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ORIGINAL PAPER

Which role can arbuscular mycorrhizal fungi play in the facilitation of *Ambrosia artemisiifolia* L. invasion in France?

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Abstract *Ambrosia artemisiifolia* L. (common ragweed), an annual invasive plant, was introduced more than 100 years ago from North America to Europe. Like the majority of other invasive plants in Europe, it develops in open, disturbed areas such as fields, wastelands, roadsides, and riverbanks. Recently, arbuscular mycorrhizal fungi (AMF) have been suspected to play a role in some plant invasion processes. As the common ragweed is known to be colonized by AMF in its native range, the intensity of mycorrhizal root colonization was studied in 35 natural populations in eastern France. About 94% of the *A. artemisiifolia* populations sampled were mycorrhizal. Root colonization levels varied from 1 to 40% depending on the ecological sites, with lower levels for agricultural habitats and higher levels in disturbed sites, such as wastelands or roadsides. A subsequent greenhouse experiment showed positive impacts of AMF on the growth and development of *A. artemisiifolia*. It is proposed that the spread of this invasive plant species could be facilitated by AMF, underlining the need to integrate symbiotic interactions in future work on invasive plant processes.

Keywords *Ambrosia artemisiifolia* · Arbuscular mycorrhizal fungi · Invasive plant · Growth · Development

Introduction

The invasion of alien plants can cause a serious threat to native ecosystems and economics (Pimentel 2002), and is the second cause of biodiversity losses after habitat destruction (Vitousek et al. 1997). Species invasions are favored by the vulnerability of the invaded ecosystems (Sakai et al. 2001), the multiplication of introductions by human exchanges (Williamson 1996), or due to their own genetic, biological, physiological, and ecological attributes (Roy 1990; Prinzing et al. 2002). Works focussing on ecological attributes base invasion success by alien plants on an enemy release hypothesis, where introduced species escape their natural enemies, thus giving them an advantage (Maron and Vila 2001; Keane and Crawley 2002). Other studies (Richardson et al. 2000; Klironomos 2002; Rudgers et al. 2005) have dealt with the potential role of the arbuscular mycorrhizal (AM) symbiosis to facilitate plant invasion in new areas. Most invasive plants are mycotrophic and thus preadapted to successfully establish in habitats containing AM fungi (AMF). Given the widespread distribution of AMF and their low host-plant specificity, mutualistic associations could favor invasion of many ecosystems by alien plants (Richardson et al. 2000).

In Europe, one of the most problematic invasive species is *Ambrosia artemisiifolia* L. (common ragweed), an annual plant belonging to the Asteraceae family. Introduced from North America to Europe in the middle of the 19th century (Heckel 1906), this species is now widespread in numerous countries (Clot et al. 2002; Török et al. 2003), and particularly in France (Guillerm et al. 1990; Muller 2000). *Ambrosia artemisiifolia* has been introduced in France at various times since 1865 and is now largely widespread along the Rhone valley on a North–South axis (Chauvel et al. 2006). It not only invades a broad range of open disturbed areas, such as wastelands, roadsides, and riverbanks, but also field crops such as sunflower, soybean, maize, cereal crop stubble, or

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set-asides (Chollet et al. 1999). This plant species is also a serious threat to human health because of its abundant allergenic pollen release (Bassett and Crompton 1975; Déchamp and Méon 2002; Laaidi et al. 2003). *Ambrosia artemisiifolia* was extensively studied in its native area, but very few studies focused on the invasive process of the species in Europe, and particularly in France (Genton et al. 2005a,b; Chauvel et al. 2006).

Crowell and Boerner (1988) describe *A. artemisiifolia* as an obligately mycorrhizal plant in the USA. Koide and Li (1991) demonstrated that AMF facilitates phosphorus uptake in *A. artemisiifolia*, and Schreiner and Koide (1993) showed that this plant species was able to stimulate germination of *Glomus intraradices* spores. The ability to form mycorrhiza could explain why *A. artemisiifolia* is one of the dominant species in disturbed ecosystems in the USA (Medve 1984). Caussanel et al. (2000) and Déchamp and Caussanel (2002) demonstrated that *A. artemisiifolia* introduced in France is colonized by AMF and discussed their potential role in the context of plant control. Moreover, Fumanal et al. (2004) pointed to the role of AMF in facilitating *A. artemisiifolia* invasion. The aim of this work was to evaluate the mycorrhizal status of natural populations of *A. artemisiifolia* in France and the effect of AMF on their growth and development.

Materials and methods

Field study

Roots of 671 individuals over 35 natural *A. artemisiifolia* populations were collected in 2004 from adult plants before the reproductive stage. These populations were sampled from six habitat types in French areas where *A. artemisiifolia* was at first introduced and from more recent spread areas (Table 1). Populations were located in plains, except population #41, which was situated at 1,100 m of altitude.

Samples of about 2 g of fresh fine roots were collected on uprooted *A. artemisiifolia* plants, washed with water, cleared in 10% KOH for 1 h at 90°C, and then stained for 15 min in a lactoglycerol solution containing 0.05% of acid fuchsin (Berch 1979) or trypan blue (Phillips and Hayman 1970). For each sample, about 50 stained root fragments were randomly selected and checked through binoculars (50×). The intensity of AMF colonization (%*M*) was scored for each root sample, as the percentage of root fragment showing mycorrhizal structures (vesicles, intercellular hyphae, and arbuscules) using %*M*=(Nm/Nr)×100, where Nm is the number of root segments with mycorrhizal fungi and Nr is the total number of root segments scored. This technique, quite similar to the gridline-intersect method (Giovannetti and Mosse 1980), was chosen due to the high number of root samples to be analyzed. For comparison purposes, both methods were used on several root samples, and showed similar results (data not shown). When the identification of arbuscules structures was difficult with binoculars, they were checked with a microscope (200×). The frequencies of AMF colonized plants (%*P*) were also calculated for each *A. artemisiifolia* population according to %*P*=(Np/*N*)×100, where Np is the number of *A. artemisiifolia* showing AMF root colonization and *N* is the total of analyzed plants per population.

Subsequently, ten core samples of soil (0.2 L) were randomly collected around ten *A. artemisiifolia* plants in the site showing the most mycorrhizal plant population (#25), with the aim of identifying the AMF. Spores of AMF were extracted by wet sieving on a 50-µm-mesh sieve and by the flotation-bubbling method (Furlan and Fortin 1975) for identification.

The effect of habitat type on %*M* and %*P* was checked using a one-way ANOVA, after arcsin transformation of values before statistical analyses to homogenize the residual variance. The correlation between %*M* and %*P* among all populations was analyzed using the Pearson product-moment correlation coefficient. All statistical analyses were performed with SYSTAT 11® software for Windows ($p<0.05$).

Greenhouse experiment

Plant and fungal materials

Four natural populations of *A. artemisiifolia* (#40, #14, #4, and #27), among the 35 analyzed previously (Table 1), were chosen to analyze AMF–*A. artemisiifolia* relations. Populations were selected according to their geographical distribution (from center to periphery of the *A. artemisiifolia* distribution in France) and to their contrasted habitat of origin (crop field, ruderal, and natural habitats). Mature seeds of the four populations used in the experiment were collected plant-by-plant from 20 mother plants in October 2003. Fifteen seeds from each mother plant were put on germination paper in Petri dishes and stratified for 2 weeks (wet dark stratification at 4°C) to break primary dormancy. After this treatment, seeds were placed in a germination room (24°C/11 h day, 15°C/13 h night). Two young seedlings from each mother plant were transplanted in noninoculated (NM) and AMF-inoculated (M) substrate. Only the seedlings at the same early stage of development (cotyledon), and germinating at the same time, were transplanted.

The AMF *G. intraradices* Schenck & Smith (DAOM 181602, Ottawa Agricultural Herbarium) was chosen as the inoculant fungus for its ability to form abundant internal vesicles and its growth-promoting effect on various plants

Table 1 Locations and habitats in France of natural populations of *Ambrosia artemisiifolia* analyzed for AM colonization (*N* number of plants sampled)

Population ID	Location	Habitat	Longitude (N)	Latitude (E)	*N*
8	Saint-Donat	Orchard	4° 54′ 10″	45° 08′ 24″	24
10	Salindres	Orchard	4° 12′ 11″	44° 11′ 29″	21
12	Cabiac	Field crop	4° 19′ 55″	44° 17′ 22″	20
40	Concoeur et corboin	Field crop	4° 57′ 44″	47° 11′ 07″	20
39	Urcy	Field crop	4° 51′ 02″	47° 15′ 33″	20
33	Nievroz	Field crop	5° 03′ 59″	45° 48′ 39″	20
3	Romans-sur-Izère	Field crop	5° 00′ 04″	45° 03′ 10″	18
7	Allex	Field crop	4° 56′ 28″	44° 44′ 27″	19
11	Barjac	Field crop	4° 19′ 11″	44° 18′ 40″	20
14	Mézieu	Field crop	5° 04′ 43″	45° 43′ 57″	19
17	Mézieu	Field crop	5° 04′ 28″	45° 44′ 05″	20
36	Asnan	Field crop	3° 32′ 25″	47° 18′ 29″	20
28	Bey	Field crop	4° 56′ 03″	46° 47′ 34″	20
6	Allex	Fallow	4° 55′ 16″	44° 44′ 10″	16
35	Asnan	Fallow	3° 32′ 56″	47° 18′ 27″	20
30	Bey	Fallow	4° 56′ 28″	46° 47′ 43″	20
29	Bey	Fallow	4° 56′ 25″	46° 47′ 39″	20
2	Romans-sur-Izère	Fallow	5° 00′ 04″	45° 03′ 10″	20
4	Allex	Riverbank	4° 56′ 40″	44° 44′ 27″	32
5	Allex	Riverbank	4° 56′ 40″	44° 44′ 27″	14
9	Alès	Riverbank	5° 05′ 52″	44° 06′ 17″	17
31	Nievroz	Riverbank	5° 03′ 59″	45° 48′ 39″	20
24	Labergement	Wasteland	5° 14′ 27″	47° 14′ 34″	9
23	Labergement	Wasteland	5° 14′ 27″	47° 14′ 34″	20
26	Spoy	Wasteland	5° 11′ 46″	47° 26′ 41″	20
22	Labergement	Wasteland	5° 14′ 27″	47° 14′ 34″	10
27	Lux	Wasteland	5° 12′ 56″	47° 27′ 03″	22
38	Magnien	Wasteland	4° 26′ 19″	47° 06′ 37″	20
25	Labergement	Wasteland	5° 14′ 27″	47° 14′ 34″	20
37	Brinon sur Beuvron	Wasteland	3° 28′ 21″	47° 17′ 49″	20
20	Mézieu	Wasteland	5° 04′ 45″	45° 43′ 41″	20
21	La Boisse	Wasteland	5° 01′ 49″	45° 49′ 41″	19
32	Miribel	Roadside	4° 56′ 41″	45° 49′ 03″	20
41	Col du Cucheron	Roadside	5° 50′ 11″	45° 22′ 07″	11
13	Rivieres	Roadside	4° 16′ 30″	44° 14′ 8″	20

(Furlan et al. 1983). The fungus was propagated on *Allium porrum* L. grown for 12 weeks under greenhouse conditions on calcined clay, Oil-Dri US-special Typ/IIIR (Oil-Dri Company, Chicago, USA), which is an attapulgite commonly used for the propagation of AMF (Plenchette et al. 1996). *Allium porrum* plants were uprooted and washed, and root systems were cut into pieces 0.5 cm long. A subsample of the total root inoculum was cleared and stained as previously described. The root colonization level obtained with the gridline-intersect method and the number of vesicles per root centimeter were checked to ensure inoculum quality.

Experimental design

Pots (1.2 L) were filled with the Oil-Dri substrate. A slow release fertilizer (Nutricote, N–P–K/14–8–8) was added and mixed to each pot at a rate of 3.6 g/L (Waterer and Coltman 1989). Mycorrhizal *A. porrum* root pieces (250 mg dry weight), air-dried 24 h, were placed 3–4 cm below the substrate surface for inoculated (M) pots. The same quantity of autoclaved (25 min at 110°C) AMF inoculum was placed in NM pots to introduce the same quantity of organic matter in control and treated pots. After transplantation, plants were grown in a greenhouse from 28th March to 8th June 2004 (mean temperature= 19.67±3.94°C, mean humidity=45±16%) with a natural photoperiod. Pots were watered twice a week with tap water without adding any fertilizer, and randomized twice per month.

Plant growth analyses

During the experiment, plant height, width (maximal plant diameter), number of leaves, and nodes were recorded at 16, 31, 58, and 72 days after transplanting. After 72 days of growth, plants were uprooted when the release of pollen

began and before the production of mature seed. The root systems, cut from the shoots, were gently washed. The final plant height, width, diameter of the main stem, shoot and root dry mass, and number of leaves were recorded. Dry weight was determined after drying at 80°C for 48 h. Mycorrhizal colonization (%*M*, %*P*) was recorded from a sample of each root system (about 1.2 g of fresh root collected randomly) after acid fuchsin staining, as described above. Plants treated with AMF that did not develop symbiosis were removed from the analysis of results.

The colonization levels of *A. artemisiifolia* by *G. intraradices* (%*M*, %*P*) in the populations were compared using one-way ANOVA and generalized linear model procedures (GLM). Growth parameters were analyzed as a dependant variable by two-way ANOVA (GLM), with AMF and populations considered as fixed effects and individuals (nested within the population) as a random effect. The evolution of plant height, width, and number of leaves over time was analyzed using a two-way ANOVA with repeated measures (GLM). The models were the same as those used previously, but included repeated measures of height integrating unequally spaced measurements (16, 31, 58, and 72 days). In addition, a general contrast analysis was performed on these models to determine the stage at which curves of M/NM plant development began to differ.

Relative growth rate (RGR) was calculated to compare the relative performance of plants as RGR=(ln LHWt_2−ln LHWt_1)/(t_2−t_1), where LHW is a function of number of leaves×plant height×plant width, expressed in centimeters squared at final (t_2) and initial (t_1) time (in days after the planting out of seedlings). The LHW was used to calculate RGR instead of plant dry mass because it was a nondestructive measure that was highly positively correlated to shoot dry mass at harvest time (F=311.71, r^2=0.82, p<0.001). The RGR was analyzed with two-way ANOVA (GLM procedure) with AMF treatment and populations as fixed effects and individuals (nested within the population) as a random effect. Linear regression was applied to determine whether significant differences in root and shoot dry mass were due to functional shifts in allocation patterns or due to differences in growth among AMF treatments. Slope differences were tested using the analysis of covariance with AMF treatments as fixed effect and shoot dry mass as a covariate.

Developmental analysis

The rate of leaf appearance on the main axis (phyllochron), defined as the time interval between the appearance of two successive levels of leaves, was used to characterize the vegetative development of *A. artemisiifolia*. Phyllochron (P) was calculated as the mean number of appeared leaves, and time was expressed in degree-days, P=(Ldd$_2$−Ldd$_1$)/(dd$_2$−dd$_1$), where L is the average of leaf nodes (one leaf node=two opposite leaves or one leaf when they become alternate), at thermal time dd$_2$ and dd$_1$ (cumulative degree-days). The base temperature used for phyllochron calculation was Tb=0°C (Deen et al. 2001). The number of leaf nodes until the appearance of the first male raceme was used to calculate the phyllochron.

The number of plants with male racemes used as an indicator of the beginning of reproductive phase was recorded 58 days after transplanting. Data were analyzed as previously using two-way ANOVA (GLM) with populations and treatments as fixed effects and individuals (nested within the populations) as a random effect.

Results

Field observations

Three AMF species: *Glomus mosseae*, *Glomus constrictum*, and *Scutellospora* sp. (Fig. 1a–c), were identified by Y. Dalpé (Agriculture and Agri-Food Canada, Ontario, Canada) from spores collected around mycorrhizal *A. artemisiifolia* plants in site #25. The AMF colonizing *A. artemisiifolia* roots developed vesicles, intercellular hyphae (Fig. 1d), and arbuscules (*Arum*-type) in cortical cells (Fig. 1e,f).

Almost 94% of *A. artemisiifolia* populations sampled were mycorrhizal, and only two from a crop field and fallow showed no evidence of an AM association. *Ambrosia artemisiifolia* had, on average, 13.2±1.9% of %*M* (from 0 to 40%) and 66±4.4% of %*P* (from 0 to 100%) (Table 2). The averages of %*M* and %*P* by habitat types are presented in Fig. 2. Levels of %*M* were significantly different (F=16.299, p<0.001) between habitat types. The lowest values for %*M* were found for *A. artemisiifolia* populations in orchard and crop field habitats (1.9±0.9, 6.8±1.7%), whereas those from roadsides showed highest values (27±5.8%), and those from fallow, riverbank, and wasteland habitats were intermediate (11.1±5.8, 18.5±3.7, and 17.6±3.9%). Even if values for %*P* seemed to follow a similar pattern of variation, no significant differences were found between habitat types (F=1.983, p=0.111), due to high intra- and interpopulation variations. However, when all populations were considered together, a positive correlation between %*M* and %*P* was found (r=0.67, p<0.001).

The most colonized *A. artemisiifolia* populations were found in the only altitudinal habitat (#41, %*M*=38.4±7.5%) and in a wasteland (#25, %*M*=40.2±7.3%). When different habitat types were considered in a similar geographical locality, populations showed high differences in their %*M* or %*P* (Tables 1 and 2). For example, in the locality of Labergement (populations #22, #23, #24, and #25), four different wasteland habitats were distinguished and

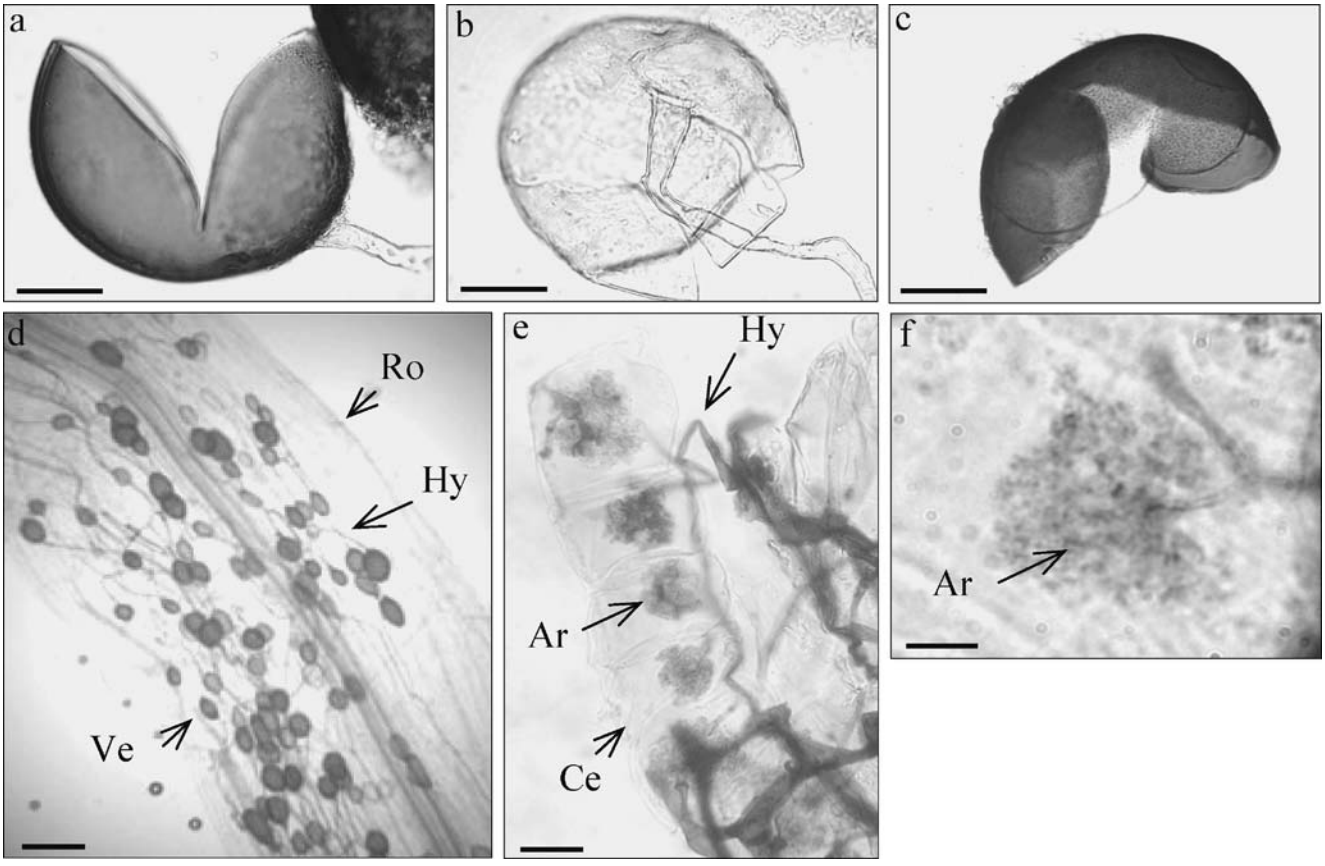

Fig. 1 Microscope observations of AMF associated with *Ambrosia artemisiifolia* in a wasteland habitat (#25) from northern France. Spores isolated from soil surrounding *A. artemisiifolia* roots: **a** *Glomus constrictum*, **b** *Glomus mosseae*, **c** *Scutellospora* sp.; *bars*=50 μm. AMF colonization observed on *A. artemisiifolia* roots: **d** vesicles and intercellular hyphae (stained with acid fuchsin), *bar*=100 μm; **e**, **f** arbuscules in cortical cells (stained with trypan blue), *bar*=20 μm (**e**), 9 μm (**f**). *Ar* arbuscule, *Ce* cortical cell, *Hy* intercellular hyphae, *Ro* root, *Ve* vesicle

A. artemisiifolia sampled from these habitats showed high differences in AMF colonization (%*M*, from 3±1.2% to 40.2±7.3%; %*P* from 44 to 95%). Also, for populations from a same area with equivalent %*P* but habitat differences, large differences in %*M* were found (Asnan, #35, #36; Nievroz, #33, #31; Allex #6, #7, #4, #5). The same trend was observed for Bey populations (#28, #29, #30) with the same %*P* but with higher %*M* levels for an old fallow (#30) compared to a young fallow (#29) and a sunflower field crop (#28).

Greenhouse experiment

The inoculum used for AMF treatments contained about 163 vesicles of *G. intraradices* per root centimeter of *A. porrum*, with a colonization level of 75%. Positive colonization by the AMF in *A. artemisiifolia* roots (%*M*) was checked at the end of the experiment, when the plants were harvested (Table 3). Levels of %*M* and %*P* ranked, respectively, from 3 to 8% and from 77 to 90%, and were not statistically different between populations (%*M*: F=1.66, p=0.189; %*P*: F=0.407, p=0.751).

Growth response of A. artemisiifolia to G. intraradices

Colonization by *G. intraradices* of *A. artemisiifolia* showed a positive effect on final growth parameters, but no effects of populations or treatment×population interaction were observed (Table 4). In each population tested, AMF treatment had a positive impact on the *A. artemisiifolia* growth parameters measured (Fig. 3). Positive impact of *G. intraradices* on plants was higher for root and total plant dry mass than for shoot dry mass or number of leaves, stem diameter, plant height, and width.

The repeated-measures analysis showed significant differences between M/NM plants for three growth parameters over time (height: F=518.352, p<0.001; width: F=1,043.066, p<0.001; number of leaves: F=1,178.179, p<0.001). Significant positive effects of *G. intraradices* inoculation on height (F=4.328, p=0.007), width (F=7.010, p<0.001),

Table 2 Average levels of root colonization of *Ambrosia artemisiifolia* by AMF (%*M*±standard errors) and frequencies of colonized *A. artemisiifolia* individuals (%*P*) in natural populations in France

Population ID	%*M*	%*P*
8	3.9±2.4	42
10	2±0.6	19
12	1.4±0.3	55
40	0	0
39	1±0	10
33	3.9±0.8	80
3	9.3±3.6	56
7	3.8±0.8	63
11	4.7±1.7	60
14	4.4±1	79
17	1±0	20
36	11.8±4.9	50
28	13.1±3.4	95
6	0	0
35	2.7±0.6	50
30	26.8±6.3	100
29	12.7±4.4	95
2	2.2±0.7	25
4	7.8±1.9	50
5	20.2±4.3	64
9	25.6±9.3	59
31	21.5±5.3	95
24	3±1.2	44
23	10.3±5.2	80
26	32.3±5.8	100
22	3.4±1.2	70
27	13.9±3.6	100
38	12.2±6.6	65
25	40.2±7.3	95
37	31.2±7.1	85
20	15.4±4.4	40
21	15.7±6.7	68
32	25±5.6	100
41	38.4±7.5	100
13	17.5±4.2	65

and number of leaves (F=8.907, p<0.001) were observed over time, whereas no effect of populations (height: F=1.973, p=0.051; width: F=1.780, p=0.082; number of leaves: F=1.845, p=0.070) or AMF treatment-by-population interaction (height: F=1.536, p=0.146; width: F=1.025, p=0.426; number of leaves: F=0.898, p=0.530) were detected, suggesting similar growth responses among populations in N/NM treatments. Differences concerning plant height were observed in the last two measures (58–72 days, p=0.016), whereas differences in width (31–58 days, p=0.002) and number of leaves (16–31 days, p<0.001) appeared earlier.

The RGR was significantly higher for M plants, but no effect of population or interaction between population and treatment was found (Table 4, Fig. 4a). The root/shoot relationship between all M and NM plants was allometrically analyzed by linear regression (Fig. 4b). Root/shoot weights were highly correlated, and the slope for M plants (slope=0.4560, r^2=0.82, p<0.0001) was higher than the slope for NM plants (slope=0.3193, r^2=0.90, p<0.0001). Slopes of M/NM linear regressions were significantly different (F=12.11, p=0.001), suggesting that the differences observed are due to the allometric shifts in response to the AMF treatment. Moreover, no significant differences were found between regression line slopes among populations of M (F=0.56, p=0.6454) or NM plants (F=0.42, p=0.7391). Phyllochron values differed between M and NM plants. The M plants produced leaves significantly faster than the NM plants (F=4.455, p=0.043), but no differences among populations (F=0.866, p=0.469) or population/treatment interaction (F=1.535, p=0.225) were found. The number of M plants with male racemes at 55 days after germination (representing flowering phenology) was significantly lower than in NM plants (F=4.780, p=0.032) and was significantly different between populations (F=7.056, p<0.001), but no population/treatment interaction (F=0.719, p=0.544) was observed.

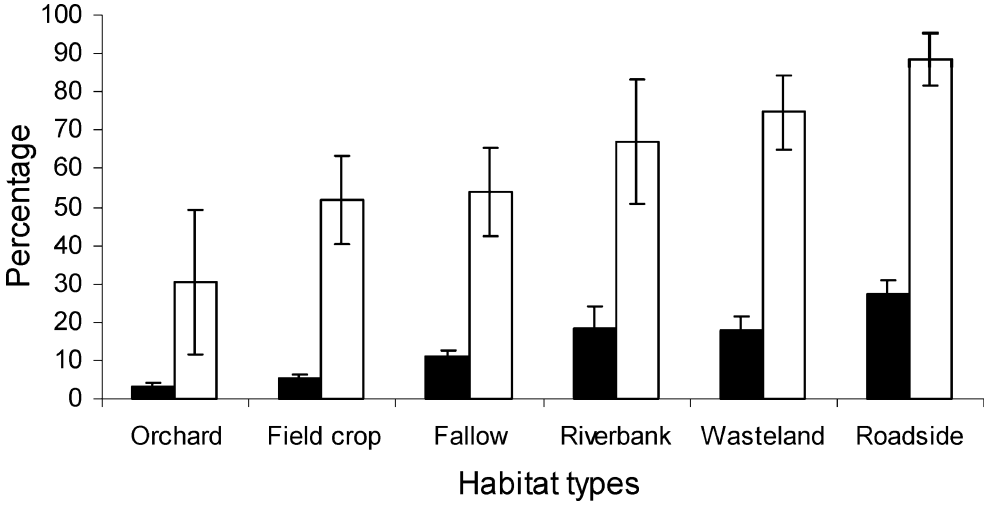

Fig. 2 Natural root colonization of *Ambrosia artemisiifolia* by AMF (%*M*) (*black bars*) and frequencies of colonized *A. artemisiifolia* individuals (%*P*) (*white bars*) in different habitat types (average percentage±standard error)

Table 3 Average levels of root colonization (%*M*±standard errors) by *Glomus intraradices* in individual inoculated *Ambrosia artemisiifolia* plants and frequency of plants colonized (%*P*) in each population 72 days after inoculation and growth in a greenhouse

Population ID	%*M*	%*P*
40	5.4±1.1	78.6±11.4
4	7.1±1.6	90.0±6.9
27	7.9±2.7	77.8±14.7
14	3.4±0.6	87.5±8.5

Discussion

Introduced *A. artemisiifolia* populations in France were found to have AMF root colonization, confirming previous reports from Europe (Caussanel et al. 2000; Kovacs and Bagi 2001; Kovacs and Szigetvari 2002). The data obtained from the present field survey showed that the majority of *A. artemisiifolia* populations were mycorrhizal (94%), with the frequency of individuals colonized within populations (%*P*) being important (66±4.4%). Individual plant root colonization (%*M*) was significantly different between habitat types, whereas the frequencies of AMF colonized plants within populations (%*P*) were not so different. Populations of *A. artemisiifolia* growing in orchard habitats were least colonized, whereas fallow land populations were more colonized than field crops. Moreover, %*M* was higher in older fallows than in younger ones, which could be linked to the increase in floristic richness and equitability in the community (Barni and Siniscalco 2000). *Ambrosia artemisiifolia* populations developing in wasteland and riverbank habitats were higher than those growing in cultivated lands. Finally, *A. artemisiifolia* developing along roadsides represented the most highly mycorrhizal populations. There was therefore a general tendency for AMF colonization (%*M* and %*P*) of *A. artemisiifolia* to increase from cultivated to noncultivated lands, which may be related to differences in the physico-chemical properties of soils (soil texture, moisture, pH, nutrients) or to cessation of agricultural practices, such as fungicide applications or soil tillage (Read and Birch 1988; Plenchette 1989).

Gradient in AMF colonization among habitat types of *A. artemisiifolia* is consistent with what has been observed in other plant species (Read et al. 1976) and may contribute to the capacity of common ragweed to invade heterogeneous habitats. However, Medve (1984) found contrasting results for American *A. artemisiifolia* developing in early-disturbed habitats. The AMF *G. mosseae* and *G. constrictum* found in one field site (#25) are considered as frequent and generalist species. Interestingly, Jin et al. (2004) reported these two AMF species to be associated with the invasive history of *Solidago canadiensis* on Chongming Island in China.

Table 4 Two-way ANOVA (GLM) of *Ambrosia artemisiifolia* growth parameters with population effect, treatment effect (NM or M), and individual effect (nested within populations)

Source of variation	df	Height		Width		Leaf number		Stem diameter		Shoot dry mass		Root dry mass		Total dry mass		RGR	
		Mean square	*p*	Mean square	*p*	Mean square	*p*	Mean square	*p*	Mean square	*p*	Mean square	*p*	Mean square	*p*	Mean square	*p*
Population (Po)	3	288.976	0.167	84.487	0.094	27.377	0.152	0.045	0.100	5.307	0.453	2.116	0.113	12.270	0.360	<0.001	0.120
Treatment (T)	1	728.765	0.041*	351.271	0.004*	84.863	0.022*	0.089	0.041	27.707	0.038*	24.304	<0.001*	94.232	0.006*	<0.001	0.008*
Po×T	3	269.757	0.190	13.010	0.784	14.507	0.406	0.011	0.631	2.851	0.696	0.672	0.568	6.353	0.636	<0.001	0.152
Individual (Po)	32	168.227	0.446	54.497	0.129	17.166	0.320	0.025	0.272	7.303	0.277	1.200	0.287	14.098	0.251	<0.001	0.020*
Error	32	160.313		36.349		14.524		0.020		5.902		0.981		11.061		<0.001	

*Significant difference for $p<0.05$

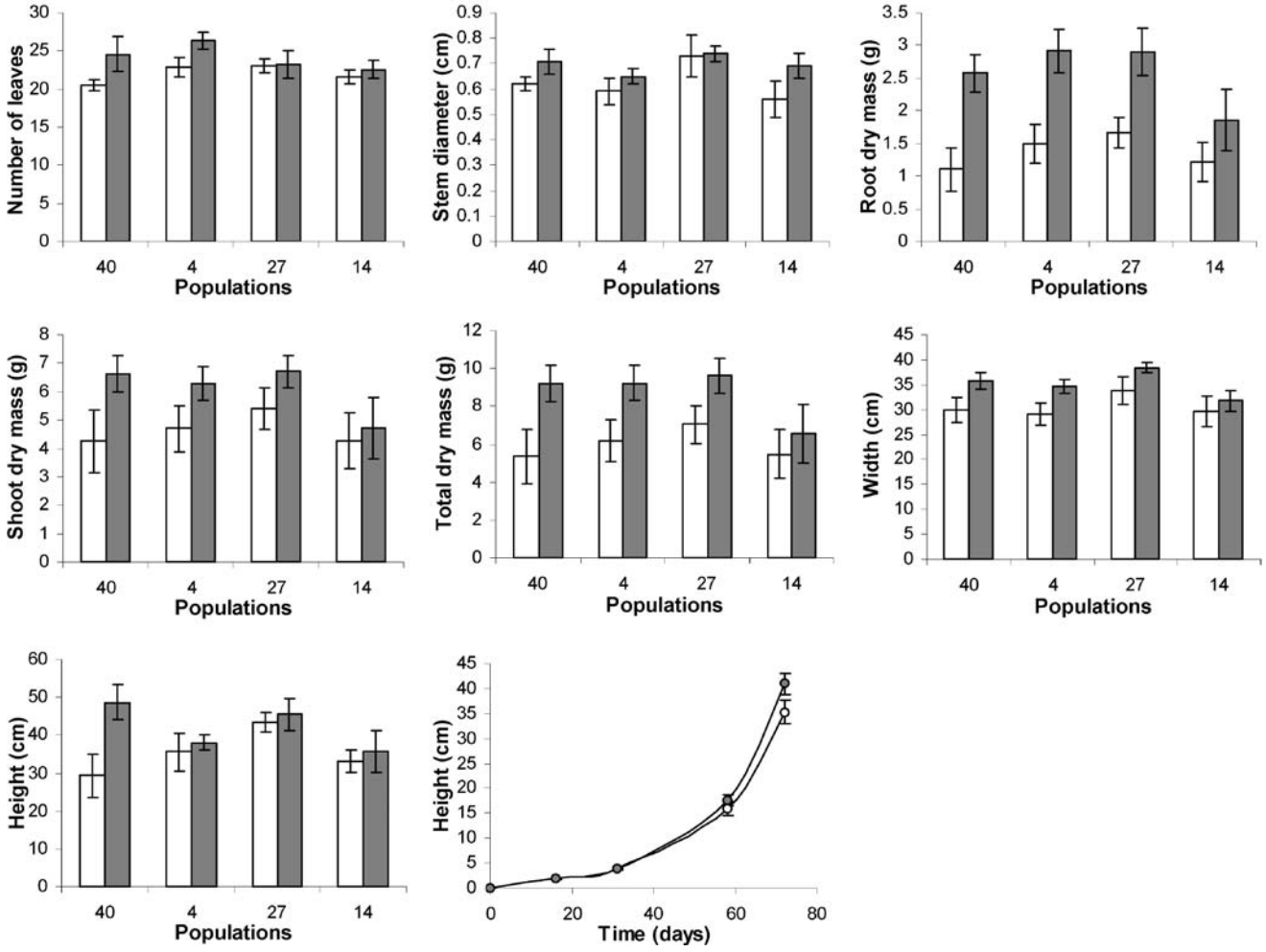

Fig. 3 Growth parameters of *Ambrosia artemisiifolia* after 72 days at the end of the greenhouse experiment (number of leaves, stem diameter, root, shoot, total dry mass, width, and height) and change in plant height through time according to the four populations studied across two treatments: NM (*white bars*) and M (*black bars*). *Error bars* indicate standard error of the mean

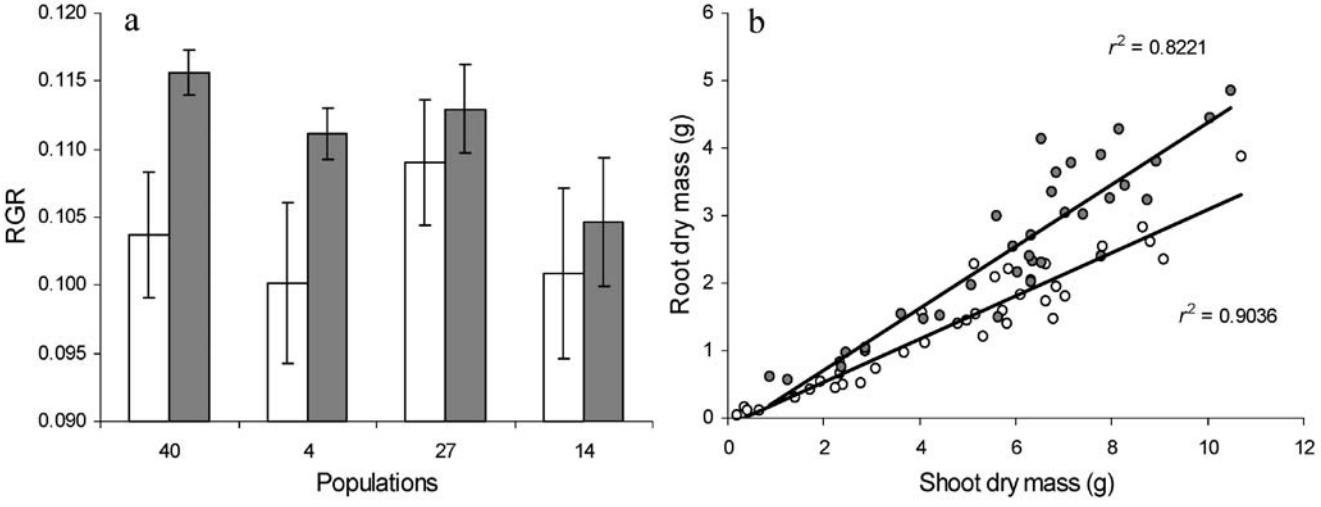

Fig. 4 Effect of *Glomus intraradices* on **a** mean RGR per *Ambrosia artemisiifolia* population and **b** allometric relationship of root and shoot plant dry mass. Treatments are represented by NM (*white circles*) and M (*black circles*) and corresponding (*line*) linear regression line. *Error bars* indicate standard error of the mean

Because of the frequent association of *A. artemisiifolia* with AMF in natural habitats, controlled experiments were conducted to study the effect of such an association on host plant development. Average root colonization of individuals (%*M*) by *G. intraradices* in greenhouse experiments was similar that frequently found in natural habitats. A significant positive impact of *G. intraradices* on *A. artemisiifolia* growth was observed. Mycorrhizal plants were taller, produced more leaves, and had higher shoot and root dry mass than nonmycorrhizal plants. Increase in root dry mass following AMF inoculation was higher than for shoot dry mass. Crowell and Boerner (1988) found similar effects of AM on *A. artemisiifolia* plant mass, but they observed an opposite pattern for root-to-shoot ratios under conditions of plant competition. Nevertheless, increases in root dry mass in AM plants have previously been recorded for other AMF–host plant combinations (Nuortila et al. 2004). The linear regression analysis of the root and shoot dry mass confirmed that inoculation with *G. intraradices* promoted a shift in the resource allocation from shoots to roots in *A. artemisiifolia*, which was probably not due to resource limitations (Coleman et al. 1994; Marler et al. 1999). Differences in the number of leaves between M/NM *A. artemisiifolia* plants were observed soon after germination, whereas differences in plant height and width appeared later. These increases in growth parameters with time would provide a competitive advantage to the mycorrhizal plants. According to Crowell and Boerner (1988), the competitive ability of *A. artemisiifolia* would be severely reduced when AMF are absent or sparse in soil.

Beyond a general positive impact on *A. artemisiifolia* growth, changes in resource allocation could strongly affect plant population biology through reproductive allocation. Caussanel et al. (2000) previously pointed out the need to investigate the potential impact of AMF on pollen and seed production of *A. artemisiifolia*. A positive correlation has previously been demonstrated between shoot dry mass and pollen (r^2=0.83) and seed (r^2=0.90) production of French *A. artemisiifolia* plants (Fumanal et al. 2005). The increased shoot dry mass observed in *G. intraradices*-colonized plants suggests that pollen and seed numbers could also be increased by AM formation, as has been observed for other plant species (Stanley et al. 1993; Koide et al. 1994; Philip et al. 2001). Such reproductive parameters are key factors for invasive plants (Williamson and Fitter 1996) because an increase in seed production would favor the persistence of plants in perturbed environments (via the soil seed bank), increase the individuals population and their local colonization capacities, and also their capacity to colonize new areas by high propagule pressure (Williamson 1996). Furthermore, a higher pollen production allows the gene flow to increase among individuals and populations, thus increasing their genetic diversity, and may play a role in local habitat adaptations (Sakai et al. 2001). In the case of allergenic plants, such as *A. artemisiifolia*, an increase in pollen production could also be a threat to human health.

The vegetative development of *A. artemisiifolia* (expressed by phyllochron values) was significantly enhanced in mycorrhizal plants, and the phenology of reproduction (male inflorescence formation) was delayed. Such a pattern could influence the intrapopulation gene flow by affecting plant reproduction. Moreover, the RGR of *A. artemisiifolia* was also increased by inoculation with *G. intraradices*. Recent studies using *G. intraradices* and *Atriplex nummularia* showed similar positive impacts on plant growth (Plenchette and Duponnois 2005). From the ecological aspect, high RGR may promote rapid occupation of a large space, which could be an advantage for invasive annual plants under competitive conditions. High RGR may also facilitate the rapid completion of the plant life cycle and could be an advantage for ruderal plants, such as *A. artemisiifolia*, developing in disturbed habitats (Grime and Hunt 1975).

With regards to the invasive status of *A. artemisiifolia*, increases in growth and development caused by AMF colonization could facilitate the spread of this species. Mycorrhizal interactions could be a key point in invasive plant processes, not only by facilitating local adaptation and/or reducing environmental stress, but also through their direct or indirect effects on interplant competition. Moreover, AM are now recognized as a major factor in structuring plant communities, as plant competition processes (Richardson et al. 2000). Even if increasing abundance of some invasive plants species has been attributed to their ability to associate with AMF (Klironomos 2002; Stampe and Daehler 2003), the facilitation of invasive organisms by mutualist organisms had received less attention by researchers compared to competition or predation processes (Bruno et al. 2005). The observations from the present study highlight the need to better evaluate the potential role played by AMF in invasive species dynamics.

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