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Allelochemicals: Role in Agriculture and Forestry

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Chapter 17

Allelopathic Effects on Mycorrhizae

Influence on Structure and Dynamics of Forest Ecosystems

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Ectomycorrhizal species differ in their sensitivity to allelochemicals originating in litter and soil organic material. This phenomenon produces a successional change in mycorrhizal species as ecosystems rebuild litter layers following disturbance, and possibly acts to structure rooting zones in such a way that competition among higher plants is decreased. Mycorrhizae alter foliage chemistry, and thus potentially form a closed loop in which they both act on, and are acted upon by, system biochemistry.

A mycorrhiza (literally, fungus-root) is a symbiotic association between a fungus and a plant. Mycorrhizae occur most frequently on plant roots, but may be found on any tissue involved in uptake of elements from soil. Mycorrhizae, formed by numerous fungi in the orders Phycomycetes, Basidiomycetes, and Ascomycetes, can be divided into two broad groups: those that penetrate host cells (endomycorrhizae) and those that do not (ectomycorrhizae). A few fungal species defy this neat classification, penetrating the cells of one host but not those of another.

Among the endomycorrhizae, the most common are those that, because of distinctive structures produced by the fungus, are called vesicular-arbuscular mycorrhizae (VAM). VAM are Phycomycetes of the family Endogonaceae, and may occur on trees, shrubs, or herbs, and in any plant phyla (1). Ectomycorrhizae (EM), formed by numerous species of Basidiomycetes, Ascomycetes, and Phycomycetes, are distributed less widely in the Plant Kingdom than VAM. Although EM have been reported on herbs, principal hosts are trees and woody shrubs, particularly Gymnosperms and Angiosperms of the families Betulaceae and Fagaceae (1). Most of the important commercial tree species of the temperate zone are ectomycorrhizal. Despite their relatively narrow host range, EM fungi are highly diverse. At least 5000 species of fungi form EM, compared to fewer than 30 forming VAM (2).

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Over 90 percent of the roughly 200 plant families so far investigated form mycorrhizae and for many the relationship is obligate (3). Mycorrhizae directly benefit host plants by increasing water and nutrient (particularly phosphorus) uptake, increasing the lifetime of feeder roots, and protecting against root pathogens. The ecological role of mycorrhizae is in general poorly understood, but they are likely to mediate competitive relations between higher plants (4-6), alter plant palatability to herbivores (5, 7), and influence the physical structure of soils (8). The latter point is particularly important in soils with low clay content, in which virtually all aggregation and consequently water-holding capacity and porosity may result from the activity of mycorrhizae and associated rhizophere organisms (e.g., 9, 10).

For most of the world's plants mycorrhizae are the primary interface between physiologically active areas of the root and the external environment. Hence it is likely that many, perhaps most, allelochemical interactions involving higher plant roots are mediated by the fungal symbiont. Herein we briefly review past research dealing with allelopathic effects on mycorrhizae, and discuss how interaction between mycorrhizae and chemicals may influence structural and functional aspects of ecosystems. Most studies to date have dealt with EM in forest ecosystems, and this is where our discussion will focus.

Allelopathic Effects on Mycorrhizae

Allelopathic effects on mycorrhizae have been known for 40 years. Melin (11) demonstrated that EM were inhibited by ethanol-soluble, heat-stable, substances contained in litter of Scots pine (*Pinus sylvestris*) and various deciduous tree species. Since then various workers have shown inhibition of EM by organic matter or water-soluble extracts from litter or roots (12-19). EM may also be inhibited by some *Streptomyces* species (20). Mikola (21) found that the degree of inhibition of EM by leaf and litter leachates varied with concentration: low concentrations actually stimulated growth of *Cenococcum geophilum* and *Lactarius tomintosus*. Allelopathic effects on EM vary not only with concentration, but are highly specific to fungal species and nature of the allelochemical as well. Tan and Nopamornbodi (22) found that several fulvic acids and soil extracts stimulated growth of *Pisolithus tinctorius*, a fungus that is also inhibited by leachates from a number of litters. Rose et al (23), studying the influence of water-soluble leachates from various types of litter on growth of four EM fungi, found a highly significant interaction between EM species, litter type, and concentration.

Allelopathic effects on EM *may produce* striking changes in plant communities. Widespread failure of Sitka spruce (*Picea sitchensis*) plantations in Scotland was attributed to inhibition of spruce EM by substances leaching from heather (*Calluna vulgaris*) roots and/or raw humus (12). In Finland, unidentified substances leached from reindeer lichen (*Cladina* sp.) inhibit EM formation on various tree species (24). Trees growing in the absence of lichen cover may be up to 20 times as large as trees growing with lichen (25). In other cases effects are more subtle, with allelopaths altering EM composition rather than causing outright failure of EM formation. Schoeneberger and Perry (19) found that leachates from the litter of an Oregon forest reduced formation of a single EM type on Douglas-fir (*Pseudotsuga menziesii*), while EM of Western hemlock (*Tsuga heterophylla*) were unaffected. Litter from a nearby, very similar, forest had no effect. This disparity in results is not unusual; while most studies have found that organic material inhibits EM, some find just the opposite--stimulation of EM by organic material (26, 27). Such differences are probably due largely to the highly specific nature of the allelopath-EM relationship, although physiological vigor of the allelopath-producing plant also plays a role (12).

Our work has consistently shown that trees of a given species form different proportions of EM types depending on whether they are grown in soils from undisturbed forest or from clearcuts, and that the relative frequency of types formed in the latter depends on whether logging slash was burned or not. More research is needed, but the most likely explanation for these observations is that soils from plant communities in different stages of succession, or that have experienced different types of disturbance, vary in their characteristic chemical signature, and this in turn influences the type of EM forming on seedlings.

Apparently a variety of chemical compounds inhibit EM. Olsen et. al. (15) identified benzoic acid and catechol as the EM inhibitors present in aspen leaves (*Populus tremula*). They also showed that fungi decomposing wood and litter were less sensitive to these compounds than EM, and speculated that this may be due to greater production of extracellular phenol oxidases by the decomposer groups. Harley and Smith (1) suggest that the differential susceptibility to allelochemicals that occur among EM species may be related to production of phenol oxidases. Melin and Krupa (28) found inhibition of EM by several mono- and sesquiterpenes present in Scots pine (*Pinus sylvestris*) roots, although the two fungi they tested differed in their response to individual compounds.

Relatively little work has been done on allelopathic effects on VAM. Tobiessen and Werner (29) found reduced VAM formation in hardwood tree seedlings growing under pines, and spores of VAM fungi are absent from soil beneath living ponderosa pines, although they are abundant under dead trees (30). Members of the nonmycorrhizal family Cruciferae sometimes inhibit VAM formation in associated plants though this doesn't always happen (31-34).

Influence on Succession

Temperate forests are characterized by periodic catastrophic disturbance. Often this is due to fire, but various other agents such as wind and volcanic eruption also are responsible. Disturbance may influence the allelochemical environment in various ways. Living biomass is reduced, and fire reduces or eliminates litter and humus layers as well. Hence we should expect the total production of allelochemicals to be lowered following disturbance. As succession proceeds and total biomass and litter layers rebuild, allelochemicals should become an increasingly important factor in the ecosystem.

There are likely to be qualitative as well as quantitative changes in allelochemicals during succession. Incomplete combustion of organic matter produces various polynuclear aromatic hydrocarbons, many of which are mutagens, carcinogens, or protocarcinogens (35). Fire dramatically alters the microflora of forest soils, increasing the ratio of bacteria to fungi and altering the proportion of Streptomycetes spp. that inhibit root pathogens or mycorrhizal fungi (20). Concentrations of hydroxymate siderophores (HS), an important class of iron chelators, are reduced by disturbance, particularly fire (36). Because iron oxides play a role in the breakdown of phenolic compounds (H. H. Chang, personal communication), lower levels of HS may well influence allelochemical interactions. Finally the change in plant species that defines a successional sequence undoubtedly produces temporal differences in the nature of allelochemicals.

The diverse nature of mycorrhizal response to allelochemicals suggests that the changing biochemical environment during succession may drive a successional sequence of mycorrhizal fungi. It is well established that a sequence of mycorrhizal species occurs on a given tree as it matures (37, 38), and in one case this has been linked to the buildup of litter around trees (39). Sensitivity of mycorrhizal species to litter leachates correlates well with what we know about their ecological role. For example, Rose et al (23) found that growth of Pisolithus tinctorius in pure culture was inhibited by a wide range of litter types, while that of Cenococcum geophilum was stimulated. The former species is a rapid grower that greatly aids survival and growth of trees on highly disturbed sites such as mine spoils while the latter has characteristics, such as an ability to infect roots of plants grown under low light, that make it particularly suited for mature forest conditions. It is difficult to avoid concluding that P. tinctorius is an early successional fungus, adapted to the low allelochemical environment of recently disturbed sites, while C. geophilum is a late successional species that thrives, and perhaps even depends, on allelochemicals.

In our studies we find that, where more than one mycorrhizal fungus occurs in the system, disturbance invariably results in a shift in the proportion of types that are formed on tree seedlings (5, 19, 40). For example, in high-elevation forests growing on granitic soils in southern Oregon, two mycorrhizal types predominate on Douglas-fir seedlings, an unidentified species that we call "brown," and Rhizopogon vinicolor. Ninety-five percent of the mycorrhizae on seedlings grown in soils from mature forests are "brown". When seedlings are grown under the same conditions, but with soil from an adjacent clearcut in which logging slash had been burned (standard practice), the proportion changes to 22% "brown" and 68% R. vinicolor. Evidence suggests that this change is at least partially related to destruction of the upper soil layers, which contain a large proportion of organic matter. When Rose et. al. (23) added litter leachates to Douglas-fir seedlings grown in soil containing both R. vinicolor and "brown," colonization of roots by R. vinicolor was inhibited while "brown" was either unaffected or stimulated. Comparison of soils between mature forest and disturbed sites suggests at least one mechanism by which this change in fungus occurs. The former is characterized by a heavily organic surface layer 20 to 30 cm deep, overlying a sandy mineral zone. The boundary between the two is quite distinct, and is emphasized by a predominance of roots and hyphae in the upper zone. Bioassays show that "brown" is the predominant mycorrhizal fungus in this organic layer. The clearcut soil, although its total carbon content differs little from the forest, has no coherent surface organic layer; texturally its surface is very similar to the soil that is found at depth in the forest. R. vinicolor is its predominant mycorrhizal fungus (at least for Douglas-fir). Apparently, the buildup of the organic layer over time in these forests results in a partitioning of the two fungal species, the allelochemical-insensitive "brown" occupying the organic layers and the sensitive R. vinicolor the lower mineral layers. Logging and fire destroys the organic layer, leaving R. vinicolor in the mineral soil to serve as the primary fungus colonizing roots of invading tree seedlings. As litter and humus layers rebuild, "brown" becomes the dominant mycorrhizae-former, at least in the top 20 to 30 cm of soil.

Empirical evidence suggests that, in at least some cases, allelochemical-insensitive mycorrhizal fungi preferentially colonize late-successional trees. In pure culture experiments Cenococcum geophilum, which is stimulated by litter leachates, readily forms mycorrhizae both with early-successional Douglas-fir and late-successional western hemlock (41). However when these tree species are grown in soil from either disturbed areas or mature forest, C. geophilum forms a much higher proportion of the total mycorrhizae of western hemlock than of Douglas-fir (19). This raises the intriguing possibility that the shift in mycorrhizal fungi that occurs during succession facilitates change in higher plants.

Effects on Rooting Structure and Interactions Among Plants

Studies discussed earlier clearly show that allelopathic influences on mycorrhizae may alter the structure of communities by inhibiting one or more plant species. The diversity of response to allelochemicals among mycorrhizal species suggests that rooting pattern may also be influenced by the biochemical background of soils. Such structuring, rather than increasing the level of interference among individual plants, could produce the opposite effect, distributing roots throughout the soil volume and hence decreasing competition among individuals for water and nutrients. Such patterning would be strongest where plant species grown together tend to form different mycorrhizal types. For example, in a mixture of Douglas-fir and Western hemlock the former, with a relatively high percentage of allelochemical-sensitive mycorrhizae such as Rhizopogon vinicolor, would tend to root in lower soil layers, while hemlock, with its allelochemical-insensitive symbiont Cenococcum geophilum, would root in or close to surface organic layers. Because a single tree species may form several types of mycorrhizae that differ in sensitivity to allelochemicals, this mechanism of root dispersion and subsequent decrease in belowground competition could occur in monospecific stands as well.

In order to test these ideas, we are growing Douglas-fir and ponderosa pine (Pinus ponderosa) in a Replacement Series. In this standard design the total number of individuals in a pot remains constant (12 in our case) while the proportion of each species varies. Our experiment actually involves four Replacement Series, one in unpasteurized soil, one in pasteurized soil in which no mycorrhizae were added, one in pasteurized soil with a species-specific mycorrhiza synthesized on each tree species (Rhizopogon vinicolor on Douglas-fir and Rhizopogon oechorubens on ponderosa pine), and one with four mycorrhizae in the system, the two Rhizopogons plus the two generalists Laccaria laccata and Hebeloma crustiforme. Soils are from a mixed Douglas-fir - ponderosa pine forest in southwestern Oregon. A and B soil layers, plus litter, were lifted separately in the field and reconstructed in the pots to simulate natural conditions as closely as possible. Five replications are being grown in a greenhouse with pots rotated weekly so that all treatments cycle periodically through all bench locations.

This experiment is in progress; however initial results clearly show that interference between seedlings of the two tree species is altered by addition of mycorrhizae to the system. Six months after being transplanted into the same pots, height growth of ponderosa pine is suppressed by Douglas-fir in the absence of mycorrhizae (Figure 1a). When each tree has its own mycorrhizal symbiont, however, this trend is reversed, ponderosa pine increasing in height with increasing proportions of Douglas-fir in the mix (Figure 1b).

Growth of Douglas-fir is lower in unpasteurized than in pasteurized soil (Figure 1d); In previous experiments we have found that Douglas-fir growth may be either inhibited or stimulated by sterilization, depending on soil type and disturbance history, and that this may vary from one year to the next in a single soil (20, 42). Reinoculation of pasteurized soil indicates that this is not an artifact of pasteurization. In contrast to Douglas-fir, ponderosa pine, when grown only with other ponderosa pine, performs at least as well in unpasteurized as in pasteurized soil. However, the presence of Douglas-fir has a much more depressing effect on pine height growth in unpasteurized than in pasteurized soil. Reduced height growth of Douglas-fir may indicate greater root growth, and this could create more competition between the two tree species, however this is pure speculation--at this point we cannot explain why responses are so different between pasteurized and nonpasteurized soil.

Initial results of the experiment support the hypothesis that mycorrhizal diversity influences interactions between plants. Isotope labeling now in progress will tell us whether this is due to different rooting patterns, and future experiments will specifically address the role of allelochemical diversity on rooting pattern. Our results in nonpasteurized soils simply emphasize what every soil biologist knows -- patterns occurring in nature are complex and include interactions among numerous organisms. There is little question that the belowground dynamic

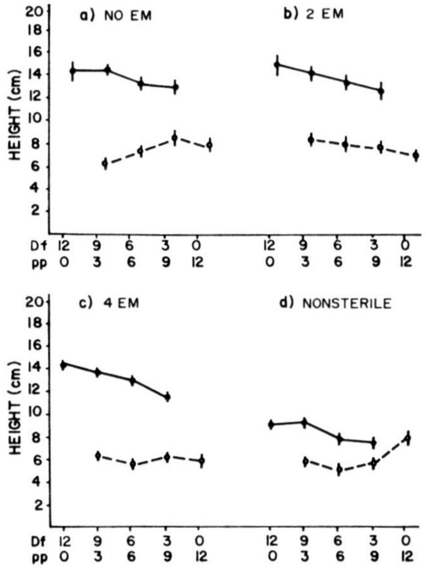

Figure 1. Height of Douglas-fir and ponderosa pine seedlings as a function of species mix and mycorrhizal infection.

Solid line = Douglas-fir
Dashed line = Ponderosa pine
Vertical bar = Standard error

a) Pasteurized soil with no added mycorrhizae
b) Pasteurized soil with two mycorrhizal species added
c) Pasteurized soil with four mycorrhizal species added
d) Unpasteurized soil

is strongly influenced by allelochemicals, although the task of unraveling mechanisms is likely to be a long one.

The Ecological Demon

The diversity of response to allelochemicals that exists within the community of mycorrhizal fungi has considerable implication for structure and functioning of terrestrial ecosystems. The repertoire of behaviors available to a given plant is greatly enhanced if it forms different mycorrhizal types, as is that of plant populations and communities forming a variety of mycorrhizae. The fungi translate information contained in allelochemicals into pattern within the ecosystem, and in this sense are reminiscent of James Clerk Maxwell's imaginary Demon, which could sort fast molecules from slow and thus impose structure on a gas. This allelochemically driven information processing ability suggests that mycorrhizae, like Maxwell's Demon, may play a far more important role in the ecosystem than indicated by their biomass or energy use.

Among the many questions remaining is the degree to which mycorrhizal fungi themselves influence the allelochemical background of a system. It is well known that mycorrhizae alter the chemistry of plant roots. Preliminary work in our laboratory shows that they influence leaf chemistry as well, producing a shift away from common monoterpenes toward unidentified compounds that may be di- or sesquiterpenes (5). The effect that such shifts may have on herbivory, decomposition and allelochemical interactions is not known. Mycorrhizae have been shown to retard litter decomposition (43), an effect that could alter the production of allelochemicals, particularly those that result from microbial activity. It seems almost certain that mycorrhizal fungi are active participants in, rather than passive prisoners of, the allelochemical dynamics of ecosystems.

Applications in Forestry and Agriculture

Because of their role as interfaces between plant roots and the soil environment, mycorrhizae have the potential to contribute significantly to the success or failure of agroecosystems. Selection of the proper fungal symbiont may reduce allelopathic effects of weeds or of one crop plant on another in mixed-species systems. Cultivation of mycorrhizal diversity and selection of specific host-fungal combinations could enhance exploitation of soil resources. Genetic selection for allelochemical-tolerant strains of mycorrhizal fungi is probably feasible. Before mycorrhizae can become a significant management tool, however, much research is needed on the basic biology and ecology of their interaction with allelochemics, and how this influences community development.

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Literature Cited

1. Harley, J. L.; Smith, S. E. "Mycorrhizal Symbiosis"; Academic Press; London, New York, 1983; p. 433.
2. Pirozynski, K. A. Can. J. Bot. 1981, 59, 1824-1827.
3. Trappe, J. M.; Fogel, R. D. In "The Belowground Ecosystem: A Synthesis of Plant"; Associated Processes: Range Sci. Dept., Sci. Ser. No. 26, Colorado State U., Fort Collins, 1977, p. 205-214.
4. Bowen, G. D. In "Tropical Mycorrhiza Research"; P. Mikola, Ed.; Oxford University Press: Oxford, 1980; pp. 165-190.
5. Perry, D. A. Proc. 6th North American Conference on Mycorrhizae, 1985, p. 104-106.
6. Read, D. J.; Francis, R.; Finlay, R. D.. In "Ecological Interactions in Soil"; Fitter, A. H., Ed.; Blackwell Sci. Pubs.: Oxford, 1985; p. 193.
7. Pacovsky, R. S.; Rabin, L. B.; Montllor, C. B.; Waise, Jr., A. C. Proc. 6th North American Conference on Mycorrhizae, 1985, p. 288.
8. Tisdall, J. M.; Oades, J. M. Australian J. Soil Res. 1979, 17:429-441.
9. Forster, S. M.; Nocolson, T. H. Soil Biol. Biochem. 1979, 13, 205-208.
10. Clough, K. S.; Sutton, J. C. Can. J. Microbiol. 1978, 24, 326-333.
11. Melin, E. Symb. Bat. Upsaliens. 1946, 8, 1-116.
12. Handley, W. R. C. "Mycorrhizal associations and Calluna heathland afforestation"; Bull. For. Commun. London, No. 36.
13. Persidsky, D. J.; Lowenstein, H.; Wilde, S. A. Agron. J. 1965, 57,311-312.
14. Hillis, W. E.; Ishikura, N.; Foster, R. C. Phytochemistry. 1968, 7, 409-410.
15. Olsen, R. A.; Odham, G.; Lindeerg, G. Physiol. Plant. 1971, 25, 122-129.
16. Robinson, R. K. J. Ecol. 1972, 60, 219-224.
17. Chu-Chou, M. Ann. Appl. Biol. 1978, 90, 407-416.
18. Alavarez, I. F.; Rowney, D. L.; Cobb, F. W., Jr.; Can. J. For. Res. 1979, 9, 311-315.
19. Schoeneberger, M. M.; Perry, D. A. Can. J. For. Res. 1982, 12, 343-353.
20. Perry, D. A.; Rose, S. L. In "Soil Biology and Forest Productivity: opportunities and constraints"; Ballard, R.; Gessel, S., Eds.; USDA Forest Service General Tech. Rep. PNW-163, Symposium on Forest Site and Continuous Productivity: Portland, Oregon, 1983, pp. 229-238.
21. Mikola, P. Communs, First. For. Fenn. 1948, 36, 1-104.
22. Tan, K. H.; Nopamornbodi, V. Soil Biol. Biochem. 1979, 11, 651-653.
23. Rose, S. L.; Perry, D. A.; Pilz, D.; Scheenberger, M. M. J. Chem. Ecology. 1983, 9, 1153-1162.
24. Brown, R. T.; Mikola, P. Acta Forest. Fenn. 1974, 141, 1-22.
25. Brown, R. T. In Proc. 6th North American Conference on Mycorrhizae; Molina, R., Ed.; Forest Res. Lab., Oregon State U., Corvallis, 1985, p. 277.

26. Harvey, A. E.; Larsen, M. J.; Jurgensen, M. F. For. Sci. 1979, 25, 350-358.
27. Parke, J. J.; Lindermann, R. G.; Trappe, J. M. Can. J. For. Res. 1983, 13, 666-671.
28. Melin, E.; Krupa, S. Physiol. Plant. 1971, 25, 337-340.
29. Tobiessen, P.; Werner, M. B. Ecology 1980, 61, 25-29.
30. Kovacic, D. A.; St. John, T. V.; Dyer, M. I. Ecology. 1984, 65, 1755-1759.
31. Hayman, D. S.; Johnson, A. M.; Ruddlesdin, I. Plant Soil. 1975, 43, 489-495.
32. Iqbal, S. H.; Qureshi, K. S. Biologia (Bratislava). 1976, 22, 287-298.
33. Ocampo, J. A.; Martin, J.; Hayman, D. S. New Phytol. 1980, 89, 27-35.
34. Ocampo, J. A.; Hayman, D. S. New Phytol. 1981, 87, 333-343.
35. Sullivan, T. J.; Mix, M. C. Bull. Environ. Contam. Toxicol. 1983, 31, 208-215.
36. Perry, D. A.; Rose, S. L.; Pilz, D.; Schoenberger, M. M. Soil Sci. Sco. Am. J. 1984, 48, 379-382.
37. Ford, E. D.; Mason, P. A.; Pelham, J. Trans. Brit. Mycol. Soc. 1980, 75, 287-296.
38. Mason, P. A.; Wilson, J.; Last, F. T.; Walker, C. Plant Soil. 1983, 71, 247-256.
39. Gardner, J. H.; Malajczwk, N. In Proc. 6th North American Conference on Mycorrhizae; R. Molina, Ed.; Forest Res. Lab., Oregon State U., Corvallis, 1985, p. 265.
40. Pilz, D. P.; Perry, D. A. Can. J. For. Res. 1984, 14, 94-100.
41. Molina, R.; Trappe, J. For. Sci. 1982, 28, 423-458.
42. Perry, D. A. Proc. 33rd Annual Western Forest Disease Work Conference., 1985, pp. 32-34.
43. Gadgil, R. L.; Gadgil, P. D. New Zealand J. Forest Sci. 1975, 5, 33-41.

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