Invasion by alien *Anthemis cotula* L. in a biodiversity hotspot: Release from native foes or relief from alien friends?

Invasion by alien taxa is a burgeoning global problem with significant socio-economic and ecological costs[1–3]. Though the relative abundance of alien species in introduced plant communities is by and large an inexplicable problem[4,5], escape from the enemies associated with such taxa in their native range (escape from enemy hypothesis) has received considerable attention[6]. However, the role of soil biota, particularly arbuscular mycorrhizas (AM), in promoting exotic plant invasion is the most recent proposition[7,8]. The present investigation indicates that release from native herbivory together with relief from alien mycorrhizal mutualists synergistically promote invasiveness of an exotic species in Kashmir Himalaya.

Anthemis cotula (Stinking Mayweed, Asteraceae) is an annual herbaceous plant native to southern Europe–west Siberia[9], where the above-ground herbivores of the species are dominated by aphids (Aphidae), spittlebugs (Cercopidae), bugs (Heteroptera), moths, slugs and snails[9]. In the Himalayan valley of Kashmir, a recently declared biodiversity hotspot[10], this fast-spreading species is emerging as a major threat to native biodiversity and ecosystem processes. Invasion of ruderal habitats by this species has been attributed to its protracted recruitment pattern aided by habitat disturbance and favourable moisture, temperature, light and nutrient regimes, and high population size even after seedling mortality[11] and allelopathic activity of its aqueous leaf leachate[12]. In western Europe, the species is attacked by about 68 insect pathogens, of which 13 specifically use it as their host (S. Benvenuti, pers. commun.). 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[13]. Notwithstanding our intensive surveys of innumerable populations since 2000, we are yet to record any associated herbivore or pathogen that may be limiting its spread in virgin areas.

In view of the significant influence of AM mutualists on different attributes of plants[14,15] in general, and invasiveness of some species[16] in particular, the present study was carried out to document the extent of mycorrhizal colonization across different populations of *A. cotula* in the Kashmir Valley and to ascertain the effect of mycorrhization on its growth and fitness through controlled and ecologically relevant experiments.

During the field surveys undertaken from March to September (2005), thirty seedlings from each of the fourteen populations (Table 1) were collected for determining the extent of mycorrhization of *A. cotula*. Populations that fall within geographical coordinates of 34°50′–34°26′N lat. and 74°5′–75°45′E long., and altitudinal range of 1380–3050 m asl, were supported by habitats varying in exposure, disturbance and moisture status. Roots from each sample of 30 seedlings were cut into 1 cm pieces and pooled. One hundred root pieces from each of these pooled samples were cleared in 15% KOH and subsequently stained with trypan blue, followed by destaining in 50% lactic acid. The per cent colonization was estimated following McGonigle *et al.*[17]. Since most of the AM fungi were of *Glomus* type, a pot trial was set up to evaluate the influence of AM on the growth and fitness of *A. cotula* using *Glomus mossae* spores (obtained from School of Life Sciences, Jawaharlal Nehru University, New Delhi) as inoculum. Healthy achenes of *A. cotula*, collected by the authors from the field populations during the previous year, were sown during April 2005, in 9 inch diameter earthen pots containing sterilized silt loam soil (clay, 28%; silt, 50%; sand, 22%; pH 7.5 and organic carbon, 1.6%). The pots were maintained in the Botanic Garden of the University of Kashmir, Srinagar. Plants growing in pots not inoculated with spores of *G. mosseae* served as controls. After emergence, three seedlings per pot were retained and were watered to field capacity twice a week. Data on various morphological and reproductive attributes were recorded at flowering stage and subsequently, the inoculated and uninoculated plants were harvested and oven-dried at 70°C to constant weight to determine the dry mass. Data were statistically analysed through multivariate procedures using General Linear Model of SPSS (Version 10.0). All independent variables except number of branches per plant were $\log_{10}$ transformed to attain normality and to overcome heteroscedasticity of data.

The study revealed that almost all the populations of *A. cotula* collected from different areas of the Kashmir Valley harbour AM mutualists. However, the extent of root colonization by these mutualists varied across populations (Table 1). Plant samples drawn from populations of Mirzabagh, Zukura and Hokersar (all falling within Srinagar) showed high per cent root length colonization (85, 84.6, and 80.6 respectively) and the same decreased (11.1, 14 and 8.5) in populations distant from Srinagar, viz. Uri, Drass and Bandipora respectively (Table 1).

Experimental studies revealed that inoculation of *A. cotula* with *G. mossae* significantly enhances both morphological and reproductive attributes of the host species (Table 2). While morphological traits like stem height, shoot biomass and number of branches were favourably influenced by mycorrhization (Table 2), the favourable effect was statistically significant in fitness attributes, such as number of inflorescences per plant and number of achenes per inflorescence ($P < 0.01$). However, length and dry mass of roots was more in the case of uninoculated control plants. On the basis of extent of increase in growth due to mycorrhizal inoculation[18], the mycorrhizal dependency in *A. cotula* was 116.95%.

Thus, the present investigation reveals favourable influence of AM mutualists on reproductive attributes (Table 2), which is of special importance in *A. cotula* because of it being an annual plant. Any factor that increases achene production in this plant could be considered as a contributory factor in its spread and invasion. Earlier reports of AMF-mediated increase in fecundity and seed quality in a number of agricultural weeds and invasive species[14,19] support the present observations. Such benefits result from enhanced uptake of soil nutrients, such as P, NH_4–N, K, Ca, Mn, Fe, Cu, Zn and Ni in various plants[19,20] which in turn allows mycorrhizal plants to tolerate wide variations in biotic and abiotic factors[21]. In *A. cotula*, the AMF-mediated benefits translate into enhanced fitness as number of capitula per plant and number of

Table 1. Per cent root length colonization (% RLC) in different populations of *Anthemis cotula*

Sampling site*	% RLC	Sampling site	% RLC
Mirzabagh (13)	85.1	Lalbazar (14)	50
Zukura (17)	84.6	Hazratbal (12)	41.1
Shikarghat (12)	80.6	Ferozpur (30)	35.2
Nagbal (19)	67.5	Baramulla (50)	34.8
Narbal (18)	64.5	Uri (80)	11.1
Gondhasibat (16)	54.1	Bandipur (55)	8.5
Pampore (9)	50	Drass (140)	14

*Distance (km) from Srinagar is given in parentheses.

Table 2. Effect of *Glomus mosseae* on vegetative and reproductive attributes of *A. cotula* (statistical analysis based on log-transformed data except for number of branches)

Plant attributes	Control Mean ± SE	*Glomus*-treated Mean ± SE	*F* ratio Attribute × treatment
Root length (cm)	15.1 ± 1.053	13.866 ± 1.374	0.518
Stem length (cm)	36.633 ± 2.451	45.066 ± 3.547	3.849
Root biomass (g)	0.32 ± 0.055	0.176 ± 0.028	5.668
Shoot biomass (g)	2.133 ± 0.135	2.693 ± 0.386	1.820
Root : shoot ratio	0.236 ± 0.088	0.066 ± 0.02	6.062
No. of branches/plant	18.400 ± 1.907	21.766 ± 1.299	2.172
No. of inflorescences/plant	4.400 ± 0.115	24.000 ± 4.932	53.945*
No. of achenes/capitulum	39.200 ± 2.666	59.066 ± 3.644	20.398**

$*P < 0.001$ and $**P < 0.01$.

achenes per capitulum increased significantly in comparison with control (Table 2), thereby supporting similar findings of earlier authors[22]. Increase in reproductive output in this species upon removal of competitors and herbivores has been also reported[9], and the increased fecundity observed during the present study could be explained both on the basis of direct influence of AMF on growth and indirect influence of changed secondary metabolite production, which is thought to alter plant–insect herbivore interaction[16]. Richardson *et al.*[23] also reported that many introduced plant species rely on mutualisms in their new habitats to overcome barriers to establishment and become naturalized and, in some cases, invasive.

In conclusion, the present investigation not only supports enemy release hypothesis[6], but also the Klironomos hypothesis[5] which posits that some invaders in their non-native range drive the soil biota to their advantage. In addition, invasive plants like *A. cotula* enjoy a positive feedback from soil mycorrhizas in their invaded range in contrast to possible negative feedback in their home range[24]. The mycorrhizal mutualists not only help such invasive species in overcoming biotic resistance in their invaded range, but also help them in avoidance of damage caused by insect herbivory as suggested by Gange *et al.*[16]. Thus, the successful spread of invasive *A. cotula* in Kashmir Himalaya is due to its release from the enemies associated with it in its native range and the friendly mycorrhizal associates that produce a synergistic effect on its morphological and fitness attributes. However, future studies need to include comparisons of both belowground and aboveground pathogens and mutualists between and within natural and introduced ranges of the species for improved understanding of its invasiveness.

1. Didham, R. K., Tylianakis, J. M., Hutchinson, M. A., Ewers, R. M. and Gemmell, N. J., *Trends Ecol. Evol.*, 2005, **20**, 470–474.
2. Perrings, C., Dehnen-Schmutz, K., Touza, J. and Williamson, M., *Trends Ecol. Evol.*, 2005, **20**, 212–215.
3. Pimentel, D., Zuniga, R. and Morrison, D., *Ecol. Econ.*, 2005, **52**, 273–288.
4. Blumenthal, D., *Science*, 2005, **310**, 243–244.
5. Klironomos, J. N., *Nature*, 2002, **417**, 67–70.
6. Keane, R. M. and Crawley, M. J., *Trends Ecol. Evol.*, 2002, **17**, 164–170.
7. Callaway, R. M., Thelen, G. C., Rodringuez, A. and Holben, W. E., *Nature*, 2004, **427**, 731–733.
8. Wolfe, B. E. and Klironomos, J. N., *Bioscience*, 2005, **55**, 477–487.
9. Erneberg, M., *Oecologia*, 1999, **118**, 203–209.
10. http.//biodiversityhotspots.org/kp/hotspots/himalaya/
11. Allaie, R. R., Reshi, Z. and Wafai, B. A., *Trends Life Sci.*, 2005, **20**, 49–56.
12. Allaie, R. R., Reshi, Z., Rashid, I. and Wafai, B. A., *J. Agron. Crop Sci.*, 2006, **192**, 186–191.
13. www.ceh.ac.uk/sections/brc/projects/PhytophagousInsectDatabase.htm
14. Jordan, N. R., Zhang, J. and Huerd, S., *Weed Res.*, 2000, **40**, 397–410.
15. Allen, M. F., Swenson, W., Querejeta, J. I., Egerton-Warburton, L. M. and Treseder, K. K., *Annu. Rev. Phytopathol.*, 2003, **41**, 271–303.
16. Gange, A. C., Brown, V. K. and Aplin, D. M., *Ecology*, 2005, **86**, 603–611.
17. McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. S. and Swan, J. A., *New Phytol.*, 1990, **115**, 495–501.
18. Menge, J. A., Johnson, E. L. and Platt, R. G., *New Phytol.*, 1978, **81**, 553–559.
19. Heppell, K. B., Shumway, D. L. and Koide, R. T., *Funct. Ecol.*, 1998, **12**, 171–175.
20. Marschner, H. and Dell, B., *Plant and Soil*, 1994, **159**, 89–102.
21. Smith, W. E. and Read, D. J., *Mycorrhizal Symbiosis*, Academic Press, CA, USA, 1997.
22. Shah, M. A., Reshi, Z. and Irfan, R., *J. Appl. Ecol. Environ. Res.*, 2006, **4**, (in press).
23. Richardson, D. M., Allsopp, N. D., Antonio, C., Miller, S. J. and Rejmanek, M., *Biol. Rev.*, 2000, **75**, 65–93.
24. Newsham, K. K., Fitter, A. H. and Watkinson, A. R., *J. Ecol.*, 1995, **83**, 991–1000.

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