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Detection of *Tricholoma Matsutake* in Soil After FOREST Fire in a *Pinus Densiflora* Forest in Korea

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We examined *T. matsutake* survived in soil after forest fires in *T. matsutake*–produced forest in the pre–fire period and the situation of damages from forest fire in stands. We established a square plot of (50 m×50 m) in a burned site and divided it into 100 square quadrats of (5 m×5 m). Tree density of *P. densiflora*, the dominant tree species, in plot was 2,104/ha, with a basal area accounting for 99%. The percentage of dead pine trees was 52.1%, which is less than in other species as 88.6% and 100% of *Quercus* spp. and *Prunus sargentii*, respectively. In PCR amplification using the *T. matsutake*–specific primer pair, the 400–bp fragment was found in 29 of 100 soil sample s from each quadrat and in control cultures. The relationship to the percentage of crown damage differed significantly (p=0.0026), with means between Tm and nTm of 59% and 70.5%, respectively. Moreover, the difference between BLT and nBLT on crown damage was highly significant in the U–test (p=0.0008). Therefore, *P. densiflora* in inner quadrats with low crown damage had *T. matsutake* on their roots. *T. matsutake* favor *P. densiflora* and mineral soils with low litter rather than under BLT with a thick humus layer. The area of *T. matsutake* from a forest fire in crown damage to *P. densiflora* will eventually decrease in relation to the increase in BLT. Hence, the species distribution in understory vegetation such as shrub and BLT is an important factor for overstory tree survival from forest fires. In conclusion, the management of *T. matsutake* forest by controlling tree density and taking out understory vegetation is considered a good method for decreasing damage from forest fires.

INTRODUCTION

Tricholoma matsutake (S. Ito et Imai) Sing. is an ectomycorrhizal basidiomycete that produces economically important edible mushrooms "matsutake" in association with *Pinus* spp. plants in the Northern Hemisphere (Ogawa, 1975; Hosford *et al*., 1997). The prices of this mushroom as a seasonal delicacy favored by the Japanese, Chinese and Korean are so expensive. But the annual yield of *T. matsutake* in Japan has dramatically decreased since the 1940s. The yield of 52 tons in 2002 was only 0.4% of the 12,000 tons recorded in 1941 (Ministry of Agriculture, Forestry, and Fisheries of Japan). So, almost volumes of consumption in Japan are imported mainly from Korea, China and North America. A similar decline in *T. matsutake* production has occurred in China (Xu and Ribot, 2004) and Korea (Lee, 2008) probably because of recent global climate change leading to drought and warming as well as due to post–World War Ⅱ urban development and pine wilt disease (Hosford *et al*., 1997; Hall *et al*., 2003).

In recent, forest fires occurred in east cost regions of Korea in Goseong in May 1996, in Donghean in April 2000, and in Yangyang in May 2005 and burned 3762 ha, 23794 ha, and 973 ha, respectively, of forested land (Chun, 2005). These fires led to tremendous economic losses of 202.7 (Goseong), 100 (Donghean), and 18.3 (Yangyang) million US dollars. Because *Pinus densiflora* Sieb. et Zucc. which had been associated with mainly *T. matsutake* production is the dominant species in those forests and destroyed by these fires. Therefore, the forest fires damaged not only the pine trees but also reduced wood resources and local income. Specific countermeasures for the recovery of *T. matsutake* in forests are needed in these regions.

In fungal molecular methods, polymerase chain reaction (PCR) analysis of the internal transcribe spacer (ITS) region of nuclear rDNA has proven to be a reliable methods for identification at species level (Burn *et al*., 1991; Bridge *et al*., 1998). This method is also acceptable to ectomycorrhizal fungi and has been used in both fields and laboratories (Henrion *et al*., 1992; Gardes and Burns, 1993; Karen *et al*., 1997). In recent, Kikuchi *et al*. (2000) developed a specific ITS primers pairs for *T. matsutake* detection in soils. This method is useful for the identification of *T. matsutake* from the field research (Guerin–Laguette *et al*., 2005).

Therefore, our objectives in this study were 1) to examine *T. matsutake* population that survived in soils after fires using PCR analysis of ITS region of nuclear rDNA, and 2) to clarify the situation of individual damages of *P. densiflora* associated with surviving *T. matsuteke* in *T. matsutake* produced forest in the pre–fire period. This information will be useful in managing *T. matsutake*–producing forests for the conservation of the fungal resources and reforestation of *T. matsutake* forests after fires.

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MATERIALS AND METHODS

Study site and field survey

The study site (38° 05' N, 128° 35' E) was in the region of the Yangyang forest fire of May 2005, close to the East Sea in Kangwon Province, Korea, partly on the eastern slope of the Baekdu–Tekgan range (Fig. 1). A field survey was carried out in February 2006, 10 months after the fire. We established a square plot of (50 m× 50 m) in a burned site on the southeastern slope and divided it into 100 square quadrats of (5 m×5 m). We measured overall tree height and diameter at breast height (dbh, 1.3 m) of trees more than 1.3 m tall. Trees >1.3 m were divided into two classes, dying and surviving, and individually scored according to the percentage of fire damage to stems and crowns.

Soil sampling

On hundred soil samples of 50–100 g were collected from the 10–15–cm layer of soil in each quadrat after the field survey in February 2006. Samples were kept in a freezer at −80 °C until DNA extraction.

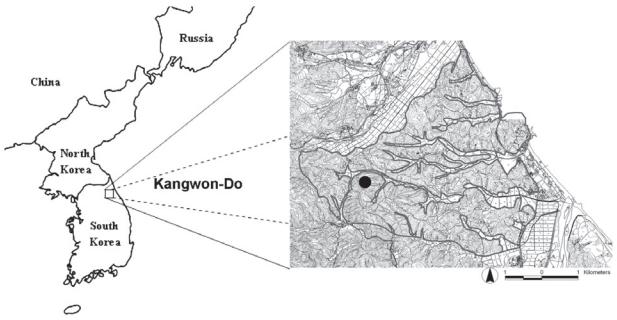

Fig. 1. Location of the study site (●).

DNA extraction and polymerase chain reaction (PCR)

Fungal DNA was extracted from 0.25–1.00 mg of each soil sample using the UltraCleanTM Soil Isolation Kit (MO BIO), according to the manufacturer's instructions. The internal transcribed spacer (ITS) region, including the 5.8S ribosomal DNA (rDNA) segment, was amplified using primers specific for *T. matsutake* TmF and TmR (Kikuchi *et al*., 2000) regions. Each 50–μl amplification reaction mixture contained 5 μl 10x G–Taq buffer, 4 μl 2.5 M dNTP, 5 μl 5x Tuning buffer (Cosmo Genetech, Seoul, Korea), and 0.5 μl G–Tag DNA polymerase (Cosmo Genetech, Seoul, Korea). Amplifications were performed using a 2720 Thermal Cycler (Applied Biosystems). Initial denaturation started at 95 °C for 10 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 59 °C for 30 s, and extension at 72 °C for 1.5 min; a final extension was performed at 72 °C for 8.5 min. The presence or absence of a specific fragment band was visualized by 2% agarose gel electrophoresis and ethidium bromide staining.

RESULTS

Characteristics of the forest structure of a post–fire stand

Table 1 shows stand condition after the forest fire. The forest was composed of *P. densiflora*, *Quercus mongolica* Fisch, *Q. aliena* Blume, *Q. serrata* Thunb., *Q. variabilis* Blume, and *Prunus sargentii* Rehd. Tree

Table 1. Stand condition of the study site

Tree species	Density (trees/ha)	DBH* (cm)	H* (m)	BA (m^2/ha)	Dead tree (%)
Pinus densiflora	2104 (0.92)**	12.28 (1–3)	4.31 (1.3–12)	30.29 (0.99)**	52.1
Quercus mongolica	124	4.97 (0.8–18)	3.1 (1.5–6.7)	0.35	74.2
Q. aliena	4	3.6 (2.5–2.5)	1.6 (1.6–1.6)	0.002	100
Q. serrata	12	4.1 (1.2–7)	3.3 (1.7–5.6)	0.021	100
Q. variabilis	20	4.9 (3.5–8)	3.4 (2–6.5)	0.042	80
Prunus sargentii	13	3.56 (2–4.7)	3.93 (1.8–2.8)	0.013	100

* Values are mean and range ** Ratio of *P. densiflora*.

Table 2. Percentage of dead trees among the ranges of height

Tree species / Height (m)	*P. densiflora* dead no./total no.	(%)	*Quercus spp** dead no./total no.	(%)	*P. sargentii* dead no./total no.	(%)
<5	235/358	66	27/35	77.1	3/3	100
5–10	39/169	23.1	4/5	80	–	–
10<	0/1	0	–	–	–	–
Total	274/526	52.1	31/40	77.5	3/3	100

* Inculding *Quercus* species as 31 of *mongolica*, 1 of *aliena*, 3 of *serrata*, and 5 of *variabilis*.

Table 3. Percentage of dead trees among the ranges of DBH

Tree species / DBH (cm)	*P. densiflora* dead no./tatal no.	(%)	*Quercus spp** dead no./total no.	(%)	*P. sargentii* dead no./total no.	(%)
<10	160/193	82.9	30/38	78.9	3/3	100
10–20	112/292	38.4	1/2	50	–	–
20<	2/41	4.9	–	–	–	–
Total	274/526	52.1	31/40	77.5	3/3	100

* Same in Table 2.

Table 4. Relationship between dead trees and the percentage of stem damage in ranges

Tree species / Damage**(%)	*P. densiflora* dead no./tatal no.	(%)	*Quercus spp** dead no./total no.	(%)	*P. sargentii* dead no./total no.	(%)
<30	89/258	34.5	0/8	0	–	–
30–60	142/219	64.8	0/1	0	–	–
60<	43/49	87.8	31/31	100	3/3	100
Total	274/526	52.1	31/40	77.5	3/3	100

* Same in Table 2. ** Stem damage.

Table 5. Relationship between dead trees and the percentage of crown damage in ranges

Tree species / Damage**(%)	*P. densiflora* dead no./tatal no.	(%)	*Quercus spp** dead no./total no.	(%)	*P. sargentii* dead no./total no.	(%)
<30	0/148	0	0/8	0	–	–
30–60	0/88	0	0/1	0	–	–
60<	274/290	94.5	31/31	100	3/3	100
Total	274/526	52.1	31/40	77.5	3/3	100

* Same in Table 2. ** Crown damage.

density of *P. densiflora*, the dominant tree species, was 2,104/ha, with a basal area accounting for 99%. The mean height of *P. densiflora* was larger than that of the other tree species, and the percentage of dead pine trees was 52.1%, which is less than in other species. The relationship between dying trees and tree height is shown in Table 2. The percentage of dying *P. densiflora* in relation to tree height was 66% of trees <5 m tall and 23.1% of trees 5–10 m tall. There was a high percentage of dying trees with a DBH <10 cm (Table 3), and a high percentage of trees of *P. densiflora* and *Quercus* spp. had damaged stems (Table 4). In terms of crown damage, although there were no dead trees in the <30% and 30–60% crowns, almost all trees of over 60% died (Table 5).

Detection of *T. matsutake* in post–fire soils

In PCR amplification using the *T. matsutake*–specific primer pair, the 400–bp fragment was found in 29 of 100 soil samples and in control cultures (Fig. 2). In quadrat nos. 6, 10, 11, 14, 16, 19, 23, 29, 30, 33, 38, 39, 42, 43, 45, 49, 52, 56, 57, 58, 60, 61, 64, 65, 67, 88, 90, 92, and 93, *T. matsutake* with gray–colored cell were detected (Fig. 3). The percentage of dead *P. densiflora* in each quadrat to the total pine number was assessed. All detected quadrats containing *T. matsutake*, except nos. 16, 43 and 52, had surviving *P. densiflora*, while only one tree survived in quadrats 38, 61, 88, and 92. There was no difference between the percentage of dead trees per quadrat and detection of *T. matsutake*. More than half of the quadrats (55.5%) containing *T. matsutake* were on stony soils.

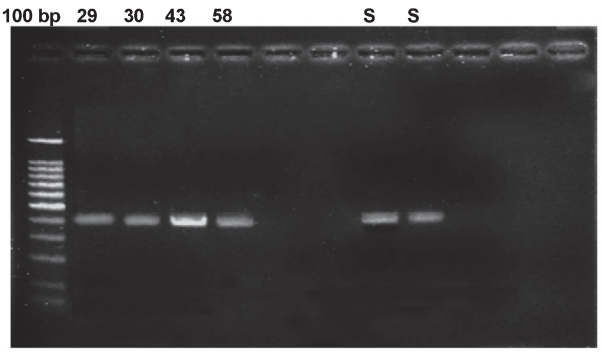

Fig. 2. Amplication with the *T. matsutake* specific primer pair TmF/TmR (29, 30, 43, 58, quadrat nos,; S, hyphae of *T. matsutake* from pure culture).

50 m

5m

10	9	8	7	6	5	4	3	2	1
1/3(33.3)	4/7(57.1)	4/5(80)	2/5(40)	4/7(57.1)	1/2(50)	1/4(25)	0/5(0)	0/4(0)	1/7(14.3)*
20	19	18	17	16	15	14	13	12	11
3/5(60)	0/3(0)	6/9(66.7)	3/7(42.3)	5/5(100)	0/5(0)	1/6(16.7)	1/5(20)	1/5(20)	0/5(0)
30	29	28	27	26	25	24	23	22	21
2/6(33.3)	1/4(25)	4/7(57.1)	1/4(25)	3/4(75)	1/5(20)	1/8(12.5)	1/3(33.3)	0/1(0)	0/8(0)
40	39	38	37	36	35	34	33	32	31
2/5(40)	1/5(20)	3/4(75)	0/3(0)	5/9(55.6)	4/5(80)	1/3(33.3)	0/3(0)	0/6(0)	1/6(16.7)
50	49	48	47	46	45	44	43	42	41
3/6(50)	2/5(40)	2/4(50)	1/3(33.3)	1/3(33.3)	1/3(33.3)	4/5(80)	4/4(100)	3/9(33.3)	2/3(66.7)
60	59	58	57	56	55	54	53	52	51
0/5(0)	1/6(16.7)	1/3(33.3)	1/5(20)	2/4(50)	3/5(60)	5/8(62.5)	4/7(57.1)	4/4(100)	4/6(66.7)
70	69	68	67	66	65	64	63	62	61
3/6(50)	4/6(66.7)	5/7(71.4)	2/5(40)	1/4(25)	0/4(0)	2/5(40)	10/10(100)	5/5(100)	9/10(90)
80	79	78	77	76	75	74	73	72	71
1/3(33.3)	6/6(100)	4/5(80)	3/5(60)	1/2(50)	3/6(50)	3/6(50)	5/5(100)	9/9(100)	6/8(75)
90	89	88	87	86	85	84	83	82	81
2/5(40)	4/4(100)	3/4(75)	1/1(100)	0/3(0)	2/5(40)	5/6(83.3)	3/3(100)	7/8(87.5)	5/5(100)
100	99	98	97	96	95	94	93	92	91
2/4(50)	12/12(100)	7/7(100)	2/4(50)	2/6(33.3)	0/4(0)	4/8(40)	4/6(66.7)	7/8(87.5)	2/5(40)

50 m

Fig. 3. Quadrats of survival *T. matsutake* with grayish cells.
* Number of dead tree/total number of tree (% of dead tree)

DISCUSSION

Surviving *T. matsutake* were detected in 29 of 100 quadrats after the forest fire in this former *T. matsutake* production forest. *Pinus densiflora* is the dominant tree, occupying 99% of total basal area, and is the symbiont for *T. matsutake*, but only 252 trees (47.9%) survived the fire. Although four of the quadrats in which *T. matsutake* were detected had only one living tree, living *T. matsutake* hyphae were found in the soil. Thus the presence of the host tree may be the most important factor for *T. matsutake* remaining in soils after disturbances such as forest fires. However, only three quadrats (nos. 16, 43, and 52) in which *T. matsutake* were detected contained none living trees. It is interesting that trees with a DBH of 25.5 cm and 20.83 cm were found in the quadrat next to that in which *T. matsutake* were detected. The living *T. matsutake* hyphae appear to be associated with the lateral roots of the nearest big *P. densiflora* tree. Mycorrhizal fungi in soil are usually found at depths of 10–15 cm, and *T. matsutake* can form ectomycorrhizal associations with the lateral roots of juvenile *P. densiflora* seedlings (Yamada *et al.*, 2006). Guerin–Lagutte *et al.* (2005) succeeded in establishing *T. matsutake* mycorrhizae on long root segments of 50–year–old *P. densiflora* trees. Therefore, some large trees from the populations in the forest are very important for the preservation of the biota.

In terms of height and DBH of surviving trees, there was no significant difference in a *U*–test between Tm (inner tree of quadrats containing *T. matsutake*) and nTm (inner tree of quadrats without *T. matsutake*) (Table 6) Therefore, mycorrhizal infection by *T. matsutake* may not be influenced by the growth of adult *P. densiflora* in nature. Seedlings of *P. densiflora*, on the other hand, show better growth with symbiont *T. matsutake* than non–infected seedlings (Yoshimura, 2004).

Table 6. Relationship between Tm and nTm, and the height, DBH and the percentage of stem and crown damage caused by the forest fire in average and U–test

	Average*		*p*–value
	Tm**	nTm***	
Height (m)	5.41 (2.2–9.7)	5.09 (1.7–12)	0.0839
DBH (cm)	16.21 (6–30)	15.1 (4.5–28)	0.0871
Stem damage (%)	30.33 (2–82.61)	34.34 (0.33–100)	0.2067
Crown damage (%)	59.04 (5–100)	70.47 (5–100)	0.0026

* Values are mean and range (Min.–Max.). ** Inner tree of quadrats containing *T. matsutake*. ***Inner tree of quadrats without *T. matsutake*.

Table 7. Relationship between BLT and nBLT, and the percentage of stem and crown damage caused by the forest fire in U–test

	Average*		*p*–value
	BLT**	nBLT***	
Stem damage (%)	37.27 (4.26–100)	31.87 (0.33–100)	0.0568
Crown damage (%)	76.18 (5–100)	64.41 (5–100)	0.0008

* Values are mean and range (Min.–Max.). ** Inner tree of quadrats with BLT. ***Inner tree of quadrats without BLT.

Although the relationship between Tm and nTm and the percentage of stem damage caused by the forest fire was not significant in the *U*–test, the relationship to the percentage of crown damage differed significantly (p=0.0026), with means between Tm and nTm of 59% and 70.5%, respectively (Table 6). Although the relationship between broadleaf trees (BLT; inner trees of quadrats with BLT) and nBLT (inner trees of quadrats without BLT) and the percentage of stem damage caused by the forest fire was not significant in the *U*–test, the difference between BLT and nBLT was highly significant in the *U*–test (p=0.0008) (Table 7). The mean crown damage in BLT was 76.2%, and that in nBLT was 64.4%. Crown damage to BLT in forest fires is an important factor as these provide connecting materials from the ground to the crown fire. This is particularly important in the studied forest where the mean height of BLT was 2.99 m (range 1.5–6.7 m) while the mean height of *P. densiflora* was 4.3 m (1.3–12.0 m). Moreover, almost all oak trees as BLT in this study area were resprouting from stumps. *Pinus densiflora* in inner quadrats with low crown damage had *T. matsutake* on their roots. *T. matsutake* favor *P. densiflora* and mineral soils with low litter rather than BLT with a thick humus layer. The area of *T. matsutake* from a forest fire in crown damage to *P.*

densiflora will eventually decrease in relation to the increase in BLT. Hence, the species distribution in understory vegetation such as shrub and BLT is an important factor for overstory tree survival from forest fires.

In conclusion, the management of *T. matsutake* forest by controlling tree density and taking out understory vegetation is considered a good method for decreasing damage from forest fires. Moreover, the *T. matsutake* production from managed pine forests is higher than that from abandoned forests. Yoshimura (2004) suggested removing BLT, shrubs, herbs, and the humus layer for the improvement of *T. matsutake* forests. If *T. matsutake* forests that were productive before a fire contain *P. densiflora*, it is recommended that reforestation with this pine should be undertaken soon after the fire because *T. matsutake* hyphae were found to survive in soils after the fire. Choung *et al*. (2004) reported that pre–fire pine forests have been converted to oak–dominated stands immediately after the fire on the east coast. This could lead to the suppression of *P. densiflora* seedlings by taller BLT sprouts (Choung, 2002). Changes in soil pH after a fire should also be considered, and Sawamoto *et al*. (1997) reported that the soil pH became alkaline in Siberian forests after fire. Alkaline soils containing Douglas–fir, sequoia, and beech seedlings also suffer from increased damping–off caused *Rhizina undurata* Fr. ex Fr. more than those in acidic soils (Schonhar, 1955). Other soil–borne diseases caused by *Rhizoctonia solani* Kuhn and *Fusarium* spp. can result in damping–off of coniferous seedlings on neutral to alkaline soils (Tint, 1945; Ito, 1950; Sato and Shoji, 1964). *Pinus densiflora* saplings should be grown in nurseries for immediate artificial plantation in recovering *T. matsutake* forests after forest fires.

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