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Variation in Mycorrhizal Infection of the epiphytic orchid *Ionopsis utricullariodes* (Orchidiaceae) on different substrata

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Epiphytic habitats are poor in nutrients for plants to grow. Nonetheless, epiphytic plants have developed strategies to absorb scarce nutrients in this environment (Lesica and Antibus, 1990; Lüttge, 1997). Epiphytic orchids use mycorrhizal relationships with a group of fungi generally designated as *Rhizoctonia*-like fungi. Cortical cells of epiphytic orchid roots are infected by the fungi and form agglomerations of hyphae known as pelotons and the cortical cells digest those pelotons and incorporate nutrients from the fungi into the plant (Arditti 1992). Mycorrhizal relationships in orchids are crucial for seedling development as orchid seeds are dust-like and possess little nutrients for germination and development. Consequently, mycorrhizal interactions are needed for successful establishment (Arditti, 1992). An understanding of the orchid mycorrhizal symbiosis is important because distribution and abundance of the symbiotic fungi in the environment may play a key role in the distribution and diversity of orchids (Otero, 2002).

The epiphytic environment is not homogeneous and aerial roots may grow on different substrata; for example, some epiphytic roots are associated with epiphytic mosses. The effect of this variation on the mycorrhizal interactions of epiphytic plants is not well understood. In the present study we determine whether or not the percentage of cells infected by mycorrhizal fungi varies among roots that grow on different substrata. Additionally, we determine whether or not the developmental stage of the epiphytic orchid *Ionopsis utricularioides* (Sw.) Lindl. is correlated with percentage of cells infected by mycorrhizal fungi. Given that mycorrhizal fungi are required for successful orchid seed germination (Rasmussen, 2002), but mycorrhizal fungi may not be essential for adult orchids (Bayman et al., 2002), we expected that small plants will have proportionally more cells infected with mycorrhizal fungi than those of larger plants.

This research was done at La Selva Biological Station, Costa Rica (Longitude: 84°00′ W, Latitude: 10°26′ N). We choose 13 adult plants of *Ionopsis utricularioides* (Swartz) Lindley (Orchidacecae), growing on *Psidium guajaba* L. (Myrtaceae), that had roots growing in four substrata: bark, dry leaves, mosses, and dry leaves with mosses. Four roots per plant, each associated with a different substrate, were chosen to estimate percentage of cells infected with pelotons. Ten transverse sections were stained with toluene blue and mounted on a microscope slide (Otero et al, 2002). Each transverse section was categorized according to the percentage of cortical cells with pelotons [i = 0 %, ii = 1-25%, iii = 26-50%, iv = 51-75% and v= 76-100%]. Average percentage of cortical cells infected by mycorrhizal fungi per root was calculated and data were arc-

sine transformed for normalization. We measured the length of the longest leaf, an effective surrogate for plant size in this species (Montalvo and Ackerman, 1987).

There were significant differences in the percentage of infected cells among roots on all different substrata (Nested ANOVA $F_{3, 32} = 47.60$, $p < 0.001$, Fig. 1). The trophic status of orchid mycorrhizal fungi is not clear (Bayman et al., 2002). Roots growing on mosses had a higher percentage of cells infected by the fungi. *Ionopsis utricularioides* in Puerto Rico is specifically associated to a group of *Rhizoctonia*-like fungi in the genus *Ceratobasidium* (clade B) (Otero et al., 2002; Otero et al 2004). The same group of fungi was found associated with this species in Panama and Costa Rica (Otero et al., 2007). Some *Ceratobasidium* spp., such as *C. bicorne* from northern-Europe, are moss pathogens (Hietala et al., 2001). Possibly some orchid mycorrhizal fungi are moss pathogens that infect the orchid roots and seed but are controlled and utilized by orchids. Alternatively, the high percentage of root cells with mycorrhizal fungi growing in mosses may be a consequence of microclimatic conditions (e.g. moisture) conducive to fungal growth.

There were significant differences in the percentage of infected cells among plants in different host trees (Nested ANOVA, $F_{3, 41} = 21.60$, $p < 0.0001$), even though the trees were the same species and close to each other. The percentage of infected cells by mycorrhizal fungi in roots of *I. utricularioides* may be affected by abiotic factors that influence the growth and availability of the fungi such as humidity, temperature, pH, irradiance and substrata and by the presence of competitors, such as pathogenic parasites and fungivorous arthropods. These factors likely vary over time and at small spatial scales creating different microhabitats that affect the distribution and composition of the fungal community. Established epiphytic orchids, not needing the fungal association, would be immune to such changes. It is possible that the fungal community around the orchid root changes over time and that the mycorrhizal fungi become locally extinct, but the orchid is able to survive such events and persist having low mycorrhizal infection.

The percentage of infected cells was not correlated with plant size (Pearson correlation, $r = 0.34$, $N = 13$ $p = 0.25$, Fig. 2). Plants of similar sizes had considerable differences in the percentage of infected cells and vice versa. We expected small plants to have a higher percentage of infection than bigger ones. Nonetheless, our data did not fit this prediction; the percentage of cells infected by mycorrhizal fungi was independent of plant size. While of different sizes, all plants considered in this study might be adults that do not represent the stages in which fungi is particularly important (e.g. seedlings and protocorms). Infrequent mycorrhizal infections have been documented for epiphytic orchids (Hadley and Williamson, 1972; Lesica and Antibus, 1990; Rivas-Rossi et al., 1998; Otero et al., 2002; but see, Goh et al., 1992;).

Our results show that roots associated

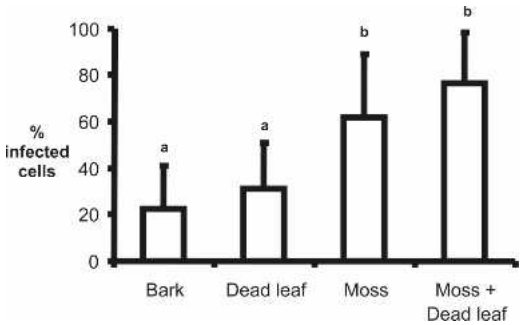

FIG. 1. Percentage of cells infected by mycorrhizal fungi of roots of *I. utricularioides* growing in different substrates. Error bars are *sd*. letters indicate statistically significant differences.

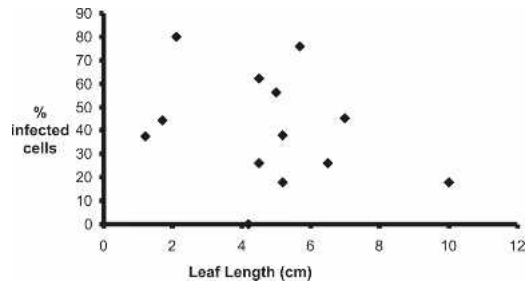

FIG. 2. Relationship between the length of the longest leaf and the percentage of cells infected by mycorrhizal fungi in *I. utricularioides*. Leaf size is an estimator of plant size.

with mosses had higher percentage of cortical cells infected with mycorrhizal fungi suggesting two alternative explanations: orchid mycorrhizal fungi may be moss parasitic fungi; and micro-clime conditions in moss, such as humidity, may promote orchid mycorrhizal formation.

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