

LETTERS TO NATURE

to extrapolate to a composition of primary Hawaiian tholeiitic melt. □

Received 18 April; accepted 30 July 1991.

1. Maaloe, S. *Lithos* **12**, 59-72 (1979).
2. Wright, T. L. *J. geophys. Res.* **89**, 3233-3252 (1984).
3. Clague, D. A., Holcomb, R. T., Torresan, M. & Ross, S. *US Geol. Surv. Open-file Report* 89-109, (1988).
4. Moore, J. G. *Am. J. Sci.* **263**, 40-52 (1965).
5. Garcia, M. O., Muenow, D., Aggrey, K. & O'Neil, J. *J. geophys. Res.* **94**, 10525-10538 (1989).
6. Dixon, J. E., Clague, D. A. & Stolper, E. M. *J. Geol.* **99**, 371-394 (1991).
7. Clague, D. A., Moore, J. G., Dixon, J. E. & Friesen, W. B. *J. Petrol.* (in the press).
8. Wright, T. L. *US Geol. Surv. Prof. Paper* **735** (1971).
9. Helz, R. T., & Thornber, C. R. *Bull. Volcanol.* **49**, 651-668 (1987).
10. Helz, R. T. in *Volcanism in Hawaii US Geol. Surv. Prof. Paper* 1350 eds Decker, R. W., Wright, T. L. & Stauffer, P. H. 691-722 (1987).
11. Hofmann, A. W., Feigenson, M. D. & Raczek, I. *Contrib. Mineral. Petrol.* **88**, 24-35 (1984).
12. Wilkinson, J. F. G. & Hensel, H. D. *Contrib. Mineral. Petrol.* **98**, 326-345 (1988).
13. Nicholls, J. & Stout, M. Z. *J. Petrol.* **29**, 1031-1057 (1988).
14. Schwindinger, K. R. & Anderson, A. T. Jr *Contrib. Mineral. Petrol.* **103**, 187-198 (1989).
15. Moore, J. G., Clague, D. A., Ludwig, K. R. & Mark, R. K. *J. Volcan. geotherm. Res.* **42**, 273-284 (1990).
16. Hart, S. R. & Davis, K. E. *Earth planet. Sci. Lett.* **40**, 203-219 (1978).
17. Mysis, B. O. & Kushi, I. *Earth planet. Sci. Lett.* **42**, 383-388 (1979).
18. Shaw, H. R. *Am. J. Sci.* **272**, 870-893 (1972).
19. Bottinga, Y., Weill, D. & Richet, P. *Geochim. cosmochim. Acta* **46**, 909-919 (1982).

Herbivore-driven mycorrhizal mutualism in insect-susceptible pinyon pine

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THE mutualistic ectomycorrhizal fungi associated with the roots of woody perennials can enhance nutrient uptake and provide protection from pathogens in exchange for up to 40% of the photosynthate produced by host plants¹⁻⁹. By removing photosynthetic tissue, herbivores could reduce the amount of photosynthate available for maintaining this mutualism^{10-15,29}. Here we examine how ectomycorrhizal levels vary between trees resistant and susceptible to an insect herbivore, and demonstrate how mycorrhizal levels respond to the experimental removal of a native herbivore under natural conditions. We find that pinyon pine trees susceptible to chronic insect attack have 33% fewer ectomycorrhizae than resistant trees, demonstrating that the herbivore-mycorrhizae-host plant interaction differs between resistant and susceptible trees. We removed insects from susceptible trees and find that the mycorrhizal levels of these trees increased to a level comparable to that of resistant trees. This demonstrates that herbivores negatively affect the mutualism between ectomycorrhizal fungi and susceptible trees, and that mycorrhizal levels can rebound after herbivore removal. The dynamics of these interactions on resistant and susceptible plants could be important for understanding plant-pest interactions in natural and managed systems.

To determine whether the amount of herbivore attack was associated with the amount of mycorrhizal colonization, we examined the mycorrhizae of mature, 150-year-old pinyon pine (*Pinus edulis*) that were resistant to herbivory ($\bar{x} \pm 1$ s.e. annual shoot mortality from 1982 to 1990 = 8.6% \pm 0.74, $n = 15$), and susceptible to herbivory ($\bar{x} \pm$ s.e. = 25.6% \pm 1.60, $n = 15$) by a stem- and cone-boring moth, *Dioryctria albobitella*¹⁶. The larvae of this moth feed in pinyon pine stems where their chronic attack causes reduced tree growth, loss of female reproductive function, and an abnormal shrub-like architecture¹⁶. Resistant and susceptible trees grow adjacent to one another in intermixed stands and differ genetically¹⁷.

The mycorrhizal levels of resistant and susceptible trees were estimated by visually determining the percentage of short roots that were actively mycorrhizal¹⁸ on a total of 80 cm of root (representing 160-200 short roots) excavated from two locations

and two sampling times for each tree. These estimates were verified by examining 10% of the roots microscopically for the presence of characteristic ectomycorrhizal structures, the Hartig net and fungal mantle¹⁹. Sampled trees grew 10-20 m apart and the complete excavation of the root systems of 10 trees demonstrated that pinyon roots from different trees rarely intermingled.

Resistant trees had significantly higher levels of mycorrhizal colonization than susceptible trees in both samples ($F_{1,28} = 13.08$, $P = 0.001$) (Fig. 1). The lower mycorrhizal levels of susceptible trees could be explained in three ways. First, although

TABLE 1 Levels of soil nutrients, moisture, and pH for resistant and susceptible trees

	NO ₃ * N	NH ₄ * N	PO ₄	% H ₂ O	pH
Resistant	6.74 \pm 2.43	4.57 \pm 0.98	4.94 \pm 0.93	4.48 \pm 0.30	7.00 \pm 0.05
Susceptible	5.73 \pm 1.23	4.61 \pm 1.06	5.54 \pm 0.99	4.31 \pm 0.34	7.00 \pm 0.09
t-statistic	0.203	0.220	-0.443	-0.394	-0.045
P value	0.841	0.983	0.663	0.697	0.964

Values are expressed as means \pm 1 s.e. The units for soil nutrient data are μ g nutrient per g soil. All data were analysed using a Student's *t*-test with 18 degrees of freedom²⁷. $n = 10$ per group.

resistant and susceptible trees are intermixed in a site, microsite differences in soil nutrients and/or moisture could affect mycorrhizae^{1,5}. But we found no differences between resistant and susceptible trees in soil moisture, nitrate, ammonium, and phosphate, and soil pH (Table 1). Second, high herbivore loads could drive the mutualism by suppressing mycorrhizae on susceptible trees, or third, high mycorrhizal levels could directly or indirectly enhance resistance^{15,20} such that higher mycorrhizal densities result in greater resistance to herbivory.

To help distinguish between these two hypotheses, we examined the mycorrhizal levels of susceptible trees from which the moth had been removed for a minimum of 4 years using an annual application of the systemic insecticide Cygon, and compared them with the mycorrhizal levels of resistant and susceptible control trees. Because of the difficulty of spraying large trees, a second set of mature trees, 2-4 m tall and 60 years old, were used for this experiment. Cygon reduced moth herbivory on insecticide-treated trees (1990 $\bar{x} \pm 1$ s.e. shoot mortality for susceptible treated trees = 1.3% \pm 0.38; susceptible control trees = 16.4% \pm 1.82; resistant trees = 2.3% \pm 0.69, $F_{2,42} = 57.15$, $P < 0.0001$, $n = 15$ per group). Soil microarthropod (for example mites, collembola) densities did not differ between resistant, susceptible, and susceptible treated trees demonstrating that Cygon had no effect on fungivores that might feed directly on mycorrhizae²¹⁻²⁴ ($\bar{x} \pm$ s.e. soil arthropods per litre of soil for susceptible-treated trees = 87 \pm 6.3; susceptible control trees = 78 \pm 4.5; resistant trees = 87 \pm 15.4, Kruskal-Wallis test statistic = 0.909, $P = 0.635$, $n = 10$ trees per group).

Susceptible trees with herbivores removed had mycorrhizal levels significantly higher than susceptible trees with herbivores, but not significantly different from resistant trees in either April or August samples ($F_{2,42} = 25.31$, $P < 0.0001$) (Fig. 2). This experiment demonstrates that herbivory suppresses mycorrhizae in susceptible trees and that herbivores can negatively affect this important mutualism. These data also show that the mycorrhizal differences observed between older resistant and susceptible trees also occur in the same groups of younger trees, indicating a general pattern independent of age.

Because herbivores both consume plant tissue and negatively affect an important plant-fungal mutualism, the cumulative impact of herbivory on susceptible plants is far greater than just their immediate feeding. As a result of herbivore feeding, for example, 150-year-old susceptible trees lose an average of 26% of their annual shoot production and 33% of their mycorrhizae

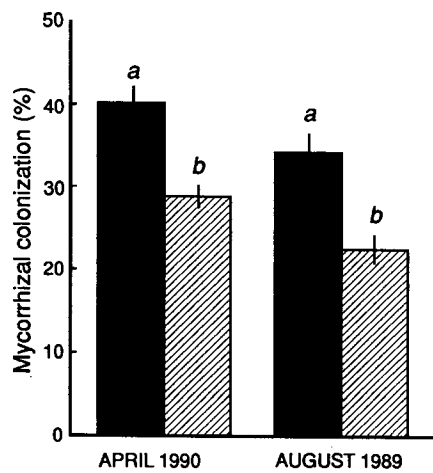


FIG. 1 Mycorrhizal levels of moth-resistant (solid bars) and moth-susceptible 150-year-old trees (hatched bars) in August 1989 and April 1990. Bars are means ($n=15$ per group) ± 1 s.e. Bars with different letters (a, b) are significantly different from one another at $P < 0.05$. Data were analysed using a one-way analysis of variance with repeated measures²⁸.

relative to resistant trees. The cumulative loss of both leaf tissue and mycorrhizae may make plants more susceptible to pathogens and other pests.

Although we have shown that herbivores mediate the mycorrhizal mutualism in susceptible trees, we do not know what drives the herbivore-plant-fungal interaction in resistant trees. The genetic differences between resistant and susceptible trees may not only lead to differential responses to herbivory through plant defenses, but also to entirely different mycorrhizal dynamics. Resistant trees, for example, could provide sufficient photosynthate to maintain mycorrhizae when they experience moderate herbivory. The improved plant nutrition resulting from mycorrhizae may then help prevent resistant plants from experiencing further insect attack. Because host-plant genetics are known to affect ectomycorrhizal formation in other pine species²⁵, the potential for mediation of plant resistance to insect attack by mycorrhizal fungi should be considered.

Our research on the relationship between amount of herbivory

and degree of mycorrhizal colonization in pinyon pine has two general implications. First, we have shown that herbivores reduce the mycorrhizal levels of both young and old susceptible plants by an average of 33% (range 27–42%) relative to resistant trees. Because the benefits plants receive from mycorrhizae are often proportional to mycorrhizal abundance^{9,25,26}, even small herbivore-induced mycorrhizal reductions could have significant impacts on plants, particularly those growing in stressful environments. Second, we have demonstrated that the herbivore-mycorrhizae-host plant interaction differs between resistant and susceptible trees. Because this interaction differs on insect-resistant and insect-susceptible trees, and because mycorrhizae are essential for plant growth in stressful environments⁹, future studies should integrate this mutualism into the body of theory dealing with plant-herbivore interactions. □

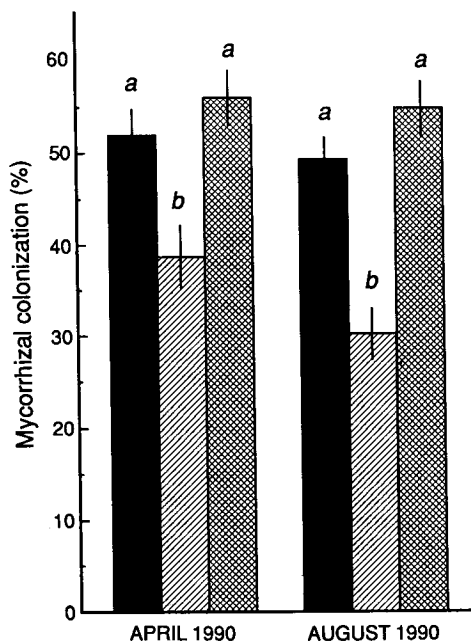


FIG. 2 Mycorrhizal levels of moth-resistant (solid bars), moth-susceptible (hatched bars), and susceptible moth-removal 60-year-old trees (cross-hatched bars) in April and August 1990. Bars are means ($n=15$ per group) ± 1 s.e. Bars with different letters (a, b) are significantly different from one another at $P < 0.05$. Data were analysed using a one-way analysis of variance with repeated measures followed by *a priori* comparisons to determine significant differences between treatments²⁸.

Received 29 April; accepted 30 August 1991.

- Meyer, F. H. in *Ectomycorrhizae: Their Ecology and Physiology* (eds Marks, G. C. & Kozlowski, T. T.) 79–105 (Academic, London, 1973).
- Marx, D. H. in *Ectomycorrhizae: Their Ecology and Physiology* (eds Marks, G. C. & Kozlowski, T. T.) 351–381 (Academic, London, 1973).
- Bowen, G. D. in *Ectomycorrhizae: Their Ecology and Physiology* (eds Marks, G. C. & Kozlowski, T. T.) 151–205 (Academic, London, 1973).
- Hacskaýlo, E. in *Ectomycorrhizae: Their Ecology and Physiology* (eds Marks, G. C. & Kozlowski, T. T.) 207–230 (Academic, London, 1973).
- Reid, C. P. P. in *The Rhizosphere* (ed. Lynch, J. M.) 281–315 (Wiley, New York, 1990).
- Scott, G. D. *Plant Symbiosis* (St. Martins, New York, 1969).
- Dehne, H. W. *Phytopathology* **70**, 293–296 (1982).
- Fogel, R. & Hunt, G. *Can. J. For. Res.* **9**, 245–256 (1979).
- Harley, J. L. & Smith, S. E. *Mycorrhizal Symbiosis* (Academic, London, 1983).
- Bethlenfalvay, G. J. & Dakessian, S. *J. Range Mgmt* **37**, 312–316 (1984).
- Bethlenfalvay, G. J., Evans, R. A. & Lesperance, A. L. *Agron. J.* **77**, 233–236 (1985).
- Borowicz, V. A. & Fitter, A. H. *Oecologia* **82**, 402–407 (1990).
- Daft, M. J. & El-Ghahmi, A. A. *New Phytol.* **80**, 365–372 (1978).
- Trent, J. D., Wallace, L. L., Svejcar, T. J. & Christiansen, S. *Can. J. Plant Sci.* **68**, 115–120 (1988).
- Jones, C. G. & Last, F. T. in *Microbial Mediation of Plant-Herbivore Interactions* (eds Barbosa, P., Krischik, V. A. & Jones, C. G.) 65–104 (Wiley, New York, 1991).
- Whitham, T. G. & Mopper, S. *Science* **228**, 1089–1091 (1985).
- Mopper, S., Mitton, J. B., Whitham, T. G., Cobb, N. S. & Christensen, K. M. *Evolution* **45**, 989–999 (1991).
- Harvey, A. E., Larsen, M. J. & Jurgensen, M. F. *For. Sci.* **22**, 393–398 (1976).
- Wilcox, H. E. in *Methods and Principles of Mycorrhizal Research* (ed. Schenck, N. C.) 103–114 (American Phytopathological Society, St. Paul, 1982).
- Rabin, L. B. & Pacovsky, R. S. *J. Econ. Ent.* **78**, 1358–1363 (1985).
- Findlay, R. in *Ecological Interactions in Soil: Plants, Microbes and Animals* (eds Fitter, A. H., Atkinson, D., Read, D. J. & Usher, M. B.) 319–331 (Blackwell Scientific, Palo Alto, 1985).
- McGonigle, T. P. & Fitter, A. H. in *Mycorrhizae in the Next Decade: Practical Applications and Research Priorities* (eds Sylvia, D. M., Hung, L. L. & Graham, J. H.) 209 (Institute of Food and Agricultural Studies, University of Florida, Gainesville, 1987).
- McGonigle, T. P. & Fitter, A. H. *Proc. R. Soc. Edinb.* **B94**, 25–32 (1988).
- Rabatin, S. C. & Stinner, B. R. in *Microbial Mediation of Plant-Herbivore Interactions* (eds Barbosa, P., Krischik, V. A. & Jones, C. G.) 141–168 (Wiley, New York, 1991).
- Cline, H. L. & Reid, C. P. P. *For. Sci.* **28**, 237–250 (1982).
- Last, F. T., Wilson, J. & Mason, P. A. *Agric. Ecosyst. Environ.* **28**, 293–298 (1990).
- Zar, J. H. *Biostatistical Analysis* (Prentice-Hall, Englewood Cliffs, 1984).
- Snedecor, G. W. & Cochran, W. G. *Statistical Methods* (Iowa State University Press, Ames, 1980).
- Last, F. T., Peilham, J., Mason, P. A. & Ingleby, R. *Nature* **280**, 168–169 (1979).

ACKNOWLEDGEMENTS. We thank the US Forest Service for their cooperation in using public lands and J. States and C. Grier for their expertise with mycorrhizae and soils, M. Caouette, T. DelVecchio, S. Mopper and T. Theimer for assistance in the field and K. Christensen, N. Cobb, K. Floate, C. Grier, P. Keim, S. Shuster, J. States and T. Theimer for critically reviewing the manuscript. Financial support was provided by the NSF, USDA, the ARCS Foundation, and Sigma Xi.