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Full Length Research Paper

Synergistic effect of herbivory and mycorrhizal interactions on plant invasiveness

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Amongst many novel interactions that alien plants forge with their new associates in the introduced range, the role of Arbuscular mycorrhizal (AM) mutualism and herbivory has been hitherto studied separately. Since these associations operate concurrently in nature to influence plant performance, we attempted to investigate their interactive effect on invasiveness of Mayweed Chamomile (*Anthemis cotula* L.), a highly invasive species in the Kashmir Himalaya, India. Survey of some field populations of *A. cotula* in native (European) and introduced (Kashmir Himalayan) regions revealed high incidence of phytophagous parasites on the species in native range, in contrast to almost enemy-free populations in the introduced range. However, occasional association of an aphid herbivore with some individuals of *A. cotula* in the introduced range was found to have positive influence on traits contributing to invasiveness of the species. We subsequently established a pot experiment in which *A. cotula* was grown with and without mycorrhizas and herbivores, both in isolation and combination. Results revealed that mycorrhizal inoculation and herbivory, both in isolation and in combination, influenced significantly growth and fitness, hence invasiveness, of *A. cotula.* While our simple study point towards likely synergistic influence of below- and above-ground interactions on plant invasiveness, we stress for the need of comprehensive multifactor interaction analysis while dealing with plant invasions.

Key words: *Anthemis cotula*, arbuscular mycorrhizal fungi, alien invasive species, herbivory, overcompensatory growth.

INTRODUCTION

Introduced plant populations often lose interactions with enemies and mutualist from their native ranges and gain interactions with new species, under new abiotic conditions. Many of the current hypotheses on the role of biotic interactions in plant invasions focus exclusively on enemies, mutualist or competitors. For instance the enemy release hypothesis argues that the loss of interactions with natural enemies allows introduced populations to attain greater abundances (Colautti et al., 2004; Torchin and Mitchell, 2004), while the mutualisms facilitation hypothesis posits that the replacement of lost mutualist from plants native ranges with new mutualist in their introduced ranges is key to invaders establishment and spread (Richardson et al., 2000). However invader success may be better understood by considering the joint effects of enemies and mutualist, including interactions between them, on introduced populations; because alien plants, upon their introduction in the non-native range, inevitably interact with multitude of organisms, such as competitors, predators, pathogens, and mutualist which can both positively and negatively affect their growth (Seifert et al., 2009). Herbivores, for example, can reduce photosynthetic area, plant fitness, alter plant allocation patterns, increase plant defences, and even lead to plant death, while mutualists, such as mycorrhizal fungi can form symbiotic associations that often improve plant growth and survival (Bennett et al., 2009). Moreover, plants usually interact with herbivores or mutualists at the same time. Individual plants, for example, may associate with many species of mycorrhizal fungi which differ greatly in their effect on

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plant growth (Bever, 2002). For instance, Vannette and Hunter (2011) revealed that while one species of AM fungus (Scutellospora pellucid) has positive effect on host plant species, another species (*Glomus etunicatum*) appeared to act as a parasite on the same host species, thereby indicating variability in the costs and benefits of resource mutualisms.

Aboveground foliage consumers may reduce photosynthate translocation to the root system and decrease its availability to mycorrhizal fungi, resulting in reduction of mycorrhizal colonization and reduced development of the symbiosis (Gehring et al., 1997; Hetrick et al., 1990; Trent et al., 1988). Mycorrhizas, in turn, may affect herbivores through alteration of plant growth or foliar chemistry (Goverde et al., 2000), and they may have large effects on plant responses to herbivores by influencing anti-herbivore defences and/or herbivory tolerance (re-growth capacity). However, recently mycorrhizal colonization has been shown not to affect tolerance to defoliation of an annual herb in different light availability and soil fertility treatments (Aguilar-Chama and Guevara, 2011). Garrido et al. (2010) reports that variation in AMF colonization modifies the expression of tolerance to above-ground defoliation and that a negative interaction between AMF concentration and plant tolerance to defoliation does exist. It is important to note that the grazing tolerance and mycorrhizal colonization of plants are linked to resource manipulation and plant size, as shown for a biennial plant species, *Gentianella campestris*, by Piippo et al. (2011). The role of mycorrhizas in plant invasions has been reviewed by Shah et al. (2009) to highlight the paradoxically conflicting observations in the context of mutualism-commensalism-parasitism gradient that characterizes the relationship between AM fungi and their alien vs. native hosts. In the said review authors have dealt with tripartite AMF-host plant-herbivore interactions also.While both mycorrhizas and herbivores have been shown to influence plant invasions, their effects have been hitherto studied independently. This is so despite the likely interactions between herbivores and arbuscular mycorrhizal (hereafter AM) fungi because both depend upon and influence important plant resources. The response of arbuscular mycorrhizal fungi to stimulate herbivory is reported to be species specific and is also relatively difficult to generalize even when dealing with the same plant species (Klironomos et al., 2004). Gehring and Witham (1991) showed herbivore driven mycorrhizal mutualism in insect-susceptible pinyon pine but did not know what drives such an interaction in resistant trees. Notwithstanding these sporadic attempts, the dynamics of tripartite (herbivore-plant-AM) interaction with respect to invasive alien plants is still lacking. The present study is continuation of our series of previous studies that demonstrated extensive association of AM fungi with *Anthemis cotula*, a highly invasive species in the Kashmir Himalaya, across different habitats (Shah and Reshi, 2007), differential influence of mycorrhizal source and neighbour identity on its invasiveness (Shah et al., 2008 a, b) and the over compensatory response of this species after herbivore damage (Rashid et al., 2006). The simple study was designed to compare the extent of enemies associated with *A. cotula* in its native and invaded regions and investigate the effect of AM fungi and herbivory, both in isolation and combination, on invasiveness of this species.

The specific questions asked in the present study, carried out from 2008 to 2010, were (a) whether *A. cotula* has been released from native enemies in the introduced range, and (b) how herbivores interact with this species *viz-a-viz* mycorrhizal associates to influence its invasiveness.

MATERIALS AND METHODS

Study species

Mayweed Chamomile (*Anthemis cotula* L.), which is a member of Asteraceae, is an annual herbaceous plant native to southern Europe-west Siberia (Erneberg, 1999) where its most important herbivores are aphids (Aphidae), spittlebugs (Cercopidae), bugs (Heteroptera), moths, slugs and snails. In its native range the species is attacked by about 68 herbivores, mainly insects, of which 13 specifically use it as their host (Shah and Reshi, 2007). In addition, a stem mining agromyzid fly (*Napomyza sp.*) and two insect species, namely *Cochylidia implicitana* (Tortricidae) and *Homeosoma nimbella* (Pyralidae) are also reported to feed on its shoots, flowers, fruits and seeds (Shah and Reshi, 2007) keeping the species under check. However, no such bio-control is available in its invaded region that is, Kashmir Himalaya.

In the Himalayan valley of Kashmir, this fast spreading species is emerging as a major threat to native biodiversity and ecosystem processes.

Large-scale invasion of the ruderal habitats facilitated by AM mutualists (Shah and Reshi, 2007) of this species has been attributed to its extended recruitment pattern aided by habitat disturbance, high population size even after seedling mortality (Allaie et al., 2005), allelopathic activity of its aqueous leaf leachate (Allaie et al., 2006), herbivore induced over-compensatory growth (Rashid et al., 2006) and profuse production and synchrony between germination of achenes and favourable environmental conditions (Rashid et al., 2007).

Field observations

Intensive surveys were carried out during the present study in different parts of the Kashmir Valley for observing any herbivores associated with this species. In order to assess whether or not *A. cotula* was released from its native enemies, information was previously obtained about the enemies associated with this species in its native range from Dr. S. Benvenuti, Agricultural Research Institute, National Resource and Environment, University of Pisa, Italy. Moreover, 5 European populations of *A. cotula* were surveyed in Gothenburg, Sweden, for the assessment of native enemies associated with this species. 5 Kashmir Himalayan populations of this specie were also surveyed for comparison. Hundred individuals from each of the five populations were screened for the extent and type of aphid associations. The damaging effect on *A. cotula* due to herbivores was recorded in terms of number of branches infested/individual and accordingly assessing the percentage damage.

Figure 1. A view of invasion by *Anthemis cotula* in the Kashmir Himalaya (left) and its association of black aphids within Europe, the native range of the species (right).

Inoculation experiment

To verify the role of AM fungi and the commonly associated herbivore invasiveness of *A. cotula*, a pot trial with three replications per treatment was established in a fully factorial design with two factors (herbivory and mycorrhizas with 2 levels each) in the Botanical Garden of the University of Kashmir, Srinagar, Jammu and Kashmir, India, wherein pre-germinated achenes of *A. cotula* (collected during previous year from the field populations) were transferred to earthen pots (22cm × 28cm) filled with sterilized garden soil (clay = 28%, silt = 50%, sand = 22%, pH = 7.5 and organic carbon = 1.6%) mixed with sand in a ratio of 2:1. The soil was sterilized by autoclaving thrice at 85°C and 115 lb for 90 min with a 12 h interval between autoclaving. 3 seedlings per pot and 3 pots per treatment were maintained till maturity under natural light conditions of 12 to 14 h, relative humidity between 58.5 to 79.3 and temperature ranging from 7.2 to 30.9°C from March to September, 2006. *A. cotula* was grown in presence of the herbivore and mycorrhiza, both separately and in combination. Treatments without herbivore and mycorrhizas served as control. The Herbivores were collected from the field populations of *A. cotula* and 18 individuals were placed on the foliage of 3 week old plants in 6 pots to ensure sufficient number for appropriate interaction. Three of these pots were treated with AM fungi in addition to herbivore and 3 pots (other than those treated with herbivore) were treated with AM alone. Plants were watered evenly on alternate days. Plants in all the inoculated pots were screened for AM colonization, at the end of the experiment, to verify the infectivity of the inoculum. The mixed AM fungal inoculum used consisted of five species of *Glomus* (*G. mosseae, G. etunicatum, G. fasciculatum, G. caledonium, G. proliferum*) and one species of *Gigaspora* (*G. margarita*). These AM isolates were obtained from the School of Life Sciences, Jawahar Lal Nehru University, New Delhi, India. The AM inoculants were chosen on the basis of their abundant association with natural populations of *A. cotula* in the region (Shah and Reshi, 2007). Prior to utilization the inoculum was stored at -4°C. The data on vegetative and reproductive attributes of *A. cotula* under different treatments were subjected to analysis of variance, with herbivory, AM and their interaction as the main effects. Basic statistics, such as trait means and variances were calculated using SPSS 10.

RESULTS

The field survey of five European populations of *A. cotula* at different sites in its native region (Gothenburg, Sweden) revealed high incidence of black aphids associated with this species (Figure 1). Mean of 100 individuals from five different populations revealed about 85% incidence of aphid association (Table 1). For each infested individual plant more than 70% of the branches were affected with evident perforations of foliage due to phytophagy (Table 1), thereby showing the damaging effects on this species. Contrastingly, our intensive surveys during past three years of numerous populations of *A. cotula* across different habitats of Kashmir Himalaya did not yield any pathogen or parasite associated with it. However, our field observations revealed only a specific herbivore larva associated with some individuals of this species. De-topping of plant individuals by the herbivore larva was found to result in very profuse branching pattern of herbivored than the un-herbivored individuals (data not given).

Results of the pot trial to verify the role of AM fungi and the herbivory, individually and in combination, in invasiveness of *A. cotula* are given in Figure 1. Screening of pot grown *A. cotula* for AM association revealed absolutely no colonization in un-inoculated control plants and quite significant colonization in all the inoculated pots (data not presented). Effect of mycorrhizal inoculation

Table 1. Brief description of sampling sites in the native European and the introduced Kashmir Himalayan regions together with number of individuals of *Anthemis cotula* damaged in a population (for each 100 individuals studied randomly per population) and the extent of damage caused to the individual plant (estimated on the basis of number of branches infested/individual and presented in %age terms).

Sites in native and non-native regions	Habitat type	No. of individuals damaged/100 individuals in population	Extent of damage/individual
European sites			
E1	Dry, exposed, highly disturbed	86	75
E2	Dry, shady, moderately disturbed	82	68
E3	Dry, exposed, highly disturbed	92	72
E4	Dry, exposed, highly disturbed	79	70
E5	Wet, shady, moderately disturbed	85	78
Kashmir sites			
K1	Dry, exposed, moderately disturbed	0	0
K2	Dry, protected, undisturbed	2	0
K3	Dry, exposed, highly disturbed	0	0
K4	Wet, exposed, moderately disturbed	3	0
K5	Wet, exposed, highly disturbed	0	0

and herbivory, both in isolation and in combination, showed significant (P<0.001) influence on the growth and reproductive attributes of this species. Perusal of the results indicates a significant interaction effect of herbivory and mycorrhizal association on the stem length (Figure 2a), stem biomass (Figure 2c) and the number of capitula per plant (Figure 2f). However no significant differences were observed in other parameters such as root length (Figure 2b), root biomass (Figure 2d) and the number of branches per plant (Figure 2e) between treatment means and the means of their interaction.

DISCUSSION

The positive impact of AM fungi on almost all the parameters of the target species investigated during the present study pointed towards the potential of invasive species to draw these soil fungi in the introduced range to their favour. The results are in conformity with some recent studies (Fumanal et al., 2006; Shah et al., 2008a, b). Besides, the herbivore-induced promotion of fitness (overcompensation) by increasing the number of branches per plant that resulted in an increase in the number of capitula per plant in the present study corroborated our previous results (Rashid et al., 2006). Almost similar results have been obtained in *Ipomopsis aggregate* by Paige (1999). The overcompensation in monocarpic herbs is usually associated with apically dominant shoot architecture of intact plants, increased lateral branching following herbivory, and increased reproductive success as a consequence of damage (Routio et al., 2005). However, the effect of herbivory was significantly influenced by the presence of AM fungi, which can be explained by compensatory continuum hypothesis (Maschinski and Whitham, 1989) that expects overcompensation to be more prevalent in resource rich environments, as created by AM fungi in the present study. While apical dominance is rather insensitive to changes in resource availability, yet overcompensation is possible in conditions where plants experience meristem limitation (due to apical dominance) in relation to available resources (Routio et al., 2005). A recent study has also shown that the grazing tolerance and mycorrhizal colonization of plants are linked to resource manipulation and plant size (Piippo et al., 2011). However, mycorrhizal colonization has been shown not to affect tolerance to defoliation of an annual herb in different light availability and soil fertility treatments (Aguilar-Chama and Guevara, 2011).

Since mycorrhizal species are reported to differentially alter plant growth and response to herbivory by multiple mycorrhizal species is driven by the inclusion of a super-fungus (Bennett and Bever, 2007), we used a mixed inoculum containing all those AM which have been

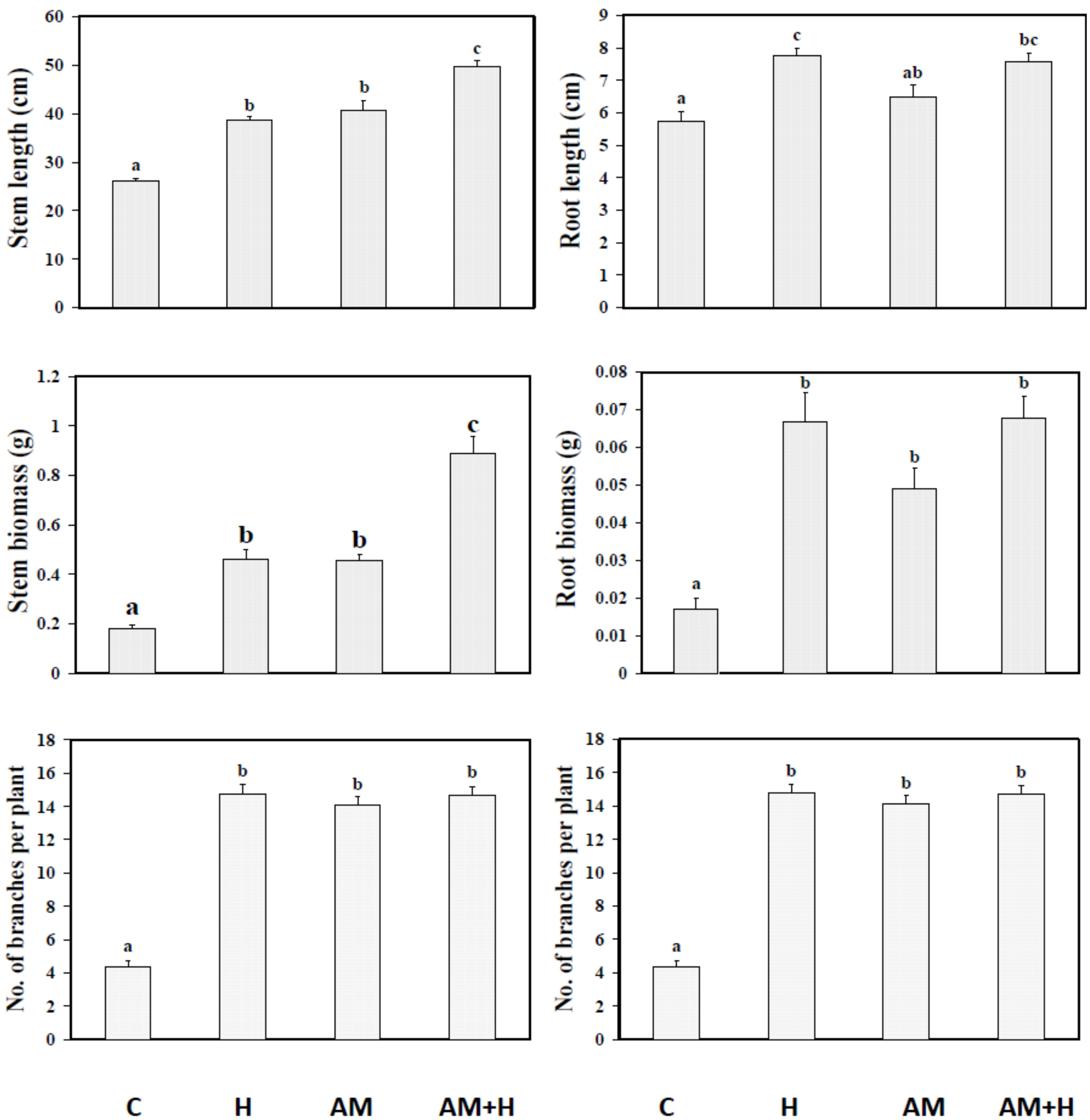

Figure 2. Effect of Herbivory (H), mychorrizal inoculation (AM) and their combination (AM+H), in comparison to control (C), on stem length , root length, stem biomass, root biomass, number of branches per plant and number of capitula per plant of *Anthemis cotula.* Errors bars represent standard eeror of the mean. Bars that share letters were not different at P<0.05 (post-ANOVA) Tukey test.

previously reported to promote growth and fitness of *A. Cotula* (Shah and Reshi, 2007; Shah et al., 2008a, b). Whilst the effect of herbivores and mycorrhizas in relation to plant invasion has been hitherto studied independently, the present study on their interactions to influence fitness of alien species provides a framework for further studies in this direction. Herbivores, consuming aboveground foliage, may reduce photosynthate translocated to the root-associated mycorrhiza, thereby reducing their extent of colonization and impairing symbiosis development (Gehring et al., 1997; Hetrick et al., 1990; Trent et al., 1988). Mycorrhizas, in turn, may have many potential effects on plant herbivore interactions. Under certain conditions, up to 40 to 50% of a plant's net production may be allocated to its fungal symbiont (Fogel and Hunt, 1979; Harris and Paul, 1987). Because mycorrhizal fungi both consume photosynthate and at the same time enhance mineral nutrient acquisition and growth capacity, the cost–benefit relationships among mycorrhizal fungi, herbivores and host plants are likely to be complex. Mycorrhizas may affect herbivores through alteration of plant growth or foliar chemistry (Goverde et al., 2000) and they may have large effects on plant responses to herbivores by influencing anti-herbivore defenses and/or tolerance to herbivory (re-growth capacity). The significant influence of mycorrhizal inoculation and herbivory, both in isolation and in combination, on invasiveness of *A. cotula* in the present study point towards the fact that altered interactions of alien species with enemies, mutualists and competitors in the introduced range may jointly influence their invasive success and need to be understood in unison. The present study provides some useful clues for tripartite (herbivore-host-AM) interactions for further experiments to gain further insights into the top-down and bottom-up plant interactions that facilitate alien plant invasions.

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