

# An overwintering cover crop increases inoculum of VAM fungi in agricultural soil

L. Galvez, D.D. Douds, Jr., P. Wagoner, L.R. Longnecker, L.E. Drinkwater, and R.R. Janke

**Abstract.** *We conducted a field experiment within a low-input reduced tillage trial to determine how a cover crop affects inoculum levels of vesicular-arbuscular mycorrhizal (VAM) fungi. Plots with and without the hairy vetch cover crop were established on September 30, 1993, under moldboard plow (MP), chisel-disk (CD), and no-till (NT) treatments in low-input (LI) management, and MP in conventional (CONV) management. We conducted a 3-week colonization assay in the greenhouse with bahiagrass seedlings to assess the relative colonization potential of the soils in the fall and following spring. Hairy vetch roots were colonized by indigenous VAM fungi by 65 days after planting, with plants from NT being more colonized than plants from MP or CD plots. Spore populations were greater in the LI than in the CONV system. The beneficial effect of the cover crop on VAM spore populations in soil was manifested in the spring, with the *Glomus* type group more abundant in plots with cover than without it. The greenhouse bioassay showed that colonization potential of spring 1994 soil samples was higher in plots with cover than without cover for both the LI and CONV systems. Just one season of an overwintering cover crop of hairy vetch increased the inoculum of VAM fungi the following spring before the next cash crop was planted.*

**Key words:** cover crop, hairy vetch, sustainable agriculture, crop rotations, crop diversity

## Introduction

Vesicular-arbuscular mycorrhizal (VAM) fungi are ubiquitous soil fungi that form a mutualistic symbiosis with the roots of most crop plants. Greenhouse experiments long have shown increased growth of mycorrhizal plants compared with nonmycorrhizal controls (Harley, 1989). The fungal hyphae outside the root act as extensions of the root

system to aid in the uptake of immobile mineral nutrients such as P (Rhodes and Gerdemann, 1975). The fungi receive carbon compounds in return and are believed to be obligately dependent upon this source to produce new spores and hyphae and colonize more roots. Other research suggests VAM fungi also enhance their host's water relations (Davies et al., 1992), N uptake (Frey and Schuepp, 1993) and disease resistance (Schenck, 1987). The demonstrated benefits of VAM fungi mean that they should be crucial for adequate plant growth and economically acceptable yields when no chemical fertilizers and pesticides are used.

Compared with conventional practices, larger and more diverse populations of VAM fungi occur in low-input (LI) agricultural systems that do not use chemical inputs but that use diverse crop rotations and conservation tillage (Limonard and Ruisen, 1989; Sattelmacher et al., 1991; Douds et al., 1993). Various cultural practices contribute to this observation. Tillage af-

fects both the distribution and efficacy of VAM fungi (An et al., 1990; McGonigle et al., 1990; Douds et al., 1995). Disruption of hyphae in the soil can decrease colonization of roots and mycorrhizae-mediated P uptake (McGonigle and Miller, 1993). Nonmycorrhizal plants in a crop rotation can decrease populations of VAM fungi (Harinikumar and Bagyaraj, 1988). Diversity of plant species in a crop rotation enhances VAM fungal diversity (Rabatin and Stinner, 1989). Greenhouse and field experiments have shown that chemical fertilizers, notably P, suppress colonization by VAM fungi and therefore their populations (Douds and Schenck, 1990; Kurle et al., 1991). Long-term following suppresses populations of VAM fungi to the point of inhibiting the growth of the subsequent crop (Thompson, 1987).

Another factor that distinguishes LI from conventional agriculture is its use of overwintering cover crops to compete with weeds, retard soil erosion, supply fixed N and retain soil N. Overwintering cover crops potentially are very beneficial for VAM fungi. Since VAM fungi are obligate symbionts, warm, moist soil is not conducive to keeping them viable without plant hosts (Nemec, 1987; Douds and Schenck, 1991). Respiration rates of hyphae would increase and spores might be stimulated to germinate in warm soil, depleting their metabolic reserves. These conditions are possible in the autumn following crop senescence and in spring before sowing. Cover crops also are a reason that soils in LI farming systems are covered with living plants a significantly longer portion of the year than in conventional systems. This was suspected to be largely responsible for the higher populations of VAM fungi measured in LI than in conventionally managed soils (Douds, et al., 1993).

Mention of a brand or firm name does not constitute an endorsement by the USDA over others not mentioned.

L. Galvez is a postdoctoral research associate and D.D. Douds, Jr. is a microbiologist, U.S. Department of Agriculture, Agriculture Research Service, Eastern Regional Research Center, 600 E. Mermaid Lane, Philadelphia, PA 19118; P. Wagoner is an agronomist, L.R. Longnecker is a technician, L.E. Drinkwater is Agronomy Coordinator, and R.R. Janke was Center Director, Rodale Institute Research Center, 611 Siegfriedale Road, Kutztown, PA 19530. R.R. Janke currently is Associate Professor, Department of Agronomy, Kansas State University, Manhattan, KS 66506-5501.

The objective of this study was to compare the VAM fungus colonization potentials of LI and conventionally farmed soils, each with and without an overwintering cover crop of hairy vetch (*Vicia villosa* Roth.). The cover crop would be an available host plant during periods of normally bare soil in conventional agriculture and should yield higher inoculum levels than plots without overwintering plant cover.

## Materials and Methods

Experiments were conducted within the low-input reduced tillage trial at the Rodale Institute Research Center, Kutztown, Pennsylvania (Wagoner et al., 1993). Conventional (CONV) and LI wheat (*Triticum aestivum* L.)-corn (*Zea mays* L.)-soybean (*Glycine max* [Merr.] L.) rotations were established in 1988. Each farming system contains tillage treatments ranging from no-till to moldboard plowing. The CONV system relies on chemical fertilizers and pest control. Nutrient management and weed control in the LI system are achieved by cover crops grown between cash crops. Part of the experiment was used for this study. The dominant soil type was a Berks shaley silt loam (Typic Dystrochrept) with 160–200 kg/ha available P (Bray I).

Winter wheat was harvested from 12×30 m plots in July 1993 in fields under CONV and LI management. Hairy vetch was mechanically sown (260 seeds/m<sup>2</sup>, row spacing 17.5 cm) on August 24 in moldboard plowed (MP), chisel disked (CD), and no-tilled (NT) plots as part of the LI rotation. Subplots (1-m<sup>2</sup>) with and without cover were established by hand weeding on September 30, 1993, to learn if a lack of cover would depress inoculum levels. Four subplots per cover treatment were established in each of four blocks to yield 16 subplots per tillage × cover treatment combination in the LI system. Plots without cover were maintained by reweeding on October 5, 18, and 28, November 16, and April 20. In addition, on Oct. 1, 1993, hairy vetch was hand-sown into eight hand-hoed 1-m<sup>2</sup> subplots in a conventionally managed plot in the same field that had been moldboard plowed before the previous crop of winter wheat was sown. We also established eight subplots without cover by hand-hoeing, and maintained them as above.

We collected soil samples from the center of all subplots on October 5, 1993. The top 9 cm of soil was sampled using an 8-cm bucket auger. We isolated spores of VAM fungi by wet sieving (Gerdemann and Nicolson, 1963) and centrifugation (Jenkins, 1964), and characterized and quantified them. We counted only healthy-looking spores, that is, those whose contents were multi-vacuolate. The remaining soil then was mixed 1:1 [v/v] with vermiculite and potted into 165-cm<sup>3</sup> conical plastic pots ("Super cell C-10", Steuwe and Sons, Canby, Oregon). Bahiagrass (*Paspalum notatum* Flugge) seedlings were transplanted into the pots (one pot per subplot) and grown in a greenhouse under natural photoperiods and a temperature range of 10–30°C for three weeks. Entire root systems were cleared and stained for VAM fungi (Phillips and Hayman, 1970). We quantified colonization using the gridline intersect method (Newman, 1966). Hairy vetch roots were collected from the field on October 28, 1993, and assayed for VAM fungus colonization as above. We estimated ground cover for all plots at this time. On May 5, 1994, we collected soil samples and roots of hairy vetch, and assayed colonization, spore counts, and inoculum potential, as above. Data were also collected on ground cover, soil moisture (gravimetric after drying at 60°C for 48 h), and soil temperature.

We analyzed the data with ANOVA after transformation with arcsin (for percentage root length colonized) or SQRT(X+1) (for spore count). Characteristics for which significant treatment effects were seen were characterized further using Tukey's method of multiple comparisons ( $\alpha=0.05$ ).

## Results

### VAM fungus colonization of hairy vetch

Hairy vetch roots were well colonized by VAM fungi 65 days after planting (Fig. 1). Plants in the LI/NT treatment were significantly ( $p<0.005$ ) more colonized than plants in the LI/MP and LI/CD treatments in the fall sample. Plants in the conventionally managed soils had the lowest colonization. Colonization levels for plants in the LI treatments in the spring sample were lower than those from the fall sample because of increased root growth, but maintained their

relative rankings among the three different tillage regimes. In spring, colonization of hairy vetch roots in CONV management was as high as in the LI/MP and LI/CD treatments.

### Plant cover, soil temperature and moisture

The percentage of ground covered by living plants (hairy vetch and weeds) estimated at the end of October showed how tillage affected plant establishment. The NT, CD, and MP plots had 100, 83 and 69% plant cover, respectively. Plots with a cover crop in CONV averaged only 3.5% because of their later planting date. All LI plots with hairy vetch showed full cover in May, while plots with a cover crop in CONV ranged between 10 and 60% cover (average of 39%). LI plots without a cover crop had between 0 and 20% cover (average of 3%) in the spring. Most of this was Canada thistle (*Cirsium* spp.) or dandelion (*Taraxacum officinale*). CONV plots without a cover crop remained free of plants.

Soil temperature was significantly ( $p < 0.001$ ) greater in subplots without cover on May 5, 1994 (Fig. 2). No significant differences in soil moisture were found between covered and bare soil (24.3 and 23.9%, respectively).

### VAM fungal spore populations

Spores isolated from soil samples were classified as *Glomus* spp. and placed in four groups: *Glomus occultum* Walker and *G. occultum*-like spores (LOCT); *Glomus etunicatum* Becker and Gerdemann and *G. etunicatum*-like spores (LETC); *Glomus fasciculatum* Thaxter and *G. fasciculatum*-like spores (LFSC); and other *Glomus* spp. as described by Douds et al. (1995). No spores were found that could definitively be placed in other genera.

Spore populations in the autumn 1993 samples reflected only tillage and farming system treatments because cover treatments were imposed only one week before collection of soil samples. Thus the results were pooled across cover treatment. Overall, populations of all spore type groups were lower in CONV than LI (Table 1). Among LI treatments, the LOCT and LFSC groups were more abundant in NT, while LETC and *Glomus*-type groups were more abundant in the CD treatment.

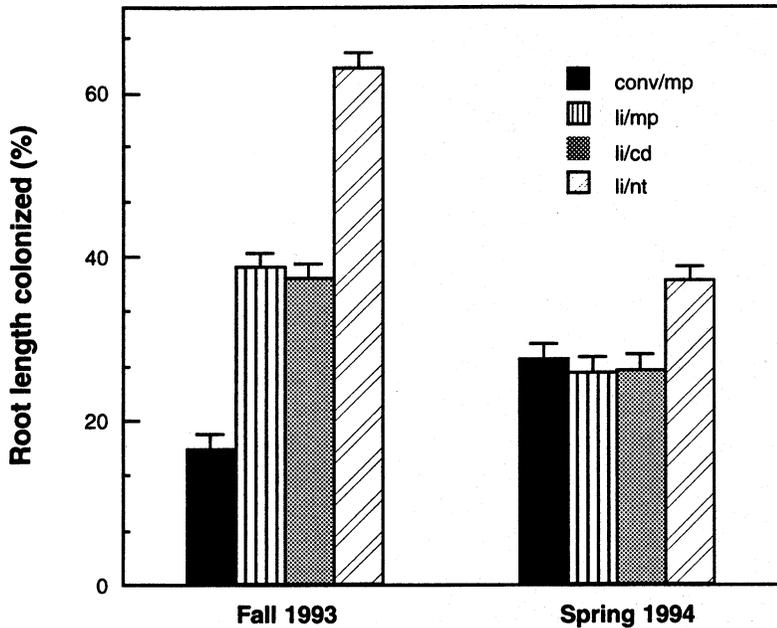


Figure 1. Colonization of roots of hairy vetch by VAM fungi. Roots were collected from the field on October 28, 1993 and May 5, 1994. Boxes represent means of 16 observations + SEM (LI/MP, LI/CD, LI/NT) or 8 observations + SEM (CONV/MP).

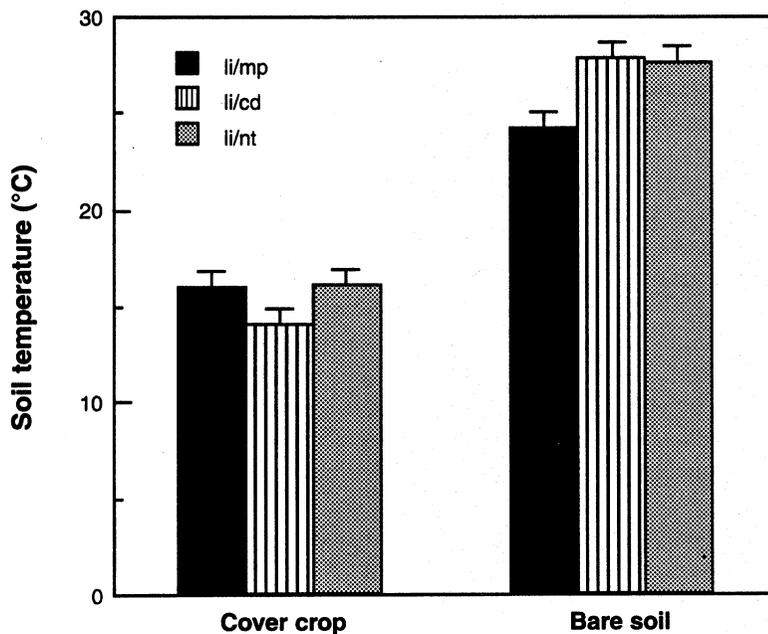


Figure 2. Soil temperature on May 5, 1994 in subplots with and without a cover crop of hairy vetch. Bars represent means of 16 observations + SEM.

Populations of all spore types in the spring 1994 samples were significantly affected by tillage regime in the LI system ( $p = 0.0001$  to  $0.023$ ) (Table 2). Cover treatment had a significant effect only in the *Glomus*-type group ( $p=0.0016$ ). The LETC group was more numerous ( $p=0.081$ ) in covered plots in LI/NT and CONV/MP.

Spores of the LFSC group exhibited a clumped pattern of distribution, resulting in large standard errors.

#### Colonization potential of the soil

The greenhouse bioassay with soil collected in fall, 1993 showed no significant dif-

ferences in colonization of bahiagrass between plots with and without a cover crop in any LI tillage regime (Table 3) or in CONV management (Fig. 3). The soil from the conventional system had the lowest colonization potential of all samples. The colonization potential of the soil collected in spring, 1994 was higher in plots with cover crops than in those without them in both the LI and CONV systems. The colonization of bahiagrass was higher (sometimes almost double) for soil collected in the spring than in the fall.

#### Discussion

An overwintering cover crop of hairy vetch significantly increased the inoculum of VAM fungi in soils from LI and CONV management compared with soils without a cover crop. Hairy vetch roots provided living plant hosts for VAM fungi during autumn and early spring growth periods. In addition, the colonization potential of all soils, as measured by the greenhouse bioassay, was greater in the spring than the previous autumn. Seasonal differences in environment in the greenhouse and relief of spore dormancy (Douds and Schenck, 1991) may have contributed to this observation. The cover crop also affected this phenomenon. The differences in colonization of bahiagrass between autumn and spring bioassays were much larger in soils from the hairy vetch-covered plots than in soils from bare plots. The largest increase from fall to spring occurred in the conventional plots, with 125 and 100% increases for the plots with and without cover, respectively.

Spore populations showed three general trends: larger numbers in the spring sample in plots with cover crops than in those without them; lower numbers in the spring than in the fall; and greater populations in LI than CONV. The beneficial effect of the cover crop was especially marked for the *Glomus*-type and LETC groups. The lower numbers of spores in the spring than in the fall may reflect normal mortality, since we counted only healthy-appearing spores. Other work at this site and elsewhere has shown higher populations of spores of VAM fungi under low-input than conventional management (Douds et al., 1993;1995).

The rapid colonization of hairy vetch roots by indigenous VAM fungi in the NT plots shows the importance of an intact

**Table 1. Populations of VAM ( $50 \text{ cm}^{-3}$ ) spores in the soil of the cover crops experiment at the Rodale Research Center, (samples taken Oct. 5, 1993).<sup>1</sup>**

Treatment	LOCT <sup>2</sup>	LETC <sup>3</sup>	<i>Glomus</i>	LFSC <sup>4</sup>
LI/MP	14.5c,A	11.8b,A	19.6b,A	0.3b,A
LI/CD	20.0b	18.4a	26.6a	0.5b
LI/NT	79.0a	8.1c	14.3c	9.3a
CONV/MP	8.6A	7.7B	4.9B	0.2A

<sup>1</sup> LI = low-input; CONV = conventional; MP = moldboard plow; CD = chisel-disk; NT = no-till. Lower case letters for comparisons within LI (means of 32 observations) by Tukey's method of multiple comparisons ( $\alpha = 0.05$ ); upper case letters to compare CONV/MP (means of 16 observations) with LI/MP by the Bonferroni multiple comparison method ( $\alpha = 0.05$ ).

<sup>2</sup> *Glomus occultum* and *G. occultum*-like spores (hyaline, <100  $\mu\text{m}$  diameter).

<sup>3</sup> *Glomus etunicatum* and *G. etunicatum*-like spores (yellow, <130  $\mu\text{m}$  diameter).

<sup>4</sup> *Glomus fasciculatum* and *G. fasciculatum*-like spores (brownish, <85  $\mu\text{m}$  diameter).

**Table 2. Populations of VAM spores ( $50 \text{ cm}^{-3}$ ) in the soil of the cover crops experiment at the Rodale Research Center (samples taken May 5, 1994).<sup>1</sup>**

Treatment	Cover	LOCT	LETC	<i>Glomus</i>	LFSC
LI/MP	yes	17.2±5.0	5.8±1.1	13.2±2.3	0.8±0.5
	no	13.0±1.6	6.2±1.0	9.0±1.6	0.5±0.3
LI/CD	yes	14.0±2.3	16.2±1.0	32.0±0.4	0.3±0.2
	no	12.0±0.8	18.2±6.7	19.8±3.1	0.3±0.2
LI/NT	yes	44.5±4.6	9.0±2.3	8.8±1.0	10.0±9.0
	no	72.2±9.3	3.8±0.8	7.5±1.6	1.8±0.8
CONV/MP	yes	5.8±1.0	6.5±1.5	4.5±1.4	0
	no	4.9±1.0	3.4±0.8	2.2±0.6	0

<sup>1</sup> Abbreviations as in Table 1. Means of 16 observations ± SEM.

**Table 3. Colonization of bahiagrass roots (% root length colonized) with soil inoculum from the cover crops experiment.<sup>1</sup>**

Treatment	Cover	Fall 1993	Spring 1994
LI/MP	yes	14.5±1.6	26.6±1.5
	no	13.7±1.7	21.6±1.9
LI/CD	yes	16.2±1.6	29.4±1.4
	no	15.9±1.9	26.2±1.8
LI/NT	yes	15.0±2.2	23.4±1.9
	no	17.5±3.0	18.5±1.0

<sup>1</sup> Abbreviations as in Table 1. Means of 16 observations ± SEM.

hyphal network in the soil as a significant component of the inoculum of VAM fungi. Fungal mycelium separated from the host plant survives only two to four weeks (Sieverding, 1991). Soil tillage disrupts fungal mycelium networks, which explains why, in the fall, colonization of hairy vetch roots was greatest under NT. The following spring, after a few months without soil disturbance, VAM hyphal networks recovered in cover crop plots, reducing the differences in colonization among the different tillage regimes.

Cover crops used as mulch maintain soil moisture, but under certain climates and management regimes they can deplete soil water needed for subsequent crops (Sarrantonio, 1994). In this experiment, the cover crop did not affect the soil moisture. Water loss from hairy vetch by transpiration may

have counteracted the reduction in soil evaporation, leaving no net benefit. We collected soil only once for this measurement, and the outcome might be different for data collected throughout a growing season.

To recycle and use nutrients efficiently is a goal of sustainable farming systems. To achieve it, the system relies on diversified crop rotations that include cover crops, and on active microbial populations. Cover crops may have symbioses that fix atmospheric N or take up soluble, leachable nutrients and keep them in organic form until the cover is plowed or mowed. Also, we have shown that the cover crop serves as a host for VAM fungi during periods when the fungi might become nonviable. This study found that an overwintering cover crop of hairy vetch increased the VAM inoculum after just one season. Careful planning and management of farming systems to include the right sequence of cash and cover crops, as well as conservation tillage, will enhance levels of VAM fungi and contribute to the long term health of the soil and sustainability of the agricultural system.

Acknowledgment. We thank E. Boswell and E. Chang for technical assistance. This work was supported by USDA-CSRS NRICGP grant no. 92-37101-7439.

## References

1. An, Z.-Q., J.H. Grove, J.W. Hendrix, D.E. Hershman, and G.T. Henson. 1990. Vertical distribution of endogenous mycorrhizal fungi associated with soybean as affected by soil fumigation. *Soil Biology and Biochemistry* 22:715-719.
2. Davies, F.T., J.R. Potter, and R.G. Linderman. 1992. Mycorrhiza and repeated drought exposure affect drought resistance and extraradical hyphae development of pepper plants independent of plant size and nutrient content. *J. Plant Physiology* 139:289-294.
3. Douds, D.D., L. Galvez, R.R. Janke, and P. Wagoner. 1995. Effect of tillage and farming system upon populations and distribution of vesicular-arbuscular mycorrhizal fungi. *Agric., Ecosystems and Environment* 52:111-118.
4. Douds, D.D., R.R. Janke, and S.E. Peters. 1993. VAM fungus spore populations and colonization of roots of maize and soybean under conventional and low-input sustainable agriculture. *Agric., Ecosystems and Environment* 43:325-335.

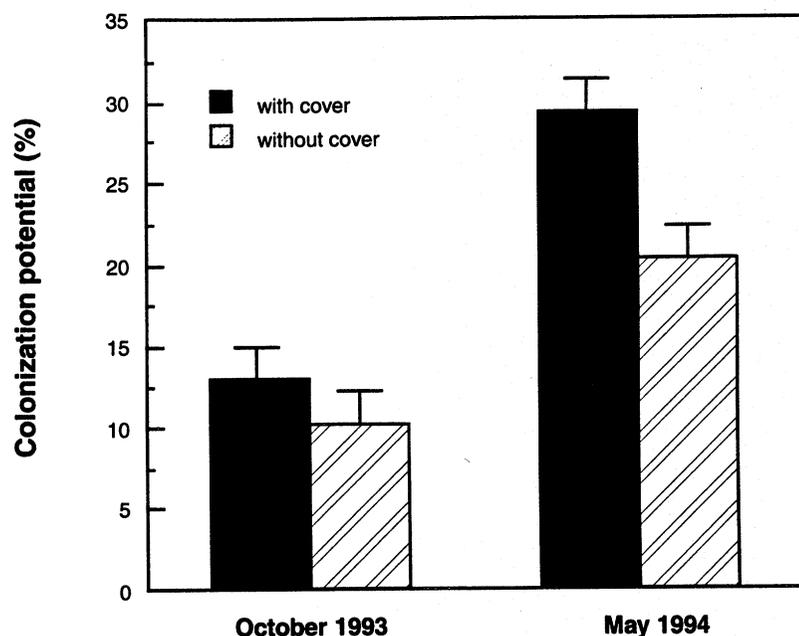


Figure 3. Percentage root length of bahiagrass colonized while grown in soil from CONVMP plots with and without a cover of hairy vetch. Soil was collected on October 5, 1993 and May 5, 1994. Bars represent means of 8 observations + SEM.

5. Douds, D.D., and N.C. Schenck. 1990. Increased sporulation of vesicular-arbuscular mycorrhizal fungi by manipulation of nutrient regimes. *Applied and Environmental Microbiology* 56:413-418.
6. Douds, D.D., and N.C. Schenck. 1991. Germination and hyphal growth of VAM fungi during and after storage in soil at five matric potentials. *Soil Biology and Biochemistry* 23:177-183.
7. Frey, B., and H. Schuepp. 1993. Acquisition of nitrogen by external hyphae of arbuscular mycorrhizal fungi associated with *Zea mays* L. *New Phytologist* 124:221-230.
8. Gerdemann, J.W., and T.H. Nicolson. 1963. Spores of mycorrhizal Endogone species extracted by wet sieving and decanting. *Trans. British Mycological Soc.* 46:235-244.
9. Harinikumar, K.M., and D.J. Bagyaraj. 1988. Effect of crop rotation on native vesicular-arbuscular mycorrhizal propagules in soil. *Plant and Soil* 110:77-80.
10. Harley, J.R. 1989. The significance of mycorrhiza. *New Phytologist* 92:129-139.
11. Jenkins, W.R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Disease Reporter* 73:288-300.
12. Kurle, J.E., F.L. Pflieger, and R.K. Crookston. 1991. The effect of management and edaphic factors on vesicular-arbuscular mycorrhizal (VAM) populations in a corn-soybean rotation. *Phytopathology* 81:1210 (abstract).
13. Limonard, T., and M.A. Ruissen. 1989. The significance of VA-mycorrhiza to future arable farming in the Netherlands. *Netherlands J. Plant Pathology* 95 (suppl. 1):129-136.
14. McGonigle, T.P., D.G. Evans, and M.H. Miller. 1990. Effect of degree of soil disturbance on mycorrhizal colonization and phosphorus absorption by maize in growth chamber and field experiments. *New Phytologist* 116:629-636.
15. McGonigle, T.P., and M.H. Miller. 1993. Mycorrhizal development and phosphorus absorption in maize under conventional and reduced tillage. *Soil Sci. Soc. Amer. J.* 57:1002-1006.
16. Nemeček, S. 1987. Effect of storage temperature and moisture on *Glomus* species and their subsequent effect on citrus rootstock seedling growth and mycorrhiza development. *Trans. British Mycological Soc.* 89:205-212.
17. Newman, E.I. 1966. A method of estimating the total length of root in a sample. *J. Applied Ecology* 3:139-145.
18. Phillips, J.M., and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. British Mycological Soc.* 55:158-160.
19. Rabatin, S.C., and B.R. Stinner. 1989. The significance of vesicular-arbuscular mycorrhizal fungi-soil macroinvertebrate interactions in agroecosystems. *Agric., Ecosystems and Environment* 27:195-204.
20. Rhodes, L.H., and J.W. Gerdemann. 1975. Phosphate uptake zones of mycorrhizal and non-mycorrhizal onions. *New Phytologist* 75:555-561.
21. Sarrantonio, M. 1994. *Northeast Cover Crop Handbook*. Soil Health Series. Rodale Institute, Emmaus, Pennsylvania.
22. Sattelmacher, B., S. Reinhard, and A. Pomikalko. 1991. Differences in mycorrhizal colonization of rye (*Secale cereale* L.) grown in conventional or organic biological-dynamic farming systems. *J. Agronomy and Crop Sci.* 167:350-355.
23. Schenck, N.C. 1987. Vesicular-arbuscular mycorrhizal fungi and the control of fungal root diseases. In I. Chet (ed). *Innovative Approaches to Plant Disease Control*. John Wiley and Sons, Inc., New York, N.Y. pp. 179-191.
24. Sieverding, E. 1991. *Vesicular-arbuscular Mycorrhiza Management in Tropical Agrosystems*. Agency for Technical Cooperation (GTZ), Eschborn, Germany. p. 31.
25. Thompson, J.P. 1987. Decline of vesicular-arbuscular mycorrhizae in long fallow disorder of field crops and its expression in phosphorus deficiency of sunflower. *Australian J. Agric. Research* 38:847-867.
26. Wagoner, P., L.R. Longnecker, and R.R. Janke. 1993. *The Low-input Reduced Tillage Trial at the Rodale Research Center. Four-year Summary 1988-1991*. Rodale Institute Research Center, Kutztown, Pennsylvania.