luc & Goodey, 1964					
<i>gracilis</i> de Man, 1880	A1	6	6.0 ± 0.0	4	O

A1- migratory endoparasites: A2- semi endoparasites, A3-ectoparasites, A4-sedentary parasites, V- vectors of plant viruses.

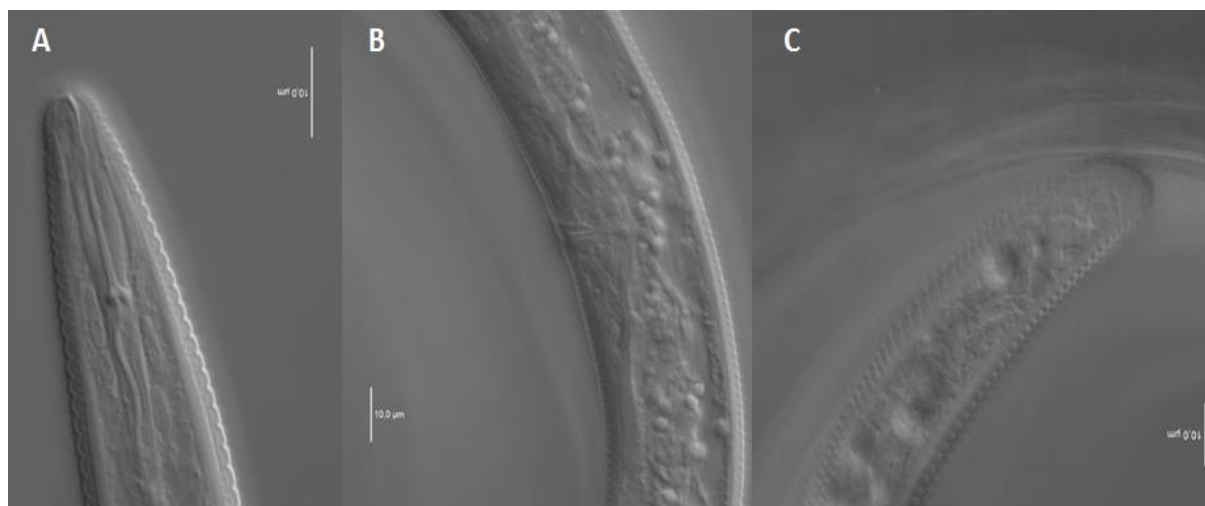


Fig.1. *Tylenchorhynchus maximus* (Allen, 1955). A-head, B-vagina, C- tail.

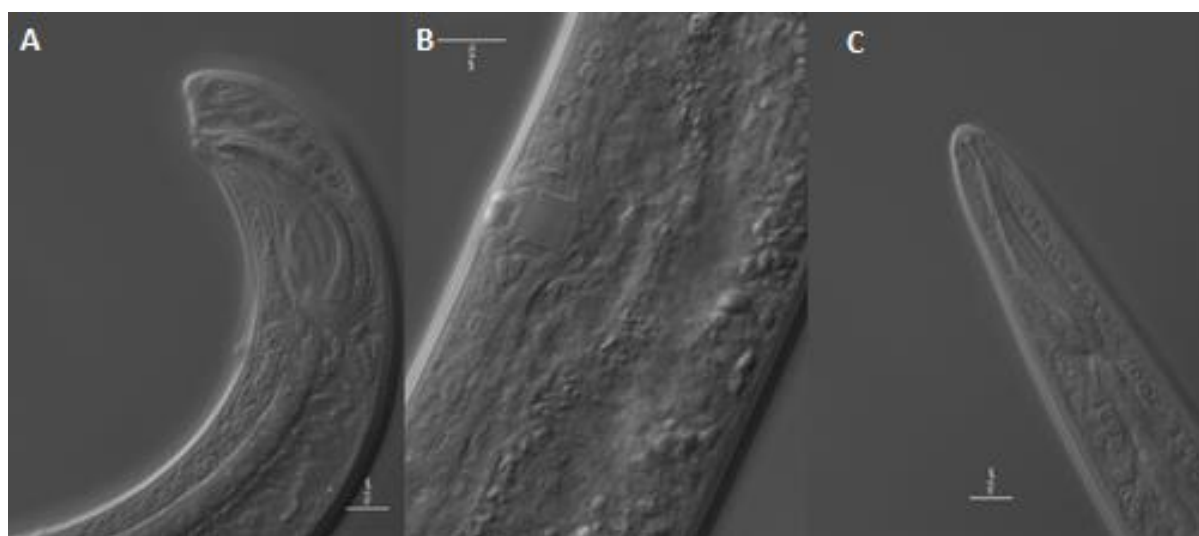


Fig.2. *Trichodorus sparsus*. A-spicule, B-vagina, C-stylet

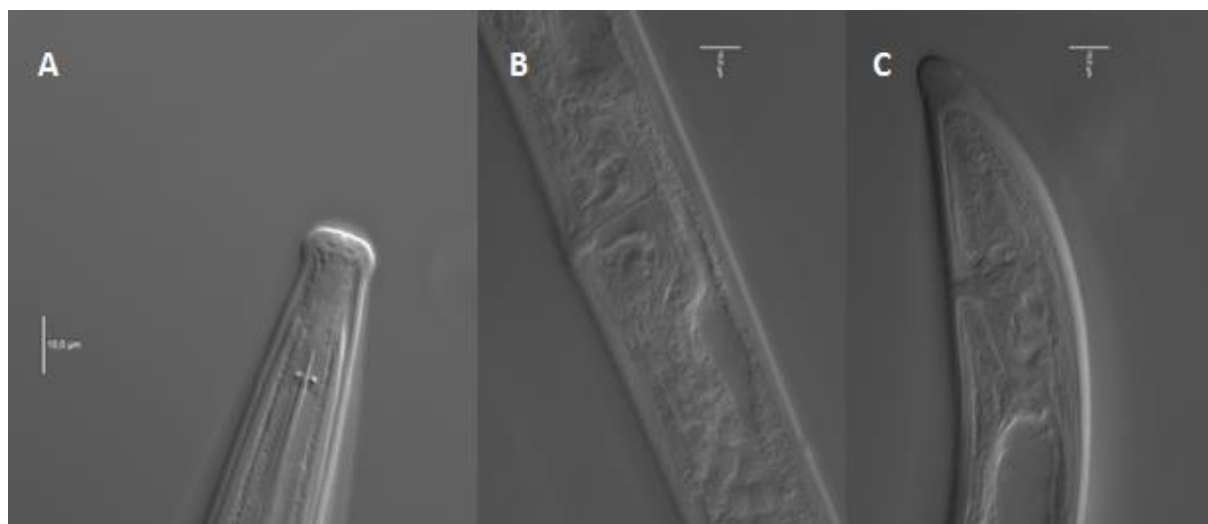


Fig.3. *Longidorus attenuatus* Hooper, 1961. A-head B-vagina, C-tail

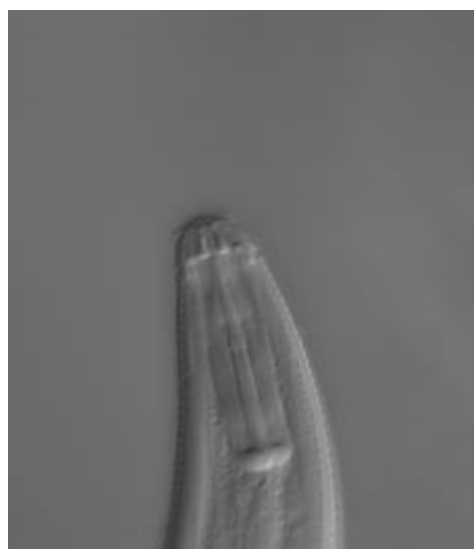


Fig.4. *Rotylenchus buxopilus* (stylet)

Discussion

Meloidogyne spp. are some of the most destructive nematodes in agriculture, having a wide host range. About ten species of *Meloidogyne* are known to be a serious pest of major food crops, vegetables, fruit and ornamental plants in temperate, tropical and subtropical regions [42], [43], [44], [45]. However, several species of root-knot nematode were already noted in the roots of *Paulownia* spp. plants in Europe, Asia and America [46], but there is still a lack of information on migratory soil nematode species. *Meloidogyne* spp. are difficult to control due to their high rate of reproduction. Symptoms are seen in the roots, leaves, and the overall growth of the infected plant. Abnormal small and spherical growths called galls are formed, situated near small roots. They are formed when nematodes enter the root and release chemicals on the root cells on which they feed. Defective roots can no longer transport water

and nutrients properly and the end result is stunted, wilted, yellowing plants and a decreased 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 increases as well. However, the use of a combination of control practices, known as an integrated pest management plan, has proved to be effective.

Paulownia spp. are susceptible to witches' broom disease caused by a mycoplasma-like organism (MLO). Whereas little was known regarding potential disease issues for *Paulownia* spp. in Australia [26], those in China were attacked by witches' broom where the MLO was observed in phloem [28]. It was reported that *Paulownia*'s witches' broom disease in Asian plantations was vectored by the insect *Halymorpha halys* Uhler (Brown-marmorated stink bug) [27], however this disease can also be caused by fungi, oomycetes, insects, mistletoe, dwarf mistletoes, mites, phytoplasmas, viruses and nematodes. The effects of *Paulownia* witches' broom disease vary depending on the *Paulownia* species. It mostly affects branches, the trunk, flowers and roots, leading to premature death. *Paulownia* spp. are susceptible to classic virus diseases like CMU (cucumber mosaic virus) [47] TRV (tomato rattle virus) and EMV (elm mottle virus) [48] previously vectored by aphids and secondarily from diseased roots by nematodes from the virus vector groups: Longidoridae, Xiphinema and Trichodorus which frequently inhabit Polish soils.

Anthracnose, a fungal disease known as leaf blight, *Sphaceloma Paulowniae* and Mistletoe (*Loranthus* spp.) can also cause some considerable damage in *Paulownia* spp. (Lyons, 1993).

The *Paulownia tomentosa* L. revealed a susceptibility to cucumber mosaic virus and tomato rattle virus. Even though the semi-endoparasitic nematodes *Rotylenchus agnetis* Szczygieł, 1968 were frequent in the root zone, the 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 *Paulownia* plantations in Poland.

Paulownia tomentosa L. has been cultivated in the south-eastern region of Poland for a long time. The most common species within the genus are *P. fortunei*, *P. kawakamii*, *P. taiwaniana*, and *P. tomentosa*. Out of a dozen root-knot nematode species which parasitise *Paulownia* spp. the most often mentioned are: *M. incognita*, *M. javanica* and *M. arenaria* [49], [50], [51], [52], [53], [54]. Galled roots of *Paulownia tomentosa* Steud were collected from a plantation in Poland during the growing season. A morphological, morphometric and molecular (28S rDNA, species-specific SCAR primers and 5S-18S rDNA primers) study revealed the presence of *Meloidogyne hapla* in root systems and root zones. In a pot

experiment conducted under controlled conditions, *M. hapla* completed its life cycle within the root tissues of *Paulownia*. To date, this is the first record of a nematode parasitising *Paulownia* in Poland after a routine inspection of *Paulownia* plantations where roots with outgrowths, typical of the presence of *Meloidogyne*, were observed.

The purpose of this study was to determine the plant parasitic nematode fauna in Polish plantations. Based on our knowledge of their susceptibility to virus diseases, the plant virus vectors inhabiting the root zone, i.e. the genus *Longidoridae* and *Trichodoridae*, were surveyed for the first time. This should draw the attention of agricultural practitioners and plant protection specialists to the interaction of *Paulownia* cropping and *M. hapla* population density in the soil.

Conclusions

1. It is necessary to analyse the soil for nematode fauna which are potential pests of *Paulownia* plantations.
2. A wide-ranging search of nematode species which are vectors of plant virus diseases needs to be carried out before planting new plantations (PCR analysis could find mycoplasmas or virus diseases in plants).

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