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THE ECOLOGICAL SIGNIFICANCE OF ARBUSCULAR MYCORRHIZAL FUNGAL EFFECTS ON CLONAL REPRODUCTION IN PLANTS

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Abstract. The population ecology of clonal plants depends on the number and distribution of ramets formed during growth. Variation in clonal reproduction has previously been explained by variation in effects of abiotic resource heterogeneity and by plant genotypic variation. Different co-occurring species of the mutualistic arbuscular mycorrhizal fungi (AMF) have been shown to differentially alter growth traits of *Prunella vulgaris*, which we hypothesize would lead to changes in clonal reproduction. Two experiments were carried out to test whether different co-occurring mycorrhizal fungi significantly influence clonal reproduction of *P. vulgaris*, whether this effect also occurs when *P. vulgaris* is growing in an artificial plant community, and how the effects compare with plant genotype effects on clonal growth of *P. vulgaris.*

In the first experiment, the number of ramets of *P. vulgaris*, growing in a plant community of simulated calcareous grassland, was significantly affected by inoculation with different mycorrhizal fungi. The number of ramets produced by *P. vulgaris* differed by a factor of up to 1.8 with different mycorrhizal fungi. The fungal effects on the number of new ramets were independent of their effects on the biomass of *P. vulgaris.*

In a second experiment, 17 different genotypes of *P. vulgaris* were inoculated with different mycorrhizal fungi. There were significant main effects of genotypes and mycorrhizal fungi on clonal reproduction of *P. vulgaris.* The effect of different mycorrhizal fungi contributed more than the effect of plant genotype to variation in size and ramet production. However, mean stolon length and spacer length, which determine the spatial arrangement of ramets, were only significantly affected by plant genotype. There were no mycorrhizal fungal $\times$ plant genotype interactions on clonal growth of *P. vulgaris* indicating that there is no obvious evidence that selection pressures would favor further coevolution between *P. vulgaris* and mycorrhizal fungal species.

In natural communities plants can be colonized by several different AMF at the same time. The effect of the mixed AMF treatment on the growth and clonal reproduction of *P. vulgaris* could not be predicted from the responses of the plants to the single AMF. To what extent, however, the patterns of colonization by different AMF differ among plants in a natural community is unknown.

Since the effects of AMF on growth and clonal reproduction occur on a population of *P. vulgaris* in a microcosm plant community and because the effects are also as great as those caused by plant genotypic variation, we conclude that the effects are strong enough to potentially affect population size and variation of clonal plants in communities.

Key words: arbuscular mycorrhizal fungi (AMF); clonal reproduction; genotype; Glomales; mycorrhizal symbiosis; population biology; Prunella vulgaris; *ramet; resource heterogeneity.*

INTRODUCTION

In many habitats such as grasslands, clonal plants are the dominant growth form (Turkington and Cavers 1978, Klimes et al. 1997, Marshall and Price 1997). Through growth and clonal reproduction, plants can give rise to genetically identical offspring, known as ramets, which have the potential to grow independently of the parent (Kays and Harper 1974). The most common way that plants clonally reproduce is by the formation of stolons or rhizomes, on which new ramets are formed (Jónsdóttir and Watson 1997). It is through clonal reproduction that genet size, the number of individual plants which originate from the same zygote, is determined in a population (Harper 1977, Schmid 1984, 1986, 1990, Klimes et al. 1997, Oborny and Cain 1997). The extent and pattern of clonal reproduction can determine the spatial distribution of ramets and genets in a community, and can also determine the population size of plant species (Harper 1977). Furthermore, the degree of clonal reproduction is important for the survival and fitness of plants in well-es-

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tablished communities, where successful establishment of new plants from seeds may be very rare (Jónsdóttir and Watson 1997, Schläpfer and Fischer 1998). Both ramet size and ramet number can be considered as fitness related traits (Sackville-Hamilton et al. 1987, Charlesworth, 1994). This is because both of these traits directly affect ramet population growth rate. Sexual reproduction is also directly proportional to clone size, i.e., number × biomass of ramets, even though many vegetative cycles of the plant may have to be observed before the full effects on sexual reproductive output are also observed (Schmid et al. 1995, Gardner and Mangel 1999, Meyer and Schmid 1999 *a*, *b*, Winkler and Fischer 1999, Winkler et al. 1999, Van Kleunen et al. 2000). Therefore, effects on clonal reproduction affect the success of future populations of a given plant species.

In clonal plants there is usually a great phenotypic variability found in natural populations. Variation in clonal growth patterns is thought to be determined by genotypic variation in the population (Schmid 1985*a*, Cheplick 1997, Skálová et al. 1997), by heterogeneity of the environment that the plant encounters (Schmid 1985*b*, Hutchings and de Kroon 1994, McLellan et al. 1997), or by an interaction of these two factors (Via and Lande 1985). The existence of plant genotype × environment interactions is thought to be important for the prolonged coexistence of genotypes (Silander 1985, Gillespie and Turelli 1989, McLellan et al. 1997) and, therefore, for the maintenance of genetic diversity in a population. However, most studies on plant genotype × environment interactions have concentrated on abiotic factors such as light or nutrient availability (de Kroon et al. 1994).

One part of the biotic environment for >80% of the world's plant species is the arbuscular mycorrhizal fungi (AMF) with which they form mutualistic symbioses (Smith and Read 1997). Natural ecosystems comprise diverse AMF communities, with typically 5–15 morphologically distinct AMF taxa per site (Walker et al. 1982, Johnson et al. 1992). The distribution of the different AMF within plant roots in natural ecosystems is not known. Multiple colonization by several different AMF taxa has been observed, although the extent of its occurrence is unknown (Rosendahl et al. 1990, Clapp et al. 1995). Different AMF species from a natural community have recently been shown to differentially alter the growth of different plant species (Streitwolf-Engel et al. 1997, van der Heijden et al. 1998*a*) and through these effects AMF may determine plant diversity and community structure (van der Heijden et al. 1998*b*). In a previous study conducted in pots, we have shown that clonal growth traits of *Prunella vulgaris* and *Prunella grandiflora*, namely the number and length of stolons, were significantly influenced by different AMF taxa originating from the same calcareous grassland (Streitwolf-Engel et al. 1997). These effects occurred independently of plant size, measured as dry mass, and of plant phosphorus concentration. Thus, effects of different AMF on clonal growth traits cannot be explained merely by AMF effects on plant size. Both the number of stolons and stolon length are important traits determining the number and spatial distribution of ramets in a population (de Kroon and Hutchings 1995). Observed effects on stolon number and length by different AMF isolates lead us to the hypothesis that, through their effects on clonal growth, different co-occurring AMF in a grassland affect the population size (number of ramets) of plants. However, in the study of Streitwolf-Engel et al. (1997), the formation of new ramets was not measured and the plants were growing singly in pots rather than with a surrounding plant community.

Other neighboring plants in a community, with which there could be competition for available space and resources, are also potentially strong determinants of the amount and variation in clonal reproduction. To assess whether different AMF could potentially affect the amount and variation in clonal reproduction, it is therefore necessary to test whether such effects still occur when *P. vulgaris* is growing in a plant community. It is already known that variation in clonal reproduction of *P. vulgaris* is affected by plant genotypic variation (Schmid 1985*a*, *b*). Genotypic variation in clonal reproduction could be so large that the effects of AMF may be ecologically insignificant in comparison. For the AMF effects on clonal reproduction to be important for populations of *P. vulgaris*, the effects would therefore have to be as large or larger than genotypic effects and this has not previously been tested.

The combined effects of different AMF and plant genotypic variation on clonal growth may also be important for the evolutionary ecology of clonal plants. Clonal reproduction, measured as the formation of new ramets, has been considered as a measure of genet fitness, where fitness is defined as the rate of change of units carrying a certain allele or allele complex (Schmid 1990, Wikberg 1995). A strong effect of different AMF isolates on clonal reproduction would suggest that selection pressures exist favoring symbioses that lead to the greatest fitness in the plants. To our present knowledge, there is no specificity in the colonization of plant roots by different AMF taxa. To our knowledge, there are no published studies where the interaction between different AMF taxa and plant genotypes on fitness related traits has been tested. A significant interaction would mean that different AMF could significantly and differentially affect the fitness of different genotypes. It would also provide some supporting evidence that selection pressures could potentially favor the coevolution of specific combinations of plant genotypes and AMF taxa, since the success or failure of certain genotypes to reproduce would be dependent on which AMF is present in its roots. Such coevolution would, however, only be the case if a given AMF taxa, which results in the increased fitness of a

given plant genotype, would also be fitter when forming a symbiosis with the same plant genotype.

We conducted an experiment to test the hypothesis that different co-occurring AMF taxa affect the clonal reproduction and population size of *P. vulgaris* in a plant community, either through their direct effects on clonal growth or indirectly through effects of AMF on the growth of other plants in the community. In this experiment, clonal reproduction of *P. vulgaris* was measured in experimental microcosms containing plant communities simulating dry, calcareous grasslands. The microcosms were separately inoculated with one of four different AMF or a mixture of all four AMF isolates. All the AMF isolates and plants originally came from a calcareous grassland in Switzerland. In a second experiment, we investigated and compared the effects of different co-occurring AMF taxa on variation in growth and clonal reproduction in 17 different genotypes of *P. vulgaris.* In this experiment, we also tested whether there were significant plant genotype × AMF taxa interactions on the growth and clonal reproduction of *P. vulgaris.* The 17 genotypes originated from the same calcareous grassland and were separately inoculated with three of the AMF used in experiment 1 or with a mixture of these three AMF isolates.

Methods

Site description, plant and fungal material

The different AMF isolates used in our experiments, as well as *P. vulgaris* genotypes, originated from the same field site, Nenzlinger Weide, a calcareous grassland in the Jura Mountains, Switzerland (grid reference 255 609 of the Landeskarte der Schweiz 1990). The site is 450 m above sea level, supports a diverse flora characteristic of calcareous grasslands and fits the phytosociological description of mesobrometum (Ellenberg 1988). The four AMF isolates, *Glomus geosporum* (BEG 18), *Glomus* sp. (BEG 19), *Glomus* sp. (BEG 21), and *Glomus* sp. (Basle Pi) were isolated as described by Streitwolf-Engel et al. (1997) and van der Heijden et al. (1998*a*). These AMF are the commonest morphological types to occur in the soil at the study site, although this does not necessarily mean that they are the commonest AMF in the roots of the plants in that community. All cultures are maintained in the Botanical Institute of the University of Basel. All AMF isolates are both morphologically and genetically different from each other. Genetic differences among isolates were established by PCR amplification of the ITS1, ITS2, and 5.8S regions of rDNA from spores of these isolates, followed by cloning and sequencing (I. R. Sanders, *unpublished data*), according to the method of Sanders et al. (1995). Because three of the four isolates are undescribed, they are subsequently referred to as AMF taxa rather than species. Further information regarding these isolates can be obtained from the Banque Européenne des Glomales.[3]

Forty samples of *P. vulgaris* were collected on 2–3 October 1995 from an area measuring 20 × 200 m at the field site. Intersections on a 1 × 1 m grid were selected using a random number procedure. The nearest *P. vulgaris* plant to each of the selected intersections was collected and taken to the greenhouse. In order to diminish the chance of taking ramets of the same genotype, plants were not collected from a selected position if a plant had previously been taken within a 1 m radius of that position. The plants were assumed to be different genotypes, based on their spatial distance and their morphological differences (Schmid 1985*b*). The genotypes were successively cloned by meristem culture (Birrer 1994). Each genotype was subcultured 11 and 16 times in meristem culture (experiments 1 and 2, respectively) over a period of eight months. Meristem culture was performed in order to grow sufficient replicate ramets of each genotype, but also to remove potential maternal effects (Lynch and Walsh 1997). While the removal of maternal effects is necessary for any study of genotypic variation, there was an additional rationale for propagating the plants using meristem culture. All *P. vulgaris* individuals that are collected from the field are already colonized by AMF. Since it is already known that AMF taxa differentially affect the growth of *P. vulgaris,* these maternal effects can only be removed by growing the plants in an AMF-free environment. During meristem culture the plants were kept in growth chambers (day 16 h/22°C, night 8 h/20°C). Following the subculturing, the plants were grown on hormone medium for a further 14 d. They were then transferred to growth medium containing no hormones. Following the appearance of the first roots, 14 d later, they were planted into trays containing sterilized sand. After 11 d of gradual hardening in the greenhouse, they were planted into microcosms (experiment 1) or into single pots (experiment 2). Several of the genotypes either did not survive the meristem culturing or could not be propagated in meristem culture in sufficient numbers required for the experiments. Eighteen genotypes of *P. vulgaris* were successfully propagated in sufficient numbers to be used for the experiments. One of the genotypes was removed from the experiment later because after a careful observation of its morphology it was identified as a hybrid between *P. vulgaris* and *P. grandiflora.*

AMF inoculation and plant growth conditions

Experiment 1.—Experiment 1 was set up to test the hypothesis that different co-occurring AMF taxa affect the clonal reproduction and population size of *P. vulgaris* in a plant community. *P. vulgaris* were planted into experimental microcosms containing artificial

[3] URL: ⟨http://wwwbio.ukc.ac.uk/beg/BEGDatabase/amfreq-reg.htm⟩

plant communities of simulated calcareous grassland and inoculated with one of four different AMF isolates or a mixture of these four AMF isolates (van der Heijden et al. 1998*b*). The 40 microcosms were planted in containers measuring $26.5 \times 17 \times 18$ cm^3 filled with 7.38 kg of a mixture of autoclaved quartz sand and autoclaved calcareous grassland soil (2:1 volume/volume). They were inoculated with 100 g of soil inoculum containing spores and hyphae of one of the four AMF isolates or with a mixture of all four AMF isolates. A nonmycorrhizal control treatment was established by inoculating with autoclaved mixed inoculum. The microcosms (with and without AMF) received 380 mL of a filtered washing of mixed soil inoculum to correct for possible differences in soil bacterial and fungal communities among the different AMF inocula, which could potentially cause non-AMF related effects on plant growth (Koide and Li 1989). AMF spores are the largest fungal propagules in the soil and filtering removes AMF spores but not other soil bacteria and fungi (Koide and Li 1989). In each microcosm, 70 seedlings of 11 calcareous grassland plant species were planted at fixed distances from each other. The number of seedlings of each plant species corresponded to their natural abundance at the field site (Leadley and Stöcklin 1995). The positions of the plants were randomized among blocks but not within blocks, meaning that the position of the plants was the same among microcosms within a block. Seven meristem-cultured *P. vulgaris* plants were planted per microcosm 6 wk after the inoculation and establishment of the microcosms. Thus, the *P. vulgaris* plants had the possibility of being colonized by spores and hyphae of the original inoculum but also by the extraradical hyphal network of the AMF that had already colonized the plant community. In natural communities, plant roots are more likely to be colonized by hyphae of the existing hyphal network so this inoculation method mimics, as near as possible, the natural situation. The seven *P. vulgaris* were planted at predetermined positions that were randomized among blocks but not within blocks. Of these seven plants, five were of different genotypes that were selected randomly from the pool of 18 genotypes. The same five genotypes were planted in all microcosms. The remaining two plants per microcosm were both of one genotype that also originated from the pool of 18 genotypes. They were planted into each microcosm to make up the full complement of *P. vulgaris* plants in the community. They were not of mixed genotypes because at the time of setting up the experiment there were not enough replicates of seven different genotypes. Plants were selected for uniformity in size and development.

The experiment was set up as a randomized block design with eight blocks and one replicate of each of the five mycorrhizal treatments in each block. The position of the blocks, and of the treatments within a block, were randomized every 2 wk. Thus, differences in a given variable among blocks represent potential effects of environmental differences caused by the position of the block and also the effects of the spatial arrangement of plants in the microcosm. The microcosms were maintained in the greenhouse for two growing seasons and received a winter period from January to March 1997. Details regarding fertilization of the microcosms are given in van der Heijden et al. (1998*b*). Aboveground parts were cut in four regular harvests (mowing level 2.5 cm above the soil surface). The *P. vulgaris* plants were harvested in December 1998, after 17 mo of growth.

During the experiment the positions of *P. vulgaris* plants and of new ramets were regularly mapped so that all new ramets in the microcosms could be attributed to each of the seven original plants that were planted. Before the final harvest, the number of new ramets per plant was counted. Stolons that had formed roots and leaves were counted as new ramets as these would have the potential to be physiologically independent from the mother plant (Kays and Harper 1974). At the final harvest aboveground parts of the plants were dried at 80°C and weighed.

Experiment 2.—Experiment 2 was set up to investigate and compare the effects of different co-occurring AMF taxa on variation in growth and clonal reproduction in 18 different genotypes of *P. vulgaris*. In natural communities, plant roots are more likely to be colonized by hyphae of the existing hyphal network so we used an inoculation method that mimics, as near as possible, the natural situation. *P. vulgaris* was inoculated by a hyphal network that was previously established in pots on donor *Bromus erectus* plants. *B. erectus* was chosen as a donor plant because it is a dominant species at the field site (and in the microcosms of experiment 1) and does not vary in its growth with the different AMF isolates used in this experiment (van der Heijden et al. 1998*a*, *b*). This means that differences in AMF effects on *P. vulgaris* are not likely to be due to differential effects of the AMF isolates on the growth of *B. erectus*. The inoculation method was also chosen so that it would be as similar as possible to the inoculation method used in experiment 1. Thus, colonization of *P. vulgaris* roots in both experiments could occur from an existing AMF hyphal network. *B. erectus* seeds were sown in trays containing sterilized sand and inoculum of one of the three AMF isolates used in experiment 1, namely *Glomus* sp. (BEG 19), *Glomus* sp. (BEG 21), and *Glomus* sp. (Basle Pi). Three preinoculated *B. erectus* plants were planted into each of the 364, 11 cm diameter pots containing a mixture of autoclaved quartz sand and autoclaved calcareous grassland soil (1:1 volume/volume). For the single AMF treatments, three *B. erectus* plants were pre-infected with one of the three isolates. For the mixed AMF treatment the *B. erectus* plants were pre-infected with all three AMF isolates. After 5 wk of growth, the *B. erectus* plants were killed by cutting just below the

soil surface. Plants of the 18 genotypes of *P. vulgaris* were then planted singly into the pots that contained the roots of the *Bromus* plants but no other plant species. The plants were selected for uniformity in size and development.

A small pilot experiment was conducted to follow the colonization of *P. vulgaris* by the different AMF during the first stages of the experiment with the different AMF isolates. For this pilot experiment 16 additional *P. vulgaris* plants were inoculated with the three AMF isolates in the same way and under the same conditions as the plants in experiment 2. The plants were harvested 4, 10, and 20 d after planting. After 4 d, AMF hyphae were visible in the roots of all three AMF treatments. Ten days after planting, mycorrhizal structures were visible in plants inoculated with all three AMF isolates, indicating that the time point of initial colonization was the same for all three AMF isolates (I. R. Sanders, *unpublished data*).

The plants in experiment 2 were arranged in the greenhouse (day 14 h/26°C, night 10 h/20°C) in a complete randomized block design, with five blocks and one replicate of each of the 72 treatment combinations in each block. The position of blocks was randomized every week. Thus, differences in a given variable among blocks represent differences due to environment caused by the position of the blocks. Plants were watered every second day and were harvested 126 d after planting.

During experiment 2, the growth of the plants was followed for each clone by the following nondestructive measurements: the number of leaves, number of stolons, total stolon length, the spacer length, and the number of new ramets. Measurements were made 0, 30, 63, and 126 d after planting. The spacer length was determined by measuring the distance from one branching node to the next branching node. Ramet number was determined as described for experiment 1. Relative growth rates (RGR) were calculated for every plant from the leaf number taken from two time points and divided by the number of days of the time interval. Leaf number could be used as a predictor of RGR because there was a significant positive correlation between leaf number and plant dry mass of the plants at the final harvest of experiment 2 ($r^2 = 0.85$, $P \leq 0.001$). At the final harvest, 126 d after planting, leaves, stolons, and roots were separated, dried at 80°C, and weighed. The variables of plant dry mass and leaf number were chosen because they give a measurement of plant size. Stolon number, stolon length, and spacer length were measured because they are important traits determining the number of ramets that can potentially be formed, and these traits affect their spatial arrangement (de Kroon and Hutchings 1995). Ramet number was chosen because it is a direct measurement of clonal reproduction. The following variables were calculated from the measured variables: total dry mass, mean stolon length, and the number of ramets formed per gram of plant tissue.

A subsample of the dried roots was stained with trypan blue (Phillips and Hayman 1970) and percentage of AMF colonization was estimated using a gridline intersection method (Giovannetti and Mosse 1980).

Statistical analysis

The biomass and ramet number were analyzed as a complete randomized block design in experiment 1, with eight levels for the factor block and five levels for the factor AMF treatment. In a second ANOVA model, where the factor plant genotype was included, the values for the two plants that were not different genotypes were excluded. Thus, there were five levels for the factor plant genotype. The factors block, AMF and plant genotype were treated as random effects. A significant block × AMF effect was not expected and the AMF effect was tested using the residual mean square as denominator to calculate the *F* ratio (Newman et al. 1997). In the second model, the mean squares of the AMF × plant genotype interaction was used as the denominator for calculating the *F* ratio (Zar 1984). AMF colonization is obligatory for the growth of *P. vulgaris* in these experimental conditions. As a consequence, inclusion of the nonmycorrhizal treatment in the analysis can produce a highly significant mycorrhizal effect in ANOVA that does not reflect differences among plants caused by effects of different AMF. The nonmycorrhizal treatment in experiment 1 was, therefore, excluded from the analysis. Because AMF taxa differentially affected the structure of the plant community in the microcosms, other than the dominant plant *B. erectus* (van der Heijden et al. 1998*b*), any AMF effects on clonal reproduction of *P. vulgaris* could be direct or indirect. Although it is not possible to separate these effects, we tested whether AMF effects could possibly be explained by alterations in the biomass of any of the single plant species by correlation of *P. vulgaris* dry mass with the dry mass of each of the other plant species in the microcosms.

In experiment 2, there were five levels for block, 17 levels for plant genotype, and four levels for AMF treatment. One genotype was removed from the analysis because it was later identified as a hybrid between *P. vulgaris* and *P. grandiflora*, based on its growth characteristics. During the course of experiment 2, 19 plants died and were excluded from the analysis. These plants were evenly distributed across treatments. MANOVA was performed on 11 variables of plant growth, with the main effects of block, AMF, and plant genotype. The percentage colonization of fungal growth was not included in the MANOVA because, on the basis of results from previous experiments, we did not expect this variable to be correlated with variables of plant growth. Principal component analysis was also performed on the 11 plant growth variables. Principal component scores were calculated for each individual

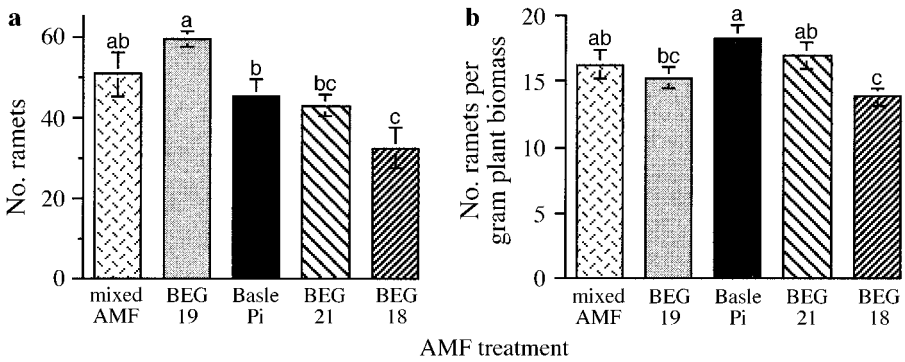

FIG. 1. (a) Mean number of ramets per microcosm and (b) mean number of ramets per gram aboveground biomass of *P. vulgaris* per microcosm of five *P. vulgaris* plants growing in microcosms of simulated calcareous grassland (experiment 1). The microcosms had been inoculated with one of three AMF *Glomus* isolates or a mixture of all four AMF *Glomus* isolates. Bars show ±1 SE; $n = 8$ per AMF treatment. Different letters above bars indicate a significant difference ($P \leq 0.05$) according to the LSD test.

plant for the first two principal components that explained the majority of variance in the data set. These two components are not correlated and the principal component scores for each individual were used in an ANOVA to test for AMF and genotype effects between uncorrelated variables.

Because the MANOVA showed significant effects, a protected ANOVA was performed on the individual plant and AMF variables (Scheiner 1993). Block, AMF, and plant genotype were considered as random effects. The significance of the AMF and genotype effects was tested using the mean squares of the AMF × plant genotype interaction as the denominator to calculate the *F* ratio (Zar 1984). Bonferroni correction was not performed to adjust the significance levels of the protected ANOVA results because the MANOVA had shown significant AMF and plant genotype effects within the data set (Scheiner 1993) and because the Bonferroni correction is not appropriate in the case where some of the measured traits could be correlated (Lynch and Walsh 1997). Mean contrasts were carried out using the least significant difference (LSD) and were only performed where a significant AMF treatment or plant genotype effect occurred. Although this is not valid for comparisons among different AMF in the single treatments, because AMF is treated here as a random factor, it is useful for making the comparison between single AMF treatments and the mixed treatments. The variables leaf number, total stolon length, leaf mass, root mass, total dry mass, stolon mass, spacer length, and the number of ramets per gram plant tissue were square-root transformed to give a normal distribution. The variables stolon number and mean stolon length were natural log-transformed to give a normal distribution. Analysis was carried out on the transformed variables but graphical representation of the results is shown for the untransformed data. Variance components were calculated for the factors in the ANOVA model and expressed as a percentage of the total variance (Sokal and Rohlf 1995).

RESULTS

Experiment 1: Effects of AMF taxa on growth and clonal reproduction of P. vulgaris *in microcosms*

There were significant effects of different AMF on aboveground biomass in *P. vulgaris* in the microcosms ($F_{4,28} = 3.51$, $P \leq 0.019$). The aboveground biomass of *P. vulgaris* and the other plant species growing in the microcosms is presented in van der Heijden et al. (1998*b*). AMF affected the structure of the plant community, through AMF × plant species interactions on the biomass of each plant species (van der Heijden et al. 1998*b*). Thus, AMF effects on biomass of *P. vulgaris* could be an indirect effect of AMF effects on some of the other plant species in the microcosms. We constructed a correlation matrix of the biomass of each plant species in the microcosms to determine whether the biomass of *P. vulgaris* was negatively correlated with the biomass of any of the other plant species. There were no significant negative correlations between the biomass of *P. vulgaris* and any of the other plant species in the microcosms, although there was a positive correlation of *P. vulgaris* biomass with that of *Lotus corniculatus.* This does not eliminate the possibility of indirect AMF effects on the growth of *P. vulgaris* but suggests that it was not due to a change in the biomass of one plant species in the microcosms.

There was a significant AMF treatment effect on the formation of new ramets by *P. vulgaris* in the microcosms (Fig. 1a). This could also be interpreted as a direct or an indirect AMF effect or a combination of both direct and indirect effects. *P. vulgaris* inoculated with *Glomus* sp. (BEG 19) formed more ramets compared to plants inoculated with any of the other single AMF isolates. There was a twofold difference in ramet formation among the AMF treatments; plants inocu-

TABLE 1. Results of multivariate analysis of variance (MANOVA) performed on 11 variables of plant growth with four AMF treatments and 17 plant genotypes.

Source of variation	Pillai trace†	*F*	df (num.)	df (den.)	*P*
Block	0.83	5.74	44	964	≤0.0001
AMF	0.51	5.54	33	720	≤0.0001
Plant genotype	2.19	3.85	176	2728	≤0.0001
AMF × plant genotype	1.69	0.94	528	2728	0.8006

Note: MANOVA was executed with the following variables, which were measured at the end of the experiment: total dry mass, stolon dry mass, leaf dry mass, root dry mass, leaf number, total stolon number, total stolon length, mean stolon length, spacer length, ramet number, and the ratio of ramet number to plant biomass.

† Pillai's trace test statistic. This value was used to calculate an approximate *F* ratio with accompanying numerator (num.) and denominator (den.) degrees of freedom.

lated with *Glomus* sp. (BEG 19) produced 59 ramets per microcosm, while only 33 ramets became established when inoculated with *Glomus geosporum* (BEG 18). The plants inoculated with the mixture of AMF isolates formed an intermediate number of ramets compared to the single AMF treatments, but significantly more compared to plants inoculated with *Glomus geosporum* (BEG 18) (Fig. 1a). There was a significant effect of different AMF on the number of ramets produced per gram of aboveground *P. vulgaris* biomass ($F_{4,28} = 4.086$, $P \leq 0.0099$; Fig. 1b). Thus, the effects of different AMF isolates on the formation of ramets were independent of AMF effects on biomass of *P. vulgaris.*

TABLE 2. (A) Results of ANOVA on principal components from principal component analysis (PCA) and (B) eigenvalues for each variable used in the PCA.

A) ANOVA results

Source of variation	Principal component 1†	Principal component 2†
AMF treatment	7.06‡ (14.6%)	7.34‡ (13.0%)
Plant genotype	3.89‡ (1.6%)	13.15‡ (5.9%)

B) Eigenvalues

Variable	PC1 eigenvalues§	PC2 eigenvalues§
Total dry mass	0.400	−0.412
Stolon dry mass	0.370	−0.150
Leaf dry mass	0.345	−0.268
Root dry mass	0.225	−0.588
Leaf number	0.346	0.151
Total number of stolons	0.367	0.205
Total stolon length	0.355	0.281
Mean stolon length	0.110	0.180
Spacer length	0.240	0.400
Number of ramets	0.354	0.151
Number of ramets: plant biomass	−0.07	0.11

† *F* ratios and levels of significance from the analysis of variance for the effect of AMF treatment (4 levels) and plant genotype (17 levels) on two variables that were composed of the scores for each plant calculated using the first two principal components from a principal component analysis comprising 11 variables of plant growth. Numbers in parentheses represent the percentage of total variation in the data set that is explained by the effect.

‡ Significance for the *F* ratios are $P \leq 0.0005$ with 3 and 16 degrees of freedom as numerator for the AMF and plant genotype effect, respectively. Denominator degrees of freedom was 48 for both effects.

§ The PCA was performed on 11 variables, and the eigenvalue for each of the variables for the first two principal components is shown.

The main aim of experiment 1 was to test whether different AMF affect the clonal reproduction of *P. vulgaris* when coexisting with other plants in a community. Although it was not a central aim of experiment 1 to test for main plant genotype or genotype × AMF interactions, it was possible because measurements were made on individual plants. There was a significant plant genotype effect on both the number of ramets per plant and the number of ramets formed per gram of aboveground *P. vulgaris* biomass. *F* ratios for the ANOVA were $F_{4,12} = 24.42$, $P \leq 0.0001$ and $F_{4,12} = 15.32$, $P \leq 0.0001$, for ramet number and ramet number per gram of aboveground dry mass, respectively. There were no significant AMF × plant genotype interactions for these variables. A more detailed investigation of different AMF and plant genotype effects on the growth of *P. vulgaris* was made in experiment 2.

Experiment 2: Effects of different AMF and P. vulgaris *genotypes on clonal growth*

MANOVA revealed highly significant main effects of block, AMF treatment, and plant genotype on the 11 variables of plant growth (Table 1). There was no significant AMF × plant genotype interaction. Principal component analysis was performed on the 11 variables of plant growth and the first two principal components explained 57% and 15% (sum = 72%) of the variation, respectively. The eigenvectors showed that the weightings of variables for plant dry mass were high in principal component 1 and that weightings for spacer length, stolon length, and stolon number were high for principal component 2 (Table 2). Scores for each plant for the first two principal components, which are not correlated, were used as dependent variables for ANOVA. The ANOVA showed highly significant main AMF and plant genotype effects (Table 2). For both of these variables, the AMF effect accounted for a greater percentage of the total variation than the plant genotype effect. There was no significant AMF × plant genotype effect on the two variables constructed from

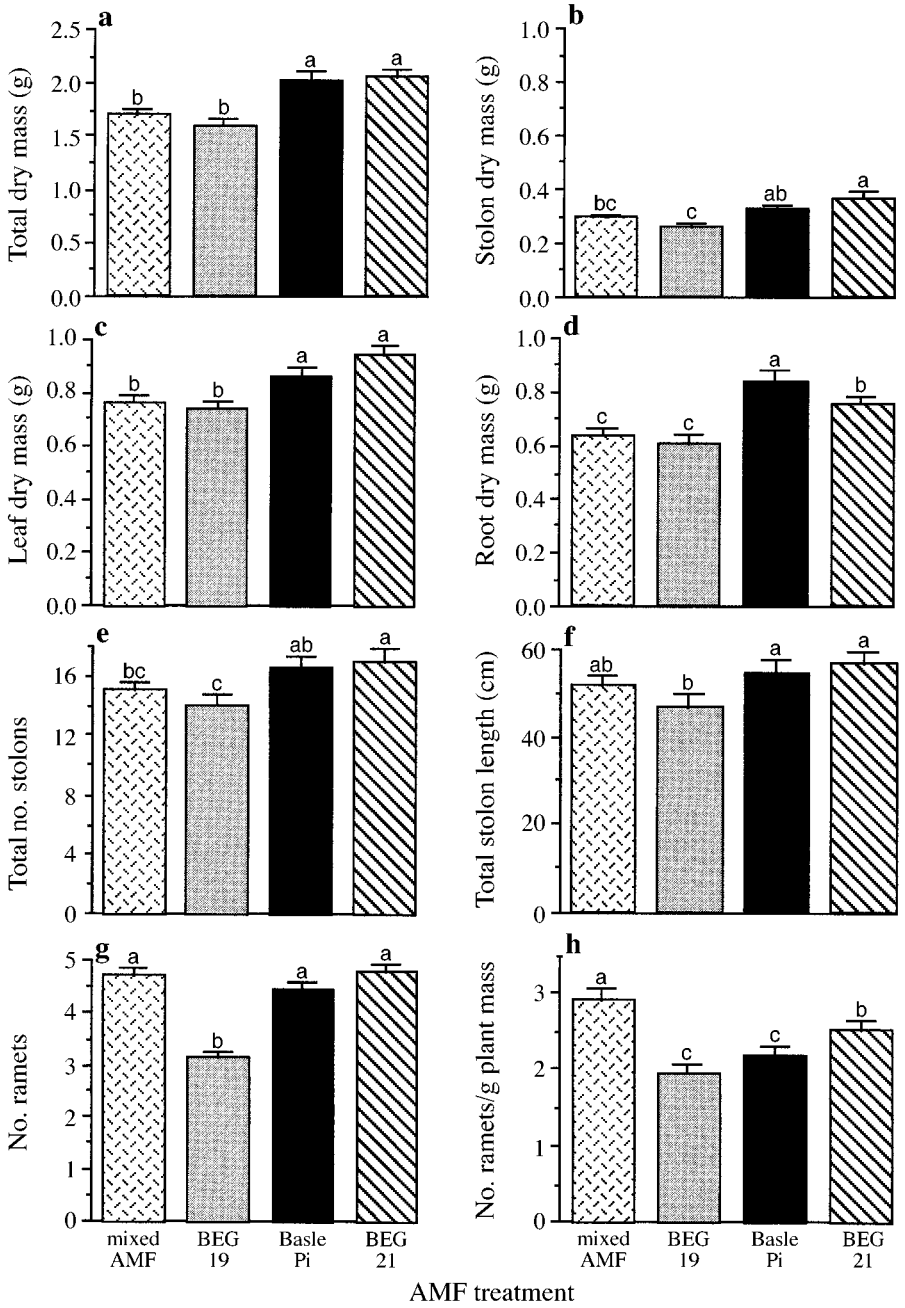

FIG. 2. Mean (a) total plant mass, (b) stolon mass, (c) leaf mass, (d) root mass, (e) number of stolons, (f) total stolon length, (g) number of ramets, and (h) number of ramets per gram aboveground biomass in *P. vulgaris* inoculated with one of the three AMF *Glomus* isolates or a mixture of all three AMF *Glomus* isolates in experiment 2. Bars show +1 SE. Different letters above bars indicate significant difference according to the LSD test ($P \leq 0.05$).

the principal component analysis (*F* ratios from the ANOVA were $F_{48, 248} = 0.81$ and 0.84, $P \leq 0.81$ and 0.76, for principal components 1 and 2, respectively).

The biomass and formation of ramets by different genotypes of *P. vulgaris* were strongly and significantly influenced by the different AMF isolates (Fig. 2, Table 3). At the time of harvest, plants inoculated with *Glomus* sp. (Basle Pi) and *Glomus* sp. (BEG 21) were larger than plants inoculated with either *Glomus* sp. (BEG 19) or the mixed AMF treatment (Fig. 2a). The differential effect of AMF isolates was also observed in the allocation of biomass to the stolons and leaves of *P. vulgaris* (Fig. 2b, c). Allocation of biomass to roots was higher in *P. vulgaris* plants inoculated with *Glomus* sp. (Basle Pi) than in plants of any other AMF treatment (Fig. 2d). The significant effects of inoculation with

TABLE 3. *F* ratios and levels of significance from the analysis of variance for the effect of AMF treatment (4 levels) and plant genotype (17 levels) on 11 variables of growth of *P. vulgaris* and on variables of root colonization by AMF in experiment 2.

Variable	AMF treatment	Plant genotype
Total dry mass	10.14*** (22.4%)	2.58* (1.0%)
Stolon dry mass	10.31*** (18.2%)	1.84^{NS} (0.5%)
Leaf dry mass	6.84*** (21.3%)	2.89* (1.8%)
Root dry mass	13.90*** (22.1%)	4.08*** (1.1%)
Leaf number	6.77*** (16.4%)	8.45*** (4.9%)
Total number of stolons	3.22* (7.0%)	6.98*** (4.4%)
Total stolon length	4.12* (7.9%)	5.25*** (2.5%)
Mean stolon length	2.44^{NS} (5.5%)	4.54*** (3.5%)
Spacer length	0.83^{NS} (0%)	12.49*** (8.7%)
Number of ramets	12.26*** (35.3%)	4.08*** (2.3%)
Number of ramets: plant biomass	14.46*** (31.9%)	4.06*** (1.7%)
Mycorrhizal colonization (% root length)	38.74*** (55.5%)	0.55^{NS} (0%)

Notes: Numbers in parentheses represent the percentage of total variation in the data set that is explained by the effect.

* $P \leq 0.05$; *** $P \leq 0.001$; NS, not significant. The significance levels are for the *F* ratios, with 3 and 16 degrees of freedom as numerator for the AMF and plant genotype effect, respectively. Denominator degrees of freedom were 48 for both effects.

different AMF isolates were also manifested in the number and length of stolons, although these effects were only marginally significant (Table 3; Fig. 2e, f). Inoculation with different AMF strongly affected the number of ramets and this followed the same trend as the effects of stolon number and length. *P. vulgaris* plants inoculated with *Glomus* sp. (BEG 19) produced the fewest stolons, the smallest total stolon length, and the smallest number of ramets. Plants inoculated with *Glomus* sp. (Basle Pi) and *Glomus* sp. (BEG 21) produced more stolons and a greater total stolon length than plants inoculated with *Glomus* sp. (BEG 19). Plants in the mixed AMF treatment produced an intermediate number of stolons, an intermediate total stolon length compared to single AMF treatments, and a significantly larger number of ramets than plants inoculated with *Glomus* sp. (BEG 19). The significant effect of different AMF isolates on clonal reproduction, as measured by ramet number, was independent of the effects of the AMF isolates on biomass (Fig. 2h). Plants inoculated with *Glomus* sp. (BEG 21) produced a greater number of ramets per gram plant biomass than plants inoculated with the other two single AMF isolates. Plants in the mixed AMF treatment were small in size but produced a significantly greater number of ramets per gram of plant biomass than plants in any of the single AMF treatments. Two growth traits that are considered important determinants of the distribution of new ramets are spacer length and mean stolon length. Neither of these two variables were significantly affected by AMF treatment.

Multivariate analyses were used to test whether the AMF effects were the same throughout the duration of the experiment (von Ende 1993). MANOVA was performed on three leaf number variables recorded at three times: 30, 63, and 126 d following inoculation. MANOVA was also performed on RGR calculated for the three time intervals when measurements were made between the beginning of the experiment and the final harvest. The analyses showed a highly significant AMF effect on leaf number and RGR, meaning that leaf number and RGR differed according to AMF treatment when the different measurement times are considered simultaneously. There was a significant AMF effect on leaf number and RGR in this analysis. The Pillai's trace test gave approximate-$F_{9, 762} = 12.75$, $P \leq 0.0001$ and approximate-$F_{9, 762} = 10.97$, $P \leq 0.0001$, for leaf number and RGR, respectively. A profile analysis was used to test whether the response curves (change in leaf number and change in RGR) were the same with each AMF treatment throughout the experiment (von Ende 1993). This is also known as a test of parallelism. Profile analysis was performed by running MANOVA using two variables that were transformed to reflect the differences or contrasts in leaf number and RGR between times (von Ende 1993). The Pillai's trace test gave approximate-$F_{6, 508} = 5.03$, $P \leq 0.0001$ and approximate-$F_{6, 508} = 16.84$, $P \leq 0.0001$, for leaf number and RGR, respectively. A significant effect in this analysis is analogous to a significant AMF treatment $\times$ time interaction in a repeated-measures ANOVA and indicates that the effect of the different AMF changed over the duration of the experiment. There was a significant AMF treatment effect on leaf number at 30, 63, and 126 d after inoculation according to ANOVA performed on the individual variables of leaf number at each time of measurement (Fig. 3). Thirty days after inoculation the effect of the mixed AMF treatment was not significantly different from the effect of *Glomus* sp. (BEG 21). At this time, the leaf number of plants in these two treatments was significantly higher than in plants inoculated with *Glomus* sp. (Basle Pi) or with *Glomus* sp. (BEG 19). Sixty-three days after inoculation plants inoculated with the mixture of AMF isolates were most similar in leaf number to plants inoculated with *Glomus* sp. (Basle Pi). They had significantly few-

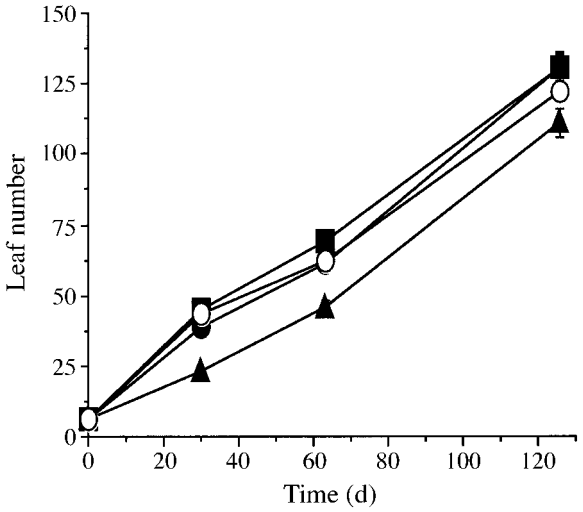

FIG. 3. Mean leaf number of *P. vulgaris* during experiment 2. Plants were inoculated with one of the three AMF isolates or a mixture of all three AMF isolates: ○ = mixed AMF, ▲ = *Glomus* sp. (BEG 19), ● = *Glomus* sp. (Basle Pi), ■ = *Glomus* sp. (BEG 21). Bars show ±1 SE. Some error bars are too small to be shown. *F* ratios for the AMF treatment effect were $F_{3,48} = 39.03$, $P \leq 0.0001$; $F_{3,48} = 21.28$, $P \leq 0.0001$; and $F_{3,48} = 6.77$, $P \leq 0.001$ (at 30, 63, and 126 d after planting, respectively).

er leaves compared to plants inoculated with *Glomus* sp. (BEG 21), but significantly more leaves than plants inoculated with *Glomus* sp. (BEG 19). At the final harvest, after 126 d, plants inoculated with the mixture of AMF isolates, *Glomus* sp. (BEG 21) or *Glomus* sp. (Basle Pi) had significantly more leaves than *Glomus* sp. (BEG 19).

The fungi in the different AMF treatments grew significantly differently in the roots of *P. vulgaris* (Table 3, Fig. 4). Colonization in the mixed AMF treatment was not significantly different from colonization by *Glomus* sp. (BEG 21), the AMF with the highest level of colonization. The fungi in these treatments grew significantly more than *Glomus* sp. (BEG 19) or *Glomus* sp. (Basle Pi). The levels of AMF colonization with the different AMF isolates were not significantly correlated with any of the variables of plant growth.

All the variables of plant growth were signifcantly affected by plant genotype, except for stolon dry mass (Table 3). The effect of plant genotype on total dry mass and leaf dry mass was only marginally significant and accounted for a very small percentage of the variation in these two variables. Mycorrhizal colonization was also not significantly different among plant genotypes (Table 3). Plant genotype effects were also shown to differ during the experiment. A profile analysis was used to test whether the response curves (change in leaf number and change in RGR) were the same with each genotype throughout the experiment (von Ende 1993). This was performed by running MANOVA using two variables constructed to reflect the differences or contrasts in leaf number and RGR between times (von Ende 1993). The Pillai's trace test gave approximate-$F_{32, 508} = 2.32$, $P \leq 0.0001$ and approximate-$F_{32, 508} = 1.46$, $P \leq 0.048$, for leaf number and RGR, respectively. This is analagous to a treatment × time interaction in a repeated-measures ANOVA (von Ende 1993). This indicates that the effect of the different genotypes on leaf number and RGR changed over the duration of the experiment.

Means among plant genotypes are not presented for the variables where a significant plant genotype effect occurred since this was an expected result, which has previously been documented for this species in other publications (Schmid 1985*b*, Schmid et al. 1996). The inclusion of this factor in the experiment allows a comparison of the relative variation in these traits that is accounted for by effects of different AMF and plant genotypes and to establish whether interactions between these two factors occur.

Effects of plant genotype vs. AMF effect on plant growth

The amount of variance in the experiment that was explained by AMF effects was greater than that explained by plant genotypic effects for the principal component scores and all of the individual variables except for spacer length (Tables 2 and 3). Variables for which AMF accounted for a particularly large part of the variation were those reflecting the size of *P. vulgaris* and those that are a measurement of clonal reproduction, namely dry mass, ramet number, and the number of ramets formed per gram plant tissue. The amount of variation accounted for by different AMF was lower in variables that determine the position of new ramets, namely stolon number, total stolon length, mean stolon length, and spacer length, than for the other variables.

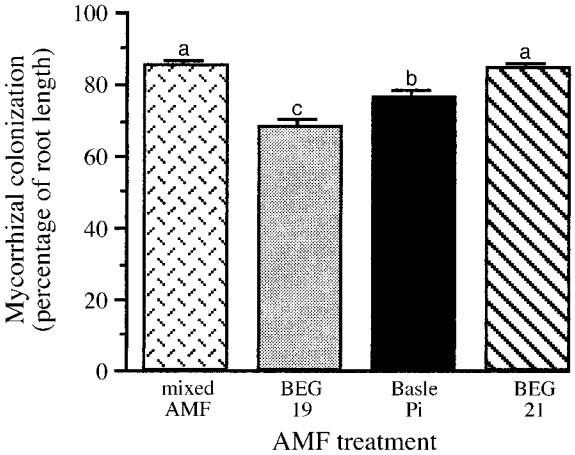

FIG. 4. Mean percentage root length of *P. vulgaris* colonized by AMF in experiment 2. Bars show +1 SE. Different letters above bars indicate significant difference according to the LSD test ($P \leq 0.05$).

There were no significant interactions between the different AMF and plant genotypes for any of the variables of plant or fungal growth from either the ANOVA or from any of the MANOVA.

Discussion

Effects of different AMF on clonal reproduction

The number and size of ramets and, therefore, the fitness of *P. vulgaris* was strongly determined by different co-occurring AMF. AMF are known to affect plant mineral nutrition and improve plant growth (Smith and Read 1997), and the biomass of *P. vulgaris* was significantly affected by different AMF in both experiments. An obvious explanation for the AMF effects on clonal growth is that AMF affect plant biomass and that the number of ramets is dependent on plant biomass. However, the effects of different AMF on ramet number were independent of AMF effects on plant biomass in both experiments.

Previous published work showing AMF effects on clonal growth traits only considered the responses of *P. vulgaris* growing individually in pots (Streitwolf-Engel et al. 1997). This study not only confirms the results of this earlier work, it also demonstrates that the AMF effects on ramet formation were large enough to significantly affect the population size of *P. vulgaris* ramets growing in a heterogeneous environment, where each ramet was surrounded by neighboring plants of the microcosm community. One possible explanation is that the differential effect of AMF on the growth of *P. vulgaris* was due to a reduced growth of some of the other plant species in the communities with certain AMF, i.e., that the AMF effect on *P. vulgaris* was indirect. The lack of any significant negative correlations between the biomass of *P. vulgaris* and the other single species in the microcosms suggests that such a simple indirect effect of AMF on the growth of *P. vulgaris* is not the explanation and that indirect effects are more complex.

The observed AMF effects were on the number and size of ramets. Thus, AMF affected the reproduction and the architecture of *P. vulgaris.* These architectural effects could be important for *P. vulgaris* to occupy open spaces in a plant community by placing new ramets in available sites and to compete with other plants in the community. An obvious mechanistic explanation for the results is that AMF improve the resource capture of *Prunella,* leading to improved growth and consequently improved clonal reproduction. However, in this experiment and in experiment 2, the effects cannot clearly be explained by improved growth leading to improved clonal reproduction. The architectural effects were independent of the size of *Prunella.* Thus, the effects of AMF on clonal growth of *Prunella* cannot simply be attributed to AMF effects on resource capture.

Variation in clonal growth explained by AMF differences vs. plant genotypes

We found that the amount of variance in *P. vulgaris* growth and reproduction caused by different AMF treatments was greater than the amount explained by plant genotypic variance for most variables. AMF were found to be a more important factor explaining total phenotypic variance in size and ramet formation than variation between the different plant genotypes. The most important variables determining clonal growth and its spatial distribution are the number of ramets, the size of ramets, spacer length, and stolon length. The number of ramets, as well as the mean size of the ramets, was more strongly affected by AMF treatment, whereas spacer length and mean stolon length were only determined by the genotype of the plant. This indicates that some patterns of clonal growth are genetically determined and are not modified by different AMF while others are. The results indicate that AMF strongly determine the number of ramets and their size, but that spacer and stolon are likely to be determined by the genotype of the plant, through genotypic effects. By the joint effects of AMF and genotypes on ramet formation and spacer lengths, the architecture of clones may be affected, leading some genets to have tightly packed ramets while others have few, loosely aggregated ramets. These characteristics have been shown to influence the competitive and colonization ability of clonal plants (Harper 1985, Schmid 1986).

The results of experiment 2 show that a potentially large portion of the total phenotypic variance observed in a clonal plant population is due to effects of different AMF. To what extent the different AMF add to the total variance in clonal reproduction in natural communities depends strongly on the distribution of AMF in the field. If the different AMF in an ecosystem are not homogeneously distributed, due to varying microhabitats or to varying interactions with host plants, then through their spatial distribution AMF have the potential to cause variation in clonal reproduction. Little information exists on diversity of AMF in natural communities or its spatial arrangement. However, evidence exists that different plant species differentially affect life history traits of different AMF species, namely degree of colonization and rates of sporulation (Sanders and Fitter 1992, Bever et al. 1996). Thus, in a plant community the relative distribution of AMF taxa could be heterogeneous due to the distribution of the different plant species in the community and their effects on different AMF taxa. At present, more information is required on AMF community structure to make further interpretations of these effects.

Response of different genotypes to different AMF isolates

Genotypes of clonal plants have been shown to vary in their response to their abiotic and biotic environment

(Cheplick 1995, 1997, Skálová et al. 1997). In a heterogeneous environment this effect is important for the maintenance of intraspecific genetic diversity (Silander 1985, Gillespie and Turelli 1989). In our study the effect of different AMF isolates on clonal growth of *P. vulgaris* was independent of the genotypic effects. This was shown by the lack of any significant AMF × genotype effect in experiment 2. Different co-occurring AMF will, therefore, not directly contribute to the maintenance of intraspecific diversity of *P. vulgaris.*

Coevolutionary aspects

The lack of an AMF × plant genotype interaction also has consequences for understanding coevolution in the mycorrhizal symbiosis. A prerequisite for coevolution of AMF and *P. vulgaris* to occur is the existence of a mechanism by which a plant's clonal offspring would be colonized by the same AMF or its offspring. This mode of transmission of AMF to the plant's clonal offspring is indeed possible since the AMF used in this experiment have been shown to grow up to 14 cm away from the roots of *P. vulgaris* (Sanders et al. 1998), a distance that is well within the range of new ramet formation from the mother plant. However, a genetic basis for further coevolution in the symbiosis would require that certain genotypes in the population would be at a selective advantage, i.e., fitter, when forming a symbiosis with certain AMF. Our experiments provide no evidence of this since there was no AMF × plant genotype interaction on growth or clonal reproduction. Furthermore, a beneficial combination of plant genotype and AMF would also have to provide an increased fitness to the fungus, as well as the plant, for the two partners to coevolve. One possible measure of fungal fitness is the amount that it colonizes roots. In experiment 2, there were no plant genotypic effects on AMF growth in the roots of *P. vulgaris.* We conclude that there is no evidence for selection pressures that would currently favor rapid coevolution in this symbiosis. However, the absence of an AMF × plant genotype interaction does not mean that coevolution is not proceeding, only that it is unlikely to be proceeding rapidly. One further possibility is that because AMF are thought to colonize many different individual plants simultaneously, other species in the community could have stronger influences on the coevolution of AMF with plants. *P. vulgaris* co-occurs with the dominant plant of calcareous grasslands, *Bromus erectus.* This species can also be colonized by the AMF used in this study and therefore, due to its higher frequency, the interactions with this species may exert a stronger effect on coevolution than does *P. vulgaris.*

Effects of an AMF community on clonal growth

In natural communities plants are confronted with a mixture of different AMF species and indeed multiple colonization of roots is known to occur (Rosendahl et al. 1990, Clapp et al. 1995). In both experiments, when plants were inoculated with a mixture of AMF isolates, they reached an intermediate biomass compared to the single AMF treatments. This indicates that the effects of the different isolates in the roots of an individual may give an effect that is an average of the effects of the individual isolates. However, ramet number and the number of ramets per gram plant biomass in plants inoculated with the mixed AMF treatment in experiment 2 were significantly greater than in plants inoculated with some of the single AMF, rather than being an average of the single AMF isolate effects. The effects of the mixed AMF treatment compared to single AMF treatments on growth of *P. vulgaris* also changed during the course of the experiment, indicating that a community of AMF induces a more variable effect over time on plant growth than the single AMF isolates. The effect of a community of AMF on plant clonal growth cannot, therefore, be predicted from the effect of single AMF isolates on plant growth.

Conclusions

Different co-occurring AMF have a strong effect on the clonal growth and fitness of *P. vulgaris.* We conclude that these effects are of potential ecological significance for the population biology of *P. vulgaris* since the effect of different co-occurring AMF on clonal growth occurs when *P. vulgaris* is growing in a plant community and because the effect is as strong as the plant genotypic effect for important fitness traits. The AMF in an ecosystem should, therefore, not be considered as a uniform group of fungi as they differ so strongly in their fitness effects. The composition of AMF populations, their diversity, and the distribution of AMF have the potential to determine clonal growth and propagation of clonal plants.

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