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Patterns of arbuscular mycorrhiza colonisation of the roots of *Hyacinthoides non-scripta* after disruption of soil mycelium

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Abstract Early-season colonisation of new roots of *Hyacinthoides non-scripta* (L.) Chouard ex Rothm. was investigated to determine how arbuscular mycorrhizal symbiosis is re-established after the annual root system is shed. During the rootless phase in summer, colonies of bulbs were removed and replanted after the soil around and below the bulb had been mixed (major disturbance) so as to disrupt the external mycelium of arbuscular mycorrhizal (AM) fungi. As a minor disturbance treatment, top soil was removed, bulbs were turned or not in their growth position with as little other disturbance as possible, and the top soil replaced. Control plants were left undisturbed. Half of the plants were harvested 3–4 weeks after the onset of root emergence. Populations of all AM fungi in roots were greatly reduced by major disturbance, whilst those in other treatments and controls were unaffected. At the second harvest, in spring, when shoots had emerged, root colonisation by fine endophytes and *Scutellospora* morphotypes developed in all treatments, whereas that of *Acaulospora* morphotypes remained low after major disturbance. Disturbance treatments delayed the appearance, at the second harvest, of mycorrhizas with degenerate arbuscules. Leaf phosphorus concentration was unaffected by soil disturbance, possibly due to partial recovery of AM fungal populations or buffering by resources stored in the bulb.

Key words *Acaulospora* · *Hyacinthoides non-scripta* · Bluebell · Disturbance · *Glomus* · Fine endophytes · Phosphorus · *Scutellospora*

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Introduction

Most mature soils contain extensive mycelia of arbuscular mycorrhizal (AM) fungi, which function not only as a nutrient foraging system (Smith and Read 1997) but also as a source of inoculum for new roots (Brundrett et al. 1985; Read et al. 1976). An alternative source of root colonisation may be glomalean spores, but spore viability in the field is often very low (Read et al. 1976; JW Merryweather, unpublished data) and the mycelium may be the predominant inoculum. This hypothesis is supported by a number of field and glasshouse investigations demonstrating that soil disturbance, which fragments the existing hyphal network, reduces or almost eliminates AM development. (Miller 1987; Read and Birch 1988; Evans and Miller 1988; Jasper et al. 1989) in glasshouse and agricultural maize crops. Reduced shoot phosphorus (P) and plant growth have been shown to be related to reduced AM fungal colonisation resulting from disruption of native mycelium when compared to undisturbed soil (Evans and Miller 1988, 1990; McGonigle et al. 1990a; McLellan et al. 1995). Disruption of soil structure in a mature ecosystem may, therefore, affect plant productivity, nutrient uptake and fungal infectivity, and may have a considerable damaging impact on soil ecosystem functioning (Read and Birch 1988).

Bluebell [*Hyacinthoides non-scripta* (L.) Chouard ex Rothm.] is obligately mycorrhizal (Merryweather and Fitter 1995) and has an annual root system. Early colonisation of the developing root system is, therefore, essential. Since bluebells develop synchronously and all individuals in a population are rootless immediately prior to colonisation, colonisation cannot derive from other living bluebell roots but must arise from spores or mycelium, either surviving in soil or connected to root systems of other plants. It is known that AM fungi can survive for several weeks either within dead roots (Tommerup and Abbott 1981) or disconnected and independent of host roots (Addy et al. 1994). Several dif-

ferent fungal taxa can colonise bluebell roots (Clapp et al. 1995; Merryweather and Fitter 1998*a*), and each may employ a different strategy for survival and recolonisation.

We employed mechanical soil disturbance to test the hypothesis that early-season mycorrhiza formation in bluebell growing in a natural ecosystem depends on the integrity of the mycelial network. Distinct AM fungal morphotypes were characterised in order to assess whether glomalean taxa associated with bluebell respond in different ways to soil disturbance. Leaf P concentration was measured in order to determine whether effects on colonisation had any impact on the plant. Less severe disturbance treatments were also employed to test the extent to which bluebell roots follow channels left by shrivelled roots from the previous year (Chilvers 1981; Merryweather and Fitter 1995) and whether AM fungi invade new roots from mycelium surviving within the old root channels.

Materials and methods

Experimental design

The experiment was conducted at Pretty Wood, Castle Howard, UK. In early May 1995, 40 individual clumps of flowering bluebells of similar size and separate from others (>30 cm) were each marked with canes. Six weeks later, after the above-ground plants had senesced, the marked bluebell clumps were selected at random for four treatments, each replicated 10 times:

1. Major disturbance: upper soil and bulbs were removed, the soil surrounding the colony was thoroughly mixed and bulbs were replanted as originally configured.
2. Undisturbed.
3. Bulbs turned: upper soil was removed intact, each bulb was carefully turned arbitrarily 90–360° without further disturbance and the soil plug replaced.
4. Topsoil disturbed: upper soil was removed intact, and the bulbs were not moved, but left in position relative to the old roots, and soil was replaced.

Any mycelial connection between the old roots and the upper soil would have been severed by treatments 1, 3 and 4 but unaffected in treatment 2.

Root colonisation

Root samples were taken at two harvests in September 1995 (2 months after treatment and shortly after root emergence) and March 1996 (8 months after treatment). On each occasion, 5 replicate clumps of plants from each treatment were dug up and approximately 20 root fragments (>5 cm) were randomly selected. Roots were cleared in 10% potassium hydroxide (10 min at 90 °C) and twice stained with 0.01% acid fuchsin (30 min at 90 °C) using a phenol-free modification of the method of Kormanik and McGraw (1982). Percentage root length colonised by AM fungi (%RLC) was assessed using the magnified intersection method (McGonigle et al. 1990b) on 100 intersections per slide.

The presence of four different fungal morphotypes was investigated, based on the anatomical features described by Merryweather and Fitter (1998*a*) and checked against published descriptions (Abbott 1982; Brundrett et al. 1996) of pure cultures of each genus: *Scutellospora* morphotype, *Acaulospora* morphotype, *Glomus* morphotype 1, fine endophytes. The *Glomus* morphotypes were rare and data are not included. A fifth distinction was made in which arbuscules (and hyphae) were degenerate but recognisable as AM fungi. It was not possible to separate them into morphotypes nor to confirm senescence/death by biochemical methods.

Leaf phosphorus

Two leaf samples (approximately 200 mg dry tissue) were taken from each bluebell clump in March 1996 (leaves were not sufficiently developed at the first harvest), dried at 80 °C and phosphorus analysed by the molybdenum blue method (Allen 1974).

Statistical methods

Data of root colonisation by AM fungi (%) were arcsine-square root transformed and analysed using the general linear model (GLM) command for factorial (treatment, harvest) analysis of variance (ANOVA) of unbalanced data using the Minitab 10 statistics software. Duncan's Multiple Range Test was applied to treatment and harvest means to identify differences significant at the $P<0.05$ level using the SPSS 6.1 statistics package. Because data for different fungal taxa came from the same root samples, a multivariate ANOVA (SPSS 6.1) was used to determine the validity of the factorial ANOVA; no differences in the significance tests were found. Leaf phosphorus data were tested by a one-way (treatment) ANOVA.

Results

The minor disturbance treatments 3 and 4 had no effect on root colonisation by the *Scutellospora* morphotype, the *Acaulospora* morphotype and fine endophytes ($P=0.541$, 0.444 and 0.973 respectively by ANOVA) when compared to the undisturbed control. There was no treatment × harvest interaction. Data for senescent/dead AM fungi varied significantly among the three control treatments (treatment: $F_{2,23}=4.59$, $P=0.021$; harvest: $F_{1,23}=5.67$, $P=0.026$).

Root colonisation by the *Scutellospora* morphotype was the most frequent at both harvests (Fig. 1a), and increased from harvest 1 to harvest 2 ($F_{1,31}=11.67$, $P=0.001$). Colonisation was unaffected by turning the bulbs or removing the topsoil. Major soil disturbance reduced colonisation by the *Scutellospora* morphotype at harvest 1 to 4% of the undisturbed control (treatment 2) but this had recovered at harvest 2. The *Acaulospora* morphotype colonised roots at a much lower frequency than the *Scutellospora* morphotype and data were very variable (Fig. 1b). Colonisation increased from harvest 1 to harvest 2 ($F_{1,31}=7.93$, $P=0.008$). Major soil disturbance reduced colonisation by *Acaulospora* morphotypes at harvest 1 and to a greater extent at harvest 2, when compared to the other treatments. Major soil disturbance did not affect root colonisation by fine endophytes (Fig. 1c) at harvest 1 ($P=0.431$). There was an increase between harvests only after major disturbance (harvest: $F_{1,31}=7.69$, $P=0.009$; interaction treatment × harvest: $F_{3,31}=3.82$, $P=0.019$). At harvest 2, colonisation had increased from <1% to 33% in the disturbed treatment and was greater than in other treatments.

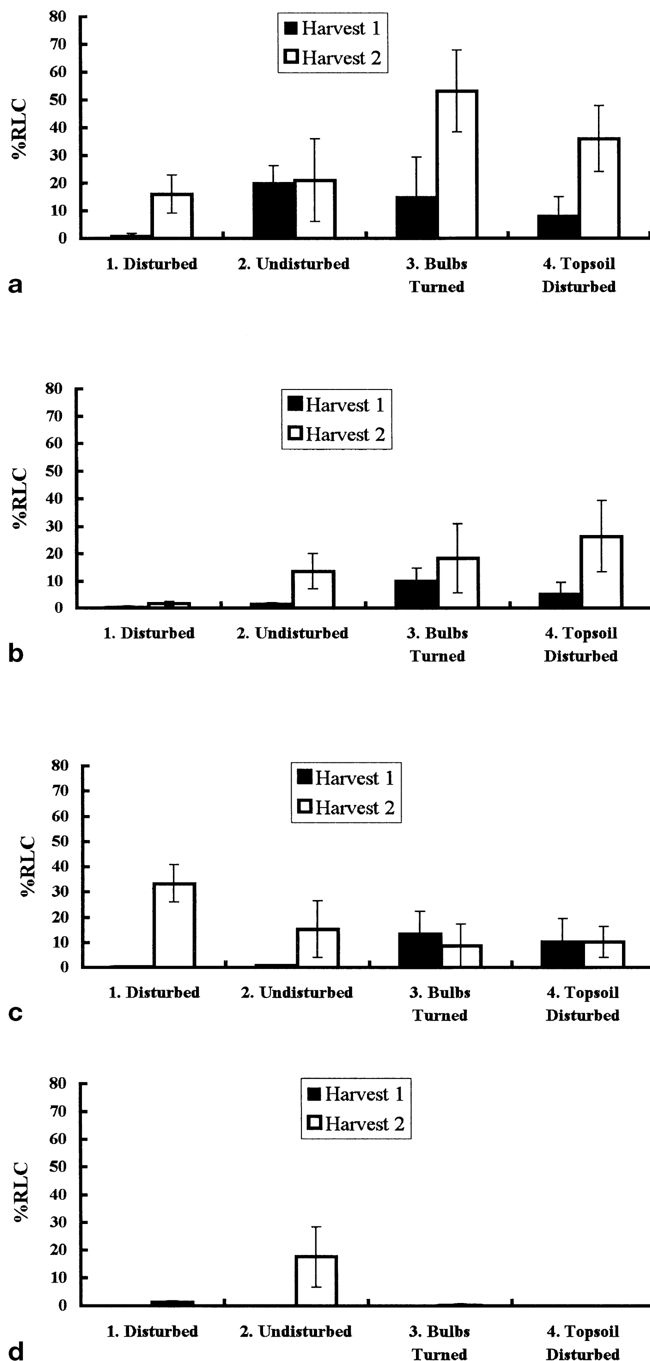

Fig. 1 Root colonisation (%RLC) of *Hyacinthoides non-scripta* by AM fungi: **a** *Scutellospora* morphotype; **b** *Acaulospora* morphotype; **c** fine endophytes; **d** apparently senescent/dead AM fungi; *bars* are standard errors

AM fungi considered from their appearance to be senescent or dead were not found at harvest 1 (Fig. 1d) and occurred in significant quantities only in the roots of control plants at harvest 2. There were very small amounts in roots from the major disturbance and bulbs turned treatments, and they were absent from the topsoil disturbed treatment ($F_{3,31}=3.99$; $P=0.016$). Comparison of means by Duncan's test revealed significant differences between the undisturbed treatment and the other treatments.

The P concentrations in pairs of leaf samples were very similar and were combined. Mean leaf P concentrations were all between 5.2 and 5.4 mg g^{-1} and there were no differences between treatments ($F_{3,36}=0.08$, $P=0.972$).

Discussion

Colonisation of bluebell roots by AM fungi at the beginning of the season in the Pretty Wood site was rendered ineffective by major soil disturbance and was consequently dependent upon an established inoculum. Root colonisation was dramatically reduced and populations of AM fungi were affected, especially the *Scutellospora* morphotype, the main component of the bluebell mycorrhiza and the first to colonise during the annual cycle (Merryweather and Fitter 1998*b*). Destruction of the soil mycelium has been reported to reduce mycorrhizal inoculum potential under glasshouse and agricultural field conditions (Read and Birch 1988; Jasper et al. 1989; Evans and Miller 1990) and is confirmed here for a natural ecosystem. Recovery may take more than one season, as previously demonstrated by Miller (1987) in a very different natural ecosystem.

Rapid early-season colonisation by the *Scutellospora* morphotype and the profound effect on its root population caused by major disturbance suggest that there is an established network of this fungus in the soil at Pretty Wood, with which bluebell interacts during most of its growing season. Spores probably contribute in a minor way to the process of colonisation of the new bluebell roots in this system because, in most seasons, spore production occurs well after the roots emerge from the bulb (Merryweather and Fitter 1998b) and field spore viability is usually low (JW Merryweather, unpublished data). Mycelium of the *Scutellospora* morphotype may become inactive during the bluebell's rootless phase in summer or may be supported by associated plants in the vicinity. The latter is certainly possible since spores of *Scutellospora dipurpurescens* occur in a region of the field site where bluebells are not found, and where bracken (*Pteridium aquilinum*), which normally accompanies bluebell at the Pretty Wood site, is the only AM plant species (Merryweather and Fitter 1998*b*).

Since bluebell is an obligately mycorrhizal plant (Merryweather and Fitter 1995) and the *Scutellospora* morphotype mycorrhiza is the earliest in the season to colonise bluebell roots, at a time when P uptake is very active (Merryweather and Fitter 1995), it may be the taxon that contributes most to P acquisition at the Pretty Wood site (Merryweather and Fitter 1998*b*), particularly during the host's subterranean growth phase between September and late January (Merryweather and Fitter 1995). In this experiment, populations of the *Scu-*

tellospora morphotype partially recovered following severe reduction by disturbance and leaf P remained unaffected. Either this or other fungi made up for a temporary reduction in P inflow, or else tissue P content was buffered from bulb resources. However, in the first year of a 2-year study of the effect of the fungicide benomyl on P content of bluebell at the Pretty Wood site, we found that the fungicide reduced root colonisation to <10% and leaf P concentration was consequently reduced by 15% (Merryweather and Fitter 1996).

The *Acaulospora* morphotype mycorrhiza generally contributed less to the AM fungal population in the bluebell roots. It occurred very infrequently at the beginning of the season, and by the second harvest was established at a relatively low %RLC in control plant roots. It was virtually absent from roots of plants in the main disturbance treatment, indicating that the inoculum potential had been severely reduced and root populations failed to recover by harvest 2.

The fine endophyte named as *Glomus tenue* (Greenhall) Hall (Hall 1977) is not easily defined in bluebell. What was recorded here was a morphologically diverse array of root fungi, all bearing arbuscules (or arbuscule-like organs) on hyphae less than 1.5 μm in thickness. The characteristic fan-like hyphal structures characteristic of *Glomus tenue* were not always present, and the Pretty Wood fine endophytes may be *Glomus tenue,* as previously described, plus other fungi from which it cannot be separated with confidence.

Apparently senescent or dead AM fungi were recorded where there was sufficient structural evidence of similarity to known AM fungi. They occurred only late in the season at harvest 2, when both roots and internal mycelium might be expected to be in the early stages of senescence, and only in the roots of control plants. In the roots of plants in all three disturbed treatments, senescence may have been delayed and the mycorrhizas remained active, whereas in the undisturbed control the fungi may have naturally reached the end of their cycle in the absence of external interference.

Our hypothesis that early root colonisation takes place via old root channels was not supported by the data. Turning the bulbs gently, so that roots might be unable find the old channels, had no effect upon early colonisation. Either the new roots were able to locate root channels or, simply entering the soil, they were colonised as they passed through an existing glomalean mycelium.

In conclusion, the present data provide field evidence that taxa of AM fungi differ in their response to disturbance. Root colonisation by the *Scutellospora* morphotype and the fine endophytes was initially much reduced in bluebell roots by soil disturbance, but recovered to control levels later in the growing season. Colonisation by the *Acaulospora* morphotype, a late coloniser, did not recover. Few studies of AM fungi populations have investigated the morphology of the fungi in roots in the field to enable some degree of identification (Abbott 1982; Brundrett and Abbott 1994; Braunberger et al. 1997) and none has done so entirely in a natural plant community. However, it is now proving possible to distinguish between AM fungi, at least to the genus level (Abbott 1982; Abbott and Robson 1991; Merryweather and Fitter 1998b), and this together with molecular probes should enable an improved understanding of the dynamics of arbuscular mycorrhiza in the field.

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