Population Dynamics of *Bursaphelenchus lignicolus* (Nematoda : Aphelenchoididae) and *B. mucronatus* in Pine Seedlings¹

Kazuyoshi FUTAI

Laboratory of Applied Botany, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan

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Two species of *Bursaphelenchus* nematode which were different in their pathogenicity to pine trees were inoculated onto the seedlings of *Pinus thunbergii* and *P. densiflora*, and dispersion and population density of both nematodes in the inoculated seedlings were examined once a week for 8 weeks. Pathogenic *B. lignicolus* dispersed and propagated rapidly in all inoculated seedlings of *P. thunbergii* but failed to disperse and propagate in some of the inoculated seedlings of *P. densiflora*. On the other hand, non-pathogenic *B. mucronatus* hardly propagated in both pines except in a few seedlings though nematodes of this species could disperse as well as *B. lignicolus*. Rapid dispersion accompanied with propagation of nematodes always resulted in weakening or death of hosts irrespective of the species of pine and nematode examined, though a few dead seedlings without so many nematodes were observed 6–8 weeks after inoculation.

INTRODUCTION

In order to compare the resistance of a particular plant to different species of nematodes, or to compare the pathogenicity of a particular species of nematode to different plants, the rate of reproduction of the nematode has been examined with many nematode species (JONES, 1956; SEINHORST, 1967; VANGUNDY and KIRKPATRICK, 1964). Population growth of *Bursaphelenchus lignicolus*, the causal agent of the wilting disease of pines, in seedlings of *Pinus thunbergii* and *P. densiflora* has been studied by MAMIYA (1974) in relation to the water content of the hosts. Dispersion of inoculated *B. lignicolus* in wood of *P. thunbergii*, *P. densiflora* or in *P. taeda* was also investigated in connection with the reduction of oleoresin exudation which is a well known symptom of the tree diseased by *B. lignicolus* (HASHIMOTO and DOSONO, 1975). In the present investigation, the population growth and the distribution pattern of *B. lignicolus* in seedlings of *P. thunbergii* or *P. densiflora* were compared with those of *B. mucronatus*, a non-pathogenic nematode closely related to *B. lignicolus*.

MATERIALS AND METHODS

Three-year-old seedlings (*P. thunbergii* and *P. densiflora*) were inoculated with *B. lignicolus* or *B. mucronatus* in the following manner. After peeling a small part of the

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bark 3 to 6 cm from the base of the stem (about 5 mm wide and 3 cm long), a piece of absorbent cotton was placed on the wound, and a water suspension of nematodes, 2,000 per seedling was poured on it. The nematodes which were reared on *Botrytis cinerea*, extracted with a Baermann funnel, and filtered through a 325 mesh to remove small individuals were used as the inocula. The nematode inoculation was conducted on the 19th of June, 1978 using 60 seedlings for each host-parasite combination, and 5 seedlings of both species inoculated with either nematode were harvested once a week for 8 weeks. After removing leaves, twigs and roots, the surface of the stem was washed, and wiped with tissue paper. The part of the stem 15 cm from the base was weighed for its fresh weight and cut into five short pieces each 3 cm long. The dry weight and the water content of these pieces were calculated from the fresh to dry weight ratio of the remaining part of the stem, on the assumption that the water content at any part of the stem was the same. Every short piece was cut longitudinally into halves. One-half was soaked in 0.5% 2,3,5-triphenyltetrazolium chloride (TTC) solution for 12 hours, and the other half set on a Baermann funnel for 24 hours to extract nematodes. The number of nematodes recovered was counted for each half piece. Using the numbers of nematodes thus counted for five stem pieces, the population density of both pine wood nematodes was calculated for the gram dry weight of each host stem. The viability of the stem was determined from the stainability to TTC (PARKER, 1957). Furthermore, changes in the appearance of inoculated seedlings were observed every three to seven days for 8 weeks with all seedlings not yet served for the experiments on the nematode population and distribution, and the results are shown in Fig. 1 as the percentage of the weakened or dead seedlings to all remainders.

The nematodes used in the present experiments were the same as those mentioned in my previous report (FUTAI, 1980).

RESULTS

Symptoms of inoculated seedlings

As shown in Fig. 1, the inoculation of *B. lignicolus* caused more rapid damages in seedlings of *P. thunbergii* than in those of *P. densiflora*. That is, complete death occurred 49 days after inoculation in *P. thunbergii* but even 57 days after inoculation some seedlings of *P. densiflora* were still alive. On the other hand, the inoculation of *B. mucronatus* caused far less damages in both pine species, i.e. only four seedlings weakened or died in *P. thunbergii*, no symptoms appeared in *P. densiflora* except for one seedling weakened to death (so the results of the latter host-parasite combination was excluded from Fig. 1).

Dispersion and population growth of nematodes in pine stems

Figure 2 illustrates the dispersion pattern of nematodes in the inoculated seedlings. The extent of damage in each seedling examined was judged from appearance and by TTC tissue stainability and represented by 'W' (=weakened) or 'D' (=dead) on each column in Fig. 2. The population density of *B. lignicolus* and *B. mucronatus* in host stem 15 cm long was plotted against the time after inoculation (Fig. 3a) in relation to the changes of water content in the stem (Fig. 3b).

When *B. lignicolus* was inoculated onto *P. thunbergii* every seedling was damaged to death, and such host weakness or death was always accompanied with the rapid

Fig. 1. Changes in percentage of the weakened or dead pine trees after inoculation of *B. lignicolus* and *B. mucronatus*.

■: dead ▨: leaves are almost browned ▩: leaves are faded as a whole ▤: leaves are vaguely faded

Fig. 2. Changes in distribution and population density of *B. lignicolus* and *B. mucronatus* in stems of inoculated seedlings.

Nematode density* is shown in five grades as follows, : 0, : 0—9,

: 10—99, : 100—999, : 1000—

Arrows means the inoculation points. (*: nematode number recovered from each stem piece of 3 cm length)

dispersion and full propagation of nematodes inoculated. Figure 3a,b show that population density of *B. lignicolus* increased with the lowering of water content in the stem of *P. thunbergii*, but when water content dropped to 20 to 40%, population density began to decrease.

In the stem of *P. densiflora*, *B. lignicolus* rapidly dispersed as in *P. thunbergii*, but its propagation was often suppressed and the population density greatly differed from seedling to seedling.

In any seedling of *P. thunbergii* and of *P. densiflora*, *B. mucronatus* dispersed in a similar manner to *B. lignicolus* but failed to propagate in most of such seedlings resulting in gradual decrease in number. Only two seedlings, one each of *P. thunbergii* and *P. densiflora* allowed propagation of *B. mucronatus*, and these seedlings were dead. Another seedling of *P. thunbergii* inoculated with *B. mucronatus* was found to be dead at the 7th week (Fig. 2), but no nematodes were recovered from it.

DISCUSSION

The rate of nematode reproduction in a particular host is determined by various factors concerned in the relationship between the nematode and the plant. Avail-

Fig. 3. (a): Population change of *B. ligniculus* (●) and *B. mucronatus* (○) in the stems of *P. thunbergii* and *P. densiflora*, and (b): changes of water content in stems after inoculation of nematodes. Every seedling of both pine species was inoculated with about 2,000 nematodes and the numbers of extracted nematodes per gramme dry weight of wood at each harvest time are presented.

ability of food, its quality, and the ability of the parasite to secure and ingest it may affect the rate of reproduction (NUSBAUM and BARKER, 1971). The reproductive rates of *B. ligniculus* on different substrates, i.e. cultured fungus (*Botrytis cinerea*), callus tissues of alfalfa or pine, and seedlings of pines in the field are plotted on the same graph (Fig. 4). The data on propagation of the nematode on callus tissues in this figure were quoted from the results of TAMURA and MAMIYA (1975, 1976). This figure shows that the reproductive rate of *B. ligniculus* in pine seedlings is more similar to that on callus tissues than to that on fungus culture (*Botrytis cinerea*). On the other hand, *B. mucronatus*, which can reproduce on *Botrytis cinerea* with a reproductive rate slightly lower than *B. ligniculus* (FUTAI, 1980) could not propagate on pine seedlings.

Reproductive rate of *B. ligniculus* in *P. densiflora* greatly differed individually, but was lower on the average than that in *P. thunbergii* (Fig. 3). This seems to be in rela-

Fig. 4. Population growth of *B. lignicolus* on various substrates, i.e., cultured fungus *Botrytis cinerea* (at 29°C: ○---○ at 26°C: ●—●), callus tissues of alfalfa (×AC) or of *P. densiflora* (×PC), and seedlings in the field (*P. thunbergii*: ○—○).

tion with the results that the symptoms advanced more slowly in *P. densiflora* than in *P. thunbergii* (Fig. 1), and might be in support to the suggestion that *P. densiflora* has a slightly higher resistance to *B. lignicolus* than *P. thunbergii* (OHYAMA et al. 1974; OHBA et al. 1977; FUTAI and FURUNO, 1979). MAMIYA (1974) reported that *B. lignicolus* can propagate in seedlings of *P. densiflora* and in those of *P. thunbergii* at a similar rate, but the present data with *P. densiflora* slightly differed from MAMIYA's. While the cause of such difference is unknown, the fact that the inoculation density used here (2,000/seedling) was lower than that used by MAMIYA (5,000/seedling) might be taken into consideration.

Whether or not endoparasitic nematodes such as *B. lignicolus* can disperse in host plant seems to be another important factor related to the resistance of host. HASHIMOTO and DOSONO (1975) found that *B. lignicolus* rapidly dispersed in the stem of *P. thunbergii* and *P. densiflora* but its dispersion was apt to be suppressed in *P. taeda*. Results obtained in the present study also showed rapid dispersion of *B. lignicolus* in seedlings of *P. thunbergii* and *P. densiflora*. The early dispersion of *B. mucronatus* in such seedlings was similar to that of *B. lignicolus*.

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REFERENCES

- FUTAI, K. (1980) Developmental rate and population growth of *Bursaphelenchus lignicolus* (Nematoda: Aphelenchoididae) and *B. mucronatus*. *Appl. Ent. Zool.* **15** : 115–122.
- FUTAI, K. and T. FURUNO (1979) The variety of resistances among pine-species to pine wood nematode, *Bursaphelenchus lignicolus*. *Bull. Kyoto Univ. For.* **51** : 23–36 (in Japanese with an English summary).
- HASHIMOTO, H. and Y. DOSONO (1975) Dispersion and reproduction of *Bursaphelenchus lignicolus* in resistant or in susceptible pines. *Trans. Mtg. Jap. For. Soc.* **86** : 301–302 (in Japanese).¹
- JONES, F. G. W. (1956) Soil populations of beet eelworm (*Heterodera schachtii* Schm.) in relation to cropping. *Ann. appl. Biol.* **44** : 25–56.
- MAMIYA, Y. (1974) Population increase of *Bursaphelenchus lignicolus* in wood of pine seedlings in relation with the time after inoculation. *Trans. Mtg. Jap. For. Soc.* **85** : 249–251 (in Japanese).
- NUSBAUM, C. J. and K. R. BARKER (1971) Population dynamics. in *Plant Parasitic Nematodes* (ed. ZUCKERMANN, B. M., W. F. MAI, and R. A. ROHDE) New York: Academic Press, Vol. I, 345 pp.
- OHBA, K., K. NISHIMURA and T. TODA (1977) Difference in survival rates among various pine races inoculated with pine wood nematodes (*Bursaphelenchus lignicolus*). *Trans. Mtg. Kyushu Branch Jap. For. Soc.* **30** : 69–70 (in Japanese).¹
- OHYAMA, N., K. KAWANOBE and A. SAITO (1974) The resistance of *Pinus densiflora*, *P. thunbergii*, *P. taeda* and *P. elliotii* to pine wood nematode (*Bursaphelenchus lignicolus*). *Trans. Mtg. Kyushu Branch Jap. For. Soc.* **27** : 77–78 (in Japanese).¹
- PARKER, J. (1957) Seasonal changes in some chemical and physical properties of living cells of *Pinus ponderosa* and their relation to freezing resistance. *Protoplasma* **48** : 147–163.
- SEINHORST, J. W. (1967) The relationship between population increase and population density in plant parasitic nematodes. (III), (IV) *Nematologica* **13** : 429–442.
- TAMURA, H. and Y. MAMIYA (1975) Reproduction of *Bursaphelenchus lignicolus* on alfalfa callus tissues. *Nematologica* **21** : 449–454.
- TAMURA, H. and Y. MAMIYA (1976) Reproduction of *Bursaphelenchus lignicolus* on pine callus tissues. *Trans. Mtg. Jap. For. Soc.*, **86** : 229–230 (in Japanese).
- VANGUNDY, S. D. and J. D. KIRKPATRICK (1964) Nature of resistance in certain citrus rootstocks to citrus nematode. *Phytopathology* **54** : 419–427.

¹ Title translated by the present author,