Annu. Rev. Plant Physiol. Plant Mol. Biol. 1992. 43:557–81

REGULATION OF THE VESICULAR-ARBUSCULAR MYCORRHIZAL SYMBIOSIS

Roger T. Koide and R. Paul Schreiner

Department of Biology, The Pennsylvania State University, University Park, Pennsylvania 16802

KEY WORDS: vesicular-arbuscular mycorrhizal fungus, regulation of infection, signaling and recognition, phosphorus, carbohydrates, anti-fungal compounds, plant defense mechanisms

CONTENTS

INTRODUCTION

The vesicular-arbuscular mycorrhizal (VAM) symbiosis is exceptionally common among terrestrial flowering plants. Some researchers (e.g. 95) estimate that from 85–90% of the approximately 231,000 species of angiosperms (144) form this symbiosis despite there being only approximately 120 described species of VAM fungi (128). This indicates that the VAM fungi are somehow undeterred by the vast array of constitutively produced plant secondary

0066-4294/92/0601-0557$02.00

metabolites that may serve to prevent fungal infections. One might argue, then, that the biotrophic VAM fungi have been extremely successful in utilizing a diverse assemblage of host plants as sources of nutrition.

It is easy to see that fitness of the VAM fungi is improved by their association with plants. Most, if not all, of the reduced carbon used for VAM fungal growth and metabolism is derived from the host plant. Indeed, the relationship of the fungus with host plants is obligatory. The symbiosis may also increase nutrient (particularly phosphorus) uptake into the host plant and may thereby increase host fitness. "Benefit" to the host plant, however, is not necessarily an inevitable result of mycorrhizal infection (50, 51, 100, 101). Thus, while we generally regard the symbiosis as mutualistic, we can only be sure that the fitness of the fungus is consistently increased as a consequence of participation in the association.

We do not intend to review every aspect of the physiology of the mycorrhizal symbiosis here. Recent reviews on various aspects of the physiology of the mycorrhiza include those by Cooper (35), Gianinazzi-Pearson & Gianinazzi (59), Hayman (74), Smith & Gianinazzi-Pearson (139), and Koide (90). One of the focal points of this paper is the determination of compatibility between VAM fungi and their host plants. A second focal point concerns the mechanisms by which hosts plants regulate the extent of mycorrhizal infection.

COMPATIBILITY

The Role of Signaling and Recognition

In this contribution we refer to compatibility of VAM fungus and host plant as competence on the part of both symbionts to engage in a fully functioning symbiosis. We regard a fully functioning mycorrhiza as one in which the fungus penetrates the host root and forms arbuscules across which materials (phosphate and carbohydrates) may be exchanged, and in which propagation of the fungus is effected. Penetration and limited infection of a root by mycorrhizal fungi without arbuscule formation or subsequent sporulation does not constitute full participation in the symbiosis.

It seems likely that for the symbiosis to be established, molecular signaling events must occur that lead to various physiological and anatomical changes in both symbionts. There are likely several necessary steps leading to the formation of a functioning mycorrhiza, each of which may require the sending out of appropriate signals by one member of the symbiosis followed by their recognition by the other member. A failure either to signal properly or to recognize the signals would result in the failure to establish the symbiosis. These signals may be exchanged between fungus and host plant in the rhizosphere (135), at the point of attachment (139), or within the root itself

(6). Diffusible host signals in the rhizosphere may affect spore germination, germ tube extension rate, and the direction of germ tube extension. Signals at the root surface may regulate adhesion and penetration. Signals within the root may influence the formation of arbuscules, the rate of carbohydrate transfer to the fungus, the rate of fungal growth within the root, the likelihood for reinfection, and the production of vesicles and spores.

Roots produce a wide variety of water soluble and volatile organic compounds, some of which are directly available to organisms in the rhizosphere. These compounds may serve as attractants, nutrient sources, and even as genetic regulatory signals for VAM fungi prior to infection. Many of the compounds produced by roots also nourish a well-developed rhizosphere microflora (127), which may also release compounds that affect mycorrhizal fungi. Indeed, soil microbes may produce substances that stimulate the rate of VAM fungal spore germination (9, 37). Since roots maintain a relatively high concentration of microbes in the rhizosphere compared to the bulk soil (127), it is possible that stimulation of VAM fungi by roots in nature is due indirectly to the effects of one or more of these microorganisms.

The symbioses involving autotrophic and parasitic plants may be analogous, in some ways, to the VA mycorrhiza. For some parasitic plants, multiple signals may be involved in the establishment of the symbiosis. In the association between parasitic angiosperm *Striga* spp. and their hosts, distinct, species-specific phenolic compounds at very low concentrations are required for seed germination as well as haustorium formation, both of which are necessary for successful parasitism (97, 146). We do not intend to imply by analogy that a host factor is necessary for VAM fungal spore germination or germ tube extension. We note below that host factors may be stimulatory without being essential. The *Striga* system, however, may still serve as a useful model. The compound that induces germination of *Striga* seeds is exuded by the roots of the host. Since germination requires a relatively long exposure (hours) to the compound, and because the compound is chemically labile, the seeds of the parasite must be fairly close to the living host to germinate (97). The signal for haustorium production by *Striga asiatica,* on the other hand, cannot be detected in host exudates, suggesting that the molecule is tightly bound to roots or occurs inside the host root (146). Haustorium formation can therefore only occur after contact with roots.

Mycorrhizal fungi may also undergo morphological differentiation very near the host root, a phenomenon that suggests a role for labile signals. Hyphal branching, the production of fan-like structures (118) or "tufts" (63), may occur when germ tubes closely approach host roots. The need for labile factors may also partially explain the current inability to culture mycorrhizal fungi in the absence of living host roots. Tester et al (149) proposed that recognition at the cell wall or middle lamella may control subsequent mycor-

rhizal fungal penetration. This reminds us of the interior root signal that induces formation of the *Striga* haustorium. Others (115) have similarly proposed that "intrinsic" or internal factors control mycorrhizal infection.

There is some evidence that mycorrhizal fungal spores both before and immediately after germination are incapable of synthesizing DNA (29, 157). The host may supply signals that initiate this process and control other genetic events. Nodule formation by hosts of *Rhizobium* may serve as a useful model. Nodule formation is controlled by the *Rhizobium* genes *nod*ABC. Transcription of these genes is regulated by products of the regulatory gene *nod*D and a phenolic factor in root exudate, either a flavone or flavanone (97). Whether regulatory metabolites produced by roots or rhizosphere microbes also serve to control gene activity in the mycorrhizal fungi remains an intriguing but largely untested possibility. Recent indications that flavonoid compounds increase mycorrhizal fungal spore germination, hyphal growth, and the extent of mycorrhizal infection (58, 109, 135) hint at that possibility.

At low concentrations, phenolic compounds appear to be nearly universal signaling molecules. In addition to their use in communication between host plants and mutualistic bacteria such as *Rhizobium*, they are used for communication between host plants and parasitic bacteria such as *Agrobacterium* and parasitic angiosperms (42, 97). At high concentrations, however, many of these signal molecules are allelopathic or antimicrobial (phytoalexins). This suggests that many symbionts associated with autotrophic plants have come to utilize host defense compounds as recognition cues.

The difference between VA mycorrhizal fungi and parasitic angiosperms or *Rhizobium* is that the fungi exhibit little host specificity. If chemical cues are involved in regulating mycorrhizal infections such as for parasitic angiosperms, those regulating mycorrhizas must differ in some fundamental ways. Lack of specificity might occur if the specific signal molecules necessary at the various stages of infection were produced by all mycotrophic plant species (66, 139). Alternatively, a variety of signals may be capable of initiating the same signal transduction pathways leading to the formation of the symbiosis. To a limited extent, the broad host range of the pathogenic *Agrobacterium tumefaciens* may be due to the suitability of many related compounds as effective cues (97).

Successful parasitism is often associated with either resistance to host defenses or the failure to trigger the defense response (23, 39). It would appear that from the limited research available on peroxidase, chitinase, and phytoalexins that mycorrhizal fungi somehow do not trigger full host defense responses.

Phenolic defenses employed by plants against fungi appear to be common. Many phenolic compounds have antimicrobial properties and may either be constitutively produced or induced by specific bacteria or fungi (98, 145).

Phenolics may inactivate fungal enzymes and, if they are polymerized, may act as physical barriers to penetration (23, 141). Many of these phenolic defenses are dependent on peroxidase, which catalyzes the oxidation of important cell wall phenolic compounds. Spanu & Bonfante-Fasolo (141) found that mycorrhizal fungal inoculation of leeks resulted in a transiently higher level of peroxidase activity compared to uninoculated controls. These results suggested that the mycorrhizal fungus initiated a defense reaction. Later, however, peroxidase activities were actually lower in mycorrhizal plants, suggesting that a full defense response was circumvented.

The production of chitinase is a defense response commonly induced in plants by invading fungi (104, 166). Mycorrhizal infection of leek roots caused a transient increase in the level of chitinase activity followed by a depressed level of activity compared to uninoculated controls (140). This, again, suggested that mycorrhizal fungi initiated a host defense response that was later depressed.

Another common defense against invasion by pathogens is the production and rapid local accumulation of phytoalexins, compounds whose formation is induced by elicitor molecules from invading fungi or bacteria (147). In a study by Morandi et al (107), mycorrhizal infection of soybeans was associated with an increased concentration of isoflavonoid phytoalexins. The concentrations were still low in comparison to those induced by pathogens or abiotic elicitors so, again, it would appear that the mycorrhizal fungi failed to elicit the full defense response.

Thus, while infection by mycorrhizal fungi appears to initiate some plant defense responses, these defenses may not progress to their fullest expression in mycotrophic plant species. Recognition and signaling events may serve to truncate a full-blown defense, allowing the symbiosis to become established. The situation in some nonmycotrophic species, however, may be different. Recently, Allen et al (3) showed that inoculation with VAM fungi initiated a defense response in *Salsola kali* (Chenopodiaceae) that is typical of defenses against pathogenic fungi by many plant species. Portions of the roots around infection points turned brown and were autofluorescent, developments that suggest a role for phenolic defenses. This phenomenon may represent the lack of recognition of the VAM fungus by the plant.

Nonmycotrophic species, although relatively rare, may be useful exceptions to the mycotrophic "rule" and may shed significant light on the nature of the signaling processes used by mycotrophic species. Are such species nonmycotrophic because they fail to provide the correct sequence of chemical cues for the fungi leading to the symbiotic state, or do they fail to recognize signals coming from the fungi? Comparing mycotrophic with nonmycotrophic species could be instructive.

The species shown not to form fully developed arbuscular infections are

frequently confined to a few families either in the Caryophyllales (Phytolaccaceae, Nyctaginaceae, Chenopodiaceae, Amaranthaceae, Portulacaceae, Caryophyllaceae) or the Capparales (Brassicaceae, Capparaceae, Resedaceae; 57, 149). There are also some notably exceptional nonmycotrophic species from largely mycotrophic families, such as *Lupinus* spp. (7, 108, 155) in the Fabaceae (Leguminosae). One of the best pieces of evidence supporting the idea that mycorrhizal fungi can increase phosphorus uptake is that nonmycotrophic species appear to have evolved alternative means of increasing their ability to acquire phosphorus. These include a high degree of root hairiness (81, 86), production of proteoid roots and accompanying chelators and acids (70), rhizosphere acidification (71, 99), and high rhizosphere phosphatase activity (86). Mustards (Brassicaceae) may also reduce competition for phosphate with other plant species by producing allelopathic substances (31, 85). *Urtica dioica,* a nonmycotrophic member of the Urticaceae, apparently employs yet another strategy. It grows naturally only in high-phosphorus soils (117, 126).

Mycorrhizal fungal hyphae often grow on or around the roots of nonmycotrophic species without significant penetration (summarized in 149). Could this lack of penetration result from a lack of positive stimuli or the lack of sufficient nutrients? The presence of roots of mycotrophic species may, in some cases, increase the penetration and limited internal growth of VAM fungi in the roots of nonmycotrophic species. This increase occurs in members of the Chenopodiaceae (2, 105, 163), the Brassicaceae (summarized in 149), and in *Lupinus* (108, 155). This observation is consistent with the lack of some positive cues in these nonmycotrophic species, which can be supplied by nearby roots of mycotrophic species. An alternative explanation, however, is simply that when the fungus infects a host root, it can acquire a carbon source and thereby have more energy with which to attempt to infect the nonmycotroph.

It seems probable that different evolutionary scenarios have led to a range of mechanisms responsible for the nonmycotrophic status. Some of these mechanisms may, indeed, involve the failure to provide correct chemical cues leading to the symbiotic state. Another possible reason for nonmycotrophy in some species, however, may be the production of compounds that directly inhibit mycorrhizal fungi. The plant taxa utilizing anti-fungal compounds would obviously be poor research tools for the investigation of chemical signals necessary for full expression of the symbiosis. Use of such taxa may not allow one to detect positive signals because of the presence of active fungal inhibitors. For example, Glenn et al (63) found that when VAM fungal hyphae came into close proximity to *Brassica* roots, the number of germ tube branches and hyphal tufts was reduced compared to when they approached roots of tobacco or tomato. These data are consistent with the lack of a

stimulatory compound in mustards as they proposed, but they are also consistent with the presence of an inhibitor. A more tractable approach toward elucidating the signals and recognition events necessary for establishment of the VAM symbiosis would be to employ either mutants of mycotrophic plant species or mutant mycorrhizal fungi that are, for some reason, incapable of engaging in the symbiosis.

Since we cannot yet grow the fungi in pure culture, investigations into the plant signals involved in the establishment of the symbiosis will be greatly aided by the use of mutant plants. Recently, Duc et al (45) reported on some nod$^-$ mutant pea lines, 14 of which were incapable of forming mycorrhizas. It would appear that some components of the plant/*Rhizobium* and VAM symbioses are shared. Indeed, Wyss et al (165) found that antibodies against nodulins were cross reactive with mycorrhiza-specific "mycorrhizins." Gianinazzi-Pearson & Gianinazzi (60) suggested that mechanisms similar to those involved in signaling between hosts and *Rhizobium* bacteria may be involved in the early events of mycorrhiza formation. Mutants such as the nonmycotrophic pea lines promise to be useful in the determination of genetic and physiological events that initiate and maintain the mycorrhizal symbiosis.

Production of Anti-fungal Compounds by Nonmycotrophic Species

Research on the mechanisms responsible for the nonmycotrophic status of plants has often focused on members of the Brassicaceae because they produce mustard oils (isothiocyanates) that have potent insecticidal (94), antibiotic (103), allelopathic (31, 164), and fungicidal (44, 69, 80, 106, 161) activities. Isothiocyanates are enzyme-mediated (myrosinase, a thioglucosidase) hydrolysis products of sulfur-containing secondary metabolites called glucosinolates; they are often volatile but may also be nonvolatile (89, 106, 125). In addition to isothiocyanates, glucosinolates may yield a variety of other compounds when hydrolyzed, including nitriles, thiocyanates, and others depending on the structure of the glucosinolate and the conditions of hydrolysis (33).

Glucosinolates and myrosinase appear to coexist in a type of idioblast called a myrosin cell. In the root, these cells are apparently confined to the cortex (72, 79, 151). The water soluble glucosinolates appear to be compartmented in the vacuole while myrosinase appears to be associated with various membranes in the cytoplasm (72, 96, 151). This arrangement is referred to as the isothiocyanate "bomb" (96). Compartmentation is obviously necessary if production of toxic compounds upon hydrolysis is to be controlled.

A question remains about whether sufficient concentrations of isothiocyanates exist in the rhizosphere and on the rhizoplane to account for observed

inhibition of mycorrhizal fungi by intact roots. It is usually assumed that only mechanical disruption of myrosin cells due to herbivory, for example, will allow the enzyme and substrate to mix and release isothiocyanates and other potentially toxic compounds. The "bomb," however, may be a bit leaky. That is, a small level of hydrolysis may occur in the absence of tissue disruption. Constitutive production of volatile isothiocyanates actually serves as an attractant for some specialized isothiocyanate-resistant insects (73). Tang & Takenaka (148) and R. P. Schreiner & R. T. Koide (unpublished) have also shown that isothiocyanates are released into the rhizospheres of *Carica papaya* and *Brassica kaber,* respectively, even in the absence of root damage. Because isothiocyanates are rather nonpolar compounds, they may accumulate on the rhizoplane, particularly those that are nonvolatile. Volatile isothiocyanates would be expected to have farther-reaching effects but, being nonpolar, would still tend to accumulate in the rhizosphere. Thus, despite having a low rate of constitutive release, isothiocyanates may accumulate sufficiently to have significant biological activity (148). Mustard plants themselves appear to be less susceptible to the ill effects of toxic hydrolysis products than species outside the Brassicaceae (85). In several instances mustards have been shown to have no significant effect on infection of host plants growing in the same soil (19, 111, 114, 120; R. P. Schreiner & R. T. Koide, unpublished data). The production of nondiffusing or chemically unstable isothiocyanates is consistent with this observation. There is evidence that anti-fungal substances produced by mustards may be degraded by rhizosphere microorganisms or rendered ineffective by adsorption onto soil particles (113). In a few cases, however, the presence of mustards has been shown to inhibit infection in host species growing nearby (46, 75, 119), a finding consistent with the production and accumulation of significant concentrations of anti-fungal compounds.

Glenn et al (62) concluded that glucosinolates were not involved in the lack of infection of *Brassica* species by mycorrhizal fungi. They examined a number of *Brassica* cultivars differing in glucosinolate concentrations and also attempted to vary glucosinolate concentration by placing seedlings on agar with or without sulfur. Nearly all combinations of sulfur treatment and cultivar resulted in penetration of roots by fungi, but arbuscular infections never developed. Glucosinolate concentrations, however, were only examined in separate hydroponically grown 4–6 week old plants, not in the actual test seedlings. Moreover, even if grown on sulfur-deficient media, seedlings would probably not be glucosinolate deficient because adequate sulfur may be supplied by the cotyledons. Indeed, the ultrastructural work of Glenn et al (62) indicated that mycorrhizal fungal penetrations occurred only in dead cortical cells, those that would be incapable of producing isothiocyanates. In addition, hyphae growing near healthy cells had retracted cytoplasm,

consistent with the presence of inhibitory compounds. Moreover, it is not clear how the concentration of the nontoxic glucosinolates (80, 106, 159, 161) they measured is related to the concentration of the active isothiocyanates.

Tester et al (149) concluded that isothiocyanates were probably ineffective in preventing mycorrhizal infections because species producing them had been reported to be infected by VAM fungi and because of the belief that intact tissues do not release isothiocyanates. As indicated above, however, isothiocyanate release from intact tissues does occur. Furthermore, the mycorrhizal infections of isothiocyanate-producing species they reported, for example *Carica papaya,* were very slight, lacked arbuscules, and did not result in a host growth response even in poor soils (82, 121; but see 116, which indicates the presence of arbuscules in *Carica*).

The importance of isothiocyanates to the nonmycotrophic status of many species may be variable because different plant species produce different combinations of isothiocyanates and because variation in biological activity among the many isothiocyanates is great (44, 106, 161). It is also possible that some mycorrhizal fungal isolates have developed a limited capacity to detoxify some isothiocyanates, as have certain insects (156, 160). Certainly there does appear to be variation among mycorrhizal fungal species in their susceptibility to antifungal compounds (108). Isothiocyanate production is tissue specific (48), and it is not clear whether all isothiocyanate-producing species make substantial quantities in the roots. Moreover, the poorly developed infections or attempted infections in several *Brassica* species are consistent with an isothiocyanate-mediated resistance, since myrosin cells only make up a small proportion of the cells in a given tissue (72, 79, 151). VAM fungi could penetrate quite a few cortical cells before encountering a myrosin cell.

There does appear to be evidence for the production of inhibitory compounds by mustards that are effective in deterring mycorrhizal fungi. Extracts of radish and cabbage plants applied to alfalfa roots significantly reduced mycorrhizal infection and reduced germination of *Glomus mosseae* spores (113). The volatile fraction of cabbage root extracts alone was also shown to have inhibitory activity on *Glomus mosseae* spore germination (46, 158). Vierheilig & Ocampo (159) also demonstrated that reaction of the glucosinolate sinigrin with myrosinase produced a strong volatile inhibitor of VAM fungal spore germination. From these studies and others presenting consistent results (154), it is clear that some isothiocyanates are capable of inhibiting mycorrhizal fungi.

We indicate here that there is currently little reason to dismiss isothiocyanates as effective anti-mycorrhiza compounds. The use of mutant mustard plants incapable of producing isothiocyanates would help to determine with

greater surety whether such compounds are involved in the nonmycotrophic status of many mustards.

Anti-fungal compounds may also be employed by nonmycotrophic species from other plant families. Infections in the roots of host plants were abnormal in the presence of *Lupinus* roots, which caused unusual anatomical distortions of the hyphae (108). Further experiments showed that both *Lupinus* roots and seed coats contain soluble compounds that induce the fungal abnormalities and that the active substances were stable in the soil (108). Apparently the proportion of mycorrhizal fungal spores finally germinating was not influenced by these substances, although the rate of germination may have been (7). A recent report (60) also indicated that grafts of *Lupinus* shoots to mycotrophic pea roots completely inhibited arbuscule formation and reduced the development of hyphae within the pea roots. It was suggested that this was evidence of a shoot factor produced by *Lupinus* that inhibits mycorrhiza formation (60), but this factor's existence has yet to be demonstrated.

Urtica dioica L. (stinging nettle) is a nonmycotrophic member of the largely nonmycotrophic Urticaceae (149). *Urtica* produces a chitin-binding lectin (*Urtica dioica* agglutinin, UDA), a potent fungal inhibitor (24). Other plant lectins, such as wheat germ agglutinin and potato lectin, apparently have less antifungal activity (24). It is interesting that both wheat and potato are mycotrophic species. Whether UDA is sufficiently effective against mycorrhizal fungi to prevent infection is unknown.

Certain compounds produced by nonmycotrophic species may corrupt necessary positive cues or signals. For example, isothiocyanates have antimicrobial activity because they have a high affinity for amino acids and proteins, particularly their thiol, sulfide, and terminal amino groups (87, 88). If peptides, proteins, or other compounds with thiol, sulfide, or amino groups were involved in any of the signaling processes necessary to initiate and maintain mycorrhizal infections, the presence of isothiocyanates could prevent infection independent of any direct antifungal action.

In one report, soluble root extracts of spinach (Chenopodiaceae) were shown to inhibit VAM fungal spore germination slightly (158). In most cases, however, neither soluble root exudates nor root volatiles have been shown to have antifungal activity in the Chenopodiaceae (14, 129, 159). Volatiles from beet roots may even stimulate VAM hyphal extension (14) and may also be active chemotropically (56). Indeed, the simple lack of positive cues is more consistent with some members of the Chenopodiaceae that do not appear to express anti-fungal activity constitutively (R. P. Schreiner & R. T. Koide, unpublished). It may also be significant that the incidence of the symbiosis appears to be much greater in the Chenopodiaceae than in the Brassicaceae (78, 110).

It is difficult to make generalizations about the nature of compatibility

between mycorrhizal fungi and their hosts. We suspect that many mechanisms are employed by nonmycotrophic species that make them incapable of participating in the mycorrhizal symbiosis. One mechanism may involve the lack of positive cues (signaling). Another may involve the production of fungal inhibitors. Some nonmycotrophic species may employ both mechanisms. We stress that whatever the mechanism employed, the nonmycotrophic status need not be a consequence of direct selection against mycorrhizal fungi. For example, production of "anti-fungal" compounds such as isothiocyanates may have been a response to herbivory.

REGULATION OF INFECTION IN HOST PLANTS

If the fungus grew unchecked in its host, the "cost" of infection (in the transfer of reduced carbon, for example) could easily outweigh any "benefit" derived from an enhanced nutrient uptake. The net result could be a decrease in plant fitness. Depressions of plant performance caused by mycorrhizal infections may occur in some controlled experiments, particularly when soil phosphorus availabilities are high (reviewed in 138); but unequivocal cases of such depressions, to our knowledge, have not been demonstrated under realistic field conditions. Indeed, growth depressions of host plants by mycorrhizal fungi may actually be artifacts of inappropriate control inoculations (92). There is, instead, ample evidence that VAM fungal infections are regulated in a way that lessens the likelihood of reduction in the fitness of host plants.

Regulation of the symbiosis involves a combination of mechanisms that either limit or promote infection by the fungi, depending upon environmental conditions. Regulation of infection by host species may not be mediated by the same cues that are necessary to establish the fully functioning symbiosis, and we concern ourselves here with the means of regulating the extent of fully functioning mycorrhizal infections. From a phytocentric viewpoint, our premise is that hosts have developed mechanisms that optimize their fitness by adjusting the extent of mycorrhizal infection according to changes in the environment. This kind of adjustment appears to be common to many other mutualistic symbioses (136). However, it is clear that in the optimization of its fitness, the fungus may employ strategies that actually diminish host fitness. We examine both evidence of host-advantageous regulation of the fungus and evidence of fungus-advantageous regulation of the host.

Evidence that the host plant actively regulates infections in a way that optimizes its fitness comes from several types of studies in which the effect of the environment on infection was investigated. For example, if the host plant regulated infection so as to acquire phosphorus most efficiently for a given expenditure of carbon, we would predict that mycorrhizal infections might be

limited if photosynthetic carbon acquisition were limited by some factor other than phosphorus. Indeed, when photosynthesis is limited by light, the rate at which mycorrhizal infection is established or the number of spores produced are often reduced (18, 40, 41, 49, 54, 150).

Much of the research on host regulation of infection has focused on the role of plant phosphorus status. Many researchers have shown that the phosphorus status of the host plant is negatively correlated with the degree of mycorrhizal infection. Additions of phosphorus have been shown to reduce the rate of growth of internal hyphae or the rate of extension of infection fronts (1, 5, 152), the rate of growth of external hyphae, and the production of secondary infection points (1, 4, 5, 131, 152). Indeed, some have called the symbiosis "self regulatory" (74), because when phosphorus availability increases, the fungus may become less important to the phosphorus economy of the host and the potential for uncompensated carbon drain increases. Additions of nitrate may increase the extent of mycorrhizal infection (for example, 76) by reducing tissue phosphorus concentrations in a manner consistent with known "dilution" of phosphorus caused by growth.

Host plants may use many mechanisms to regulate mycorrhizal infections. First, chemical signals may be produced by the host to increase the rate of infection. Second, defensive chemicals or structures may be employed selectively to limit infection. Finally, the host may regulate, by one means or another, the rate at which carbohydrates are passed to the fungus.

Regulation by Chemical Stimuli

It stands to reason that VAM fungal spore germination, germ tube extension, and even the directionality of germ tube extension are sensitive to a number of compounds typically found in rhizospheres. Indeed, germination, hyphal extension, and direction of extension are sensitive to CO_2, other root volatiles, root exudates, and bacterial products (8, 13, 37, 56, 143). Any of these "signals" could indicate the presence of a potentially suitable root and might thereby function to increase the chances of initiating mycorrhizal infection in much the same way that host signals influence *Striga* seed germination. While none of these compounds appears to be absolutely necessary for spore germination or hyphal extension (10, 36, 118), a number of them may regulate the rate at which roots initially become infected.

Root exudates have been shown to increase VAM fungal spore germination (58, 65). As the germ tube approaches the root, elongation may also be improved by root exudates and other compounds associated with roots. For example, water soluble exudates from the roots of Troyer citrange, sudangrass, (65) and clover (47, 58) increased germ tube elongation of VAM fungi. Others have shown that soluble root exudates, either alone or in concert with root volatiles, increase germ tube elongation (12, 13, 14, 77).

Not all infections are initiated through spores (28), and infected root pieces containing viable hyphae and vesicles may also be infective. Work comparable to that performed on spores investigating the role of exudates on regrowth of hyphae from root pieces has not been carried out. There is some evidence that the effect of root exudates on germ tubes from spores is different from that on hyphae regrowing from root pieces (10).

It is becoming increasingly clear that exudate quality (in addition to its quantity) may be important in the regulation of mycorrhizal infection. Early attempts to correlate specific components of exudate with degree of infection, however, were unsuccessful. Variation in phosphorus amendment influenced total exudation but had little effect on the ratios of sugars, carboxylic acids, and amino acids in exudate (132). More recent research, however, suggests that flavonoid compounds exuded by roots may strongly stimulate VAM fungi (58, 109, 135), and variation in the quantity of these compounds due to variation in plant phosphorus status should be investigated (see 47). In many plants, phosphate deficiency symptoms include anthocyanin accumulation in the leaves and stems. Whether a similar phenomenon occurs in the roots and whether such compounds regulate mycorrhizal infection have yet to be determined.

The flavonoids apigenin, naringenin, and hesperitin, all of which may induce the genes *nod*ABC (123), also increased mycorrhizal fungal germ tube extension (58). In independent studies, other flavonoid compounds have been shown to have regulatory activity. Quercetin may stimulate germination and hyphal extension (G. Becard, personal communication), and the isoflavonoids formononetin and biochanin-A and the flavone chrysin may stimulate hyphal extension and infection of clover by *Glomus* species (109, 135). These compounds are effective at concentrations from 0.5–5 μg ml^{-1} so they are not likely to stimulate by serving as sources of carbon.

There is likely to be some variation among VAM fungal species and isolates in the degree to which they are stimulated by compounds in the rhizosphere. Demonstrations of limited host specificity in nature (61, 84, 102) could be explained by such phenomena.

In soil, volatile compounds may diffuse away from roots more rapidly and for greater distances than do soluble exudates. They may, therefore, be better suited for some kinds of host/fungus communication. Indeed, volatiles do have many regulatory roles for various components of the soil microflora (52, 53). There is now convincing evidence that volatiles produced by roots influence the rate and extent of growth of mycorrhizal hyphae as well as the direction of growth (12, 13, 14, 56, 143).

Carbon dioxide may be one root volatile with important regulatory activity. A volatile factor that stimulates hyphal elongation was produced by both mycotrophic (carrot) and nonmycotrophic (beet) species (13, 14). Use of

KOH traps eliminated the effects of the root volatiles (13). Although KOH may neutralize some organic volatiles, use of $KMnO_4$ (which oxidizes many organic compounds) did not have any effect, suggesting that CO_2 was the active agent. Further, additions of 0.5% CO_2 greatly stimulated hyphal extension when exudates from host roots were present (13). The positive effects of carbon dioxide on hyphal extension suggests that observed seasonal shifts in carbon dioxide concentration in soils (64) could modify the rate of establishment of infection.

Carbon dioxide is probably not the only volatile compound with significant effects on the VA mycorrhiza. Gemma & Koske (56) and St. John et al (146) showed that use of $KMnO_4$ traps eliminated any chemotropism of aerial germ tubes of *Gigaspora* to roots of mycotrophic species, and decreased infection, respectively. [It is interesting to note that in their study, Gemma & Koske (56) found that the nonmycotrophic kohlrabi in the Brassicaceae did not exhibit the same phenomenon. See the section on Production of Antifungal Compounds by Nonmycotrophic Species, above.] When Gemma & Koske (56) used KOH traps, chemotropic responses to roots of mycotrophic species were also eliminated. These data may indicate that while organic volatiles exert some control over phenomena such as hyphal extension and chemotropism, CO_2 may be necessary for their expression. Indeed, Becard & Piche (13) showed that stimulatory root exudates were only effective in the presence of CO_2, suggesting the need for CO_2 before other regulatory compounds are effective.

Regulation by Defensive Chemicals and Anatomies

We indicated, above, a possible role for defensive chemicals such as chitinase, phenolics (as indicated by peroxidase activity), and phytoalexins in regulating mycorrhizal infections. It is apparent, however, that in many cases mycorrhizal fungi either fail to elicit these defenses or are able to prevent their full deployment.

While chitinase could be employed to inhibit mycorrhizal fungi, host chitinase might also be necessary for the production of arbuscules. Arbuscules are surrounded by very thin, largely nonchitinous walls (22). We wonder whether host chitinase is necessary for this condition. It might be instructive to follow arbuscule frequency and host root chitinase activity. Transiently high levels of chitinase activity during the initial phases of infection (140) would appear to correspond to the period of maximum arbuscule formation.

In addition to the possible role of defensive chemicals, root anatomy may also play a role in determining the rate at which internal infections develop. Papilla formation around invading hyphae may be one method for preventing or slowing mycorrhizal infection (55). Brundrett & Kendrick (26, 27) have also shown that several other aspects of root anatomy are important. In

particular, air channels within roots allow rapid growth of internal hyphae and hence rapid movement of the infection front along the root (see also 28). Drew et al (43) indicated that phosphorus deficiency resulted in increased aerenchyma formation in maize. Whether this can influence the rate of internal root colonization by mycorrhizal hyphae is unknown, but its investigation would prove to be worthwhile. Suberization of cell walls, such as those of the exodermis, may prevent invasion into particular cells (21, 25) and could thus be employed to reduce infection. Whether plants can alter the degree of tissue suberization according to environmental variables such as light or phosphorus availability in order to control internal infections is unknown.

Regulation by Control of Carbohydrate Transfer

Ultimately, control over the activity of mycorrhizal fungi by the host plant could occur via regulation of carbohydrate transfer because the fungus relies entirely or nearly entirely on the host as its source of reduced carbon. This may happen in a number of different ways including: (*a*) alteration of carbohydrate allocation to the root system, (*b*) alteration of the rate of carbohydrate exudation from the roots, (*c*) control of arbuscule number, and (*d*) regulation of carbohydrate transfer at the arbuscule.

When phosphorus availability increases, the allocation of carbon to the root relative to the shoot often declines (20). In fact, in some cases the absolute allocation of carbon to the root system may actually decline. In other instances, however, locally high availabilities of phosphorus may cause a local proliferation of roots and mycorrhizal hyphae (142) which, of course, would require an increase in carbon allocated for root growth. How the carbon allocation strategy within root systems affects mycorrhizal fungal growth and infection has not yet received much attention. Such studies, however, could prove important in our understanding of how efficiently carbon is deployed belowground for the acquisition of resources there (91).

It has been well demonstrated in several studies that root membranes become leaky as phosphorus status declines. Ratnayake et al (122), Schwab et al (133), and Graham et al (67, 68) proposed that increased exudation due to decreased phosphorus status stimulated fungal activity, which led to enhanced infection. Because phosphorus status might influence the level of infection independent of its effects on root exudation rate, means of altering exudation independent of phosphorus status have been sought. Root exudation has been shown to be affected by soil temperature (68), host developmental state (83), and shading (153). In each case, regardless of host phosphorus status, the rate of exudation was correlated with the extent of mycorrhizal infection (percentage infection).

Most studies investigating the relationships among phosphorus status, membrane leakiness, and exudation have been performed on uninfected

events is promising. Although we cannot yet grow VA mycorrhizal fungi in pure culture and thus cannot produce uninfective mutant fungi, the use of nonmycotrophic mutant host lines (45) would allow one to elucidate specific host genetic lesions responsible for their nonmycotrophic status. When coupled with the use of transformed roots in pure two-member cultures (11), comparison of mutant and wild-type root systems would allow one to identify specific cues necessary for establishing the symbiosis. If, by analogy to other symbiotic systems, we assume there are multiple necessary signaling and recognition steps, the analysis of a variety of mutants with lesions at various steps will be most useful. Because flavonoid molecules would appear to function as signals in a number of symbioses, their role in the mycorrhizal symbiosis should be examined carefully. However, the rather nonspecific associations VAM fungi have with their hosts indicate that some aspects of the signaling process are different from those in the *Rhizobium* system, for example.

The possibility that the fungus controls host function independent of phosphorus physiology remains an intriguing yet largely unexplored area of research. Berta et al (16) showed that in leeks, mycorrhizal infection was associated with a lower degree of chromatin condensation and nuclear hypertrophy in root cortical cell nuclei. This observation suggested that fungal infection somehow regulated host nuclear DNA transcription. Nuclear hypertrophy is often associated with arbuscule formation (32), but the mechanism by which host DNA transcription is coordinated with arbuscule formation is unknown. Arbuscule formation is also associated with a large increase in the volume of host cytoplasm and an increase in the number of organelles, both of which decrease when arbuscules disintegrate (32). In addition, the host membrane surrounding the arbuscule may possess new enzymatic activities (60). The nature of the signaling and recognition events leading to these alterations is unknown but will prove to be an important and fascinating future research topic.

Adventitious roots were more numerous in mycorrhizal leeks than in uninoculated controls (15). Associated with this increased branching, and perhaps its cause, was a significant depression of the mitotic index in apical meristems of infected roots (see also 17). Although modification of branching patterns need not influence total root system length, it may influence the three-dimensional architecture of the roots within the soil. Whether mycorrhizal fungi function only as simple appendages to existing roots or whether they actually alter the architecture of the host root system in a significant way is a question that has much relevance to breeding programs designed to maximize the efficiency of nutrient capture.

Finally, the mechanism of carbohydrate and phosphate transfer at the arbuscular interface is largely unknown. Schwab et al (133) recently proposed

that phosphate translocation from fungus to host and carbohydrate movement from host to fungus are linked by a transport system similar to the Pi-translocator of the chloroplast membrane, where a phosphate is exchanged for a triose-phosphate. As Schwab et al (133) pointed out, such a system would explain why plants in extremely low-phosphorus soils exhibit low levels of colonization (5, 38, 93): There is too little Pi to drive the translocator for the fungus to obtain sufficient carbohydrate. Whether this or other mechanisms are involved and whether the fungi themselves are capable of altering carbohydrate transfer at the arbuscule as suggested above are questions left for future research.

ACKNOWLEDGMENTS

We acknowledge financial support from the National Science Foundation and the Andrew W. Mellon Foundation. Discussions with Drs. Robert H. Hamilton, Ian Sanders, and David Gustine were useful and are gratefully acknowledged.

Literature Cited

1. Abbott, L. K., Robson, A. D., De Boer, G. 1984. The effect of phosphorus on the formation of hyphae in soil by the vesicular-arbuscular mycorrhizal fungus, *Glomus fasciculatum*. *New Phytol.* 97:437–46
2. Allen, M. F. 1983. Formation of vesicular-arbuscular mycorrhizae in *Atriplex gardneri* (Chenopodiaceae): seasonal response in a cold desert. *Mycologia* 75(5):773–76
3. Allen, M. F., Allen, E. B., Friese, C. F. 1989. Responses of the nonmycotrophic plant *Salsola kali* to invasion by vesicular-arbuscular mycorrhizal fungi. *New Phytol.* 111:45–49
4. Amijee, F., Stribley, D. P., Tinker, P. B. 1986. The development of endomycorrhizal root systems. VI. The relationship between development of infection, and intensity of infection in young leek roots. *New Phytol.* 102:293–301
5. Amijee, F., Tinker, P. B., Stribley, D. P. 1989. The development of endomycorrhizal root systems. VII. A detailed study of effects of soil phosphorus on colonization. *New Phytol.* 111:435–46
6. Anderson, A. J. 1988. Mycorrhizae-host specificity and recognition. *Phytopathology* 78(3):375–78
7. Avio, L., Sbrana, C., Giovannetti, M. 1990. The response of different species of *Lupinus* to VAM endophytes. *Symbiosis* 9:321–23
8. Azcon, R., Ocampo, J. A. 1984. Effect of root exudation on VA mycorrhizal infection at early stages of plant growth. *Plant Soil* 82:133–38
9. Azcon-Aguilar, C., Diaz-Rodriguez, R. M., Barea, J. M. 1986. Effect of soil micro-organisms on spore germination and growth of the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae*. *Trans. Br. Mycol. Soc.* 86:337–40
10. Barea, J. M. 1986. Importance of hormones and root exudates in mycorrhizal phenomena. In *Physiological and Genetical Aspects of Mycorrhizae*, ed. V. Gianinazzi-Pearson, S. Gianinazzi, pp. 177–87. Paris: INRA. 832 pp.
11. Becard, G., Fortin, J. A. 1988. Early events of vesicular-arbuscular mycorrhiza formation on Ri T-DNA transformed roots. *New Phytol.* 108:211–18
12. Becard, G., Piche, Y. 1989. New aspects on the acquisition of biotrophic status by a vesicular-arbuscular mycorrhizal fungus, *Gigaspora margarita*. *New Phytol.* 112:77–83
13. Becard, G., Piche, Y. 1989. Fungal growth stimulation by CO_2 and root exudates in vesicular-arbuscular mycorrhizal symbiosis. *Appl. Environ. Microbiol.* 55(9):2320–25
14. Becard, G., Piche, Y. 1990. Physiological factors determining vesicular-arbuscular mycorrhizal formation in host and nonhost Ri T-DNA transformed roots. *Can. J. Bot.* 68:1260–64
15. Berta, G., Fusconi, A., Trotta, A.,

Scannerini, S. 1990. Morphogenetic modifications induced by the mycorrhizal fungus *Glomus* strain E3 in the root system of *Allium porrum* L. *New Phytol.* 114:207–15
16. Berta, G., Sgorbati, S., Soler, V., Fusconi, A., Trotta, A., et al. 1990. Variations in chromatin structure in host nuclei of a vesicular arbuscular mycorrhiza. *New Phytol.* 114:199–205
17. Berta, G., Tagliasacchi, A. M., Fusconi, A., Gerlero, D., Trotta, A., et al. 1991. The mitotic cycle in root apical meristems of *Allium porrum* L. is controlled by the endomycorrhizal fungus *Glomus* sp. strain E3. *Protoplasma* 161:12–16
18. Bethlenfalvay, G. J., Pacovsky, R. S. 1983. Light effects in mycorrhizal soybeans. *Plant Physiol.* 73:969–72
19. Black, R., Tinker, P. B. 1979. The development of endomycorrhizal root systems. II. Effect of agronomic factors and soil conditions on the development of vesicular-arbuscular mycorrhizal infection in barley and on the endophyte spore density. *New Phytol.* 83:401–13
20. Bloom, A. J., Chapin, F. S., Mooney, H. A. 1985. Resource limitation in plants—an economic analogy. *Annu. Rev. Ecol. Syst.* 16:363–92
21. Bonfante-Fasolo, P., Fontana, A. 1985. VAM fungi in *Ginkgo biloba* roots: their interactions at cellular level. *Symbiosis* 1:53–67
22. Bonfante-Fasolo, P., Grippiolo, R. 1982. Ultrastructural and cytochemical changes in the wall of a vesicular-arbuscular mycorrhizal fungus during symbiosis. *Can. J. Bot.* 60:2303–12
23. Brett, C., Waldron, K. 1990. *Physiology and Biochemistry of Plant Cell Walls,* pp. 137–54. London: Unwin Hyman. 194 pp.
24. Broekaert, W. F., Van Parijs, J., Leyns, F., Joos, H., Peumans, W. J. 1989. A chitin-binding lectin from stinging nettle rhizomes with antifungal properties. *Science* 245:1100–2
25. Brundrett, M., Kendrick, B. 1988. The mycorrhizal status, root anatomy, and phenology of plants in a sugar maple forest. *Can. J. Bot.* 66:1153–73
26. Brundrett, M., Kendrick, B. 1990. The roots and mycorrhizas of herbaceous woodland plants. I. Quantitative aspects of morphology. *New Phytol.* 114:457–68
27. Brundrett, M., Kendrick, B. 1990. The roots and mycorrhizas of herbaceous woodland plants. II. Structural aspects of morphology. *New Phytol.* 114:469–79
28. Brundrett, M., Piche, Y., Peterson, R. L. 1985. A developmental study of the early stages in vesicular-arbuscular mycorrhiza formation. *Can. J. Bot.* 63:184–94
29. Burggraaf, A. J. P., Beringer, J. E. 1989. Absence of nuclear DNA synthesis in vesicular-arbuscular mycorrhizal fungi during in vitro development. *New Phytol.* 111:25–33
30. Buwalda, J. G., Stribley, D. P., Tinker, P. B. 1984. The development of endomycorrhizal root systems. V. The detailed pattern of development of infection and the control of infection level by host in young leek plants. *New Phytol.* 96:411–27
31. Campbell, A. G. 1959. A germination inhibitor and root growth retarder in Chou Moellier (*Brassica oleracea* var.). *Nature* 183:1263–64
32. Carling, D. E., Brown, M. F. 1982. Anatomy and physiology of vesicular-arbuscular and nonmycorrhizal roots. *Phytopathology* 72(8):1108–14
33. Chew, F. S. 1988. Biological effects of glucosinolates. In *Biologically Active Natural Products: Potential Use in Agriculture,* ed. H. G. Cutler, pp. 155–81. Washington, DC: Am. Chem. Soc.
34. Deleted in proof
35. Cooper, K. M. 1984. Physiology of VA mycorrhizal associations. In *VA Mycorrhiza,* ed. C. L. Powell, D. J. Bagyaraj, pp. 155–88. Boca Raton, FL: CRC Press
36. Daniels, B. A., Trappe, J. M. 1980. Factors affecting spore germination of the vesicular-arbuscular mycorrhizal fungus, *Glomus epigaeus. Mycologia* 72:457–71
37. Daniels Hetrick, B. A. 1984. Ecology of VA mycorrhizal fungi. See Ref. 35, pp. 35–55
38. De Miranda, J. C. C., Harris, P. J., Wild, A. 1989. Effects of soil and plant phosphorus concentrations on vesicular-arbuscular mycorrhiza in sorghum plants. *New Phytol.* 112:405–10
39. Deverall, B. J. 1972. Phytoalexins. In *Phytochemical Ecology,* ed. J. B. Harborne, pp. 217–33. London/New York: Academic
40. Diederichs, C. 1983. Influence of light on the efficacy of vesicular-arbuscular mycorrhiza in tropical and subtropical plants. II. Effect of light intensity under growth chamber conditions. *Angew. Bot.* 57:45–53
41. Diederichs, C. 1983. Influence of light on the efficacy of vesicular-arbuscular

mycorrhiza in tropical and subtropical plants. III. Influence of daylength. *Angew. Bot.* 57:55–67
42. Dixon, R. A., Lamb, C. J. 1990. Molecular communication in interactions between plants and microbial pathogens. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 41:339–67
43. Drew, M. C., He, C., Morgan, P. W. 1989. Decreased ethylene biosynthesis, and induction of aerenchyma, by nitrogen or phosphorus starvation in adventitious roots of *Zea mays* L. *Plant Physiol.* 91:266–71
44. Drobnica, L., Zemanova, M., Nemec, P., Antos, K., Kristian, P., et al. 1967. Antifungal activity of isothiocyanates and related compounds. I. Naturally occurring isothiocyanates and their analogues. *Appl. Microbiol.* 15(4):701–9
45. Duc, G., Trouvelot, A., Gianinazzi-Pearson, V., Gianinazzi, S. 1989. First report of non-mycorrhizal plant mutants (myc-) obtained in pea (*Pisum sativum* L.) and fababean (*Vicia faba* L.). *Plant Sci.* 60:215–22
46. El-Atrach, F., Vierheilig, H., Ocampo, J. A. 1989. Influence of non-host plants on vesicular-arbuscular mycorrhizal infection of host plants and on spore germination. *Soil Biol. Biochem.* 21(1):161–63
47. Elias, K. S., Safir, G. R. 1987. Hyphal elongation of *Glomus fasciculatus* in response to root exudates. *Appl. Environ. Microbiol.* 53(8):1928–33
48. Elliott, M. C., Stowe, B. B. 1971. Distribution and variation of indole glucosinolates in woad (*Isatis tinctoria* L.). *Plant Physiol.* 48:498–503
49. Ferguson, J. J., Menge, J. A. 1982. The influence of light intensity and artificially extended photoperiod upon infection and sporulation of *Glomus fasciculatus* on sudan grass and on root exudation of sudan grass. *New Phytol.* 92:183–91
50. Fitter, A. H. 1985. Functioning of vesicular-arbuscular mycorrhizas under field conditions. *New Phytol.* 99:257–65
51. Fitter, A. H. 1986. Effect of benomyl on leaf phosphorus concentration in alpine grasslands: a test of mycorrhizal benefit. *New Phytol.* 103:767–76
52. French, R. C. 1985. The bioregulatory action of flavour compounds on fungal spores and other propagules. *Annu. Rev. Phytopathol.* 23:173–99
53. Fries, N. 1973. Effects of volatile organic compounds on the growth and development of fungi. *Trans. Br. Mycol. Soc.* 60(1):1–21
54. Furlan, V., Fortin, J. A. 1977. Effects of light intensity on the formation of vesicular-arbuscular endomycorrhizas on *Allium cepa* by *Gigaspora calospora*. *New Phytol.* 79:335–40
55. Garriock, M. L., Peterson, R. L., Ackerley, C. A. 1989. Early stages in colonization of *Allium porrum* (leek) roots by the vesicular-arbuscular mycorrhizal fungus, *Glomus versiforme*. *New Phytol.* 112:85–92
56. Gemma, J. N., Koske, R. E. 1988. Pre-infection interactions between roots and the mycorrhizal fungus *Gigaspora gigantea:* chemotropism of germ-tubes and root growth response. *Trans. Br. Mycol. Soc.* 91(1):123–32
57. Gerdemann, J. W. 1968. Vesicular-arbuscular mycorrhiza and plant growth. *Annu. Rev. Phytopathol.* 6:397–418
58. Gianinazzi-Pearson, V., Branzanti, B., Gianinazzi, S. 1989. In vitro enhancement of spore germination and early hyphal growth of a vesicular-arbuscular mycorrhizal fungus by host root exudates and plant flavonoids. *Symbiosis* 7:243–55
59. Gianinazzi-Pearson, V., Gianinazzi, S. 1983. The physiology of vesicular-arbuscular mycorrhizal roots. *Plant Soil* 71:197–209
60. Gianinazzi-Pearson, V., Gianinazzi, S. 1989. Cellular and genetical aspects of interactions between hosts and fungal symbionts in mycorrhizae. *Genome* 31: 336–41
61. Giovannetti, M., Hepper, C. M. 1985. Vesicular-arbuscular mycorrhizal infection in *Hedysarum coronarium* and *Onobrychis viciaefolia:* host-endophyte specificity. *Soil Biol. Biochem.* 17(6): 899–900
62. Glenn, M. G., Chew, F. S., Williams, P. H. 1985. Hyphal penetration of *Brassica* (Cruciferae) roots by a vesicular-arbuscular mycorrhizal fungus. *New Phytol.* 99:463–72
63. Glenn, M. G., Chew, F. S., Williams, P. H. 1988. Influence of glucosinolate content of *Brassica* (Cruciferae) roots on growth of vesicular-arbuscular mycorrhizal fungi. *New Phytol.* 110:217–25
64. Glinski, J., Stepniewski, W. 1985. *Soil Aeration and Its Role for Plants,* pp. 91–104. Boca Raton, FL: CRC Press
65. Graham, J. H. 1982. Effect of citrus root exudates on germination of chlamydospores of the vesicular-arbuscular mycorrhizal fungus, *Glomus epigaeum*. *Mycologia* 74(5):831–35
66. Graham, J. H. 1988. Interactions of mycorrhizal fungi with soilborne plant pathogens and other organisms: an in-

troduction. *Phytopathology* 78(3):365–66
67. Graham, J. H., Leonard, R. T., Menge, J. A. 1981. Membrane-mediated decrease in root exudation responsible for phosphorus inhibition of vesicular-arbuscular mycorrhiza formation. *Plant Physiol.* 68:548–52
68. Graham, J. H., Leonard, R. T., Menge, J. A. 1982. Interaction of light intensity and soil temperature with phosphorus inhibition of vesicular-arbuscular mycorrhiza formation. *New Phytol.* 91:683–90
69. Greenhalgh, J. R., Mitchell, N. D. 1976. The involvement of flavour volatiles in the resistance to downy mildew of wild and cultivated forms of *Brassica oleracea*. *New Phytol.* 77:391–98
70. Grierson, P. F., Attiwill, P. M. 1989. Chemical characteristics of the proteoid root mat of *Banksia integrifolia* L. *Aust. J. Bot.* 37:137–43
71. Grinsted, M. J., Hedley, M. J., White, R. E., Nye, P. H. 1982. Plant-induced changes in the rhizosphere of rape (*Brassica napus* var. Emerald) seedlings. I. pH Change and the increase in P concentration in the soil solution. *New Phytol.* 91:19–29
72. Grob, K., Matile, P. 1979. Vacuolar location of glucosinolates in horseradish root cells. *Plant Sci. Lett.* 14:327–35
73. Harborne, J. B. 1988. *Introduction to Ecological Biochemistry*. San Diego, CA: Academic. 356 pp. 3rd ed.
74. Hayman, D. S. 1983. The physiology of vesicular-arbuscular endomycorrhizal symbiosis. *Can. J. Bot.* 61:944–63
75. Hayman, D. S., Johnson, A. M., Ruddlesdin, I. 1975. The influence of phosphate and crop species on endogone spores and vesicular-arbuscular mycorrhiza under field conditions. *Plant Soil* 43:489–95
76. Hepper, C. M. 1983. The effect of nitrate and phosphate on the vesicular-arbuscular mycorrhizal infection of lettuce. *New Phytol.* 92:389–99
77. Hepper, C. M. 1984. Isolation and culture of VA mycorrhizal fungi. See Ref. 35, pp. 95–112
78. Hirrel, M. C., Mehravaran, H., Gerdemann, J. W. 1978. Vesicular-arbuscular mycorrhizae in the Chenopodiaceae and Cruciferae: Do they occur? *Can. J. Bot.* 56:2813–17
79. Hoglund, A. S., Lenman, M., Falk, A., Rask, L. 1991. Distribution of myrosinase in rapeseed tissues. *Plant Physiol.* 95:213–21
80. Holley, R. A., Jones, J. D. 1985. The role of myrosinase in the development of toxicity toward *Nematospora* in mustard seed. *Can. J. Bot.* 63:521–26
81. Itoh, S., Barber, S. A. 1983. A numerical solution of whole plant nutrient uptake for soil-root systems with root hairs. *Plant Soil* 70:403–13
82. Janos, D. P. 1980. Mycorrhizae influence tropical succession. *Biotropica* 12(Suppl.):56–64
83. Johnson, C. R., Graham, J. H., Leonard, R. T., Menge, J. A. 1982. Effect of flower bud development in chrysanthemum on vesicular-arbuscular mycorrhiza formation. *New Phytol.* 90:671–75
84. Johnson, N. C., Pfleger, F. L., Crookston, R. K., Simmons, S. R., Copeland, P. J. 1991. Vesicular-arbuscular mycorrhizas respond to corn and soybean cropping history. *New Phytol.* 117:657–63
85. Ju, H. Y., Bible, B. B., Chong, C. 1983. Influence of ionic thiocyanate on growth of cabbage, bean, and tobacco. *J. Chem. Ecol.* 9(8):1255–62
86. Jungk, A. 1987. Soil-root interactions in the rhizosphere affecting plant availability of phosphorus. *J. Plant Nutr.* 19(9–16):1197–1204
87. Kawakishi, S., Kaneko, T. 1985. Interaction of oxidized glutathione with allyl isothiocyanate. *Phytochemistry* 24(4):715–18
88. Kawakishi, S., Kaneko, T. 1987. Interaction of proteins with allyl isothiocyanate. *J. Agric. Food Chem.* 35:85–88
89. Kjaer, A. 1960. Naturally derived isothiocyanates and their parent glucosides. *Fortschr. Chem. Org. Naturst.* 18:122–76
90. Koide, R. T. 1991. Tansley Review No. 29. Nutrient supply, nutrient demand and plant response to mycorrhizal infection. *New Phytol.* 117:365–86
91. Koide, R., Elliott, G. 1989. Cost, benefit and efficiency of the vesicular-arbuscular mycorrhizal symbiosis. *Funct. Ecol.* 3(2):4–7
92. Koide, R. T., Li, M. 1989. Appropriate controls for vesicular-arbuscular mycorrhiza research. *New Phytol.* 111:35–44
93. Koide, R. T., Li, M. 1990. On host regulation of the vesicular-arbuscular mycorrhizal symbiosis. *New Phytol.* 114:59–74
94. Larson, P. O. 1981. Glucosinolates. In *The Biochemistry of Plants*, ed. P. K. Stumpf, E. E. Conn, 7:501–25. London: Academic
95. Law, R. 1985. Evolution in a mutualistic environment. In *The Biology of*

Mutualism, Ecology and Evolution, ed. D. H. Boucher, pp. 145–70. New York: Oxford Univ. Press
96. Luthy, B., Matile, P. 1984. The mustard oil bomb: rectified analysis of the subcellular organisation of the myrosinase system. *Biochem. Physiol. Pflanzen.* 179:5–12
97. Lynn, D. G., Chang, M. 1990. Phenolic signals in cohabitation: implications for plant development. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 41:497–526
98. Mansfield, J. W. 1983. Antimicrobial compounds. In *Biochemical Plant Pathology,* ed. J. A. Callow, pp. 237–65. New York: John Wiley & Sons
99. Marschner, H., Romheld, V., Cakmak, I. 1987. Root-induced changes of nutrient availability in the rhizosphere. *J. Plant Nutr.* 10(9–16):1175–84
100. McGonigle, T. P. 1988. A numerical analysis of published field trials with vesicular-arbuscular mycorrhizal fungi. *Funct. Ecol.* 2:473–78
101. McGonigle, T. P., Fitter, A. H. 1988. Growth and phosphorus inflows of *Trifolium repens* L. with a range of indigenous vesicular-arbuscular mycorrhizal infection levels under field conditions. *New Phytol.* 108:59–65
102. McGonigle, T. P., Fitter, A. H. 1990. Ecological specificity of vesicular-arbuscular mycorrhizal associations. *Mycol. Res.* 94(1):120–22
103. McKay, A. F., Garmaise, D. L., Gaudry, R., Baker, H. A., Paris, G. Y., et al. 1959. Bacteriostats. II. The chemical and bacteriostatic properties of isothiocyanates and their derivatives. *J. Am. Chem. Soc.* 81:4328–35
104. Metraux, J. P., Boller, T. 1986. Local and systemic induction of chitinase in cucumber plants in response to viral, bacterial and fungal infections. *Physiol. Plant Pathol.* 28:161–69
105. Miller, R. M., Moorman, T. B., Schmidt, S. K. 1983. Interspecific plant association effects on vesicular-arbuscular mycorrhiza occurrence in *Atriplex confertifolia. New Phytol.* 95:241–46
106. Mithen, R. F., Lewis, B. G., Fenwick, G. R. 1986. In vitro activity of glucosinolates and their products against *Leptosphaeria maculans. Trans. Br. Mycol. Soc.* 87(3):433–40
107. Morandi, D., Bailey, J. A., Gianinazzi-Pearson, V. 1984. Isoflavonoid accumulation in soybean roots infected with vesicular-arbuscular mycorrhizal fungi. *Physiol. Plant Pathol.* 24:357–64
108. Morley, C. D., Mosse, B. 1976. Abnormal vesicular-arbuscular mycorrhizal infections in white clover induced by lupin. *Trans. Br. Mycol. Soc.* 67(3): 510–13
109. Nair, M. G., Safir, G. R., Siqueira, J. O. 1991. Isolation and identification of vesicular-arbuscular mycorrhiza-stimulatory compounds from clover (*Trifolium repens*) roots. *Appl. Environ. Microbiol.* 57(2):434–39
110. Newman, E. I., Reddel, P. 1987. The distribution of mycorrhizas among families of vascular plants. *New Phytol.* 106:745–51
111. Ocampo, J. A. 1980. Effect of crop rotations involving host and non-host plants on vesicular-arbuscular mycorrhizal infection of host plants. *Plant Soil* 56:283–91
112. Ocampo, J. A., Azcon, R. 1985. Relationship between the concentration of sugars in the roots and VA mycorrhizal infection. *Plant Soil* 86:95–100
113. Ocampo, J. A., Cardona, F. L., El-Atrach, F. 1986. Effect of root extracts of non host plants on VA mycorrhizal infection and spore germination. In *Physiological and Genetical Aspects of Mycorrhizae,* ed. V. Gianinazzi-Pearson, S. Gianinazzi, pp. 721–24. Paris: INRA. 832 pp.
114. Ocampo, J. A., Hayman, D. S. 1981. Influence of plant interactions on vesicular-arbuscular mycorrhizal infections. II. Crop rotations and residual effects of non-host plants. *New Phytol.* 87:333–43
115. Ocampo, J. A., Martin, J., Hayman, D. S. 1980. Influence of plant interactions on vesicular-arbuscular mycorrhizal infections. I. Host and non-host plants grown together. *New Phytol.* 84:27–35
116. Peterson, R. L., Ashford, A. E., Allaway, W. G. 1985. Vesicular-arbuscular mycorrhizal associations of vascular plants on Heron Island, a great barrier reef coral cay. *Aust. J. Bot.* 33:669–76
117. Pigott, C. D. 1971. Analysis of the response of *Urtica dioica* to phosphate. *New Phytol.* 70:953–66
118. Powell, C. L. 1976. Development of mycorrhizal infections from endogone spores and infected root segments. *Trans. Br. Mycol. Soc.* 66(3):439–45
119. Powell, C. L. 1979. Spread of mycorrhizal fungi through soil. *New Zeal. J. Agric. Res.* 22:335–39
120. Powell, C. L. 1981. Inoculation of barley with efficient mycorrhizal fungi stimulates seed yield. *Plant Soil* 59:487–90
121. Ramirez, B. N., Mitchell, D. J., Schenck, N. C. 1975. Establishment and

growth effects of three vesicular-arbuscular mycorrhizal fungi on papaya. *Mycologia* 67:1039–41
122. Rao, A. S. 1990. Root flavonoids. *Bot. Rev.* 56(1):1–84
123. Ratnayake, M., Leonard, R. T., Menge, J. A. 1978. Root exudation in relation to supply of phosphorus and its possible relevance to mycorrhizal formation. *New Phytol.* 81:543–52
124. Read, D. J., Koucheki, H. K., Hodgson, J. 1976. Vesicular-arbuscular mycorrhiza in natural vegetation systems. I. The occurrence of infection. *New Phytol.* 77:641–53
125. Robinson, T. 1980. *The Organic Constituents of Higher Plants: Their Chemistry and Interrelationships*, pp. 318–39. North Amherst, MA: Cordus Press
126. Rorison, I. H. 1968. The response to phosphorus of some ecologically distinct plant species. I. Growth rates and phosphorus absorption. *New Phytol.* 67:913–23
127. Rovira, A. D., Bowen, G. D., Foster, R. C. 1983. The significance of rhizosphere microflora and mycorrhizas in plant nutrition. In *Inorganic Plant Nutrition*, ed. A. Lauchli, R. L. Bieleski, pp. 61–93. Berlin: Springer-Verlag
128. Schenck, N. C., Perez, Y. 1987. *Manual for the Identification of VA Fungi*. Gainesville, FL: INVAM
129. Schmidt, S. K., Reeves, F. B. 1984. Effect of the non-mycorrhizal pioneer plant *Salsola kali* L. (Chenopodiaceae) on vesicular-arbuscular mycorrhizal (VAM) fungi. *Am. J. Bot.* 71(8):1035–39
130. Deleted in proof
131. Schwab, S. M., Menge, J. A., Leonard, R. T. 1983. Comparison of stages of vesicular-arbuscular mycorrhiza formation in sudangrass grown at two levels of phosphorus nutrition. *Am. J. Bot.* 70(8):1225–32
132. Schwab, S. M., Menge, J. A., Leonard, R. T. 1983. Quantitative and qualitative effects of phosphorus on extracts and exudates of sudangrass roots in relation to vesicular-arbuscular mycorrhiza formation. *Plant Physiol.* 73:761–65
133. Schwab, S. M., Menge, J. A., Tinker, P. B. 1991. Regulation of nutrient transfer between host and fungus in vesicular-arbuscular mycorrhizas. *New Phytol.* 117:387–98
134. Secilia, J., Bagyaraj, D. J. 1988. Fungi associated with pot cultures of vesicular-arbuscular mycorrhizas. *Trans. Br. Mycol. Soc.* 90(1):117–19
135. Siqueira, J. O., Safir, G. R., Nair, M. G. 1991. Stimulation of vesicular-arbuscular mycorrhiza formation and growth of white clover by flavonoid compounds. *New Phytol.* 118:87–93
136. Smith, D. C. 1987. Regulation and change in symbiosis. *Ann. Bot.* 60(Suppl. 4):115–27
137. Smith, D. C., Douglas, A. E. 1987. *The Biology of Symbiosis*, pp. 32–63. London: Edward Arnold. 302 pp.
138. Smith, S. E. 1980. Mycorrhizas of autotrophic higher plants. *Biol. Rev.* 55:475–510
139. Smith, S. E., Gianinazzi-Pearson, V. 1988. Physiological interactions between symbionts in vesicular-arbuscular mycorrhizal plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 39:221–44
140. Spanu, P., Boller, T., Ludwig, A., Wiemken, A., Faccio, A., et al. 1989. Chitinase in roots of mycorrhizal Allium porrum: regulation and localization. *Planta* 177:447–55
141. Spanu, P., Bonfante-Fasolo, P. 1988. Cell-wall-bound peroxidase activity in roots of mycorrhizal *Allium porrum*. *New Phytol.* 109:119–24
142. Stebbins, G. L. 1974. *Flowering Plants; Evolution Above the Species Level*. Cambridge, MA: Harvard Univ. Press
143. Stewart, A., Mansfield, J. W. 1985. The composition of wall alterations and appositions and their role in the resistance of onion bulb scale epidermis to colonization by *Botrytis allii*. *Plant Pathol.* 34:25–37
144. Stewart, G. R., Press, M. C. 1990. The physiology and biochemistry of parasitic angiosperms. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 41:127–51
145. St. John, T. V., Coleman, D. C., Reid, C. P. P. 1983. Growth and spatial distribution of nutrient-absorbing organs: selective exploitation of soil heterogeneity. *Plant Soil* 71:487–93
146. St. John, T. V., Hays, R. I., Reid, C. P. P. 1983. Influence of a volatile compound on formation of vesicular-arbuscular mycorrhizas. *Trans. Br. Mycol. Soc.* 81(1):153–54
147. Stone, B. A. 1989. Cell walls in plant-microorganism associations. *Aust. J. Plant Physiol.* 16:5–17
148. Tang, C. S., Takenaka, T. 1983. Quantitation of a bioactive metabolite in undisturbed rhizosphere-benzyl isothiocyanate from *Carica papaya* L. *J. Chem. Ecol.* 9(8):1247–53
149. Tester, M., Smith, S. E., Smith, F. A. 1987. The phenomenon of "nonmycorrhizal" plants. *Can. J. Bot.* 65:419–31
150. Tester, M., Smith, S. E., Smith, F. A.,

Walker, N. A. 1986. Effects of photon irradiance on the growth of shoots and roots, on the rate of initiation of mycorrhizal infection and on the growth of infection units in *Trifolium subterraneum* L. *New Phytol.* 103:375–90
151. Thangstad, O. P., Iversen, T. H., Slupphaug, G., Bones, A. 1990. Immunocytochemical localization of myrosinase in *Brassica napus*. L. *Planta* 180:245–48
152. Thomson, B. D., Robson, A. D., Abbott, L. K. 1986. Effects of phosphorus on the formation of mycorrhizas by *Gigaspora calospora* and *Glomus fasciculatum* in relation to root carbohydrates. *New Phytol.* 103:751–65
153. Thomson, B. D., Robson, A. D., Abbott, L. K. 1990. Mycorrhizas formed by *Gigaspora calospora* and *Glomus fasciculatum* on subterranean clover in relation to soluble carbohydrate concentrations in roots. *New Phytol.* 114:217–25
154. Tommerup, I. C. 1984. Development of infection by a vesicular-arbuscular mycorrhizal fungus in *Brassica napus* L. and *Trifolium subterraneum* L. *New Phytol.* 98:487–95
155. Trinick, M. J. 1977. Vesicular-arbuscular infection and soil phosphorus utilization in *Lupinus* spp. *New Phytol.* 78:297–304
156. Van Emden, H. F. 1972. Aphids as phytochemists. In *Phytochemical Ecology*, ed. J. B. Harborne, pp. 25–43. London/New York: Academic
157. Viera, A., Glenn, M. G. 1990. DNA content of vesicular-arbuscular mycorrhizal fungal spores. *Mycologia* 82(2): 263–67
158. Vierheilig, H., Ocampo, J. A. 1990. Role of root extract and volatile substances of non-host plants on vesicular-arbuscular mycorrhizal spore germination. *Symbiosis* 9:199–202
159. Vierheilig, H., Ocampo, J. A. 1990. Effect of isothiocyanates on germination of spores of *G. mosseae*. *Soil Biol. Biochem.* 22(8):1161–62
160. Wadleigh, R. W., Yu, S. J. 1988. Detoxification of isothiocyanate allelochemicals by glutathione transferase in three *Lepidopterous species*. *J. Chem. Ecol.* 14(4):1279–88
161. Walker, J. C., Morell, S., Foster, H. H. 1937. Toxicity of mustard oils and related sulfur compounds to certain fungi. *Am. J. Bot.* 24:536–41
162. Williams, P. G. 1985. Orchidaceous rhizoctonias in pot cultures of vesicular-arbuscular mycorrhizal fungi. *Can. J. Bot.* 63:1329–33
163. Williams, S. E., Wollum, A. G., Aldon, E. F. 1974. Growth of *Atriplex canescens* (Pursh) Nutt. improved by formation of vesicular-arbuscular mycorrhizae. *Soil Sci. Soc. Am. Proc.* 38:962–65
164. Wolf, R. B., Spencer, G. F., Kwolek, W. F. 1984. Inhibition of velvetleaf (*Abutilon theophrasti*) germination and growth by benzyl isothiocyanate, a natural toxicant. *Weed Sci.* 32:612–15
165. Wyss, P., Mellor, R. B., Weimken, A. 1990. Vesicular-arbuscular mycorrhizas of wild-type soybean and nonnodulating mutants with *Glomus mosseae* contain symbiosis-specific polypeptides (mycorrhizins), immunologically cross-reactive with nodulins. *Planta* 182:22–26
166. Young, D. H., Pegg, G. F. 1981. Purification and characterization of B-1,3-glucan hydrolases from healthy and *Verticillium albo-atrum* infected tomato plants. *Physiol. Plant Pathol.* 19:391–417