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Ammonia Fungi — A Chemoecological Grouping of Terrestrial Fungi*

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CONTENTS

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INTRODUCTION

In a series of experiments to observe the response of higher fungi to chemical disturbance of forest soil (ground), urea application brought about a restricted and luxuriant occurrence (formation of reproductive structures on the soil surface)* of a few peculiar fungi, with all others disappearing, and some striking changes in soil properties (SAGARA & HAMADA, 1965). To elucidate the ecological meanings or causes of these phenomena, the following investigations were carried out.

I. The urea effect was ascertained with the soils of various vegetations in many parts of Japan and the fungi which showed the same or similar responses as above were listed. Further, modification of the urea effect by the region or by the type of vegetation was studied.

II. Various kinds of chemicals or agents were applied to soil to clarify the substitute for urea, the factors responsible for the urea effect, and the limits or extent of the phenomena in question.

III. Natural habitats of the fungi obtained by the treatment with urea and its related materials were searched for in the field.

IV. Responses of soil and other organisms to the chemical treatments and in the natural habitats detected by III were examined to determine the characteristics of the place of occurrence of the fungi in question.

V. Taxonomic positions of these fungi were studied and their known habitats, which may suggest their ecological or physiological characters, were cited and discussed to delimit the findings.

In these studies, the soils of uncultivated lands, especially of forests, were dealt with. Only the reproductive stages are discussed, though it has been observed on many occasions that a considerable vegetative growth do precede reproduction (Pl. 1, C; footnote on p. 269). Through these studies, I will propose an experimental-ecological

* The words "occurrence (*or* to occur)" and "appearance (*or* to appear)" are used in this sense. The words "reproductive structures" indicate basidiocarps in Basidiomycetes, ascocarps in Ascomycetes, conidiophores and conidia in Deuteromycetes, and sporangiophores and sporangia in Zygomycetes. Further, the words "to yield (to produce, *or* to obtain) a species (*or* a fungus)" mean "to bring about the occurrence of a fungus species".

grouping of soil fungi (terrestrial fungi) as a step towards further studies.

Succession (sequential appearance) of the fungi, fruit body production, effects of temperature (or season) and of concentration of the chemicals, etc. will not be discussed in detail in the present paper. An outline of the present contents was preliminarily published in SAGARA (1973).

HISTORICAL

It has not been well recognized that some fungi appear in response, direct or indirect, to chemical treatment of soil, and, despite considerable fertilization research, there has been relatively little literature on the occurrence of fungi. The information available is shown in Table 1.

Excluded from Table 1 are the papers by GILBERT (1875), RAUTAVAARA (1950), and FIEDLER & HUNGER (1963). GILBERT observed, after repeated application of fertilizers to meadow-land for twenty years, a luxuriant occurrence of "*Marasmius oreadum*" in fairy rings exclusively "on the plot with superphosphate of lime, sulphate of soda and magnesia but without potass for fourteen years". RAUTAVAARA recognized that lime dust discharged from a nearby factory had some effects on the flora of larger fleshy fungi in a spruce forest. These are somewhat outside the scope of the present studies because their studies are concerned with the effects of long-term or continuous applications of chemicals (see p. 248 for the significance of "repeated application"). FIEDLER & HUNGER observed the effect of CaO and dolomite on the fungus flora in a spruce forest eight years after their application. Since they did not deal with the immediate effect of the treatment, their work too is difficult to compare with the present work, though I myself have observed that the effect of lime continues for many years.

Together with those listed in Table 1, this information may serve to claim that some species of fungi appear as a result of chemical treatment. The present studies are not only to establish this knowledge by presenting some new facts but also to emphasize the specificity of susceptible species, the constancy of their occurrence, the secondary effects of treatment, and the importance of "experiment" as an approach to the ecology of soil fungi in general.

Effects of the treatment with urea or its related nitrogen compounds on soil have been studied from various viewpoints, e.g. by LEES & QUASTEL (1946*b*), FRANZ (1956), JUNG (1958), COOKE (1962), COURT, STEPHEN & WAID (1962), TINSLEY & HANCE (1963), and ROBERGE & KNOWLES (1966, 1967). However, the occurrence of fungi after the treatment had never been reported before our work (SAGARA & HAMADA, 1965; SAGARA, 1973). In his studies of fireplace fungi, after treatment with K_2CO_3, PETERSEN (1970*b*) obtained four of the species which, in the present studies, had been obtained with ammonia-related agents or alkalis. But he only described them as "with a different ecology" (Table 1). His question was answered preliminarily in my previous paper (SAGARA, 1973) and will be discussed more fully in the present paper.

Table 1. Reports of fungi obtained by the chemical treatment of forest soils

Authors	Agents	Species[a]	Character
LOHWASSER, 1953	Limes	*Lactarius deliciosus*	
HORA, 1958, 1959	$Ca(OH)_2$	*Omphalia maura*	"Of burnt ground"
		Galactinia praetervisa	
		Aleuria lilacina	
HORA, 1959	"Growmore"	*Lactarius rufus*	
	$(NH_4)_2SO_4$	*Paxillus involutus*	
	Superphosphate		
	$Ca(OH)_2$	*Clitocybe dicolor*	In fairy ring
FRANZ & LAUB, 1959	$CaCO_3$	*Laccaria amethystina*	"Zellulosezersetzer"
HINTIKKA, 1960	Acetone	*Pholiota carbonaria*	"Pyrophile Arten"
		Coprinus boudieri	
		Lachnea sp. (close to *L. melaloma*)	
	Butanol	*Coprinus boudieri*	
		Pholiota carbonaria	
SAGARA & HAMADA, 1965	Urea	*Ascobolus denudatus*	"Unknown" (="Proteophilous fungi", SAGARA, 1973)
		Lyophyllum plexipes f. *typicum* (?)	
		An unidentified discomycete	
PETERSEN, 1970*a*	$CaCO_3$	*Humaria hemisphaeroides*	"Fireplace fungi"
		Peziza praetervisa	
		Lamprospora dictydiola	
	$Ca(OH)_2$	*Octospora* sp.	
PETERSEN, 1970*b*	$CaCO_3$	*Ascobolus pusilus*	"Fireplace fungi"
		Fayodia maura	
		Humaria hemisphaeroides	
		Lamprospora dictydiola	
		Octospora spp. (three)	
		Peziza endocarpoides	
		Peziza praetervisa	
		Iodophanus carneus	"At least common on burnt ground"
		Omphalia pyxidata	
		Peziza granulosa	
	Na_2CO_3	*Lyophyllum tylicolor*	"With a different ecology" (different from fireplace group)
		Peziza sp.	
	K_2CO_3	*Ascobolus denudatus*	
		Coprinus sp.	
		Lyophyllum gibberosum	
		Lyophyllum tylicolor	
		Peziza sp.	
		Peziza palustris	

Authors	Agents	Species[a]	Character
LAIHO, 1970	NH_4-fertilizers, Urea	*Paxillus involutus*	"Ectomycorrhizal fungus"
HORA, 1972	Na_2CO_3	*Coprinus echinosporus*	
		Peziza sp.	
		Tephrocybe tesquorum	
	$(NH_4)_2SO_4$	*Lactarius rufus*	
		Paxillus involutus	
		Russula emetica	
	KNO_3	*Clitocybe langei*	
		Hygrophorus hypothejus	
SAGARA, 1973	Nitrogenous materials liberating ammonia, or strong alkalis	Twenty-five species to be again mentioned in the present paper	"Proteophilous fungi"
	Calcium cyanamide	*Octospora* sp.	Fireplace fungi
		Peziza atrovinosa	
		Peziza praetervisa	
		Plicaria trachycarpa	
		Pulvinula sp. nos. 2, 3	
		Coprinus gonophyllus	
		Helvella lacunosa	
	$Ca(OH)_2$	*Octospora* sp.	
		Peziza praetervisa	
		Pulvinula sp. no. 2	
		Coprinus gonophyllus	
	$CaCO_3$	*Pulvinula* sp. nos. 1, 2	
	Calcium acetate	*Peziza echinospora*	
LEHMANN, 1973	Urea	*Cladosporium herbarum*	
		Phoma sp.	
		Epicoccum purpurascens	
		Verticillium sp.	
		Tephrocybe tesquorum	
		Coprinus echinosporus	
		Ascobolus denudatus	
		Pseudombrophilla deerata	
		Mycena sp.	

a. Some are synonymous: *Galactinia praetervisa*=*Peziza praetervisa; Lyophyllum plexipes* f. *typicum*=*Lyophyllum tylicolor*=*Tephrocybe tesquorum; Omphalia maura*=*Fayodia maura;* The "unidentified discomycete" in SAGARA & HAMADA (1965)=*Gelatinodiscus* sp. in SAGARA (1973) and in the present paper.

I OCCURRENCE OF FUNGI AFTER TREATMENT WITH UREA

Methods

In the first experiment with urea, only a few species were recorded (SAGARA & HAMADA, 1965). It was then supposed that some other fungi would be obtained due to the same or similar response to the urea treatment, and it was found, in the meantime, that at least some of them could be obtained by treating a small amount of soil with urea in the laboratory. Thereupon, I continued the urea treatment with the soils of various vegetations in the field or in the laboratory under different conditions.

Experimental stands

The stands for the field experiments or for collecting soil samples used in the laboratory experiments were chosen so as to cover a wide variety of conditions in Japan (Fig. 1). They were distributed in the warm temperate zone, the cool temperate zone, and the subarctic zone (in higher altitudes), and numbered consecutively from south to north. Their descriptions — vegetation type, locality, and altitude — are given below in the list. The vegetation types are designated by the names of the dominant plants of the strata. In cases where some account of the vegetation can be found in the paper by NUMATA, MIYAWAKI & ITOH (1972), the heading number of the paragraph containing the account is shown in the parentheses immediately after the designation of vegetation type. These descriptions are followed by the date on which the urea treatment in the field was conducted (F) or on which the soil sample was collected (C).

List of experimental stands

1. *Ardisia sieblodii-Cinnamomum japonicum-Alpinia intermedia-Polystichopsis pseudoaristata* forest** (1. 1. 1. 2). Sata, Kagoshima; 80 m. C: 3. iv. 67.
2. *Schefflera octophylla-Ardisia sieboldii-Colysis pothifolia-Piper kazura* forest** (1. 1. 1. 2). Sata, Kagoshima; 70 m. C: 3. iv. 67.
3. Roadside planting of *Livistona subglobosa*. Sata, Kagoshima; 30 m. C: 3. iv. 67***.
4. Roadside planting of *Musa* ×*paradisiaca*. Sata, Kagoshima; 60 m. C: 3. iv. 67***.
5. *Cryptomeria japonica* artificial forest. Sata, Kagoshima; 70 m. C: 3. iv. 67.
6. *Pinus thunbergii* stand on sea-coast (1. 1. 4). Sata, Kagoshima; 10 m. C: 3. iv. 67.
7. *Cinnamomum camphora-Quercus ?glauca-Pleioblastus simoni* forest (? 1. 1. 2. 1). Kagoshima City; 100m. C: 4. iv. 67.
8. Solitary growth of *Ficus wightiana* on sea-coast. Kagoshima City; near 0 m. C: 4. iv. 67.
9. *Pinus densiflora* forest (1. 2. 1. 2). Mt. Kirishima, Kagoshima; 780 m. C: 31. iii. 67.
10. *Chamaecyparis obtusa* artificial forest. Mt. Kirishima, Kagoshima; 780 m. C: 31. iii. 67.
11. *Tsuga sieboldii-Pinus densiflora-Pleioblastus ?distichus* var. *nezasa* forest (1. 1. 2. 2). Mt. Kirishima, Kagoshima; 1200 m. C: 1. iv. 67.
12. *Rhododendron kiusianum-Pleioblastus ?distichus* var. *nezasa-Miscanthus sinensis* wind-blown scrub* (2. 2. 2). Mt. Kirishima, Kagoshima; 1400 m. C: 1. iv. 67.
13. *Picea polita-Fagus crenata-Pleioblastus ?distichus* var. *nezasa* forest (? 2. 1. 1). Mt. Kirishima, Kagoshima; 1330 m. C: 1. iv. 67.

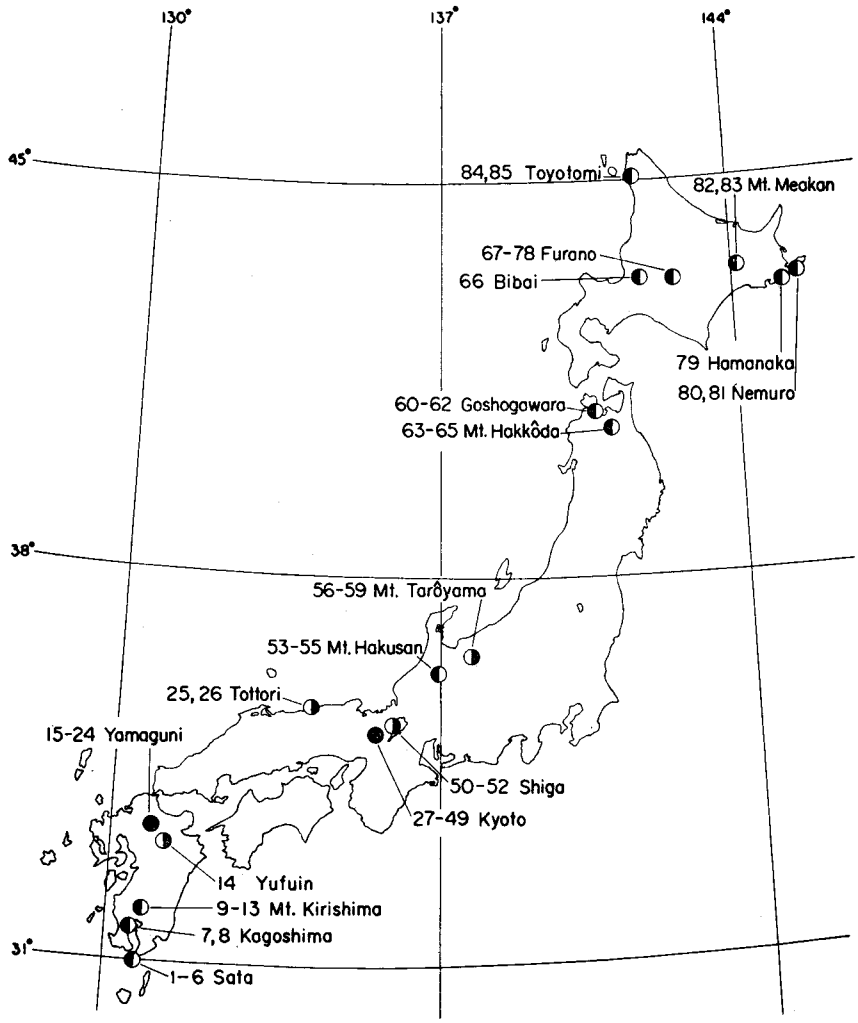


Fig. 1. Location of experimental stands throughout Japan. The arabic numbers indicate stand numbers. ◑: The stands where field experiments were carried out. ◐: The stands where soil samples for laboratory experiments were collected. ●: The stands where field experiments were carried out and soil samples collected.

14. *Plelioblastus ?distichus* var. *nezasa* grassland* (2. 2. 2). Yufuin, Ôita; 950 m. F: 6. ix. 68.
15. *Quercus acutissima* artificial forest*. Yamaguni, Ôita; 220 m. F: 21. xi. 67; 20. vii. 68. C: 5. iv. 67.
16. *Quercus glauca* coppice (1. 2. 1. 1). Yamaguni, Ôita; 230 m. F: 21 & 23. xi. 67; 20. vii. 68. C: 5. iv. 67.
17. *Pinus densiflora* artificial stand. Yamaguni, Ôita; 320 m. F: 21 & 23. xi. 67; 20. vii. 68. C: 5. iv. 67.
18. *Quercus serrata* coppice (? 1. 2. 1. 3). Yamaguni, Ôita; 340 m. F: 21. xi. 67; 20. vii. 68. C: 5. iv. 67.
19. *Pinus densiflora* stand (natural; 1. 2. 1. 2). Yamaguni, Ôita; 350 m. F: 21. xi. 67; 20. vii. 68.
20. *Chamaecyparis obtusa* artificial forest. Yamaguni, Ôita; 270 m. F: 21. xi. 67; 20. vii. 68. C: 6. iv. 67.

21. *Cryptomeria japonica* artificial forest. Yamaguni, Ôita; 250 m. F: 23. xi. 67; 20. vii. 68. C: 6. iv. 67.
22. *Quercus glauca* coppice (1. 2. 1. 1). Yamaguni, Ôita; 210 m. F: 23. xi. 67; 20. vii. 68.
23. *Phyllostachys heterocycla* f. *pubescens* (bamboo) stand (1. 2. 1. 4). Yamaguni, Ôita; 180 m. F: 27. xi. 67; 20. vii. 68. C: 6. iv. 67.
24. *Fagus crenata* forest* (2. 1. 1). Yamaguni, Ôita; 990 m. F: 22. xi. 67; 19. vii. 68. C: 6. iv. 67.
25. *Pinus thunbergii* artificial stand on sand dune of sea-coast (dwarfish; the growth very poor and the ground exposed to sun-light). Tottori City; 30 m. F: 20. viii. 69.
26. *Pinus thunbergii* artificial stand on sand dune of sea-coast (rather tall; the growth better than that in no. 25 and the ground shaded from sun-light). Tottori City; 35 m. F: 20. viii. 69.
27. *Castanopsis cuspidata* forest (? 1. 1. 2. 1). Kyoto City; 120 m. F: 24. ii. 67; 13. viii. 67.
28. *Phyllostachys bambusoides* (bamboo) stand (1. 2. 1. 4). Kyoto City; 95 m. F: 27. ii. 67; 13. viii. 67.
29. *Castanopsis cuspidata* forest (? 1. 1. 2. 1). Kyoto City; 150 m. F: 24. ii. 67; 13. viii. 67.
30. *Castanopsis cuspidata* forest (? 1. 1. 2. 1). Kyoto City; 150 m. F: 24. ii. 67: 13. viii. 67.
31. *Castanopsis cuspidata* forest (? 1. 1. 2. 1). Kyoto City; 140 m. F: 24. ii. 67; 13. viii. 67. C: 4. x. 67.
32. *Pinus densiflora-Chamaecyparis obtusa* forest (1. 2. 1. 2). Kyoto City; 190 m. F: 19. ii. 67; 13. viii. 67. C: 4. x. 67.
33. *Pinus densiflora-Chamaecyparis obtusa* forest (young) (1. 2. 1. 2). Kyoto City; 190 m. F: 20. ii. 67; 13. viii. 67.
34. *Cryptomeria japonica* artificial stand. Kyoto City; 170 m. F: 25. ii. 67; 13. viii. 67. C: 4. x. 67.
35. *Chamaecyparis obtusa* artificial stand. Kyoto City; 150 m. F: 25. ii. 67; 13. viii. 67. C: 4. x. 67.
36. *Quercus acutissima-Q. serrata* stand* (? 1. 2. 1. 3). Kyoto City; 140 m. F: 25. ii. 67; 14. viii. 67. C: 4. x. 67.
37. *Cryptomeria japonica* artificial forest. Kyoto City; 220 m. F: 26. ii. 67; 14. viii. 67.
38. *Quercus acutissima* artificial stand*. Kyoto City; 230 m. F: 26. ii. 67; 14. viii. 67.
39. *Quercus acutissima* artificial stand*. Kyoto City; 210 m. F: 26. ii. 67; 14. viii. 67.
40. *Chamaecyparis obtusa* artificial stand. Kyoto City; 180 m. F: 26. ii. 67; 14. viii. 67.
41. *Phyllostachys nigra* f. *henonis* (bamboo) stand (1. 2. 1. 4). Kyoto City; 160 m. F: 25. ii. 67; 14. viii. 67. C: 11. x. 67.
42. *Pinus densiflora* forest (young) (1. 2. 1. 2). Kyoto City; 110 m. F: 27. ii. 67; 14. viii. 67.
43. *Pinus densiflora* forest (1. 2. 1. 2). Kyoto City; 110 m. F: 27. ii. 67; 14. viii. 67.
44. *Castanopsis cuspidata* forest (? 1. 1. 2. 1). Kyoto City; 160 m. F: 24. ii. 67; 13. viii. 67.
45. Weed community in the campus of Kyoto University*; 60 m. F: 2. iii. 67; 15. viii. 67.
46. Weed community in the campus of Kyoto University*; 60 m. F: 2. iii. 67; 18. viii. 67.
47. *Cinnamomum* stand in the Botanical Garden, Kyoto University; 70 m. F: 15. viii. 67.
48. *Castanopsis-Pasania-Quercus* stand in the Botanical Garden, Kyoto University; 70 m. F: 15. viii. 67.
49. *Aphananthe-Ulmus* stand in the Botanical Garden*, Kyoto University; 70 m. F: 15. viii. 67.
50. *Pinus densiflora* forest (young) (1. 2. 1. 2). Shiga, Shiga; 120 m. F: 25. xi. 66.
51. *Quercus serrata-Q. variabilis* forest* (? 1. 2. 1. 3). Shiga, Shiga; 120 m. F: 5. xi. 66.
52. *Fagus crenata* forest* (2. 1. 1). Mt. Hira, Shiga; 1000 m. F: 5. xi. 66.
53. *Fagus crenata* forest* (2. 1. 1). Mt. Hakusan, Ishikawa; 1200 m. C: 17. ix. 67.
54. *Abies mariesii* stand (3. 1). Mt. Hakusan, Ishikawa; 2000 m. C: 17. ix. 67.
55. *Pinus pumila* thicket (4. 1). Mt. Hakusan, Ishikawa; 2400 m. C: 17. ix. 67.
56. *Fagus crenata* forest* (2. 1. 1). Mt. Tarôyama, Toyama; 1400 m. F: 10. viii. 68.
57. *Abies mariesii-Sasa ?kurilensis* forest (3. 1). Mt. Tarôyama, Toyama; 1750 m. F: 9. viii. 68.
58. Subalpine grassland* (4. 2). Mt. Tarôyama, Toyama; 2040 m. F: 9. viii. 68.
59. *Pinus pumila* thicket (4. 1). Mt. Tarôyama, Toyama; 2340 m. F: 9. viii. 68.
60. *Quercus dentata-Q. mongolica* var. *grosseserrata* forest* (? 2. 2. 1. 2). Goshogawara City, Aomori; 140 m. C: 3. ix. 67.
61. *Pinus densiflora* forest (1. 2. 1. 2). Goshogawara City, Aomori; 200 m. C: 3. ix. 67.
62. *Thujopsis dolabrata* var. *hondai* forest. Goshogawara City, Aomori; 180 m. C: 3. ix. 67.
63. *Fagus crenata* forest* (2. 1. 1). Mt. Hakkôda, Aomori; 800 m. C: 4. ix. 67.

64. *Abies mariesii* stand (3. 1). Mt. Hakkôda, Aomori; 1060 m. C: 5. ix. 67.
65. *Pinus pumila* thicket (4. 1). Mt. Hakkôda, Aomori; 1550 m. C: 5. ix. 67.
66. Drained high-moor land (see no. 84 for the intact state). Bibai City, Hokkaidô; 18 m. C: 29. viii. 67 (Sample *a* was taken at 13–25 cm deep, and Sample *b* at 50–60 cm deep; both were peat).
67. *Picea abies***** artificial forest. Furano City, Hokkaidô; 240 m. C: 21. viii. 67.
68. *Pinus sylvestris***** artificial forest. Furano City, Hokkaidô; 230 m. C: 23. viii. 67.
69. *Pinus strobus***** articifial forest. Furano City, Hokkaidô; 260 m. C: 23. viii. 67.
70. *Larix olgensis* var. *koreana***** artificial forest*. Furano City, Hokkaidô; 400 m. C: 23. viii. 67.
71. *Betula maximowiczii-Sasa ?senanensis* forest*. Furano City, Hokkaidô; 400 m. C: 23. viii. 67.
72. *Abies sachalinensis-Picea jezoensis* forest (3. 6). Furano City, Hokkaidô; 400 m. C: 23. viii. 67.
73. *Betula ermani-Picea jezoensis-Sasa ?senanensis* forest (3. 6). Furano City, Hokkaidô; 730 m. C: 21. viii. 67.
74. *Betula ermani-Sasa ?senanensis* stand. Furano City, Hokkaidô; 930 m. C: 21. viii. 67.
75. *Tilia japonica-Abies sachalinensis-Sasa ?senanensis* forest (3. 6). Furano City, Hokkaidô; 430 m. C: 22. viii. 67.
76. *Betula ermani-Abies sachalinensis-Picea jezoensis-Sasa ?senanensis* forest (3. 6). Furano City, Hokkaidô; 600 m. C: 22. viii. 67.
77. *Betula ermani-Picea jezoensis-Abies sachalinensis-Sasa ?kurilensis* forest (3. 6). Furano City, Hokkaidô; 800 m. C: 22. viii. 67.
78. *Pinus pumila-Sasa kurilensis* thicket (4. 1). Furano City, Hokkaidô; 1225 m. C: 22. viii. 67.
79. High moor (2. 1. 6). Hamanaka, Hokkaidô; near 0 m. C: 27. viii. 67 (Dead plant materials at 0–20 cm deep were collected).
80. *Quercus mongolica* var. *grosseserrata-Betula platyphylla* var. *japonica* stand*. Nemuro City, Hokkaidô; 35 m. C: 26. viii. 67.
81. *Abies sachalinensis* forest (3. 6). Nemuro City, Hokkaidô; 45 m. C: 27. viii. 67.
82. *Picea glehnii-Abies sachlinensis* forest (3. 7). Mt. Me-akan, Hokkaidô; 850 m. C: 25. viii. 67.
83. *Pinus pumila* thicket (4. 1). Mt. Me-akan, Hokkaidô; 1350 m. C: 25. viii. 67.
84. High moor (2. 1. 6). Toyotomi, Hokkaidô; 4 m. C: 31. viii. 67 (Dead plant materials at 0–10 cm deep were collected).
85. *Betula platyphylla* var. *japonica-Sasa ?senanensis* stand*. Toyotomi, Hokkaidô; 5 m. C: 31. viii. 67.

* Summergreen. Others are evergreen or mixed.

** After SAKO, S.: Bull. Fac. Agr. Kagoshima Univ. No. 13, 205–220 (1963); often regarded as subtropical.

*** The leaves which had been cut down and decaying on the ground were collected.

**** Exotic.

Field experiments

Plots. The field experiments were conducted in stands located in Ôita, Tottori, Kyoto, Shiga, and Toyama Prefectures (Fig. 1). In each stand, three experimental plots, each being 0.5 m wide and 1 m long, were marked out along the slope. The untreated areas surrounding the plots were regarded as controls. The plots were labelled consecutively by data of treatment and treatment series throughout the field experiments (I, II).

Treatment. Fertilizer urea (granular form, N 46%) was spread on the ground surface by hand scattering at the rates of 40, 80, and 160 g N per plot. These are designated as treatment series i, ii, and iii, respectively.

In several stands, another treatment was added: the O horizon was removed and then urea was dressed on the A1 horizon.

In most stands the treatment was conducted under two different seasons, i.e. winter (November-February) and summer (July, August), since I had found that the fungus flora to be obtained was most typically different when treated in these two seasons.

Observation. The experimental plots were frequently visited in the early stages so as not to overlook the fungi which appear for only short periods. The intervals of visit were prolonged towards the later stages as the changes in fungus flora became slow and their occurrence was usually limited to the so-called "fungus seasons", i.e. early summer and autumn. The names of the species occurring were recorded, and the fruit bodies, especially of the basidiomycetes, were picked off after the recording.

Period of the experiment. The date of treatment was mentioned in the preceding list of the stands. Observation was continued until autumn of 1969 in Shiga (Sts. 50–52), until autumn of 1971 in Ôita (Sts. 14–24) and Kyoto (Sts. 27–49), until summer of 1972 in Tottori (Sts. 25, 26), and until autumn of 1973 in Toyama (Sts. 56–59).

Laboratory experiments

Design. a) Soil used: organic matter (plant remains) accumulated over the ground surface; in forests it formed O horizon (A_0 horizon, raw humus layer).

b) Amount of soil treated in one container: wet soil equivalent to 20 g dry soil.

c) Container used: wide-mouthed glass bottle, 6.5 or 7.5 cm diam (3.2 cm diam at the mouth) and 17.0 cm deep (Pl. 2, C, D).

d) Temperatures employed: 10 C and 25 C; the former to yield the fungi occurring after treatment in winter in the field and the latter those occurring after treatment in summer (see above).

e) Amount of urea applied: 0.2 or 0.4 g N in 20 ml solution per bottle, that is, 1 ml of aqueous solution containing 10 or 20 mg N was applied to soil equivalent to 1 g dry soil.

f) Treatment series:

	Temperature (C)	Amount of urea (g N per bottle)
Ser. i	10 (± 1)	0.2
Ser. ii	10 (± 1)	0.4
Ser. iii	25 (± 1)	0.2
Ser. iv	25 (± 1)	0.4

Controls (untreated series) were not prepared because untreated soils had not yielded any fungus in some preliminary experiments.

Collection of soil. Soil samples for the laboratory experiments were collected from the stands located in Kagoshima, Ôita, Kyoto, Ishikawa, Aomori, and Hokkaidô Prefectures (Fig. 1). In each stand, the organic layer — the soil — was cut out with an edged tool at several points and packed in a polyethylene bag. The stand number

was used as the soil number. The soils were preserved at lower temperatures (below 15 C) without drying until further treatments.

Procedures before incubation. First, each soil was stirred and mixed well to make it homogeneous. A small portion (10 g) was used for the determination of water content (oven-dry at 105 C). From the rest, soil equivalent to 20 g dry weight was put in the glass bottle. Through these procedures, all possible care was taken not to cause mutual contamination of the soils.

Urea solution was applied with a pipet to the soils in the bottles. Each bottle was covered at its mouth with filter paper (Toyo Roshi no. 6) to maintain proper aeration and to minimize the chance of contamination from the air. The bottles were then placed in a phytotron (10 C) or in incubators (25 C). Light was provided continuously from fluorescent lamps.

Managements during incubation. a) Addition of water. In the initial periods following the application of urea, the soils absorbed a large quantity of water, parallel with the increase in water-holding capacity of soil (see IV). The water given in the form of urea solution was not enough to ensure this process, so that distilled water was added at some intervals.

b) Draining. Some time after the addition of water, a certain quantity of fluid stagnated at the bottom of the bottle. It was dark reddish-brown, probably containing decomposition products, waste products, etc. It appeared to suppress the growth of fungi and therefore was poured out of the bottle. Care was taken not to destroy the texture of soil and mycelia.

c) Washing. After the initial periods mentioned above, the rate of water absorption and the rate of organic matter decomposition decreased. But it was still necessary to supply water to the soils at least to supplement the loss by natural desiccation and to clean away the undesirable materials. For these purposes, the soils were washed in the following way: (i) Each bottle was filled with water sterilized by boiling (cool when used); (ii) The soil in the bottle was stirred and kept standing for a short time to absorb the water and to release the undesirable materials; (iii) The water was then decanted leaving its small portion in the bottle, or else a small amount of sterilized water was newly added.

These treatments were more frequently conducted in Ser. iii and iv than in Ser. i and ii, because all processes, biological, chemical, or physical, appeared to proceed more rapidly at higher temperature.

Observation. The treated soils were observed every day, every second day, or every third day in the early stages when the changes in fungus flora were rapid and every seven days in the later stages. The name of the species appearing was recorded, and the matured fruit bodies were picked out of the bottle after the recording.

Period of the experiment. The urea application and the incubation were carried out on 7 May 1967 for the soils of Kagoshima and Ôita, and on 12 Oct. 1967 for the soils of the other places. The observation was continued until 23 May 1970 for the former and until 23 Apr. 1969 for the latter.

Problem of contamination. The majority of the fungi occurring after these treatments are thought to have originated from hyphae or spores which had been present in the soils since the time of collection. The following may support this view. The peats (Soils 66*a*, *b*) produced no fungus in this experiment (Table 3). When the same peats were inoculated, prior to the urea treatment, with cultured hyphae or spores of several fungi obtained beforehand by the treatment of other soils with urea, at least one of them did appear but, again, not others. This implies that the peats contained some

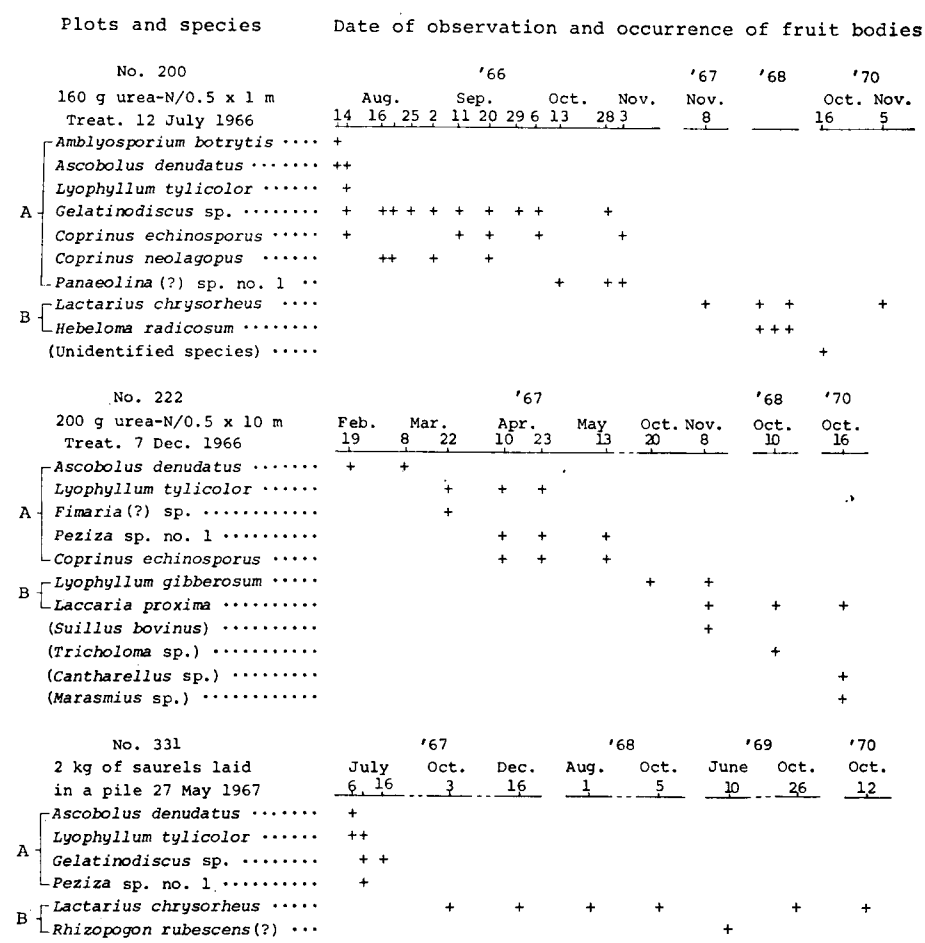


Fig. 2. Examples of the sequential occurrence of fungi on the ground treated with urea or on which dead fish were placed in the *Pinus-Chamaecyparis* forest (St. 32). In Plot 200 urea was incorporated into the O horizon and in Plot 222 urea was spread on the surface (neither mentioned in the Methods, see the text). As for Plot 331 see II and Pl. 4, A. The capitals A and B beside species names indicate the grouping within the "urea fungi" (p. 223). The species in parentheses were considered to have appeared after partial recovery of original or normal fungus flora and they are not included in the urea fungi.

nutrients, enabling the fungi to grow only if urea was added, but that they were devoid of the very fungi. Thus, the contamination from the air, if any, does not seem to cause serious confusion in the results.

Table 4 includes the fungi which were not recorded in the field experiments (footnote *a*). Some of them might occur as laboratory contaminants. But it is also probable that the laboratory experiments could produce some fungi which were not detected in the field experiments notwithstanding their hyphal growth.

Results and Discussion

Two modes of response were observed in the fungi which showed a preference (?) for the urea plot. The majority of them appeared exclusively on the experimental plot and were not found at all in the control (Pl. 1). The rest appeared relatively

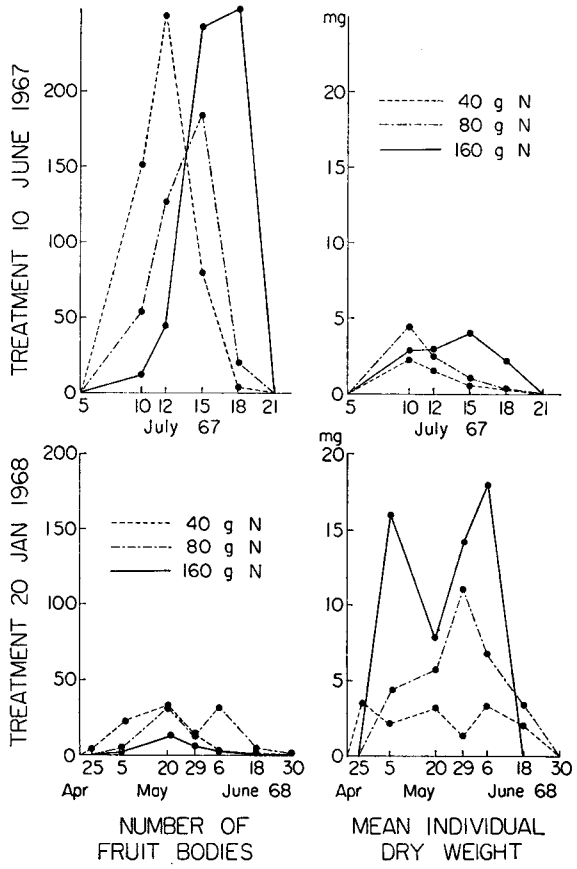


Fig. 3. Quantitative changes in the occurrence of *Lyophyllum tylicolor* after urea treatment under two different seasons. See II for the methods of treatment (Table 5; Plots 339–341 for the upper graphs, and Plots 567–569 for the lower ones).

Table 2. Occurrence of fungi on the ground in

Occurrence in any of Treatment ser. i–iii is represented by one of the following symbols: W, treatments both in winter and summer; A, occurred in experiments not mentioned in the in Sts. 50–52 only in winter. The symbols by lower case indicate occurrences needing confir- further experiments.

Stand no.	14	15	16[c]	17[c]	18	19	20	21	22	23	24[c]	25	26	27	28	29	30	31
Deuteromycetes																		
Amblyosporium botrytis	S	W	W	W	W				W	W	W		S	S		S	WS	S
Cladorrhinum foecundissimum															S	S		S
Ascomycetes																		
Ascobolus denudatus		W		W	W									WS	W	WS	WS	WS
Ascobolus sp. no. 2																	A	
Chaetomium globosum[d]																		
Fimaria(?) sp.		W	W	W	W	W	W	WS	W	W	W			W	W	W	W	W
Gelatinodiscus sp.				S							S		S	S		S	S	S
Humaria velenovskyi																		
Melastiza sp.																		
Peziza sp. no. 1		W	W	WS	W	W	W	W	W	W	W		S	W	W	W	W	W
Trichophaea gregaria				W														
Basidiomycetes																		
Collybia cookei		WS		W							S							
Collybia(?) sp.						w												
Coprinus echinosporus		W	W	W	W				W	W	W		W	WS	W	WS	W	W
Coprinus lagopus															S			
Coprinus neolagopus			S					S			S			S	S	S	S	S
Coprinus phlyctidosporus		S	S		S				S	W	S		s	S		S	S	
Coprinus sp. no. 7																		
Hebeloma radicosum														WS		WS	WS	WS
Hebeloma spoliatum	S	W	W	WS	W				WS		WS		S	W		W	WS	WS
Hebeloma vinosophyllum	S	WS	WS	S	WS				S				S	WS		S	WS	WS
Laccaria proxima		S									WS							
Lactarius chrysorheus																		
Lepista tarda																		
Lyophyllum constrictum (?)[a]																		
Lyophyllum gibberosum														W		W		W
Lyophyllum tylicolor	S	W	WS	WS		WS					WS		S	WS		WS	WS	WS
Panaeolina rhombisperma										S					S			
Panaeolina(?) sp. no. 1																		
Rhizopogon rubescens (?)																		
Rhodophyllus babingtonii[b,d]																		

a. Or *L. leucocephalum* (?). *b.* Forma *japonicus*. *c.* Including the plots where O horizon was

various vegetations of Japan after treatment with urea

occurred after treatment in winter; S, occurred after treatment in summer; WS, occurred after
Methods. In Sts. 14, 25, 26, 47–49, and 56–59 treatment was conducted only in summer and
mation or questions remaining on the identifications, and blanks possibilities of occurrence in

32[c]	33[c]	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	56	57	58	59
S	A			W				W		WS	S	S	S								S			S
S							S						S	S	S	S								
WS	W	W	W	WS	WS	WS	S	W	W	WS	WS	WS			A		A	W	W	W	S	S		S
																S								
W	A	W	W	W	WS	WS	WS	WS	W	W	W	W	W	W			A	W	W	W				
S	WS	S	S	S	S			S		S	S	S										S		
WS																		W			S	S		S
													W	W	A						S			
W	A	WS	W		WS		W	W	W	W	A	W	W	W				W		W	S	S		S
A																								
A	W	W	W	W				w		W	W	WS						W		W		S		S
							S							S										
S		S		S	S	S	S	S	S	S		S	S	S	A	S	S							
A		S	S	WS		S	WS	WS				WS	S		S	S	S				S			
								W																
A	A	S		WS						WS	W	W												
A				WS			WS	W		WS	A							W	W	W	S			
A		S		WS				S		S		WS							W					
WS	WS						W			W														S
A										W														S
													W											
					W	W	W	W																
W	W			W								W								W				
WS	WS	WS	WS	WS				S		WS	WS	WS								W	S	S		S
													S	S										
A																								
W	A																							
															A									

removed prior to the urea application. *d.* "Doubtful species"; p. 223, Table 4 (footnote *f*).

luxuriantly on the plot, that is, they were sometimes or often met on the untreated place but, when they occurred on the plot, the fruit bodies were more abundant (dense, frequent) or larger (thick), or both, than in the control (Pl. 2, B). The latter appear in the later stages of succession (see below), and hence their appearance may be regarded as a process of recovery of fungus flora after the disturbance (see p. 243).

A few examples of sequential occurrence are shown in Fig. 2 to explain an overall picture of the fungus succession. The first two of the three data used in Fig. 2 (Plots 200, 222) were from experiments not mentioned in the Methods, but they were in principle the same as those obtained in the present experiments. The picture may be drawn as follows, though the phases are not clear-cut:

Deuteromycetes→Ascomycetes→Basidiomycetes (→Deuteromycetes).

Table 3. Occurrence of fungi on soils collected from various

L, occurred at 10 C (Treatm. ser. i or ii, or both); H, occurred at 25 C (Treatm. ser. iii or iv, or Methods. The symbols by lower case indicate occurrences needing confirmation or questions

Soil no.[a]	1	2	3	4	5	6	7	8	9	10	11	12	13	15	16	17	18	20	21	23	24	31	32	34
Deuteromycetes																								
Amblyosporium botrytis																LH							A	L
Cladorrhinum foecundissimum										H									H		H		A	H
Doratomyces purpureofuscus	L		L	L		LH							L	L		L				L	L	L	L	
Doratomyces putredinis	L	L	L	LH	LH	LH	L	LH		L					L			L	L				A	
Oidiodendron truncatum							L		L	L	L	L	L		L			L						L
Ascomycetes																								
Ascobolus denudatus	L	l			H				Lh	LH	Lh	LH	L	L		LH	LH	L	L		LH	LH	LH	LH
Ascobolus sp. no. 2											LH		H											
Fimaria(?) sp.		L			L	LH		l	L	l				L		L	L	L	L		L			
Gelatinodiscus sp.									h		h		h										H	
Melastiza sp.		L																	L					
Peziza sp. no. 1											l					L		L					A	L
Basidiomycetes																								
Coprinus echinosporus													L			l	L			L		L	L	l
Coprinus lagopus	H	H	H	H		H	H	H							H							h		
Coprinus narcoticus[c]																							A	L
Coprinus neolagopus									H	H	h	H		H	H	H	H	H	H	H	H	H	A	H
Coprinus phlyctidosporus	LH	LH	LH	LH	LH		LH		LH		LH	L	L		L		LH	L		L	LH	L		L
Coprinus stercorarius																								
Coprinus sp. no. 7																								
Coprinus sp. no. 8[c]															l		L			l				
Lyophyllum tylicolor									L			LH	LH								L		LH	L
Panaeolina(?) sp. no. 3[c]																								

a. Identical with the stand number. *b*. Including the samples *a* and *b*. *c*. "Doubtful species";

Treatment in the laboratory did not yield the larger basidiomycetes which appeared in the later stages in the field, but had a second deuteromycete phase after the basidiomycete phase.

Some quantitative aspects of the succession are shown in Fig. 3 with one of the fungi which appear in the early stages. An actual succession consists of overlapping changes of these kinds in plural species.

The results of the field experiments are shown in Table 2 and those of the laboratory experiments in Table 3, irrespective of successional and quantitative aspects. As for the *Pinus densiflora-Chamaecyparis obtusa* forest in Kyoto (St. 32), the general mushroom flora of the untreated state, i.e. the original flora before the treatment, may be seen in SAGARA (1964), where, among the larger fleshy fungi to be discussed in the present

vegetations of Japan and treated with urea in the laboratory

both); LH, occurred both at 10 C and 25 C; A, occurred in experiments not mentioned in the remaining on the identifications, and blanks indicate possibilities of occurrence in further experiments.

35	36	41	53	54	55	60	61	62	63	64	65	66[b]	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85
H	H	H				H	H	H	H																	H				
	L		L					L											L					LH						L
L	L	H												L	L											L				
L					L		L			L			L	L																
LH	LH	l	LH	LH	LH	l	LH	L	L	LH	LH		Lh			Lh			LH			l h	LH	Lh	L	Lh	LH	L	LH	L	Lh
			H				H			H																		H		
			L			L		L																						
						L																								
L	L			L	L	L	L		L	L	L						L		L	L				L	l	L		L	L	
		h	H										H				H		H		H									
				L						l	L		H							L			L	L				L			
	H	H				H		H	H					H	H		H			H	H	H	H	H	H	H	H		H	H	H
L	L		L			L	L							L			H	L	LH	L						L			1		
	H																						L								
		L				L												LH	L	L	L			L							
L	L		L		LH					L	LH													LH				LH	L		
																				L											

p. 223, Table 4 (footnote *f*).

paper, only *Lactarius chrysorheus* was listed. It would mean that the fungi in question are, generally, not common.

To be added to the list of species obtained in the field experiment are *Cantharellus minor*(?) and *Rhodophyllus lampropus*. In an experiment not mentioned in the Methods, these appeared relatively abundantly on a urea plot (and a calcium cyanamide plot) in a young *Pinus-Chamaecyparis* forest near by St. 32. And, as shown in Tables 2 and 3, some urea treatments conducted outside the present study brought about the occurrence of some fungi which were missed in the present experiments. These mean that, in some places, the number of species which appear after the urea treatment will increase to certain extent, and hence the symbols to indicate occurrence as in these tables will increase if further treatments are undertaken under a wider variety of conditions.

Universality of the phenomena

The occurrence of special fungi and the changes in soil properties after the urea treatment were generally observed in almost all the stands and soils studied (see IV for the changes in soil properties). This result and the fact that many of the identified species have been known in the flora of Europe and North America (see V) suggest that, even though there may exist some difference in the flora of fungi to respond, these phenomena will be universally observed, at least all over the warm temperate to subarctic regions of the Northern Hemisphere, if proper environmental conditions are provided. The results by LEHMANN (1973) support this presumption.

Exceptions and their possible causes are as follows.

a) In the laboratory experiment, the peats collected from deep layers of drained high moor (Soils 66*a*, *b*) yielded no fungus. This would be due to lack of the fungus flora to respond to the urea treatment (p. 216) (the surface layers of natural [undrained] high moors [Soils 79, 84] did yield a few of the fungi in question).

b) In the field experiment, the *Pinus thunbergii* plantation on sand dune (St. 25) produced no fungus. In this case, severe desiccation owing to the poor growth of the pine and little accumulation of organic matter must have suppressed the appearance of the fungi.

c) The subalpine grassland (St. 58) also produced no fungus. But, when the soil sample from this stand was treated in the laboratory, some of the fungi in question were obtained (not shown in Table 3). This implies that these fungi were potentially present in the soil but failed to appear in the field, probably due to the wind-blown or sun-exposed condition.

Specificity of the flora: *"urea fungi"*

The number of species which responded to the urea treatment by developing reproductive structures on the soil surface did not increase greatly when the experiments were extended from the Kyoto district to other parts of Japan. Only two species were not obtained in Kyoto but obtained in other places: *Trichophaea gregaria* (St. 17) and

Collybia cookei (Sts. 15, 17, 24). This may mean that the majority of the fungi in question can be obtained within a small district, if the treatment is repeated with a variety of vegetations and in different seasons (both treatment and observation were most frequently done in the Kyoto district) and that the assemblage of the species does not differ largely from district to district. Thus, the occurrence-inducing or growth-promoting effect of urea treatment, whether it is a primary or secondary one, seems to be observed in a limited number of fungal species. On the basis of this specificity and the constancy of occurrence, these species were provisionally termed "urea fungi" (SAGARA, 1973).* At present, thirty-five species are included in this group, excluding the five doubtful species for which further confirmation is needed (Tables 2, 3).

Groupings within the urea fungi

A set of suffixes, "-biont", "-philous", "-xenous", and "-phobous", were adopted by MOSER (1949) for the fungi of burnt ground and then by COOKE (1957) for the fungi of polluted water and sewage. These are applicable also to the urea fungi. The fungi whose occurrence was restricted to the urea-treated soils may be termed *ureobiont* species. The others, occurring relatively luxuriantly on the treated soils, may be termed *ureophilous* species (Table 4, footnote *e*). The urea fungi comprises these two groups. Fungi to be termed *ureoxenous* species, which were indifferent to urea treatment and appeared by chance on urea plot, were rare. On the other hand, there were many fungi to be termed *ureophobous* species, those in which occurrence (and hyphal growth too?) was suppressed by the urea treatment: e.g. *Russula densifolia* in the *Castanopsis cuspidata* forests of Kyoto (Sts. 30, 31), *Lactarius hatsudake* in the *Pinus thunbergii* plantation of Tottori (St. 26), and some species of the genera *Marasmius* and *Mycena* in the *Pinus-Chamaecyparis* forest of Kyoto (St. 32). Generally speaking, no fungus seemed unaffected by the urea treatment.

The urea fungi can be divided into two groups in another way (Table 4): one comprises the species which occurred in the laboratory experiment (Group A) and another those which did not (Group B). The characters of the fungi leading to this grouping seem to be correlated with some other features. Generally in the field experiments, the fungi of Group A appeared earlier and lasted for shorter periods than those of Group B in the succession. The reproductive structures of the former are generally smaller and more perishable than those of the latter. All of the former are ureobiont whereas some of the latter are ureophilous. Some physiological characters will also be related to this grouping (see p. 269, footnote).

The fact that the larger basidiomycetes which appear in the later stages of succession did not occur in the laboratory experiments could be attributed, at least to a certain extent, to the facts that, in the field, the treated soils were open to invasion by all kinds of organisms living in the surrounding soils, and also, the amount of soil used in the laboratory experiments was rather small.

* I use this term until they are re-defined as "ammonia fungi".

Table 4. Grouping of the urea fungi after their occurrence in the laboratory or field experiment

Group A: The fungi obtained in the laboratory, the majority being obtained also in the field	Group B: The fungi not obtained in the laboratory but only in the field
Deuteromycetes	**Ascomycetes**
Amblyosporium botrytis	*Humaria velenovskyi*[c]
Cladorrhinum foecundissimum	*Trichophaea gregaria*
Doratomyces purpureofuscus[a]	
Doratomyces putredinis[a]	
Oidiodendron truncatum[a]	**Basidiomycetes**
Ascomycetes	*Cantharellus minor*(?)[d,e]
Ascobolus denudatus	*Collybia cookei*
Ascobolus sp. no. 2	*Collybia*(?) sp.
Chaetomium globosum[b,f]	*Hebeloma radicosum*
Fimaria(?) sp.	*Hebeloma spoliatum*
Gelatinodiscus sp.	*Hebeloma vinosophyllum*
Melastiza sp.	*Laccaria proxima*[e]
Peziza sp. no. 1	*Lactarius chrysorheus*[e]
Basidiomycetes	*Lepista tarda*[e]
Coprinus echinosporus	*Lyophyllum constrictum* or *L. leucocephalum* (?)
Coprinus lagopus	*Lyophyllum gibberosum*
Coprinus narcoticus[a,f]	*Rhizopogon rubescens*(?)[e]
Coprinus neolagopus	*Rhodophyllus babingtonii* f. *japonicus*[f]
Coprinus phlyctidosporus	*Rhodophyllus lampropus*[d,e]
Coprinus stercorarius	
Coprinus sp. no. 7	
Coprinus sp. no. 8[a,f]	
Lyophyllum tylicolor	
Panaeolina rhombisperma[b]	
Panaeolina(?) sp. no. 1[b]	
Panaeolina(?) sp. no. 3[a,f]	

a. Obtained only in the laboratory.
b. Obtained in a laboratory experiment not mentioned in the Methods.
c. Obtained in a laboratory experiment using a larger amount of soil (4 kg in dry wt.).
d. Obtained in a stand not mentioned in the Methods (see p. 222)
e. "Ureophilous". Others are all "ureobiont". See p. 223.
f. Requiring further confirmation before determination as urea fungus ("doubtful species", p. 223).

Regional differences in the composition of urea fungi

The findings pointed out under this heading should be re-examined by further experiments since repetition of the treatment is expected to increase the record of occurrence (see p. 222). In the discussion of the results, some unpublished data will be taken into account.

Amblyosporium botrytis. Not obtained from the soils of Kagoshima (Table 3).

Cladorrhinum foecundissimum. Not recorded in Ôita (Table 2): the time of observation may not have been adequate as it appears for a short period.

Fimaria(?) sp. Not obtained from the soils of northern Japan (Mt. Hakkôda and Hokkaidô; Table 3).

Peziza sp. no. 1. Not obtained from the soils of northern Japan (Mt. Hakkôda and Hokkaidô) and those of Kagoshima (except Soil 11) (Table 3).

Coprinus neolagopus. Not obtained at higher altitudes in Toyama (Sts. 56–59; Table 2). This is plausible as, in the laboratory experiments, this species could appear only when the soils were placed under relatively high temperatures (20–30 C) at least during an initial period, but see p. 226.

Coprinus echinosporus. Not obtained from the soils of Kagoshima except Soil 11 (Table 3).

Coprinus narcoticus. Not obtained from the soils of Kagoshima and Ôita (Table 3).

Collybia cookei. Obtained only in Yamaguni, Ôita (Table 2). The substratum of this fungus was decaying fruit bodies, probably, of some urea fungi which had occurred previously and not been picked off. Some other urea fungi appearing towards later stages also might grow on dead hyphae of the urea fungi grown in the early stages. Namely, it is possible that some of the urea fungi are fungicolous. This is the reason for including this species in the group of urea fungi despite the fact that it did not appear on the soil itself.

Hebeloma radicosum. Not obtained in Ôita and Toyama (Table 2).

Lyophyllum constrictum or *L. leucocephalum* (?). Obtained only in a limited area in Kyoto (Table 2).

Lyophyllum gibberosum. Not obtained in Ôita (Table 3): temperature at the time of treatment (November) might be slightly inadequate for its occurrence as this species appears exclusively after treatment in winter.

Relationships with the type of vegetation

Field experiments were rather intensively carried out in Kyoto, so that this problem can be discussed more thoroughly than the preceding one.

Characterization of vegetation by the flora of urea fungi. The forests of *Cryptomeria, Chamaecyparis,* and *Phyllostachys* (bamboo) in Kyoto and Ôita produced very few or none of the fungi of Group B (Table 2). The *Fagus* forests in Shiga and Ôita (and Toyama also?) yielded two species in common, *Hebeloma spoliatum* and *Laccaria proxima* (Table 2). The *Pinus densiflora* forests at lower altitudes in Kyoto (Sts. 32, 42) and the *Pinus pumila* thicket at higher altitude in Toyama (St. 59) produced two species in common, *Laccaria proxima* and *Lactarius chrysorheus* (Table 2). These results may be interpreted in a different way, as follows. *Laccaria proxima* was obtained in the forests of Pinaceae and deciduous species of Fagaceae only; *Lactarius chrysorheus* was obtained in the forests and thicket of Pinaceae only. Thus, the same type of vegetation may be characterized by some species of urea fungi, even when they are located far away from each other.

Natural (original) fungus flora and the urea fungi. The fungi of Group B occurred relatively frequently or restrictedly in the forests of *Pinus, Quercus, Castanopsis,* and *Fagus* but not in those of *Cryptomeria, Chamaecyparis,* and *Phyllostachys* (see the preceding paragraph). This reminds us of our empirical knowledge that the general mushroom flora under natural conditions is relatively poor in the latter forests. For example, in Kyoto and Shiga, *Lactarius chrysorheus* is not rare in pine forests but is seldom met in other forests. After the urea treatment too, this fungus did not appear in the latter. That is, the occurrence of this fungus caused by the urea treatment was consistent with its natural distribution. Some other urea fungi (ureophilous ones) showed the same

tendency. The differences in the natural flora seem, to a certain extent, to be persistent even with urea treatment. In other words, the flora to be obtained by urea treatment seems not to exceed that which is present, fruiting or not fruiting, under the natural conditions.

Mycorrhizal relations. Species of the genera *Pinus, Abies, Quercus, Castanopsis,* and *Fagus* usually form ectomycorrhizas, whereas those of *Cryptomeria, Chamaecyparis,* and *Phyllostachys* form endomycorrhizas. The above-mentioned tendencies, namely that the flora of terrestrial fleshy fungi is richer in the forests of the former trees and that the urea-to-fungus reaction system is affected by the natural fungus flora, are considered to be related, at least to a certain extent, to the type of mycorrhiza formation. Some of the urea fungi are likely to form mycorrhiza facultatively or to live on in rhizosphere under natural conditions (III). I observed, however, that they did not always accompany plant roots when they grew on a urea plot.

Level land and hills. From the results of the field experiments in Kyoto, a discrepancy in the flora of urea fungi was observed between the vegetations of level land (dwelling area of man, Sts. 45–49) and those of hills (Sts. 27, 29–40, 42–44). The former vegetations did not yield *Gelatinodiscus* sp., *Coprinus echinosporus, Hebeloma radicosum, Hebeloma spoliatum, Hebeloma vinosophyllum, Lyophyllum gibberosum,* and *Lyophyllum tylicolor,* which were common in the latter vegetations (Table 2). On the contrary, the former yielded *Melastiza* sp., *Lepista tarda,* and *Panaeolina rhombisperma* which were not obtained in the latter (but see p. 227 for *Melastiza* sp.). As they are actually located in a transition between level land and hills, the bamboo stands (Sts. 28, 41; see also St. 23) seem to fall in an intermediate position, yielding *Coprinus echinosporus* and *Panaeolina rhombisperma* which were characteristic of both the hills and the level land, respectively, but not the other fungi mentioned above.

In this connection it is worth noting that the general mushroom flora under natural conditions also differs in hills and level lands.

Age of a stand. In a young *Pinus-Chamaecyparis* stand developing close to St. 32 (twenty years old; not mentioned in the list of the stands), the parent rock being the same, *Cantharellus minor*(?) and *Rhodophyllus lampropus* occurred in appreciable quantities on the urea plot (p. 222). But they did not so occur in St. 32 (about eighty years old). The age of the stand might have some significance in this.

Notes on some species. The following may be added to the above and should be re-examined in further studies.

Cladorrhinum foecundissimum. Not obtained from the vegetations of higher altitudes (Tables 2, 3). This may coincide with the fact that, in the laboratory experiments, this species could be obtained only when the soils were placed under high temperatures (20–30 C) at least during an initial period following the treatment.

Oidiodendron truncatum. Obtained rather more frequently from the soils of coniferous forests than from those of other forests (Table 3).

Coprinus lagopus. Obtained from the soils of the forests growing at lower altitudes rather than those at higher altitudes (Tables 2, 3). This is plausible because this fungus had the same habit of occurrence as *Cladorrhinum foecundissimum* in relation to temperature.

Coprinus neolagopus. Obtained from the soils of higher altitudes (Table 3). This is strange because,

as in *Cladorrhinum foecundissimum* and *Coprinus lagopus*, this species required, for its occurrence, high temperatures at least during an initial period and such a condition cannot be expected at the very places where the soil samples were collected (cf p. 225).

Melastiza sp. Characteristic of level land in Kyoto (p. 226) but obtained in the *Fagus* forest in Toyama (St. 56; Table 2).

II EFFECTS OF SOME OTHER AGENTS ON FUNGUS FLORA

Methods

The agents used

Urea exerts diverse effects when it is applied to soil; it serves as nitrogen sources, alkalizes the soil, kills soil organisms, and stimulates organic matter decomposition. These effects were separately examined by substituting other agents for urea. Some materials unrelated to urea were also used. They may be grouped as follows.

Standard (control): urea.

Agent group 1: aqua ammonia, calcium cyanamide.

Agent group 2: ammonium salts yielding alkalinity in soil.

Agent group 3: dead bodies of animals, dead plant materal with high protein content.

Agent group 4: final products of nitrogen metabolism in animals.

Agent group 5: proteins, peptone, amino acids.

Agent group 6: aliphatic amines.

Agent group 7: ammonium salts not yielding alkalinity in soil (cf Agent group 2).

Agent group 8: nitrites.

Agent group 9: nitrates.

Agent group 10: miscellaneous nitrogen compounds that may be related to urea, ammonia, or amine.

Agent group 11: carbohydrates, oils, lipoid.

Agent group 12: carboxylic acids, alcohols, phenols, aldehydes, mercaptan.

Agent group 13: alkali and alkaline-earth substances yielding alkalinity in soil.

Agent group 14: alkali salts not yielding alkalinity in soil.

Agent group 15: mineral acids (cf Agent group 13).

Agent group 16: fungicides, herbicide, fire, and heat.

Agent group 17: organic solvents.

Field experiments

The experiments were carried out in the *Pinus densiflora-Chamaecyparis obtusa* forest in Kyoto (St. 32, p. 212).

Details of the treatments are shown in Table 5. The methods were nearly the

Table 5. Treatments and periods of observation in the field experiments

Agents	Plot no.	Amounts (for 0.5×1 m)	Periods A[a]	Periods B[b]
Urea	120–122[c]	10, 20, 40 g N	5. iv. 66	15. vii. 70
	339–341	40, 80, 160 g N	10. vi. 67	6. x. 73
	567–569	40, 80, 160 g N	20. i. 68	16. vii. 72
	709–711	40, 80, 160 g N	4. vi. 70	14. x. 74
	735	150 g N	2. v. 71	3. x. 74
	772–776	100, 500, 1000, 2000, 4000 g N	15. ii. 72	20. x. 74
	777	140 g N	15. ii. 72	20. x. 74
	820	160 g N	17. viii. 72	20. x. 74
	887	115 g N	2. ii. 74	20. xi. 74
	932	500 g (230 g N)	15. viii. 74	20. xi. 74
	961	500 g (230 g N)	30. xii. 74	5. v. 75
Group 1[d]				
Aqua ammonia	345–347	40, 80, 160 g N in 1 l	10. iv. 67	6. x. 73
	564–566	40, 80, 160 g N in 1 l	20. i. 68	16. vii. 72
	778	1 kg of 25% soln. (205 g N)	15. ii. 72	20. x. 74
	869	950 g of 28% soln. (219 g N)	17. viii. 72	20. x. 74
	888	500 g of 28% soln. (115 g N)	2. ii. 74	20. xi. 74
	933	1 kg of 28% soln. (230 g N)	15. viii. 74	20. xi. 74
	962	1 kg of 28% soln. (230 g N)	30. xii. 74	5. v. 75
Calcium cyanamide	123–125[c]	10, 20, 40 g N	5. iv. 66	14. x. 73
	342–344	40, 80, 160 g N	10. vi. 67	6. x. 73
	573–575	40, 80, 160 g N	20. i. 68	2. x. 73
Group 2				
$(NH_4)_2CO_3$	779	500 g (130 g N)	15. ii. 72	20. x. 74
	943	1 kg (260 g N)	15. viii. 74	20. xi. 74
Ammonium formate	850	1 kg (222 g N)	17. viii. 72	20. x. 74
	906	500 g (111 g N)	2. ii. 74	20. xi. 74
Ammonium acetate	348–350	40, 80, 160 g N	10. vi. 67	6. x. 73
	685–687	40, 80, 160 g N	5. ii. 69	2. x. 73
	780	450 g (82 g N)	15. ii. 72	20. x. 74
	854	1 kg (181 g N)	17. viii. 72	20. x. 74
Ammonium oxalate	858	1 kg (197 g N)	17. viii. 72	20. x. 74
	907	500 g (98 g N)	2. ii. 74	20. xi. 74
Group 3				
Saurel	331, 332[e]	2 kg (12–13 fish)	27. v. 67	6. x. 73
Mackerel	871, 873[f]	3.5 kg (4 fish)	18. viii. 72	20. x. 74
	872[f]	4.3 kg (5 fish)	18. viii. 72	20. x. 74
Tôfu (curds from soybean)	520, 522[e]	10 pieces (98 g proteins)	25. xii. 67	12. x. 70

Agents	Plot no.	Amounts (for 0.5 × 1 m)	Periods A[a]	B[b]
Group **4**				
Urea, ammonia	See p. 228			
Uric acid	736	150 g N	2. v. 71	3. x. 74
	902	190 g (63 g N)	2. ii. 74	20. xi. 74
Hippuric acid	737	150 g N	2. v. 71	3. x. 74
	831	2 kg (156 g N)	17. viii. 72	20. x. 74
	903	580 g (45 g N)	2. ii. 74	20. xi. 74
Group **5**				
Albumin from eggs	821	1 kg	17. viii. 72	20. x. 74
	889	500 g	2. ii. 74	20. xi. 74
	963	1 kg	30. xii. 74	5. v. 75
Zein	822	1 kg	17. viii. 72	20. x. 74
Casein from milk	823	1 kg	17. viii. 72	20. x. 74
	890	500 g	2. ii. 74	20. xi. 74
Peptone	740	150 g N	2. v. 71	3. x. 74
	824	1 kg (130 g N)	17. viii. 72	20. x. 74
	891	370 g (48 g N)	2. ii. 74	20. xi. 74
	964	1 kg (130 g N)	30. xii. 74	5. v. 75
Sodium glutamate	739	150 g N	2. v. 71	3. x. 74
	829	1 kg (136 g N)	17. viii. 72	20. x. 74
	894	750 g (102 g N)	2. ii. 74	20. xi. 74
	967	1.5 kg (204 g N)	30. xii. 74	5. v. 75
L-Glutamic acid	827	1.5 kg (95 g N)	17. viii. 72	20. x. 74
	893	500 g (31.7 g N)	2. ii. 74	20. xi. 74
	966	1.5 kg (95 g N)	30. xii. 74	5. v. 75
L-Arginine	828	500 g (160 g N)	17. viii. 72	20. x. 74
	895	250 g (80 g N)	2. ii. 74	20. xi. 74
L-Cystine	826	1 kg (175 g N)	17. viii. 72	20. x. 74
Group **6**				
Ethylenediamine	833	500 g (230 g N)	17. viii. 72	20. x. 74
	896	500 g (230 g N)	2. ii. 74	20. xi. 74
Trimethylamine	834	2 kg of ca. 30% soln. (142 g N)	17. viii. 72	20. x. 74
	897	1 kg of ca. 30% soln. (71 g N)	2. ii. 74	20. xi. 74
Group **7**				
$(NH_4)_2SO_4$	126–128[c]	10, 20, 40 g N	5. iv. 66	20. x. 70
	680, 681	160, 320 g N	12. xii. 68	6. x. 73
	935	2 kg (420 g N)	15. viii. 74	20. xi. 74
NH_4NO_3	129–131	10, 20, 40 g total N	6. iv. 66	20. x. 70
	682, 683	160, 320 g NH_4-N	12. xii. 68	6. x. 73
	936	2 kg (344 g NH_4-N)	15. viii. 74	20. xi. 74
NH_4Cl	712–714	40, 80, 160 g N	4. vi. 70	14. x. 74
	937	2 kg (500 g N)	15. viii. 74	20. xi. 74
Group **8**				
$NaNO_2$	715–717	40, 80, 160 g N	4. vi. 70	14. x. 74
Amyl nitrite	859	500 g (60 g N)	17. viii. 72	20. x. 74
Group **9**				
$NaNO_3$	718–720	40, 80, 160 g N	4. vi. 70	14. x. 74

Agents	Plot no.	Amounts (for 0.5×1 m)	Periods A[a]	B[b]
KNO_3	783	750 g (104 g N)	15. ii. 72	20. x. 74
$Ca(NO_3)_2 \cdot 4H_2O$	784	500 g (59 g N)	15. ii. 72	20. x. 74
NH_4NO_3	See Group 7			
(KNO_3 + KOH)	722–724	40, 80, 160 g N	4. vi. 70	
(cf KNO_3, KOH)		+ 1 l KOH soln.[g]	+ 12. vi. 70[g]	14. x. 74
	742–744	40, 80, 160 g N		
		+ 250 g KOH	2. v. 71	3. x. 74
Group **10**				
Urethane	738	150 g N	2. v. 71	3. x. 74
Thiourea	782	500 g (184 g N)	15. ii. 72	20. x. 74
Hydrazine hydrate				
(N_2H_2 80%)	787	500 g (350 g N)	15. ii. 72	20. x. 74
	940	500 g (350 g N)	15. viii. 74	20. xi. 74
Hydroxylamine				
hydrochloride	788	500 g (101 g N)	15. ii. 72	20. x. 74
	938	1 kg	15. viii. 74	20. xi. 74
Sulfamic acid	789	1 kg (144 g N)	15. ii. 72	20. x. 74
Formamide	843	500 g (155 g N)	17. viii. 72	20. x. 74
	900	500 g (155 g N)	2. ii. 74	20. xi. 74
Acetamide	844	500 g (119 g N)	17. viii. 72	20. x. 74
	901	500 g (119 g N)	2. ii. 74	20. xi. 74
	944	1 kg (238 g N)	15. viii. 74	20. xi. 74
Acetonitrile	845	500 g (171 g N)	17. viii. 72	20. x. 74
Aniline	860	1 kg (150 g N)	17. viii. 72	20. x. 74
	899	500 g (75 g N)	2. ii. 74	20. xi. 74
	941	500 g (75 g N)	15. viii. 74	20. xi. 74
	942	1 kg (150 g N)	15. viii. 74	20. xi. 74
	943	2 kg (300 g N)	15. viii. 74	20. xi. 74
	968	1 kg (150 g N)	30. xii. 74	5. v. 75
Nitrobenzene	790	1 kg (150 g N)	15. viii. 74	20. xi. 74
Group **11**				
Starch, soluble	791	1 kg	16. ii. 72	20. x. 74
D(+)-Sucrose	875	1 kg	4. ix. 72	20. x. 74
D(+)-Sucrose	792	1 kg	16. ii. 72	20. x. 74
	876	1 kg	4. ix. 72	20. x. 74
D(+)-Glucose	793	1 kg	16. ii. 72	20. x. 74
	877	1 kg	4. ix. 72	20. x. 74
Whale oil	794	1 kg	16. ii. 72	20. x. 74
	878[h]	1 kg	4. ix. 72	20. x. 74
Olive oil	795	1 kg	16. ii. 72	20. x. 74
	879[h]	1 kg	4. ix. 72	20. x. 74
Lecithin from soybeans	796	1 kg (N 1.76%)	16. ii. 72	20. x. 74
Group **12**				
Formic acid	847	500 g of 90% soln.	17. viii. 72	20. x. 74
Acetic acid	806	1.5 l of 75% soln.	16. ii. 72	20. x. 74
	851	500 g (glacial)	17. viii. 72	20. x. 74
n-Butyric acid	837	500 g	17. viii. 72	20. x. 74
Pyruvic acid	834	500 g	17. viii. 72	20. x. 74

Agents	Plot no.	Amounts (for 0.5×1 m)	Periods A[a]	B[b]
Oxalic acid	810	1 kg	16. ii. 72	20. x. 74
	855	500 g	17. viii. 72	20. x. 74
Succinic acid	799	1 kg	16. ii. 72	20. x. 74
Fumaric acid	798	1 kg	16. ii. 72	20. x. 74
DL-Malic acid	839	500 g	17. viii. 72	20. x. 74
Benzoic acid	830	1 kg	17. viii. 72	20. x. 74
	911	500 g	2. ii. 74	20. xi. 74
Acetone	See Group 17			
Ethanol	543	99.9%, 2 l	18. i. 68	2. x. 73
	544	70%, 2 l	18. i. 68	2. x. 73
	545	70%, 2 l, incorporated	18. i. 68	2. x. 73
	749	70%, 1 l	3. v. 71	14. x. 74
	750	99.9%, 1 l	3. v. 71	14. x. 74
	765	70%, 1 l, incorportaed	3. v. 71	14. x. 74
	766	99.9%, 1 l, incorporated	3. v. 71	14. x. 74
Methanol	808	1 kg	16. ii. 72	20. x. 74
	880	1 kg	4. ix. 72	20. x. 74
n-Amyl alcohol	800	1 kg	16. ii. 72	20. x. 74
iso-Amyl alcohol	801	1 kg	16. ii. 72	20. x. 74
n-Butyl alcohol	802	1 kg	16. ii. 72	20. x. 74
iso-Butyl alcohol	803	1 kg	16. ii. 72	20. x. 74
n-Propyl alcohol	804	1 kg	16. ii. 72	20. x. 74
iso-Propyl alcohol	805	1 kg	16. ii. 72	20. x. 74
Glycerine	861	500 g	17. viii. 72	20. x. 74
Phenol	835	550 g of 90% soln.	17. viii. 72	20. x. 74
Cresol	755, 756	1 l of 5, 10 vol % emulsion with water	3. v. 72	14. x. 74
	836	350 g	17. viii. 72	20. x. 74
Formaldehyde	761, 762	1 l of 3.7, 7.4% soln.[i]	3. v. 71	14. x. 74
	841	500 g of 37% soln.[j]	17. viii. 72	20. x. 74
Acetaldehyde	842	500 g of 80% soln.	17. viii. 72	20. x. 74
Methyl mercaptan	832	500 g of ca. 5% soln.	17. viii. 72	20. x. 74
Group **13**				
NaOH	785, 786	250, 500 g	15. ii. 72	20. x. 74
	868	500 g	17. viii. 72	20. x. 74
KOH	351–353	0.5 l of 1, 2, 4 N soln.	10. vi. 67	6. x. 73
	560–562	1 l of 1, 2, 4 N soln.	20. i. 68	14. x. 72
	721	See footnote *g*	12. vi. 70	14. x. 74
	729	500 g	4. vi. 70	14. x. 74
	741	250 g	2. v. 71	3. x. 74
Potassium formate	849	1 kg	17. viii. 72	20. x. 74
	913	500 g	2. ii. 74	20. xi. 74
Potassium acetate	853	1 kg	17. viii. 72	20. x. 74
	914	500 g	2. ii. 74	20. xi. 74
Potassium oxalate	857	1 kg	17. viii. 72	20. x. 74
	915	500 g	2. ii. 74	20. xi. 74

Agents	Plot no.	Amounts (for 0.5×1 m)	Periods A[a]	B[b]
MgO	726	1 kg	4. vi. 70	14. x. 74
$Mg(OH)_2$	728	1 kg	4. vi. 70	14. x. 74
CaO	725	1 kg	4. vi. 70	14. x. 74
$Ca(OH)_2$	141–143[c]	43, 86[k], 172 g	9. iv. 66	14. x. 73
	546–548	305, 610, 1220 g	18. i. 68	14. x. 72
	727	1 kg	4. vi. 70	14. x. 74
$CaCO_3$	144–146[c]	50, 100, 200 g	9. iv. 66	16. x. 71
Calcium formate	848	1 kg	17. viii. 74	20. x. 74
	918	500 g	2. ii. 74	20. xi. 74
Calcium acetate	852	1 kg	17. viii. 72	20. x. 74
	919	500 g	2. ii. 74	20. xi. 74
Calcium oxalate	856	1 kg	17. viii. 72	20. x. 74
	920	500 g	2. ii. 74	20. xi. 74
Sodium borate	747, 748	0.5, 1 l of 0.01 M soln.	3. v. 71	3. x. 74
Group **14**				
K_2SO_4	7–9[c]	10.2, 20.4, 40.8 g	22. vi. 65	14. vii. 66
	15, 18, 21[c]	10.2, 20.4, 40.8 g	23. vi. 65	3. i. 66
$NaNO_3$, KNO_3	See Group 9			
Group **15**				
H_2SO_4	354–356	0.5 l of 1, 2, 4 N soln.	10. vi. 67	6. x. 73
	867	500 g (min 95%)	17. viii. 72	20. x. 74
HNO_3	357–359	0.5 l of 1, 2, 4 N soln.	10. vi. 67	6. x. 73
	866	500 g (69–71%)	17. viii. 72	20. x. 74
HCl	865	500 g (min 35%)	17. viii. 72	20. x. 74
H_3PO_4	555–557	1 l of 0.5, 1, 2 M soln.	18. i. 68	16. vii. 72
Group **16**				
$CuSO_4 \cdot 5H_2O$	751, 752	250, 500 g	3. v. 71	14. x. 74
$Ca(ClO)_2$	753, 754	250, 500 g	3. v. 71	14. x. 74
$NaClO_3$	763, 764	250, 500 g	3. v. 71	14. x. 74
Propylene oxide	757, 758	250, 500 g	3. v. 71	14. x. 74
	767, 768	250, 500 g, incorporated	3. v. 71	14. x. 74
Paraformaldehyde	759, 760	250, 500 g	3. v. 71	14. x. 74
Some other poisons	See Group 12			
Burning (bonfire)[l]	326, 327	3 h	8. iii. 67	6. x. 73
	328, 329	2 h	16. iii. 67	6. x. 73
	513, 514	3.5, 3 h	20. xii. 67	6. x. 73
	583	2.5 h	31. i. 68	6. x. 73
	633, 634	2.5 h	1. viii. 68	6. x. 73
	817, 818	2.5 h	24. ii. 72	20. xi. 74
Autoclaving	558[m]	2 kg/cm², 40 min	19. i. 68	14. x. 72
	559[n]	2 kg/cm², 40 min	19. i. 68	14. x. 72
	812[o]	1.2 kg/cm², 20 min	24. ii. 72	20. x. 74
Bathing[p]	811	60 C, 2–4 h[q]	24. ii. 72	20. x. 74
Steaming	813	100 C, 5 min	24. ii. 72	20. x. 74
Oven-drying[r]	814	105 C, 4 h[s]	24. ii. 72	20. x. 74
	815	130–140 C, 3 h[t]	24. ii. 72	20. x. 74
Dry-distilling[u]	816	4–6 h[v]	24. ii. 72	20. x. 74

Agents	Plot no.	Amounts (for 0.5×1 m)	Periods A[a]	B[b]
Group **17**				
Acetone	809	1 kg	16. ii. 72	20. x. 74
	881	1 kg	4. ix. 72	20. x. 74
Ethyl ether	862	2 kg	17. viii. 72	20. x. 74
Benzene	863	2 kg	17. viii. 72	20. x. 74
Xylene	864	2 kg	17. viii. 72	20. x. 74
Alcohols	See Group 12			

a. Date of treatment. *b*. Date of final observation (see p. 276). *c*. 0.5×10 m. *d*. See the text for the grouping of agents. *e*. Not 0.5×1 m; the fish were placed in a pile. *f*. Not 0.5×1 m; the fish were laid side by side. *g*. 0.5 l of 0.5 N soln. was applied to the upper half and 0.5 l of 1 N soln. to the lower half of each plot on 12 June 1970. *h*. 0.5×0.5 m. *i*. Containing 0.6–0.8% methanol. *j*. Containing 6–8% methanol. *k*. Nearly the same rate as the one adopted by Hora (1958). *l*. Different amounts of dead wood and twigs were burnt at various intensities over various areas (not 0.5×1 m but around or below 0.5 m^2). Consequently, the duration of burning in hours is of little significance. *m*. The soil of O horizon was collected from 0.5×0.5 m, treated in the laboratory, and returned to the original place. *n*. The soil of O-B horizons was collected from 0.3×0.3×0.2 (depth) m, treated in the laboratory, and returned to the original place. *o*. Plot nos. 811–816 were all 0.5×1 m, and the soil of O horizon was treated in the laboratory and returned to the original place. *p*. The soil was packed in tins and placed in a bath. *q*. Temperatures of the inner part of each tin were lower than 40 C at the end of heating. *r*. The soil was packed in wire gauze baskets and dried in electric ovens. *s*. Temperatures of the inner part of each basket must have been lower than 100 C even at the end of heating. *t*. Temperatures of the inner part of the basket were 65–70 C at the end of heating. *u*. After a suggestion by Hintikka (1960). The soil was packed in tins and placed over a gas flame. *v*. Few ashes were produced.

same as those described in I: the agents were dressed on the surface of 0.5×1 m or 0.5×10 m plots marked out on the slope. In cases where only one plot could be prepared for each agent, a higher rate was adopted since it had been found in the experiments with urea and alkalis that quite heavy rates were rather successful for detecting the response of the fungi.

Laboratory experiments

The methods were fundamentally the same as those described in I. The soil samples were collected from the O horizon of the *Pinus-Chamaecyparis* forest in which the field experiments were carried out. Details of the treatments are shown in Tables 6 and 7. The unglazed pots of one series were placed in a row on a wooden shelf within a large water-proof case. The shelf was set so as to leave a gap between the pots and the bottom of the case where drained-off water stagnated. The case was covered with a transparent sheet of vinyl polymers, leaving an opening at lateral sides for proper aeration and humidity.

Table 6. Treatments in the laboratory experiments

Agents	Amounts (per wet soil equivalent to 1 g dry soil)	Exp. no. (Table 7)
Control	No application of the agents	9 b, d
Urea	2.5, 5, 10, 20, 40 mg N in 0.67 ml[a] water	9 a–d
	10, 20 mg N; 1 ml water added	40 a, b
Group 1[b]		
Aqua ammonia	2.5, 5, 10, 20, 40 mg N in 0.67 ml	9 a, c
Calcium cyanamide	2.5, 5, 10, 20, 40 mg N, incorporated	9 b, d
Group 2		
Ammonium acetate	2.5, 5, 10, 20, 40 mg N in 0.67 ml water	9 a, c
Group 4		
Uric acid	10, 20 mg N; 1 ml water added	40 a, b
Hippuric acid	10, 20 mg N; 1 ml water added	40 a, b
Group 5		
Peptone	10, 20 mg N; 1 ml water added	40 a, b
Sodium glutamate	10, 20 mg N; 1 ml water added	40 a, b
Group 7		
$(NH_4)_2SO_4$	2.5, 5, 10, 20, 40 mg N in 0.67 ml water	9 a, c
NH_4Cl	2.5, 5, 10, 20, 40 mg N in 0.67 ml water	9 a, c
$[(NH_4)_2SO_4 + KOH]$	After 101 days in Exp. 9a, 0.67 ml of 0.5 N KOH soln. was added to each soil	9' a
	After 52 days in Exp. 9c, 0.67 ml of 0.5 N KOH soln. was added to each soil	9' b
$[NH_4Cl + KOH]$	The same as in $[(NH_4)_2SO_4 + KOH]$	9' a, b
Group 9		
$NaNO_3$	2.5, 5, 10, 20, 40 mg N in 0.67 ml water	9 b, d
KNO_3	2.5, 5, 10, 20, 40 mg N in 0.67 ml water	9 b, d
$Ca(NO_3)_2 \cdot H_2O$	2.5, 5, 10, 40 mg N in 0.67 ml water	9 b, d
Group 13		
NaOH	0.67 ml of 0.25, 0.5, 1, 2, 4 N soln.	9 a, c
	0.67 ml of 0.1, 0.25, 0.5, 1 N soln.	12 a, b
KOH	0.67 ml of 0.25, 0.5, 1, 2, 4 N soln.	9 a, c
	0.67 ml of 0.1, 0.25, 0.5, 1 N soln.	12 a, b
$Mg(OH)_2$	4.9, 9.8, 19.5, 38.9, 77.8 mg; 0.67 ml water added	9 a, c
$Ca(OH)_2$	6.2, 12.4, 24.7, 49.4, 98.7 mg; 0.67 ml water added	9 a, c
	2.5, 6.2, 12.4, 24.7 mg; 0.67 ml water added	12 b, c

a. =2/3 ml: dissolved in 10 or 16.7 ml water and then applied to the wet soil equivalent to 15 or 25 g dry soil, respectively (see Table 7). *b.* See the text for the grouping of agents.

Results and Discussion

The results are shown in Tables 8 and 9, Figs. 2 and 3, and Pls. 2 (A, B), 3 and 4 (A, C). As regards the original fungus flora, see p. 221. The calcium compounds (Agent groups 13, 1) and burning (Agent group 16) produced many pyrophilous (fireplace) fungi (SAGARA, 1973; Table 1) but they are not discussed here. An identified deuteromycete was obtained after treatment with olive oil and whale oil (Agent group 11), but this is excluded from the present discussion because it has not

Table 7. Conditions in the laboratory experiments

Exp. no.	Ser.	Sort of container	Soil per container[a]	Temp (C)	Light sources	Date of soil collection	Date of application & incubation	Date of final observation
9	a	Unglazed pot[b]	15 g	18.5–22	Sun[c]	11. v. 66	14. v. 66	26. viii. 66
	b			19–22	Sun[c] and glow-lamps[d]			26. viii. 66
	c			25–28	Sun[c]			5. vii. 66
	d			25.5–28	Sun[c] and glow-lamps[d]			5. vii. 66
9'	a	Additional treatment to 9a		9–11	Fluorescent lamps[e]		26. viii. 66	20. ii. 67
	b	Additional treatment to 9c					5. vii. 66	3. xi. 66
12	a	Unglazed pot[b]	15 g	19–22				19. viii. 66
	b	Unglazed pot[b]	15 g	25.5–28	Sun[c] and glow-lamps[d]	31. v. 66	1. vi. 66	19. viii. 66
	c	Unglazed pot[f]	25 g	19–22				20. ii. 67
40	a	Glass bottle[g]	20 g	9–11	Fluorescent lamps[e]	26. iv. 71	5. v. 71	23. x. 72
	b			24–25				

a. In dry weight: the portion to be used in the experiment was not dried (see I). *b.* 9 cm diam at the mouth, 5 cm diam at the bottom, and 7 cm deep. *c.* Through the glass roof of a phytotron and mostly shaded by its structures. *d.* During night. *e.* Continuous illumination. *f.* 11.5 cm diam at the mouth, 6.5 cm diam at the bottom, and 9 cm deep. *g.* The same as those used in I.

been obtained after treatment with the nitrogenous materials and because the purity of the oils is questionable (this fungus was mis-identified as "an yeast" in my previous paper [SAGARA, 1973]). *Trichoderma viride*(?) appeared after almost any kind of treatment in summer but this too is excluded from the present discussion because of its obscure character.

Effects of each Agent group, with special reference to nitrogen and alkalinity

Agent groups **1–6** (urea-related agents). Aqua ammonia was almost equivalent to urea in its effects on the fungus flora. It seems safe to say at least that the flora to be obtained with aqua ammonia does not exceed that to be obtained with urea. Other agents also were generally as effective as urea. Comments on an exception and on some other results are as follows:

L-Cystine was very resistant to decomposition (see IV) and a small portion of that applied to Plot 826 remained in the form of crystals even two and a half years after the application. It did not yield any fungus. Zein, L-glutamic acid, and hippuric acid decomposed very slowly and yielded only a few species of urea fungi in small quantities.* Some part of zein on Plot 822 remained undecomposed even two and a half years after

* In connection with these results, the followings may be pointed out of unpublished data: (i) keratin and human hair, both containing larger quantities of cystine and glutamic acid, were completely ineffective; (ii) collagen, containing larger quantities of glycine, proline, and glutamic acid, was very slightly effective; (iii) glycine was effective only when it was applied at relatively high rates (20 mg N/1 g dry soil in the laboratory, 373 g N/0.5×1 m in the field).

Table 8. Occurrence of fungi on the forest ground

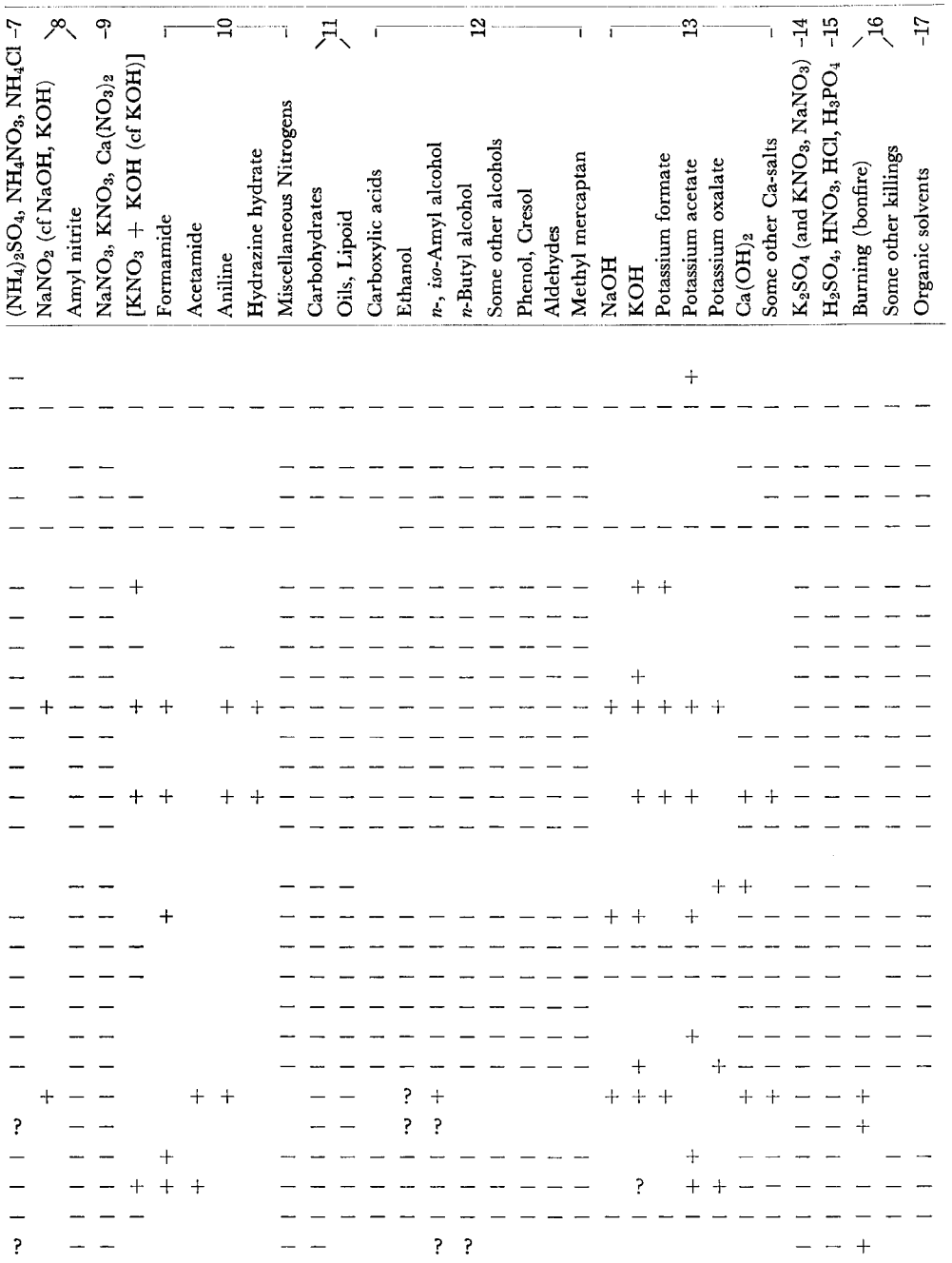
Frequency of treatment not equal for the agents. +: occurrence confirmed. —: no occurrence or smaller prospect of occurrence in further experiments. ?: occurrence needing confirmation, or

Group no.	1			2				3		4				5						6	
Agents	Urea as the standard	Agua ammonia	Calcium cyanamide	$(NH_4)_2CO_3$	Ammonium formate	Ammonium acetate	Ammonium oxalate	Saurel (sea-fish)	Mackerel (sea-fish)	Tôfu (bean-curds)	Uric acid	Hippuric acid	Albumin	Zein	Casein	Peptone	Sodium glutamate	L-Glutamic acid	L-Arginine	L-Cystine	Ethylenediamine	Trimethylamine
Zygomycetes																						
Mucor spp.[a]						+			+							+						
Rhopalomyces strangulatus	—	—	—	—	—	—	—		+		—	—		—			—	—		—	—	—
Deuteromycetes																						
Amblyosporium botrytis	+	+									+				+	+	+		+	—	+	
Cladorrhinum foecundissimum	+																			—	+	
Penicillium lividum	—	—	—	—	—	—	—	—	—	—	—	+	—		—	—	—		—	—	—	—
Ascomycetes																						
Ascobolus denudatus	+	+	+	+	+	+	+	+	+	+	+		+		+	+	+		+	—	+	
Ascobolus sp. no. 2				+				+	+		+		+		?	+			+	—		?
Byssonectria aggregata						+														—		
Fimaria(?) sp.	+	+	+												+				+	—	+	
Gelatinodiscus sp.	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	—	+	+
Humaria velenovskyi	+	+		+							+					+			+	—	+	+
Iodophanus carneus			+																	—		
Peziza sp. no. 1	+	+	+	+	+	+	+	+		+	+		+		+	+	+		+	—	+	
Scutellinia scutellata									+											—		
Basidiomycetes																						
Collybia(?) sp.	+		+						+													
Coprinus echinosporus	+	+	+	+	+	+	+	+		+	+		+	+	+	+	+	+	+	—	+	+
Coprinus neolagopus	+										+				+					—		
Coprinus phlyctidosporus	A		A												+					—		
Hebeloma radicosum	+	+	+		+		+								+	+	+		+	—		
Hebeloma spoliatum	+	+		+		+	+	+							+	+	?		+	—		
Hebeloma vinosophyllum	+	+										+				+	+			—		
Laccaria proxima	+	+	+	+	+	+			+			+	+		+	+	+		+		+	+
Lactarius chrysorheus	+	+				+		+	+	+			+		+	+						
Lyophyllum gibberosum	+	+	+	+	+	+													+	—		
Lyophyllum tylicolor	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	—	+	+
Panaeolina(?) sp. no. 1	+										+									—		
Rhizopogon rubescens(?)	+	+				+		+	+							+						

a. Sp. no. 8 with potassium acetate, no. 10 with ammonium acetate, and nos. 11–13 with mackerel;

after treatment with urea and some other agents

and with no prospect of occurrence in further experiments. Blanks: not occurring but with a larger question remaining on the identification. A: occurred in experiments not mentioned in the Methods.



distinction between them not yet clarified.

Table 9. Occurrence of fungi on the forest soil after treatment with urea and some other agents in the laboratory

See Table 8 for explanation of symbols.

Agents	Group no.	1		2	4		5		7			9			13	
	Urea as the standard	Aqua ammonia	Calcium cyanamide	Ammonium acetate	Uric acid	Hippuric acid	Peptone	Sodium glutamate	$(NH_4)_2SO_4$, NH_4Cl	$[(NH_4)_2SO_4 + KOH]$	$[NH_4Cl + KOH]$	$NaNO_3$, KNO_3, $Ca(NO_3)_2$	NaOH	KOH	$Mg(OH)_2$	$Ca(OH)_2$
Zygomycetes																
Mucor spp.[a]	−	−	−	+			+		−	−	−	−	−	−	−	−
Deuteromycetes																
Amblyosporium botrytis	+					−			−			−	−	−	−	−
Cladorrhinum foecundissimum	+	+				−			−			−	−	−	−	−
Doratomyces purpureofuscus	A															
Doratomyces putredinis	+							+	−			−	−	−	−	−
Penicillium lividum	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
Ascomycetes																
Ascobolus denudatus	+	+	+	+	?		+	+	−	+	+	−	+	+		?
Ascobolus sp. no. 2					+		?		−			−				
Gelatinodiscus sp.	+	+	+	+	+	+	+		−	+	+	−	+	+	?	
Peziza sp. no. 1	+	+		+					−	+	+	−				
Basidiomycetes																
Collybia(?) sp.																+
Coprinus echinosporus	+				+	+	+	+	−	+	+	−			−	−
Coprinus narcoticus	A								−			−				
Coprinus neolagopus	+	+	+						−			−	−	−	−	−
Lyophyllum tylicolor	+	+		+					−	+	+	−			−	−

a. Sp. no. 1 with ammonium acetate, and sp. no. 2 with peptone; distinction between the specimens not yet clarified.

the application. 500 g of albumin (Plot 889), 370 g of peptone (Plot 891), 500 g of L-glutamic acid (Plot 893), 750 g of sodium glutamate (Plot 894), all for 0.5×1 m, were unexpectedly ineffective. In such materials, these rates of nitrogen seem not high enough to induce the fungus succession. The carbon-to-nitrogen ratio might affect this aspect.

The species not obtained after treatment with urea but obtained with other agents of these groups are: *Mucor* spp. with ammonium acetate, mackerel, and potassium acetate; *Rhopalomyces strangulatus* with mackerel; *Penicillium lividum* with hippuric acid; *Byssonectria aggregata* with ammonium acetate; *Iodophanus carneus* with calcium cyanamide; *Scutellinia scutellata* with mackerel. Their occurrence was restricted to the treated soils, so that they would be grouped under some terms having the suffix "-biont", if we continue such a grouping as that applied to the urea fungi (p. 223).

Some more species were obtained after treatment with dead animal bodies, proteins, and peptone, mostly in the early stages of succession, though they are not yet confirmed or identified. These results may mean that nitrogenous materials more complex than urea have their own flora to be added to the urea fungi as extra members. At the same time the possibility that these complex materials fail to yield some of the urea fungi can not be denied.

Since the additional species named above were not obtained after treatment with the nitrogen-free materials (see below), they are provisionally integrated with the urea fungi and discussed together in the present paper. This integrated group was termed "proteophilous fungi" in my previous paper (SAGARA, 1973).*

Agent group **7** (non-basic NH_4-N). *Lactarius chrysorheus* was recorded after the treatment with NH_4NO_3, and *Rhizopogon rubescens*(?) with $(NH_4)_2SO_4$ and NH_4NO_3 at their higher rates (Plots 680, 682), but it was not clear whether or not their growth was stimulated by the treatment. The results will be discussed again later (p. 242). It can at least be said that the agents of this group will never yield the "ureobiont" species (p. 223). However, they became as effective as urea when KOH was added after their application (Table 9). These results suggest that an alkaline condition itself or liberation of ammonia is necessary.

Agent group **8** (NO_2-N). Effect of $NaNO_2$ was similar to that of NaOH or KOH. It would be reasonable to consider that $NaNO_2$ reacted like NaOH in its effect on the fungus flora since NO_2 was discharged as gas immeadiately after the treatment. Consequently, this will be discussed under Agent group 13. Amyl nitrite did not produce any fungus.

Agent group **9** (NO_3-N). No particular fungus was obtained. Some urea fungi were obtained by the addition of alkali (Table 8), but no more than those produced by the effect of alkali itself (see Agent group 13).

Agent group **10** (miscellaneous N). Formamide, acetamide, hydrazine hydrate, and aniline were somewhat effective. It has not been determined whether these chemicals reacted as ammonia, as bases, or in any other form in soil. The effect of

* I use this term until the group is re-defind as "ammonia fungi" in a broad sense.

free hydroxylamine is yet to be studied.

Agent group **11** (carbohydrates, oils, lipoid). No particular fungus was obtained except for one deuteromycete on oil plots (p. 234). These materials can be regarded as non-nitrogenous components of an organism body. Consequently, this result may imply that the urea effect of dead fish (Agent group 3) is attributable to its proteinacious parts.

It the starch plot (791), a small portion remained undecomposed even three years after the treatment, and the remnants showed an iodine reaction. This may mean that the forest soil is not deficient in carbon source.

Agent group **12** (N-free compounds). Some of the alcohols seem to be capable of yielding some ureophilous fungi but not any ureobiont fungi. This point will be discussed again later (p. 242). Others were not effective at all. These substances can be regarded as the nitrogen-free by-products of protein decomposition. The result, therefore, may suggest that nitrogen-free compounds are not primarily concerned in the urea effect of some proteins, amino acids, and peptone (Agent group 5).

Agent group **13** (bases). The potassium alkalis, NaOH, and $NaNO_2$ (Agent group 8) yielded many or a few of the urea fungi. The result with K_2CO_3 (and Na_2CO_3) by PETERSEN (1970*b*) and with Na_2CO_3 by HORA (1972) (see Table 1) may fall in this category.* The calcium (and magnesium?) compounds were scarcely effective; they rather produced pyrophilous fungi (SAGARA, 1973). Similar tendencies have been observed also by PETERSEN (1970*b*). 0.5 or 1 l of 0.01 M sodium borate solution (pH 9.18 at 25 C, a buffer solution for pH measurement; Plots 747, 748) caused no discernible change.

PETERSEN (1970*b*) considered that "the succession of species on the K_2CO_3-treated plots may be explained by qualitative and quantitative changes of the organic matter in the time after the treatment and a different ability of the individual species to utilize the various fractions". This explanation is all-inclusive and not misleading. Agreeing to his view, I would attempt further interpretation.

The occurrence of the urea fungi after the treatment with the alkalis may be attributed, primarily, to ammonia that must have been chemically released from the soil. This view may be supported by the following evidence (see footnote on p. 269): when the soil sample from the O horizon, quivalent to 20 g dry soil, was treated with 100 ml of 1 N KOH solution, gaseous ammonia was at once distinctly detected with Nessler's reagent; with 3.7 g of $Ca(OH)_2$ suspended in 100 ml water, it was only slightly detected; with 100 ml of 0.01 M sodium borate solution, it was not detected at all. Bases, limes above all, have been known to promote the decomposition of organic matter (through microbial activities) and the production of ammonia in soil (ALEXANDER, 1961; MITSUI, 1955; see IV). The strong bases may kill soil organisms, hydrolize proteins, and enhance the decomposition of soil organic matter. These effects also might have contributed to the production of ammonia or urea-related

* In further experiment in our *Pinus-Chamaecyparis* forest, K_2CO_3 and Na_2CO_3 showed almost the same effect as KOH and NaOH.

nitrogens like those of the Agent groups 2, 5, and 6. The quantity of ammonia released from the soil by the alkali treatment would, however, be relatively small. This view may be substantiated by the following: (i) Except for a few species such as *Gelatinodiscus* sp. and *Laccaria proxima,* the fruit bodies obtained on the alkali plots were much poorer in number or size, or in both, than those obtained on the plots of urea or its related agents; (ii) The fungi which require a higher concentration of urea or ammonia to appear, e.g. *Amblyosporium botrytis, Cladorrhinum foecundissimum,* and *Coprinus neolagopus,* did not occur on the alkali plots. The photographs in the paper by PETERSEN (1970*b*) show a luxuriant or normal appearance of *Lyophyllum gibberosum* and *L. tylicolor* on K_2CO_3 plot. Such has not been seen in my experiment with alkalis. This may imply that the accumulation of nitrogen was richer in the area of his study than here.

At any rate, it may be asserted that a high pH value by itself is not effective unless accompanied by some change in the situation of nitrogen.

Agent group **14** (non-basic K or Na). No special fungus was obtained. This would mean that K^+ or Na^+ ions themselves were, primarily, not necessary for the occurrence of urea fungi after the alkali treatment. PETERSEN (1970*b*) followed a similar logic: on the basis of the fact that "neither treatment with KCl nor treatment with Na_3PO_4 brought about the appearance of any of the species" obtained after the treatment with K_2CO_3 or Na_2CO_3, he considered it "improbable that changes in water soluble K or Na were the causes of the change of the fungus flora after the treatment".

Agent group **15** (mineral acids). No particular fungus was obtained. The acids might kill some soil organisms including fungi. Proteins in soil may be hydrolyzed by the acids too. If there had not been acid conditions, therefore, some urea fungi might have been able to grow there.

Agent group **16** (killings). As regards alcohols, see Agent group 12 and p. 242. Bonfire sites yielded three ureophilous species. As on the urea plot, they appeared in the later stages of succession (SAGARA, 1973). This point will be discussed again later (p. 242). Others did not produce any particular fungus. It seems safe to say that organism-killing treatment itself does not yield a full series of urea fungi.

"Partial sterilisation" (RUSSELL & HUTCHINSON, 1909) has been known to induce an increase of ammonia in soil (SUZUKI, 1961). With paddy soil it has been clarified that elevation of soil temperature, mixing soil layers, preliminary drying, and heating (and lime application) accelerate the mineralization of organic nitrogens under flooded conditions and can be a substitute for nitrogenous fertilizers (MITSUI, 1955). COOKE (1958 and personal communication 11 Sep. 1970) considered that the restriction of some fungi to burnt ground was probably attributed to certain unexplained changes in available nitrogen. Except for burning and $Ca(ClO)_2$, the killings conducted in the present work were probably not accompanied with a significant elevation of pH value (IV). There is still a question as to what fungi occur when the soil is partially sterilized and an alkaline condition is prepared.

Agent group **17** (organic solvents). No fungus was obtained (see Agent group 12

as for alcohols).

The essential factor for the succession of urea fungi

From the above results it is clear that the agents substitutive for urea are confined to the nitrogenous materials which are basic themselves (e.g. aqua ammonia, amines, L-arginine) or yield basic substances on decomposition (e.g. ammonium acetate, peptone, albumin) (see IV). In the following text we will call these agents (including urea) **ammonia-related** materials. Among these, aqueous ammonia has the simplest chemical structure and proteins have the most complex. When the alkali effect (p. 240) is taken into account, it seems that a sufficient supply of NH_4-N together with an alkaline condition is essential for the sequential occurrence of the urea fungi. If enough water or moisture is always present in soil, it may be said that the key substance is free ammonia, which is inevitably hydrated and reacts as a base in soil ($NH_3+H_2O \rightleftharpoons NH_4^+ + OH^-$). The ammonia-related materials decompose to ammonia on or in soil, but it has not been clarified at what stage of decomposition they serve as an effective cause of the phenomena under discussion.

Some secondary effects of the treatment

Three ureophilous fungi, i.e. *Laccaria proxima, Lactarius chrysorheus,* and *Rhizopogon rubescens*(?), all occurring in the later stages of the fungus succession on the plots treated with ammonia-related materials, draw special attention because they were obtained after the treatment with some agents unrelated (superficially) to ammonia (Table 8). Thus:

Laccaria proxima with aniline (cf p. 239), ethanol(?), *n*- and *iso*-amyl alcohol (Pl. 3, A), alkalis (Pl. 3, B), and burning (Pl. 3, C);

Lactarius chrysorheus with NH_4NO_3 (?), ethanol (?), *n*-amyl alcohol (?), and burning;

Rhizopogon rubescens(?) with $(NH_4)_2SO_4$ (?), NH_4NO_3 (?), *n*-butyl alcohol (?), and burning. (see Table 8 for the meaning of the question marks.)

Laccaria proxima was most prominent for its abundant occurrence, although the fruit bodies were not so thick as on the plots of ammonia-related materials. The cause of the occurrence of these fungi with these agents is still obscure. The sole aspect probably common in the treatments with these agents and the ammonia-related materials may be partial sterilization (p. 241). If so, ammonia cannot be excluded as the cause. But it is clear that initial alkalinity (see IV) is not necessary, at least for *Laccaria proxima.* This may suggest that the occurrence of some urea fungi (proteophilous fungi) is not dependent on ammonia or ammonia-related agents applied but on certain secondary effects. Killing and selection of soil organisms, metabolites of the organisms which predominated in the early stages, or their dead cells may form a part of the secondary effects (see *Collybia cookei* on p. 225).

Laccaria proxima is likely to appear rather more often on disturbed grounds in forests, such as footpaths, small broken grounds lacking O horizon for some reasons, etc. In the mode of response to chemical or mechanical disturbance or as a facultative symbiont of trees, this fungus may be similar to *Paxillus involutus* as studied by LAIHO (1970). *Laccaria proxima* and some other ureophilous fungi could be those which are able to colonize the disturbed soil earlier than others, gaining the advantage in the course of recovery of fungus flora to normal (?) state (see p. 220).

III NATURAL HABITATS OF THE UREA (PROTEOPHILOUS) FUNGI

Methods

From the results in I and II, excretions or dead bodies of some terrestrial animals were expected to give rise to such changes in soil conditions and fungus flora as those observed after the treatment with the ammonia-related agents (p. 242). Consequently, the spots in nature where these matters were added by chance were examined repeatedly and the fungi occurring there were recorded. The spots examined are as follows:

a) 21 spots where human urine was deposited;
b) 14 spots where human feces (omnivorous) were deposited;
c) 2 spots where the dead body of a cat was abandoned;
d) 5 spots where the dead body of a dog was abandoned;
e) 5 spots where a human body lay or hung after suicide;
f) 1 spot where night soil was illegally dumped.

These were encountered in various forests in Kyoto and Shiga.

In addition, the dung of the wild boar (*Sus scrofa leucomystax**, omnivorous) was examined, three samples in the laboratory and one *in situ*.

In the case of the identified fungi, their habitats can be known from taxonomic literature. The habitats known from this source will be quoted in V.

Results and Discussion

The results are shown in Table 10. It should be emphasized that, usually, the fungi in question appeared not on the feces or the dead bodies themselves but on the soils after they were so decomposed as to leave nothing but bone, hair, or a little skin (Pl. 4).

The species which were obtained on these natural habitats but not after the treatment with the ammonia-related materials are: *Lepista nuda* (FR.) COOKE on the night

* Prof. M. ASAHI, Hyôgo Medical College, kindly identified mammalian dung, to whom I am very grateful.

Table 10. Occurrence of fungi on the forest ground where some natural matter happened to be placed and decomposed

See Table 8 for explanation of symbols.

	Human urine	Human feces	Cat carcass	Dog carcass	Human corpse	Night soil	Wild boar dung
Zygomycetes							
Mucor sp. nos. 4, 5, 14[a]							+
Rhopalomyces strangulatus	−				+		−
Ascomycetes							
Ascobolus denudatus	+	+	+	+	+		
Ascobolus sp. no. 2		+		+			
Gelatinodiscus sp.	+	+		+	+		−
Humaria velenovskyi						+	−
Peziza sp. no. 1	+	+					+
Basidiomycetes							
Coprinus neolagopus			+	+			−
Coprinus stercorarius	−	+					
Hebeloma spoliatum	+		+	+		+	−
Hebeloma vinosophyllum				+		+	−
Laccaria proxima	+	+				+	
Lactarius chrysorheus	+	+	+				
Lepista nuda						+	−
Lyophyllum tylicolor	+	+	+	+			−
Panaeolina rhombisperma						+	−

a. Distinction between these and those obtained in II are not yet clarified.

soil-dumped ground; *Mucor* spp., *Ascobolus crenulatus* P. KARST.(?), *Chaetomium brasiliense* BATISTA & PONTUAL, *Chaetomium murorum* CORDA, and many unidentified coprophilous(?) fungi on the remnants of boar dung.

RICHARDSON & WATLING (1968) included *Lepista nuda* in their list of fungi on dung, but they described its habitat as "on compost and well-manured plots." Prof. T. HONGO (personal communication) found this fungus growing on night soil-dumped ground in a bamboo stand. Thus, it seems not unreasonable to include it in the group

of proteophilous fungi (p. 239). The fungi obtained on the boar dung, except *Peziza* sp. no. 1 (and *Mucor* spp. ?), have been known as coprophilous fungi, according to the list by RICHARDSON & WATLING (1968), and they were not obtained after the treatment with the ammonia-related materials (I, II). Therefore, these will not be discussed further in this paper.

According to PARK (1968), WEBSTER (1970), and HUDSON (1972), the term "coprophilous fungi" usually refers to those occurring on the dung of herbivores. Some of the proteophilous fungi have been recorded from "dung", although the sort of dung, i.e. whether of herbivore, of omnivore, or of carnivore, was not mentioned in many literature (V). It is supposed, however, that they appear more often on, or, after the decomposition of, the dung of omnivore or carnivore than on that of herbivore, for the former may be richer in nitrogen. If the omnivore or carnivore dung contains some amount of decay-resistant materials, such as mud, plant remains, skin, etc., the proteophilous fungi may appear *on the dung*. The boar dung mentioned above contained a large amount of mud, and this could be the reason why *Peziza* sp. no. 1 appeared *on* it. Laying the boar dung aside because it rather yielded so-called coprophilous fungi, the matters studied may be included in the ammonia-related materials.

It is strange that the human corpses yielded only a few urea fungi. The reasons may be considered as follows: (i) the quantity of nitrogen released from the corpse was too large for many of the fungi to grow; (ii) the decomposition period was long enough to bring about biological or chemical situations similar to those which develop under continuous presence or "repeated application" of urea (see IV); (iii) some of the decomposition products disturbed the normal succession. At any rate, the fungus succession on soil after the decomposition of dead animal remains has not been known in forensic medicine, although, according to TISDALE & NELSON (1966), the effect that dead bodies had on increasing the growth of crops was known around 700 B.C. or even earlier in Old Testament.

Urine-deposited ground has drawn little attention as a habitat of fungi (see V). In this work, only the urine of man was studied. Urine of other animals will also be effective though it is very difficult to detect the spots (the litter turns black, so that it is not impossible; see IV). Even if it is possible, the reproductive structures of the fungi will rarely be obtained unless the animals are large enough to deposit large amounts of urine. With a small amount of urine, the fungi will not be able to fructify on the soil surface or their reproductive structures will be too small or scanty to detect.

Dead bodies of wild animals as well would become the source of ammonia or ammonia-related materials. Actually, however, dead bodies or decomposition residues of larger vertebrates are rarely encountered in the field in Japan. Hence there seems to be little chance for our fungi to appear in appreciable quantities under natural conditions. These would be a part of the reasons why many of the fungi, especially those which are small in their reproductive structures and appear in the early stages of succession, are new species or new to the Japanese flora (see V).

There may be some other sources for their living. Fecal matters and dead bodies

of soil animals would be enough for their hyphal growth. The ability to develop basidiospores on "mycelial basidia" (SINGER, 1962, p. 16) in *Lyophyllum tylicolor* (SAGARA & HAMADA, 1965) may have significance under the conditions in which ammonia-related materials are supplied in very small amounts. Exudates of plant roots contain amino acids and other nitrogen compounds, and the fungi in question may form mycorrhiza (p. 226) or live on in rhizosphere. A little data in this respect were obtained in a laboratory experiment: when the fine roots of various trees collected from a *Pinus densiflora* forest were washed with sterilized water and treated with urea in an unglazed pot, *Ascobolus denudatus*, *Peziza* sp. no. 1, *Gelatinodiscus* sp., *Fimaria*(?) sp. and *Lyophyllum tylicolor* appeared on the roots. Dead cells or waste products of other microbes also may become the source of nutrient for these fungi (p. 242). As discussed in II, however, ammonia-related substances may not be of primary importance in the occurrence of some urea fungi.

IV CHANGES IN SOIL PROPERTIES AND OTHER ORGANISMS AFTER THE DISTURBANCES

Methods

Examination of soil properties

In the course of studies I-III, besides observation with the naked eye, changes in pH value and water content were examined. The soil samples for these measurements were taken from the transitional zone clearly recognizable between L layer and F layer in the O horizon. The pH value was determined with the suspension of 4 g wet soil in 20 ml distilled water, using a Hitachi-Horiba pH Meter Model M-5. The water content was determined with 10 g wet soil after oven-drying at 105 C for 5 h.

Observations on other organisms

Responses of plant roots were most carefully studied. The roots placed under observation were the terminal rootlets developing laterally and creeping through the surface layers of the soil (O or A1 horizon). These are considered to be the "humus strivers" in the root system schematically presented by LYR & HOFFMAN (1967).

Responses of other organisms were recorded only when they were conspicuous.

Results and Discussion

The changes observed after the urea treatment will be described first, since they were most thoroughly studied and were typical of those observed after the treatment with the ammonia-related materials (p. 242).

Changes after urea treatment

Soil properties. In the initial stage, the O horizon smelled of ammonia and turned

black (Pl. 5, A). Its aqueous extract was reddish-brown just like that of compost (farm manure), whereas that of the control was almost colorless and transparent. Elevation of soil temperature, as is often observed in a compost heap, was not recorded after the urea treatment. The odor then turned to that of compost. The color change and the occurrence of the urea fungi were generally confined to within the plot (Pl. 1; see also Pl. 2, A and Pl. 3), showing that there was little movement of urea or its successive transformation products. The black color was discernible for two years or more.

The pH value rose to around 9 within a few days, possibly due to ammonia pro-

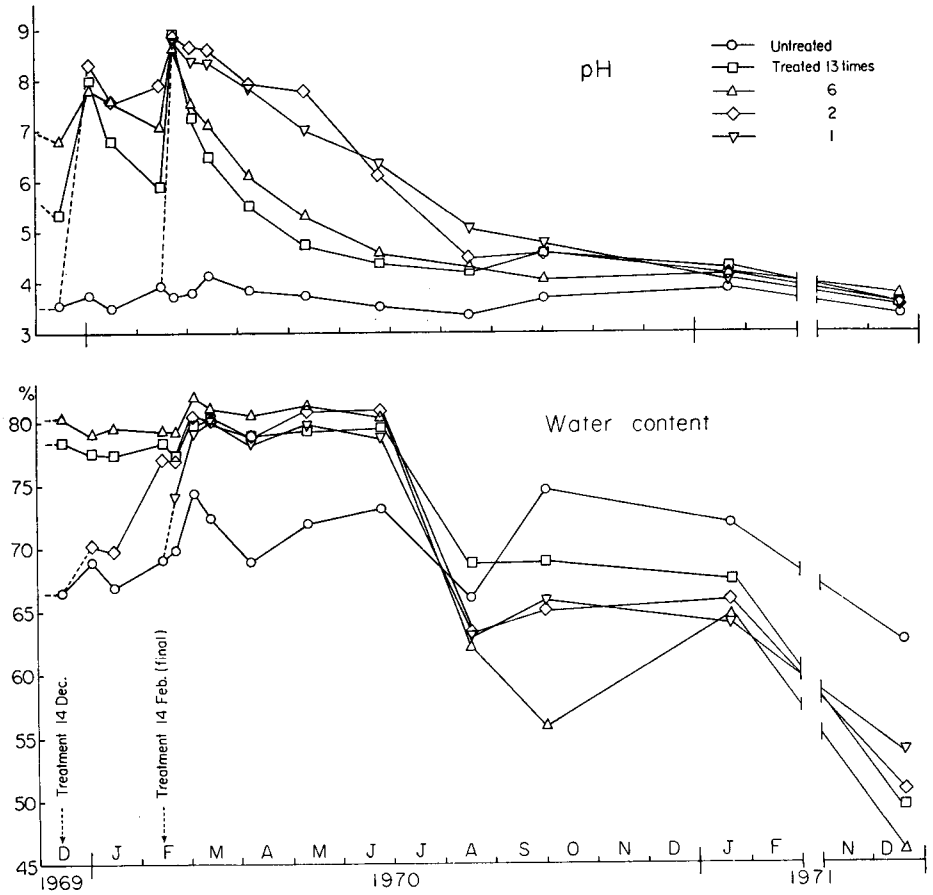


Fig. 4. Changes in pH value and water content in the O horizon of the *Pinus-Chamaecyparis* forest (St. 32) after repeated or single application of 200 g urea-N to 0.5 × 10 m plot. —□— After monthly applications from Jan. 1969 to Feb. 1970 except Jan. 1970 (13 times). —△— After monthly applications from Aug. 1969 to Feb. 1970 except Jan. 1970 (6 times). —◇— After double applications, one in Dec. 1969 and another in Feb. 1970. —▽— After a single application in Feb. 1970. —○— Control (untreated). Means of three samples from each plots.

duced by the decomposition of urea, and then lowered slowly, remaining slightly higher than that of the control even two years or more after the treatment (Figs. 4, 5). The fungus succession in question began even when the pH value was above 7.

The water content also increased, possibly owing to the increase in water-holding capacity of organic matter by enhanced decomposition (Fig. 4; Pl. 5, A) and to the increase in fresh weight of the microbial population. In the later stages, however, it often decreased to below that of the control. Breakdown of the organic matter (raw humus) dramatically proceeded in the early stages, but some raw parts resistant to decay were left undecomposed.

Effects of repeated treatment. Repeated application of urea to the same plot accelerated the rate of lowering in pH value (Fig. 4).* This repetition suppressed the occurrence of the urea fungi, and, after being treated six or thirteen times, the urea fungi, especially those expected to appear in the early stages (Group A), rarely occurred (Table 11). The same effect was observed in a laboratory experiment too (Table 12). A large amount of urea like 160 or 320 g N/0.5×1 m (equivalent to 1600 or 3200 g N/0.5×10 m, respectively) did not exert such an effect when it was applied at one

Table 11. Occurrence of fungi after single or repeated application of urea to soil in the field

See the explanation of Fig. 4 for the methods of treatment. Number of fruit bodies appearing are presented roughly by +~+++ in Group A and by the total number in Group B (see I for this grouping).

Number of times of treatment	Occurrence of the fungi of Group A		Occurrence of the fungi of Group B	
13	None		*Hebeloma radicosum*	1
			Hebeloma spoliatum	5
			Laccaria proxima	79
6	*Coprinus echinosporus*	+	*Laccaria proxima*	4
2[a]	*Ascobolus denudatus*	+	*Lyophyllum gibberosum*	409
	Lyophyllum tylicolor	++	*Laccaria proxima*	1
	Fimaria (?) sp.	++		
	Coprinus echinosporus	+++		
	Peziza sp. no. 1	+		
1	*Ascobolus denudatus*	+	*Lyophyllum gibberosum*	193
	Fimaria (?) sp.	+	*Laccaria proxima*	1
	Lyophyllum tylicolor	+		
	Peziza sp. no. 1	+		
	Coprinus echinosporus	+		

a. Probably almost equivalent to a single treatment of 400 g N/0.5×10 m, since the days between these two applications were so dry and cold that the biological activity would have been negligible.

* Thanks are due to Miss Y. WAKAMATSU of our Laboratory, who was then studying protozoans in the urea-treated soil, for her observation which led to this finding.

Table 12. Occurrence of fungi after single and double applications of urea to soil[a] in the laboratory

Number of fruit bodies appearing are presented roughly by +~+++ in Ascomycetes and by the total number in Basidiomycetes.

Pot no.	First treatment[b]: mg N /1 g soil[d]	Occurrence after the first treatment		Second treatment[c]: mg N/1 ml /1 g soil[d]	Occurrence after the second treatment	
1	2.5	None		10	*Ascobolus denudatus* *Lyophyllum tylicolor* *Coprinus echinosporus*	+ 7 5
2	5	*Gelatinodiscus* sp.	++	10	*Ascobolus denudatus* *Lyophyllum tylicolor*	+ 3
3	10	*Ascobolus denudatus* *Gelatinodiscus* sp. *Lyophyllum tylicolor*	+ ++ 5	10	*Ascobolus denudatus*	+
4	20	*Ascobolus denudatus* *Lyophyllum tylicolor* *Gelatinodiscus* sp.	++ 8 +	10	None	
5	40	*Ascobolus denudatus* *Gelatinodiscus* sp.	++ ++	10	None	

a. Collected from the O horizon of the *Pinus-Chamaecyparis* forest in Kyoto (St. 32). *b.* Conducted as Exp. 9c in Tables 6 and 7. *c.* Conducted as a part of Exp. 9' in Table 7: when the fungal occurrence after the first treatment ceased or almost ceased, this treatment was added to the same pots. *d.* In dry weight; see the Methods of I.

time (see Fig. 4 for the case of 160 g N/0.5 × 1 m). Repeated addition of urea seemed to shift the urea-to-fungus reaction system. Decrease in the carbon-to-nitrogen ratio (or exhaust of some carbon sources) in soil or prevalence of certain soil organisms adapted to such conditions (cf "saturation of soil with nitrifying bacteria", LEES & QUASTEL, 1946*a*) might be involved in these consequences. A single treatment, in other words, "sudden addition" (HORA, 1959), may be essential to cause an ordinary succession of urea fungi.

In the case of a single treatment in the field, it is not yet known whether there is any upper limit in amount of urea above which the urea fungi cannot appear. A few of them occurred even on the plot of 4000 g N/0.5 × 1 m (Plot 776, Table 5). The lowering in pH value on this plot was as slow as the cases in the single treatments shown in Figs. 4 and 5. In the laboratory, the upper limit may often be observed.

Other organisms. Damage to higher plants, particularly to herbs, grasses, or seedlings or younger plants of forest trees, were conspicuous when urea was applied at rates higher than 80 g N/0.5 × 1 m. The rootlets of forest trees were damaged, but the heavier the damage in the initial period, the more striking the growth of new rootlets in the subsequent period (Pl. 5, B, C).

In the *Pinus thunbergii* plantation on the sand dune in Tottori (St. 25), the new fine roots developing after the initial damage were often devoid of ectotrophic fungal sheath and were equipped with root hairs (Pl. 5, E). Such naked fine roots could not be found in untreated places (Pl. 5, D). In the *Pinus-Chamaecyparis* forest (St. 32), on the other hand, the new fine roots of the pine developing after the treatment were not naked but the variety of the mycorrhizas, being distinguishable by color and form, had been reduced. These could be attributed to the killing effect of the treatment.

A dense colonization of green and blue-green algae was observed on another urea plot in the sand dune pine stand mentioned above.

Changes after treatment with some other agents (cf II)

Soil properties. a) Agent groups **1–6** (urea-related materials). Except L-cystine, all the agents brought about fundamentally the same changes as urea did. The measurements were not done for some of the agents, but blackening of the soil, which must be caused by their basic reaction and attained when the pH value rises to the alkaline range, could be observed for these also, at least at the rate where some urea fungi occurred. FRANZ (1956) recognized similar changes in O horizon after treatment with anhydrous ammonia. But he reported disappearance of fungi; he might be correct if he observed only the initial stage. At the L-cystine plot (826), the pH value was 2.45 (one sample) in contrast to 4.0–4.3 of the untreated places (24 Oct. 1972) (this compound did not yield any fungus). After the treatment with calcium cyanamide, the water content of soil was lower than the control even in the early stages, possibly owing to its lime or excessive carbon component, or both (the fertilizer-grade calcium cyanamide contains excessive carbon).

b) Agent groups **7–9** (non-basic NH_4-N, NO_2-N, NO_3-N). Except $NaNO_2$ of Agent group 8 (see below), none of the agents induced those color change and dramatic breakdown of organic matter, the first symptoms for the occurrence of the urea fungi. The difference between urea and non-basic ammonium-N in the effect on humification has also been recognized by some other workers (JUNG, 1958; TINSLEY & HANCE, 1963). After the treatment with NH_4Cl and $NaNO_3$, the pH value slightly rose but still remained in acid range (Fig. 5). The water content also increased but it did not reach the level attained by urea treatment (Fig. 5). The reason for these increases has not been studied.

c) Agent group **10** (miscellaneous N). Hydrazine hydrate and the amides slightly changed the color to black. Aniline clearly brought about the blackening and considerably promoted the organic matter decomposition. These probably increased the water content. Others did not cause such changes.

d) Agent groups **11** and **12** (N-free compounds). None of the agents caused the color change and the breakdown of organic matter as mentioned above. Alcohols seemed to induce an increase in water content. With oils, lipoid, phenol, and cresol, the O horizon turned blackish, but this color was different from that with ammonia-related agents: the litter appeared as if it had been soaked in oil. With some carbox-

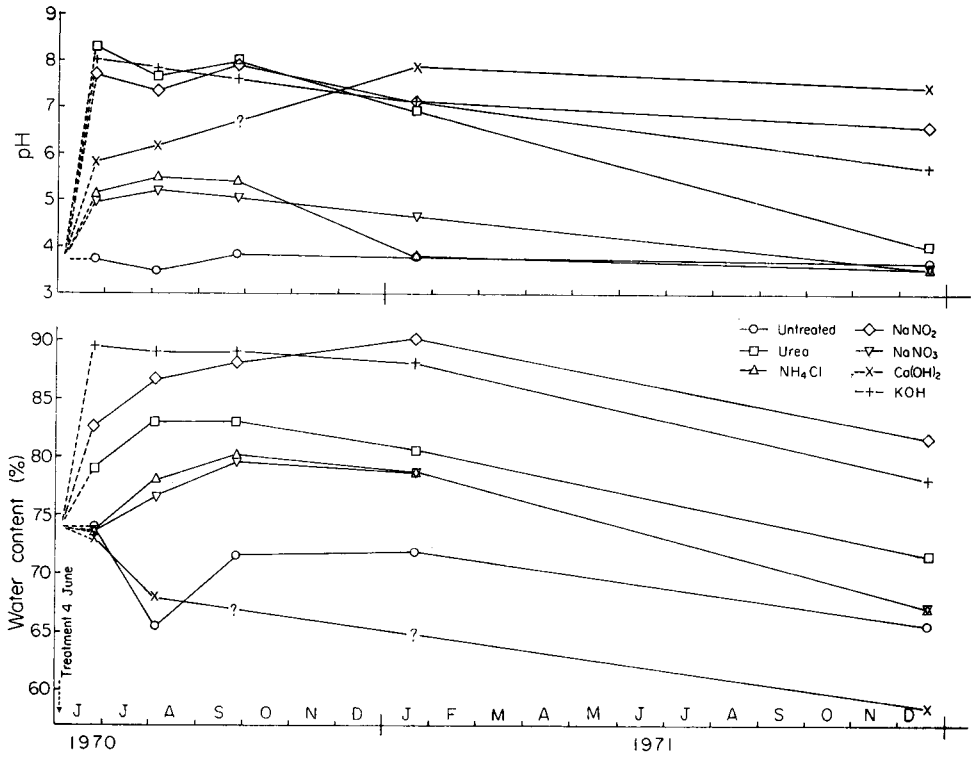


Fig. 5. Changes in pH value and water content in the O horizon of the *Pinus-Chamaecyparis* forest (St. 32) after the treatment of 0.5×1 m plots with urea and some other agents (cf II). —□— 160 g urea-N (Plot 711). —△— 160 g NH_4Cl-N (Plot 714). —◇— 160 g $NaNO_2$-N (Plot 717). —▽— 160 g $NaNO_3$-N (Plot 720). —×— 1 kg $Ca(OH)_2$ (Plot 728). —+— 500 g KOH (Plot 729). —○— Control (untreated). Single samples from each plots.

ylic acids, the litter was considerably decolored.

e) Agent group **13** (bases). $NaNO_2$ (Agent group 8), NaOH, and KOH exerted as strong influence as urea did (Fig. 5). PETERSEN (1970*b*) also reported similar changes in the raw humus layer after treatment with K_2CO_3 (and Na_2CO_3). Effects of $Ca(OH)_2$ and $CaCO_3$ were similar but much weaker, and these brought about drier conditions (Fig. 5). $Mg(OH)_2$ appeared to rank between KOH (or NaOH) and $Ca(OH)_2$ in its effect on the soil.

f) Agent groups **14–17** (non-basic K or Na, mineral acids, killings, organic solvents). The color change and the enhanced decomposition of organic matter as mentioned above were not seen with any of these agents. HNO_3 (Plot 866) changed the color to yellow. H_2SO_4 (Plot 867) caused a burn of the surface of the O horizon. After the burning (Agent group 16), the raw humus remaining unburnt beneath the layer of ash and charcoal showed similar changes in pH value and water content to those observed after the treatment with limes (Agent group 13).

Other organisms. a) Agent groups **1–6** (urea-related materials). The development

of new rootlets after the initial damage was as vigorous and luxuriant as after the urea treatment (Pl. 5, C). An exception was calcium cyanamide: the new root growth was suppressed, probably owing to lime or excessive carbon, or both, contained in it, or to drier conditions of the soil brought about by this treatment (p. 250). L-Cystine remained in the form of crystals for a long period (p. 235) and the new root growth in the above-mentioned sense was not observed.

b) Agent group **7** (non-basic NH_4-N). The new root growth was fairly good at the higher rates. Their nutritional values for higher plants seem not to differ greatly from those of the ammonia-related materials; nevertheless, their effect on fungi and soil properties distinctly differed from that of the latter.

c) Agent group **8** (NO_2-N). See Agent group 13 for $NaNO_2$. Amyl nitrite might slightly stimulate root growth.

d) Agent group **9** (NO_3-N). No particular response was found in the roots.

e) Agent group **10** (miscellaneous N). The agents other than urethane, hydrazine hydrate, and nitrobenzen appeared to promote root growth slightly.

f) Agent group **13** (bases). $NaNO_2$ (Agent group 8), NaOH, and KOH heavily damaged the root, suppressing the subsequent development also. The effect of other bases on plant roots was not conspicuous: it might be parallel with the intensities of basicity of the chemicals (see p. 251) or, in the case of liming, the roots might show a similar response to that after burning (cf p. 251 and below).

g) Agent groups **11**, **12**, and **14–17** (N-free compounds, non-basic K or Na, mineral acids, killings, organic solvents). The roots were damaged in the initial periods to various degrees depending on the characters of the agents. Some of the agents slightly promoted root growth in the subsequent period. After the burning (Agent group 16), the growth of roots was good. But the new rootlets in the burnt ground were not so thick as those in the plots treated with the ammonia-related agents (such was also the case in the fungal fruit bodies, see p. 242). Sterilizing treatments stimulate the liberation of ammonia in soil (p. 241). Therefore, the "slightly better growth" of roots after treatment with some agents belonging to these groups and some of the Agent groups 8 and 10 might be attributed to their toxicity.

Snails often appeared on the plots treated with ammonia-related agents and limes, and on bonfire sites. These are the very places where at least some of the fungi in question or the fireplace fungi occurred. Some special species of moss colonized commonly on the plots of calcium cyanamide or limes and on the burnt grounds, as was reported by PETERSEN (1970*a*, *b*).

Changes in the natural habitats (cf III)

Soil properties. The changes were similar to those in the plots treated with urea or aqua ammonia. Some data are shown in Table 13.

Other organisms. The damage to higher plants and the response of root was similar to that observed after treatment with urea or aqua ammonia. This was true

Table 13. pH values and water contents recorded in forest soils (O horizon) on which some natural matter happened to be placed and decomposed

Matters	Date of placement	Date of measurement	pH		Water content	
			The spot	Outside	The spot	Outside
Human urine	16. iv. 66	23. v. 66	7.0	3–4[a]	—	—
Human urine	23. iv. 66	23. v. 66	7.3	3–4[a]	—	—
Cat carcass	ii? 66	6. vi. 66	7.3	3–4[a]	—	—
Human corpse	12? viii. 68	17. ix. 68	5.3	3.6	78.8	72.5
		17. x. 68	3.9	3.5	80.0	70.0
		7. viii. 72	3.7	3.6	50.0	54.0
Human corpse	iv? 68	17. x. 68	5.9	3.2	76.3	70.0
		7. viii. 72	3.5	3.5	41.5	45.0
Human corpse	ix? 68	17. x. 68	7.0	4.6	81.3	61.3

a. Estimated; see the control values in Figs. 4 and 5.

even on the human corpse sites where only a few urea fungi (or proteophilous fungi) were obtained (p. 245).

V TAXONOMIC NOTES ON EACH SPECIES AND EXAMINATION OF THEIR KNOWN HABITATS

The proteophilous fungi (p. 239, 244), including the "doubtful species" (p. 223), are enumerated under the Classes Zygomycetes, Deuteromycetes, Ascomycetes, and Basidiomycetes. Within each Class, they are arranged in alphabetical order.

In cases where the taxonomic status is uncertain, they are morphologically described or taxonomically discussed. Colors of the reproductive structures are usually described after the designations by RIDGWAY (1912) and in these cases the color terms begin with capital letters. In cases where the identifications have been settled or approximated, the habitats mentioned in the taxonomic literature, on which the identifications are based, are listed, mostly by direct quotation.*

The specimens are preserved in my personal herbarium at the Biological Laboratory, Yoshida College, Kyoto University, Kyoto.

Zygomycetes

Mucor spp.

Identifications are not yet done (see Tables 8–10, footnotes). Sp. no. 2 appears to be close to *M. hiemalis* WEHMER.

Rhopalomyces strangulatus THAXT.

This fungus was reported as new to the Japanese flora by TUBAKI (1973) on the basis of the specimens obtained in the present studies.

Habitats: "on old bones and other decaying animal matter" (THAXTER, 1891).

* Translations form the literature not in English are also shown under quotation marks.

Deuteromycetes

Amblyosporium botrytis FRES.

Habitats: "on decaying basidiomycetes, well-rotted wood and plant debris, bone, dung, and isolated from heathland soil" (PIROZYNSKI, 1969).

Cladorrhinum foecundissimum SACC. & MARCH.

Habitats: "on boar dung", "isolated from soil and textile samples buried in soil" (VON ARX & GAMS, 1967).

Doratomyces purpureofuscus (FRIES) MORTON & SMITH

Habitats: on various kinds of plant debris, "pig feces, rabbit dung, deer dung, mushroom compost", or "as air contaminant" (MORTON & SMITH, 1963); "on dung" (TUBAKI, 1954, under the name *Stysanus medius*).

Doratomyces putredinis (CORDA) MORTON & SMITH

Habitats: "laboratory contaminant", "on decayed onions" (MORTON & SMITH, 1963).

Oidiodendron truncatum BARRON

Habitats: "isolated from soil of mixed wood and ceder bog" (BARRON, 1962); "in soil" (TOKUMASU, 1973).

Penicillium lividum WESTLING

Habitats: "isolated from soil, stored cereal products, and other organic materials subject to air or soil borne contamination" (RAPER & THOM, 1949).

Ascomycetes

Ascobolus denudatus FR.

Apothecia sessile or with a short stalk, up to 5 mm diam, yellowish-green. Excipulum covered with groups of subglobular cells with yellowish or almost hyaline walls. Asci 170–270 × 17.5–20(–22.5) μm. Ascospores 15–17.5 × 7.5–9 μm, when swollen reaching 20–21 × 11–12.5 μm, ornamented with longitudinal subparallel ridges that only occasionally anastomose.

The ascospores of *A. denudatus* have been described as (16–)18–22(–23) × (8.5–)9.5–11.5 μm (VAN BRUMMELEN, 1967) or 17–20 × 8–9 μm (DENNIS, 1968). They are slightly larger than the present materials. Cells of excipular warts are rust-brown according to VAN BRUMMELEN, whereas in the present materials they are yellowish or almost hyaline. These differences pose a question about the identity. However, PETERSEN (1970*b*) reported "*Ascobolus denudatus*" from a K_2CO_3-treated plot and LEHMANN (1973) from a urea-treated plot (I, II). I take this information to back up the identification by Prof. R. P. KORF, who reported this fungus as new to the Japanese flora (KORF, 1965).

Habitats: "on rotten wood and branches, rotten straw and leaves, composted bracken, humid soil, manure pile, tan refuse, honey comb of wasp nest, old carpet, rarely on dung" (VAN BRUMMELEN, 1967).

Ascobolus sp. no. 2 (Fig. 6; Pl. 6, A–C)

Apothecia cup-shaped, disc up to 2 mm diam, concave then flat, yellowish-green,

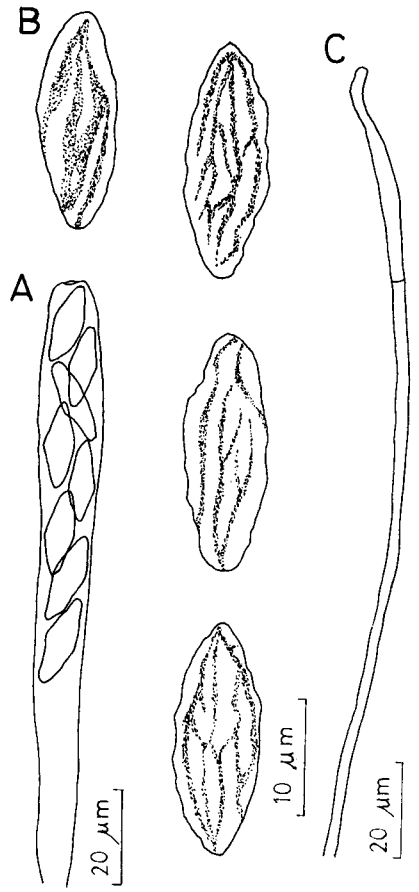


Fig. 6. *Ascobolus* sp. no. 2. A, ascus; B, ascospores; C, paraphysis.

becoming dark violet to almost black at maturity; outer surface yellowish-green to grayish green, finely mealy with groups of subglobular or pear-shaped cells. Asci up to 220×16 µm, not blued by iodine. Ascospores fusiform, 18–21×6.5–7.5(–8) µm, when swollen reaching 22.5×10 µm, purple then purplish brown, finally almost brown, ornamented with longitudinal thick ridges which can be counted up to several on one side and only occasionally anastomose. Paraphyses simple, almost straight, scarcely enlarged above, 2.5–3 µm thick.

This species appears to be near *A. epimyces* (COOKE) SEAVER, but the spore ornamentation seems to be somewhat different from those described by SEAVER (1942) and VAN BRUMMELEN (1967):

SEAVER stated, "spore-sculpturing consisting of delicate lines (apparently ridges) which anastomose and give the spore a decidedly striate appearance"; VAN BRUMMELEN described more abundant and thinner ribs than the present materials and stated, "ornamented with longitudinal anastomosing lines".

Habitats of *A. epimyces*: "on old *Corticium*, but apparently on the remains of some slime mould"

(SEAVER, 1942); "on rotten wood, rotten leaves of trees and old paper" (VAN BRUMMELEN, 1967).

Byssonectria aggregata (BERK. & BR.) ROGERSON & KORF

Habitats: "on plant debris on moors and wet ground" (DENNIS, 1968, under the name *Octospora carbonigena*).

Chaetomium globosum KUNZE ex FR.

Habitats: isolated from or collected on almost all kinds of substrata including dung of various animals (SKOLKO & GROVES, 1953; UDAGAWA, 1960).

Fimaria(?) sp. (Fig. 7; Pl. 6, D)

Apothecia usually less than 6 mm diam, rarely more than 1 cm across, cup-shaped, sessile, disc flat or slightly concave, Orange-Pink, Pale Flesh Color, Pale Salmon Color, or Seashell Pink; outer surface slightly paler, often dotted upwards with brown, obtuse, septate, thin-walled, adpressed hairs which tend to occur in bunches at the margin, giving it a minutely dentate, brown appearance. Asci operculate, 110–160 × 8–12 μm, not blued at the tip by iodine. Ascospores uniseriate, elliptical (oval), 10–12.5 × 5.5–6.5 μm, warted with very fine papillae, hyaline, white in mass, with two guttles in certain cases. Paraphyses slender, straight, branched, scarcely enlarged upwards and up to 2.5 μm thick, septate, green in iodine.

According to Prof. KORF (personal communication 4 May 1971), the spore markings do not stain in cotton blue and DE BARY bubbles are prominent. He suggests

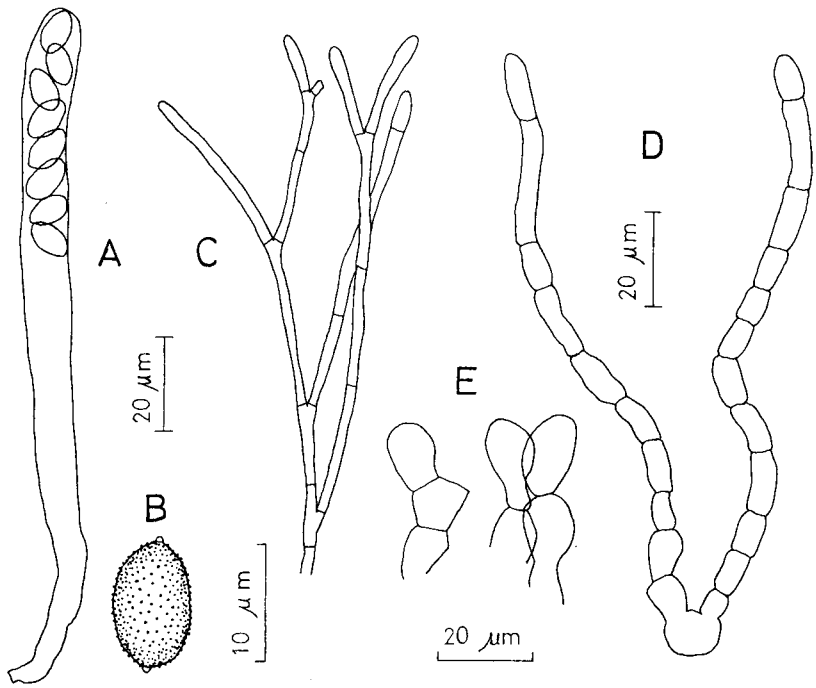


Fig. 7. *Fimaria*(?) sp. A, ascus; B, ascospore; C, paraphyses; D, hairs of outer surface; E, marginal hairs.

that this fungus may be a new species.

Gelatinodiscus sp. (Fig. 8; Pl. 6, E, F)

Apothecia cup-shaped, often short-stalked (helotioid), expanded to sessile, disc concave, Parrot Green, Oil Yellow, Yellowish Oil Green, Citrine or Dark Citrine, Sulphine Yellow when a little dried, hymenium Citron Yellow to Strontian Yellow when longitudinally sectioned; flesh rather thick; outer surface concolorous with some shade of brown, darker towards the base, finely scurfy with groups of thin-walled, globose cells. Asci operculate, 160–200 × 8.5–11 μm, blued at the tip (sometimes all

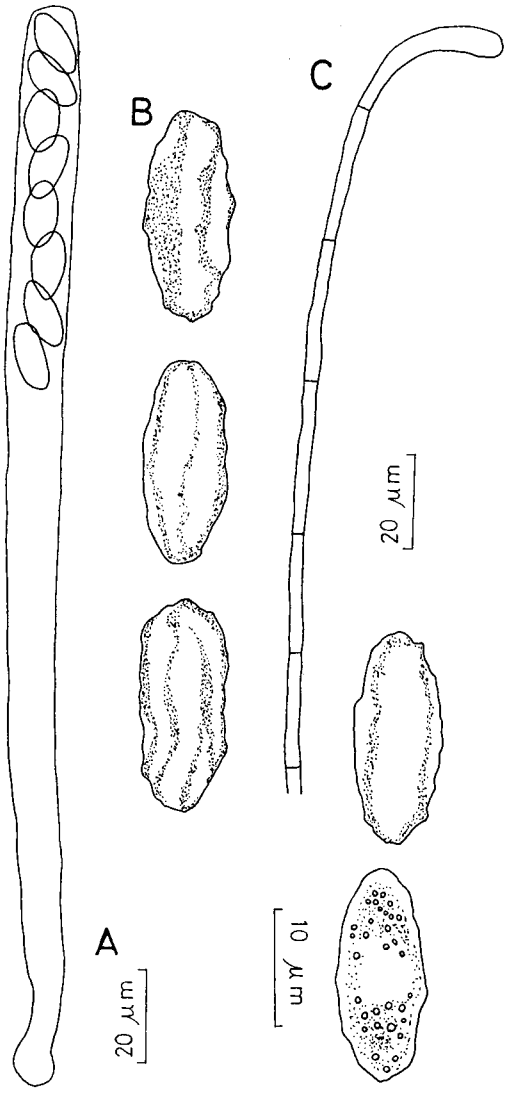


Fig. 8. *Gelatinodiscus* sp. A, ascus; B, ascospores; C, paraphysis.

over) by iodine. Ascospores uniseriate, oblong-elliptic, 15–17.5×6–7.5 μm, ornamented with low, irregular ridges. Paraphyses simple or forked at the base, about 2.5 μm wide, curved, slightly clavate and up to 5 μm thick at the tip, septa many.

Prof. KORF suggests that this fungus may be a new species.

Humaria velenovskyi (VACEK in SVRČEK) KORF & SAGARA

This combination was published, together with some descriptions, on the basis of the materials obtained in the present studies (KORF & SAGARA, 1972).

Habitats: on burn site, humid soil among mosses and conifer needles (SVRČEK, 1948, under the name *Lachnea velenovskyi*).

Iodophanus carneus (PERS.) KORF

Habitats: "on dung, rotting vegetable matter, including textiles and rope, and on soil" (DENNIS, 1968).

This species has been known as a coprophilous fungus (RICHARDSON & WATLING,

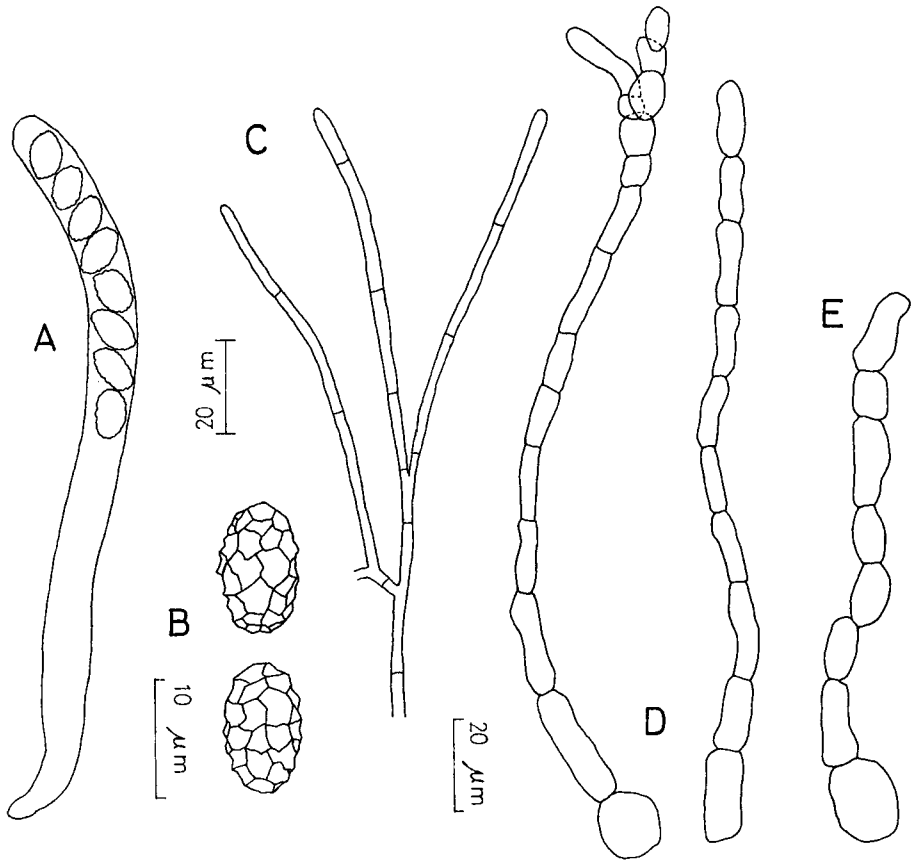


Fig. 9. *Melastiza* sp. A, ascus; B, ascospores; C, paraphyses; D, hairs of outer surface; E, marginal hair.

1968), but recently it was found on burnt ground (PETERSEN, 1970*a*) and on $CaCO_3$-treated plots (PETERSEN, 1970*b*).

Melastiza sp. (Fig. 9).

Apothecia up to 7 mm diam, sessile or very short-stalked under certain conditions, disc concave then flat or convex on aging, Capucine Buff, Salmon Buff, Light Ochraceous-Salmon, Buff Pink or Pinkish Buff; outer surface dotted with brown, obtuse, scarcely thick-walled, septate, adpressed hairs which are wider, shorter and more closely set at the margin, giving it a brown, minutely dentate appearance. Asci 140–230 × 10–11 μm, not blued by iodine. Ascospores elliptical, 11–13.5 × 6.5–7.5 μm, marked with an irregular reticulum, hyaline, with two small guttles in certain cases. Paraphyses slender, less than 2.5 μm thick, straight, branched, apex not clavate.

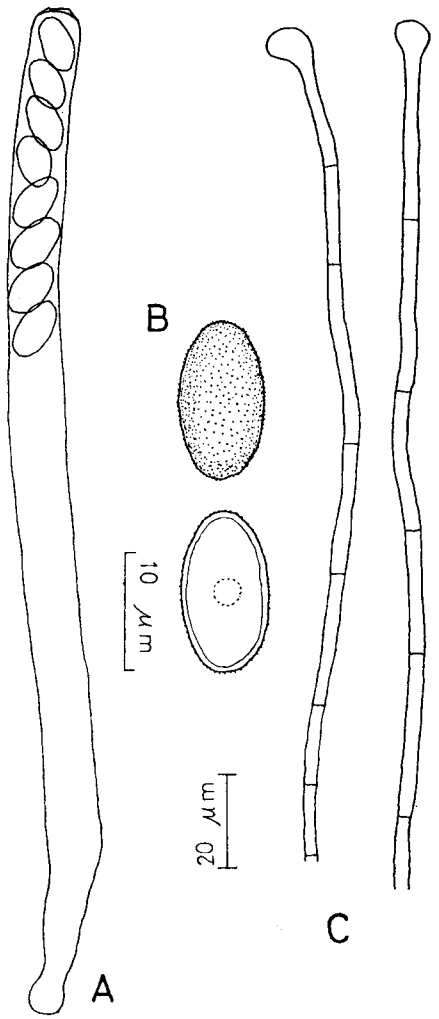


Fig. 10. *Peziza* sp. no. 1. A, ascus; B, ascospores; C, paraphyses.

Prof. KORF suggests that this fungus may be near *Melastiza flavorubens* (REHM) PFISTER & KORF. Viewed from the outside, it is difficult to distinguish this species from the *Fimaria*(?) sp.

Peziza sp. no. 1 (Fig. 10; Pl. 6, G)

Apothecia up to 3 cm across, usually less than 1 cm, cup-shaped then expanded, often substipitate, disc almost colorless when young, Pinkish Buff at maturity; outer surface almost colorless to Ivory Yellow, minutely scurfy. Asci 200–230 × 11–12 μm, blued at the tip by iodine. Ascospores elliptical, 12.5–15.0 × 7.5–8.5 μm, hyaline, white in mass, very finely warted, without oil drops. Paraphyses simple, septate, 2.5–3.5 μm wide, distinctly clavate and up to 7.5 μm thick at the tip.

Prof. KORF suggests that this fungus may be a new species. The "*Peziza* sp." obtained by PETERSEN (1970*b*) after treatment with K_2CO_3 (and Na_2CO_3) seems to be a different species.

Scutellinia scutellata (LINNAEUS ex St. AMANS) LAMBOTTE

Habitats: "on wet ground or on sodden wood" (DENNIS, 1968); "on decaying wood or on humus-rich soil" (IMAZEKI, HONGO & TUBAKI, 1970).

Trichophaea gregaria (REHM) BOUD.

Habitats: "on footpaths in forests of sandy soil, also on burnt ground, etc." (MOSER, 1963).

Basidiomycetes

Cantharellus minor PECK (?)

According to Prof. E. J. H. CORNER (personal communication 1 May 1969), this may be a color-form of the species.

Habitat of *C. minor*: "on the ground in woods" (CORNER, 1966).

Collybia cookei (BRES.) J. D. ARNOLD

Habitats: "on the ground in forests and rotting fruit bodies of fleshy fungi" (IMAZEKI & HONGO, 1965).

Collybia(?) sp. (Pl. 7, A)

Pileus strongly pulvinate when young, plane to expanded at maturity, often zonate.

Coprinus echinosporus BULLER (=*C. insignis*[?] in SAGARA [1973])

This fungus is identical with *C. insignis* PECK (?) *sensu* IMAZEKI & HONGO (1965). As they themselves added question mark, this fungus is different from *C. insignis* in some aspects. Meanwhile, HORA (1972) and LEHMANN (1973) reported *C. echinosporus* from a Na_2CO_3-treated plot and a urea-treated plot, respectively. Its morphology and behavior are very similar to the one we found. Therefore, I follow their identification. The "*Coprinus* sp." obtained after the treatment with K_2CO_3 by PETERSEN (1970*b*) seems to be the same species.

Habitats: "on refuse heaps" (IMAZEKI & HONGO, 1965); "on the forest ground where night soil (?) was dumped" (AOKI, 1968*b*); "on sticks dredged from a pool" (BULLER, 1920).

Coprinus lagopus (FR.) FR.

Habitats: "on refuse heaps and among leaf litter in forests" (IMAZEKI & HONGO, 1965); "on the ground in woods and bushes" (MOSER, 1967); "on refuse heaps, arable fields rich in organic matter and flowerbeds" (AOKI, 1970*a*).

Coprinus narcoticus (FR.) FR.

Habitats: "on decaying wood" (AOKI, 1970*c*); "on decaying straw-mat" (HONGO, 1971); "on soil" (MOSER, 1967).

Coprinus neolagopus HONGO & SAGARA (1967)

This fungus was reported as a new species on the basis of the specimens obtained in the present studies.

Coprinus phlyctidosporus ROMAGN.

Habitats: "on old burn sites" (MOSER, 1967); "on vegetable manure heaps and burnt ground" (IMAZEKI & HONGO, 1965); "on vegetable manure heaps and on the forest ground" (AOKI, 1970*b*).

Coprinus stercorarius FR.

Habitats: "on dung, manured soil, and refuse heap" (IMAZEKI & HONGO, 1965).

Coprinus sp. no. 7 (Pl. 7, B, C)

Epicutis of cap slightly green when young, the cells not spherical. Stipe not thick at the base, developing down to a long pseudorhiza which does not have a sclerotium. Spores elliptic-navicular, 7.5–11.5 × 3–6 μm, light brown to brown under the microscope.

Mr. M. AOKI (personal communication 20 July 1972) suggests that this fungus may be a new species or at least new to the Japanese flora.

Coprinus sp. no. 8 (Pl. 7, D)

Spores elliptic-oval, 8.7–12 × 3–4 μm.

According to Mr. AOKI, the shape of the spores is similar to that of *C. cortinatus* LANGE or *C. flocculosus* FR. but the position of the germ pore is different from the latter. Namely, the pore is apical in *C. cortinatus* and strongly eccentric in *C. flocculosus,* whereas in the present materials it is only slightly eccentric. Mr. AOKI suggests that this fungus may be a new species or at least new to the Japanese flora.

Hebeloma radicosum (FR.) RICKEN

Habitats: "in broad-leaved forest" (MOSER, 1967); "in the close vicinity of stumps in broad-leaved forests" (IMAZEKI & HONGO, 1965).

Hebeloma spoliatum (FR.) KARST.

Habitats: "in mixed forest" (MOSER, 1967); "in mixed forest of pine and broad-leaved trees" (IMAZEKI & HONGO, 1965).

Hebeloma vinosophyllum HONGO

This species appears to be close to *H. sarcophyllum* PECK which is said to be larger and stouter with different shaped cystidia (HONGO, 1965). Revision of these two species may be necessary after observing the response of the latter to urea treatment, since larger and stouter specimens were obtained after the urea treatment.

Habitats: "in broad-leaved and conifer forests" (HONGO, 1965); "refuse heaps, particularly of animal matter garbage" (AOKI, 1968*a*).

Laccaria proxima (BOUD.) PAT.

Habitats: "on the ground or among the sphagna in forests" (IMAZEKI & HONGO, 1965).

This species was found to be a fireplace fungus (SAGARA, 1973; II). On burnt ground, MOSER (1949) collected *Laccaria laccata* (SCOP.) BERK. var. *rosella* (BATSCH) SING. and PETERSEN (1970*a*) collected *L. laccata, L. proxima,* and *L. tortilis* ([BOLT.] S. F. GRAY) COOKE. These authors did not, however, come to the conclusion that these Laccarias prefer burnt ground, although MOSER (1949) mentioned in a footnote that *L. laccata* var. *rosella* would probably be placed under the group "anthracophilous fungi". FRANZ & LAUB (1959) obtained *L. amethystina* on a limed plot. All these species are very similar to each other in their morphological characters. Consequently, their classification should be reviewed, if possible, on the basis of world-wide data from experiments using the same methods as described in I and II.

Lactarius chrysorheus FR.

Habitats: "on the ground in forests" (IMAZEKI & HONGO, 1957); "in broad-leaved forests, especially of oaks and chestnuts" (MOSER, 1967).

Lepista nuda (FR.) COOKE

Habitats: "on the ground in forests and bamboo stands" (IMAZEKI, HONGO & TUBAKI, 1970); "in humus under hardwoods, under conifers, or on piles of trash or decaying leaves" (BIGELOW & SMITH, 1969, under the name *Clitocybe nuda*); "on compost and well-manured plots" (RICHARDSON & WATLING, 1968).

Lepista tarda (PECK) MURRILL (=*L. subnuda* in SAGARA [1973])

Habitats: "on arable fields rich in organic matter" (IMAZEKI & HONGO, 1965, under the name *L. subnuda*); "on lawns, cultivated soil, pastures, old fields, etc. but also on compost piles, manure, or occasionally sawdust heaps," "... fruitings took place during August and September following use o various fertilizers (chemical or manure) in May and June" (BIGELOW & SMITH, 1969, under the name *Clitocybe tarda* var. *tarda*).

Lyophyllum constrictum (FR.) SING. or ***L. leucocephalum*** (FR.) SING. (?) (Pl. 7, E, F)

Carpophores almost pure white all over. Pilei 1.5–5 cm. A couple of stipes often develop from a long pseudorhiza. Smell strongly of meal. Spores echinulate, elliptic-oval (in dried specimens; the spore shape in fresh specimens was not examined).

Whether the carpophore has a partial veil or not has not been determined. The former species has the veil, but the latter does not. On the other hand, the latter forms a long pseudorhiza whereas the former does not. Thus, whether this fungus is *L. constrictum, L. leucocephalum,* or any other species allied to these cannot be decided.

Habitats of *L. constrictum*: "pastures, especially where the grass is scorched by urine, and amongst short grass under conifers" (REA, 1922, under the name *Lepiota constricta*); "grassy pastures, in spaces where the grass is scorched by horse-urine" (LANGE, 1935–40, under the name *Tricholoma constrictum*).

Habitats of *L. leucocephalum*: "deciduous woods" (REA, 1922, under the name *Tricholoma leucocephalum*).

Lyophyllum gibberosum (J. SCHAEFF.) M. LANGE

This fungus was reported as new to the Japanese flora by HONGO (1972) on the basis of the specimens obtained in the present studies.

Habitats: "on footpaths in pine forests, together with *Omphalia maura* (charcoal remnants no longer discernible)" (SCHÄFFER, 1942); "on humid places in coniferous forests, particularly among the mosses which are often demolished by the fungus, but also on the needle litter" (LANGE, 1954).

MOSER (1949) collected this fungus from burnt places, but LANGE (1954) and LANGE & SIVERTSEN (1966) denied that occurrence on burnt ground was its general character. On the other hand, it is hard to understand LANGE's observation, namely "mosses being often demolished by the fungus" (see above; translated from his French text). I cannot imagine that this fungus demolishes mosses; the death of the mosses might have been caused by some other (ammonia-related?) agents as supposed from the present studies (I-IV).*

Lyophyllum tylicolor (FR. ex FR.) LANGE & SIVERTSEN

This fungus was reported as new to the Japanese flora by HONGO & SAGARA (1967) under the name *L. tesquorum* on the basis of the specimens obtained in the present studies.

Habitats: "wood of *Picea* (boarders of drives, etc.)" (LANGE, 1935–40, under the name *Collybia erosa*); "inside and outside of woods of *Fagus*, on the ground covered with short moss" (LANGE, 1935–40, under the name *Collybia tylicolor*); "on what apparently were the very decayed remains of some fleshy fungus" (SMITH, 1941, under the name *Collybia olympiana*): "among mosses in the mixed forest of *Pinus densiflora* and broad-leaved deciduous trees" (AOKI, 1970*d*). See the footnote below.

Panaeolina rhombisperma HONGO (Fig. 11, A, D)

This fungus was described as a new species by HONGO (1973) on the basis of the specimens obtained in the present studies.

Panaeolina(?) sp. no. 1 (Fig. 11, B, E; Pl. 7, G)

This fungus is undistinguishable from *Panaeolina rhombisperma* when viewed from the outside but differs in the shapes of the spores and cystidia. See the next species for further discussions.

Panaeolina(?) sp. no. 3 (Fig. 11, C, F)

This fungus is undistinguishable from the preceding two species when viewed from the outside but differs in the shapes of the spores and cystidia. One of the characteristics common in these three species is that the pilei are often covered with drops of water(?). Sp. nos. 1 and 3 may also be new species belonging to *Panaeolina* or its related genera.

Rhizopogon rubescens (TUL.) TUL. (?)

The fruit bodies are harder and more elastic with thicker peridia (ca. 500 μm) than those of *R. rubescens* which are common in pine forests along the sea coast. The present material may be an upland form of the species. The "*Rhizopogon* sp." described by AOKI (1972) appears to be the same fungus.

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* Dr. R. WATLING (personal communication 10 Feb. 1975) suggests that "the death of mosses mentioned by LANGE is because of excess urine". Further he says that "both this fungus and *Lyophyllum tylicolor* can be found near former sites of bonfire and on edges of paths in conifer woodland where urine has been deposited".

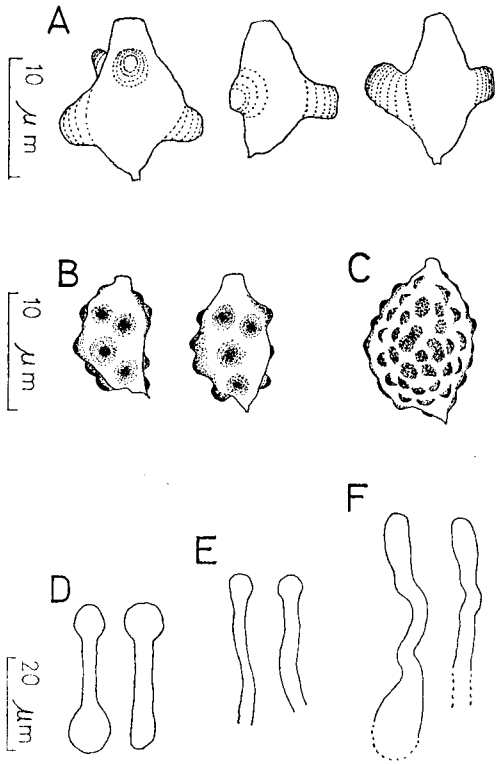

Fig. 11. *Panaeolina rhombisperma*, *Panaeolina*(?) sp. no. 1, and *Panaeolina*(?) sp. no. 3. A–C, basidiospores of respective species in this order. D–F, cheilocystidia of respective species in this order.

Habitats of *R. rubescens*: "embedded in the ground of pine forests on coastal sand dunes" (IMAZEKI & HONGO, 1965).

Rhodophyllus babingtonii (BLOX.) QUÉL. f. ***japonicus*** HONGO

Habitats: "under trees in garden" (IMAZEKI & HONGO. 1965).

Rhodophyllus lampropus (FR. ex FR.) QUÉL. *sensu* HESLER (1967)

Neither *R. lampropus sensu* LANGE (1935–40) nor that *sensu* KÜHNER & ROMAGNESI (1953).

Habitats: "grassy places" (MOSER, 1967, under the name *R. sodalis*); "on soil in deciduous woods" (HESLER, 1967).

Some findings

Taxonomical

Among the fungi obtained in the present studies (I-III), the following were reported or will be reported as new species:

Ascobolus sp. no. 2, *Fimaria*(?) sp., *Gelatinodiscus* sp., *Melastiza* sp., *Peziza* sp. no. 1, *Coprinus neolagopus*, *Coprinus* sp. nos. 7 and 8, *Panaeolina rhombisperma*, *Panaeolina*(?) sp. nos. 1 and 3.

And the followings were reported or will be reported as new to the Japanese flora:

Rhopalomyces strangulatus, *Amblyosporium botrytis*, *Cladorrhinum foecundissimum*, *Doratomyces putredinis*, *Penicillium lividum*, *Ascobolus denudatus*, *Byssonectria aggregata*, *Humaria velenovskyi*, *Iodophanus carneus*, *Trichophaea gregaria*, *Collybia*(?) sp., *Lyophyllum constrictum* or *L. leucocephalum* (?), *Lyophyllum gibberosum*, *Lyophyllum tylicolor*, *Rhodophyllus lampropus*.

Thus, the present studies have been of value in discovering new species or latent flora.

Autoecological

As for the following species, their occurrence after the application of ammonia-related materials (I, II) are not only the new facts but also can hardly be expected from what their known habitats exhibit or suggest:

Oidiodendron truncatum, *Penicillium lividum*, *Byssonectria aggregata*, *Humaria velenovskyi*, *Trichophaea gregaria*, *Cantharellus minor*(?), *Hebeloma radicosum*, *Hebeloma spoliatum*, *Laccaria proxima*, *Lactarius chrysorheus*, *Lyophyllum gibberosum*, *Rhizopogon rubescens*(?), *Rhodophyllus babingtonii* f. *japonicus*, *Rhodophyllus lampropus*.

Thus, the present studies may have contributed towards unveiling the latent character of known species.

In some other fungi, it is not impossible to explain the experimental results by their known habitats, if we regard the habitats as ammonia- or nitrogen-rich places. These cases are as follows:

Amblyosporium botrytis	decaying basidiomycetes, bone, dung;
Cladorrhinum foecundissimum	boar dung;
Doratomyces purpureofuscus.	dung;
Doratomyces putredinis	decayed onions;
Ascobolus denudatus	manure piles, dung;
Chaetomium globosum	dung of various animals;
Collybia cookei	rotting fruit bodies of fleshy fungi;
Coprinus echinosporus	refuse heaps, night soil(?)-dumped ground;
Coprinus lagopus	refuse heaps, arable field rich in organic matter;
Coprinus narcoticus	decaying straw-mats;
Coprinus phlyctidosporus	vegetable manure heaps;
Coprinus stercorarius	dung, manured soil, refuse heaps;
Hebeloma vinosophyllum	refuse heaps;
Lepista nuda	piles of trash, compost, well-manured plots;
Lepista tarda	arable fields rich in organic matter, compost piles, manure, fertilized soil;
Lyophyllum constrictum (?)	horse urine-deposited ground (?);
Lyophyllum gibberosum	death of mosses (by urine?);
Lyophyllum tylicolor	decayed remains of fleshy fungus.

This is the first time that a part of the so-called coprophilous fungi were obtained by the treatment of soil with chemicals related to ammonia. Thus, the present studies may have thrown light upon the physiological character of known species, or in other words, may have contributed to an analysis of the chemical character of some natural habitats through fungal appearance.

Morphological or phylogenical

Many of the fungi under discussion have spores with rough surfaces, either with spines, warts, ridges, etc. They are:

Amblyosporium botrytis, Oidiodendron truncatum, Ascobolus denudatus, Ascobolus sp. no. 2, *Fimaria*(?) sp., *Gelatinodiscus* sp., *Humaria velenovskyi, Melastiza* sp., *Peziza* sp. no. 1, *Scutellinia scutellata, Trichophaea gregaria, Coprinus echinosporus, Coprinus narcoticus, Coprinus phlyctidosporus, Hebeloma radicosum, Hebeloma spoliatum, Hebeloma vinosophyllum, Laccaria proxima, Lactarius chrysorheus, Lepista nuda, Lepista tarda, Lyophyllum constrictum* (?), *Lyophyllum gibberosum, Lyophyllum tylicolor, Panaeolina rhombisperma, Panaeolina*(?) sp. nos. 1 and 3, *Rhodophyllus babingtonii* f. *japonicus, Rhodophyllus lampropus.*

The group proteophilous fungi, particularly urea fungi, seems to be rather well characterized by the predominance of rough-spored species, though *Cladorrhinum foecundissimum, Coprinus lagopus,* and *Coprinus neolagopus* with smooth spores are also important for their higher specificities to urea treatment. No biological interpretation for this is yet prepared.

All the discomycetes (of Ascomycetes) enumerated above are operculate. Most of the fireplace (pyrophilous) discomycetes are also operculate (SEAVER, 1942; MOSER, 1949; PETERSEN, 1970*b*; SAGARA, 1973). WEBSTER (1970) noticed that there were few inoperculate discomycetes among the coprophiles, and he thought it curious. SAGARA (1973) pointed out a correspondence (parallelism) of fungus flora between the fireplace group and the present group. The effects of fire, dung (of herbivores), and ammonia-related materials (urine, feces of man [or of omnivores or carnivores ?]), dead animal body) on the ecosystem are, more or less, sudden and passing, and bring about striking changes in the situation of nitrogen. Thus, the three places (habitats) where these three kinds of agents are added may offer similar conditions to fungal colonization and may have been similar objects for fungal speciation. This might be the reason why we find some related flora among these places.

GENERAL DISCUSSION

A general view of the phenomena

The phenomena described in I-IV may be summarized as Table 14, which schematically presents the situations under which the urea fungi (p. 223) or the proteophilous fungi (p. 239) occurred. Alkalinity appears to be necessary as an initial condition to induce their occurrence but it seems not sufficient. The presence or supply of a considerable amount of ammonium-N or related nitrogens would be necessary. Both conditions can be fulfilled by an addition of aqueous ammonia or free ammonia (p. 242). The application of some strong alkalis to soil may also satisfy such conditions (II, IV), but it is hard to imagine that any of these alkalis would happen to be added to soil under natural conditions. Therefore, the soils to which a large amount of ammonia-related materials (p. 242, 245) are added by chance should be the proper place for these fungi to occur sequentially in nature.

Table 14. A schematic presentation of the phenomena observed in the organic matter layer of soil after treatment with various agents

Phenomena	NH_4OH[a]	$(NH_4)_2SO_4$[b]	NO_3-N	N-frees[c]	KOH[d]	Killings[e]
Color change to black	+	−	−	−	+	−
Alkaline reaction	+	−	−	−	+	−
Increase in water content	+	±	±	±	+	?
Enhanced decomposition of organic matter	+	−	−	−	+	−
Compost odor	+	−	−	−	±	−
Stimulated root growth	+	+	−	?	−	?
Occurrence of the ammonia fungi	+	−	−	−	±	−

a. Representing the ammonia-related materials (p. 242), which are basic in their own forms or become basic on decomposition. *b.* Representing the ammonium salts of strong acids which do not react as bases. *c.* Carbohydrates, oils, lipoid, carboxylic acids, alcohols, phenols, aldehydes, and mercaptan. *d.* Representing alkalis including some K- and Na- salts of weak acids *e.* Propylene oxide, acetic acid, alcohols, heat, etc.

The simplest form of the effective nitrogens was ammonia (aqueous) and the most complex was protein (II). Ammonia can well represent the chemical character of these nitrogens in relation to soil (IV). Thus, ammonia may be considered as essential, although it is not clear at what stage of transformation these nitrogens become effective as the cause of the phenomena in question (p. 242). On this line of thinking, the fungi obtained in I-III may be summarized as Table 15.

The survey of known habitats (V) suggests that the proteophilous fungi may appear separately or independently on some other places, i.e. not in the succession as described in I (Fig. 2). Even on the natural habitats mentioned in III and on some experimental plots treated with ammonia-related materials, they often appeared individually, although hyphal growth might have occurred there together or sequentially. (According to unpublished data, the composition of fungus species to appear is strongly controlled by the initial conditions, such as temperature during some early days following the treatment and concentration of nitrogen.) Therefore, ac omplete succession by all members of the group should be considered as only ideal or potential. Historically, it has not been known that these fungi may form a succession, except for the recent studies by PETERSEN (1970*b*) and HORA (1972) who observed it with only several of our species.

Relationships with known ecological groups

Some of the proteophilous fungi appear in the fungus successions on some special habitats so far recognized, such as dung (of herbivores), compost, refuse heaps, decaying plant materials, etc. (V). This would indicate that the same or similar processes, chemical, physical, or biological, take place in the ammonia-treated soil and these habitats, and makes it difficult to distinguish the present group from the known ecological groups (habitat groups) by the species composition. But, still, the

Table 15. A listing of some agents and the fungus floras obtained by treatment of soil with them—a chemoecological view of the specificity between the agents and the susceptible species.

Agents	Species (in alphabetical order)
Ammonia (aqueous)	Ammonobiont fungi[a]: *Amblyosporium botrytis, Ascobolus denudatus, Cladorrhinum foecundissimum, Coprinus echinosporus, Coprinus neolagopus, Fimaria*(?) sp., *Gelatinodiscus* sp., *Hebeloma radicosum, Hebeloma spoliatum, Hebeloma vinosophyllum, Humaria velenovskyi, Lyophyllum gibberosum, Lyophyllum tylicolor, Peziza* sp. no. 1 Ammonophilous fungi[a]: *Laccaria proxima, Lactarius chrysorheus, Rhizopogon rubescens*(?) .....**Ammonia fungi** in the narrow sense
Urea[b]	(Ureobiont fungi): Ammonobiont fungi + *Collybia*(?) sp., *Coprinus narcoticus, Coprinus phlyctidosporus, Doratomyces putredinis, Panaeolina*(?) sp. no. 1, *Doratomyces purpureofuscus,* (+ *Ascobolus* sp. no. 2, *Chaetomium globosum, Collybia cookei, Coprinus lagopus, Coprinus stercorarius, Coprinus* sp. nos. 7 & 8, *Lyophyllum constrictum* or *L. leucocephalum* (?), *Melastiza* sp., *Oidiodendron truncatum, Panaeolina rhombisperma, Panaeolina*(?) sp. no. 3, *Rhodophyllus babingtonii* f. *japonicus, Trichophaea gregaria*)[c] (Ureophilous fungi): Ammonophilous fungi + *Cantharellus minor*(?), (+ *Lepista tarda, Rhodophyllus lampropus*)[c]
Uric acid	Most of the ammonia fungi
Hippuric acid	A part of the ammonia fungi + *Penicillium lividum*
Calcium cyanamide	Most of the ammonia fungi + *Iodophanus carneus* + many pyrophilous fungi
Ammonium acetate	Most of the ammonia fungi + *Byssonectria aggregata, Mucor* sp.
L-Glutamic acid ...	A part of the ammonia fungi
L-Arginine	Most of the ammonia fungi
Ethylenediamine ...	Most of the ammonia fungi
Peptone	Most of the ammonia fungi + *Ascobolus* sp. no. 2, *Mucor* sp.
Zein (corn protein)	A part of the ammonia fungi
Casein (from milk)	Most of the ammonia fungi
Animal carcass	Most or a part of the ammonia fungi + *Ascobolus* sp. no. 2, *Mucor* spp., *Rhopalomyces strangulatus, Scutellinia scutellata*
Human urine	Most of the ammonia fungi
Human feces	Most of the ammonia fungi + *Ascobolus* sp. no. 2, *Coprinus stercorarius*
Wild-boar dung ...	One ammonia fungus (*Peziza* sp. no. 1) + many coprophilous fungi
KOH, NaOH	Most of the ammonia fungi
$Ca(OH)_2$	A small part of the ammonia fungi + some pyrophilous fungi
Burning (bonfire) ...	Many pyrophilous fungi including the three species (*Laccaria proxima, Lactarius chrysorheus, Rhizopogon rubescens*[?]) which also appear as ammonia fungi

a. The suffixes "-biont" and "-philous" are used in the same sense as explained in I.
b. The experimental data with urea are richer than those with aqua ammonia.
c. Obtained from vegetation other than the *Pinus-Chamaecyparis* forest (St. 32).

following points tend to encourage the establishment of this conceptual group.

a) As seen from the recent reviews by PARK (1968) and HUDSON (1972), it has never been recognized that urine, human feces, or dead animal bodies (except the durable parts, such as hair, feathers, skin, and bone) yield a particular array of fungi

on soil after they are decomposed. The significance of such readily-disappearing materials in fungus ecology should be stressed.

The "keratinophilous fungi" (HUDSON, 1972) may be an ecological group close to the present one. COOKE (1958) suggested that fungi growing on many types of proteinacious materials should be placed in the group of fungi more or less restricted to keratin. But the flora known as keratinophilous fungi (DOMINIK & MAJCHROWICZ, 1965, 1970; MAJCHROWICZ & DOMINIK, 1968, 1969; HUDSON, 1972) is completely different from the present one. And the present results with L-cystine and keratine (and collagen?) (II; footnote on p. 235) suggest that it is better to separate the effect of scleroproteins and that of other readily-decomposing proteins.

b) ALEXANDER (1961) stated, "Without question, the fungi occupy a dominant position in proteolysis in certain soils, particularly in acid localities. .....The microbiology of protein breakdown in soil is inadequately understood." He himself or Dojô-biseibutsu Kenkyû-kai (1966, pp. 288–289) named none or few fungi in this connection. At least a part of the present group of fungi are thought to be the very ones to have been mentioned by these authors, as it seems most probable from the results of the present studies and from some preliminary observations* that they take part in the transformations (immobilization or mineralization) of ammonia, proteins (except scleroproteins ?), or nitrogen compounds degraded from the proteins or excreted by animals as the final products of nitrogen metabolism. It should be emphasized at least that there exist some special fungi to be studied in relation to the above-mentioned phases of the nitrogen cycle.

HORIKOSHI (1971, 1974) proposed "alkalophilic microorganisms" for some bacteria, actinomycetes, and molds (his main interest has been with bacteria). His proposal does not conflict with ours since the former has been defined *in vitro* while the latter is going to be defined *in situ*. In some physiological aspects, however, these two will possibly meet and provide us with a deeper understanding of the microbial system in soil, as it is not improbable that the alkalophilic microorganisms are those which colonize on or in soils where the proteophilous fungi occur.

New grouping of fungi

The proteophilous fungi cannot be considered as a physiological group, since they have little been studied *in vitro* and since some or many of them may not "be produced", in the strict sense, by the primary effect of ammonia or ammonia-related materials (p. 242). They cannot also be proposed as a habitat group since they have

* i) In a test-tube culture on HAMADA's medium (tap water 1000 ml, glucose 20 g, dry yeast 5 g, 1.0 N HCl 1.6 ml, agar 20 g), many of the fungi, particularly those appearing in the early stages of the succession, showed striking growth when urea was added.

ii) In *Coprinus phlyctidosporus*, the basidiospores showed a high rate of germination in ammonia water (initial pH 11.0) but no germination in aqueous solutions of KOH. According to Mr. Akira SUZUKI, then at Kyoto University, spore germination in *Hebeloma vinosophyllum* was induced by urea, ammonia, and ammonium salts but not by KOH and NaOH (read at the 18th Annual Meeting of the Mycol. Soc. of Japan, 1974).

not all been identified as the inhabitants of such natural habitats as studied in III. But it seems reasonable to take them as an experimental- (or, chemical-) ecological group on the basis of their confined, constant, and luxuriant occurrence on certain characteristic places prepared by the experimental (chemical) treatment. That is, they can be defined as **a chemoecological group of fungi which sequentially develop reproductive structures exclusively or relatively luxuriantly on the soil after a sudden addition of ammonia, some other nitrogenous materials which react as bases by themselves or on decomposition, or alkalis.** The general term **ammonia fungi** may be proposed for them.

In the narrow sense it refers to the species to be obtained with aqueous ammonia and in the board sense it includes the additional flora to be obtained with the nitrogenous materials more complex than ammonia or urea (Table 15). In this definition I would not say anything about the causal relationships. It is a descriptive and rudimentary understanding like the recognition of "fireplace fungi" or "coprophilous fungi" which have been originally based on *place* (habitat) but not on physiological characteristics. In the present case, I have failed in finding out a word which should properly represent "places treated with ammonia or ammonia-related materials" and could be used as the prefix or adjective in the name of the group. This may be a matter of course: if such a word were present, my work could not be original.

The term "urea fungi" is now abandoned in the prospect that urea can almost completely be replaced by ammonia as the effective agent on fungus flora, and that if any species remains which can be obtained with urea but not with ammonia, it can be included in the definition in the broad sense. The term "proteophilous fungi" is also abandoned because it cannot express the difference within proteins in the effect on fungi (p. 269), because it cannot indicate the ineffectiveness of nitrogen-free compounds deriving from proteins on decomposition (p. 240), and because it cannot clearly refer to the essential thing, ammonia. In my doctoral thesis (SAGARA, 1974), the same group was called "ammonogenous fungi" and defined as "the group of fungi which sequentially develop reproductive structures exclusively or relatively luxuriantly on the soil after ammonia or nitrogenous materials which release ammonia and cause alkaline conditions are added suddenly". This term should again be abandoned because it too strongly implies causal relationships. And the statement of the definition should be changed as above because, for some of the effective agents, measurements to confirm release of ammonia or occurrence of alkaline conditions have not been carried out (see p. 250).

One may be opposed to assigning some of the above-mentioned species in the present group, on the grounds that he once collected them from "untreated" or "normal" places. This criticism will be significant only if he can prove that these very places had never received the equivalent of an application of ammonia or ammonia-related materials. Generally speaking, it is rather difficult to determine by observation whether or not any spot in the natural surroundings has been "untreated" or "treated". On one occasion, for example, I came across a luxuriant occurrence of

Hebeloma vinosophyllum on a *normal* place in the *Castanopsis cuspidata* forest (St. 30), which is situated near human dwellings. From the results of the experiments with ammonia-related materials, I suspected that something to release ammonia had been added there. Digging up the soil under the fruit bodies, I found the bones of a dog, which must have been buried by somebody. Thus we could say confidently that the place had certainly been *treated* and that the fungal occurrence there was reasonable. It is on the basis of such constancy and specificity in the occurrence of our fungi that they can be attributed to the group defined, even if they occasionally appear separately (i.e. not in the succession), and are the source of physiological and evolutionary interest.

CONCLUSIONS

A unique group of fungus species appeared sequentially on the soil (raw humus) of uncultivated land after an application of aqua ammonia, some other nitrogens reacting as bases, or some alkalis. This was accompanied by characteristic changes in color, odor, pH value, water content, organic matter, and plant roots in soil. These phenomena, which may fall in the category of chemical ecology, have not been described as a whole and deserve further studies. I propose, therefore, to raise the fungi as a chemoecological group under the general term *ammonia fungi*, taking ammonia as the representative of the effective agents.

SUMMARY

1) A particular array of fungus species appeared exclusively or relatively luxuriantly on soil (raw humus layer) treated with urea. This phenomenon was generally observed with a wide variety of vegetations distributed throughout Japan. The fungus flora was dependent, to a certain extent, on the type of vegetation.

2) Aqua ammonia, aliphatic amines, and some other nitrogenous materials which react as bases in their own forms or on decomposition (*ammonia-related* materials) were generally as effective as urea. Non-basic NH_4-N, NO_3-N, and N-free compounds were not effective. Alkalis were somewhat effective, probably owing to their ability to liberate ammonia from soil. The occurrence of NH_4-N together with an alkaline condition, or in short NH_4OH, seems to be the essential factor for the above effect. Certain secondary causes were also suggested.

Some of the nitrogenous materials that are more complex than urea or ammonia yielded some species additional to the flora obtained with the latter. They are discussed together.

3) In nature, most of these fungi appeared on the ground in forests where human urine, human feces, or dead bodies of mammals had been placed by chance and decomposed. These matters also can be considered as ammonia-related materials (see above).

4) Characteristic changes commonly observed in the soil to which these effective

nitrogenous materials had been added were: color change to black, increases in pH value (generally over 7) and water content, enhancement of organic matter decomposition, ammonia or compost odor in the initial or early stages; and stimulated root growth in the subsequent stages.

Repetition of treatment accelerated the decrease of pH value after its initial increase and suppressed the occurrence of many of the urea fungi. A single application is essential.

5) Each of the 47 fungus species in question were taxonomically examined and questions on the identities of some species were pointed out. Eleven of all were determined to be or seem to be new species and 15 new to the Japanese flora. The unidentified fungi were morphologically described.

In the 32 species identified, their habitats mentioned in the taxonomic literature range from decaying basidiomycetes, decaying plant material (including manure and compost), dung, refuse heap, manured soil, etc. to non-special places. In 18 species, occurrence after chemical treatment is conceivable if we regard their habitats as ammonia- or nitrogen-rich places, but in the rest occurrence is very difficult to explain by their known habitats.

6) The fungi in question can neither be attached to the habitat groups so far known nor be established as a new habitat group: they are an assemblage of fungus species obtainable by chemical treatment. Thus, they are proposed as a chemo-ecological group under the general term "ammonia fungi".

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* May be translated as "Illustrations of Japanese Mushrooms", containing monochromatic drawings and morphological descriptions in Japanese. It has been edited by Mr. Minoru AOKI and circulated among the members of Nihon Kinoko Dôkôkai, which may be translated as "The Amateur Association for Mushroom Research, Japan".

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Addenda

1) The *Rhizopogon rubescens*(?) (p. 263) has been identified as *R. superiorensis* A. H. SMITH by Dr. J.M. TRAPPE, Forestry Sciences Laboratory, U. S. Department of Agriculture, Oregon, to whom I am imdebted.

2) Observation on most experimental plots listed in Table 5 has been continued until November 1975. From this, the following relations have been found, which should be added to Table 8 by the mark + or ?:

Collybia(?) sp. occurs after treatment with calcium formate;
Laccaria proxima occurs after treatment with zein (?), formamide, potassium oxalate, phenol (?);
Lyophyllum gibberosum occurs after treatment with peptone.

The meanings of the question mark are the same as those in Table 8.

PLATE 1

Exclusive occurrence of the fungi on the experimental plots (cf I)

A. *Lyophyllum tylicolor* on plot treated with urea. 1.6 kg N was applied to 4×5 m in the *Pinus densiflora-Chamaecyparis obtusa* forest in Kyoto (St. 32) on 2 May 1971 (Plot 746, not mentioned in the Methods). Photo on 29 May 1971. *Amblyosporium botrytis, Ascobolus denudatus,* and *Gelatinodiscus* sp. also occurred but cannot be seen in this photograph.

B. *Hebeloma vinosophyllum* on plot treated with urea. 160 g N was applied to 0.5×1 m in the *Quercus glauca* coppice in Ôita (St. 16) on 20 July 1968 (Plot 611). Photo on 7 July 1969. Arrow indicates the upper left corner of the plot, being marked with a peg.

C. Mycelial mat, most probably of *Hebeloma vinosophyllum,* developed beneath L horizon inside Plot 611 (see B). Scale in centimeter. Left of the scale untreated and right of it a portion of the plot. Photo on 31 Dec. 1968, i.e. about five months after treatment and about six months before the fungus occurrence shown in B. The L horizon was temporarily removed for photographing. The mycelia appeared white.

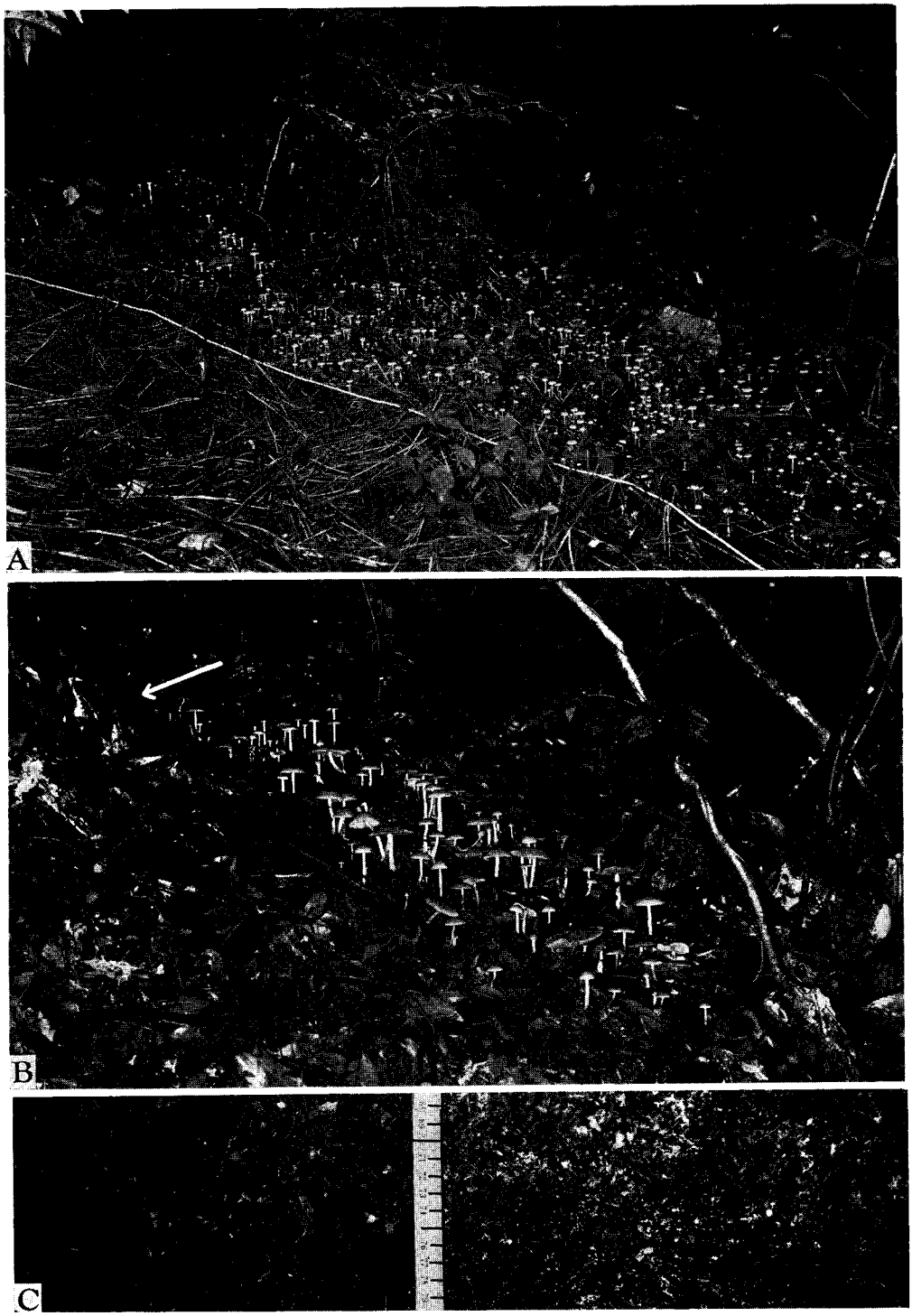

A
B
C

PLATE 2

Relatively luxuriant occurrence of the fungi on experimental plots (cf I, II)

A. *Laccaria proxima* on plot treated with ammonium acetate. 160 g N was applied to 0.5×1 m in the *Pinus-Chamaecyparis* forest (St. 32) on 10 June 1967 (Plot 350). Photo on 6 Oct. 1968.

B. The same fungus on an untreated place (arrow), just outside Plot 350 (see A). Photo on the same day as that of A.

Occurrence of the fungi in laboratory experiments (cf I)

C. *Coprinus phlyctidosporus* on soil sample treated with urea. 20 g (in dry weight) of fallen leaves of *Musa* ×*paradisiaca* collected at Sata, Kagoshima (St. 4), were treated with 0.2 g N in a glass bottle (7.5 cm diam) and incubated at 10 C on 7 May 1967. Photo on 16 Oct. 1967.

D. *Lyophyllum tylicolor* on soil sample treated with urea. 20 g (in dry weight) of raw humus collected from the *Abies mariesii* stand of Mt. Hakkôda, Aomori (St. 64), were treated with 0.2 g N in a glass bottle (6.5 cm diam) and incubated at 10 C on 12 Oct. 1967. Photo on 1 Dec. 1967.

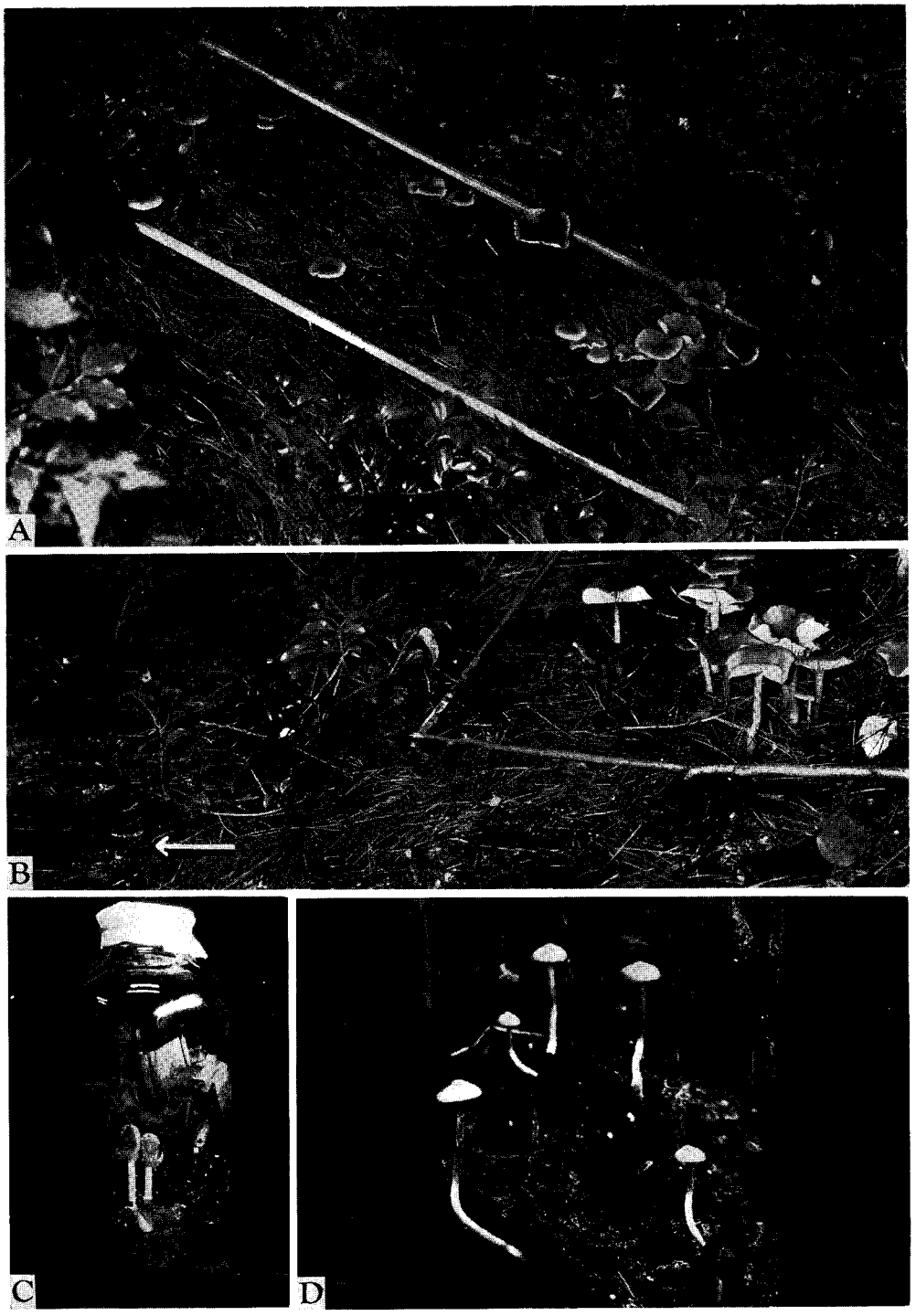

A
B
C
D

PLATE 3

Occurrence of Laccaria proxima after treatment with some nitrogen-free agents in Pinus-Chamaecyparis *forest* (St. 32) (cf II)

A. 1 kg of *iso*-amyl alcohol was applied to 0.5 × 1 m on 16 Feb. 1972 (Plot 801). Photo on 1 Oct. 1973.

B. 1 kg of potassium formate was applied to 0.5 × 1 m on 17 Aug. 1972 (Plot 849). Photo on 1 Oct. 1973.

C. A bonfire was burnt for 2.5 h on 24 Feb. 1972 (Plot 817). Photo on 3 Oct. 1974.

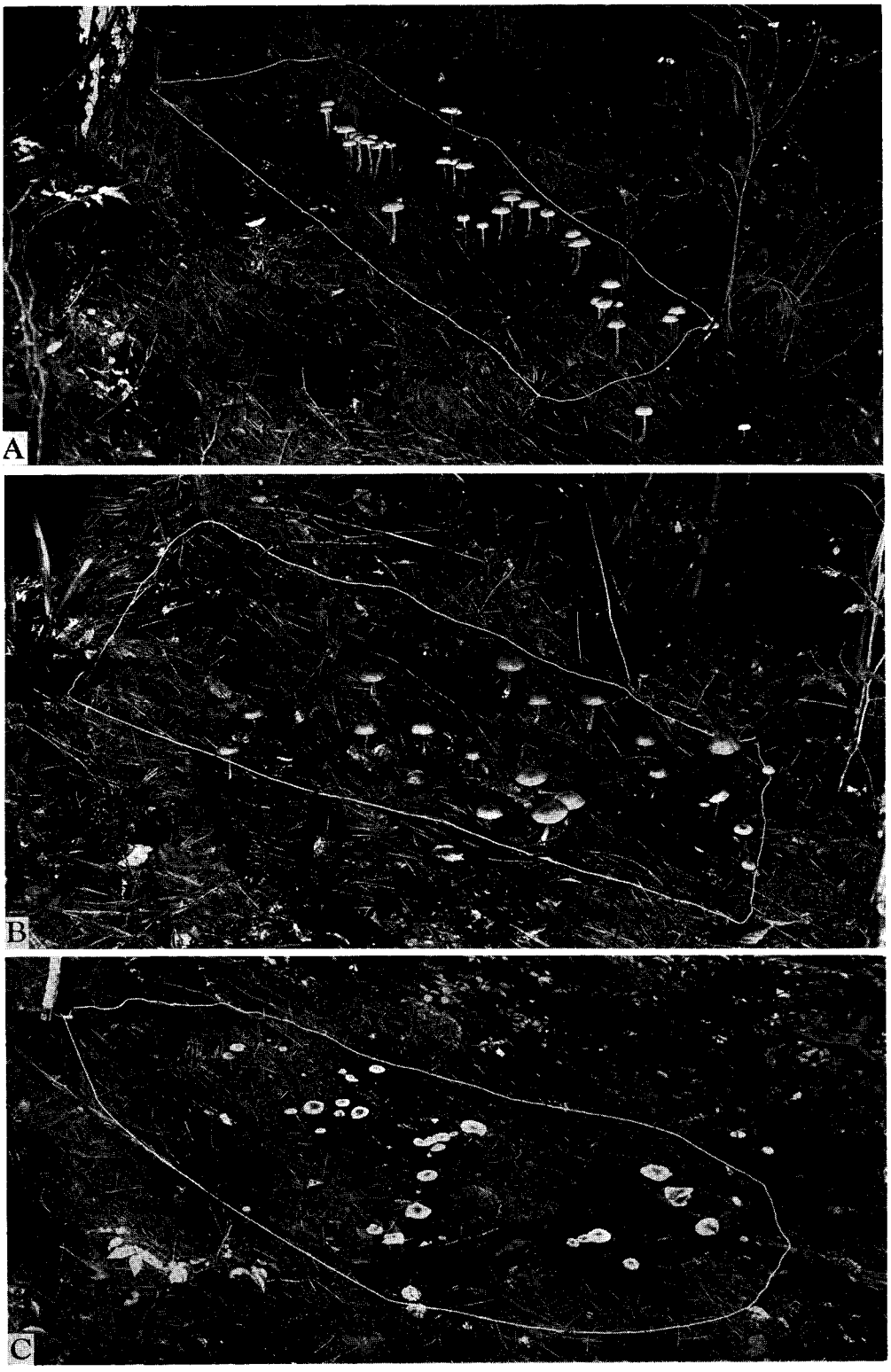

A
B
C

PLATE 4

Occurrence of the fungi on the ground after the addition of some natural materials

A. *Lyophyllum tylicolor* on ground treated with fish. 2 kg of saurels were placed on the ground in the *Pinus-Chamaecyparis* forest (St. 32) on 27 May 1967 (Plot 331). Photo on 6 July 1967. Cf II and Fig. 2.

B. *Ascobolus denudatus* (arrows) and *Lyophyllum tylicolor* on human urine-deposited ground. Urine was deposited in the *Quercus acutissima-Q. serrata* stand in Kyoto (St. 36) on 13 Sep. 1967. Photo on 9 Oct. 1967. Cf III.

C. *Lactarius chrysorheus* occurring on the peripheral part of Plot 331 (see A). A pencil in the central part, where the saurels were placed. This fungus later invaded the central part. Photo on 3 Oct. 1967, i.e. about three months after the occurrence of *Lyophyllum tylicolor*. Cf II and Fig. 2.

D. *Hebeloma spoliatum* (arrows) occurring after the decomposition of a dog carcass. It was abandoned on the ground in the *Quercus serrata-Q. variabilis* forest in Shiga (St. 51), probably, in Nov. 1966. Photo on 26 Oct. 1967. Cf III.


A
B
C
D

PLATE 5 (cf IV)

A. A profile of the surface layers of forest ground, showing the change of color to black in the O horizon after the treatment with urea. 200 g N was applied to 0.5×10 m in the *Pinus-Chamaecyparis* forest (St. 32) on 8 Sep. 1967 (Plot 462, not mentioned in the Methods). Photo on 15 Nov. 1967. The color change was accompanied by an increase in water content and an alkaline condition. *Al* indicate the A1 horizon. Bar 5 cm.

B. New rootlets of *Chamaecyparis obtusa* invading the urea-treated plot. 320 g N was applied to 0.5× 1 m in the young *Pinus-Chamaecyparis* forest in Kyoto (St. 33) on 20 Feb. 1967 (Plot 245, not mentioned in the Methods). Photo on 17 July 1967. The broken line indicates the margin of the plot at its upper left corner. Bar 5 cm. The rootlets of the pine developed there later.

C. Rootlets of *Chamaecyparis obtusa* which developed in the O horizon of the plot treated with aqua ammonia, showing the difference in luxuriance to be parallel with the amount of nitrogen applied. From left: control, Plot 564 (40 g N), Plot 565 (80 g N), Plot 566 (160 g N), all 0.5×1 m in the *Pinus-Chamaecyparis* forest (St. 32). Application on 20 Jan. 1968, collection of the roots on 10 Mar. 1969. Each block 2 g in fresh weight. Bar 10 cm.

D, E. Formation of fungal sheath-free roots in *Pinus thunbergii* after urea treatment. D, one of its ectomycorrhizas collected from an untreated place in its plantation on a sand dune in Tottori (St. 25). E, a fungal sheath-free root of the pine which developed after the treatment in the same stand: 1.6 kg N was applied to 0.5×10 m on 14 July 1971 (Plot 770, not mentioned in the Methods). Collection of the roots on 27 Nov. 1971. Bars 1 mm. Such terminal fine root as in E should otherwise have developed to ectomycorrhizas like the one in D or of some other forms. Note that the root in E is equipped with numerous root hairs, whereas the one in D has none at all.

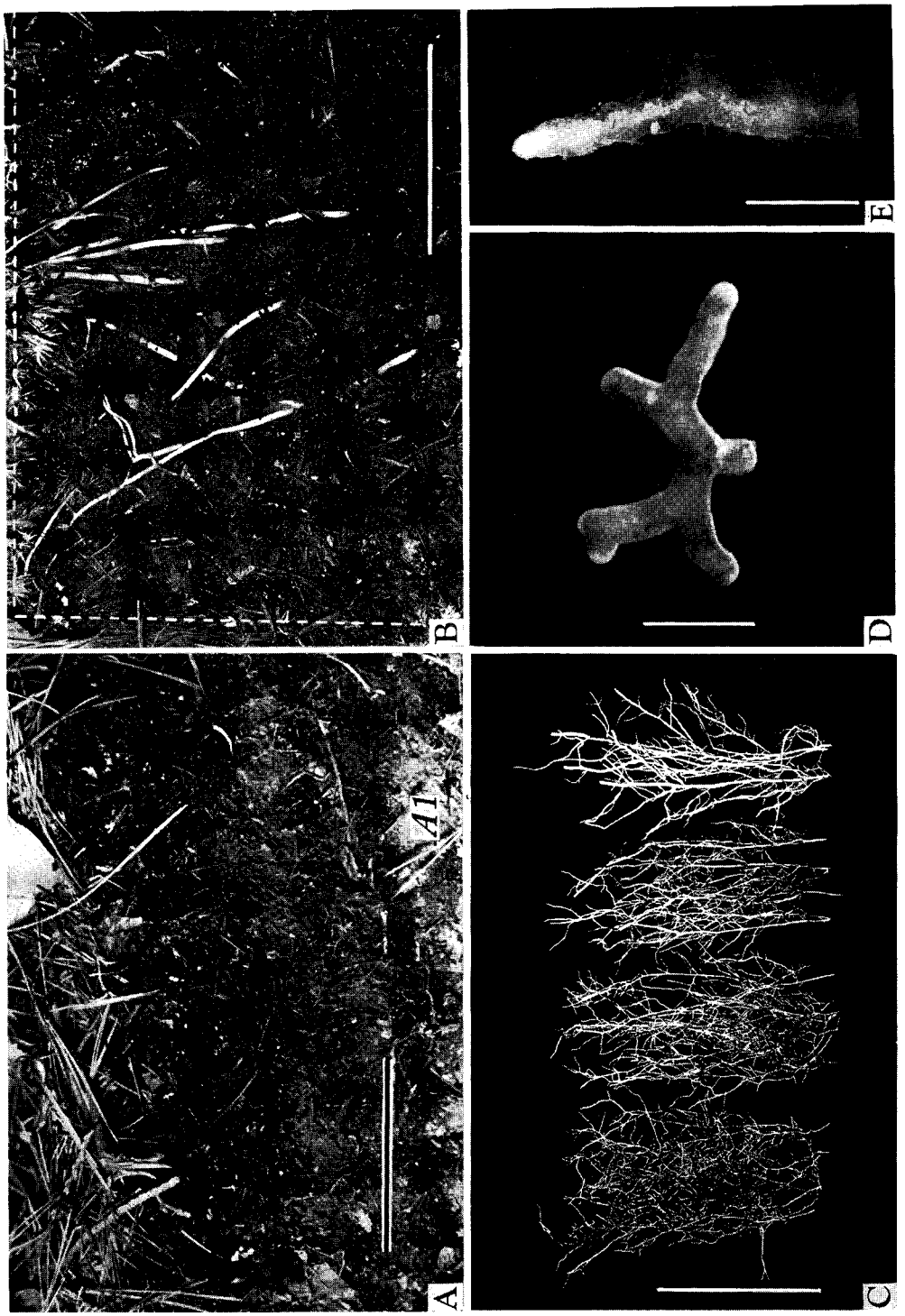

A
A1
B
C
D
E

PLATE 6 (cf V)

A-C. *Ascobolus* sp. no. 2. A, asci and paraphyses in unstained water mount, bar 20 μm. B, ascospores in unstained glycerine mount, bar 20 μm. C, a group of cells on the outer surface of apothecium in unstained water mount, bar 20 μm.

D. *Fimaria*(?) sp. Apothecia. Bar 1 cm.

E, F. *Gelatinodiscus* sp. E, apothecia, bar 1 cm (stipes of these specimens are longer than those usually obtained in the field experiment). F, outer surface of apothecium in unstained water amount, bar 100 μm.

G. *Peziza* sp. no. 1. Apothecia. Bar 1 cm.

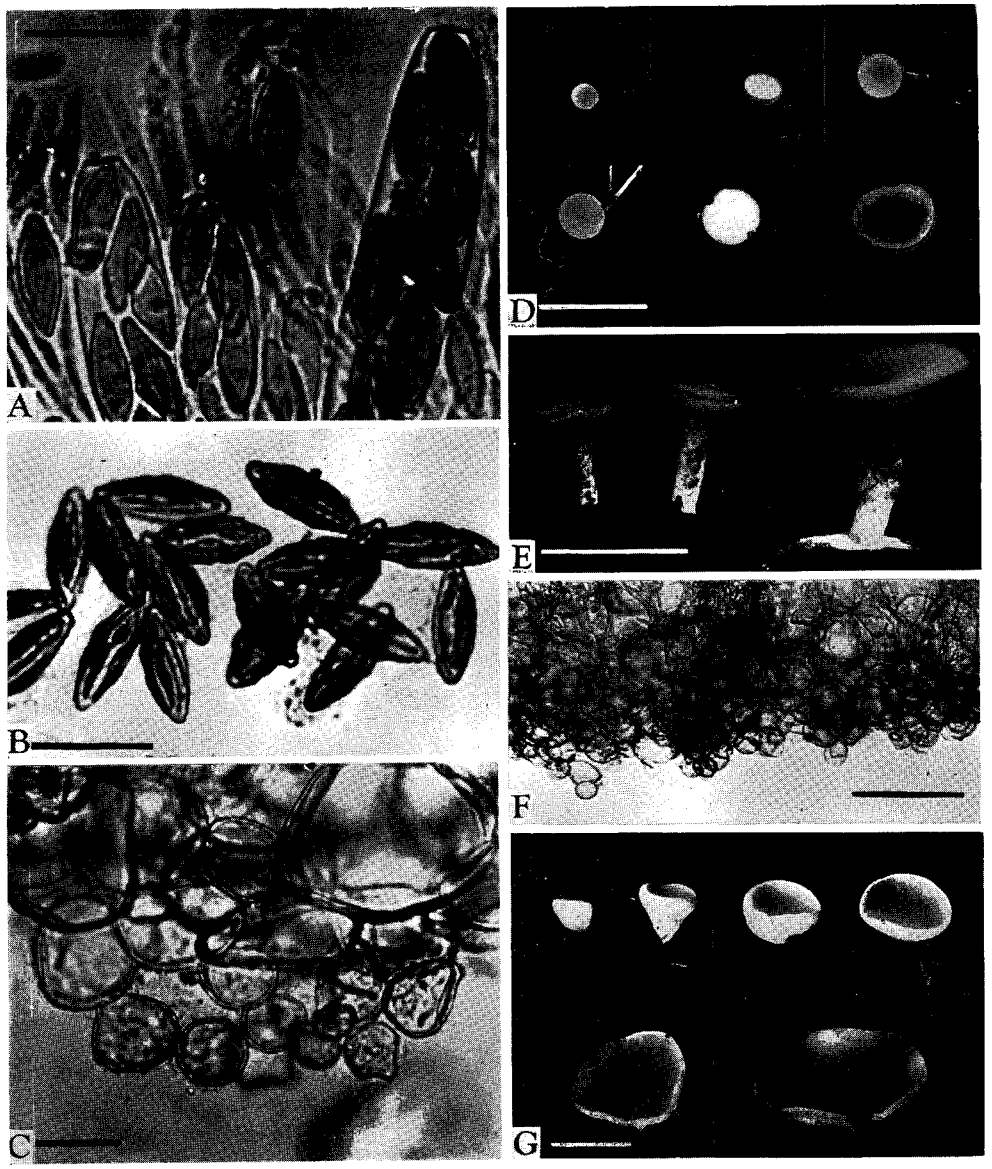

A
B
C
D
E
F
G

Plate 7 (cf V)

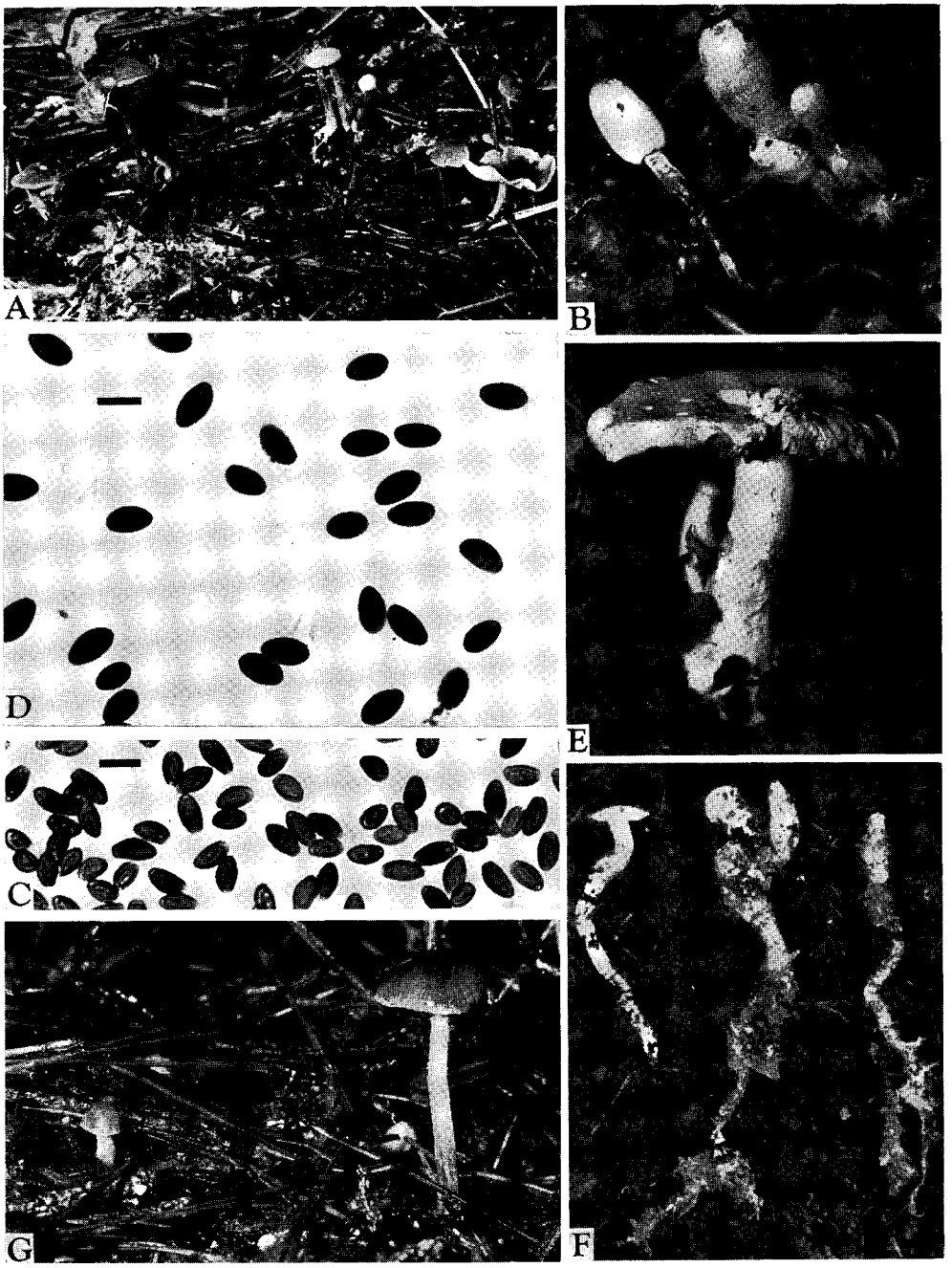
A. *Collybia*(?) sp. Basidiocarps. ×1/2.

B, C. *Coprinus* sp. no. 7. B, young basidiocarps, with long pseudorhiza. ×3/4. C, basidiospores in unstained glycerine mount, bar 10 μm.

D. *Coprinus* sp. no. 8. Basidiospores in unstained glycerine mount. Bar 10 μm.

E, F. *Lyophyllum constrictum* or *L. leucocephalum* (?). E, a mature basidiocarp (front) and a young one developing from the same pseudorhiza, ×3/4. F, basidiocarps with long pseudorhiza, ×2/3; note the branching in the development of the basidiocarps (center).

G. *Panaeolina*(?) sp. no. 1. Basidiocarps. ×3/4.


A
B
D
E
C
G
F