クロマツ林におけるマツ材線虫病罹病木の外部病徴の変異

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論　文

Variation in External Symptom Development of Pine Wilt Disease in Field Grown *Pinus thunbergii*

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TOGASHI, Katsumi: **Variation in external symptom development of pine wilt disease in field grown *Pinus thunbergii* J. Jpn. For. Soc. 71: 442〜448, 1989** To determine the temporal relationship between the tree decline and the death process of trees naturally infected with *Bursaphelenchus xylophilus* (STEINER et BUHRER) NICKLE, the degrees of oleoresin exudation from artificial wounds were determined on all trees in a *Pinus thunbergii* PARL. stand each month from May to October, and the foliage coloration was observed each week from June through October and one to three times each month from November to May. Of the trees first weakened from June through September, a large proportion of them died, and all of their foliage had become red-brown or brown in the year when they were first weakened (Pattern A). The time required for them to die was longer in trees first weakened in June or July than in trees first weakened in August or September. In some trees first weakened in September or October, nearly all of their foliage became discolored during the year in which they were first weakened, and all of their foliage became red-brown or brown during the following year (Pattern B). In some trees first weakening between August and October, a part of their foliage became discolored during the year when they first weakened; nearly all of their foliage became discolored during the next year, and soon after that all of their foliage became red-brown or brown (Pattern C). In a few trees first weakened in October, a part of their foliage became discolored the following April, and all of their foliage became red-brown or brown by mid-June at the latest (Pattern D).

富樫一巳：**クロマツ林におけるマツ材線虫病罹病木の外部病徴の変異　日林誌 71：442〜448, 1989**　材線虫病に自然感染したクロマツの衰弱時期と枯死過程の関係を明らかにするために，1林分の全クロマツに対して樹脂滲出能の調査を5月から10月まで毎月行った。また，マツの葉色の調査を6月から10月までは毎週，11月から5月までは毎月1〜3回行った。6月から9月までの間に衰弱しはじめたマツの場合，その大部分は衰弱した年に全葉が茶色〜赤茶色になって枯れた(パターンA)。枯死過程の完了までの平均時間は8, 9月に衰弱しはじめたマツより6, 7月に衰弱しはじめたマツのほうが長かった。9月または10月に衰弱しはじめたマツの場合，衰弱した年にほとんどすべての葉が変色し，翌年全葉が茶色〜赤茶色に変色するものがあった(パターンB)。8月から10月の間に衰弱しはじめたマツには，衰弱した年に一部の葉が変色し，翌年になってほとんどの葉が変色し，その後すぐに全葉が茶色〜赤茶色になる場合が見られた(パターンC)。10月に衰弱しはじめたマツのうち少数のものは，衰弱の翌年の4月に一部の葉が変色し，遅くとも6月中旬までに全葉が茶色〜赤茶色に変色した（パターンD)。

I. Introduction

Japanese black pine, *Pinus thunbergii* PARL., and Japanese red pine, *P. densiflora* SIEB et ZUCC., have been damaged greatly by the pine wilt disease in Japan. This disease is induced by a nematode, *Bursaphelenchus xylophilus* (STEINER et BUHRER) NICKLE, which is vectored by a cerambycid, *Monochamus alternatus* HOPE (KIYOHARA and TOKUSHIGE, 1971; MAMIYA and ENDA, 1972; MORIMOTO and IWASAKI, 1972).

After inoculation with *B. xylophilus*, pine trees are first weakened, which is judged as being complete or partial cessation of oleoresin exudation from artificial wounds (cf., MAMIYA, 1983). At this stage of disease development or after, *M. alternatus* females lay eggs on the trees. After the cessation of oleoresin exudation,

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the foliar coloration begins to change. It is only after an appreciable proportion of the foliage has become discolored that it is recognized that the trees are infected with *B. xylophilus*. Accordingly, it is important to understand the temporal relationship between tree decline and foliar coloration change (death process) if this epidemic is to be controlled.

KISHI (1988) stated that the period between nematode inoculation and foliage discoloration was more uniform in artificially inoculated trees than in naturally infected trees. However, there have been few reports concerning the temporal relationship between tree decline and the death process in trees under field conditions. MAMIYA *et al.* (1973) examined the time of tree decline, but they felled about two-thirds of the trees during the course of foliage discoloration. KISHI (1988) examined a temporal relationship between the time of tree decline and the time when he found an appreciable change of foliage coloration, but he observed foliage coloration only once a month. Therefore, it was difficult to show the progressive rate of death process. Although MALEK and APPLEBY (1984) distinguished four stages of foliar coloration change of *P. sylvestris* L. in the USA, they did not relate them to the time when trees were first weakened.

This paper deals with the temporal relationship between the initial tree decline and the death process based on weekly observations in a *P. thunbergii* stand, where almost all of the trees were killed by the pine wilt disease (TOGASHI, 1989). In this study, the death process was expressed by three stages of foliar coloration change.

II. Study Site, Materials and Methods

The study stand, as well as materials and methods have already been described in detail by TOGASHI (1988). A brief outline is provided here.

The study stand, which was about 9.2 a in area, was located on the landward fringe of a huge littoral forest of *P. thunbergii* at Oshimizu in Ishikawa Prefecture. There were 249 live trees on the study site early in June 1980. They averaged 8 m tall and 9 cm in dbh. This stand was in an early stage of infestation by *M. alternatus* and *B. xylophilus*. Aerial applications of insecticide were not made in the study area during this study. All dead trees were removed from the study site before the start of beetle emergence. Consequently, *M. alternatus* adults moved into the study stand from the surrounding pine stands.

Each month from May through October, the degrees of oleoresin exudation on all trees were determined using a method modified from that of ODA (1967) (TOGASHI, 1988). Observations were made on the appearance of both trees which had completely or almost had stopped oleoresin exudation from artificial wounds and on trees with foliage that was discolored partially. Records were kept for the color of foliage obtained by field observations which included location and relative quantity of discolored foliage. These observations were made once a week from June through October and one to three times each month from November through May.

The study was conducted between June 1980 and June 1983. Some trees were felled before all of their foliage became red-brown or brown to examine the survival rate and the mortality factors of *M. alternatus* within them. Thus, the times of all foliage changes to red-brown or brown were not recorded for all trees. Trees were judged as dead when nearly all of the foliage was discolored.

III. Results

Some branches may be killed by *B. xylophilus*, but entire trees may not (ZINNO *et al.*, 1987; KISHI, 1988). In this study stand, only one sample was observed in 1982; a tree 4.06 m tall was normal in oleoresin exudation from wounds on tree trunks at breast height during 1982. The tree trunk and its branches were alive up to 2.4 m from the ground, but those were dead from the height of 2.4 m up to the tree top by November 1982. The dead branches contained *B. xylophilus*, and they had feeding scars of *M. alternatus* on barks. There was a larval tunnel of *M. alternatus* in the dead trunk. Because the tree trunk was broken off in June 1983 at 2.5 m above the ground where it had become small in diameter owing to larval feeding, nearly all dead parts were lost from it. Consequently, it still was surviving in the autumn of 1984.

When the trees were first weakened from June through October and were dead by the next May, the

Table 1. Death process of *Pinus thunbergii* killed by *Bursaphelenchus xylophilus* in relation to the time of tree decline

Time when trees were first weakened	June	July	August	September	October
No. of trees killed	14[b]	21[c]	34[d]	35	24
Trees required over 1 week for nearly all foliage to be discolored from green[a]					
No. of trees examined	12	19	26	23	19
Discoloration of a part of the foliage					
Time of discoloration	Late June to mid-August	Early July to late August	Mid-August to mid-October	Late August to mid-November	Early October to late April
Coloration of discolored foliage	Yellowish green, brown	Yellowish green, pale brown, reddish brown	Pale brown, bright yellow, yellowish green, reddish brown	Yellowish green, reddish brown, brown, yellow	Bright yellow, pale brown, brown
Location of discolored foliage	Lower crown in many cases	Upper or lower crown, sporadically all over the crown	Upper crown, sporadically all over the crown	Sporadically all over the crown, lower crown	Sporadically all over the crown
Discoloration of nearly all of the foliage					
Time of discoloration	Mid-July to early October	Late July to mid-December	Mid-August to mid-March	Mid-September to late May	Early December to late May
Coloration of discolored foliage	Yellowish green, pale brown, brown	Pale brown, yellowish green, reddish brown	Yellowish green, pale brown, reddish brown	Yellowish green, reddish brown, pale brown, brown	Pale brown, yellowish green, reddish brown
Trees required 1 week for nearly all foliage to be discolored from green					
No. of trees examined	1	1	7	12	5
Time of discoloration	Late July	Late July	Mid-August to mid-September	Early September to early November	Late October to early November, and late April
Coloration of discolored foliage	Yellow, yellowish green	Pale brown, yellowish green	Yellowish green, pale brown	Yellowish green, pale brown, brown	Yellowish green, pale brown, brown, yellow
Time for all foliage to be discolored to reddish brown or brown					
No. of trees examined	12	14	21	16	7
Time of discoloration	From early August	From mid-August	From early September	From late September	From early March

[a] Not including trees which stopped oleoresin exudation in the previous year. [b] Not including a suppressed tree. [c] Not including a tree losing nearly all of its branches by snowfall. [d] Not including a tree whose trunk was broken below the crown by snowfall.

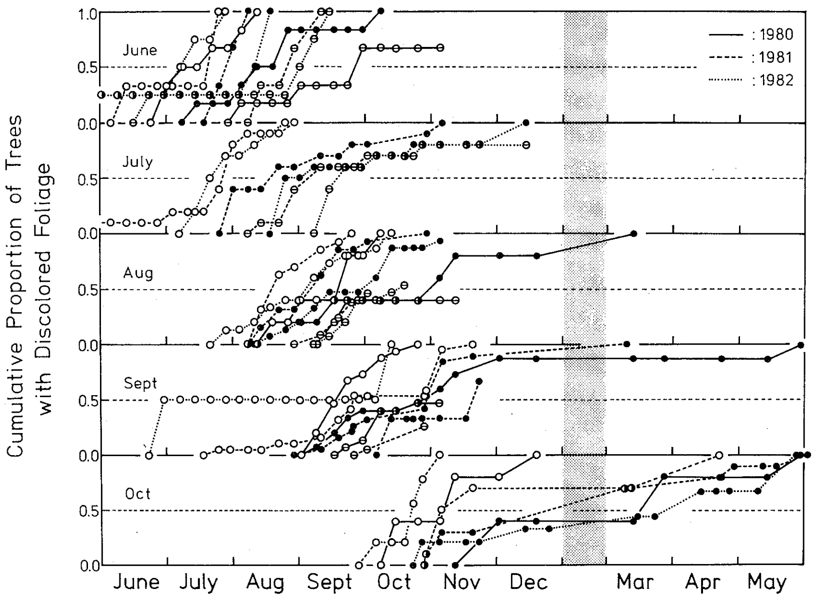

Fig. 1. Death process of *Pinus thunbergii* in relation to the time when the trees were first weakened

Months in the figure represent the time when trees were first weakened. Cumulative proportion sometimes did not attain 1.0 because some trees were felled before all of their foliage changed to red-brown or brown. The death process is shown in three stages of the relative quantity of discolored foliage which the trees attained (○ : Discoloration of a part of the foliage, ● : Discoloration of nearly all of the foliage, ⊖ : Discoloration of all foliage, becoming red-brown or brown).

primary mortality factor was *B. xylophilus* (TOGASHI, 1989). Table 1 and Fig. 1 show the death process in such trees in relation to the time when they were first weakened.

A part of the foliage began to change color at the time of the cessation of oleoresin exudation or soon thereafter. First, discolored foliage was yellowish green, pale brown, or bright yellow in many cases, and brown or red-brown in some cases. There was some variation in the location of first discolored foliage ; the discolored foliage occurred in the upper crown, at the bottom of the crown, or sporadically over the entire crown. In many cases, the 1- or 2-year-old foliage became discolored sooner than the current-year's foliage. Progressively, nearly all foliage became discolored. At this stage, the crowns were composed of foliage of different colors such as yellow-green, pale brown, red-brown, and brown. All foliage, however, eventually became red-brown or brown. When tree decline was delayed, each stage of foliage discoloration, i.e. discoloration of a part of the foliage, of nearly all of the foliage, and of all of the foliage to red-brown or brown, was also delayed.

General patterns of the tree death process described above were not observed in two cases. One was when almost all of the foliage became discolored in one week, although all of the foliage was green a week before the observations. This occurred on trees first weakened from August through October (Table 1). The other case was when all discolored foliage fell during one week, resulting in the seeming recovery of the tree. This case was observed with two trees just after the passage of Typhoon # 10 in 1982 when the discolored foliage of five trees decreased remarkably. All discolored foliage was observed to have temporarily fallen from a tree in April, July, August, and September each, regardless of typhoons. In some trees first weakened in October, all discolored foliage was lost during the winter. Such was observed on two trees in 1980 and four trees in 1982. Possibly, the northwest seasonable wind in winter caused the shedding of the discolored foliage. In such trees, the date when a part of the foliage became discolored was designated as the date when the discolored foliage was first observed before defoliation.

Table 2. Periods of time required for early and late stages of the death process in *Pinus thunbergii* infected with *Bursaphelenchus xylophilus* (days)

Time when trees were killed[a]	Time when trees were first weakened	Pattern of death process[b]	Period between when a part of the foliage became discolored and when nearly all of the foliage became discolored		Period between when nealy all of the foliage became discolored and when all of the foliage became red-brown or brown	
			No.[c]	Mean±S. E.[d]	No.[c]	Mean±S. E.[d]
Year when trees were first weakened	June	A	13	29.1±6.1	12	25.5±4.5
	July	A	20	50.7±7.7	15	19.2±2.7
	August	A	30	18.5±2.7	19	20.0±2.2
	September	A	30	22.6±5.4	12	15.8±3.6
	September	B	2	7.0±7.0	2	118.0±6.0
	October	B	8	19.9±8.2	1	124
Year after trees were first weakened	August	C	2	154.5±1.5	1	15
	September	C	3	189.0±40.2	0	
	October	C	13	155.1±12.5	6	16.0±5.3
	October	D	3	16.0±12.7	2	10.5±10.5

[a] Trees were judged to be dead when nearly all of their foliage was discolored. [b] See the text for an explanation of the patterns of the death process. [c] No. of trees examined. [d] S. E. =standard error.

So far, the death process of pine trees has been shown by the time when they attained a given stage of foliage discoloration as shown in Table 1, or by the percentage of the cumulative number of trees attaining a given stage in Fig. 1, for each of the tree groups first weakened in different months. This representation cannot show the characteristics of different death processes of trees first weakened in the same month. Thus, the death process was divided into two parts (early and late) by the date when a part of the foliage became discolored, the date when nearly all of the foliage became discolored, and the date when all of the foliage changed to red-brown or brown. Table 2 gives the periods of time required for the two parts of the death process for each tree group first weakened in different months and with different patterns of the death process.

For trees first weakened from June through September, large proportions of them were killed and all of their foliage changed to red-brown or brown in the year when they were first weakened (Pattern A in Table 2). In this case, the early part of the death process averaged longer in trees first weakened in June or July than in trees first weakened in August or September. The late part of the death process was the longest in trees first weakened in June, and the shortest was in trees first weakened in September. Consequently, the period of time required for complete death was longer in trees first weakened in June or July than in trees first weakened in August or September. This led to many trees first weakened from June through September to change all of their foliage to red-brown or brown between late August and late October.

In some trees, all of the foliage became red-brown or brown the year after they were first weakened. This occurrence was divided into three patterns of the death process. The first pattern was that nearly all of the foliage became discolored in the year when the trees were first weakened (Pattern B in Table 2). Such a pattern was observed in trees first weakened in September or October. The early part of their death process required 7～20 days, which was not different from that in trees first weakened in August or September (Pattern A). However, the late part of the death process required about 120 days, all of the foliage changing to red-brown or brown in early and mid-March. Another pattern occurred when a part of the foliage became discolored in the same year that the trees were first weakened, and nearly all of the foliage became discolored the following year (Pattern C in Table 2). This pattern was observed in trees first weakened in August, September, or October. In such trees, nearly all of the foliage became discolored from March through May of the following year (the early part of the death process being 150～190 days), and soon after that, all of the foliage changed to red-brown or brown (the late part of death process being about 15 days).

The third pattern was that of a part of the foliage becoming discolored the next April (Pattern D in Table 2). This was observed in trees first weakened in October. In this case, nearly all of the foliage became discolored between April and May, and all of the foliage changed to red-brown or brown by mid-June at the latest. Because the foliage coloration did not change between December and February, it was judged that the death process ceased during winter. Consequently, all of the foliage changed to red-brown or brown primarily between March and May of the following year.

IV. Discussion

Field inoculation experiments with *B. xylophilus* show that pine trees tend to be killed the year after they are inoculated when the inoculation time is delayed or when the inoculum quantity is small (SHOJI *et al.*, 1983 ; SHOJI and ZINNO, 1985). Inoculation experiments in controlled environmental chambers show that the symptom development of pine wilt disease is promoted both by temperatures between 25 and 30°C and by the small water content of a soil, and that it is inhibited both by temperatures of 15 to 20°C and by a sufficient supply of water within the soil (KIYOHARA, 1973 ; SUZUKI and KIYOHARA, 1978).

In studies on trees naturally infected with *B. xylophilus* in the forest, MAMIYA *et al.* (1973) stated that *P. densiflora* trees weakened by August were killed by October, but that trees first weakened in September or October were killed between January and March of the next year in Chiba Prefecture adjoining the Pacific Ocean. KISHI (1988) showed that the period between tree decline and foliage discoloration averaged longer as the time of tree decline was delayed. This trend was reconfirmed in this study. MAMIYA (1983) stated that delayed disease development of naturally infected pine trees in northern areas can be attributed mainly to the effect of low temperatures.

In this study, the death process was classed into four patterns. For trees with Pattern A, the period between initial tree decline and all foliage becoming red-brown or brown was longer for trees first weakened in June or July than for those first weakened in August or September. This result may be ascribed to the difference in water content of the soil because there is a rainy season between June and July in Japan.

KISHI (1988) pointed out that there were large variations in the period between initial tree decline and foliage discoloration of trees first weakened in the same month. In this study, there were different death processes in the trees which were first weakened in the same month between August and October. These results suggest that factors other than air temperature and water content of the soil also may affect the death process. The inoculum quantity of *B. xylophilus* and the susceptibility of *P. thunbergii* are also known to be related to the development of this disease (HASHIMOTO and SANUI, 1974 ; FUTAI and FURUNO, 1979). Thus, the death process of naturally infected trees was probably affected significantly both by the rate and the total number of *B. xylophilus* entering the trees before and after the disease incidence, and by the difference in susceptibility of individual *P. thunbergii* trees to *B. xylophilus*.

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