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Arbuscular mycorrhizal fungi influence life history traits of a lepidopteran herbivore

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Abstract Results from pot and microcosm studies in the greenhouse have shown that plant growth and foliar chemistry is altered by the presence and species composition of arbuscular mycorrhizal fungi (AMF). The growth and survival of herbivores which feed on plants could, as a consequence, also be affected by these mutualistic soil fungi. Consequently, interactions between AMF, plants and herbivores could occur. To test this, larvae of the common blue butterfly, *Polyommatus icarus* (Lycaenidae), were fed with sprigs of *Lotus corniculatus* (Fabaceae) plants which were inoculated with one of two different AMF species, with a mixture of these AMF species or with sprigs of plants which were not inoculated with AMF. Survival and larval weight of third instar larvae fed with plants colonised by AMF were greater than those of larvae fed with non-mycorrhizal plants. Survival of larvae feeding on non-mycorrhizal plants was 1.6 times lower than that of larvae feeding on plants inoculated with a mixture of AMF species and 3.8 times lower than that of larvae feeding on plants inoculated with single AMF species. Furthermore, larvae fed with non-mycorrhizal plants attained only about half the weight of larvae fed with mycorrhizal plants after 11 days of growth. These differences in larval performance might be explained by differences in leaf chemistry, since mycorrhizal plants had a 3 times higher leaf P concentration and a higher C/N-ratio. Our results, thus, show that the presence of belowground mutualistic soil fungi influences the performance of aboveground herbivores by altering their food quality. Larval consumption, larval food use and adult lipid concentrations of the common blue butterfly differed between larvae which were fed with plants inoculated with different AMF species. This suggests that the performance of herbivores is not only influenced by the presence of AMF but also depends on the identity of the AMF species colonising the host plants. Moreover, a significant interaction term between AMF species and maternal identity of the larvae occurred for adult dry weight, indicating that the performance of offspring from different females was differently influenced by AMF species composition. To our knowledge, these results show for the first time that the species composition of AMF communities can influence life-history traits of butterfly larvae and possibly herbivores in general.

Key words Herbivory · Lycaenidae · Mycorrhiza · Plant-fungal-insect interactions · *Polyommatus icarus*

Introduction

The size and structure of herbivore populations are shaped by a variety of factors such as host plant availability, the presence of predators and parasitoids and abiotic variables such as temperature and humidity (Stamp and Casey 1993). Foodplant quality is another important factor, since the concentrations of N, P, water and carbohydrates, and the presence and composition of allelochemicals in plant tissue are major determinants of insect growth and development (Table 1). These parameters are in turn affected by other factors such as soil nutrient availability, air temperature, water balance, light, atmospheric carbon dioxide or micro-organisms (Barbosa et al. 1991; Ayres 1993; Carter et al. 1997; Buse et al. 1998; Goverde et al. 1999). Thus, any factor that changes host-plant quality affects insect-plant interactions and herbivo-

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Table 1 Comparison between food plant quality measures which are affected by arbuscular mycorrhizal fungi (AMF) and which are important for herbivores (+ positive effect, – negative effect, –/+ positive or negative effect, depending on substance investigated, *0* no effect, *n.d.* no data). Effects of AMF on food plant quality depend on environmental conditions (e.g. nutrient availability, plant age)

Factor	Influenced by AMF[a]	Influence on herbivore[a]
Water	+/0[1,2]	+++[8,9]
Carbohydrates	–/+[3]	++[10]
Allelochemicals	–/+[4,5]	–-/+[9,10]
Specific leaf area	n.d.	+[9]
N	–/+/0[1,6]	+++[8,9]
P	+++[1,7]	+[11]
Micro-nutrients (e.g. Zn, Cu, Mg)	+++[1]	+[9,11]
SiO_2	n.d	–[9]

[a] References: *1* Smith and Read (1997), *2* Nelson (1987), *3* Müller et al. (1999), *4* Morandi (1996); Vierheilig et al. (1998), *5* Gange and West (1994), *6* Edathil et al. (1996), *7* Gerdeman (1964), *8* Mattson (1980), *9* Schoonhoven et al. (1998), *10* Bernays and Chapman (1994), *11* Wigglesworth (1984)

ry at the same time. Root symbionts such as N-fixing bacteria or arbuscular mycorrhizal fungi supply plants with additional N, phosphorus and other important nutrients (Barbosa et al. 1991; Smith and Read 1997). The additional nutrients provided by these symbionts change plant tissue quality which, in turn, can influence plant-herbivore interactions. However, little research has addressed effects of symbionts that co-occur in the habitat on insect-plant relationships (Briggs 1990; Gehring and Whitham 1994; Manninen et al. 1998).

One of the most common mycorrhizal symbionts that influence plant growth and foliar chemistry are the arbuscular mycorrhizal fungi (AMF). AMF are abundant in soils of most ecosystems and form a mutualistic symbiosis with the roots of approximately 60% of all terrestrial plant species, thereby acting as extensions of plant root systems, increasing nutrient uptake and plant growth (Smith and Read 1997). Plants which are colonised by AMF normally contain more P and micro-nutrients than uncolonised plants and N concentrations vary between mycorrhizal and non-mycorrhizal plants (Table 1). Furthermore, the pool sizes and distribution of non-structural carbohydrates within the plant, and the presence and composition of secondary plant metabolites, some of which might be toxic for herbivores, can also vary between mycorrhizal and non-mycorrhizal plants (Morandi 1996; Vierheilig et al. 1998; Müller et al. 1999). Some leaf-quality variables which are important for herbivores and which are influenced by AMF are summarised in Table 1. Several studies have shown that the growth of herbivores on mycorrhizal and non-mycorrhizal plants differs; some herbivore species performed better on mycorrhizal plants (Gange and West 1994; Gehring and Whitham 1994) while others performed better on non-mycorrhizal plants (Rabin and Pacovsky 1985; Gange and West 1994; Gehring and Whitham 1994; Gange and Nice 1997).

A comparison of the performance of herbivores on mycorrhizal and non-mycorrhizal plants is, in many cases, ecologically less relevant because plants are normally colonised by AMF. Therefore, most herbivores will only feed on mycorrhizal plants. Generally plants grow in communities that vary in AMF species composition and number (Walker et al. 1982; Johnson et al. 1992; Bever et al. 1995). It is assumed that AMF are non-host-specific and, consequently, that each plant can be colonised by any AMF species (Law 1988; Fitter 1990). It is, however, ecologically important which AMF species colonises a plant because both the biomass and the foliar chemistry of plants vary depending on which AMF species occupy the roots (Roldan-Fajardo 1994; Streitwolf-Engel et al. 1997; van der Heijden et al. 1998a, 1998b). Therefore, we hypothesised that the growth and survival of herbivores that feed on plants might depend on the identity of AMF species that colonise their host.

Furthermore, there may be genetic variation in the herbivores in their responses to different AMF species. For instance, offspring of different females might be differently affected by different AMF species, and AMF species could, therefore, affect the genetic composition of herbivore populations through natural selection. One of the aims of our study was to provide results allowing more general conclusions about the impact of AMF on life history traits of herbivores.

To test our hypothesis, we inoculated *Lotus corniculatus* plants with two different AMF species, with a mixture of these species or with no AMF. Sprigs of these plants were fed to larvae of the common blue butterfly *Polyommatus icarus*, a herbivore which mainly feeds on *L. corniculatus*. The plant species, the AMF species and the herbivore all co-occur in the same calcareous grassland. We recorded the development and survival of this herbivore (1) feeding on leaves of plants with AMF compared to leaves of plants not inoculated with AMF, and (2) feeding on leaves of plants colonised by different AMF species. As far as we know this is the first study which investigates the ecological relevance of the presence of different AMF species on life-history traits of herbivores.

Materials and methods

Plant, fungal and butterfly species

The plant (*Lotus corniculatus* L., Fabaceae), the AMF species (*Glomus* spp., Glomales), and the butterfly (*Polyommatus icarus* Rott., Lepidoptera, Lycaenidae), used in this study all coexist in calcareous grasslands in the Swiss Jura mountains. These calcareous grasslands (*Mesobromion*) harbour species-rich plant and butterfly communities (Baur et al. 1996; Delarze et al. 1999), and a diverse community of AMF (Sanders et al. 1995).

We started the experiment with three different AMF species (hereafter referred to as AMF species I, II or III) isolated from a calcareous grassland (Nenzlingen, Switzerland) and propagated on *Hieracium pilosella* plants growing in an autoclaved (121°C; 30 min) loamy sand. These three AMF will be called AMF species, although the fungi have not yet been taxonomically de-

scribed. The three AMF species: AMF species I (*Glomus* sp.; isolate BEG 21); AMF species II (*Glomus* sp.; isolate Basle Pi) and AMF species III (*Glomus* sp.; isolate BEG 19) were morphologically different from each other. The same AMF species were used in previous experiments and further information about these three species is given there (Streitwolf-Engel et al. 1997; Sanders et al. 1998; van der Heijden et al. 1998a,1998b) and from the Banque Européen des Glomales at http://wwwbio.ukc.ac.uk/beg/contents.htm. Unfortunately the plants inoculated with AMF species III did not become colonised by this AMF species. Therefore, we excluded this treatment from the experiment. AMF species III was included in the mixed treatment. However, we assume that this AMF species did not colonise the plant roots and hence could not influence the effect of the mixed AMF treatment.

AMF inoculation, plant growth conditions, plant and fungal variables

Native seeds of *L. corniculatus* (UFA Samen, Basel, Switzerland) were surface-sterilised (5 min in 1% H_2O_2) and germinated on autoclaved quartz sand (121°C; 30 min) in a greenhouse at the University of Basel, Switzerland. On 21 June 1998 three *L. corniculatus* seedlings were transplanted to 760-ml plastic pots containing 453 g soil (dry weight) of an autoclaved (121°C; 30 min) soil mixture of calcareous grassland soil and quartz sand (2:1 v/v). The seedlings were either grown with one of the three AMF species (treatments I, II and III), with all three species together (mixed treatment), or without AMF (NM treatment) in a total of 250 pots. Because non-mycorrhizal *L. corniculatus* produce a low amount of biomass (van der Heijden et al. 1998a,1998b), additional pots of the NM treatment were included to obtain enough material for the feeding trials. Pots were either inoculated with 25 g soil inoculum of one of the three AMF species, with 25 g mixed soil inoculum comprising an equal proportion of each of the three AMF species or with 25 g mixed soil inoculum containing the three AMF species which had been autoclaved (121°C; 30 min, NM treatment). In each pot, 15 g of soil inoculum was mixed with the soil and the remaining 10 g soil inoculum was distributed between the three holes made to plant the three seedlings. After 10 days the smallest seedling was removed from each pot. The plants were watered twice a week with distilled water and additionally once a week to an amount which was equal to 28.1% soil mass. The plants were maintained in a greenhouse at the University of Basel, Switzerland (day/night regime: 16/8 h, 24/14°C). All pots received 25 ml of inoculum washing: 600 g of the mixed inoculum was sieved through a 30-µm sieve with 7.5 l water to correct for possible differences in bacterial and fungal communities between different inocula (Koide and Li 1989). At the start of the experiment, and after 31 days, all pots received a 10-ml suspension of *Rhizobium* which were derived from root nodules of *L. corniculatus* plants collected from the calcareous grassland mentioned before (Nenzlingen, Switzerland). Nodules were surface-sterilised (1% H_2O_2), crushed with a mortar and pestle and left for 24 h in 0.1M manitol in tapwater. Each pot received a pulse of additional nutrients (50 ml 25% Hoagland solution, Fink 1979) 38 days after the start of the experiment.

To measure the plant and fungal variables, 14 pots per treatment were randomly chosen and harvested at the end of the feeding experiment. The roots of each pot were washed and divided into two sub-samples. One root sub-sample and the shoots of the plants were oven-dried (48 h at 80°C) and weighed. Root, shoot and total biomass were determined. The fresh root sub-samples were cleared with 10% KOH and AMF structures inside plant roots were stained with trypan blue (Phillips and Hayman 1970). The percentage of root length colonised by AMF was estimated under the microscope by a modified line intersection method (McGonigle et al. 1990) whereby a minimum of 50 line intersections per root sample were scored for the presence of AMF.

Specific leaf area (SLA) was determined by weighing fresh leaves and measuring the leaf area using a planimeter (LI-3100 Area Meter, LI-COR, Inc., Lincoln, Neb., USA). Leaf water concentration was estimated as the proportional difference between fresh and dry leaf mass. To determine concentrations of P, N and total non-structural carbohydrates (TNC), samples were dried at 80°C and subsequently ground to powder. The P concentration was determined by using the molybdate blue ascorbic acid method (Watanabe and Olsen 1965). N concentration was determined using a CHN analyser (LECO Instruments, Model 932, St. Joseph, Mich., USA) which uses a combustion procedure. TNC were determined using a series of enzymatic digestions (Wong 1990; Körner and Miglietta 1994). Starch concentration was calculated as the difference between TNC and soluble-neutral sugar concentration.

Butterfly rearing and feeding experiment

P. icarus females were captured at different sites in the Swiss Jura mountains and kept individually in cages (20 cm×20 cm×40 cm) in the greenhouse. *L. corniculatus* plants growing in 500-ml pots were introduced into the cages as oviposition substrate (Goverde et al. 1999). Eggs laid on these plants were removed every 3 days and stored until use (but no longer than 10 days) at 10°C in a refrigerator. We obtained eggs from five different females and these were kept separated by female in petri dishes covered with nylon mesh. After hatching, batches of five to eight caterpillars per female were placed in petri dishes. Five or ten (depending on the number of caterpillars available) petri dishes per female were established. These petri dishes were assigned across the five AMF treatments, giving one or two replicate petri dishes at this stage for each female and each AMF treatment. Petri dishes contained a moistened piece of paper towel (15 mm×50 mm) to maintain air humidity and were covered with nylon mesh and the petri dish lid. Petri dishes were placed in the lab at 25.8°C (±1.5°C). Every 2nd day, a plant from the appropriate AMF treatment was randomly chosen and fresh, equally sized sprigs were removed. The sprigs were placed into a 1.5 ml tube (Eppendorf) full of water with a pierced cap, and given to the larvae. We took care to provide young sprigs and enough plant material to have the same conditions for all larvae from the different treatments. When the caterpillars moulted to the third instar they were weighed individually and a sub-sample of three larvae from each of the five females were randomly selected and transferred to individual petri dishes for continued rearing. For each treatment and female three replicates were established. Thus, we knew maternal identity and feeding history of each caterpillar throughout its life. Mortality until third larval instar was recorded. Caterpillars were fed ad libitum as above, with fresh plant material changed every 2nd day and the change in weight of this plant material was measured to determine food consumption. The caterpillars were weighed every 2nd day and the weight gain and faeces production were measured. The following parameters of larval growth were calculated:

1. Larval consumption: the estimated dry weight of total plant material consumed per larva. Sprigs were weighed before being given to the larvae and after they were exchanged. Weight loss resulted from larval consumption and evaporative water loss. To estimate the latter, control sprigs were cut at the same time and placed in a petri dish under the same conditions but without a larva. Dry weights of the extrapolated data were calculated by using the proportional difference between fresh and dry leaf mass of control plants.
2. Food used: larval consumption minus faeces production (both in dry weight).

Each pupa was weighed at the onset of pupation and placed in one of the 18 compartments of a plastic box (21 cm×12 cm) with a nylon web floor. Boxes were placed 2 cm above a pan of water to maintain high humidity (Friedrich 1975). After they had emptied their guts, emerged butterflies were killed, sexed, and weighed. Butterflies were dried at 60°C for 48 h and stored in an exsiccator. Lipids of adults were extracted with a 2:1 chloroform:methanol solvent following the method described in Goverde et al. (1999).

Experimental design and statistical analysis

The experiment was set up as a complete randomised design with four AMF treatments (species I, species II, mixed, non-mycorrhizal) with 50 replicates per treatment. The position of these 200 pots was randomised weekly. Of the 50 replicates per treatment, 14 randomly chosen pots were used to determine plant and fungal variables.

Plant variables (total biomass, percentage of root length colonised by AMF, SLA, and leaf water, P, N, C and TNC concentrations) were analysed for differences between AMF treatments using a one-way analysis of variance (ANOVA, type III sums of squares JMP 3.1, SAS 1995) where total biomass was log-transformed to improve homoscedasticity. To compare among groups *a priori* contrasts were used, comparing first the non-mycorrhiza (NM) treatment against the others, then the mixed treatment against AMF species I and II and finally comparing species I against species II (Crawley 1993).

Effects on early larval mortality were studied by using a logistic regression model, with a binomial distribution, with the factors AMF treatment, female genotype and replicate. We employed stepwise reduction of the full model, removing highest-order interactions and the least significant effect first. We ceased model reduction when no further interactions or factors could be removed and we did not remove biologically interesting main factors (AMF treatment and female genotype). As mentioned above contrasts were used to compare among AMF treatments. The analysis was performed with Genstat 5.3 (Payne 1993).

We performed a full factorial ANOVA (type III sums of squares, JMP 3.1, SAS 1995) with the fixed factors AMF treatment and maternal identity plus the factor sex of the individual. The following variables of *P. icarus* were analysed: total development time (time from first larval instar until adult eclosion), food consumption, food use, adult dry weight and lipid concentration of adults. Again *a priori* contrasts were used to compare the mixed treatment against AMF species I and II and species I against species II. Larval weights after 11 days of growth were analysed using the same type of ANOVA but without the factor sex.

If not otherwise stated, throughout this publication we give the arithmetic mean as measure of central tendency and the standard error of the mean as measure of variability.

Results

Effect of AMF colonisation on plant characteristics and larval development

The biomass of mycorrhizal *L. corniculatus* plants was 21–44 times higher than that of the non-mycorrhizal plants (Table 2). Leaf P, C and N concentrations also differed between mycorrhizal and non-mycorrhizal plants (Table 2). Non-mycorrhizal plants had significantly lower P and C but a higher N concentration (Table 2). As a result the C/N-ratio of non-mycorrhizal plants was significantly smaller than that of mycorrhizal plants (Table 2). Leaf water concentration and SLA were not significantly altered by AMF colonisation (Table 2). Roots of *L. corniculatus* plants not inoculated by AMF remained uncolonised, except for one pot (Table 2).

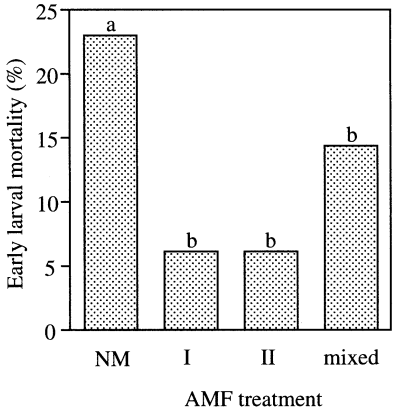

Fig. 1 Early larval mortality of *Polyommatus icarus* between first and third larval instars. Larvae were fed with plants inoculated with different arbuscular mycorrhizal fungi (AMF) species (AMF species *I*, *II* or *mixed*) or with plants not inoculated with AMF (*NM*). Different letters indicate significant differences between treatments at $P<0.05$ using *a priori* contrasts (see Table 3); $n=198$

Table 2 Foliar chemistry of *Lotus corniculatus* inoculated with different AMF species (*AMF species I, II* or *mixed*) or not inoculated with AMF (*NM*). Mean values ± 1 SE are presented. Summary of *F*-values for comparisons among the different treatments by *a priori* contrasts using JMP 3.1 (SAS 1995). For statistical analysis total plant biomass was log-transformed (*SLA* specific leaf area, *TNC* total non-structural carbohydrates)

Treatment	Total biomass (g)	Root colonisation (%)	Leaf water (%)	SLA ($cm^2\ g^{-1}$)	[P]	[C]	[N]	C/N-ratio	[TNC]
Non-mycorrhiza (NM)	0.04±0.01	1.52±1.52	80.82±0.85	291.63±26.38	3.91±0.56	40.53±0.62	5.84±0.27	7.37±0.21	[a]
AMF species I (I)	0.84±0.09	57.48±6.22	82.42±0.58	349.72±11.35	11.87±1.14	43.15±0.35	5.40±0.07	8.01±0.11	2.96±0.19
AMF species II (II)	1.41±0.14	74.42±6.87	80.50±0.70	320.26±14.25	11.64±0.93	43.40±0.24	5.24±0.10	8.32±0.18	3.38±0.23
Mixed	1.76±0.13	71.60±4.21	80.17±0.66	305.26±13.11	10.70±0.65	43.55±0.35	5.12±0.11	8.54±0.17	3.37±0.31
Contrast									
NM–I/II/Mixed	779.77***	114.42***	0.06	2.83	61.39***	33.99***	7.81**	20.67***	
Mixed–I/II	11.70***	0.79	2.32	2.13	2.22	0.46	2.33	3.32	0.32
I–II	6.27*	5.32*	3.83	1.56	0.76	0.30	1.20	1.85	1.03

* $P<0.05$, **$P<0.01$, ***$P<0.001$

[a] Not enough plant material for chemical analysis

Early larval mortality of *P. icarus* differed significantly among AMF treatments (Fig. 1, Table 3). This was because mortality of larvae feeding on non-mycorrhizal plants was 1.6 times higher than in the mixed treatment and 3.8 times higher than in the species I and II treatment. *A priori* contrasts showed that only the NM treatment differed significantly from the other treatments (Table 3). The growth of larvae feeding on non-mycorrhizal plants was much slower than on mycorrhizal plants (Fig. 2). After 11 days of growth, larvae feeding on non-mycorrhizal plants had only reached half the weight of larvae feeding on plants from the mixed treatment ($F_{3,52}$=9.21, P<0.001). For further rearing the non-mycorrhizal treatment was omitted from the analysis because mortality of caterpillars was high in this treatment and because *L. corniculatus* did not grow well enough in this treatment to provide sufficient plant material throughout the experiment.

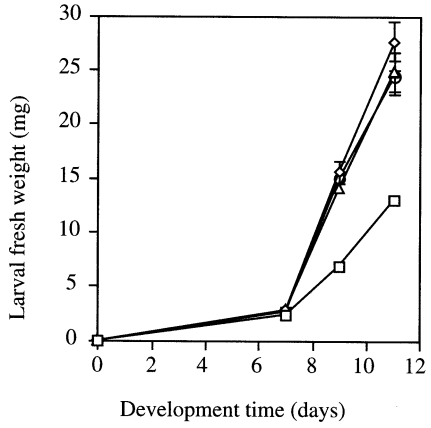

Fig. 2 Growth trajectories of *P. icarus* larvae fed with *Lotus corniculatus* plants which were not inoculated with AMF (□) compared to plants inoculated with AMF (◇ mixed, ○ species I, △ species II). Error bars ±1 SE; error bars smaller than the symbol are not depicted

Table 3 Results of logistic regression analysis on early larval mortality. *A priori* contrasts were performed in order to show differences among the AMF treatments NM, AMF species I, AMF species II and mixed

Source	*df*	Deviance	χ^2
AMF treatment	3	8.466	0.037
NM–I/II/Mixed	1	5.923	0.015
Mixed-I/II	1	2.54	0.111
I–II	1	0.000	1.000
Female genotype	4	3.517	0.475
Residual	187	133.491	

Effects of different AMF species on plant growth and life history traits of the common blue butterfly

Plant biomass differed significantly between AMF treatments with highest plant biomass found in the mixed treatment (Table 2). Several plant variables (water, SLA, P, N, TNC) differed from each other, although none of these differences was significant (Table 2). The percentage root length colonised by AMF differed between species I and II, but did not differ between these two and the mixed AMF treatment (Table 2). Furthermore, we inoculated the plants with a rhizobia inoculum that normally causes nodulation on the roots of *L. corniculatus* (M. Goverde, personal observations). At harvest *L. corniculatus* roots of all treatments were slightly nodulated but the number of nodules was not counted. Larval food consumption differed significantly between AMF treatments (Table 4, $F_{2,41}$=3.90, P=0.032) and tended to be higher for males than for females ($F_{1,42}$=3.77, P=0.063). Larval food use also differed significantly between AMF treatments (Table 4, $F_{2,41}$=4.93, P=0.015). *A priori* contrasts show that the treatment I and II differed significantly from each other for consumption and food used while the mixed treatment did not differ from the other two (Table 4). The highest food consumption was found for larvae feeding on plants colonised by AMF species II. Furthermore, on the latter, larval food use was also highest (Table 4).

Insect development time (time from first larval instar until adult eclosion) did not differ between the AMF treatments ($F_{2,43}$=0.36, P=0.70, Table 4), maternal identity ($F_{4,41}$=1.21, P=0.33) or their interaction ($F_{8,37}$=0.81,

Table 4 Larval development, consumption and food use, and adult dry weight and lipid concentration of *P. icarus* fed with *L. corniculatus* plants inoculated with different AMF species. Because of food shortage the non-mycorrhizal treatment had to be omitted. Mean values ± 1 SE are presented. Summary of *F*-values for comparisons among the different treatments by *a priori* contrasts using JMP 3.1 (SAS 1995)

Treatment	Development time (days)	Larval consumption (mg)	Larval food use (mg)	Adult dry weight (mg)	Adult lipid concentration (%)
AMF species I (I)	25.67±0.33	94.22±3.79	40.32±3.01	14.24±0.60	22.96±0.96
AMF species II (II)	25.57±0.36	109.20±3.53	53.00±3.37	14.68±0.54	20.86±0.81
Mixed	25.83±0.39	99.09±4.97	39.05±3.58	14.83±0.76	19.58±0.72
Contrast					
Mixed–I/II	0.71	0.16	3.28	1.16	8.98**
I–II	0.00	7.70**	6.81*	0.88	4.29*

*P<0.05, **P<0.01, ***P<0.001

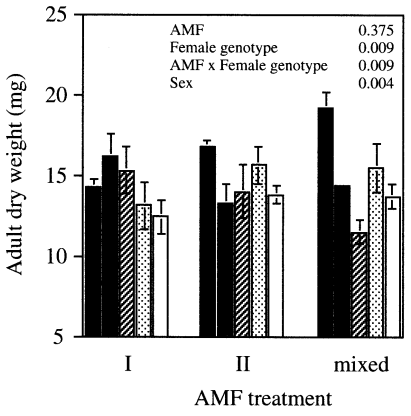

Fig. 3 Adult dry weights of *P. icarus* butterflies in relation to AMF inoculation and maternal identity (female genotype). Mean values±1 SE are presented. *P* values of the ANOVA are shown. *Density of shading of bars* indicates different individual females, from *left* (*solid*) female A, to *right* (*open*) female E; *n*=45

P=0.60). The development time differed significantly between males and females ($F_{1,44}$=7.90, *P*=0.009), with males developing 1 day faster (25.1±0.3 days) than females (26.2±0.3 days). Because growth and development of *P. icarus* differ between males and females, this could bias the results if the sexes are differently distributed among the treatments. However, in our experiment the number of males and females did not differ among the treatments (*G*=0.537, *df*=2, *P*=0.764).

There was a significant effect of gender and maternal identity on adult dry weight ($F_{1,44}$=10.08, *P*=0.004 for gender, $F_{4,41}$=4.12, *P*=0.009 for maternal identity). Additionally we observed a significant interaction between AMF treatments and maternal identity (Fig. 3). This shows that adult weight of the butterflies was affected by the different species of AMF occupying the roots, but that the effects differed according to the maternal identity (or genotype). For example, adults from female A were heaviest on plants inoculated by the mixed AMF treatment while adults from female C were heaviest when feeding on plants inoculated with AMF species I (Fig. 3). The interaction term between AMF species and maternal identity has already appeared for larval weight in the fourth instar ($F_{8,37}$=3.03, *P*=0.013), and persists marginally for pupal weights ($F_{8,37}$=2.12, *P*=0.066, data log-transformed). Finally, adult lipid concentrations differed significantly between AMF treatments ($F_{2,40}$=6.63, *P*=0.005) and sexes ($F_{1,41}$=15.80, *P*<0.001). Males contained more lipids than females, and highest adult lipid concentrations were found for larvae feeding on plants colonised by AMF species I (Table 4).

Discussion

AMF alter larval survival and development

Our results show that the colonisation of roots by AMF is important for survival and growth of larvae of the common blue butterfly feeding on their host plant *L. corniculatus*. Larvae feeding on non-mycorrhizal plants experienced 1.6 times and 3.8 times higher mortality than those feeding on the mixed AMF treatment and AMF species I and II treatment, respectively (Fig. 1). Furthermore, after 11 days of development, larvae feeding on non-mycorrhizal plants attained only half the weights of those feeding on mycorrhizal plants (Fig. 2). This variation in the performance of *P. icarus* might be explained by the observed changes in host plant quality because the larvae were fed ad libitum in all treatments. Differences in concentration of plant N, C, water and P can often explain variation in the performance of herbivores (Mattson 1980; Scriber and Slansky 1981; Schoonhoven et al. 1998). We observed that plant N, P and C concentrations differed between mycorrhizal and non-mycorrhizal plants (Table 2) and one or a combination of these factors might explain why the larvae developed better when fed on plants colonised by AMF. It is generally assumed that high tissue N concentration causes a better larval performance of insects (Mattson 1980). Our results contradict this, since the non-mycorrhizal plants had the highest N concentration which did not result in a better larval performance. We suggest that N might not have played a significant role because the N concentration of *L. corniculatus* in the our study was in general high (5.4%) compared to levels normally found in nature (2.7% N in the field, M. Goverde, unpublished work).

Furthermore, the C and P concentrations were significantly higher in mycorrhizal plants compared to non-mycorrhizal plants (Table 2). C and/or P could have positively affected larval performance since plant C concentration is related to the presence and composition of many plant compounds affecting herbivory (e.g. sugar composition, allelochemicals, phagostimulants) (Rosenthal and Berenbaum 1991; Bernays and Chapman 1994; Goverde et al. 1999; Müller et al. 1999), and since P is an important element for insect growth (Wigglesworth 1984; Schoonhoven et al. 1998).

Leaf morphology and age could have played another important role. Because non-mycorrhizal *L. corniculatus* plants hardly grew, the leaves which were fed to *P. icarus* were already old and of different shape from those of mycorrhizal plants. Although leaf water and SLA did not differ, the greater freshness of mycorrhizal plant leaves compared to the non-mycorrhizal ones might have caused the positive effect on the performance of *P. icarus*. Indeed, caterpillars of the common blue butterfly do prefer young and fresh leaves of *L. corniculatus* (M. Goverde, personal observations). Finally, the biomass of mycorrhizal *L. corniculatus* plants was 33 times higher than that of non-mycorrhizal plants of the same age (Table 2). This indicates that in the field, besides the effects of food plant quality, AMF may also greatly influence the abundance of the herbivore host plant. In our experiment, the shortage of food in the non-mycorrhizal treatment did not allow us to rear caterpillars beyond their third larval instar.

AMF species composition influences life history traits of the common blue butterfly

We hypothesised that the species composition of AMF communities affects herbivores by differentially affecting their host plants. Our results support this hypothesis because we observed that (1) larval consumption, larval food use and lipid concentrations of adult butterflies differed when larvae were fed with leaves of plants inoculated with different AMF species (Table 4) and that (2) the dry weight of adult butterflies varied, depending on the AMF species colonising their food plants and on maternal identity (Fig. 3). These results, therefore, indicate that the species composition of AMF communities present at a site is a factor that affects life-history traits of herbivores. These results are interesting because it has been shown that the species composition of AMF communities differs within and among habitats (Johnson et al. 1991; Bever et al. 1995; Helgason et al. 1998).

Although we did not find significant differences in leaf quality variables such as P, N, C and water concentrations, we observed that plants inoculated with different AMF species differed significantly in biomass and percentage of root length colonised by AMF (Table 2). This indicates that the plants obtained different, though unknown, benefits from different AMF species which might also have affected the performance of the herbivore.

The lipid concentrations of adults varied when the larvae were fed with plant material inoculated with different AMF species. This could be important, because high lipid concentrations of adult butterflies can improve their fecundity and longevity (Brown and Chippendale 1974; Tuskes and Brower 1978). Larvae feeding on leaves of plants colonised by AMF species I, which reached a higher lipid concentration than those feeding on AMF species II plants, might increase egg production in females and enhance the capacity for mate search, territorial defence and spermatophore production in males.

The observed significant interaction term between AMF species and maternal identity, for adult dry weight as well as for larval weight of the fourth instar, is important from an ecological and evolutionary point of view. Supposing that adult weight is correlated with fitness, offspring from female C, for example, would perform better in a plant community where *L. corniculatus* is colonised by AMF species I but worse on plants colonised by a combination of AMF species. On the other hand, offspring from female A would perform best on plants colonised by a combination of AMF species. The species composition of AMF communities could, therefore, influence the genetic composition of butterfly populations. Moreover, we observed that the biomass of *L. corniculatus* varied, depending on the identity of the AMF species that colonised the roots. Therefore, *L. corniculatus* could co-evolve with those AMF species which are most beneficial and, thus, host specificity could develop. Host specificity would mean that *L. corniculatus* is only colonised by certain AMF species which, as indicated above, could in turn affect the genetic composition of butterfly populations feeding on *L. corniculatus*. The AMF used in this study were all isolated from a calcareous grassland where *L. corniculatus* occurs. At the moment, however, we do not know whether these fungi normally colonise the roots of *L. corniculatus* in natural ecosystems. Molecular techniques to determine AMF species in the roots of plants are currently being developed and, therefore, this additional information will be available in the future. Recently we have shown that the species composition and diversity of AMF communities is a determinant of plant population size and community structure (van der Heijden et al. 1998b). The population size and abundance of specific plant species would, therefore, at least in part, depend on AMF species composition and this in turn would affect herbivory. For example, the biomass of *L. corniculatus* differed 5-fold depending on which AMF species was present in a simulated grassland (van der Heijden et al. 1998b). The food availability for herbivores feeding on *L. corniculatus* would, therefore, strongly depend on which AMF species are present. AMF species composition would, therefore, influence herbivore population and community structure.

Conclusion

Our results show that the presence of AMF is of importance for growth and survival of larvae of the common blue butterfly. This result indicates that microbial interactions with plants need to be considered in order to fully understand plant-herbivore interactions. Additionally, we show that different AMF species differently influence life history traits of the common blue butterfly. We conclude, therefore, that it is not only the presence of AMF that affects herbivores but also the identity of the AMF species present. This finding emphasises the complexity of AMF-plant-herbivore interactions. More research needs to be done in this field to understand the complexity of these interactions.

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