Mycoscience 38: 359–362, 1997

Note

Reflection of inside mycorrhizal status in the coloration of mycelial blocks of *Tricholoma bakamatsutake*

Yoshie Terashima

Chiba Prefectural Forest Experiment Station, 1887–1, Haniya, Sanbu-machi, Sanbu-gun, Chiba 289–12, Japan

Accepted for publication 9 June 1997

The coloration of mycelial blocks of *Tricholoma bakamatsutake* was examined in conspicuous colonies of the fungus in the field. Grayish white and gray mycelial blocks contained higher percentages of sounder stages of mycorrhizas and of root tips with attached mycelia of this fungus than dark gray and black ones. The percentage of sounder stages was higher between July and December than in the other months in grayish white mycelial blocks. A survey over time of the grayish white mycelial blocks at 113 points revealed that one point maintained gray after 77 mo.

Key Words——ectomycorrhiza; mycelial block; mycorrhizal root tip; *Tricholoma bakamatsutake*.

The ectomycorrhizal fungus *Tricholoma bakamatsutake* Hongo forms conspicuous mycelial blocks, complexes of mainly mycelia and mycorrhizal root tips, in its mycelial colonies; the mycelial blocks showed a definite color change from grayish white to dark gray from the periphery of the colony toward the center (Terashima et al., 1993). The mycelial blocks closer to the periphery consisted of sounder stages of mycorrhizal root tips than those inside, and a greater proportion of sound stages was found in June than in December (Terashima, 1993). These findings suggested that the coloration of the mycelial blocks had some relationship with the inside mycorrhizal status, and that a seasonal change occurred in mycorrhizal biomass. The objective here is to examine the relationship between the coloration of mycelial blocks of *T. bakamatsutake* and the inside mycorrhizas, and the seasonal change in the developmental stages of mycorrhizal root tips in differently colored mycelial blocks.

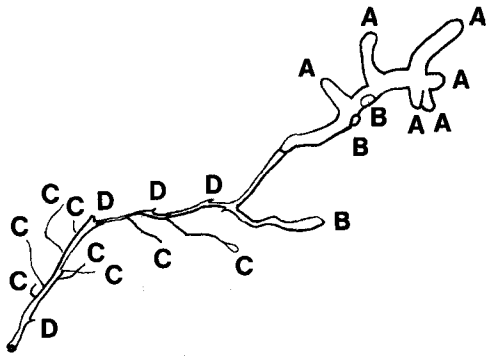

Fig. 1. Rootlet showing the four developmental stages of mycorrhizal root tips with *Tricholoma bakamatsutake*. A, sound; B, broken; C, destroyed; and D, dismissed.

The field study was conducted at a site located in a mixed forest of evergreen *Pasania edulis* Makino and *Castanopsis cuspidata* (Thunb.) Schottky var. *sieboldii* (Makino) Nakai in southern Chiba Prefecture. Five cylindrical samples, about 3 cm in diam and 5 cm depth, were randomly taken from two differently colored mycelial

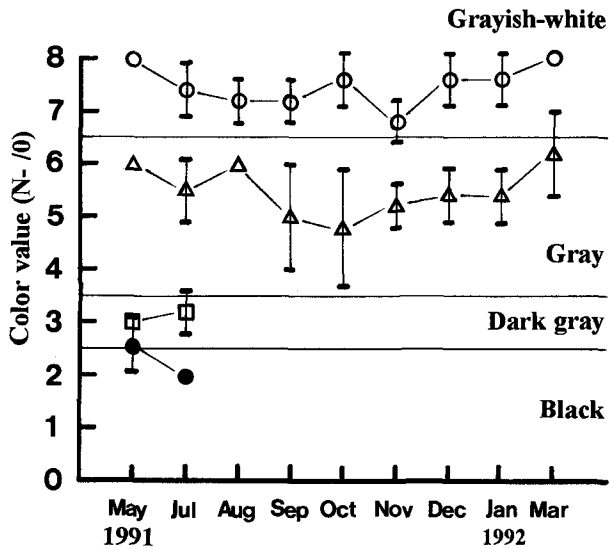

Fig. 2. Seasonal variation in color value of the samples taken from differently colored mycelial blocks of *Tricholoma bakamatsutake*.
Colors of mycelial blocks: ○, grayish white; △, gray; □, dark gray; and ●, black. The plots and bars indicate mean value and standard deviation of five samples, respectively. The color values, "grayish white," "gray," "dark gray," and "black," are in the range of hue N and chroma 0 according to the JIS color notation, discription method of colores by three attributes.

blocks, grayish white and gray, every month or two from May 1991 to March 1992. Five other samples each were also taken from dark gray and black blocks in May and July 1991. Color values of the samples were evaluated in the range of hue N and chroma 0 according to the color notation of the Japanese Industrial Standard (JIS), description method of colors by three attributes, and the presence of attached mycelia was examined. Ten segments of rootlets (about 10 mm long and 0.2–0.4 mm in diam) were then randomly selected from each sample. Mycorrhizal root tips with *T. bakamatsutake* (about 20 per segment; 0.1–0.3 mm in diam) were classified into the four developmental stages, sound, broken, destroyed and dismissed (Fig. 1). The numbers of root tips in the individual stages were counted in each segment, converted to percentages, and used as arcsine values for statistic analysis.

Differently colored pins for each year were stuck at the base of fruit-bodies of this fungus. The numbers of the pins were respectively, 113, 116, 57 and 17 in 1989, 1990, 1991 and 1992. Whenever fruit-bodies were picked, the mycelial blocks under them were observed to be grayish white. Based on this color, the coloration of the mycelial blocks under the pins of 1989 was noted as

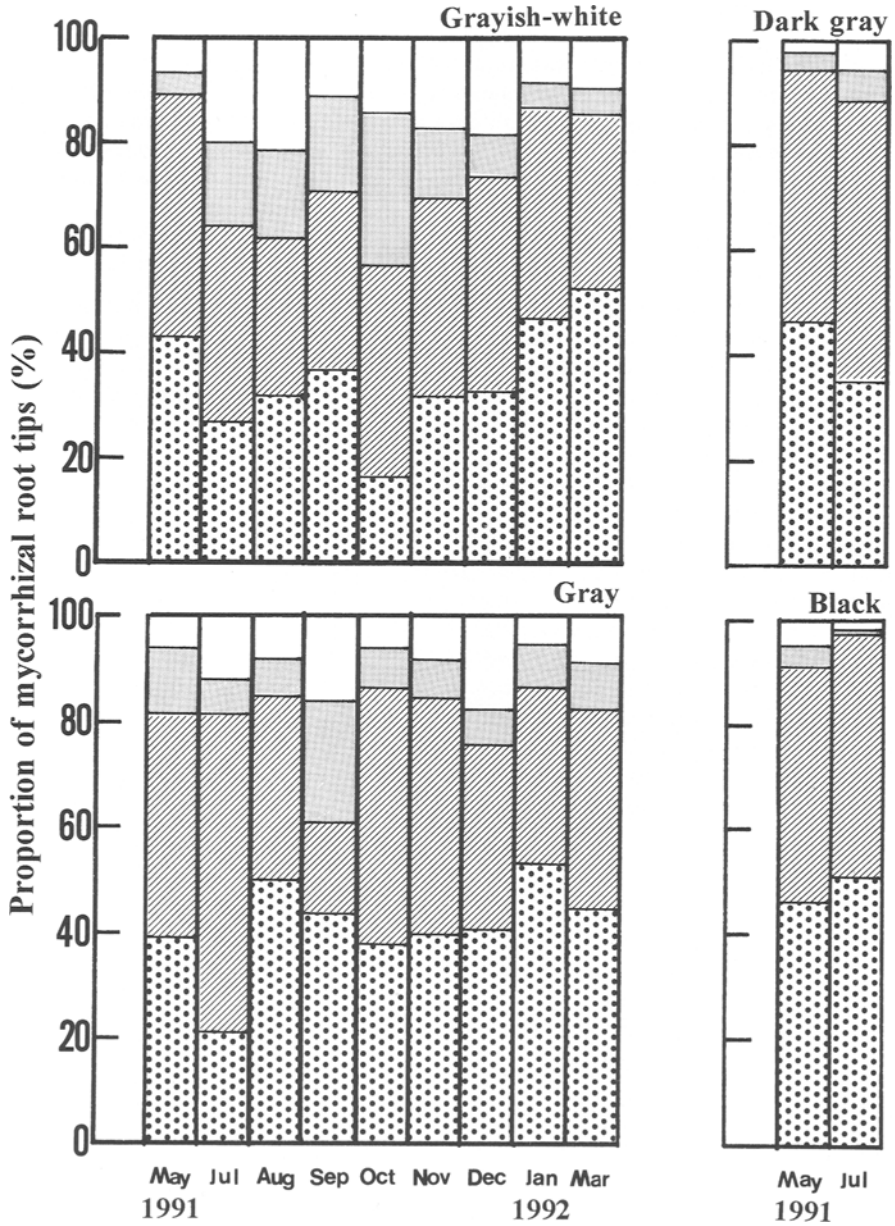

Fig. 3. Seasonal variation in proportions of the four developmental stages of mycorrhizal root tips by *Tricholoma bakamatsutake* from four differently colored mycelial blocks.
Stages: □, sound; ▩, broken; ▨, destroyed; and ▧, dismissed. Samples were taken from grayish white, gray, dark gray and black mycelial blocks.

grayish white, gray, dark gray, or black, in May, July, October and December 1992, and in March 1993, 1994, 1995 and 1996; that under the pins of 1990 and 1991 was in May, July, October and December 1992, and in March 1993; that under the pins of 1992 was in December 1992 and March 1993. Whiteness indexes were then estimated from the following equation, which is generally used for estimation of crop damage caused by insect pests:

$$\text{Whiteness index}=\frac{3N_3+2N_2+N_1}{3(N_3+N_2+N_1+No)}\times 100$$

where N_3, N_2, N_1 and N_0 are respectively the numbers of grayish white, gray, dark gray and black mycelial blocks under the pins.

Figure 2 shows the changes in the color values of the samples from the mycelial blocks of four different colors. The samples from the grayish white mycelial blocks showed a value between 6.5 and 8.0, which corresponded to the JIS color "grayish white." Values of the samples from the gray, dark gray and black mycelial blocks were between 3.5 and 6.5, between 2.5 and 3.5, and below 2.5, corresponding to the JIS "gray," "dark gray" and "black," respectively. Thus, the four colors of mycelial blocks distinguished corresponded to the color ranges of the JIS.

Figure 3 shows the seasonal variation in proportions of the four developmental stages of the mycorrhizal root tips in the four differently colored mycelial blocks. In the grayish white and gray mycelial blocks, significant differences at the 5% level by the two-way analysis were found between the blocks in the sound, broken and destroyed stages, and between the months in all the stages. In the grayish white blocks, the proportions of both sound and broken stages were significantly higher during July and December than during January and May at the 5% level by Duncan's multiple range test; there were no significant differences between months in the proportions of the destroyed stage, and the proportions of the dismissed stage were higher during January and May than July and December. In the gray blocks there were not definite inclination concerning with season in any stage. The proportion of the summation of the sound and broken stages were significantly higher in September.

The average proportions of the four stages, sound, broken, destroyed and dismissed, were respectively 13.9, 12.9, 37.4, 35.2% in the grayish white mycelial blocks; 9.5, 9.7, 47.1, 33.6% in the gray blocks; 3.8, 2.1, 45.5, 48.7% in the dark gray blocks; and 4.7, 4.3, 50.8, 40.2% in the black blocks. The dark gray and black mycelial blocks contained lower proportions of the sound and broken stages than the grayish white or gray ones.

Mycelia of this fungus were observed to be attached to 100, 90.9, 50.0 and 20.0% of the root tips in the samples taken from the grayish white, gray, dark gray and black mycelial blocks, respectively. Mycorrhizas of unidentified fungi were observed in 2.4, 62.2, 100.0 and 100.0% of the samples from the grayish white, gray, dark gray and black mycelial blocks, respectively.

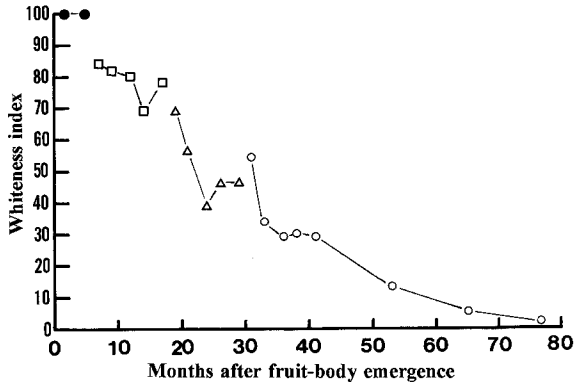

Fig. 4. Change in whiteness index of mycelial blocks under previous fruit-body positions of *Tricholoma bakamatsutake*. The mycelial blocks at the bases of fruit-bodies that emerged in 1989 (○) were examined in May, July, October and December 1992, and March 1993, 1994, 1995 and 1996; those in 1990 (△) and 1991 (□) were in May, July, October and December 1992 and in March 1993; and those in 1992 (●) were in December 1992 and March 1993. Whiteness index equation: see the text.

Figure 4 shows the change in the whiteness indexes of the mycelial blocks under the fruit-bodies that emerged from 1989 to 1992. The youngest mycelial blocks under the newest fruit-bodies in 1992 were examined two and five months later, and had a whiteness index of 100. The oldest mycelial blocks, those under the fruit-bodies of 1989, were examined from 31 to 77 mo later, and their whiteness indexes decreased from 54.0 to 1.8. After 77 mo, none point of mycelial blocks at the bases of the fruit-bodies were grayish white, one point gray, four points dark gray and 108 points black.

The finding here that the percentages of the sound and broken stages of the mycorrhizas of *T. bakamatsutake* were higher between July and December than in the other months in the grayish white mycelial blocks confirmed the distinct seasonal change in mycorrhizal status. Harvey et al. (1978) reported that active ectomycorrhizal root tips increased in May and June in Douglas fir and larch forests, and Vogt et al. (1981) noted a similar increase in September in *Abies amabilis* (Dougl.) Forbes forests.

The grayish white and gray mycelial blocks contained the sound and broken stages of mycorrhizas of *T. bakamatsutake* at a higher level and the mycorrhizas of other fungi at a lower level than the dark gray and black ones. The mycorrhizas in the sound and broken stages were surrounded by more white mycelia of this fungus than those in the other two stages. It was assumed that the quantity of the mycelia attaching to the sound and broken mycorrhizas was reflected in the coloration of the mycelial blocks.

The coloration of the mycelial blocks could be evaluated according to the JIS and each colored mycelial block contained definite proportions of the four stages of mycorrhizal root tips. It might be possible, by surveying

the color changes in the mycelial blocks, to estimate the period in which mycorrhizas with a definite composition of the stages existed in a certain location, that is, the life-span of the various stages of the mycorrhizal root tips. Of the mycelial blocks which were previously grayish white, a portion maintained gray after 77 mo. The gray blocks contained 9.5% of the sound mycorrhizas on an average. This might imply that nearly 9.5% of sound mycorrhizas were continuously present in the same location for more than 6 yr.

Literature cited

Harvey, A. E., Jurgensen, M. F. and Larsen, M. J. 1978. Seasonal distribution of ectomycorrhizae in a mature Douglas-fir/larch forest soil in western Montana. For. Sci. **24**: 203–208.

Terashima, Y. 1993. Distribution and external morphology of mycorrhizal roots at shiros of *Tricholoma bakamatsutake* in a mixed forest of *Pasania edulis* and *Castanopsis cuspidata* var. *sieboldii*. Trans. Mycol. Soc. Japan **34**: 495–505.

Terashima, Y., Tomiya, K., Takahashi, M. and Iwai, H. 1993. Distribution and characteristics of shiros of *Tricholoma bakamatsutake* in a mixed forest of *Pasania edulis* and *Castanopsis cuspidata* var. *sieboldii*. Trans. Mycol. Soc. Japan **34**: 229–238.

Vogt, K. A., Edmonds, R. L. and Grier, C. C. 1981. Seasonal changes in biomass and vertical distribution of mycorrhizal and fibrous-textured conifer fine roots in 23- and 180-year-old subalpine *Abies amabilis* stands. Can. J. For. Res. **11**: 223–229.