

**Site- and species-specific differences in endophyte occurrence in two  
herbaceous plants**

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## Summary

**1** Endophyte fungi exist within the living tissues of all plants, but compared to grasses and trees, remarkably little is known about their ecology in herbaceous species. These fungi produce an array of metabolites in culture and there is some evidence that they can increase the resistance of plants to herbivorous insects.

**2** As herbaceous plant endophytes are thought to be unspecialized, ubiquitous taxa, we hypothesized that their occurrence within two closely-related plant species would not vary between local plant communities. Furthermore, we expected to find negative relations between endophyte occurrence and that of a herbivorous insect.

**3** We tested these hypotheses by isolating endophytes from *Leucanthemum vulgare* and *Cirsium arvense* (Asteraceae) plants growing together in five populations, each about 13 km apart. Damage by the leaf mining fly, *Chromatomyia syngenesiae* was also measured on each plant.

**4** *C. arvense* harboured more species of fungi per plant and the number of isolates recovered per leaf was also higher. Several fungi showed differences in occurrence within the two plants, but these differences were not consistent between sites. The similarity in the endophyte assemblage decreased with increasing inter-site distance in *C. arvense*, but not in *L. vulgare*. We conclude that endophytes either colonize *C. arvense* more readily or have greater activity within this host (or both).

**5** Leaf miner attack was positively related to total endophyte species number in *L. vulgare*, but not so in *C. arvense*, while occurrence of *Chaetomium* species was negatively associated with insect attack in both plants. In *L. vulgare*, only 5% of relations between occurrence of different endophyte species were significant, but in *C. arvense*, this figure was 43% and all were negative.

**6** This study has important implications for understanding the factors that influence plant resistance to insects. It is the first report of endophytic fungi affecting host plant choice by insects in herbaceous plants. The abundance of unspecialized endophytes in forbs means that they are a neglected, but important aspect of plant-herbivore relations.

*Key-words:* *Chromatomyia syngenesiae*, *Cirsium arvense*, forb, fungi, insect herbivore, *Leucanthemum vulgare*, multitrophic interactions, spatial distribution

## Introduction

Endophytic fungi, defined as those species that occur within the living tissues of plants, without causing visible disease symptoms at a particular time, have been isolated from every organ of almost every plant species sampled (Stone *et al.* 2000). The definition of the term 'endophyte' has been the subject of considerable debate (Wilson 1995), and it is important to realise that it can include true endophytes and latent pathogens (Carroll 1988; Saikkonen *et al.* 2004). Endophytes are generally separated from phylloplane fungi, as the available evidence suggests that there is little, if any, similarity between the fungal assemblage that exists on the surface of, and within, a particular plant organ (Girivasan & Suryanarayanan 2004; Santamaría & Bayman 2005). The ecological roles of endophytic fungi are well studied in grasses and, to a lesser extent, trees (Saikkonen *et al.* 1998). Endophytes of the Balansiaceae are a unique group of fungi that occur within grasses, sedges and rushes. Some species are considered to be perfect mutualists, existing entirely within the plant and transmitted vertically, through the seed, from one plant generation to another. These species receive shelter and nutrients from their host and in return confer advantages in terms of herbivore or pathogen resistance, drought tolerance or competitive ability. However, such mutualism does not seem to be of universal occurrence and there are many examples where these endophytes are antagonistic or have no measurable effect on their hosts (Schardl *et al.* 2004; Saikkonen *et al.* 2006).

In contrast, non-balansiaceous endophytes in woody or herbaceous hosts are horizontally-transmitted (usually air-borne), diverse, and many are unspecialised fungi of ubiquitous occurrence (Schulz & Boyle 2005). Most species seem to have alternative life histories, being coprophilous, saprotrophic or pathogenic (as a latent infection in the host or of other, non-hosts) (Carroll 1988). These fungi produce a very wide range of metabolites in culture (Tan & Zou 2001) that may possess herbicidal, anti-microbial or insecticidal properties, offering the potential for novel pharmaceutical development (Schulz *et al.* 2002). However, in woody plants, interactions between these fungi and insects range along a continuum, from antagonistic to beneficial (Saikkonen *et al.* 1998).

While the functional ecology of endophytes in grasses and woody plants may be unclear, it is virtually unstudied in herbaceous plants. Several studies present species lists of endophytes recovered from forbs (e.g. Schulz *et al.* 1993; Peláez *et al.* 1998; Suryanarayanan *et al.* 2005). These tend to show that the majority of isolates belong to ubiquitous genera such as *Acremonium*, *Alternaria*, *Cladosporium* and *Epicoccum*. Co-occurring taxonomically

unrelated plants, or plants of the same species growing in different sites, sometimes seem to host different fungal assemblages, but this is rarely tested. An exception is Seena & Sridhar (2004) who examined endophyte infection frequency in two con-generic legumes growing in the same location. Here, no differences were found in the endophyte occurrence in the two plant species.

If the majority of herbaceous endophytes are unspecialised and ubiquitous, then one ought to find similar abundances of the dominant fungi within closely related plants (as found by Seena & Sridhar 2004) and that the pattern of abundance should be most similar between sites that are close together. In the woody plant *Theobroma cacao* L., Arnold *et al.* (2003) found that the similarity in endophyte assemblages decreased exponentially with increasing distance between sampled sites. Here we report a test of this hypothesis, involving two closely-related species within the Asteraceae, *Cirsium arvense* (L.) Scop. (creeping thistle) and *Leucanthemum vulgare* Lam. (Ox-eye daisy). We present species richness and infection frequency of the dominant endophytes, from interspersed plants of the two species at five sites along a 52 km transect.

Describing the spatial distribution and host associations of these endophytes is a critical first step towards understanding their interactions with herbivorous insects. This is because the limited evidence suggests that unspecialised endophytes may increase plant resistance to herbivores (Raps & Vidal 1998; McGee 2002; Jallow *et al.* 2004). To date, only one study has examined the co-occurrence of an insect and endophytes in the field (Gange *et al.* 2002), where it was found that *Alternaria alternata* (Fr.) Keissler was more frequent within gall tissue caused by the fly *Urophora cardui* L. than in leaves or stems. Here, we present data on the leaf mining fly, *Chromatomyia syngenesiae* Hardy, which attacks both *C. arvense* and *L. vulgare*, in relation to endophyte occurrence in each plant. Given that endophyte colonization occurs before leaf miner oviposition (Hopkins 1977; Guske *et al.* 1996; A.F. Currie unpublished), we hypothesised that plants with high levels of endophyte occurrence would show lower levels of miner attack, if the fungi are antagonists of the insect.

Unspecialised endophytes may also be antagonistic towards other microbes that occur within plants or in the soil (Domsch *et al.* 1980; Peláez *et al.* 1998; Pieckenstein *et al.* 2001). While it is reasonable to assume that if endophytes produce metabolites in culture then they may also do so in nature (Demain 1980), the consequences of this for fungal occurrence, and indeed plant reactions are unknown. It is therefore possible that if endophytes are active within the plant then certain species may exclude other fungi. This would lead to negative relations between the abundance of certain species. Meanwhile, if all endophytes within

herbaceous plants are benign, simply using the plant as a refuge, then one ought to see no relations. As Schulz & Boyle (2005) state, the distribution of endophytes within the tissues of herbaceous plants is often assumed to be restricted, but this is based on a very few studies and still does not exclude the possibility that these fungi show antagonistic interactions. As a first step towards this intriguing question, we examined relations between the occurrence of the dominant endophytes, in an attempt to determine if these fungi do show activity within hosts.

## Materials and methods

### STUDY ORGANISMS

*Cirsium arvense* and *Leucanthemum vulgare* (both Asteraceae) are perennial forbs, common in mesic grasslands in southern England. In most winters, foliage of *C. arvense* dies away and regrowth is from the rootstock in early spring. *L. vulgare* overwinters as a basal rosette of leaves. Flowering stem elongation of *C. arvense* occurs in April-May, with flowers produced June-August, while stem elongation of *L. vulgare* occurs in March-April, with flowering in May-June. In this study, sampling of leaf material took place in early July, when both species were mature.

The leaf mining fly, *Chromatomyia syngenesiae* attacks many species in the Asteraceae (Hopkins 1977). It usually has at least two generations per year, with larvae of the first generation appearing in June and the second in August.

### FIELD SAMPLING

Five separate field sites were located, at approximately 13 km intervals along a 52 km transect between Royal Holloway, University of London, Surrey and Reading University, Berkshire, UK. The locations of each were Egham (SU 998705), Sunninghill (SU 939686), Bracknell (SU 872684), Wokingham (SU 798707) and Reading (SU 738722). Each site was chosen on the basis of similar vegetation and soil type and because populations of the two study plants occurred, growing in an interspersed fashion. At each site, *C. arvense* and *L. vulgare* were the dominant and most common forbs. Grass species common to each site included *Arrhenatherum elatius* (L.) P. Beauv. ex J.S. & C. Presl, *Holcus lanatus* L. and *Agrostis capillaris* L.

At each site, 20 plants of each species were selected at random, with the criterion that each plant was within 0.5 m of an individual of the opposite species. Height, total leaf number and the number of leaves attacked by *C. syngenesiae* were recorded for each plant. Ten leaves that showed no sign of fungal infection or insect attack were selected at random from each plant, at various positions up the main stem. These were immediately placed on ice in an insulated bag, returned to the laboratory and processed within 24 h.

## ENDOPHYTE ISOLATION

Three sections, each approximately 10 mm x 10 mm were cut from each leaf, washed in water and then subjected to a surface sterilization procedure, following method III of Schulz *et al.* (1993). Briefly, this involved immersion in 100% ethanol for 30 s, washing in sterile water, immersion in 33% NaOCl for 5 min, immersion in ethanol for a further 30 s followed by four separate washings in sterile water. A fragment measuring 3 mm x 3 mm was cut from the middle of each section using a sterilised blade. Fragments of this size are optimal for fungal recovery rates (Gamboa *et al.* 2002). Fragments were placed abaxial surface downwards on potato dextrose agar (PDA) plates, containing 60 mg l<sup>-1</sup> penicillin G and 80 mg l<sup>-1</sup> streptomycin sulphate, to inhibit bacterial contamination. Fragments were incubated for 6 weeks at room temperature. Separate fragments were cut from each section after sterilization and the adaxial surface pressed onto PDA plates and then removed, as a check that the sterilization procedure had removed epiphytic fungi (Schulz *et al.* 1998). In only two cases did these presses produce colonies of a *Penicillium* sp., thus it can be assumed that the sterilization procedure was effective.

All fungal colonies growing from the fragments were sub-cultured on to potato carrot agar (PCA) and grown for a further 8 weeks under near UV light to encourage sporulation. Slide preparations were made of each and identified where possible by BCS.

## DATA ANALYSIS

All analyses were conducted using plants as replicates. Differences between sites in plant height, leaf number and the proportion of leaves mined by the fly were examined for each species using one factor analysis of variance. All percentage data were subjected to the arc sine transformation and leaf number data to the square root transformation, to meet the assumptions of ANOVA (Zar 1996). The mean number of endophyte species recovered per

leaf was calculated for each plant and this parameter, together with the total number of endophyte species per plant was subjected to two factor ANOVA, with site and plant species as main effects.

To examine the spatial structure of endophytes between sites, we calculated the Jaccard index (Magurran 2003) for all 10 possible site pairs and then plotted this index against site-site distance, following Arnold *et al.* (2003). Linear regression was used to examine the relationship separately for each plant species.

Isolation frequency (IF) of the most common endophyte species was calculated by dividing the total number of isolations (separate colonies) per plant of that species by the total number of isolations (colonies) for that plant. Following arc sine transformation, differences in IF between sites and species were examined with two factor ANOVA. Sites where a particular fungus was absent were excluded from these analyses.

Relations between plant size parameters and endophyte species richness per plant and per leaf per plant were examined with Pearson correlation. This test was also used to examine relations between the IF of the most common endophyte species. All analyses were conducted with the UNISTAT<sup>®</sup> statistical package.

## Results

### PLANT AND INSECT ATTRIBUTES

There were no significant differences in height or leaf number of both *L. vulgare* and *C. arvensis* between sites (all  $P > 0.05$ ) (data not shown). On average, across all sites, plants of *L. vulgare* were  $586.4 \pm 13.7$  mm tall and bore  $72.4 \pm 2.7$  leaves, while those of *C. arvensis* were  $650.1 \pm 29.8$  mm tall and bore  $64.9 \pm 3.6$  leaves.

The proportion of leaves attacked by *C. syngenesiae* did not differ across sites, but attack rates on *L. vulgare* were considerably higher than on *C. arvensis* (Table 1, Fig. 1). The significant interaction term between site and species shows that this difference was not consistent across sites, with the exception being site 1 (Egham), in which attack rates were similar on the two plants.

### FUNGAL ATTRIBUTES

At least one endophyte species was isolated from every one of the 200 plants sampled. A total of 11 endophyte species were isolated from *L. vulgare* and 13 from *C. arvense*. The most common fungi across both plant species (listed in decreasing order of isolation frequency) were: *Cladosporium cladosporioides* (Fresen.) De Vries, *Alternaria alternata* (Fr.) Keissler, *Chaetomium bostrychodes* Zopf, *Chaetomium cochliodes* Pall., *Gliomastix murorum* (Corda) Hughes, *Acremonium strictum* W. Gams, and *Epicoccum purpurascens* Ehrenb.: Schlecht. Other species that occurred in both plants, but which were too rare to be statistically analysed were *Aureobasidium pullulans* (de Bary) Arnaud, *Cladosporium herbarum* (Pers.) Link and *Paecilomyces variotii* Bain. One species, *Colletotrichum dematium* (Pers.: Fr.) Grove was only found in *L. vulgare*, but this was only in site 3. Three species, *Colletotrichum coccodes* (Wallr.) S. Hughes (in 1 site), *Sporormiella intermedia* (Auersw.) S.I. Ahmed & Cain ex Kobayasi (2 sites) and a species of *Phoma* (2 sites) were only found in *C. arvense*. Some sterile mycelium was recorded in all samples, but the isolation frequency was never higher than 6%.

Across all sites, there was a significant positive association between the number of endophyte species per plant and plant height in *L. vulgare* ( $r = 0.237$ ,  $P < 0.01$ ). No relations were found between the number of isolates per leaf and plant stature in this species, or between endophyte species richness or isolates per leaf and plant stature in *C. arvense* (data not shown).

Endophyte species richness (mean number of fungal species per plant) differed between plant species and across sites (Table 1). In four of the five sites, more fungal species were recovered from *C. arvense* but in site 4 (Bracknell), no difference was found, leading to a significant interaction term in the analysis. Overall, an average of  $2.4 \pm 0.1$  fungal species per plant were recovered from *C. arvense*, while  $1.6 \pm 0.1$  fungal species per plant were found in *L. vulgare*. A similar pattern was found in the mean number of isolates per leaf, and overall, the infection rate in *C. arvense* ( $1.72 \pm 0.6$  isolates per leaf) was approximately double that found in *L. vulgare* ( $0.98 \pm 0.06$ ).

When the similarity in the endophyte assemblage was plotted against inter-site distance (Fig. 2), no relation could be found for *L. vulgare*, but a significant negative relation, best fitted by a linear function ( $F_{1,8} = 6.4$ ,  $P < 0.05$ ) was found in *C. arvense*. In this species, endophyte assemblages were most similar in sites close together and became less so with increasing site separation.

Large differences were found in isolation frequencies of *C. cladosporioides*, *A. alternata*, *C. bostrychodes*, *C. cochliodes* and *G. murorum* across sites (Table 1), but these were not



consistent between the fungal species (Fig. 3). *C. cladosporioides* tended to be most common in sites 1 and 2, while *C. bostrychodes* was rare in these two sites but abundant in sites 3, 4 and 5. Meanwhile, *C. cochliodes*, which was absent from site 1, was the most frequently isolated species from *L. vulgare* in site 2 (Fig. 3d). Perhaps of more interest was the fact that *C. cladosporioides* and *G. murorum* showed differences in isolation frequency between the two plant species (Table 1). *C. cladosporioides* was more frequently isolated from *C. arvense* in every site (Fig. 3a), while *G. murorum* was commoner in *L. vulgare* at three sites (Fig. 3e). There were also significant site x species interaction terms for *C. cladosporioides*, *A. alternata* and *C. cochliodes* (Table 1). For the former species, this was because the magnitude of the difference varied between sites (Fig. 3a). For *A. alternata*, the isolation frequency was higher in *L. vulgare* at sites 1 and 3, but at site 4, the reverse was true, where it was the most abundant fungus in *C. arvense* (Fig 3b). Meanwhile, for *C. cochliodes*, the trend for higher abundance in *L. vulgare* at site 2 was reversed in site 3 (Fig. 3d).

## RELATIONS BETWEEN INSECT AND FUNGAL ATTRIBUTES

There were significant positive correlations between the proportion of leaves attacked by the fly and endophyte species number per plant and number of isolates per leaf in *L. vulgare*, while no such relations were found in *C. arvense* (Table 2). However, fly attack was negatively correlated with the isolation frequency of *C. bostrychodes* and *C. cochliodes* in *L. vulgare* and with *C. cochliodes* in *C. arvense* (Table 2).

The correlation matrices of relations between isolation frequencies of each common endophyte are presented in Table 3. The most striking result here was that in *C. arvense*, *C. cladosporioides* showed a negative relation with all other endophytes. However, in *L. vulgare*, a negative relation was only found between *C. cladosporioides* and *C. bostrychodes*. Significant negative relations were also found between *C. bostrychodes* and *C. cochliodes* and *C. bostrychodes* and *A. strictum* in *C. arvense* only (Table 3).

## Discussion

All of the common endophytes isolated in this study are ubiquitous species, with saprophytic lifestyles in soil or on decaying matter and all of them have been isolated from a very wide variety of sources (Domsch *et al.* 1980). They all occur commonly in lists of endophytes recovered from a variety of different plants (Stone *et al.* 2000). This lack of specialism led

Petrini (1986) to suggest that if closely-related plants are present in the same locality, colonization of all plants by the same endophyte will occur. To an extent, this was true in our study. We found little evidence of host specificity; few fungi seemed to be restricted to any one plant, and none of these were common. However, we did find differences in the abundance of certain fungi, as measured by isolation frequency, between the two plant species. Furthermore, the fact that we found different relations between endophytes and the insect and between the endophyte species suggests that the nature of the host-fungal associations is not the same in the two plants.

The most striking differences between the two plants was that more endophyte species per plant and higher isolation frequencies were found in *C. arvense*, and that the most common endophyte (*C. cladosporioides*) was isolated much more frequently from this plant. Given that the two plant species were growing in interspersed populations at each site, one would expect that they would be exposed equally to the air-borne spores by which these fungi infect plants (Saikkonen *et al.* 1998). The most likely explanations for these differences are either that endophytes are more successful at invading this species, or that there is more extensive fungal growth within (or both). Schulz & Boyle (2005) suggest that most endophyte colonization of foliar tissues is local and restricted, although this is based on a very few studies. At present, we do not know where *C. cladosporioides* or other endophytes occur inside leaves of our study plants. However, Cabral *et al.* (1993) found that in *Juncus* spp., *C. cladosporioides* was restricted to the sub-stomatal chamber and did not colonise internal leaf tissue. If such a situation occurs in *L. vulgare* and *C. arvense*, then the most plausible explanation for the species difference is that the fungus is more able to colonise tissues of *C. arvense*. Meanwhile, the opposite situation seems to exist for *G. murorum*, which was isolated more frequently from *L. vulgare*. Endophytes may enter plant tissue through the stomata, the plant epidermis or wounds caused by insect feeding, with the former being the most likely entry route. If there were differences in stomatal density between *L. vulgare* and *C. arvense* across our sites, then this simple morphological difference might make it statistically more likely for endophytes to enter *C. arvense*. Alternatively, it is known that endophytes do often trigger host defence responses (Schulz & Boyle 2005), existing in what these authors describe as a 'balanced antagonism' with their host. Perhaps the host response is better developed in *L. vulgare*, thus reducing the number of successful colonizations. Whatever the mechanism, these data are important because quantitative differences in colonization of closely-related hosts by the same endophyte species has not been reported

before. An investigation of why and how it occurs could be fundamental to our understanding of how these fungi can exist in plants in the balanced antagonism (Schulz & Boyle 2005).

Unspecialised endophyte assemblages within a given plant are known to vary across sites that are far apart (Collado *et al.* 1999; Arnold *et al.* 2003), while at smaller spatial scales, little habitat specificity is shown (Addy *et al.* 2000; Gange *et al.* 2002). At small scales, the availability of other habitats (soil, decaying organic material etc.) is sufficient to provide inoculum sources for colonization to be equally likely (Saikkonen *et al.* 1998). An interesting result from this study is the fact that the relation between endophyte assemblage similarity and distance between sites differed in the two plant species. In *L. vulgare*, no relation existed, suggesting that, in this plant species, the similarity of the assemblage was constant across sites. In contrast, a negative linear relation was found in *C. arvense*. Arnold *et al.* (2003) performed a similar analysis in the tropical tree *T. cacao* and found a negative curvilinear relation. The data for *C. arvense* are very similar, as the scale over which we sampled was considerably less than that of Arnold *et al.* (2003) and cover the linear part of their decline. Our data suggest that in *C. arvense*, the endophyte assemblage is merely a function of the site (i.e. availability of inoculum) in which the plant is growing and that plants are probably random samplers of the spore rain. However, this does not seem to occur in *L. vulgare*, which appears to exert some form of selection over the endophytes, so that a similar assemblage occurs within it, irrespective of site. This may be tentative evidence to further the assertion that *L. vulgare* exhibits a different (and stronger) host reaction to colonization than does *C. arvense*.

In this study, five of the seven fungal species showed no inter-site differences, as expected. However, three species showed site x plant species interactions, indicating that the pattern of abundance within the two plants was not the same at every site. If there were no differences in colonization ability or within-host activity, then one would expect the pattern to be consistent across sites. Two species (*A. alternata* and *C. cochliodes*) showed particularly interesting patterns. *A. alternata* was isolated more frequently from *C. arvense* in one site (Wokingham), while the opposite was true in two other sites (Egham and Bracknell). Meanwhile, *C. cochliodes* was much commoner in *L. vulgare* at one site (Sunninghill), but commoner in *C. arvense* in Bracknell. It is unlikely that these effects were due to abiotic variations within sites. Both plant species were of equal size and stature across sites, suggesting equality of water and nutrient availability, and the plant species composition of each site also did not vary. Even if there were differences in abiotic factors or local sources of spores, these should be manifest as minor variation in a consistent pattern across sites, as seen

in *C. cladosporioides*. If one discounts abiotic factors, then we must consider interactions between the endophytes themselves. To our knowledge, no previous study has considered the interactions between these endophytes *in planta* although there are good examples of how endophytes can enhance the resistance of plants to pathogenic fungi (e.g. Narisawa *et al.* 1998; Vilich *et al.* 1998; Pieckenstain *et al.* 2001). Perhaps of critical importance in understanding our results is that when *A. alternata* was commonest in *C. arvense*, *C. cladosporioides* occurred with relatively low abundance. The same situation occurred for *C. cochliodes*; this species was very common in *L. vulgare* in site 2 (Sunninghill), when *C. cladosporioides* was rarest. Significant negative correlations were found between *C. cladosporioides* and *C. cochliodes* in both plant species and between *C. cladosporioides* and *A. alternata* in *C. arvense*. It may be that the order of colonization of endophytes within a leaf is critical. Our observations suggest that *C. cladosporioides* is present in tissue of *C. arvense* soon after foliage emergence in spring (Currie, unpublished). If colonization by this species prevents that of later-colonizing species, either by physical or chemical means, such negative correlations may be produced. However, a most intriguing result is the fact that the pattern of negative relations between endophytes was not the same for each plant species. In *C. arvense*, 43% of relations were significant, while in *L. vulgare* it was only 5%. This may be evidence that the fungi (and particularly *C. cladosporioides*) exhibit a different level of activity within *C. arvense* and may provide further support for the notion that *L. vulgare* is able to exert more control over its endophyte assemblage, suppressing their activity. It is well known that many of these endophytes produce an array of metabolites in culture that are active against other microbes and invertebrates (e.g. Peláez *et al.* 1998, Nitao *et al.* 2002), but the consequences of such production for the host plant, and other endophytes within the plant, are unknown (Schulz & Boyle 2005). Thus, our data suggest that the order of colonization of endophyte species might be critical in determining the structure of the assemblage and their interactions with other organisms.

Insects provide good examples of how unspecialized endophytes in herbaceous plants can reduce the performance of other organisms that attack the plant, although the number of studies is small (Raps & Vidal 1998; McGee 2002; Jallow *et al.* 2004). Two of these reports (Raps & Vidal 1998; Jallow *et al.* 2004) showed that colonization of roots by *A. strictum* (one of the fungi found in the present study) reduced the performance of shoot-feeding insects. Negative effects on the insects have been explained by secondary metabolite production (McGee 2002) or alteration of phytosterol concentrations in foliage (Jallow *et al.* 2004). We found significant negative relations between leaf miner attack rates and the isolation

frequency of both *Chaetomium* species in *L. vulgare*, and of *C. cochliodes* in *C. arvense*. No reports exist of interactions between *Chaetomium* species and phytophagous insects. However, a closely related species, *C. globosum* Kunze : Steud., produces flavipin, which has activity against root-knot nematodes (Nitao *et al.* 2002). Perhaps the most surprising result was that leaf miner attack rates in *L. vulgare* showed a positive relation with the number and abundance of endophytes per plant. When *A. alternata* and *C. cladosporioides* were fed to mites on leaf surfaces, increases in mite reproduction occurred (Belczewski & Harmsen 2000). However, to date, there is no report of the interactions between these fungi and herbivorous insects. In grasses and trees, endophyte effects on insects range from negative to null to positive (Faeth & Hammon 1997; Wilson & Faeth 2001; Saikkonen *et al.* 2006), so it is reasonable to assume that similar species-specific effects occur in herbaceous plants. To date, endophytes have been almost completely neglected in studies of plant-insect interactions, but it is certain that they play a critical role. While much of the evidence for endophyte activity presented here is correlative, the examination of such relations is a crucial first step in highlighting areas that should be investigated by experiment (Wilson 2000).

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**Table 1** Summary of ANOVA  $F$  values, testing for differences in leaf miner attack and species richness and isolation frequency of endophytes, between sites, plant species, and the interactions between them. Degrees of freedom for site and site x species = 4, 190 and species = 1,190, with the exceptions of *Chaetomium cochliodes* and *Gliomastix murorum* where site and site x species = 3,152 and species = 1,152. \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$

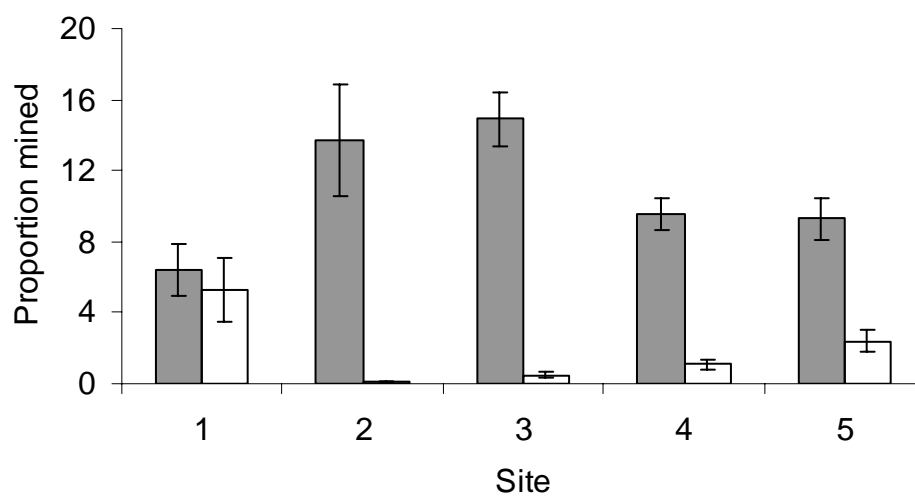
	SITE	PLANT SPECIES	SITE X SPECIES
Leaf miner attack	0.9	95.3***	7.1***
Endophytes per plant	9.7***	30.4***	3.2*
Isolates per leaf	4.9***	83.2***	5.3***
<i>Cladosporium cladosporioides</i>	6.4***	90.4***	4.1**
<i>Alternaria alternata</i>	4.9***	0.4	6.2***
<i>Chaetomium bostrychodes</i>	18.8***	0.01	1.1
<i>Chaetomium cochliodes</i>	5.2**	1.1	6.8***
<i>Gliomastix murorum</i>	8.4***	8.1**	2.2
<i>Acremonium strictum</i>	0.8	0.8	0.5
<i>Epicoccum purpurascens</i>	0.3	1.2	0.8

**Table 2** Relations between leaf mining insect attack and species richness and isolation frequency of endophytes. Values are Pearson correlation coefficients, all degrees of freedom = 98, or 78 for *Chaetomium cochliodes* and *Gliomastix murorum*. NS: not significant at  $P = 0.05$

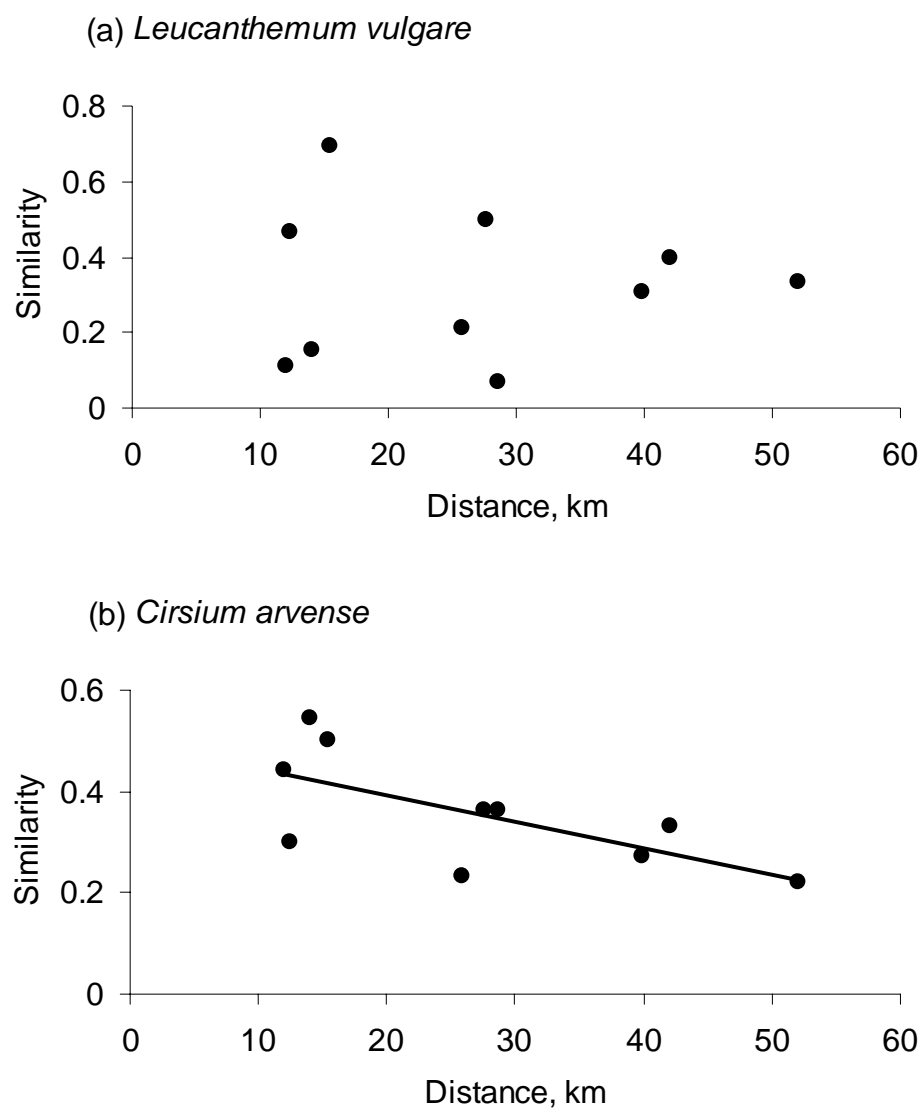
	<i>L. vulgare</i>		<i>C. arvense</i>	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Endophytes per plant	0.242	< 0.01	-0.073	NS
Isolates per leaf	0.198	< 0.05	0.026	NS
<i>Cladosporium cladosporioides</i>	-0.06	NS	0.093	NS
<i>Alternaria alternata</i>	0.041	NS	0.101	NS
<i>Chaetomium bostrychodes</i>	-0.624	< 0.001	0.101	NS
<i>Chaetomium cochliodes</i>	-0.346	< 0.01	-0.195	< 0.05
<i>Gliomastix murorum</i>	0.04	NS	0.06	NS
<i>Acremonium strictum</i>	0.097	NS	0.098	NS
<i>Epicoccum purpurascens</i>	-0.107	NS	0.064	NS

**Table 3** Correlation matrices of relations between isolation frequency of endophyte species in *L. vulgare* and *C. arvense*. Values are Pearson correlation coefficients, all degrees of freedom = 98, or 78 for *Chaetomium cochliodes* and *Gliomastix murorum*. \* $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$

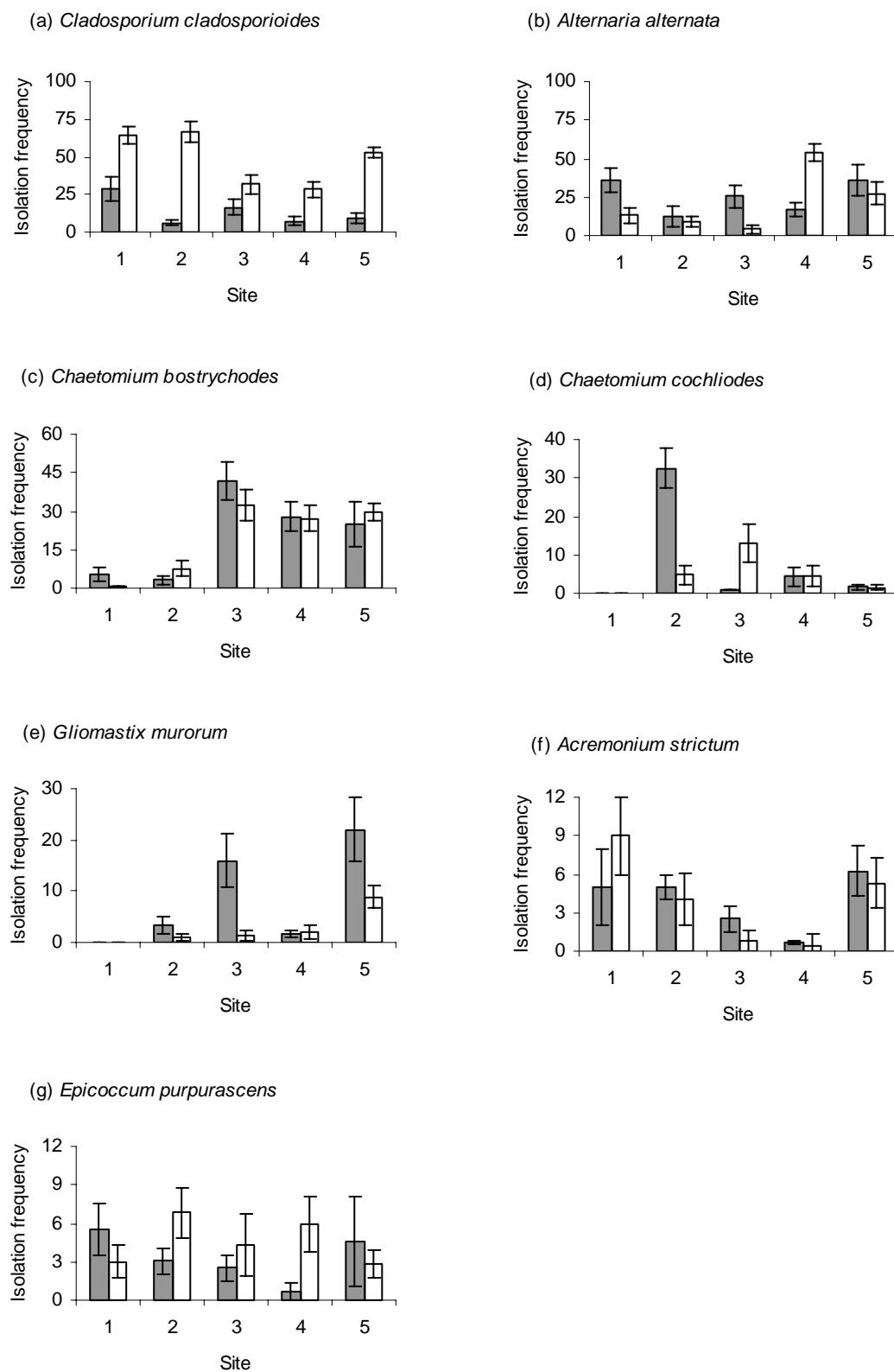
	<i>C.c.</i>	<i>A.a.</i>	<i>Ch.b.</i>	<i>Ch.c.</i>	<i>G.m.</i>	<i>A.s.</i>
<i>Leucanthemum vulgare</i>						
<i>Cladosporium cladosporioides</i> ( <i>C.c.</i> )	-					
<i>Alternaria alternata</i> ( <i>A.a.</i> )	-0.019	-				
<i>Chaetomium bostrychodes</i> ( <i>Ch.c.</i> )	-0.165*	0.118	-			
<i>Chaetomium cochliodes</i> ( <i>Ch.b.</i> )	-0.105	-0.032	-0.124	-		
<i>Gliomastix murorum</i> ( <i>G.m.</i> )	-0.012	0.06	-0.061	0.027	-	
<i>Acremonium strictum</i> ( <i>A.s.</i> )	-0.052	-0.017	-0.087	-0.102	-0.039	-
<i>Epicoccum purpurascens</i> ( <i>E.p.</i> )	-0.139	-0.118	-0.055	-0.006	-0.015	0.059
<i>Cirsium arvense</i>						
<i>Cladosporium cladosporioides</i> ( <i>C.c.</i> )	-					
<i>Alternaria alternata</i> ( <i>A.a.</i> )	-0.252**	-				
<i>Chaetomium bostrychodes</i> ( <i>Ch.c.</i> )	-0.237**	-0.066	-			
<i>Chaetomium cochliodes</i> ( <i>Ch.b.</i> )	-0.306***	-0.034	-0.177*	-		
<i>Gliomastix murorum</i> ( <i>G.m.</i> )	-0.175*	-0.085	0.02	-0.051	-	
<i>Acremonium strictum</i> ( <i>A.s.</i> )	-0.227*	-0.067	-0.195*	-0.02	0.103	-
<i>Epicoccum purpurascens</i> ( <i>E.p.</i> )	-0.317***	-0.07	-0.111	-0.072	-0.035	0.105



**Fig. 1** Mean ( $\pm$  se) proportion of leaves attacked by *Chromatomyia syngenesiae* in *Leucanthemum vulgare* (shaded bars) and *Cirsium arvense* (open bars). Key to sites: 1: Egham, 2: Sunninghill, 3: Bracknell, 4: Wokingham, 5: Reading.



**Fig. 2** Similarity (measured by the Jaccard index) of endophyte assemblages between all possible site pairs for *Leucanthemum vulgare* and *Cirsium arvense*.



**Fig. 3** Mean ( $\pm$  se) isolation frequency of endophyte species in *Leucanthemum vulgare* and *Cirsium arvense*. Key to legend as in Fig. 1.