Arbuscular mycorrhizal fungi affect phytophagous insect specialism

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Biosketch: Alan Gange's research interests include the multitrophic interactions between insects, plants and fungi. The latter involves foliar endophytes and subterranean arbuscular mycorrhizas.

Abstract

The majority of phytophagous insects eat very few plant species, yet the ecological and evolutionary forces that have driven such specialism are not entirely understood. The hypothesis that arbuscular mycorrhizal fungi can determine phytophagous insect specialism, through differential effects on insect growth, was tested using examples from the British flora. In the UK, plant families and species in the family Lamiaceae that are strongly mycorrhizal have higher proportions of specialist insects feeding on them than those that are weakly mycorrhizal. We suggest that AM fungi can affect the composition of insect assemblages on plants and are a hitherto unconsidered factor in the evolution of insect specialism.

INTRODUCTION

Many factors are thought to account for the great degree of specialism seen in the diets of most phytophagous insects. These include genetically-based trade-offs in performance on novel hosts, effects of interspecific competition, predation pressure, coevolution with plant chemical defences or constraints on the neural ability of the phytophage (Jaenike 1990; Mitter *et al*. 1991; Joshi & Thompson 1995; Bernays & Funk 1999). Specialism may also be associated with small body size, mode of overwintering, persistency of food resource and dispersal ability (Ward & Spalding 1993; Loder *et al*. 1998).

Many of these theories are founded in the response of insects to plant secondary metabolites (Harborne 1994). By specializing on a narrow diet, insects encounter fewer toxic chemicals and may even sequester these as part of their own defence. Hypotheses proposed to explain the diversity of plant secondary chemicals are generally plant- or insect-centred, but ecologists now realise that fungi existing within the roots or shoots of plants can significantly affect the chemistry of the foliage and thereby alter phytophagous insect growth (Gange & Bower 1997; Saikkonen *et al*. 1998). Arbuscular mycorrhizal (AM) fungi, which colonize the roots of most vascular plants, are known to alter plant physiology and chemistry. These changes lead to increases in growth of specialist chewing and specialist and generalist sucking insects, but decreases in growth of generalist chewers (Gange & West 1994; Borowicz 1997; Gange *et al*. 1999a; Goverde *et al*. 2000). A number of ecological correlates of mycorrhizal occurrence in the British flora have been documented (Peat & Fitter 1993), but since this work, an important ecological and evolutionary question has arisen. This is whether the differential effects of AM fungi on specialist and generalist phytophagous insects lead to different insect assemblages on mycorrhizal and non-mycorrhizal plants. Here we provide evidence that plant families or species that are strongly mycorrhizal have higher proportions of specialist insects, higher proportions of sucking insects and lower proportions of chewing insects associated with them than do those that are weakly mycorrhizal or non-mycorrhizal.

MATERIALS AND METHODS

We selected the dicotyledonous families in the British flora which only form an association with AM fungi (Harley & Harley 1987). We excluded families that contain entirely non-native species, or trees and shrubs or which had fewer than three species in the family. This produced a total of 37 families, containing 1058 species. The families were: Apiaceae, Asteraceae, Balsaminaceae, Boraginaceae, Brassicaceae, Campanulaceae, Caryophyllaceae,

Chenopodiaceae, Clusiaceae, Convolvulaceae, Crassulaceae, Dipsaceae, Euphorbiaceae, Fabaceae, Fumariaceae, Gentianaceae, Geraniaceae, Lamiaceae, Linaceae, Lythraceae, Malvaceae, Onagraceae, Oxalidaceae, Papaveraceae, Plantaginaceae, Plumbaginaceae, Polygonaceae, Polygalaceae, Portulacaceae, Primulaceae, Resedaceae, Saxifragaceae, Scrophulariaceae, Urticaceae, Valerianaceae and Violaceae. The proportion of species within each family that form an AM association was obtained from Harley & Harley (1987) and its addenda. For 102 species for which there were no previous records, we supplemented this information with our own observations. Insect host plant data were obtained from the Phytophagous Insect Data Base (PIDB), which contains over 50,000 insect-host plant records for over 6,000 species of insect and 2,000 plants (Ward & Spalding 1993). Insect data were expressed as proportions, because it is known that the absolute numbers of insects associated with any plant family is strongly dependent on the number of plant species within that family (Ward & Spalding 1993).

As families cannot be considered strictly independent data points (Harvey & Pagel 1991), we calculated standardized independent contrasts (Felsenstein 1985) using the model Comparative Analysis by Independent Contrasts (Purvis & Rambaut 1995). We used the strict consensus tree (given at http://www.cis.upenn.edu/~krice/treezilla/index.html) derived from the data matrix of analysis II (Chase *et al*. 1993), as a phylogeny for plant families. To examine the relations between degree of specialization of associated insects and the proportion of AM species within each family, we used a standard regression technique, with all proportional data being subjected to the angular transformation prior to analysis. Although statistical theory states that such regressions should be fitted without a constant (Garland *et al*. 1992), it could be argued that such a situation is biological meaningless in our data. For example, a plant family could contain no mycorrhizal species, yet one would still expect some insects to be associated with it. As a compromise, we fitted regressions with and without a constant.

The family Lamiaceae was selected for a detailed study because it is believed to be a monophyletic group (Wagstaff *et al*. 1998). To avoid confounding effects of life history, we restricted our analysis to native perennials and collected material of 21 species. These were: *Ajuga reptans, Ballota nigra, Clinopodium ascendens, C. vulgare, Glechoma hederacea, Lamium album, Lamiastrum galeobdolon ssp. montanum, Lycopus europaeus, Marrubium vulgare, Mentha aquatica, M. arvensis, Nepeta cataria, Origanum vulgare, Prunella vulgaris, Salvia pratensis, S. verbenaca, Scutellaria galericulata, Stachys officinalis, S. palustris, S. sylvatica and Teucrium scorodonia* (Nomenclature follows Stace 1991). Each species was

collected from at least 20 different localities across England, with at least 5 plants collected at each locality. All collections took place in July when plants were mature. Roots were washed and AM colonization recorded using autofluorescence microscopy (Gange *et al*. 1999b). Percent AM colonization was calculated for each species as the mean of all values obtained over all localities. Insect host plant records were obtained from the PIDB, as above. We again calculated standardized independent contrasts, using the model Comparative Analysis by Independent Contrasts, applicable when an approximate phylogeny is available (Cantino 1992). Relations between the degree of specialization of associated insects and extent of AM colonization within this family were examined using linear regression, in which relationships were fitted with and without a constant, as before.

To examine in detail the response of generalist and specialist insects to AM colonization of the Lamiaceae, we reared pairs of insect species from the same family, one of which is a specialist on Lamiaceae and one a generalist which will feed on this family. All pairs were chosen because they occurred commonly together in the natural communities from which we sampled our 21 species of plants (above). We used the chewing insects, *Scopula ornata* Scopoli (specialist) and *Idaea aversata* L. (generalist) (Lepidoptera: Sterrhinae) feeding on *Origanum vulgare* and *Pyrausta aurata* Scopoli (specialist) and *Udea prunalis* Denis & Schiffermüller (generalist) (Lepidoptera: Pyraustinae) on *Clinopodium vulgare*. The sucking insects were *Cryptomyzus ribis* L. (specialist) and *Myzus persicae* Sulzer (generalist) (Homoptera: Aphididae) feeding on *Stachys sylvatica*. Plants were collected from one locality and the mycorrhizal fungus *Glomus fasciculatum* (Thaxt.) Gerd. & Trappe isolated from the roots of all three species. Test plants were grown from seed in sterilized soil to which an inoculum of *G. fasciculatum* was added to half and sterilized inoculum to the other half, creating mycorrhizal and non-mycorrhizal individuals. A filtered soil wash was added to all pots to correct for the non-mycorrhizal microbial flora (Koide & Li 1989). Soil P content was relatively high (16 μg P g^{-1} (bicarbonate extractable)) and no supplemental nutrients were given to either treatment. Plants were grown for eight months, until mature. At this time, insects were reared singly from birth to teneral adult (suckers) or for six weeks (chewers), with one individual on 20 replicate mycorrhizal and non-mycorrhizal plants respectively. Dry weight was used as the insect growth parameter.

RESULTS

When controlling for phylogeny, there is a strong, positive relationship between the mycorrhizal status of plant families in the British flora and the percentage of associated insects which feed only on members of that family ('specialists') (without constant, $r^2 = 0.763$, $P < 0.001$; with constant, $r^2 = 0.321$, $P < 0.001$; Fig. 1a). Plant families in which the majority of species is mycorrhizal have higher proportions of specialist insects in their associated assemblages than do families in which the minority of species is mycorrhizal. A similar relationship exists for sucking insects (without constant, $r^2 = 0.605$, $P < 0.001$; with constant, $r^2 = 0.166$, $P < 0.05$; Fig. 1b). However, the reverse relationship exists for chewing insects; as strongly mycorrhizal families have associated insect assemblages with lower proportions of chewers than do families in which the minority of species is mycorrhizal (without constant, $r^2 = 0.822$, $P < 0.001$; with constant, $r^2 = 0.189$, $P < 0.01$; Fig. 1c).

Within the Lamiaceae, native perennial species in this family which are strongly mycorrhizal have an associated fauna dominated by insects which are restricted to that species ('extreme specialists') (without constant, $r^2 = 0.691$, $P < 0.001$; with constant, $r^2 = 0.485$, $P < 0.001$; Fig. 2a). Furthermore, species in the Lamiaceae which are heavily colonized by AM fungi have insect assemblages with higher proportions of sucking insect species (without constant, $r^2 = 0.654$, $P < 0.001$; with constant, $r^2 = 0.522$, $P < 0.001$; Fig. 2b) and lower proportions of chewers (without constant, $r^2 = 0.335$, $P < 0.01$, with constant, $r^2 = 0.207$, $P < 0.05$; Fig. 2c). AM colonization increased the larval growth of two specialist chewing insects by 30-40% (Fig. 3). However, generalist chewing insects responded in the opposite manner; larval growth was reduced on mycorrhizal plants. When specialist and generalist sucking insects were examined, both species grew better on the mycorrhizal plants. AM colonization therefore had a positive effect on the growth of sucking insects and specialist chewers, but a negative effect on generalist chewers.

DISCUSSION

It is clear that the composition of insect communities associated with highly mycorrhizal plant families differs from those associated with weakly mycorrhizal families. Families with a high proportion of mycorrhizal plant species have insect faunas dominated by specialist insects. Insect faunas on strongly mycorrhizal plant families have higher proportions of sucking insects and lower proportions of chewing insects. Furthermore, within the family Lamiaceae plant species that are strongly mycorrhizal have associated faunas that are also dominated by specialist and sucking insects.

In the Lamiaceae, we suggest that the enhanced performance of specialist insects and reduced performance of generalists on mycorrhizal plants, such as in our controlled experiment, has, over evolutionary time, led to the associated insect assemblage being dominated by specialists. We believe that the AM-induced effects on insect growth are due to chemical changes in the foliage. The Lamiaceae are rich in secondary metabolites, including terpenoids, flavonoids and iridoid glycosides (Simmonds & Blaney 1992). The latter group contains two chemicals in particular, aucubin and catalpol, which have activity against generalist insects, while being an important component of the diet of specialist insects (Bowers & Puttick 1988). Furthermore, AM fungi have been shown to increase concentrations of both chemicals in foliage, with resulting growth decreases in a generalist chewing insect (Gange & West 1994).

It is possible that soil fertility acts as a confounding factor in the mycorrhizal-insect relationship. For example, plants growing in resource-poor soils would be expected to invest in defence, rather than growth (Herms & Mattson 1992). Meanwhile, in such soils, mycorrhizal-induced benefits to growth should be more prevalent (Smith & Read 1997), thus resulting in a situation in which mycorrhizal plants tend to be more heavily defended by secondary metabolites. These might then be expected to support higher proportions of specialist insects (Jaenike 1990). Clearly, without further research one cannot know if this is so. However, we attempted to account for variations in soil fertility by sampling Lamiaceae from 20 different localities, encompassing a wide variation in soil fertility. We are therefore confident that our results are real and not entirely explained by soil nutrient availability.

Sucking insects are positively affected by AM colonization, irrespective of whether they are specialists or generalists. The mechanism is thought to be one in which AM fungi alter plant physiology, making the phloem elements more accessible (Gange *et al*. 1999a). This effect appears to be general across plant families, resulting in those which are strongly mycorrhizal having a greater proportion of sucking insects.

We believe that AM fungi affect the proportions of specialist insects in phytophagous assemblages because: a) growth of specialist chewing insects is enhanced on mycorrhizal plants, but growth of generalist chewers reduced; b) growth of sucking insects is enhanced and c) the majority (85%) of sucking insects in the British fauna are specialist (Ward & Spalding 1993). The underlying mechanism is one in which AM fungi alter host plant chemistry, to the advantage of specialist insects and the detriment of generalists. This conclusion does not contradict other theories of insect specialism (Mitter *et al*. 1991; Joshi &

Thompson 1995; Bernays & Funk 1999), but redefines them in that the chemical diversity on which they are based may be fungal-induced, rather than plant produced.

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FIGURE LEGENDS

Figure 1. Relationship between specialisation of associated phytophagous insects and mycorrhizal status of different plant families. Mycorrhizal status is positively related to **(a)** the proportion of the insect fauna associated with a family which feeds on only members of that family ('specialists') and **(b)** the proportion of the insect fauna which feed by sucking but negatively related to **(c)** the proportion which feed by chewing. The raw data are presented here for visual clarity, but analyses were performed with Phylogenetic Independent Contrasts.

Figure 2. Relationship between specialisation of associated phytophagous insects and mycorrhizal status of different species in the Lamiaceae. Mycorrhizal status is positively related to **(a)** the proportion of the insect fauna on a plant which feeds on only that plant ('extreme specialists') and **(b)** the proportion of the insect fauna which feed by sucking but negatively related to **(c)** the proportion which feed by chewing. The raw data are presented here for visual clarity, but analyses were performed with Phylogenetic Independent Contrasts.

Figure 3. The percentage change in growth of three phytophagous insect pairs, when reared on mycorrhizal plants, relative to the growth on non-mycorrhizal plants. On *Clinopodium vulgare*, growth of *Scopula ornata* (specialist) was increased by mycorrhizal colonization (t = 4.34, *P* < 0.001) but that of *Idaea aversata* (generalist) was decreased (t = 3.67, *P* < 0.001). On *Origanum vulgare* the specialist *Pyrausta aurata* showed increased growth on mycorrhizal plants (t = 5.55, *P* < 0.001), but the generalist *Udea prunalis* showed decreased growth (t = 2.48, *P* < 0.05). On *Stachys sylvatica*, both the specialist aphid *Cryptomyzus ribis* and the generalist aphid *Myzus persicae* showed increased growth on mycorrhizal plants (t = 3.75, *P* < 0.001 for *C. ribis* and t = 2.27, *P* < 0.05 for *M. persicae*).

Figure 1

Figure 2
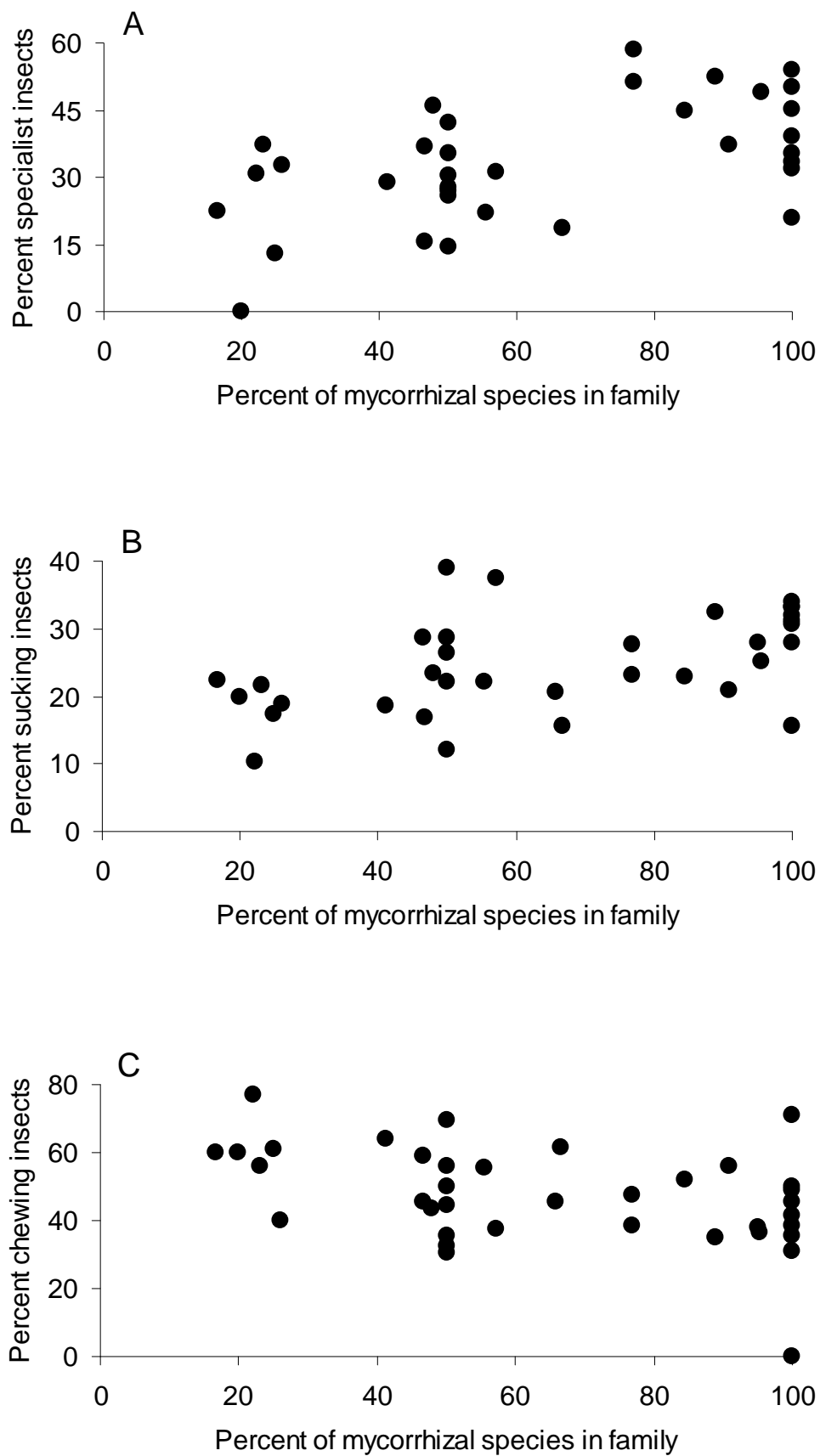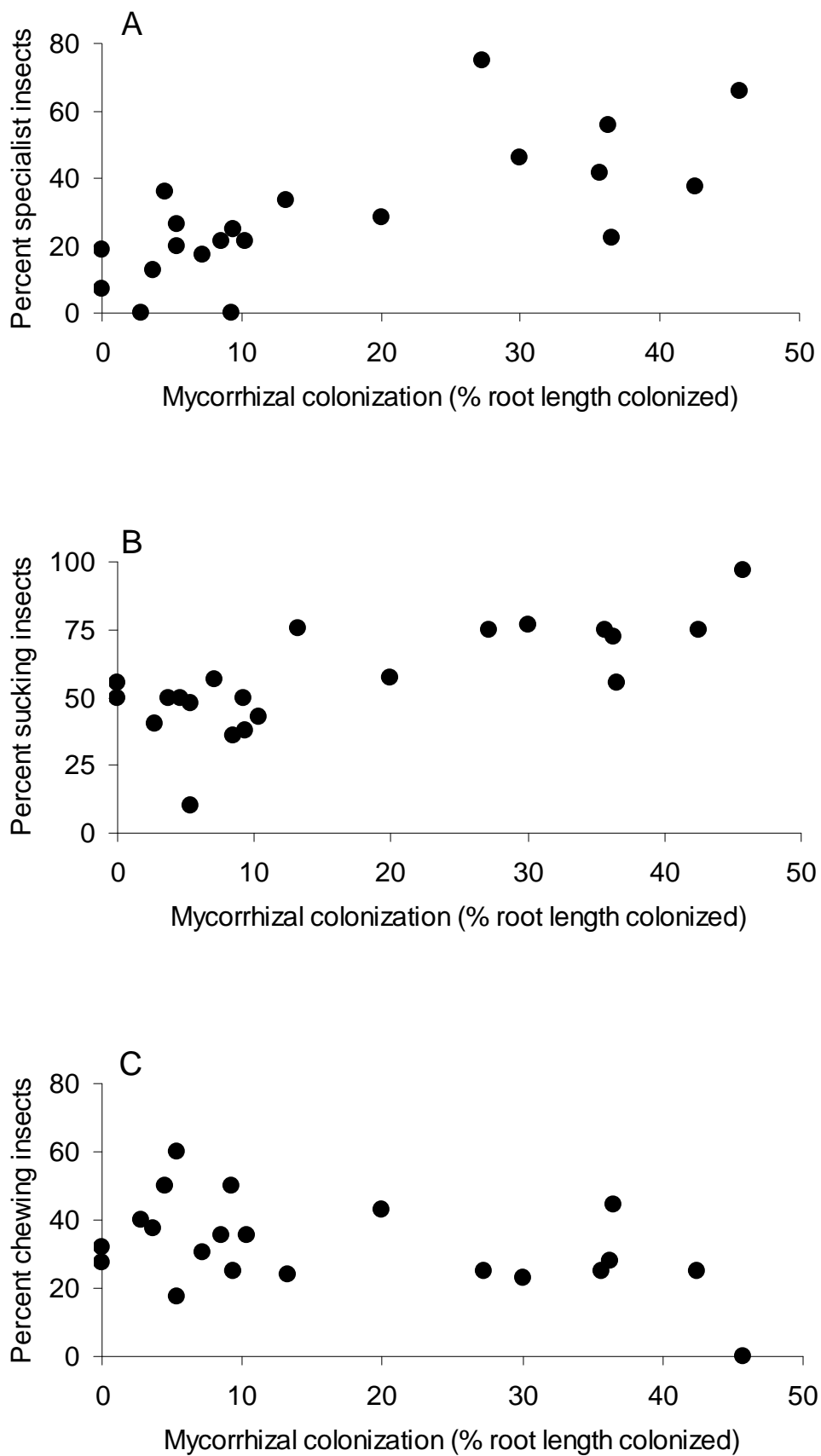
Figure 3