Abstract We investigated relationships between endophytic fungi and a leaf-mining moth, *Phyllonorycter* sp., along an elevational gradient from 2255 to 2895 m. The fungi and moth larvae inhabit leaves of *Quercus gambelii*. Fungal frequencies and larval densities varied with elevation. However, larval densities were not associated with the frequencies of infection by endophytic fungi. Survival of larvae was positively associated with the most dominant fungus, *Gnomonia cerastis*, owing to reduced parasitism of moth larvae on trees with high frequencies of *Gnomonia*.

Key words Endophytic fungi · *Quercus gambelii* · *Phyllonorycter* · Parasitism · Elevational gradient

Introduction

Host plant quality for insect herbivores is profoundly influenced by the community of micro-organisms symbiotically associated with the plant (Rabin and Pacovsky 1985; Letourneau 1988; Clay 1988). Detrimental effects of plant symbionts on herbivores result from direct effects of microbial toxins (Miller 1986; Prestidge and Gallagher 1988; Johnson and Whitney 1994; reviewed in Dahlman et al. 1991), induction of plant defenses by microbes (McIntyre et al. 1981; Karban et al. 1987), or the influence of microbes on tri-trophic interactions (Dicke 1988). Alternatively, the presence of micro-organisms may enhance plant quality for insect herbivores (Kennedy 1951; Lewis 1984; Carruthers et al. 1986; Kirkman et al. 1986; Berenbaum 1988; Johnson and Whitney 1994). In order to investigate the dynamics of plant-microbe-herbivore interactions, we have investigated direct and indirect effects of an endophytic fungus on an insect herbivore across a range of habitats.

Endophytic fungi are a widespread group of plant symbionts, some of which influence plant-insect interactions (Hammon and Faeth 1992; Clay 1990; Breen 1994). In turf and pasture grasses, endophytic fungi increase resistance to insect herbivores (Prestidge et al. 1982; Mortimer and di Menna 1983; Ahmad et al. 1986; Mathias et al. 1990; Muegge et al. 1991; reviewed in Clay 1988, 1991; Breen 1994). Increased resistance is associated with increased mortality of insects feeding on endophyte-infected grasses (Ahmad et al. 1985, 1987; Clay et al. 1985a,b; Johnson et al. 1985; Cheplick and Clay 1988; Prestidge and Gallagher 1988; Mathias et al. 1990; Clay 1991). In addition to grass-endophyte associations, a diverse assemblage of endophytic fungi have been isolated from angiosperm shrubs and trees, and from conifers (Carroll and Carroll 1978; Petrini and Carroll 1981; Carroll 1991; Butin 1992; Rollinger and Langenheim 1993). Some of these fungi also negatively affect the performance of insect herbivores (Carroll 1986, 1991; Miller 1986; Johnson and Whitney 1994).

Plant symbionts may influence trophic interactions involving host plants, herbivores, and natural enemies of herbivores (Hammon and Faeth 1992; Hunter and Price 1992). However, tri-trophic interactions involving microbial symbionts have seldom been described (but see Dicke 1988). Fungal activity in the leaves of host plants can influence trophic interactions directly through the production of mycotoxins or indirectly by the induction of plant allelochemicals (Wilson 1993). Allelochemicals can have positive or negative tri-trophic effects; they can enhance plant defenses by attracting parasitoids (Bragg 1974; Vinson 1975; Price 1981; Elzen et al. 1983; Whitman 1988; Williams et al. 1988) or they can diminish plant defenses as a result of toxic effects on parasitoids (Thurston and Fox 1972; Campbell and Duffy 1979, 1981; Barbosa and Saunders 1985; Thorpe and Barbosa 1986; Barbosa 1988; but see Gibson and Mani 1984). Mycotoxins may have tri-trophic effects which are similar to those of plant allelochemicals. However, the relationship between one major source of mycotoxins in healthy plant tissue, endophytic fungi, and par-
asitism of insect herbivores has not previously been described.

Frequencies of endophytic fungi and densities of insect herbivores often vary along elevational gradients (Carroll and Carroll 1978; Petrin and Carroll 1981; Petrin et al. 1982; but see Rolinger and Langenheim 1993). However, it is not known how elevation or other characteristics of habitats influence interactions between endophytic fungi and insect herbivores. Sources of herbivore mortality vary with elevation; mortality associated with host plant effects increases with elevation (Delucchi 1958; Randall 1982a, Paige and Capman 1993), while parasitism declines with elevation (Delucchi 1958; Whitaker 1971; Randall 1982a,b). If endophytic fungi influence these sources of mortality, elevational effects on fungal frequencies may contribute to elevational variation in herbivore performance.

We describe the effects of fungi from within the leaves of an oak, Quercus gambelii, on a leaf-mining moth Phyllonorycter sp. (Lepidoptera: Gracillariidae). We consider relationships between frequency of fungal infection and herbivore densities and performance. The evaluation of herbivore performance includes both direct associations between fungi and herbivore survival and indirect relationships between fungal frequencies and parasitism of the herbivore. We investigate these interactions across and within five sites ranging in elevation from 2255 to 2895 m.

We estimated fungal frequencies and insect densities at the scale of individual host plants, although interactions between individual fungi and insects occur at a much finer scale. Host plants were chosen as the experimental units because much of the literature on plant-endophyte-herbivore interactions suggests that endophytes benefit individual host plants by reducing herbivory (see above citations). It also has been suggested that endophytic fungi have co-evolved with plants as a component of plant defense (Clay 1988, Wilson 1993). Therefore, while finer-scale studies will contribute to mechanistic explanations of specific interactions, whole-plant analyses are essential for establishing the ecological and evolutionary context of these interactions.

**Methods**

**Study site and organisms**

The distribution and performance of a leaf-mining moth, Phyllonorycter sp., and the distributions of endophytic fungi were censused on ten Quercus gambelii trees at each of five sites. Elevations of the study sites range from 2255 to 2895 m on Mount Withington in the Cibola National Forest of western New Mexico, United States. The lowest site is in the pinyon-juniper zone near the lower distributional limit of Q. gambelii. The three intermediate sites are in the mixed-conifer zone at elevations of 2440, 2590, and 2745 m. The highest site is in the spruce-fir zone at the upper elevational limit of both Q. gambelii and Phyllonorycter.

Endophytic fungi were cultured from 6250 Q. gambelii leaves. Endophytes have been broadly defined by Hamilton and Faeth (1992) as fungi residing in host plant tissues for at least part of their life cycle. Our surface sterilization procedure (described below) did not necessarily eliminate internal stages of primarily ectophytic or pathogenic fungi. However, our analyses of individual fungal taxa are limited to those with relative frequencies greater than 4% and the fungi meeting this criteria appeared to be asymptomatic. Therefore, these fungi satisfy the more restrictive definition of endophytic fungi as asymptomatic or unapparent fungi living within healthy leaves or shoots (Carroll 1988, 1991). We excluded taxa which sexually reproduced in culture as ascomycetes. The collection was dominated by Gnomonia ceratitis (Sphaeriales: Diaporthaceae) which represented 68% of the total number of fungal infections. Only two other fungal genera individually represented at least 4% of the collection: Sordaria sp. (Sphaeriales: Sordariaceae) and Phoma spp. (Hyphomycetes: Deuteromycotina).

The leaf-mining moth examined in the current study is in the genus Phyllonorycter. Larval development of Phyllonorycter is hypermetamorphic. During the first three instars, larvae feed by slashing and sucking the contents out of cells of one or two subepidermal cell layers of the abaxial leaf surface. Hypermetamorphosis occurs in mid-August between the third and fourth instars. As a result of this hypermetamorphic change in the orientation of mouth parts, fourth and fifth instar larvae chew vertically into the mesophyll tissue. Phyllonorycter pupates in the leaf mines in late September.

**Distribution of endophytic fungi**

To determine the temporal and spatial distribution of endophytic fungi in Q. gambelii, 1250 leaves were censused every 3 weeks from 12 June 1993 through 4 September 1993. In each of the five censuses, 25 leaves were randomly collected from each of the 50 host plants. Since fungi were essentially absent during the first two censuses (Fig. 1), we limited our inferential analyses to censuses 3–5.

Endophytic fungi were cultured from Q. gambelii leaves using a surface sterilization method similar to that described by Carroll and Carroll (1978). Within 48 h of collection, leaves were dipped in a 70% ethanol solution to increase wettability, sterilized in a 10% bleach solution for 4 min, and rinsed in two sterilized water baths. A 22-mm² leaf punch was removed from the base of the lamina adjacent to the mid-rib of each sterilized leaf and placed on 2% malt extract agar. Leaf punches were examined every 2–4 days for the emergence of hyphae. Punches producing endophytes were isolated in separate petri dishes. After 5 weeks, the rate of endophyte emergence dropped to less than 1% per day, and remaining sterile leaf punches were discarded. Loss of samples due to contamination by non-endophytic fungi or bacteria during the 5-week culture periods was minimal (2.13%). All estimates of infection frequencies were based on the number of non-contaminated leaf discs per tree. Since individual leaf discs were sometimes infected by more than one fungal taxon, the total frequency of fungal infection summed across all 34 taxa was in some cases greater than one.

Endophytes were grouped on the basis of reproductive structures, vegetative morphology, and growth rate. Generic designations were obtained for all groups with relative frequencies greater than 4% of the total number of fungal infections. Specimens were identified in the Plant Pathology Laboratory at New Mexico State University, United States, and by the International Mycological Institute, United Kingdom.

Distributional patterns of the endophytic fungi among sites and through time (censuses 3–5) were analyzed with two separate repeated-measures ANOVAs. The first of these analyses investigated total fungal frequency. This estimate of fungal frequency was based on the sum of infections by all 34 fungal taxa divided by the number of non-contaminated leaf discs from each tree. Analysis of the site effect was followed by contrasts testing the significance of linear and quadratic trends in fungal frequency among sites. The second repeated measures analyses used a multivariate approach to analyze frequencies of the three most common fungal genera. Multivariate tests were followed by univariate analyses of individu-