マツノザイセンチュウに感染したクロマツ苗木に生成される異常代謝産物

誌名	日本線虫学会誌
ISSN	09196765
著者	二井, 一禎
巻/号	33巻2号
掲載ページ	p. 45-56
発行年月	2003年12月

Abnormal metabolites in pine wood nematode-inoculated Japanese black pine

Kazuyoshi Futai[1]

Spectrophotometer, paper chromatography, and liquid chromatography were used to isolate and characterize the substances associated with tissue browning of Japanese red pine, (*Pinus densiflora*) and Japanese black pine (*P. thunbergii*) inoculated with *Bursaphelenchus xylophilus* or *B. mucronatus*. Discoloration, as determined by optical density, and numbers of *B. xylophilus* was highest near the inoculation site and decreased with increasing distance from that point. Absorption spectra of water extracts from pines infected with *B. xylophilus* had a distinct peak at 278 nm. The absorption at 278 nm decreased when the extract was oxidized and shifted to 287 nm when 1N NaOH was added, suggesting that the extract contained polyphenolic substances. The increase in browning with increase in nematode population indicates that these substances were related to the nematode infection. *B. xylophilus*-inoculated seedlings contained much greater amounts of phenolics than *B. mucronatus*-inoculated and control seedlings. A catechin-like substance was the most abundant polyphenolic associated with large numbers of *B. xylophilus*. Changes in the amount of condensed tannin, the polymer of catechin, were surveyed in relation to the numbers of nematodes in pine seedlings. Jpn. J. Nematol. 33 (2), 45-56 (2003).

Key words: *Bursaphelenchus xylophilus*, condensed tannin, *Pinus densiflora*, *P. thunbergii*, tissue browning.

Polyphenolic substances that cause tissue browning are known to accumulate in injured or pathogen-infected plant tissues (Giebel, 1970; Kosuge, 1969). The pine wood nematode (PWN), *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle, 1970, the pine wilt disease pathogen, is associated with browning of pine tissues (Mamiya, 1980; Myers, 1986), suggesting that infection results in the accumulation of polyphenolic substances. When infected pine tissues were placed in distilled water overnight to extract nematodes, the water became yellowish-brown, whereas, water in which healthy pine tissues were soaked remained clear.

Oku et al. (1979) and Shaheen et al. (1984) found unique metabolites in the tissues of PWN-inoculated pines, some of which were toxic to pines. Their observations suggest that pine wilt disease may result in part from the production of such metabolites. In the present study, some of the substances associated with browning of PWN-infected pine tissues were isolated and characterized. The changes in the amount of the substances were also related to the numbers of PWNs in host tissues.

[1] Laboratory of environmental mycoscience, Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake-cho, Sakyo-ku, Kyoto 606-8502, Japan.

MATERIALS AND METHODS

Isolates of *B. xylophilus* (pathogenic) and *B. mucronatus* Mamiya and Enda, 1979 (non pathogenic) obtained from the Kansai Branch of the Forestry and Forest Products Research Institute, Kyoto, Japan, were maintained for more than 10 years on the fungus *Botrytis cinerea* (Fr.) Pers, growing on potato dextrose agar. The trees used in the experiments were Japanese red pine (*Pinus densiflora* Sieb. and Zucc.) and Japanese black pine (*P. thunbergii* Parl.) growing in field plots at Kyoto University.

Water extracts from pine wood:

Seven 8-year-old Japanese black pines were inoculated with *B. xylophilus* as follows: Four branches (7 - 9 mm d) of the same whorl on each tree were cut just below the current year's internodes; a cotton swab was placed on each cut end; and 1 ml water containing 2,000 *B. xylophilus* was pipetted onto each piece of cotton. The inoculum was extracted from 7 - 10 day-old fungus cultures by the Baerman funnel method (Baermann, 1917). One branch on each of the seven trees, located on the same whorl as the inoculated branches, served as a control. This branch was treated with 1 ml distilled water. On days 2, 4, 8, and 12 after inoculation, one inoculated branch was cut from each tree. The needles were removed and the surface of the branch was washed with water, and then the apical 20 cm of the branch was cut into 10 segments (each 2 cm long). Each segment was then sliced into small pieces and immersed for 2 days in 10 ml distilled water to extract the nematodes. The nematodes from each segment were counted. The volume of water containing pine tissue exudates (designated water extract) was adjusted to 15 ml with distilled water, and the optical density at 450 nm ($O.D._{450}$) was measured with a Shimadzu UV-100-02 spectrophoto-meter. Controls were harvested 8 days after inoculation and the optical density of their extracts determined.

Absorption spectrum of pine tissue exudates:

Five 4-year-old Japanese black pines were inoculated with 2,000 *B. xylophilus* and five with 2,000 *B. mucronatus* as in the first experiment. Five control trees received 1 ml distilled water. Three weeks after inoculation, the distal 15 cm of each inoculated terminal shoot was removed and cut into five segments (3 cm long). Each segment was sliced into thin disks and immersed for 24 hours in 10 ml distilled water. The volume of the water extracts was adjusted to 10 ml with distilled water and then diluted fivefold. The absorption spectra of the water extracts were determined at 250-700 nm using a Hitachi 305 double beam spectrophotometer.

Paper chromatography of extracts from pine tissues:

Five 8-year-old Japanese black pines and five 8-year-old Japanese red pines were inoculated with 2,000 *B. xylophilus*. Three weeks after inoculation, the inoculated branch (7-9 mm d) was collected from each tree for analysis. Five grams fresh weight of bark was peeled from each branch, sliced into 1-mm thick pieces, and homogenized for 10 minutes in 10 ml methanol. The homogenate was diluted to 100 ml with methanol and incubated for 24 hours. Each homogenate was filtered through Toyo No. 2 filter paper, and 10 ml filtrate was evaporated to dryness under reduced pressure at 40℃ using a rotary vacuum evaporator. The residue was suspended in 5 ml distilled water and partitioned with 5 ml ethyl acetate. The ethyl acetate fraction was rotary evaporated to dryness under reduced pressure at 40℃, and the residue was dissolved in 1 ml methanol. Fifty μl of this solution was examined by ascending paper chromatography developed with butanol : acetic acid : water (3 : 3 : 1) for 20 hours on 50 cm long Toyo No. 52 filter paper at room temperature. The Rf value, the color produced by spraying the paper with

2.5% FeCl, and the ultraviolet fluorescence of the separated materials were determined for each spot on the chromatograms. Control samples from similar healthy Japanese black or red pines, were examined for comparison. Chromatograms were developed in the same way as those for extracts of pine tissues, and absorption spectra of the authentic phenolics - catechin, gallic acid, flavones, protocatecuic acid, and tannic acid (10 mg of each dissolved in 10 ml of methanol and then evaporated to dryness and partitioned with ethyl acetate) - were compared with those of PWN-infected pine extracts.

Liquid chromatographic analysis of methanol extracts from nematode-inoculated pine tissue:

Five-year-old, nursery-grown *Pinus thunbergii* trees were inoculated with either 2,000 *B. xylophilus* or *B. mucronatus* nematodes as follows: the terminal shoot of a seedling was cut off below the current year's growth, and 1 ml of water containing the nematodes was pipetted onto the cut surface. Control seedlings received 1 ml of nematode-free, distilled water. The inoculations were made on 16 June, and three seedlings per treatment were harvested 3, 8, 16, 22 and 27 days after inoculation. At those times, the seedlings were cut off at ground line and the branches and needles were removed and stored at −20℃ until assayed. For the assay, the plant materials were defrosted at room temperature for 2 hours, and then beginning at the inoculation site the stem was cut into five, 3 cm-long segments. A gram of stem tissue from each segment was sliced into 1 mm thick pieces, and immersed in 10 ml of methanol for 48 hours. The methanol fraction was filtered with Toyo No. 2 filter paper, and evaporated to dryness under reduced pressure at 80 °C. The dried extract was dissolved with 5 ml of distilled water and partitioned with 5 ml of ethyl acetate. The ethyl acetate fraction was rotary evaporated to dryness. The dried residue was dissolved in 10 ml of methanol, and then 1μl of the solution was used for injection into a High Pressure Liquid Chromatography (HPLC) system (HITACHI 655 Liquid chromato-graph) equipped with a fluorometric detector (HITACHI variable wavelength UV monitor) and Zorbax ODS column (DuPont Co. Ltd). For liquid chromatographic analysis, a 1 μl sample was eluted with 45% methanol supplemented with 3% n-buthanol and 2% acetic acid at a flow-rate of 1.5 ml/minute. HPLC chromatograms of gallic acid, catechin, chlorogenic acid, cafeic acid, coumaric acid, benzoic acid, cinnamic acid and quercetin, were also examined and compared to those of the metabolites found in the nematode-inoculated and control seedlings.

Dynamics of catechol tannin and nematode populations in pines:

Four-year-old, nursery-grown *P. thunbergii* were inoculated with nematodes in June, as described, and five seedlings were harvested from each treatment and control 2, 6, 13, 20 and 27 days after inoculation. The stem was cut into five, 3 cm long segments as previously. These segments were sliced into 1 mm thick pieces and the nematodes were extracted using the Baermann funnel technique. After 18 hours the nematodes were counted and the concentration of condensed tannins in the water from the Baermann funnel was measured using the method of Ohshima and Nakabayashi (1952).

RESULTS

Water extracts from pine wood:

Both the numbers of nematodes extracted from shoot segments and the $O.D._{450}$ of the water extracts were highest in segments nearest the inoculation site and decreased with increasing distance from the site (Fig. 1). The $O.D._{450}$ value of the water extract from each segment increased with incubation time. Nematode numbers increased between 8 and 12 days. Even after 8 days water extracts from control trees

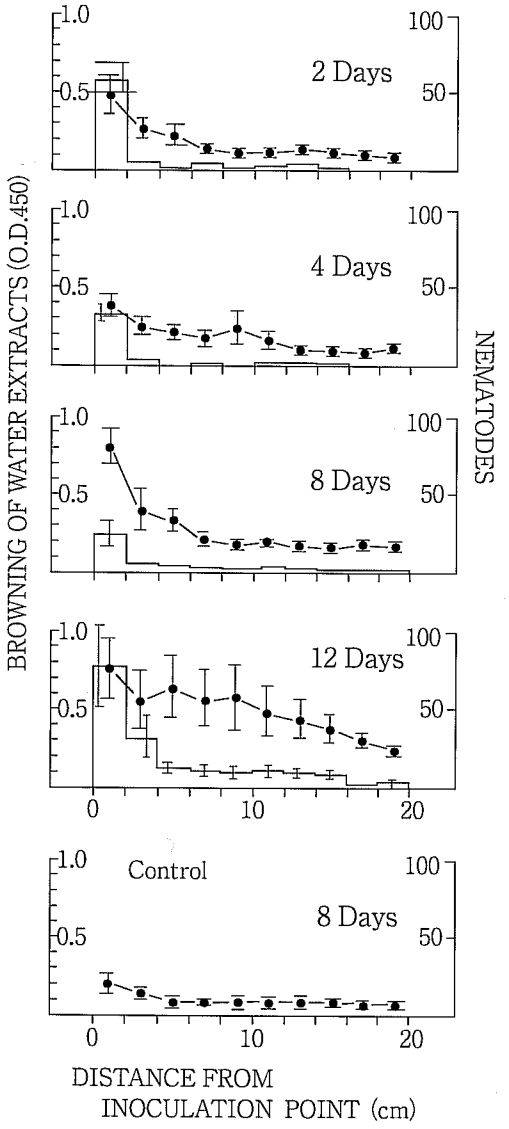

Fig. 1. Numbers of *B. xylophilus* extracted from shoot segments (columns) of *B. xylophilus*-inoculated *P. thunbergii* and O.D.$_{450}$ of water extracts (●) of the shoots (apical 20 cm from the inoculation site). Data are means of seven replicates, and the vertical lines show standard errors.

showed very low O.D.$_{450}$ values. Although there was not a close relationship between the O.D.$_{450}$ and nematode numbers (r=0.34, n=280, $p < 0.05$), the results suggest that these materials accumulate as numbers of *B. xylophilus* increase.

Absorption spectrum of pine tissue exudates:

Water extracts of tissues from infected pines had a distinctive absorption maximum at 278 nm. Extracts from segments nearest the inoculation site had the highest peak, and the peak became lower with increasing distance from the inoculation site (Fig. 2). For *B. mucronatus*, the segment containing the inoculation site had the highest peak and the peaks for

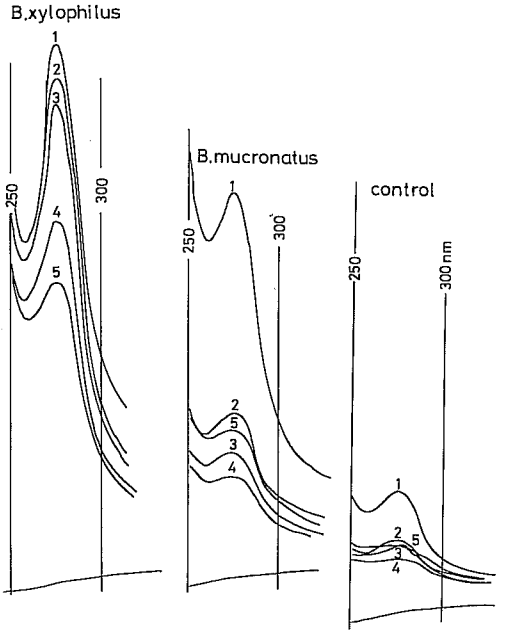

Fig. 2. Absorption spectra (at 278 nm) of extracts from *P. thunbergii* inoculated with *B. xylophilus*, *B. mucronatus* or treated with distilled water (control). The number on each peak is the segment number representing distance from the inoculation site (segment 1= 0-3 cm; 2 = 3-6 cm; 3 = 6-9 cm; 4 = 9-12 cm, 5 = 12-15 cm from the inoculation point).

other segments were much lower. Absorption spectra of water extracts from control trees that received distilled water also had a peak at 278 nm, but the absorption peak was much lower, and did not change with distance from the treatment site.

Water extracts from stem segments inoculated with *B. xylophilus* that were left for 3 - 4 days at room temperature darkened in color especially near the surface of the solution, and their absorption at 278 nm decreased. When sodium borohydride, a reducing agent with no absorption at 250 - 700 nm, was added to the solution, however, the color of the solution became pale and its absorption peak rose. This suggests the presence of some easily oxidized substance. Addition of several drops of 1N NaOH to the solution resulted in a bathochromic shift of the absorption to 289 nm, suggesting the presence of polyphenolic substance(s).

Paper chromatography of extracts from tissues:

The ethyl acetate fraction of methanol extract from the bark of infected and healthy pines produced four distinct spots on the chromatograms. The most distinct difference between the chromatograms was the larger size and deeper color (developed by a FeCl spray) in the spot of which Rf is 0.53 on chromatograms of infected pine extracts. Based on the Rf value, color, fluorescence under ultra violet light, and the absorption spectrum, the spot of the infected-pine chromatograms might be catechin; however, its Rf value (0.53) differed slightly from that of authentic catechin (0.57).

Liquid chromatographic analysis of extracts from nematode-inoculated pine tissue:

As shown in Fig. 3, the pattern of HPLC chromatograms from *B. xylophilus*- and *B. mucronatus*-inoculated and control seedlings were similar, and both chromatograms showed two distinct and many trivial peaks. After inoculation, the heights of all peaks increased simultaneously. To determine the metabolic response to nematode infection, heights of a peak (peak-1) were measured in chromatograms for *B. xylophilus*- or *B. mucronatus*- inoculated and control trees. The results are shown in Fig. 4. Eight days after inoculation, heights of peak-1 increased conspicuously throughout *B. xylophilus*-inoculated stems, while heights of peak-1 increased only slightly in the *B. mucronatus*-inoculated and control trees.

For the eight authentic phenolics examined, retention times of the peaks for catechin, gallic acid and chlorogenic acid coincided with that of the peak-1 in chromatograms for extracts from nematode-inoculated and control seedlings.

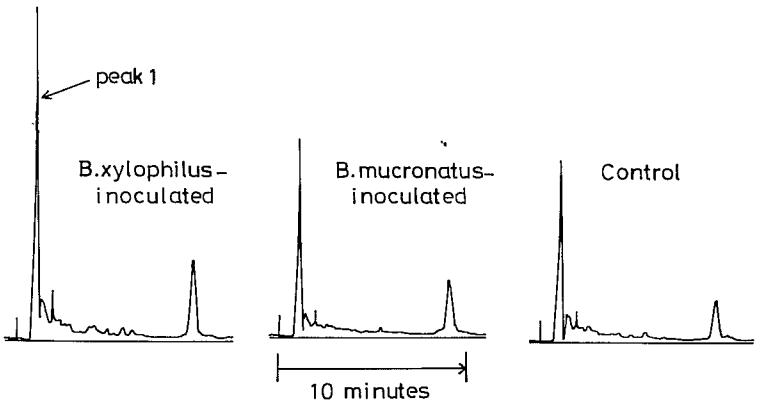

Fig. 3. HPLC chromatograms of phenolic metabolites produced in pine tissue inoculated with *B. xylophilus*, *B. mucronatus*, or distilled water.

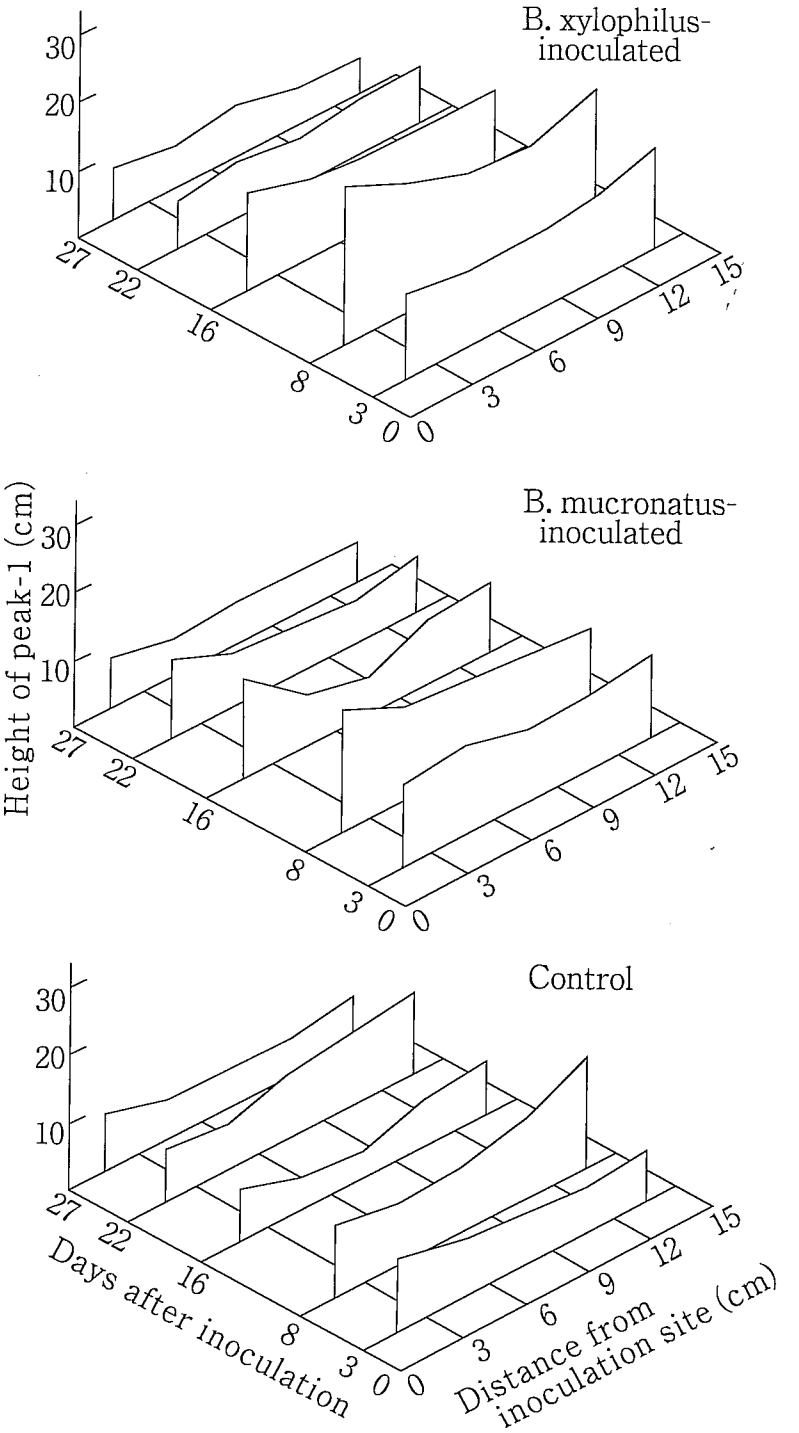

Fig. 4. Post-inoculation changes in the amount of phenolic compound(s), shown as height (cm) of peak-1 in liquid chromatograms (Fig. 3), for *B. xylophilus*-inoculated (top), *B. mucronatus*-inoculated (middle) and control (bottom) *P. thunbergii* seedlings. Measurements for the heights of peak-1 are the means of three replicates.

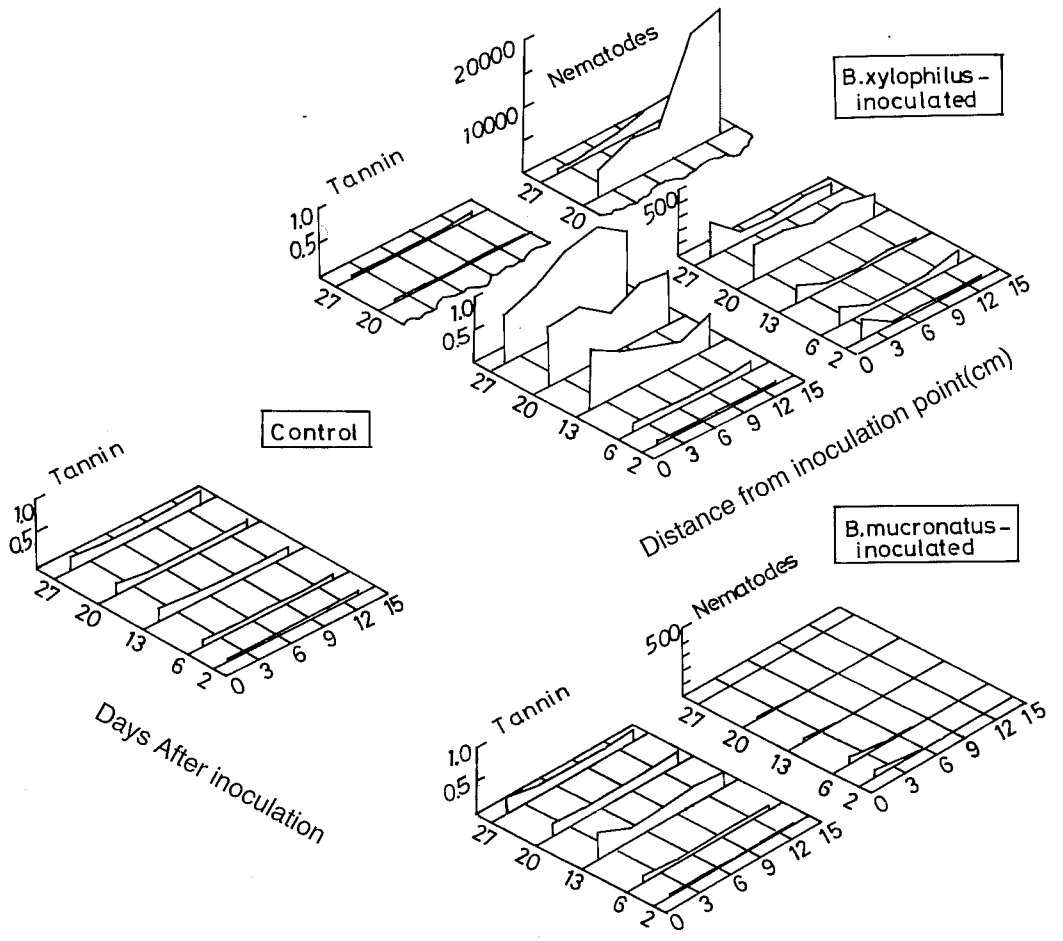

Fig. 5. Changes in the number of nematodes and chatechol tannin concentration in the stems of *B. xylophilus*-, *B. mucronatus*-inoculated and control seedlings of *P. thunbergii*. Each value is the mean of five replicates. Measurements for pine seedlings 20 and 27 days after inoculation with *B. xylophilus* are divided into two groups, i.e. severely wilted seedlings (at the rear, n=2 both on day 20 and 27) and slightly wilted seedlings (front, n=3 both on day 20 and 27).

Dynamics of catechol tannin and nematode population in pines:

The changes in catechol tannin concentration and *B. xylophilus* and *B. mucronatus* populations over time are given in Fig. 5. For the 13 days following inoculation, *B. xylophilus* numbers increased only slightly while catechol tannin levels increased steadily up to day 6 and dramatically after day 13. Tannin continued to increase up to day 27 in *B. xylophilus*-inoculated trees with slight wilt symptoms, but it decreased suddenly in trees that were severely wilted. Twenty days after inoculation, *B. xylophilus* numbers increased dramatically along the entire stem, especially in wilting trees. This increase in *B. xylophilus* populations coincided with the sudden decrease in tannin concentration in the stems.

In *B. mucronatus*-inoculated trees, catechol tannin concentrations were similar to that of control trees (Fig. 6) and showed no visible symptoms. *Bursaphelenchus mucronatus* migrated along the inoculated stems, but their populations did not increase.

To determine the relationship between *B. xylophilus* numbers and catechol tannin concentration in

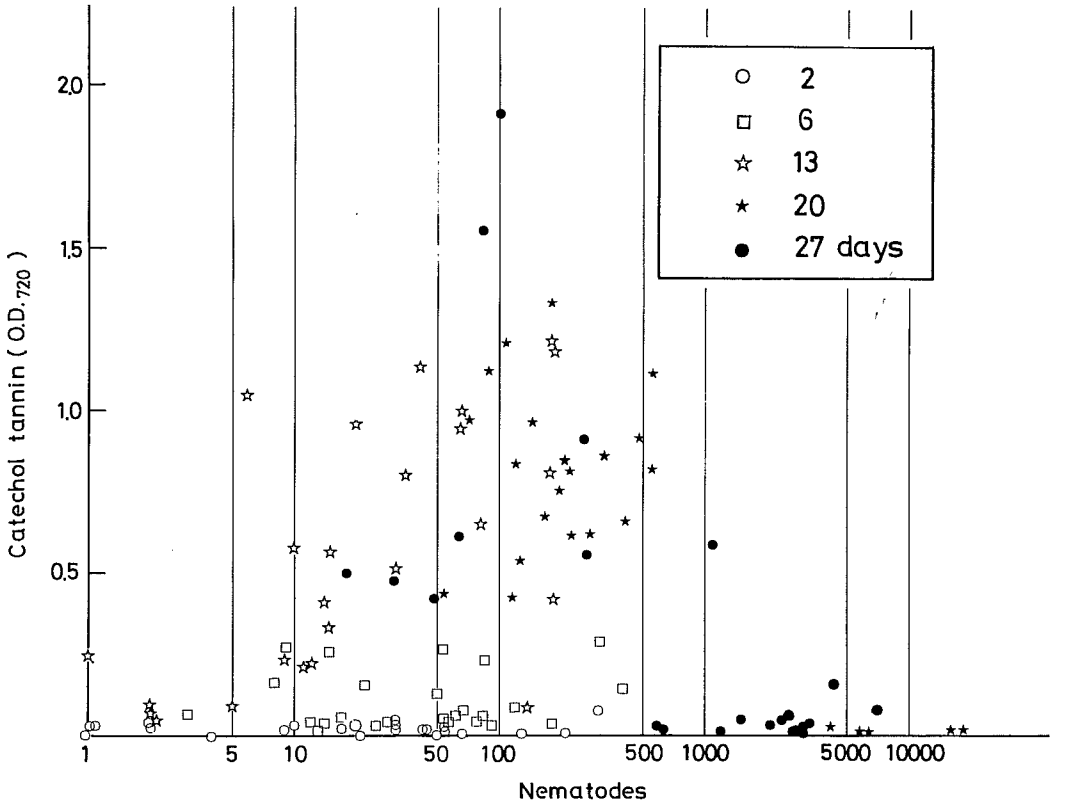

Fig. 6. The relationship between the number of *B. xylopilus* (logarithmic value) and catechol tannin concentration in nematode-invaded host tissues. Results for specific days are designated by different symbols.

nematode-infected host tissues, the value of the latter was plotted against the logarithmic value of the former (Fig. 6). Two days after inoculation, the concentration of tannin remained very low, regardless of nematode numbers. From 6 to 13 days after inoculation, the tannin concentration in each segment increased while nematode numbers were relatively low. However, at 20 and 27 days after inoculation, nematode numbers in the segments increased while the concentration of tannin decrease. Therefore, correlation coefficient values for nematode numbers and tannin concentration of stem samples 6 and 13 days after inoculation were 0.23 ($n = 25, p < 0.5$) and 0.44 ($n = 25, p < 0.05$), respectively; those for the 20- or 27-day examinations were -0.69 ($n = 25, p < 0.001$) and -0.54 ($n = 25, p < 0.01$), respectively. Thus, tannin concentration in the infected segments correlated positively with numbers of nematodes up to 13 days after inoculation, but negatively after 20 days.

DISCUSSION

Resistance to PWN is evidenced by toxicity (Bentley et al., 1985) or repulsiveness (Futai, 1979) of aqueous extracts of *P. taeda* L., which is resistant to some populations of PWN. Concentration of condensed tannin in needles or the inner bark of *P. thunbergii* appears to be associated with resistance

(Saitoh, 1970).

Inoculation of pines with avirulent PWN induces resistance to challenge inoculation with virulent PWN (Kiyohara, 1984). This induction of resistance suggests a dynamic resistance triggered by the nematode infection. Phytotoxic oxygenated monoterpenes extracted from PWN-infected *P. sylvestris* L. temporarily paralyzed the nematodes *in vitro* and suppress reproduction of the nematode, whereas extracts from uninfected *P. sylvestris* do not affect the nematode (Bolla et al., 1984). Phenolic compounds are associated with hypersensitive reactions (Klement and Goodman, 1967), which have been suggested to occur in PWN-infected tissues (Mamiya, 1980; Myers, 1986; 1988).

In order to clarify the role in hypersensitive resistance, materials synthesized in response to PWN infection must fulfill a time-site-effect relationship with nematode population (Veech, 1981). Present results indicate that 1) phenolic substances, which cause water extracts from PWN-infected tissue to turn brown (having absorption maximum at 278 nm) accumulate when numbers of PWN increase and 2) a catechin-like substance is the most abundant polyphenolic associated with large numbers of PWN.

Because PWN is a migratory nematode, it may be able to move from the site of hypersensitive reaction, thereby avoiding the effects of hypersensitivity. The nematodes could continue to trigger the hypersensitive reactions throughout the tree wherever they migrate. Substances, which might be synthesized initially in host resistance, become responsible secondarily for death of the trees (Bolla et al., 1984; Myers, 1988).

A secondary product such as catechin, which is naturally present in small amounts and is a well-known monomer of condensed tannin, may be activated or induced by *B. xylophilus* infection.

Following seedling inoculation with *B. xylophilus*, the peaks on liquid chromatograms of methanol extracts from pine tissues rose in unison, suggesting that while some specific phenolics were produced in response to the nematodes, the entire metabolic pathway of phenolic compounds was also activated. Eight days after inoculation, phenol production increased dramatically in stems of *B. xylophilus*-inoculated trees. While inoculation with *B. mucronatus* also resulted in production of phenols by the host, the response was much less dramatic. Tannins in bark of conifers are mainly condensed ones (Okamura, 1961; Sogo and Hata, 1967) and are polymers of flavanols such as catechin (Karchesy et al., 1976; Sears and Casebier, 1970). In the HPLC-chromatograms, the retention time of catechin coincides with that of the peak-1, the highest peak found in the chromatograms for pine metabolites. This coincides with the observations showing that catechin-like substances accumulate in *B. xylophilus*-inoculated tissue. Therefore, in the present study, although the substance shown as peak-1 on the chromatograms have not yet been identified, the amount of catechol (condensed) tannin in host tissue and the numbers of nematodes were monitored for 27 days after nematode inoculation. In *B. xylohilus*-inoculated trees, catechol tannins increased prior to the increase in nematode numbers. This tannin increase continued up to day 27 in trees that were slightly wilted, while tannin suddenly deceased in severely wilted trees. This reduction seems to be caused by oxidative condensation and insolubilization of tannin (Mhajan et al., 1985; Sakai et al., 1967). Concomitant with this reduction was the dramatic increase in nematode numbers, suggesting that water-soluble catechol tannin adversely affects the nematodes. Propagation of PWNs on *Botrytis cinerea*, in fact, was suppressed by exposing authentic tannins such as catechin and gallic acid (unpublished data). However, PWNs may survive or escape the effect of the tannin and successfully reproduce and spread throughout the host tissues. Fungi which proliferate in killed tissue, and serve as food for the nematodes, may also enhance nematode buildup. Concerning the role of phenolics in host

resistance to nematode invasion, Giebel (1970) characterized the susceptible host response as one in which browning and overall necrosis slowly occurs; conversely, a resistant response is one in which browning is quick and necrosis limited. Using these criteria, the response of *P. thunbergii* to *B. xylophilus* (pathogenic) is typical for a susceptible host while its reaction to *B. mucronatus* (non-pathogenic) is that of a resistant host.

The HPLC chromatograms for *B. xylophiluls*-inoculated seedlings revealed a distinct increase in phenolic compounds 3 days after nematode inoculation, while there was a conspicuous increase in seedling tannin content 13 days after the inoculation. The use of different extraction solvents might explain this discrepancy, i.e. for the HPLC samples, methanol was used, while distilled water was used for catechol tannin extraction. In a previous cytological study (Nobuchi et al., 1984), tannin-containing vacuoles started to develop in ray parenchyma cells 2 days after PWN-inoculation and the vacuoles finally burst 7 to 10 days after the inoculation. After the vacuoles burst, polyphenols might become extractable by distilled water, until then, however, only methanol would extract phenolic substances from the vacuoles.

ACKNOWLEDGEMENT

I thank Drs. J. R. Sutherland and A. Takimoto for their critical reviewing the manuscript.

LITERATURE CITED

Baermann, G. (1917) Eine einfache Methode zur Auffindung von Anchylostomum-(Nematoden)-Larven in Erdproben. Geneesk. Tijdschr. Ned.-Indie 57, 131-137.

Bentley, M. D., Mamiya, Y., Yatagai, M. and Shimizu, K. (1985) Factors in *Pinus* species affecting the mobility of the pine wood nematode, *Bursaphelenchus xylophilus*. Annals of the Phytopathological Society of Japan 51, 556-561

Bolla, R. I., Shaheen, P. and Winter, R. E. K. (1984) Effect of phytotoxin from nematode-induced pine wilt on *Bursaphelenchus xylophilus* and *Ceratocystis ips*. Journal of Nematology 16, 297-303.

Futai, K. (1979) Responses of two species of *Bursaphelenchus* to the extracts from pine segments and to the segments immersed in different solvents. Japanese Journal of Nematology 9, 54-59.

Giebel, J. (1970) Phenolic content in roots of some Solanaceae and its influence on IAA-oxidase activity as an indicator of resistance to *Heterodera rostochiensis*. Nematologica 16, 22-32.

Karchesy, J. J., Loveland, P. M., Laver, M. L., Barofsky, D. F. and Borofsky, E. (1976) Condensed tannins from the bark of *Alnus rubra* and *Pseudotsuga menziesii*. Phytochemistry 15, 2009-2010.

Kiyohara, T. (1984) Pine wilt resistance induced by prior inoculation with avirulent isolate of *Bursaphelenchus xylophilus*. In *Resistance mechanism of pines against pine wilt disease*" (Dropkin, V., ed.), Proceedings of the United States-Japan seminar, Honolulu, Hawaii, 178-184

Klement, X. and Goodman, R. N. (1967) The hypersensitive reaction to infection by bacterial plant pathogens. Annual Review of Phytopathology 5, 17-44.

Kosuge, T. (1969) The role of phenolics in host response to infection. Annual Review of Phytopathology 7, 195-222.

Mamiya, Y. (1980) Inoculation of the first year pine (*Pinus densiflora*) seedlings with *Bursaphelenchus lignicolus* and the histopathology of diseased seedlings. Journal of the Japanese Forestry Society 62,

176-183.

Mhajan, R., Singh, P. and Bajaj, K. L. (1985) Nematicidal activity of some phenolic compounds against *Meloidogyne incognita*. Revue de Nématologie 8: 161-164.

Myers, R. F. (1986) Cambium destruction in conifers caused by pinewood nematodes. Journal of Nematology 18, 398-402.

Myers, R. F. (1988) Pathogenesis in pine wilt caused by pinewood nematode, *Bursaphelenchus xylophilus*. Journal of Nematology 20, 236-244.

Nobuchi, T., Tominaga, T., Futai, K. and Harada, H. (1984) Cytological study of pathological changes in Japanese black pine (*Pinus thunbergii*) seedlings after inoculation with pinewood nematode (*Bursaphelenchus xylophilus*). Bulletin of the Kyoto University Forests 56, 224-233.

Ohshima, Y. and Nakabayashi, T. (1952) Nepherometric estimation of tannin in tea leaves. Journal of Agricultural Chemistry Society of Japan 26, 373-377.

Oku, H., Shiraishi, T. and Kurozumi, S. (1979) Participation of toxin in wilting of Japanese pine caused by a nematode. Naturwissenschaften 66, 210.

Okamura, H. (1961) On the spectra absorption of vegetable tannins. Journal of the Japanese Forestry Society 43, 34-39.

Saitoh, A. (1970) Resistance of pines against pinewood nematode and concentration of phenolic compounds in needles and inner bark. Annual Report of Kyushu branch, Forestry and Forest Products Research Institute. (in Japanese).

Sakai, R., Tomiyama, K., Ishizaka, N. and Sato, N. (1967) Phenol metabolism in relation to disease resistance of potato tubers: 3. Phenol metabolism in tissue neighbouring the necrogenous infection. Annals of the Phytopathological society of Japan 33, 216-222.

Sears, K. D. and Casebier, R. L. (1970) The reaction of thioglycolic acid with polyflavanoid bark fractions of *Tsuga heterophylla*. Phytochemistry 9, 1589-1594.

Shaheen, F., Winter, R. E. K. and Bolla, R. I. (1984) Phytotoxin production in *Bursaphelenchus xylophilus*-infected *Pinus sylvestris*. Journal of Nematology 16, 57-61.

Sogo, M. and Hata, K. (1967) On the permanganate oxidation of bark phenolic acid, tannin and Bjorkmann lignin isolated from bark of some tree species. Journal of Japanese Wood Research Society 7, 300-305.

Veech, J. A. (1981) Plant resistance to nematode. In: *Plant parasitic nematodes*, vol. 3 (Zuckerman, B. M. and Rhode, R. A. eds.), Academic Press. New York, 377-403.

Received June 23, 2003.

和文摘要

マツノザイセンチュウに感染したクロマツ苗木に生成される異常代謝産物

二井　一禎

クロマツ苗木の主軸部にマツノザイセンチュウを接種した時、異常代謝産物が生産される。これら物質の化学的な特性を、吸光スペクトル、ペーパークロマトグラフィー、液体クロマトグラフィーなどの方法を用いて調査した。また、線虫接種後のこれら物質の動態を明らかにするため、接種点からの距離と接種後の時間を変えてその量と、線虫個体数を調査した。一つの物質は吸光スペクトルのピークが278 nm 付近にあり、このピークは自動酸化により小さくなるが、１Ｎの水酸化ナトリウムを添加すると287 nm へとピーク位置が移動する。この物質は組織の褐変と関係があり、自動酸化が進むと次第に色は濃くなる。また、この物質はマツノザイセンチュウの個体数増加にともない増加するので、線虫感染に関係して生産された物質であると考えられる。これらの性質から、最も大量に生産される物質はポリフェノール類の一種で、縮合型タンニンの前駆体であるカテキンに類似の物質であることが明らかになった。