

Endophytic fungi alter foraging and dispersal by desert seed-harvesting ants

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Abstract. Endophytic fungi are thought to interact mutually with host plants by producing alkaloid metabolites that deter herbivory. Since such fungi are transmitted via seed in some grasses, the presence of endophytes may also protect plants from seed predators. We conducted seed choice experiments for two dominant seed harvesting ants, *Pogonomyrmex rugosus* in the Sonoran desert and *Pogonomyrmex occidentalis* at a higher elevation, riparian zone in Arizona, USA. Non-infected fescue (*Festuca arundinacea*) seeds and seeds infected with the endophytic fungus, *Acremonium coenophialum*, were presented to ant colonies in three different populations. Infected seeds were harvested less frequently than non-infected seed for the two populations of *Pogonomyrmex rugosus* but not for the population of *Pogonomyrmex occidentalis*. We also conducted seed dispersal experiment for one population of *Pogonomyrmex rugosus*. Of the seeds that were harvested, most of the colonies discarded more infected seeds into refuse piles than expected by chance. Seeds discarded into refuse piles have greater germination success than surrounding areas. The most important interaction of endophytes and grasses may be deterrence of seed predation and enhancing the probability of germinating in favorable sites, since these processes directly increase plant fitness.

Key words: Fungal endophytes – Harvester ants – Grasses – Granivory – Seed dispersal

Endophytic fungi, fungi that live asymptotically within plant tissues, are thought to benefit plants by deterring herbivory (Clay 1987, 1991, 1992). Infected plants have long been known to be toxic to grazing livestock (Siegel 1985). Recent research supports the hypothesis that endophytic fungi reduce herbivory of grasses by insects (Siegel et al. 1990; Clay et al. 1985; Carroll 1988; Ahmad 1985; Dahlman et al. 1991). Consumption of infected

grasses can reduce fecundity, survival, and growth of insects (Cheplick and Clay 1988, Clay 1991). Fungal alkaloids have been implicated as the mechanism for these effects (Cheplick and Clay 1988, Powell and Petroski 1992, Petroski et al. 1992).

Despite evidence that the presence of endophytic fungi in plants reduces herbivory, endophytes may have more direct effects on plant fitness if their presence alters seed predation and dispersal. Herbivory may have weak, nonexistent, or even positive effects on plant fitness and any effects on fitness may be highly variable in time and space (Maschinski and Whitham 1989; Marquis 1992, Rausher 1992). Many endophytic fungi in grasses, such as *Acremonium coenophialum* in tall fescue, *Festuca arundinacea*, are transmitted vertically, from maternal plant via seed. Concentrations of many fungal alkaloids, including *Acremonium coenophialum*, are higher in the seed than other plant parts (Clay 1991). The presence of these endophytic fungi and their mycotoxins may deter seed predators and parasites and affect seed dispersal if endophyte infected seeds are either avoided by seed harvesters or, if collected, later discarded disproportionately into favorable germination sites. Despite the potential for strong, direct effects of endophytes on plant fitness via changes in seed survival and dispersal, this aspect of the fungal endophyte – plant interaction has received only recent attention (Madej and Clay 1991).

We tested the hypothesis that the presence of the endophytic fungus (*Acremonium coenophialum*) deters harvesting of fescue seeds (*Festuca arundinacea*) by the two species of seed harvester ants, *Pogonomyrmex rugosus* and *P. occidentalis*. We also tested the hypothesis that colonies of *Pogonomyrmex rugosus* discard infected seeds non-randomly into refuse piles.

Materials and methods

Study sites and organisms

We used three study sites and two ant species in the experiments. Two sites are located in the lower Sonoran desert in central Arizona.

The Chandler (C) site (elevation 395 m) is 10 km southeast of Phoenix (Maricopa County). The Queen Creek (QC) site (elevation 610 m) is approximately 80 kilometers east of Phoenix (Maricopa County). The third, higher elevation (1675 m) site is located in the Sierra Anchas Experimental Forest (SAEF), Tonto National Forest, approximately 110 kilometers northeast of Phoenix (Gila County). This site is a riparian zone intergrading with surrounding chaparral and grassland vegetation. *Pogonomyrmex rugosus* is the dominant seed harvesting ant species at the two low elevation sites (QC and C) while *Pogonomyrmex occidentalis* is the dominant species at the higher elevation, SAEF site.

Seed harvesting experiments

To test the hypothesis that seed harvesting ants avoid endophyte-infected seeds, ants were given a choice of infected or non-infected seeds in a field experiment. We used seeds from tall fescue, *Festuca arundinacea*, in our experiments. Although *Festuca arundinacea* is not native to Arizona, several other native *Festuca* species are found in Arizona. Also, most of the grass species in Arizona, like most of North America, are long-established, non-native species. Previous work by Rissing (1981) shows that *Pogonomyrmex rugosus* readily harvests non-native *Festuca* seeds. Further, many non-native grass seeds are preferred by these ants relative to native species (Rissing 1981). Infection rates for the endophyte-infected seeds were >95%; non-infected seeds had an infection rate of <5%. The seeds were supplied and verified for endophyte infection frequency by M.R. Siegel of the University of Kentucky.

Seeds of each type were weighed and placed into 100-ml plastic graduated cylinders with 1-cm holes cut near the bottom. Two cylinders, one with each seed type, were placed by side by side and within 3 feet of the main entrance of an active ant colony, near the foraging trunk trails. We started with 50 g. in each cylinder at the C and QC sites and 30 g. of each seed type at the SAEF site. The openings of each cylinder were flush with ground level. Both cylinders were covered by a wire-mesh cage to keep rodents from consuming the seeds. Cylinders were placed at the C site colonies during the first week of May 1992, at the QC site during the first week of June 1992, and at the SAEF site during the first two weeks of July 1992. Paired cylinders were replicated at 15 different colonies at each location, and we repeated the entire experiment twice at the C site ($N=30$ paired cylinders). We returned to the colonies within 24 h at both the QC and C sites and collected all remaining seeds in the cylinders. However, at the higher elevation SAEF site, the colony foraging rates were slower and we left the cylinders for one week before we collected the remaining seeds for weighing. Ant colony foraging has been shown to be highly variable among harvester ant populations and seasons (Hollneger and Wilson 1990).

We used a one-tailed, paired t -test to test for differences in mass of seeds harvested from control (uninfected) and experimental (infected) cylinders. A one-tailed test was used because our a priori hypothesis was that ants remove more uninfected than infected seeds. We analyzed each population separately. A few replicates were not included either because wind, cattle, or rodents toppled the cylinders or ants removed all seeds of each type before the cylinders could be collected.

Seed rejection experiment

We tested the hypothesis that infected seeds are discarded more frequently than non-infected seeds after harvesting by 15 colonies of *Pogonomyrmex rugosus*. This experiment was conducted concurrently with the seed choice experiment at the C site. Seeds were colored with non-toxic, red and green vegetable dyes and presented to ants in the cylinders as described above. To avoid possible preferences due to color or chemical differences in dyes, we randomized colors between infected and non-infected seeds (i.e., some colonies were presented with red infected and green non-infected

seeds, while others were presented with green infected and red non-infected seeds). We sampled contents of the refuse piles 24 h after the placing the cylinders by randomly removing a 20 square centimeter section from the refuse pile. The refuse samples were returned to the lab and intact, whole, dyed seeds were separated, counted and weighed.

We compared the number of infected to uninfected seeds in the refuse piles to the number expected based upon the ratio of masses of uninfected to infected seeds collected by ants from the cylinders. We adjusted the number of infected and uninfected seeds in each refuse pile to numbers based on per unit seed mass, since the expected frequencies were determined from mass of seed harvested from the cylinders. One colony did not collect any seeds and was therefore excluded from analyses. We used G tests to test for differences in observed and expected frequencies of infected and uninfected seeds in the refuse pile of each colony ($N=14$). We used an $\alpha=0.001$ to compensate for experimental-wise error rate inherent in multiple comparisons (Sokal and Rohlf 1981).

Results

Both populations of *Pogonomyrmex rugosus* harvested significantly more non-endophyte infected seeds than endophyte infected seeds (Fig. 1, C site, $t=1.72$, $df=26$, $p<0.05$; QC site, $t=2.96$, $df=10$, $p<0.01$). Individuals in all colonies collected some endophyte infected seeds (range = 13% – 63.8%). These results indicate preference for non-infected seeds rather than absolute unacceptability of endophyte-infected seeds. At the higher elevation SAEF site, however, colonies of *Pogonomyrmex occidentalis* did not harvest more uninfected seed than infected seed (Fig. 1, $t=0.29$, $df=10$, $p>0.25$).

Colonies of *Pogonomyrmex rugosus* generally discarded more infected seeds than expected by chance based upon the proportion harvested (Table 1). Of the 14 colonies examined, eight discarded significantly more infected seed and two discarded more uninfected seed than expected by chance, while four randomly discarded seed types (Table 1).

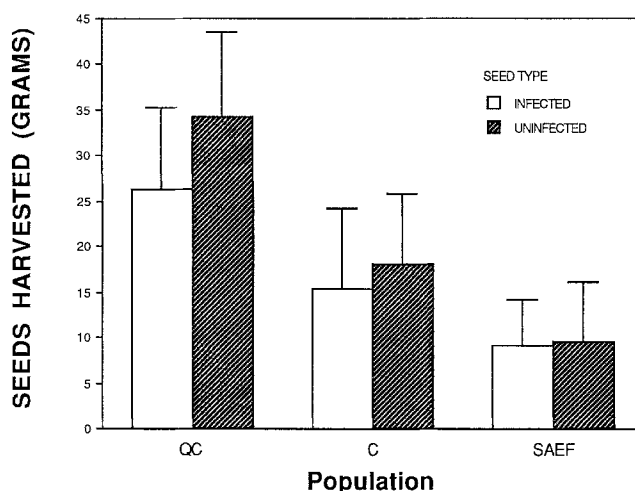


Fig. 1. Mean and standard deviation of mass of infected and uninfected seed harvested at the three study sites, Queen Creek (QC), Chandler (C), and Sierra Ancha Experimental Forest (SAEF)

Table 1. G-tests of observed and expected frequencies of uninfected and infected found in refuse piles of 14 *Pogonomyrmex rugosus* colonies

Colony	Uninfected Observed	Uninfected Expected	Infected Observed	Infected Expected	X ²	p
1	66	108.00	50	8.00	118.25	<0.001
2	69	60.72	41	49.28	6.26	N.S.
3	1206	1427.46	1366	1144.54	76.61	<0.001
4	679	624.17	1050	1104.83	7.45	N.S.
5	153	283.20	492	361.85	113.92	<0.001
6	801	1014.21	895	681.79	108.98	<0.001
7	225	339.83	517	402.16	74.18	<0.001
8	21	105.77	162	77.23	172.12	<0.001
9	618	564.03	641	694.97	9.31	N.S.
10	572	537.73	227	261.27	6.84	N.S.
11*	147	104.14	107	149.86	29.25	<0.001
12*	1804	1495.58	2021	2329.43	102.38	<0.001
13	360	443.10	416	332.90	35.87	<0.001
14	323	764.32	1025	583.68	597.92	<0.001

* Colonies where observed frequency of infected seeds in the refuse piles were significantly less than expected

Discussion

Our results support the hypothesis that endophytic fungi may benefit plants by reducing seed predation from desert seed harvesting ants, at least at low elevation sites where *Pogonomyrmex rugosus* is common. Seed harvesting ants such as *Pogonomyrmex rugosus* are major predators of seeds in the Sonoran desert (e.g., Brown et al. 1979, 1986). Thus, we predict that protection from seed predation from this ant species should directly increase fitness, and consequently frequency, of plants harboring endophytes. An interesting test of this prediction would be to compare frequency of infected and noninfected plants and plant species in areas of high and low intensity of seed predation.

Unlike *Pogonomyrmex rugosus* that inhabit the Sonoran desert, colonies of *Pogonomyrmex occidentalis* that occur at higher elevations, did not prefer uninfected seeds. This lack of preference could reflect simply an insufficient sample size to detect differences in preference (7 of 11 colonies harvested more uninfected than infected seeds, although this difference is not significant). There are however two possible biological explanations. Firstly, the two species of *Pogonomyrmex* could differ in seed preferences and ability to detect the presence of endophytic infections and associated alkaloids. Although the seed preferences of *Pogonomyrmex rugosus* are well-studied, at least relative to seed species (Rissing 1981), those of *Pogonomyrmex occidentalis* are not, and there is presently no evidence to support this explanation. The inability to discriminate infected and uninfected seeds within the time frame of the experiment may also be related to the relatively slow foraging rate of *Pogonomyrmex occidentalis*. Seed harvesting ants can learn to major on seed types (Rissing 1981). *Pogonomyrmex occidentalis* may require more time to discriminate infected seeds. Secondly, endophytes occur most often in cool season grasses (Clay 1992) and in woodlands compared to grasslands (Leuchtman 1992). Higher levels of infection are probably related to the cooler and moisture

climate. Since *Pogonomyrmex occidentalis* is a high elevation species that tends to occur in or near riparian zones, it is possible that this species frequently encounters infected seeds and has adapted to collecting and consuming them.

Our results also support our hypothesis that infected seeds, if harvested, are more likely to be discarded into refuse piles. Germination and seedling survival success are key components in plant fitness (Louda 1983) particularly in desert environments where soils are low in moisture and nutrients. Germination success and seedling recruitment are greater in refuse piles of *Pogonomyrmex rugosus* than in surrounding areas (Rissing 1986) presumably due to increased soil moisture and nutrients. We therefore predict that germination and survival of infected seeds should be greater than uninfected seeds since infected seeds are disproportionately discarded. One could argue that since fewer infected seeds are collected, fewer are likely to be dispersed into favorable germination sites relative to uninfected seeds. We note, however, that a very small fraction of intact seeds are discarded relative to those collected and consumed, and those that are, tend to be infected seeds. Although we cannot resolve the questions of relative advantages and disadvantages of predation and dispersal that are common to animal-plant systems where seed predators also act as dispersal agents (e.g., Thompson 1982; Howe and Westley 1988), we suggest the presence of endophytes benefits grasses when the fungus is transmitted via seeds.

Generally, mutualisms are predicted to be more likely in interactions where transmission of micro-organisms is directly linked to vector reproduction and fitness (Williams and Reese 1991), such as in the grass-endophyte interaction. To date, few studies have examined this aspect of the purported endophyte-plant mutualism. In one of these studies, Madej and Clay (1991) found that several species of seed-eating birds prefer to feed on uninfected to infected grass seeds. In addition to ants, the other major seed harvesters in the Sonoran desert are rodents and birds. Preliminary trials with the desert

kangaroo rat, *Dipodomys merriami*, indicate that they also reject infected seeds (T. Knoch pers. observ.).

Seed harvesters, particularly ants, can have important impacts on plant community structure via seed predation and dispersal (Brown 1979, Brown et al. 1986), especially in xeric areas (Rissing 1986, Holldobler and Wilson 1990). Seed harvesting ants can alter plant community structure, especially in deserts (Brown et al. 1979, Davidson et al. 1985, Holldobler and Wilson 1990; Crist and MacMahon 1992). Therefore, the presence of endophytic fungi may alter plant community structure by affecting selective seed predation, dispersal, and germination (Hammon and Faeth 1992), especially if the anti-predator effects extend to other groups, such as birds and rodents. Fungi have been generally overlooked by ecologists studying seed-pool and granivore-plant interactions (Reichman and Rebar 1979; Crist and MacMahon 1992). Our results suggest that one group of fungi, endophytes, can alter granivore-plant interactions. We are conducting additional tests of the hypothesis that endophytic fungi alter plant community structure via seed predation, dispersal and germination.

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