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Vesicular-arbuscular Mycorrhizae on Sapium glandulosum in pastures and light gaps

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ABSTRACT

The purpose of this study was to look at the frequency of infection and percent root infection of vesicular-arbuscular mycorrhizae present in saplings of *Sapium glandulosum* in pasture and gaps. Saplings in the pasture had a significantly higher frequency of infection than saplings in the gaps (Two-by-two contingency table, $X^2 = 27.855$, $p < 0.0001$). Saplings in the pasture also had a significantly higher mean percent infection than saplings in the gaps (Mann-Whitney U test, U = 370.00, tied p-value = 0.0013). These findings indicated that the amount of disturbance that an area has been subjected to strongly influences the levels of VAM growth on *S. glandulosum*.

RESUMEN

El propósito de este estudio fue mirar las frecuencias del porcentaje de infección de las raíces de vesicular-arbuscular micorriza presente en los arbolitos de *Sapium glandulosum* que están en el pasto y los claros del bosque. Los arbolitos del pasto tenían una frecuencia de infección que estaba significativamente mas alta que los arbolitos en los claros (Dos-por-dos contingency table, $X^2 = 27.855$, $p < 0.0001$). Arbolitos del pasto también tenían un medio porcentaje de infección que estaba significativamente mas alto que los arbolitos de los claros (Mann-Whitney U examen, U = 370.00, tied p-value = 0.0013). Estas conclusiones indican que el nivel de la alteración que una area se ha sometido tiene mucha influencia sobre los niveles de vesicular-arbuscular micorriza presente en *S. glandulosum*.

INTRODUCTION

Mycorrhizal fungi are a major component of nutrient cycles in tropical Ecosystems. Mycorrhizae form mutualistic associations with the roots of many tropical plants by providing increased uptake of scarce nutrients for plants in exchange for carbon and a host substrate on which to live. These fungi require a host plant for germination, growth, and reproduction, and cannot be grown under laboratory conditions without a substrate (Janos 1983). These symbiotic relationships are more commonly formed in areas of low soil fertility, nutrient availability, and/or water accessibility (Janos 1980). In such ecosystems, especially phosphorus-deficient soils, a plant will form a symbiotic relationship with fungi in order to obtain the nutrients and water that are necessary for survival, but present only in limited quantities. Phosphorus is essential to the transport of carbon and other photosynthetic products out of the chloroplasts and is also a necessary component of adenosine triphosphate (ATP) (Fredeen et al. 1989). Plants deprived of phosphorus have demonstrated decreased shoot growth and significantly slower leaf expansion, thus reducing photosynthetic rates. Because phosphate ions have difficulty moving through the soil, plants often are unable to absorb sufficient levels of phosphates without the help of mycorrhizae. By increasing the surface area of the roots available for absorption of nutrients from the soil, mycorrhizae may give their host plant a competitive advantage over its neighbors (Janos 1983). In return, the mycorrhizae receive carbon from the plant which is essential for spore production (Smith and Read 1997).

Vesicular-arbuscular mycorrhizae (VAM) are endomycorrhizal fungi, meaning they grow inside the roots of plants and are usually found on the adventitious roots of plants. They are characterized by the presence of vesicles and arbuscules, where phosphorus and other nutrients are exchanged for carbon (Leake and Read 1993). VAM are the symbionts that are most commonly found underground, and they can form either facultative or obligate mutualisms with plants. Whether or not the symbiosis is essential for the survival of the plant depends upon the host plant species. The probability that a facultative mycotroph, a plant which benefits from VAM relationships but does not require them for survival, will be infected by mycorrhizae is determined by soil fertility and nutrient and water availability (Smith and Read 1997). If the nutrients and water essential for survival are present in adequate amounts for the root hairs of the plant to uptake them from the soil unaided, or if the plant needs to conserve its carbon supplies for allocation toward growth and reproduction, the plant may reject the symbiosis.

VAM are important characteristics of plants in the tropics, where soil is highly infertile due to heavy rainfall, leading to the runoff and leaching of many nutrients (Terborgh 1992). In forest areas where nutrient cycling has been disrupted, such as sites of deforestation, the system becomes more open due to soil erosion, wind exposure, and the runoff of organic matter (Brady and Weil 1996). Consequently, the system loses many valuable and essential nutrients. Saplings from two different types of disturbance are examined in this study, a pasture and three light gaps. These two areas differ greatly in their nutrient cycles because although nutrients are removed in both disturbance areas due to the loss of standing trees, they are being at least partially replenished in the gaps. In the pasture, trees were merely clearcut and removed, whereas in the gaps the trees have fallen down but remain on the forest floor to decompose, thereby allowing a good proportion of nutrients to be recirculated into the system as the biomass decays. A previous study in the Monteverde Cloud Forest Preserve has shown that approximately 243.5 kg/ha of phosphorus are deposited into the ecosystem as a result of biomass decomposition (Nadkarni et al.). Therefore, the nutrient losses in the gaps can be made up by or possibly even overcompensated by decaying organic matter, whereas the pasture's only source of input comes from the weathering of the bedrock beneath it, which is inevitably poorly fertile (Nadkarni et al. 2000, Terborgh 1992). As resources become more scarce and competition for nutrients among plants increases, it is predicted in this study that there will be an increased presence of mycorrhizae in plant roots in order to acquire the necessary nutrients.

This study looks at the different levels of mycorrhizae present in the roots of *Sapium glandulosum* (Euphorbiaceae) found between pastures and gaps. *Sapium glandulosum* is a pioneer species found frequently in disturbed habitats, especially secondary growth forests succeeding gaps and pastures. In Monteverde, it is common between 1300 m and 1500m. Its seeds and saplings require sun for germination and continual growth (Haber et al. 2000).

In the gaps, where nutrient cycles have probably not suffered such severe losses as in the pastures, there are expected to be decreased amounts of VAM living in plant roots. Because nutrients are more abundant, plants should have less trouble obtaining them from the soil unaided (Brady and Weil 1996). The pasture area, on the other hand, should have a higher amount of VAM because deforestation may have caused the loss of essential nutrients. A relationship with VAM may help the saplings living in these disturbed sites that would otherwise have difficulty absorbing these nutrients on their own.

MATERIALS AND METHODS

The study was conducted in lower montane wet forest in Monteverde, Puntarenas, Costa Rica in areas of elevation 1450m – 1600m. The pasture samples were taken from the 2.5 hectare pasture located behind La Estación Biológica. The gap samples were taken from three gaps found in otherwise continuous secondary growth forest on the private reserve of La Estación Biológica.

Samples of adventitious roots of *S. glandulosum* were collected from 20 plants in the pasture and 24 plants in the forest. All samples were taken from saplings less than one meter in height. In the laboratory, they were delicately washed in water to remove soil and then stored in 2% KOH solution. Using the Kormanik et al. (1980) methodology for staining endomycorrhizae, the roots from each area were stained to indicate the presence of VAM habitation. After placing samples from each sapling in separate test tubes, approximately three mL of 10% KOH solution was added to each test tube so that the roots were completely immersed in the solution, and they were incubated in boiling water for 30 minutes. They were then rinsed with tap water inside of a screened syringe. The test tubes were also rinsed in tap water to remove residue from the basic solution. The roots were returned to test tubes and soaked in approximately three mL solution of 1% HCI for 15 minutes. After draining the 1% HCI, a staining solution heated to 75 degrees Celsius was poured into the test tubes, which were then incubated again in boiling water for 20 minutes. The mixed, heated staining solution was composed of 50% Glycerol, 1% HCI, and 0.05% Trypan Blue with a dilution of two mL Glycerol, one mL HCI, and four drops of Trypan Blue. After incubation, the staining solution was drained and the roots were immersed in approximately three mL of 50% Glycerol and 1% HCI solution. They were stored in this distaining solution until removed for examination under the compound microscope.

A minimum of five adventitious root segments were randomly chosen from each plant and cut into one cm pieces for examination. When possible, ten segments were chosen from each sample for analysis. All 20 samples from the pasture consisted of ten segments. Thirteen of the samples taken from the gaps were composed of ten segments, whereas the remaining 11 samples consisted only of five segments. Each segment exhibiting VAM was considered infected. The number of segments infected in each tree sample was summed to calculate a percent infection per tree. A Mann-Whitney U test was used to test for a difference in the percentage of segments of saplings infected from the pasture versus the gaps. A two-by-two contingency table was used to determine a difference in frequency of root infection between specimens collected in the pasture versus the gaps.

RESULTS

All of the trees examined from both ecosystems showed the habitation of VAM on at least one of their roots. A total of 185 root segments from the gap were examined, 132 of which exhibited the presence of VAM. Out of the 200 root segments examined from the pasture, 184 showed the presence of mycorrhizae. A significant difference was found in the frequency of root infection between the two sites (Two-by-two contingency table, $X^2 = 27.855$, $p < 0.0001$; Fig. 1). Saplings in the pasture showed a mean percent infection of 92.00% ($sd^2 = 1.71\%$, N = 20). Saplings in the gap exhibited a mean percent infection of 68.75% ($sd^2 = 6.97\%$, N = 24). The percent infection for saplings in the gaps versus saplings in the pasture also showed a significant difference (Mann-Whitney U test, U = 370.00, tied p-value = 0.0013; Fig. 2).

DISCUSSION

The presence of VAM in all of the saplings suggests that mycorrhizal fungi play an important role in nutrient acquisition by *S. glandulosum*, at least during its early stages of growth. Previous studies have shown that the majority of relationships formed between mycorrhizae and pioneer species are facultative (Janos 1983). Further studies would be required to determine the extent to which *S. glandulosum* depends on VAM to survive.

This study showed that saplings in the pasture and gaps housed significantly different levels of VAM (Fig. 1). The two factors that determine the extent to which VAM will be present on a mycotroph's roots are: (1) the likelihood of that species becoming infected, based solely on the affinity of its roots for the fungus, and (2) the nutrient fertility of the soil (Smith and Read 1997). Because all sapling roots examined were taken from the same species, all samples had an equivalent inherent probability of infection. Therefore, the different levels of VAM could most likely be attributed to differences in nutrient availability. Because the soil in the pasture probably has lower fertility, the samplings need the assistance of VAM to increase their nutrient uptake and had infected roots 92% of the time (Fig. 2). Previous studies have reported increased mineral absorption of plants in nutrient-unstable soils when mycorrhizae were present, and consequently, improved annual crop productivity (Janos 1983). In the gap areas, where disruption has probably been less severe and the soil contains more nutrients, the percentage of VAM encountered on roots was significantly lower, occurring only 68.75% of the time (Fig. 2).

One possible explanation for decreased infection in gaps is that increased surface area for more absorption is not necessary for essential growth and reproduction because the plant is capable of absorbing adequate nutrients with smaller levels of VAM. Previous studies in Monteverde have shown that litterfall are an important mechanism of transferring phosphorus from biomass back into the soil (Nadkarni et al 2000). Forty-two percent of the phosphorus in leaves was retranslocated from live foliage to litterfall, which rests upon the soil and releases phosphorus as it decomposes. Because the gaps are surrounded by the otherwise continuous forest of canopy trees, they can still receive a good amount of litterfall, whereas the pasture has only a few large trees still standing that are very spread out. With a lack of trees, the pasture probably does not receive nearly as much retraslocated phosphorus. Consequently, the gaps probably provide phosphorus to its plants more readily because of the continual input of nutrients, whereas the pasture probably experiences an overwhelming loss of nutrients without a source to reintroduce them in large quantities. The increased presence of mycorrhizae on sapling of gaps, then could actually be counterproductive if the plant gives away essential carbon to VAM that it needs for successful growth, when it is already receiving sufficient phosphorus.

Studies have shown that plants grown in soils with ample amounts of phosphorus contain a significantly lower percent of root infection by VAM than plants grown in phosphorus-deficient soils (McArthur and Knowles 1992). McArthur and Knowles provided evidence that when phosphorus is a non-limiting nutrient in the soil, plants may limit or even inhibit infection. Increased levels of phosphorus available to the plant increase the production of ethylene, a compound that inhibits VAM growth. As a result, the plants reject mycorrhizae in fertile soil to retain carbon for their own growth. The results of this study concurred with McArthur and Knowles (1992) findings that mycorrhizae were found in significantly lower proportions and frequencies in the less disturbed habitat. Although no soil samples were taken in this study, the results indicate that there were different levels of nutrients available in the two habitats. Future studies could confirm more accurately these varying levels of soil disturbances.

Since *S. glandulosum* is most commonly found in successional growth areas, it is probably adapted for growth in the low fertility, disturbed soils of gaps. Further studies could examine the minimum levels of phosphorus required for growth of *S. glandulosum* in these natural disturbance areas. A plant must make a decision regarding the allocation of its carbon (Smith and Read 1996), and it would be interesting to determine the minimal phosphorus threshold at which *S. glandulosum* decides to engage in nutrient trade-offs with VAM. Future studies could investigate the ways in which reliance upon VAM changes between the areas of natural disturbance, such as gaps, in which *S. glandulosum* has probably naturally evolved to live within, and the more recent, larger scale, manmade disturbances, such as pastures, that it inhabits as well.

An additional factor to consider when analyzing the results of this study is the relative abundance of VAM spores residing within the soils of gaps and pasture. Tropical soils support relatively few mycorrhizal spores, so VAM must rely heavily on the plants which support them in order to persist in a community (Janos 1996). Janos proposes that the amount of mycorrhizae present in a successional community is directly related to the time since the disturbance has occurred and the amount of root biomass present in the community. Janos suggests that as the years since the disturbance increase, the abundance of mycorrhizae in the soil increases as well. The pasture was probably initially disturbed over twenty years ago and continues to be grazed by cattle. The gaps, on the other hand, are comparatively younger, and therefore may have a lower plentitude of VAM. However, the younger ages of the gaps may be counterbalanced by the root mass from the surrounding areas and that which remains in the ground after the treefalls. Still, the VAM could be limiting in the soils of gaps and less available for symbioses with the plants. Further studies could be used to assess the different amounts of VAM spores present in gaps versus pastures.

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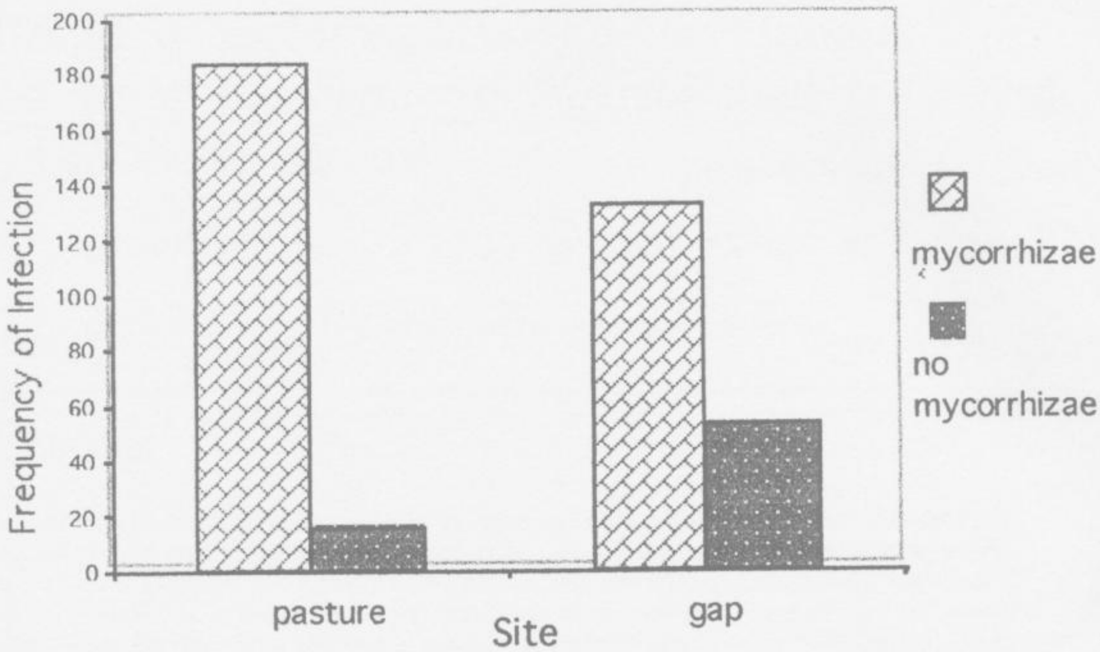

Figure 1. Frequency of *Sapium glandulosum* roots infected with mycorrhizae in two areas of different disturbance levels. Differences in infection frequency were significant (Two-by-two contingency table, $X^2 = 27.855$, $p < 0.0001$).

Figure 2. Mean proportion of *Sapium glandulosum* roots infected per site. A Mann-Whitney U test showed significant differences between the two sites (U prime = 370.00, tied p-value = 0.0013).