

The Effect of Rust Infection on Reproduction in a Tropical Tree (*Faramea occidentalis*)¹

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ABSTRACT

Fungal pathogens that infect reproductive structures of plants (*e.g.*, flowers and fruits) can reduce the seed production and seedling recruitment of host plants. We report here on the effects of a rust, *Aecidium farameae*, that infects the ovaries and pedicels of mature flowers on *Faramea occidentalis* (Rubiaceae), a small tree common on Barro Colorado Island, Republic of Panama. Rust infection of ovaries reduced the number of maturing fruit on infected trees. Trees with low rust incidence in June of 1992 had 68 percent fruit survival, compared to 17 percent fruit survival for those with high rust incidence. Infected fruits developed abnormally and were usually aborted long before uninfected fruits were mature. One hundred percent of the infected ovaries marked in July were diseased or missing in August. We conclude that infection by *A. farameae* has the potential to seriously decrease the reproductive output of *Faramea occidentalis* and may represent an important source of variation in the relative fitness of individual plants.

RESUMEN

Hongos parásitos que infectan estructuras reproductivas de plantas (flores y frutos) pueden reducir la producción de semillas y, como consecuencia, la producción de plantas de semilla de árboles huéspedes. Reportamos los efectos de una roya, *Aecidium farameae*, que infecta los ovarios y los tallos de flores maduras de *Faramea occidentalis* (Rubiaceae), un árbol común del sotobosque de la Isla Barro Colorado, República de Panamá. La infección de los ovarios por la roya redujo la cantidad de frutos maduros en árboles infectados. En los árboles que tenían pocos ovarios infectados en junio de 1992, 68 por ciento de los frutos sobrevivieron, mientras que en los árboles que tenían muchos ovarios infectados, 17 por ciento de los frutos sobrevivieron. Frutos infectados se desarrollaron anormalmente y, por lo general, fueron abortados mucho antes de que los frutos no infectados maduraran. Ciento por ciento de los ovarios que exhibían infección en julio estaban infectados o habían desaparecido en agosto. Concluimos que infección por *Aecidium farameae* tiene la capacidad de disminuir drásticamente la fecundidad de *Faramea occidentalis* y posiblemente representa una fuente importante de variación en el éxito reproductivo de árboles individuos.

Key words: *Aecidium farameae*; *Faramea occidentalis*; fruit abortion; fruit production; plant-pathogen interactions.

MATERNAL REPRODUCTIVE SUCCESS OF AN INDIVIDUAL PLANT (defined as the number of seeds produced), is limited by either resources available to the maternal plant or the amount of pollen received (Willson & Burley 1983). Animal pests, abiotic conditions (*e.g.*, weather), and disease can interfere with the production of seeds by interfering with pollination and seed development. Damage to important floral structures (*e.g.*, anthers, stigmas, ovaries) can reduce the amount of pollen received by flowers and lead to improper development of seeds, depending on the structure affected (Alexander

1987). By damaging fruit and seeds, pests and disease can also reduce the potential number of seedlings produced by a maternal plant (Stephenson 1981, Dinooor & Eshed 1984). Pathogens may also affect reproduction indirectly by weakening the plant host. The effects of fungal pathogens on reproduction in flowering plants are not well understood.

Fungi have been shown to reduce reproductive output in both cultivated and wild plant species. Studies of crop plants, fruit trees and their pests have described the effects of fungal species that attack plant reproductive structures. Both seed mass (*e.g.*, Doling 1964) and total seed output (*e.g.*, Dinooor & Eshed 1984) of crop plants have been shown to decrease as a result of infections by smuts and rusts. Far fewer studies have focused on fungal

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effects on reproduction in wild plant species. Alexander (1987; Alexander & Antonovics 1988, Alexander 1989) has studied variation in *Silene alba* resistance to a fungal smut (*Ustilago violacea*) that infects flowers and produces fungal spores within the anthers of mature flowers. This destruction of pollen-producing organs appears to reduce the number of fruits maturing in the population by half as a result of pollen limitation (Alexander 1987). Another example of fungal influence on plant reproduction involves the rust *Puccinia monoica* that reduces the seed output of host *Arabis* spp. by infecting the hosts' flowers, destroying the floral structures and forming floral mimics which produce spores (Roy 1993). In natural plant communities, such impediments to successful reproduction can profoundly influence seedling recruitment and the evolution of plant traits affecting disease resistance.

We conducted a two-month study on the effects of a fungal rust species on the fruit production of its host plant *Faramea occidentalis*, on Barro Colorado Island (BCI), Republic of Panama. The *Faramea* rust is different from the majority of fungi infecting plants on BCI, because it appears to infect almost exclusively plant reproductive structures (*i.e.*, flowers and fruits). The survivorship of mature *Faramea occidentalis* trees is not likely to be influenced by this rust, but we predicted that the production of viable seed by *F. occidentalis* would be reduced in those plants infected by the rust, due to the abnormal development of fruits and flowers. Specifically, we addressed three questions: (1) what are the natural levels of rust infection on focal individuals of *F. occidentalis*?; (2) does rust infection affect fruit maturation in *F. occidentalis*?; and (3) are heavily infected trees likely to suffer disproportionately greater losses of fruits and seeds than lightly infected trees?

MATERIALS AND METHODS

This study was conducted in the seasonal, moist forest on BCI in Gatún Lake, Panama. Detailed descriptions of the forest and environmental conditions are given in Foster and Brokaw (1996) and Windsor (1990). *Faramea occidentalis* (Rubiaceae) is a small tree common to the old-growth forest of BCI, where both populations used in this study were located. The majority of *F. occidentalis* individuals on BCI flower synchronously during June and July, with each tree producing up to several hundred white, sweet-smelling flowers (1.5–2.0 cm long; Croat 1978). The majority of flowers open

at dusk and are pollinated by moths (Sphingidae and Noctuidae, Travers, pers. obs.). Following pollination, the ovaries develop into single-seeded fruits over the next seven months (Schupp 1990). The mature fruits are eaten primarily by white-faced monkeys (*Cebus capucinus*) and howler monkeys (*Alouatta palliata*). The seeds are then dispersed following passage through their digestive tracts (Schupp 1990).

DISEASE SYMPTOMS.—In 1992, the island population of *F. occidentalis* flowered during the first two weeks of June. As the flowering period ended, symptoms of rust infection developed on ovaries and pedicels in some individuals of *F. occidentalis*. The symptoms were never observed on any species other than *F. occidentalis*. Disease incidence was very patchy across the island population of *F. occidentalis*. Symptomatic ovaries turned bright orange (in contrast to the usual yellow-green), and quickly swelled to at least twice the size of healthy ovaries. In some cases the parts of young leaves were also swollen and orange. However, typically the symptoms were confined to ovaries and pedicels. Aecial pustules formed on the swollen structures. The rust was kindly identified as the anamorph-species, *Aecidium farameae* Arthur, by Joe F. Hennen of the Botanical Research Institute of Texas, Inc., where specimens have been deposited.

ZETEK STUDY SITE: WHOLE BRANCH DATA.—On 17–18 June 1992, we chose 11 flowering *F. occidentalis* trees showing symptoms of rust infection. We chose these trees because they had branches low enough to facilitate accurate counts of developing ovaries and assessment of infection. Ten of the trees were located within a 25 × 30 m area. One additional infected tree was 20 m to the west. Between one and four accessible branches were selected from each tree (21 branches total). Branches were marked with numbered bands. On each branch we counted the total number of ovaries and the number of symptomatic ovaries. Two months later (26 Aug.), we returned to the site and counted the number of symptomatic and nonsymptomatic fruits remaining on each of the branches. We then calculated the percentage of healthy ovaries on all the focal branches per tree in June and the percentage of healthy fruits on all the focal branches per tree in August. The percentage of healthy fruits in August was regressed against the percentage of healthy ovaries in June in order to test for a significant effect of rust infection on fruit health. All

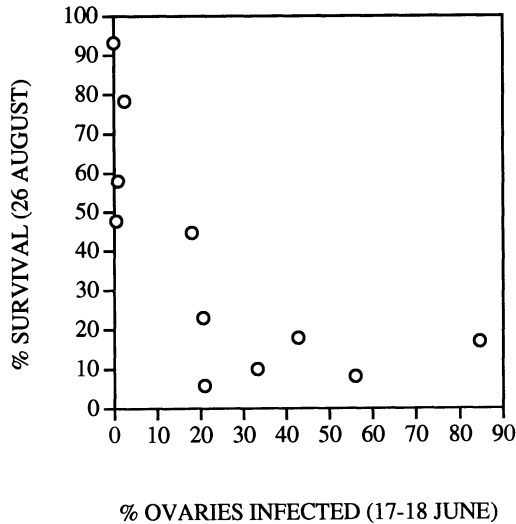


FIGURE 1. Survival of ovaries at Zetek site as a function of infection by the rust *Aecidium farameae*. Each point represents all ovaries on one to four branches on an individual of *F. occidentalis* ($N = 13-571$ ovaries/tree). Regression analysis indicated a significant negative relationship between the survival of ovaries and the percent of ovaries infected in June ($y = 69.7 - 2.4x + 0.02x^2$, $F = 12.46$, $df = 10$, $P = 0.0035$).

statistical analyses were conducted with the JMP statistical software package (SAS 1994).

ARMOUR SITE: INDIVIDUAL FRUIT DATA.—On 1 July 1992, we chose five symptomatic *Faramea occidentalis* trees at a second site 375 m southeast of the Zetek study site. We marked individual developing ovaries or clusters of ovaries with twist-ties of different lengths to indicate symptomatic or healthy ovaries. We also recorded whether the healthy ovaries were borne on symptomatic pedicels. A total of 200 developing ovaries were marked at this site. A month and a half later (26 Aug.), we returned to the site and counted the number of healthy and symptomatic fruits that had been labeled. We calculated the following: (1) the percentage of ovaries that were healthy and the percentage that were symptomatic in July; (2) the percentage of ovaries that were healthy or symptomatic in July and their fate (healthy, symptomatic or missing) in August; and (3) the percentage of ovaries that had symptomatic pedicels in July and were healthy, symptomatic or missing in August. We analyzed the independence of ovary condition in July and the status of those ovaries as fruits in August with a chi-square heterogeneity test (Zar 1974).

TABLE 1. Survival of fruits of *Faramea occidentalis* at Armour site based on symptoms of *Aecidium farameae* rust (data from 5 trees combined).

Ovary status July	Fruit status August 26 (# fruits)			Total
	Healthy	Symptomatic	Missing	
Healthy	81	4	34	119
Symptomatic	0	19	42	61
Symptomatic pedicel	0	0	20	20
Total	81	23	96	200

RESULTS

Based on the whole-branch data collected at the Zetek study site, the percentage of ovaries that showed rust symptoms in June was a good indicator ($R^2 = 0.757$) of the percentage of developing fruits surviving until August (Fig. 1). Regression analysis indicated a significant negative relationship between the percentage of fruits symptomatic in June and the percentage fruits surviving in August (Table 1). Moreover, the relationship is nonlinear as indicated by an improvement in the fit of the regression model when a quadratic term was added to the simple linear model relating the percentage of healthy fruits in August to the percentage of infected ovaries in June was added. The mean square error of the quadratic model was 47 percent lower than that for the simple linear model. Up to 95 percent of all the ovaries on focal branches were either missing (presumably aborted) or showed severe rust symptoms by August.

Symptomatic ovaries had almost no probability of surviving to produce mature fruits. None of the individually marked ovaries that showed symptoms of rust infection in July developed normally (Table 1). Thirty one percent of those ovaries which were symptomatic in June remained symptomatic on the branch in August. The other 69 percent of those ovaries were missing. In contrast, 68 percent of the ovaries appearing healthy in July had developed into healthy fruits by August; only 28 percent were missing. All ovaries that were healthy in June, but had pedicels exhibiting symptoms of rust, were missing in August. Of the healthy ovaries that did not survive until August, 90 percent ($N = 34$) were missing and 10 percent ($N = 4$) became symptomatic and remained on the plant. We assume missing ovaries fell off prematurely and failed to produce viable seed, because *F. occidentalis* fruits normally require seven months to mature (Schupp 1990). Although the overall survival rate of healthy ovaries was high, there was considerable variability

among trees (range = 58.3–100%, $x = 79.3\% \pm 18.1$, $N = 5$). A chi-square contingency test indicated that the status of fruit in August depended on the infection status of ovaries on the same branches in July ($X^2 = 111.0$, $df = 4$, $P < 0.001$).

Our results indicate that ovaries asymptomatic in June had a higher chance of subsequent infection in trees with relatively high levels of infection (>10% of ovaries on a branch infected) versus trees with low levels of infection. If the percentage of ovaries infected per tree had no effect on the probability of asymptomatic fruits remaining asymptomatic, then we would expect that the minimum percent survival of ovaries would be: $\text{Survival (\%)} = 100 - S - A$, where S is the percentage of ovaries with rust symptoms in June and A is the mean percentage abortion of fruits on low-rust branches. This second value (A) is assumed to represent an average abortion rate due to factors other than rust infection (e.g., low resource availability). The average abortion percentage due to factors other than rust (A) was 31.4 percent, as calculated from branches with very low percentages of infected ovaries in June (<10%, $N = 4$). We compared the percentage of fruits surviving on each tree to the expected survival based on the above equation by conducting a Wilcoxon sign test for paired data using only those data associated with infection percentages greater than ten percent. Tree to tree variation in fruit abortion percentages were ignored. The observed percentage survival was significantly lower than the expected survival rate in the severely infected trees (Wilcoxon's $T = 13.00$, $N = 7$, $P = 0.031$), indicating an additional reduction in fruit production due to heavy rust infection and the spread of infection to ovaries that were asymptomatic in June (Fig. 2).

DISCUSSION

Infection by the rust *Aecidium farameae* can have strong effects on reproduction in *Faramea occidentalis* trees by reducing the number of mature, healthy fruits and seeds produced by infected trees. Our results indicate that maturation of normal fruit and the production of viable seeds and, ultimately, seedlings from flowers with infected ovaries or pedicels is extremely unlikely. In our study, every ovary that was infected or borne on an infected pedicel was either missing (most likely aborted) or diseased after six weeks of maturation (Table 1). Diseased ovaries may have been aborted due to pollen limitation or damage to the infected ovaries themselves. If the rust interfered with successful

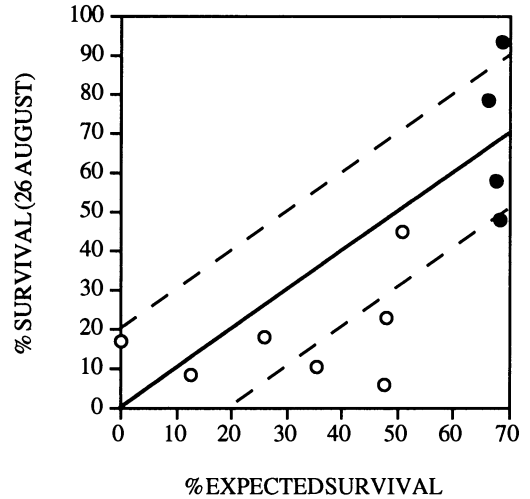


FIGURE 2. Observed survival of fruits of *Faramea occidentalis* compared to expected survival. Expected survival is the proportion of apparently healthy fruits in the first census minus the mean abortion rate among non-symptomatic fruits, as described in the text. Filled circles indicate trees with <10 percent initial infection. Line represents observed survival = expected survival (dashed lines ± 1 SD of abortion percentage).

deposition of pollen on the stigmas of infected flowers, then those ovaries might have been aborted because of the presence of unfertilized ovules (Stephenson 1981). We often observed the orange pustule material of the rust enveloping the ovary and the petals of an infected flower in such a way that pollination would have been impossible. *Faramea occidentalis* also may abort diseased ovaries just as other plants abort damaged fruits. Many studies have demonstrated that fruits or seeds damaged on the tree by seed predators (e.g., Phillips 1940, LePelley 1942, Lloyd 1980) and extreme temperatures (e.g., Addicott 1955) are more likely to be aborted than undamaged fruits. In either case, the likelihood of producing viable seeds from rust-infected fruits of *F. occidentalis* is very small.

The percentages of ovaries and fruits infected per tree were often large in the trees we observed, suggesting that this pathogen can have strong effects on the relative fitness of individual trees. During the study, infection on focal branches ranged from 0–85 percent of the ovaries (Fig. 1). High levels of infection can represent extreme limitations on the total number of viable seeds produced by a tree since the likelihood of producing viable seed from any of the infected fruits is so low. Moreover, since fruits of *Faramea occidentalis* have only one seed (Croat 1978), healthy fruits cannot produce a

surplus of viable seeds to replace those lost in infected fruits. Overall, these results imply that rust infection may have important influences on the relative fitness and year to year variation in viable seed production of *F. occidentalis* (Schupp 1990). However, it is unlikely that the rust has a significant effect on adult *F. occidentalis* survivorship, because symptoms appear to be limited to developing ovaries and only occasionally to young leaf tissue. We found no evidence of fungal attack that would be likely to result in reduced host survival. Disease distribution in the forest was extremely patchy. Infected trees and groups of trees were often separated by hundreds of meters of asymptomatic trees. Variability in resistance to infection by this rust may lead to variance in relative fitness among *F. occidentalis* individuals and the evolution of disease-resistance traits.

The infection of individual trees will depend on the resistance of the individual tree to the pathogen and on the mechanisms by which the pathogen is dispersed (Roche *et al.* 1995). We do not know how *A. farameae* is dispersed, but the pattern of infection within and among trees may provide some clues about the mechanism of rust spore dispersal. In searching for infected trees, we found patches where most *F. occidentalis* individuals suffered at least some infection. These patches were separated by broad areas in which all *F. occidentalis* individuals appeared to be healthy. This clumping of infected trees in the island population of *F. occidentalis* was similar to the dense aggregations of infected plants known from other systems where pollinators are involved in dispersal of spores. Roche *et al.* (1995) demonstrated that spore counts of a species of smut (*Ustilago violacea*) on *Silene alba* decreased with distance from an inoculum source, suggesting that pollinator dispersal of the spores leads to the aggregations of infected plants in wild populations of the host plant. We do not know if moth pollinators are distributing spores in the *Faramea*-rust system. However, the aeciospores are produced on flower parts which pollinators are likely to contact and the pollinators generally move first from flower to flower on a given tree (potential within tree spread) before moving on to the next closest tree (inter-tree dispersal; S. Travers pers. obs.).

According to an ongoing plant phenology study on BCI (J. Wright, pers. comm.), flower production in both 1991 and 1992 was much greater than usual for *Faramea*, with flower production in each of those years double the mean for the previous four years. Flower production failed almost

completely in 1993 (<4% of 1992 production), and returned to more usual levels for 1994 and 1995. The high density of flowers may have facilitated the outbreak of rust in 1992. The rust has been extremely rare on BCI in the three years following 1992 (no more than 3 infected plants found in any given year; Gilbert pers. obs.). The scarcity of rust, perhaps due to the relatively low density of flowers, has prevented further examination of *Faramea* rust biology.

Very little is known of the natural history of *Aecidium farameae*. It has been described previously on *Faramea* from Cuba (Arthur 1915, 1924), the Dominican Republic, Belize, and Panama, but only the asexual (aecial) state has ever been observed (J. Hennen, pers. comm.). Since the sexual state is unknown, the rust has the anamorphic (asexual state) name of *Aecidium*. It is not known if this rust is autecious (able to complete its entire life cycle on *Faramea* alone) or if it is heteroecious with sexual reproduction taking place on a taxonomically unrelated host. However, should *A. farameae* turn out to be heteroecious, an additional explanation for the clumped distribution of infected trees on BCI is that a secondary host for the *Faramea* rust has a clumped distribution. If the *Faramea* rust requires an alternate host, then we would expect greater infection of *F. occidentalis* individuals adjacent to higher densities of that species (Jacobi *et al.* 1993). Future studies on this plant-pathogen system will benefit from examining the basic biology of this rust in addition to the nature of dispersal among *F. occidentalis* individuals.

Our study suggests that a comprehensive understanding of limitations on fruit and seed production includes consideration of fungal pathogens. We have shown that infection by a species of rust can reduce the number of fruits produced by a host plant. However, still unexplored is the question of the relative importance of fungal pathogens versus other biotic and abiotic factors affecting plant reproduction.

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