J For Res (2005) 10:93–99
DOI 10.1007/s10310-004-0101-3

ORIGINAL ARTICLE

Tamio Akema · Kazuyoshi Futai

Ectomycorrhizal development in a *Pinus thunbergii* stand in relation to location on a slope and effect on tree mortality from pine wilt disease

Received: August 29, 2003 / Accepted: June 18, 2004

Abstract The relationship between ectomycorrhizal development and mortality from pine wilt disease was studied in an artificial *Pinus thunbergii* Parl. stand on a slope. The development of ectomycorrhizae and the survival of the trees showed the same tendency, which suggests a correlation between mycorrhizal development and resistance to pine wilt disease. The development of pine roots and mycorrhizae was greater in the upper part of the slope. The ratio of mycorrhizae to the total of mycorrhizae and fine taproots was also higher in the upper part of the slope. Tree mortality was clearly biased and more trees survived in the upper part of the slope than in the middle and the lower parts. There was no significant difference between the upper and the lower part of the slope in the number of feeding wounds made by the pine sawyer beetle, which demonstrates the opportunity of infection with this disease. There was no clear correlation between the development of mycorrhizae and the composition of the soil substrate such as total carbon, nitrogen, and phosphorus. The abundant mycorrhizae in the upper part of the slope, which mitigate drought stress, may also have decreased the rate of tree mortality.

Key words Ectomycorrhiza · Pine wilt disease · Drought stress · Tree mortality · *Pinus thunbergii*

Introduction

Japanese red pine, *Pinus densiflora* Sieb. & Zucc., and Japanese black pine, *P. thunbergii* Parl., are major components of exploited suburban forests and maritime forests in Japan, respectively. However, such pine forests, especially those in the south-western part of Japan, have been devastated by pine wilt disease during the late twentieth century, and the disease is spreading northward (Kishi 1995).

Pines are well-known ectomycorrhizal trees. Ectomycorrhizae perform many functions, such as improvement of host resistance to root diseases (Marx 1969; Buscot et al. 1992), enhancement of nutrient and water uptake (Finlay and Read 1986; Bledsoe 1992), and are generally believed to supplement and/or enhance the functions of roots. Pines are thought to be almost complete dependent upon mycorrhizal fungi (Allen 1991). Pine wilt disease itself is caused by a nematode, *Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle, and has no direct relation to mycorrhiza, although the tolerance level of the trees may be increased indirectly by mycorrhizal colonization, since mycorrhizae generally improve the physiological condition of pine trees.

The purpose of this article is to examine the abundance and distribution of pine roots and mycorrhizae in relation to their location on a slope, and then to correlate these results with the distribution of trees killed by pine wilt disease. It is said that pine trees growing near the ridge have a larger amount of mycorrhizae than the trees at the bottom of the slope, but this has been empirically attributed to the complex effects of differences in soil characteristics, including fertility, water condition, microorganisms, and so on. In an attempt to elucidate the determining factor of the mycorrhizal distribution, we also measured total carbon, total nitrogen, extractable phosphorus, and soil water conditions.

Materials and methods

Study site

The study site was an artificial pine stand in Tokuyama Experimental Station of Kyoto University Forest, Yamaguchi Prefecture (34°04′ N 131°50′ E). The annual mean temperature and precipitation in the station were

T. Akema (✉)
Forest Microbiology Group, Kyushu Research Center, Forestry and Forest Products Research Institute, 4-11-16 Kurokami, Kumamoto 860-0862, Japan
Tel. +81-96-343-3948; Fax +81-96-344-5054
e-mail: akema.tamio@ffpri.affrc.go.jp

K. Futai
Laboratory of Environmental Mycoscience, Graduate School of Agriculture, Kyoto University, Kyoto, Japan

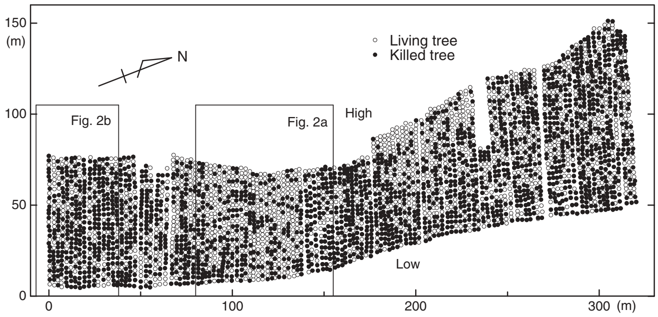

Fig. 1. The position of living pine trees in 1993 (*open circles*) and dead ones (*black circles*) between 1980 and 1993 at Tokuyama Experimental Station. The *top of each column* is the ridge line. *Frames* show the area shown in Fig. 2

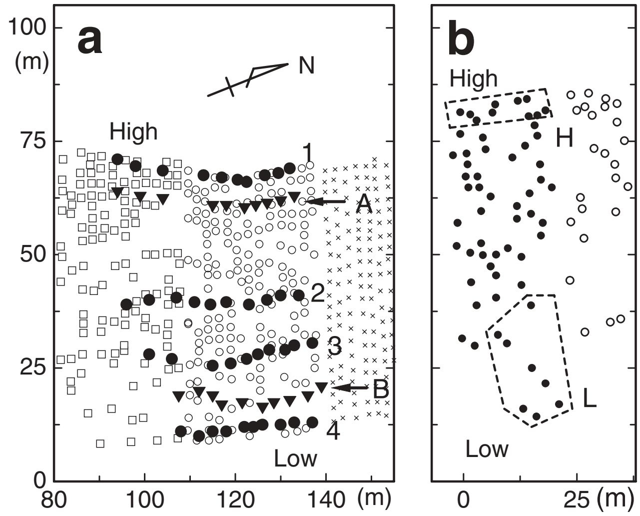

Fig. 2. The sampling points: **a** Sampling points in the first survey for roots, mycorrhizae and soil nutrients (*dots*, subplots 1–4) and for water potential (*triangles*, subplots A and B). *Squares*, *circles*, and *crosses* show the positions of surviving pine trees in early 1994 of strains 236, 241, and Shimonoseki, respectively. **b** Sampling areas for roots and mycorrhizae (subplots H and L) in the second survey. *Dots* and *circles* are the surviving pine trees in early 1997 of strains 231 and 232, respectively

14.9°C and 1915 mm, respectively. The rain falls mainly in June and July, and the drought in early spring and summer is often severe. The altitude was between 240 and 270 m and the average inclination is 25°. The top of the slope is the ridge line and the bottom end is cut vertically to make a forestry road, so the lowermost part of the slope is a bluff of 1–2 m in height. This stand was planted in 1970 with several local strains of Japanese red pine collected mainly from western Japan and 16 half-sib strains of Japanese black pine trees along the slope according to the locality where they came from.

Until 1980 most trees in this stand had been thriving, but thereafter many trees had been killed by pine wilt disease year after year, and only 28.1% of the initial population was left at the end of 1993 (Nakai et al. 1995). The locations of the surviving and dead pine trees are shown in Fig. 1. We made two field surveys in July 1993 and May 1997. The first survey was followed by some supplementary samplings in 1993 and 1994.

We analyzed the spatial data used in Nakai et al. (1995), provided by the authors, to try to find some pattern of mortality rate depending on the tree's position on a slope. As the trees were planted in columns along the slope, trees in each column were divided into three equal parts simply by the number from the top.

Sampling

First survey (1993 and 1994)

Soil samples were collected mainly from the stand of local strains of *P. thunbergii* No. 241 and No. 236, both of which survived at exceptionally high rates. These two strains seemed to have a higher tolerance than other strains, since both of the strains planted in each separate stand showed low mortality rates.

Root samples were collected from four subplots arranged at various heights on the slope; named 1 for the uppermost, 2 for the upper middle, 3 for the lower middle, and 4 for the lowermost. Ten replications for each subplot were collected and the sampling points were allocated along a level line for each height (see Fig. 2a). We put a 30 × 30 cm wooden frame on the ground as a jig and cut the roots by thrusting a knife along the frame. For each point, three soil samples were collected from different depths; 0–5, 5–10, and 10–15 cm. The soil samples were washed under running water on a 5-mm-mesh sieve and plant roots remaining on the sieve were brought to the laboratory in polyethylene bags. The thickness of the A_0 and A layers measured in subplots 1–4 in Fig. 2a are shown in Table 1.

Table 1. Thickness of A_0 and A layers (cm) measured in the first survey at subplots 1 to 4 in Fig. 2a

	Subplots			
	1	2	3	4
A_0 layer	6.0 ± 0.30	6.2 ± 0.29	5.4 ± 0.34	5.7 ± 0.40
A layer	8.8 ± 1.05	12.0 ± 1.50	14.8 ± 1.70	11.3 ± 1.17

Values shown are mean ± SE

To measure the concentrations of carbon, nitrogen, and phosphorus, we collected supplemental soil samples using a 100-ml core sampler in October 1993 from the same points as previously sampled.

We collected a further 20 soil samples, 100 ml each, to determine the water characteristics for the study site in July 1994. Ten samples came from the upper part of the slope between subplots 1 and 2, and the other ten samples were taken from the lower part, between subplots 3 and 4 (see Fig. 2a, subplots A and B, respectively). Samples were collected from a depth of 5–10 cm.

Samples used to estimate the water potential in the field were collected in October 1993 and July 1994. In 1993, four batches of ten samples each were collected in subplots 1–4.

Two batches of ten samples each were collected from subplots A and B in 1994.

Second survey (1997)

The subject of first survey was a single stand and we felt that this might be insufficient to generalize the result, so we carried out another survey in a different stand in Tokuyama Experimental Station. In May 1997, soil samples were collected from the upper and lower parts of the slope in the same stand but planted with a different local strain of Japanese black pine, No. 231 (Fig. 2b). This strain is slightly less resistant to pine wilt disease than Nos. 241 and 236, although the difference is not significant ($P > 0.1$, χ^2-test). Other strains barely survived in the lower part of the slope, and this was the only available strain in 1997. Twenty-five samples were randomly collected from the upper part (subplot H) where many trees were growing, and another 30 samples were collected from the lower part (subplot L) around the few surviving trees, for the measurement of roots and mycorrhizae only. All soil samples were contained in polyethylene bags, and brought to the laboratory.

Distribution of roots and mycorrhizae

First survey

We immersed the samples in running water, then sorted them using forceps into the roots of pines and other plants. The pine roots were then classified into mycorrhizae, taproots smaller than 2 mm in diameter which may bear mycorrhizae, and roots larger than 2 mm in diameter. The biomass in each class of roots was evaluated by fresh weight. Mycorrhizal ratio was evaluated as the proportion of the weight of mycorrhizal roots to that of fine taproots plus mycorrhizal roots.

Second survey

All pine roots were sorted out from soil samples and thick roots (over 2 mm in diameter) were discarded. The remaining fine taproots and mycorrhizae were cut into small pieces (<1 cm) in a mixer with 400 ml of water, and 100 ml of the suspension was subsampled. The root pieces in subsamples were sorted into taproots and mycorrhizae under a dissecting microscope and dried at 65°C in a drying-oven for 1 day; their dry weights were then evaluated. When the amount of roots was very small, the whole volume of the suspension was used for measurement. Mycorrhizal ratio was also calculated as described above.

Soil nutrients

We conducted all soil nutrient analysis on the samples collected in October 1993 from subplots 1–4. Total carbon and nitrogen were measured with a CN Coder (YANACO MT-600). A 0.5-g sample of soil was used for the measurement, and 4 g of cobalt oxide was used as an oxidizer for combustion of the soil. Extractable phosphorus was extracted with dilute hydrochloric acid and sulfuric acid, and the amount was determined by the molybdate-vanadate method (Nelson et al. 1953).

Water condition

Soil samples collected in July 1994 at subplots A and B were brought to the laboratory in polyethylene bags and were loaded into stainless 100-ml soil sampling tubes. After immediate measurement of fresh weight, the water contents at the potentials of 0 to -3.1, -9.8 to -98, -1.6×10^3, -3.1×10^4, and -3.1×10^5 kPa (pF 0–1.5, 2.0–3.0, 4.2, 5.5, and 6.5) were determined by the sand-column method, pressure-plate method, centrifuge method, air drying, and oven drying, respectively. The water potential of the samples in the field condition was estimated using those data. Because the sampling points in 1993 (subplots 1–4) were different from those in 1994 (subplots A and B), values for 1993 were substituted by the averages of subplots 1 and 2 for subplot A and subplots 3 and 4 for subplot B.

Number of feeding wounds made by pine sawyer beetle

The number of feeding wounds made on 1-year and 2-year internodes of the twigs by the pine sawyer beetle, *Monochamus alternatus* Hope, the vector of *B. xylophilus*, was counted to see whether the biased distribution of wilted pine trees could be ascribed to the sawyer beetle's preferred feeding behavior bringing a greater infection frequency of pine wood nematode to the lower part of the slope. For this purpose, five living trees planted both at the upper and lower parts of the stand were examined in October 1993 in the stand of strain 241. About 20 twigs were arbitrarily sampled from each of the upper and lower branches of the crown.

Results

Distribution of pine trees killed by pine wood nematode

The mortality of each group along the slope (upper, middle, and lower) between 1980 and 1997 was calculated. The mortality varied among strains ($P < 0.01$, χ^2-test), but more trees survived in the upper part than in the middle and the lower parts ($P < 0.001$, Friedman's test) regardless of strain (Fig. 3).

Distribution of roots and mycorrhizae and the mycorrhizal ratio

First survey

As shown in Fig. 4, the largest amount of pine roots was found in subplot 1 (the uppermost on the slope), while the other plant roots did not show any difference in their distri-

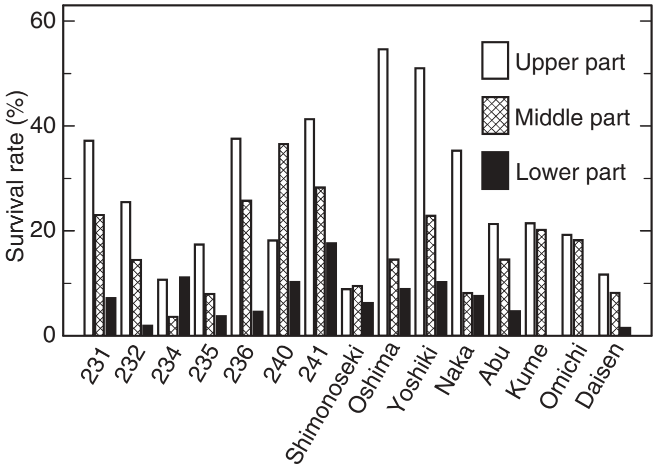

Fig. 3. The survival rate of the Japanese black pine strains on the upper (*white*), middle (*cross-hatched*), and lower (*black*) parts of the slope between 1980 and 1997

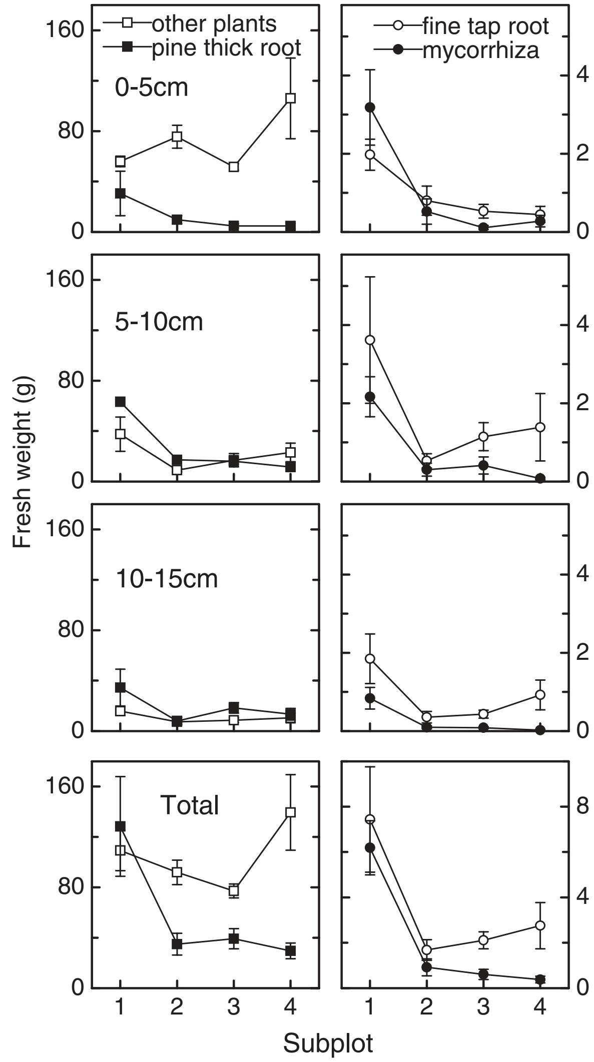

Fig. 4. Distribution of roots at different heights on the slope and at different depths from the soil surface after the removal of litter in the samples collected in the first survey from subplots 1, 2, 3, and 4 in Fig. 2a. Other plants included shrubs, grass, herbs, and ferns except tubers. Pine thick roots are thicker than 2 mm in diameter and bear only taproots. Fine taproots are pine taproots which are thinner than 2 mm and bear mycorrhizae. Mycorrhizae are ectomycorrhizae formed on pine roots. *Symbols* and *bars* are mean ± SE

bution due to location on the slope. In the pine roots, more mycorrhizae and fine taproots were distributed in subplot 1.

Irrespective of the height on the slope, the amount of mycorrhizae was smaller in the deep layer (10–15 cm) than those in surface and middle layers, though the surface layer of subplot 3 contained a smaller amount of mycorrhizae than the middle layer. In subplot 1, mycorrhizal biomass decreased with the soil depth.

The proportion of mycorrhizae to the total of fine taproots and mycorrhizae (mycorrhizal ratio) at various heights on the slope was compared (Fig. 5). Mycorrhizal development was greatest in the higher part of the slope (subplots 1 and 2). Mycorrhizal ratios in the surface and the middle layers differed among subplots (Friedman's test, $P < 0.05$), while those in the deep layer showed no difference between the subplots.

Second survey

The amount of fine taproots and of mycorrhizae, and the mycorrhizal ratios are shown in Fig. 6. Twenty samples out of 25 collected from the upper part of the slope contained pine roots, while only four samples out of 30 collected in the lower part contained pine roots. As far as the samples containing pine roots are concerned, the amount of mycorrhizal roots was significantly different between subplots H and L in Fig. 2b (Mann-Whitney's U-test, $P < 0.05$), although there was no difference in the amount of fine taproots of pines between the subplots.

Although only four samples contained pine roots in the lower part, the mycorrhizal ratios in all of them were lower than any of those in the upper part, and the difference was significant (Mann-Whitney's U-test, $P < 0.01$).

Total carbon and nitrogen, and extractable phosphorus

Irrespective of height on the slope, carbon content was highest in the surface layer (which roughly corresponded to the A_0 horizon) and decreased with depth (Fig. 7). Among four sampling subplots in Fig. 2a, the carbon content was lowest in subplot 3. The carbon content did not correlate with the amount of any of total pine roots, fine taproots, or mycorrhizae. C/N ratios showed a significant difference (Kruskal-Wallis test, $P < 0.001$ for 0–5 and 5–10 cm depth and $P < 0.05$ for 10–15 cm depth) among the four subplots, and the C/N ratio was highest in subplot 1. Extractable phosphorus content in the surface layer was not uniform (Kruskal-Wallis test, $P < 0.02$) and the samples from subplot 3 contained a lower concentration of extractable phosphorus but there was no significant difference among the samples from the deeper layers in each subplot.

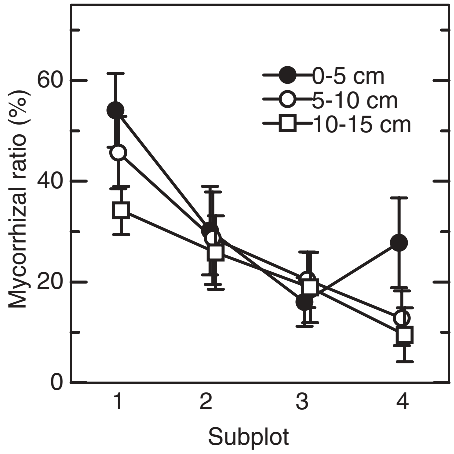

Fig. 5. The proportion of mycorrhizae to the total of fine taproots and mycorrhizae by fresh weight at each depth in each subplot: depths sampled were 0–5 (*black circles*), 5–10 (*white circles*), and 10–15 cm (*white squares*). Values are mean ± SE

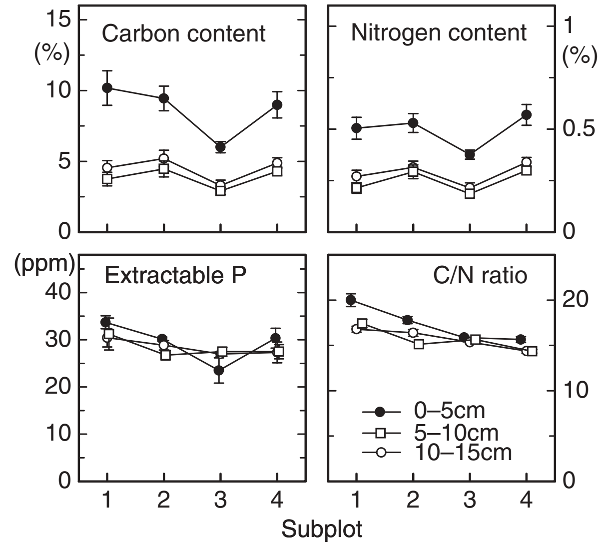

Fig. 7. The concentration of total carbon, total nitrogen, extractable phosphorus, and C/N ratio of the soil samples collected in October 1993 at each depth in each subplot. Values are mean ± SE

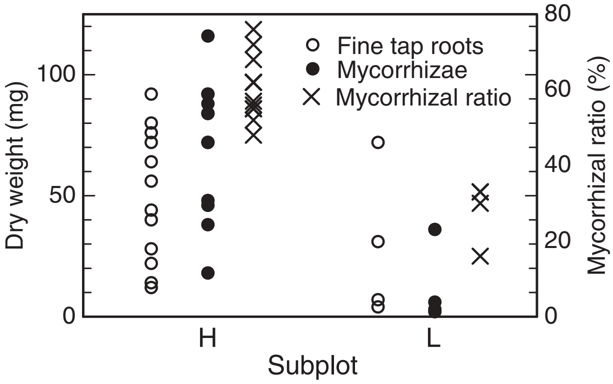

Fig. 6. The amount of fine taproots (*white circles*) and mycorrhizae (*black circles*) in the upper subplot (H) and lower subplot (L) on the slope shown in Fig. 2b. Mycorrhizal ratios (the proportion of mycorrhizae to the total of fine taproots and mycorrhizae) are also shown (*crosses*)

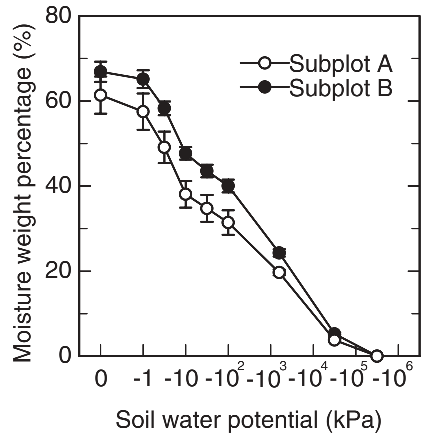

Fig. 8. Water characteristic curves in subplots A (*white circles*) and B (*black circles*). Values are mean ± SE

Water condition

Water characteristic curves of the soils in the upper and lower part of the slope are illustrated in Fig. 8. The available water capacities by weight, which are estimated by the difference in soil water content between field capacity (-6.2 kPa) and permanent wilting point (-1.6×10^3 kPa), were 25% for the upper part and 30% for the lower part, respectively, and the soil of the upper part consistently showed a higher water potential over the whole range of moisture tested.

Using these water characteristic curves, the water potential of the soil in the upper and the lower parts of the slope were estimated in October 1993 and July 1994 (Table 2). The water potential in the upper part varied more widely (-10 to -630 kPa) than in the lower part (-20 to -320 kPa).

Number of feeding wounds made by pine sawyer beetle

The numbers of feeding wounds per twig are shown in Table 3. According to a Mann-Whitney's *U*-test, there was no significant difference between the upper and the lower parts of the slope.

Discussion

Mycorrhizae are widely believed to improve the water relations of associated plants (Brownlee et al. 1983; MacFall et al. 1991). Mexal and Reid (1973) showed that *Cenococcum graniforme* (now called *C. geophilum*), which is a very common mycorrhizal fungus, can grow at a water potential of -20 bar (-2.0×10^3 kPa, which is lethal for most plants) or

Table 2. Moisture weight percentage (MWP) and water potential of the soil in the upper and the lower part of the slope

	Position on the slope	MWP (%)	Water potential (kPa)
October 1993	Upper part	38.2	−10
	Lower part	45.5	−20
July 1994	Upper part	23.2	−630
	Lower part	33.7	−320

Values in October 1993 are the average of subplots 1 and 2 for the upper part and subplots 3 and 4 for the lower part. Values in July 1994 are directly measured at subplots A and B for the upper and the lower part. Sampling points are shown in Fig. 2a

Table 3. The number of feeding wounds per twig made by pine sawyer beetle on 1-year and 2-year internodes on higher and lower positions on trees located at higher and lower parts of the slope

Position on the slope	Number of feeding wounds per twig			
	Higher twig		Lower twig	
	1-year	2-year	1-year	2-year
Upper slope	0.70 ± 0.23	0.55 ± 0.23	0.57 ± 0.22	0.76 ± 0.18
Lower slope	0.35 ± 0.13	0.55 ± 0.15	0.36 ± 0.17	0.64 ± 0.13

In the lower part of the slope, 20 high branches and 14 low branches were sampled. In the upper part of the slope, 20 high branches and 21 low branches were sampled. Values are given are mean and SE

even less. Allen (1991) suggested that fungi such as *C. geophilum* may support the water uptake of the host during the dry season. We also occasionally found *C. geophilum* at our study site regardless of the subplots (data not shown). Thus, the pine trees planted at the upper part may be tolerant to drought stress with the assistance of such mycorrhizae. Kikuchi et al. (1991) suggested that mycorrhizal fungi may facilitate the tolerance to pine wilt disease from the results of their in vitro experiments, although the difference was not statistically significant. To evaluate the role of mycorrhizae in reducing mortality from pine wilt disease, the associated mycorrhizal fungi must be examined strictly as the determinants of the symbiotic relationship.

The symptoms of pine wilt disease are caused by cessation of water flow (Ikeda and Suzuki 1984), i.e., it is caused by water deficiency. As is well known, drought stress accelerates the development of pine wilt disease (e.g., Ikeda 1996). However, more trees were killed in the lower part of the slope, where the water condition may be moderate compared with that in the upper part. There were more roots of plants other than pine trees in the lower part of the slope, which suggests that water competition between pine trees and other plants may be severe in the lower part. However, as the soil in the lower part was always more moist than in the higher part of the slope, the amount of water might be sufficient for both of pine trees and other plants in the lower part.

The biased distribution of killed trees is not attributable to a lower chance of infection in the upper part, since the number of feeding wounds of the vector insects did not show any significant difference between the upper and the lower parts. So, as Miki et al. (2001) suggested, the pine trees in the upper part of the slope might have a higher tolerance to pine wilt disease than those in the lower part. We found distinct differences in the amount of mycorrhizae between the pine trees in the upper and lower part.

In natural forests, biased distributions of pine roots and mycorrhizae are often observed, which have been attributed to differences in soil nutrient level, humidity, vegetation, mycobiota, and so on. A negative correlation has been reported between the contents of soil nitrogen or phosphorus and ectomycorrhizal development (Ruehle and Wells 1984; Daft and Nicolson 1969), although in other studies (e.g., Cordell and Marx 1994), fertilizer (both nitrogen and phosphorus) is neutral to mycorrhizal development. In this study, however, we could not explain the correlation between mycorrhizal development and soil substrates such as carbon, nitrogen, and phosphorus. The thickness of the organic layer also did not explain the difference in mycorrhizal development.

We found that the water condition was different between the upper and lower part of the slope, but could not describe in detail the water condition of the subplots; however, this seems to be very important issue. Further intensive study is necessary to elucidate the influence of soil humidity on the root and mycorrhizal development, tolerance to drought, and the susceptibility to disease. We did not evaluate the water condition of pine trees themselves in this study. To elucidate the function of mycorrhizae in dry conditions, analysis of the water condition of the host plants is important.

The absorptivity of mycorrhizae is higher than that of taproots (MacFall et al. 1991), and we found that the mycorrhizal ratio was higher in the upper part of the slope, where more pine roots were observed. This suggests that the root systems in the upper part show a higher efficiency of water uptake.

In this study, we found that the mycorrhizal development varies with height in a slope, which suggests a difference in the efficiency of the root system in water absorption. We hypothesize that the abundant mycorrhizae found in the upper part of the slope enhanced the water uptake of the pine trees, mitigated the drought stress, and thereby decreased the mortality from pine wilt disease.

Acknowledgments The authors thank Drs. T. Kosaki and J. Yanai for their help in evaluating the water characteristics. They also thank Drs. I. Nakai, Y. Akita, and S. Kitagawa for their help in sampling soils and providing data on the distribution of killed trees.

Literature cited

Allen MF (1991) The ecology of mycorrhizae. Cambridge University Press, New York

Bledsoe CS (1992) Physiological ecology of ectomycorrhizae: implications for field application. In: Allen MF (ed) Mycorrhizal functioning: an integrative plant-fungal process. Chapman Hall, New York

Brownlee C, Duddridge JA, Malibali A, Read DJ (1983) The structure and function of myccrial systems of ectomycorrhizal roots with special reference to their role in forming inter-plant connections and providing pathways for assimilate and water transport. Plant Soil 71:433–443

Buscot F, Weber G, Oberwinkler F (1992) Interactions between *Cylindrocarpon destructans* and ectomycorrhizas of *Picea abies* with *Laccaria laccata* and *Paxillus involutus*. Trees 6:83–90

Cordell CE, Marx DH (1994) Effects of nursery cultural practices on management of specific ectomycorrhizae on bareroot tree seedlings. In: Pfleger FL, Linderman RG (eds) Mychorrhizae and plant health. APS, St. Paul, MN, USA, pp 133–151

Daft MJ, Nicolson TH (1969) Effect of *Endogone* mycorrhiza on plant growth. II. Influence of soluble phosphate on endophyte and host in maize. New Phytol 68:945–952

Finlay RD, Read DJ (1986) The structure and function of the vegetative mycelium of ectomycorrhizal plants. II. The uptake and distribution of phosphorus by mycelial strands interconnecting host plants. New Phytol 103:157–165

Ikeda T (1996) Responses of water-stressed *Pinus thunbergii* to inoculation with avirulent pine wood nematode (*Bursaphelenchus xylophilus*): water relation and xylem histology. J For Res 1:223–226

Ikeda T, Suzaki T (1984) Influence of pine-wood nematodes on hydraulic conductivity and water status in *Pinus thunbergii*. J Jpn For Soc 66:412–420

Kikuchi J, Tsuno N, Futai K (1991) The effect of mycorrhizae as a resistance factor of pine trees to the pinewood nematode (in Japanese with English summary). J Jpn For Soc 73:216–218

Kishi Y (1995) The pine wood nematode and the Japanese pine sawyer. Thomas, Tokyo

MacFall JS, Johnson GA, Kramer PJ (1991) Comparative water uptake by roots of different ages in seedling of loblolly pine (*Pinus taeda* L.). New Phytol 119:551–560

Marx DH (1969) The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic fungi and soil bacteria. Phytopathology 59:153–163

Mexal J, Reid CPP (1973) The growth of selected mycorrhizal fungi in response to induced water stress. Can J Bot 51:1579–1588

Miki N, Sakamoto K, Nishimoto T, Yoshikawa K, Hada Y (2001) Relationship between the incidence of pine wilt disease and the drainage area. J For Res 6:181–186

Nakai I, Kitagawa S, Akita Y, Nakane I, Shibata S (1995) The distribution and its chronic change in a pine wilt forest stand at Tokuyama Experiment Station of Kyoto University Forest at Yamaguchi Prefecture (in Japanese). Rep Kyoto Univ For 28:1–9

Nelson WL, Mehlich A, Winters E (1953) The development, evaluation, and use of soil tests for phosphorus availability. Agronomy 4:153–188

Ruehle JL, Wells CG (1984) Development of *Pisolithus tinctorius* ectomycorrhizae on container-grown pine seedlings as affected by fertility. For Sci 30:1010–1016