Short Communication

Report on Laccaria amethystina, newly confirmed as an ammonia fungus

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Laccaria amethystina was judged to be an ammonia fungus in accordance with the definition proposed by Sagara (1975). In 72 urea-treated plots, *L. amethystina* was found to fruit in the succession of the ammonia fungi, and its fruit bodies appeared about 300 times more densely inside than outside the plots. Thus the author concluded that *L. amethystina* satisfies the definition of the ammonia fungi.

Key Words—ammonia fungi; Laccaria amethystina; urea treatment.

The "ammonia fungi" have been defined as a chemoecological group of fungi which sequentially develop reproductive structures exclusively or relatively abundantly on soil after the addition of ammonia, some other nitrogenous materials which react as bases by themselves or on decomposition, or alkalis (Sagara, 1975). The sequential development of reproductive structures is called the succession of ammonia fungi. In the ectomycorrhizal (ECM) forests in particular, there are two successional phases; saprotrophic and ectomycorrhizal. Because saprotrophic species fruit before ECM species, each of the successional stages are called the early phase (EP) and the late phase (LP) in the succession, respectively (Sagara, 1995). After the late phase in the succession, the fungal species which might have fruited before the addition of materials, recover. The ammonia fungi can be further divided into two groups according to the degree of exclusiveness of their fruiting: ammonobiont and ammonophilous species (Sagara, 1975). Thus, the timing and amount of reproductive structures developed are important characters of each ammonia fungus.

Laccaria amethystina Cooke is known to form ECM association with Quercus and Fagus in temperate deciduous or deciduous-coniferous forests of eastern North America and Europe, and with species of Quercus in Central America and northern South America (Mueller, 1992). Fruit bodies of this fungus can often be found in the forests. Cogeneric Laccaria bicolor (Marie) Orton is a known ammonia fungus (Sagara, 1995) that forms ECM association with the Pinaceae and Quercus trees (Mueller, 1992). Sagara (1995) mentioned that L. amethystina seemed to be an ammonophilous species as well as L. bicolor (see Sagara, 1992), but presented no data.

To ascertain whether *L. amethystina* can be included in the ammonia fungi, 72 urea-treated plots were established in two types of vegetation, one is dominated by *Castanopsis cuspidata* (Thunberg) Schottky and the other by *Quercus serrata* Murray and the response of ECM fungi was studied.

Urea-treatment plots were established in the forests of Iwakura, Kyoto City (35°4′N, 135°47′–48′E, 150–200 m alt.), each time in forests of two types, the one dominated by *C. cuspidata* and the other by *Q. serrata*. In the sub-canopy layer of forests, there were *Q. glauca* Thunb., *Ilex pedunculosa* Miq., *Cleyera japonica* Thunb. pro parte emend. Siebold et Zucc., *Eurya japonica* Thunb., *Pieris japonica* (Thunb.) D. Don, *Lyonia ovarifolia* (Wall.) Drude subsp. *neziki* Hara, *Acanthopanax sciadophylloides* Fr. et Sav., *Evodiopanax innovans* (Siebold et Zucc.) Nakai etc. (all the scientific names based on Kitamura and Murata (1979a, b))

To induce fruiting of the ammonia fungi, granular urea fertilizer (N 46%: Kumiai Nyouso, JA) was scattered by hand onto square plots that measuring 0.5 m in the horizontal direction and 1 m along the slope. Urea was applied in three levels: 85.8 g, 171.5 g and 343.0 g. For each level, root-severed plots were also set up to investigate the effect of absence of host-roots (see Imamura, 1999). In total, 6 urea-treated plots were established each within a square area of 6 m by 6 m, at each time of treatment in each forest stand. Treatment was conducted 6 times in 2 yr, giving a total of 72 urea-treated plots. Detailed information on the research sites is shown in Table. 1.

Plots were visited every 5–12 d for about 3 yr. All the fruit bodies developing inside the plots were counted, and then collected. The square area around plots about

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Table 1. Date of urea treatment and study sites.a)

Date of treatment	Research site	Dominant species of forest	Name of site	Direction of the slope	Ground inclination	
17 May. 1997	lwakura-kino-cho	Castanopsis cuspidata	C1	SSE	21°	
	lwakura-kino-cho	Quercus serrata	Q1	sw	29°	
10 Aug. 1997	lwakura-kino-cho	Castanopsis cuspidata	C2	SSE	25°	
	lwakura-kino-cho	Quercus serrata	02	sw	26°	
17 Feb. 1998	lwakura-kino-cho	Castanopsis cuspidata	C3	SSE	26°	
	lwakura-kino-cho	Quercus serrata	Q3	SSW	27°	
22 Feb. 1998	Iwakura-agura-cho	Castanopsis cuspidata	C4	ESE	22.5°	
	lwakura-hanazono-cho	Quercus serrata	Q4	ENE	19°	
3 Jun. 1998	lwakura-kino-cho	Castanopsis cuspidata	C5	SSE	28°	
	lwakura-kino-cho	Quercus serrata	Q5	ssw	29.5°	
12 Aug. 1998	lwakura-agura-cho	Castanopsis cuspidata	C6	ESE	14°	
	Iwakura-hanazono-cho	Quercus serrata	Q6	ENE	26°	

a) Six plots were established at each treatment in each forest stand.

 $64\,m^2$ was also inspected to see whether any ammonia fungi developed fruit bodies. Thus, in total, about 770 m^2 of the field outside the urea plots was studied.

Of the 72 urea-treated plots, *L. amethystina* fruited on 6 plots (one in *C. cuspidata* forest and the others in *Q. serrata* forest). Fruit bodies numbered 31 in all (Table 2). Only two fruit bodies of *L. amethystina* were found outside the plots throughout the research, and no other ammonia fungi fruited. One was recorded in one in *C. cuspidata* forest (C1) and the another in *Q. serrata* (Q1) forest (Table 2).

Fig. 1 shows fruit bodies of *L. amethystina* within one urea-treated plot in *Q. serrata* forest 405 d after treatment (treated on 17 May, 1997). The fruit bodies developed on the interior of the plot. The timing of fruit-

ing is shown in Table 2, *L. amethystina* fruited in LP, when other ammonia fungi, e. g. *Alnicola lactariolens* Clémençon et Hongo, *Hebeloma spoliatum* (Fr.) Karst. and *H. radicosoides* Sagara, Hongo & Y. Murak. were continuing to fruit in all the 6 plots. In one plot in *Quercus* forest (3 Nov., 1998; treated on 3 Jun., 1998), *L. amethystina* fruited first after EP (Table 2).

The above findings indicate that *L. amethystina* should be recognized as an ammonia fungus (ammonophilous species), in accordance with the definition of the ammonia fungi cited above. Although it fruited less often than *Hebeloma* species, which are principal component of LP species in the urea-treated plots, the high degree of replication afforded by the 72 plots showed conclusively that *L. amethystina* developed

Table 2. Data on plots on which fruit bodies of L. amethystina developed.

Date of treatment	Name of site	Amount of urea (g N) 40	Root severing ^{b)}	First appearance of LP species inside the plot 1998.5.26	Date of observation (No. of fruit bodies)		Putative end of LP Autumn, 1998
1997.5.17	C1				1998.6.26 (2)		
1997.5.17	Q1	40	+	1997.10.7	1998.6.17	1998.6.26 (11)	Autumn-winter, 1998
1997.5.17	Q1	80	_	1997.10.13	1998.6.26	1998.10.23	Autumn-winter, 1998
1998.2.22	Q4	80		1998.7.6	1998.10.4 (1)	,-,	Summer, 1999
1998.2.22	Q 4	160	_	1998.6.23	1998.10.4	19 98.10.19 (1)	Summer, 1999
1998.6.3	Q5	40	_	1998.11.3	1998.11.3	. ,	Summer, 1999 or later
1997.5.17ª)	C1	0	_	_	1998.6.26 (1)		_
1997.5.17ª)	Q1	0	_	<u> </u>	1998.6.26 (1)		_

a) Record of L. amethysitina outside the urea plots.

b) see Imamura (1999).



Fig. 1 Fruit bodies of *L. amethystina* in a plot of Q1 (treated with 82.8 g of urea on May 17, 1997) photographed on June 26, 1998. Eleven fruit bodies were observed on the day, as shown by arrowheads. The inset shows a magnified view.

reproductive structures in the late phase of the succession of ammonia fungi on soil after the addition of urea (Table 2). Further, *L. amethystina* developed was about 16 times more fruit bodies inside the plots than outside (Table 2). Density of fruit bodies was about 0.86 and 0.0026 per m² inside and outside the plots, respectively. Thus the fungus developed reproductive structures relatively abundantly on the soil after the addition of urea.

Lastly, *L. amethystina* appears to be associated with *Q. serrata* (Table 2) and to prefer plots treated with lower levels of urea. These issues remain for the future, however, together with on the detailed characteristics of *L. amethystina* as an ammonia fungus.

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