Positive effects of endophyte infection on sycamore aphids

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In this study, I examined the effects of infection of leaves by an endophytic fungus (Rhytisma acerinum) on the populations and individual performance of two species of aphid (Drepanosiphum platanoidis and Periphyllus acericola), living on Acer pseudo-platanus. Populations of aphids were followed through one season and numbers compared on infected and uninfected leaves. Both species of aphid aestivated during the summer. D. platanoidis aestivated as non-reproducing adults, while P. acericola did so as nymphs. Fungal abundance, as measured by stromata number, was very low compared with previous studies in the UK. Nevertheless, in contrast to other studies of arboreal endophytes and insects, fungal presence affected aphid numbers in a positive manner, and in late summer, aphid numbers were higher on infected leaves. In D. platanoidis, higher aphid numbers resulted from an increased nymphal production by adults, but in P. acericola there was an accumulation of dispersing nymphs on infected leaves.

Field rearing experiments with both aphid species demonstrated an increase in adult weight and potential fecundity in two autumnal generations. The infected leaves had higher soluble and total nitrogen and total carbon contents and the increases in aphid performance are attributed to the increase in soluble nitrogen. The numbers of sycamore aphids in one year can influence the dynamics of the population in the following season. It is suggested that increased numbers of aphids, resulting from endophytic infection, may therefore alter the between- as well as the within-year population dynamics of the species. In addition, the data appear to provide support for the hypothesis that endophyte presence may determine seasonal patterns of herbivory. The hypothesis may be interpreted with an endophyte-induced change in food quality, but other possibilities are discussed.

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There is an increasing realization among ecologists that plants are not simply discrete entities, but instead are mergers of fungal cells with plant tissues (Wilson 1993). Recent experiments have indicated that fungi are very important in mediating the interactions between insects and plants, often by changing the chemistry of their hosts. Clearly, we can no longer study insect-plant interactions without also considering pathogenic (e.g. Hatcher et al. 1994a), mycorrhizal (e.g. Gange and West 1994) or so called endophytic (Clay 1990, Carroll 1991) fungi. Over the years, the term “endophyte” has been defined several times, and recently there has been debate over the usefulness of this description. Wennström (1994) has argued that the use and abuse of the term has rendered it no longer meaningful. However, in response to this criticism, Wilson (1995a) has redefined the term as “fungi or bacteria which, for all or part of their life cycle, invade the tissues of living plants and cause unapparent and asymptomatic infections entirely within plant tissues but cause no symptoms of disease”. I have adopted this definition, and an example of a fungus that can therefore be termed an endophyte is Rhytisma acerinum (Pers.) Fries, which causes black lesions (stromata) on the adaxial surface.
of the leaves of Acer pseudoplatanus L. (sycamore). Some texts describe R. acerinum as a pathogen, for example Massee (1915) states that it causes accelerated leaf fall and reduces tree growth. Other texts consider R. acerinum to have little effect on its host (e.g. Brooks 1953) and current thinking places it more under the definition of endophyte (Minter 1995). Wilson (1995a) suggests how we may separately define endophytic and pathogenic fungi, but as he implies, we should be concentrating on the ecology of endophytes, rather than the etymology.

Endophytic fungi occur widely in grasses and they have been well studied in this family of plants. A particularly important and useful finding is that they can confer resistance to a number of insect herbivores, generally through the production of toxic alkaloids (Cheplick and Clay 1988, Breen 1994). However, the production of such chemicals means that the grasses are often poisonous to cattle and sheep (e.g. Read and Camp 1986) and much work has been devoted to finding endophyte-infected grass species which are resistant to insect herbivores, but not vertebrates. Grass endophytes belong to the family Clavicipitaceae (Clay 1989) and while endophytic fungi in woody plants belong to different families (Petrini 1986) it is of ecological relevance to examine whether these fungi also affect herbivory by insects.

In contrast to grasses, there has been comparatively little attention focused on endophytes or pathogens with long latent phases in leaves of other plants. However, the fact that whenever endophytes have been looked for, they have been found, Petrini (1986) has suggested that all living plants probably host these fungi. The majority of information on non-graminaceous endophytes concerns woody gymnosperms and there are a few examples of endophyte presence in these plants causing an increase in host resistance to phytophagous insects. For example, Carroll (1986, 1988) describes how the fungus Rhabdocline parkeri invades the galls of flies (Contarinia spp.) on Douglas fir needles. Infected galls had an average mortality rate of 68% compared with 13% in endophyte-free galls, with mortality attributed to toxins produced by the endophyte. Meanwhile, Diamandis (1981) found that larvae of the pine processionary moth, Thaumetopoea pityocampa avoided endophyte-infected needles of Pinus brutia.

A few studies have also suggested that endophytes present in the leaves of woody angiosperms can reduce insect performance. Butin (1992) demonstrated that fungi found inside insect galls on oak (Quercus robur) also occurred as endophytes. However, it is not clear from this work whether these fungi invaded galls before insect death (i.e. killing the insects) or after this event. In a more detailed study, Wilson (1995b) has shown that an endophytic fungus can grow into the leaf galls of a cynipid wasp on Quercus garryana. Insect death then results from starvation as the fungus kills the gall tissue. In addition, Hammon and Faeth (1992) discuss data which suggest that leaf miner (Cameraria spp.) growth and mortality is reduced in endophyte-infected oak (Quercus emoryi) leaves. Hammon and Faeth (1992) suggest that endophyte fungi may provide explanations for a number of patterns of insect herbivory, both spatial and temporal, that are frequently seen in nature. This paper raises many interesting questions and proposes a number of hypotheses regarding insect–endophyte–plant interactions. In particular, these authors suggest that endophyte activity may serve to determine when insects feed on a host plant. Thus, early season feeders may do so to avoid toxins from the endophyte presence which frequently builds up during a season (Wilson and Carroll 1994). In contrast however, late season feeders may actually prefer endophyte-infected leaves, because one may expect adaptations to the fungal presence by an insect species that has been consistently associated with its host plant and microbial associates in evolutionary time. This is a situation analogous to that where specialist feeders have evolved to use toxic compounds in plants as part of their diet or to sequester them for their own defence (Harborne 1988).

A detailed account of the biology of R. acerinum was published by Jones (1925). After leaves fall in autumn, fungal reproductive structures develop in the black ‘tar spots’ or stromata and in the following spring, ascospores are ejected from them. Spore production occurs in May, but stromata do not become visible until July, meaning that there is an approximate eight-week period of asymptomatic infection in the leaves, when the fungus grows endophytically. Several species of aphid also live on sycamore leaves during the summer. The most common of these is Drepanosiphum platanoidis (Schr.), and its population biology has been extensively studied (Dixon 1970, 1979). D. platanoidis overwinters as an egg which hatches in early spring (March–April) and gives rise to a first generation which feeds on the bud scales. The second generation feeds on the newly unfurled leaves but soon aestivates, without further reproduction, until August. The duration of aestivation is controlled by prevailing food quality (which drops dramatically in mid summer (Dixon 1979)) and the density of aphids on the leaves (Dixon 1975). Another species of aphid on sycamore, Periphyllus acericola (Walk.), also exhibits summer aestivation but the factors affecting the population dynamics and growth of this species are unknown.

In this paper, I use the sycamore–aphids system as a model to test part of the seasonal herbivory hypothesis of Hammon and Faeth (1992). If the hypothesis is upheld, then we might expect that post-aestivation aphids prefer infected leaves and autumnal performance of these aphids may be increased on these leaves. Although a number of factors, such as aphid density
and plant soluble nitrogen content, have been shown to affect reproduction after summer aestivation (Dixon 1975), the role of endophytic fungi has never been considered. Furthermore, this is the first report of the interactions between an endophytic fungus and an arboreal free-living aphid, as all other studies have involved gall-forming species or chewing insects.

**Materials and methods**

Twenty sapling sycamore trees were selected at random from a natural stand growing on the campus of Royal Holloway, Univ. of London, Egham, Surrey, UK. All trees were of similar height (about 2.5 m) and assumed to be of similar age (about 8 yr) at the start of the experiment. In early April 1993, 100 fully expanded leaves on each tree were selected at random from positions in the canopy between 0.3 and 2 m above ground level. These were marked by tying a small (1 cm × 1 cm) tag around the petiole. At fortnightly intervals from mid-April until mid-October, each leaf was examined and the number of aphids of *D. platanoidis* and *P. acericola* counted. The distinction was made between adults and nymphs for both species. As *P. acericola* nymphs aestival in groups, the number of groups was also recorded. A group was defined as any aggregation of more than three nymphs, with less than 1 mm between individuals. Counts of nymphs within groups was performed with the aid of a hand-held magnifier. At the end of the study, all leaves were removed and the area of each measured using the DIAS image analysis system (Delta-T Instruments, Cambridge, UK). Measurements of greatest length and width of randomly selected leaves during the season indicated that leaf size did not change over the course of the study. Therefore, it was assumed that the area of sampled leaves was the same on each date. Aphid counts were then expressed as numbers per cm² of leaf, in order to overcome any effects of leaf size determining aphid abundance. Once tar spots became evident (see below), it was possible to assign leaves to ‘infected’ or ‘uninfected’ groups. On each date, the mean number of aphids per leaf was calculated for infected and uninfected leaves for each tree, although it is important to note that sampling commenced before any leaves should have been infected (cf. Jones 1925). If data satisfied the assumptions of normality, aphid numbers were compared on infected and uninfected leaves on each date with the paired t-test, using the 20 trees as replicates. However, due to the multiple nature of these tests, the probability of Type I errors is increased. Upper bounds on probability levels were therefore set using an improved version of the Bonferroni procedure (Simes 1986), given by Miller (1981). This lowers the value of α making the tests more conservative. As different numbers of comparisons had to be made for each dependent variable, α levels vary and so individual probabilities are given in the results. In two cases, the count data were normalised by performing a square root transformation prior to the test (Zar 1984).

Tar spots first became apparent in early July and the number of spots per leaf was recorded on each sample. In order to compare fungal infection with previous work, the tar spot index (TSI) was calculated (Leith and Fowler 1987). This is simply the number of spots per 100 cm² of leaf and was calculated using the individual leaf areas (above). The frequency distributions of tar spot number and index were found to be severely skewed and thus in order to examine differences in fungal abundance between the 20 trees, a Kruskall Wallis non-parametric one way ANOVA was employed. Means were subsequently separated with a Tukey-type comparison for multiple samples (Zar 1984). Tar spots did not appear to vary greatly in size and so this factor was not controlled for in analyses.

During September and October, two generations of aphids of each species were reared on infected and uninfected leaves. Experiments were started in early September, when each aphid species ceased aestivation. A parthenogenetic generation of each species was reared in September and a sexual one in October.

During the first week of September, alate virgino parae (parthenogenetically reproducing adults) of *D. platanoidis* were placed singly in clip cages (Noble 1958) in three different positions: a) immediately over a tar spot; b) 5 cm away from a spot on an infected leaf, randomly positioned and c) on an uninfected leaf on the same tree. In all cases, the area of the clip cage (3 cm²) was greater than the tar spot, allowing a small amount of non-stroma leaf material for feeding. Ten cages of each treatment were set up on each of the 20 replicate trees. Cages were examined daily and when the adult had reproduced, she was removed and a single nymph left in each cage. Care was taken to ensure that *D. platanoidis* nymphs were reared on leaves which did not have *P. acericola* also feeding on them. The latter species can induce senescence of a leaf and thereby affect the size of *D. platanoidis* adults (Dixon 1985). When the aphids had moulted to adult, they were weighed and dissected and the number of embryos within counted as a measure of potential fecundity. The procedure was repeated (with a different set of leaves) for the next generation during October, when the majority of the aphids reared were oviparae (egg-laying females). A similar procedure was adopted for *P. acericola*, except that the first experiment was started with nymphs taken from an aestivating group. Tree means were calculated for each performance measure and paired comparisons made between a) performance on the spot vs away from the spot (within leaf) and b) away from spot vs uninfected (control) leaf (within tree). Probability levels were again corrected with the...
Bonferroni procedure. Data were tested for normality and the logarithmic transformation used when necessary, prior to the paired t-test.

At the end of September, the leaves upon which the first set of aphids had been reared were taken for chemical analysis. The 3-cm² area covered by each clip cage was cut out and freeze-dried. In the case of tar spots, the area of leaf within the cage, but not including the black stroma was used. Constraints on resources meant that it was not possible to perform analyses from all 20 trees. The samples from each of the three areas (i.e. 30 samples on each tree) were therefore pooled for all trees and ground to a powder. Seven replicate analyses for total carbon, total nitrogen and soluble nitrogen were then performed on the three treatment pools.

Total carbon was estimated by the dry combustion method and total nitrogen by semi-micro Kjeldahl digestion followed by the indophenol-blue reaction (Allen 1989). Soluble nitrogen was determined by the method of van Emden and Bashford (1969). Briefly, the leaf material was ground to a powder and 0.1 g of this material extracted with water containing 2.5% trichloroacetic acid in 0.02% phenol on an automatic shaker for twelve h. The extract was then subjected to semi-micro Kjeldahl analysis and the indophenol-blue reaction.

Because of the small sample sizes, paired comparisons between the chemical contents of the three areas were performed with the Wilcoxon matched pairs signed ranks test (Zar 1984). The comparisons made were the same as those performed for aphids (i.e. within leaves and within trees).

Results

Tar spot distribution

Tar spots were first noted on 15 July, appearing as small black dots which were surrounded by a yellow margin. These grew in size during July and all appeared to be fully formed by mid-August. Given that the vegetative phase within the leaf lasts approximately 8 weeks (Jones 1925), the likely infection time would have been about mid-May, well after the first aphid sampling date of 10 April.

Taking the overall sample of 2000 leaves, it can be seen from Fig. 1 that the majority (88%) remained uninfected during the study. The frequency distribution of tar spot occurrence was highly skewed, (not significantly different from a Poisson distribution, \( \chi^2 = 6.78, p > 0.05 \)), and the maximum number of spots recorded per leaf was 5. However, all trees had some marked leaves which were infected (Fig. 2), this figure varying from 5 to 40 of the 100 sample leaves (Fig. 2). Infection intensity varied significantly between trees (Kruskall Wallis ANOVA, \( \chi^2 = 47.2, df = 19, p < 0.001 \)), and this was caused mainly by the relatively high infection on one tree, with most trees having a similar infection rate. Variation in leaf sizes between trees resulted in a slightly different picture when infection was considered as tar spot index (TSI) (Fig. 2). The differences between trees was again significant (\( \chi^2 = 45.1, df = 19, p < 0.001 \)) with the highest TSI being recorded on the tree which had the greatest spot number.

Aphid abundance

D. platanoidis

The seasonal change in abundance of D. platanoidis is illustrated in Fig. 3. Aphid numbers reached a peak in mid-July, after which there was a steady decline until reproduction began again during September. There was no difference in the number of aphids on leaves which subsequently developed tar spots, compared with those which did not, before the estimated infection time (the first two sampling dates). In addition, no evidence could be found on any of the 20 trees for the number of spots per leaf being related to the number of aphids per leaf on either of these two dates (Spearman rank correlation, all \( p > 0.15 \)). Therefore, it appears that tar spot infection is unaffected by the presence of aphids in early spring.

Aphid abundance appeared to be affected by the presence of tar spots from early September onwards (Fig. 3a). Leaves which bore spots had significantly greater numbers of aphids than those which did not. This effect appeared to take place once the stromata
were fully formed. Spots were first observed on 15 July and all appeared to have reached their full extent by 26 August. The aphid population on uninfected leaves on this date was entirely adult (Fig. 3b, c) and therefore nymphal abundance could not be compared. However, there was a suggestion that reproduction had already begun on infected leaves (Fig. 3c) and on subsequent sampling dates, the differences in the total populations appeared to be caused by nymphs being more abundant on infected leaves.

P. acericola

Nymphs of this species were already clustered together in their aestivating groups at the start of the study (Fig. 4). Throughout the season, aphid numbers showed a gradual decline, most likely caused by the effects of predators (Fig. 4a). Anthocoris nemorum (Hemiptera: Anthocoridae) and green lacewing larvae, Chrysopa carnea (Neuroptera: Chrysopidae) were the most often encountered predators on the leaves. As with D. platanoidis, there was no evidence that P. acericola abundance differed on leaves which subsequently did, or did not, develop tar spots (dates 1 and 2). No relations could be found between aphid number or aphid group number and spot frequency either (Spearman rank, all $p > 0.25$).

The nymphs appeared to aestivate until late July, when the groups started to break up, with an increasing number of aphids being recorded as non-group members from this time (Fig. 4b, c). Throughout most of the study, the population consisted entirely of nymphs and adults were only recorded on the final sample in late October. In September, significantly more aphids were found on leaves with tar spots than those without (Fig. 4a, c). However, unlike D. platanoidis, there was no increase in the overall population at this time, because there were no adults reproducing (Fig. 4a). The lower number of non-aestivating (i.e. non-group) nymphs on uninfected leaves may therefore have been caused by

Fig. 2. Tar spot abundance over the 20 sample trees. Bars representing individual trees in the lower figure correspond to those in the upper. Bars with the same letter are not significantly different at $p = 0.05$.

Fig. 3. Seasonal changes in abundance of D. platanoidis on infected (■) and uninfected (□) leaves of sycamore. First arrow indicates estimated infection time and second arrow indicates first visible sign of infection. Values plotted are mean aphids per cm$^2$ of leaf, individual errors are omitted for clarity. Significant differences between means are indicated by stars. Note that probability levels were Bonferroni corrected and that * is equivalent to corrected 5% level, ** to 1% level and *** to 0.1% level. a) Total numbers of aphids. Significant differences were found on the following dates: (all df = 19) date 11: $t = 3.11, p = 0.0058$; date 12: $t = 3.63, p = 0.0017$; date 13: $t = 3.17, p = 0.0051$. b) Numbers of adult aphids. date 11: $t = 3.79, p = 0.0002$; date 12: $t = 6.34, p = 0.00002$; date 13: $t = 5.21, p = 0.00007$. c) Numbers of nymphs. date 11: $t = 3.79, p = 0.0002$; date 12: $t = 6.34, p = 0.00002$; date 13: $t = 5.21, p = 0.00007$.

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increased movement of nymphs from these leaves, rather than any increase in reproduction. Observations at this time indicated that a large amount of inter-leaf transfer did occur, with many aphids seen walking along the petioles. No comparisons of group number per leaf could be performed, as infected leaves had zero counts at this time (Fig. 4b). However, the final breakup of groups was six weeks later on uninfected leaves, indicating that nymphs appeared to aestivate for longer on these leaves.

**Aphid performance**

The weight and fecundity of *D. platanoidis* were significantly affected by the presence of tar spots (Fig. 5). Both alate virginoparae and oviparae were significantly heavier when reared near spots, compared with the aphids reared on uninfected leaves (virginoparae: $t = 2.43$, df = 19, $p = 0.016$; oviparae: $t = 2.49$, $p = 0.013$). There was no difference in the weight of either morph when reared in close proximity to the spot and 5 cm away from it (Fig. 5a, c). Fecundity showed a similar pattern, with virginoparae reared 5 cm away from the spot containing more embryos than those on uninfected leaves ($t = 3.56$, $p = 0.002$) (Fig. 5b). However, there was no difference in the egg content of oviparae reared near spots and those on uninfected leaves (Fig. 5d).

In general, the performance of *P. acericola* was increased by tar spot presence, in a similar manner to *D. platanoidis* (Fig. 6). However, while the weight of apterae was significantly greater on infected leaves than it was on uninfected leaves ($t = 2.89$, df = 19, $p = 0.012$) (Fig. 6a), there was no difference in the fecundity (Fig. 6b). Ovipara weight and egg content were both greater in aphids reared on leaves near spots compared with those on uninfected leaves (weight: $t = 9.34$, $p = 0.00001$; eggs: $t = 9.94$, $p = 0.00001$) (Fig. 6c, d). There was a suggestion that ovipara weight differed between the different areas on an infected leaf (Fig. 6d), but this was not significant with the corrected probability level ($\alpha = 0.02$), ($t = 2.08$, $p = 0.042$).
Plant chemistry

There was a significant difference in the soluble nitrogen content of infected and uninfected leaves (Fig. 7a). Infected leaves appeared to offer a superior food quality (i.e. higher soluble nitrogen content) compared to those which were uninfected (within tree comparison: $T = 0$, $p < 0.05$). Moreover, this effect appeared to be consistent within the different areas of the infected leaves (within leaf comparison: $T = 9$, $p > 0.05$). Total nitrogen did not differ within the leaves, but the level 5 cm away from the tar spot was significantly higher than that of uninfected leaves ($T = 0$, $p < 0.05$) (Fig. 7b). Meanwhile, infection significantly increased the total leaf carbon content (Fig. 7c) and again, the within tree comparison was significant ($T = 0$, $p < 0.05$), while the within leaf test was not. Probably as a result of these total changes, the carbon/nitrogen ratio was unaffected by fungal infection (Fig. 7d), so that both comparisons were not significant.

Discussion

In any study of this type, it is impossible to state with certainty whether tar spot presence is the cause of the observed changes in aphid growth and density, or whether tar spots happen to be correlated with some other factor which itself affects the aphids. For example, it is possible that R. acerinum successfully infects leaves of a high nitrogen content, which in turn would enhance aphid performance. However, given the consistency of the results and the fact that tar spot distribution was random, it is fair to conclude that the observed effects on the aphids are a direct result of the fungal presence. Manipulative experiments, in which leaves are artificially infected with the fungus and infested with aphids are the only way of firmly establishing cause and effect.

A feature of this study is the relative rarity of tar spots caused by R. acerinum, compared with that of previous work. Here, the maximum abundance of spots was 5 per leaf, and the maximum tar spot index of 0.4 is paltry in comparison to the value of 60 recorded by
Leith and Fowler (1987). *R. acerinum* is thought to be sensitive to air pollution, particularly sulphur dioxide (Greenhalgh and Bevan 1978) although this has been contested (Leith and Fowler 1987). It is possible that the proximity of the sampling site to London meant that pollutant levels were high, and this resulted in a low level of fungal abundance compared with the rural site of Leith and Fowler (1987). However, it is interesting that despite the rarity of the fungus, there was still a detectable effect on the populations of aphids on the sycamore leaves.

There was no evidence that leaves which became infected by *R. acerinum* had supported higher numbers of aphids in early spring. Leaves become infected by penetration through the stomata (Jones 1925) and it is highly unlikely that aphid stylet pathways act as a means of entry. The proportion of spores which enter leaves and initiate infections is unknown, but there is no evidence to suggest that aphid infestation weakens leaves in any way and makes them more susceptible to the fungus.

The presence of the endophyte appeared to affect both species of aphid in a positive manner. This is in direct contrast to other studies concerning endophytes of trees and their interactions with insects, in which the effect (sometimes indirect) is a negative one of fungus on the insect (Carroll 1986, 1988, 1991, Butin 1992, Wilson 1995b). An interesting point is that all of these studies have involved gall-forming insects, where the insect is sedentary and unable to avoid toxins produced by the fungus. Indeed, Fernandes and Price (1992) have suggested that one reason (among others) for the presence of many galling insects in apparently harsh environments is due to a lower insect mortality resulting from fewer endophytes in these habitats.

In cases where free-living insects have been studied, arboreal endophytes still appear to be antagonistic to insects (e.g. Diamandis 1981). However, there is no reason why we should expect all endophytes to act antagonistically towards insects, and indeed, it appears that endophytes may be highly variable in their interactions with herbivores in space and time (Hammon and Faeth 1992). While most endophytes in grasses confer insect resistance properties (Clay 1990), Johnson et al. (1982) found that endophytes in tall fescue deterred aphid feeding, but fungi in ryegrass had no effect on the same aphid species. Meanwhile, Kirrman et al. (1986) recorded a positive relation between endophyte infection and abundance of a number of insects in fescue grass (*Festuca spp.*).

In the case of *D. platanoidis*, there was an increase in reproduction during autumn and a subsequent increase in aphid populations on infected leaves. Adult aphids aestivate during the summer, and the reproductive activity of this aphid is controlled by food quality and the density of aphids (Dixon 1975, 1979). The aphids appear to respond to changes in the total amino acid content of leaves, rather than specific amino acids (Dixon et al. 1993), with the autumnal rise in total levels stimulating reproduction. Although the amino acid content of leaves was not measured in the current study, there was a significant increase in the soluble nitrogen content of infected leaves. This parameter can be taken as a reliable estimate of aphid food quality (McNeill and Prestidge 1982) and is a likely cause of increased aphid performance (Dixon 1979). It is noteworthy that the total nitrogen content of infected leaves also increased, and this may have significant effects on the performance of leaf chewing insects, as nitrogen is critical to the diet of most phytophagous insects (Mattson 1980).

There are a number of studies which have reported increases in leaf nitrogen contents following infection by pathogenic fungi (e.g. Paul and Ayres 1988, Ramsell and Paul 1990) and the elevated nitrogen can then lead to an increase in invertebrate herbivory (de Nooij et al. 1992). However, in many cases, preferential grazing of infected leaves occurs because the invertebrates are feeding on the infected tissue, rather than the leaf material (Ramsell and Paul 1990). Leaves infected by pathogenic fungi or viruses often show increased carbon contents, resulting from assimilate movement into infected tissues, or aberrant starch metabolism and carbohydrate accumulation (Barbosa 1991). It has been suggested that such increases in leaf carbon content can also make tissues more palatable to invertebrate herbivores (de Nooij et al. 1992). However, results with pathogenic fungi and insect herbivores can show null, negative or positive effects of the fungus on the insect (de Nooij et al. 1992, Hatcher et al. 1994a, b) and many of the mechanisms of the interactions are currently problematic. So too is the terminology, as Wennström (1994) argues against the inclusion of pathogens with long latent phases in the term endophyte, while Wilson (1995a) points to caution, but distinguishes between these terms. Although *R. acerinum* is listed in most plant pathology texts (e.g. Holliday 1992), it fits Wilson's (1995a) definition of endophyte well. Clearly, there is a continuum of infection patterns, from intermediate pathogen to complete endophyte and we cannot say if "endophytic" fungi such as *R. acerinum* alter leaf carbon and nitrogen contents in similar ways to "pathogenic" fungi (i.e. fungi which cause disease symptoms on the host plant, Wilson 1995a).

Some endophytic fungi have been shown to produce a number of enzymes, which can aid in digestion of the cuticular and epidermal layers of the leaf, during a penetration process (Petrini et al. 1992). Substrate utilization studies of endophytes from coniferous foliage have shown that these fungi are able to use most substances on or in the cell walls of the host (Carroll and Petrini 1983). Therefore, it is possible that digestive processes of the fungi alter total carbon or nitrogen contents, as compounds are moved into or out of leaves by the host. Secondly, a number of endophytes have
been shown to produce auxin-like substances in vitro (e.g. Porter et al. 1985) which may also affect the physiology of the host. Finally, endophytic fungi are becoming increasingly recognised as potential sources for secondary metabolites, which may be useful in the pharmaceutical or agricultural industries. Endophytes of grasses are renowned for producing alkaloids, which may be active against many insect herbivores (Clay 1989). However, the literature regarding arbor- real endophytes and the chemistry of their hosts is currently limited (Wilson 1993) and so these hypotheses must remain speculative.

In contrast to D. platanoidis, P. acericola populations did not increase in autumn, but numbers were significantly greater on infected leaves during September. This was not because there were more or larger groups during the aestivation period, but rather an accumulation of mobile nymphs from uninfected leaves. When the aestivating groups break up in summer, the nymphs became very active and we may speculate that group break up and activity is stimulated by changes in food quality. It is probable that increases in numbers on the infected (higher nitrogen) leaves may simply have been caused by a differential rate of departure, in a manner similar to that after flight activity, when take off is less likely, having landed on a suitable host (Kennedy et al. 1959). However, it is also possible that there may have been an attractant in infected leaves, or a deterrent or lack of certain compounds (such as amino acids) in uninfected leaves. These hypotheses could only be tested by a manipulative experiment (see above).

In both aphid species the growth and reproduction of autumnal generations was greater on infected leaves, and this was a likely reflection of the increase in food quality of these tissues. While not recorded in this study, increased reproduction in autumn will lead to increased aphid numbers the following spring (Dixon 1979). Therefore, endophyte infection in one year may also positively affect the numbers of aphids in the following season. In general, sycamore trees are continually re-infected each spring from spores ejected from leaf litter lying below the tree (Jones 1925). However, in many situations, particularly urban environments, leaves are collected and burnt, meaning that the dynamics of infection may be highly variable from year to year (Leith and Fowler 1987). Clearly, such variable infection intensities may add variance to the yearly dynamics of aphid populations (cf. Chambers 1979). It has been shown that pathogenic fungal infection of coniferous foliage can negatively affect insect populations in the following season (Krause and Raffa 1992). Therefore, in future studies of endophytes in perennial plant foliage, we must be aware of effects that may happen within and between years.

The enhanced movement and accumulation of P. acericola may have led to an intriguing, though unlikely, interaction between this species and D. platanoidis. Dixon (1985) states that P. acericola feeding in autumn can induce leaf senescence and that individuals of D. platanoidis reared near P. acericola are larger, and thus (presumably) have increased fecundity. I examined this possibility by comparing autumnal numbers of D. platanoidis nymphs on infected leaves, with and without P. acericola. No comparisons produced significant differences, but sample size was often small (generally less than 5). Care was taken in the rearing experiments to exclude P. acericola from the experimental leaves. Therefore, as a significant effect of infection was found on D. platanoidis in the absence of P. acericola, it can be assumed that this was fungal-induced, not aphid induced. However, in situations of high tar spot abundance this aphid-aphid interaction may become significant, although it may only be so in the presence of the fungus.

It is possible that, although changes in leaf nitrogen caused by infection are statistically significant, they may not be biologically significant to the aphids. For example, Gange and Pryse (1990) have found that while leaf nitrogen in alder (Alnus sp.) changes significantly through a season, this has no effect on the performance of the alder aphid. Therefore, although the most plausible explanation for these results is improved food quality, it is also possible that they provide support for the fungal endophyte hypothesis of seasonal herbivory patterns, proposed by Hammon and Faeth (1992). These authors suggest that late season feeders may show adaptations to endophyte-induced or -produced chemicals in their host plant and thus perform better on infected leaves. It is also plausible that the hypothesis may be interpreted more simply in terms of food quality, particularly if endophytes can alter nitrogen levels in tree leaves. Clearly, in order to separate these explanations, we would need to perform much more extensive analyses of leaf chemistry, with and without infections. If the known responses of D. platanoidis to food quality can be eliminated from the enquiry (Dixon et al. 1993), then we may be able to identify any direct 'endophyte factor' involved in the population dynamics of sycamore aphids. However, these results do support Hammon and Faeth's (1992) assertion that responses of insects to endophytic fungi in tree leaves may be considerably more complex and variable than those observed in grasses.

References


Van Emden, H. F. and Bashford, M. 1969. A comparison of the reproduction of *Brevicoryne brassicae* and *Myzus persicae* in relation to soluble nitrogen concentration and leaf


