Soil disturbance and infection of Trifolium repens roots by vesicular-arbuscular mycorrhizal fungi

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Abstract. Removal and storage of the surface layers of soil is known to decrease the infectivity of vesicular-arbuscular mycorrhizal (VAM) fungi. Previous studies have mostly examined the effects of profound soil disturbance on the infectivity of VAM fungi. This study examined the effects of increasing degrees of topsoil disturbance on the infectivity of VAM fungi in two sites on sandstone soils in southeastern Australia. Intact soil blocks (20 x 20 x 15 cm) were taken from each of the two sites. Increasing degrees of topsoil disturbance were achieved by cutting the blocks longitudinally into four (dist. 1), nine (dist. 2), and 25 (dist. 3) equal portions. Seeds of Trifolium repens L. were sown into the blocks and harvested 14, 21, 28, 35 and 42 days after sowing. At each sampling date, total root length, root length colonised by VAM fungi and shoot dry mass were measured. VAM colonisation had commenced by 14 days in the roots of seedlings grown in intact, dist. 1, and dist. 2 soil blocks. The initiation of VAM colonisation was delayed by up to 6 weeks for seedlings grown in the dist. 3 soil blocks. The low (i.e. dist. 1) and intermediate (i.e. dist. 2) degrees of soil disturbance did not cause a delay in the initiation of VAM, but did significantly reduce the proportion of root length colonised by VAM fungi after 21 days. After 21 days, shoot dry mass was significantly less in the seedlings grown in the dist. 3 soil blocks though not in the low and intermediate disturbance treatments. It is concluded that the most severe experimental disturbance probably disturbed the external hyphal network and root fragments (containing hyphae and vesicles), which in turn temporarily reduced the infective potential of the fungus to zero. The observed delay in the initiation of VAM in the most disturbed blocks can, therefore, be explained by the time required for hyphae to grow from other propagules in the soil which survived the disturbance event.

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Introduction

Plant roots infected with vesicular-arbuscular mycorrhizal (VAM) fungi carry a loose hyphal network extending into the surrounding soil, providing an extensive surface area for absorption of nutrients and a mechanism by which infection can be spread (Warner and Mosse 1983; Newman 1988). The external hyphal network can extend for several centimetres into the soil surrounding the plant roots they infect (Heap and Newman 1980). Sanders and Tinker (1973) found a total of about 80 cm of external hyphae for every centimetre of onion root infected by VAM fungi. The external hyphal network is considered to be particularly significant as a source of VAM infection in undisturbed soils containing few living spores (e.g. Read et al. 1976, 1985; Jasper et al. 1989a, b). Growing roots are sensitive to VAM colonisation only for a short time, and require rapid colonisation for an effective association (Brundrett 1991). The external hyphal network provides an extensive source of potentially infective propagules for actively growing roots to intercept.

Jasper et al. (1989a) argued that if the network of hyphae in undisturbed soils is an important inoculum source, then observed losses in infectivity after soil disturbance are likely to be because of damage to this network, rather than damage to the relatively robust structures of spores and root fragments colonised by VAM fungi. The external hyphal network could be disturbed in two ways: (1) the hyphae may be separated from their host roots, and/or (2) the hyphae may be physically broken up. VAM fungi are considered to be obligate symbionts in that they are dependent upon their host for an organic carbon supply (Harley and Smith 1983). However, it has been demonstrated that the external hyphae of VAM fungi can remain infective even after being separated from their host (e.g. Hepper and Warner 1983;
Jasper et al. 1989a). This suggests that physical disruption of the hyphae rather than separation from the host root, may be the cause of the loss of infectivity in disturbed soils. In a different study (Bellgard 1993) most of the propagules that initiate VAM were observed in topsoil. Additionally, very few living spores were found in the sandstone soils sampled.

It has been well documented that soil disturbance can reduce the infectivity of VAM fungi (e.g. Jasper et al. 1987, 1988; Evans and Miller 1988; Fairchild and Miller 1988; Jasper et al. 1989a, b, c). In these previous studies, only severe soil disturbance treatments were examined. In the present study, the relationship between the intensity of soil disturbance and the infectivity of VAM fungi (following McConigle et al. 1990) was examined. Additionally, it has been proposed that if VAM infection is beneficial to plants, and if this benefit is derived from increased nutrient and water uptake, then removal or reduction of VAM infection should result in decreased nutrient uptake, especially in soils with low concentrations of essential plant nutrients (Fitter 1986). The reduction in nutrient uptake could in turn lead to a reduction in plant growth. Consequently, shoot dry mass was measured to assess the impact of any soil disturbance-induced reduction of VAM infection on the growth of the bioassay seedlings.

Materials and methods

Study sites

The study sites used in this experiment were the same as those described previously (Bellgard 1993).

Design of the experiment

At each of the two sites (Avon and O’Hares), five plots were selected at random and five intact soil blocks (20 × 20 × 15 cm) were taken at each plot. These intact blocks were placed in square plastic containers and removed to a glasshouse. For each plot, two of the containers were left undisturbed (No dist.), one was divided longitudinally into four equal portions (dist. 1), another into nine equal portions (dist. 2) and the last into 25 equal portions (dist. 3).

At least 30 seeds of *Trifolium repens* were sown into each container. All containers were tap-watered daily, and received no additional nutrients. To test for potential aerial contamination of containers by VAM fungi in the glasshouse, five containers of river sand sown with 30 seeds of *T. repens* were used as a control. All pots were placed in a naturally lit glasshouse, in which the mean daily temperatures ranged between 20.0°C and 28.7°C for the duration of the bioassay. At 14, 21, 28, 35, and 42 days after planting, five randomly chosen seedlings were carefully removed from each container. Although the removal of bioassay seedlings caused some soil disturbance, less than 5% of each soil block was disturbed at each sampling occasion. In addition, each soil block was treated in the same way and the disturbance attributed to the harvesting of the seedlings was not considered to be a confounding variable. The roots of each seedling were fixed, cleared and stained, and the amount of root colonised by VAM fungi assayed using the method described by Bellgard (1993). Shoot material was dried in an at 70°C for 4 h and weighed.

Statistical analysis

The data were analysed by a “split-plot” analysis of variance (Cochran and Cox 1957) with seedlings nested within blocks, and blocks nested within plots. The analysis assumed a factorial relationship between the degree of disturbance and the elapsed time until harvesting of the seedlings, since each degree of disturbance occurred in conjunction with each harvest date. Comparisons within harvest dates are “within container” comparisons, so they are usually more precise than comparisons between different degrees of disturbance, which are “between container” comparisons (and involve more sources of variability). In addition, comparisons between degree of disturbance/harvest date combinations (e.g. No dist./ 21 days versus dist. 3/35 days) vary in precision depending on whether they have the same degree of disturbance (and are therefore “within container” comparisons) or have different degrees of disturbance (and are therefore “between container” comparisons). A consequence of this is that the analysis of variance involves more than one residual SS, and pairwise comparisons between two means will have different estimates of error depending on the particular comparison.

Results

Root growth and VAM formation

No VAM infection was found on the roots of any of the bioassay seedlings grown in the control containers to test for aerial contamination by VAM fungi. Root lengths was not affected by soil disturbance. The trends observed in the lengths of roots colonised by VAM fungi (i.e. VAM length) and the proportion of root length colonised by VAM fungi (i.e. %VAM) were identical. Consequently, only the proportion data are presented here. The trend observed in the two No dist. treatments were identical, and so the data were grouped. Consequently, the number of soil blocks for the No dist. treatment now equals 10.

In the undisturbed (No dist.), dist. 1 and dist. 2 treatments, VAM formation had commenced by 14 days (Fig. 1). In the most disturbed of the soil blocks (dist. 3), the onset of VAM formation was delayed by between 28 and 35 days in the Avon soil and between 35 and 42 days in the O’Hares soil (Fig. 1). Furthermore, even when colonisation commenced in the seedlings growing in the most disturbed blocks, the levels of VAM were significantly lower than the No dist. treatment at all stages of the experiment (Fig. 1; Table 1).

The low (i.e. dist. 1) and intermediate (i.e. dist. 2) degrees of soil disturbance had no effect on the proportion of root colonised by VAM fungi up to day 14 (Fig. 1). However, at 21 days, the low and intermediate degrees of soil disturbance significantly reduced the proportions of root length colonised by VAM fungi in both the Avon and O’Hares soils (Fig. 1; Table 1).

Shoot dry mass

The low and intermediate degrees of soil disturbance had no impact upon shoot dry mass. In the Avon soil, dist. 3 had no affect on shoot mass for the first 3 weeks. After 21 days, shoot mass was significantly lower in the