Locally Established Botrytis Fruit Rot of *Myrica faya*, a Noxious Weed in Hawaii

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ABSTRACT

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Faya (*Myrica faya*) is an introduced weedy tree threatening native ecosystems in Hawaii. Classical biological control has been investigated for several years but has yielded few insect pests or pathogens, none of which currently offers control in the field. As an alternative, attention was recently given to identify locally established insect pests and pathogens. *Botrytis cinerea* causes widespread fruit rot and is the first pathogen reported from faya in its nonnative habitat. Infection of flowers and foliage is rarely observed. Fruit rot occurs on trees of all sizes in a variety of habitats throughout the Hawaiian range of faya. In 1992, over 49 and 51% of mature fruit at two sites were infected. Similar percentages of immature fruit were infected or infested with *B. cinerea*. Adults and fruit-feeding larvae of *Amorbia emigratella* and *Cryptoblabes gnidiella* collected from infected fruit clusters in the field were heavily infested with viable conidia and may serve as vectors. Selection of more aggressive or ecologically fit strains or introduction of large numbers of *Botrytis*-infested insect vectors early in the fruiting season may enhance faya biocontrol.

Additional keywords: *Anemone hupehensis*, fungal vectors, *Penicillium dendriticum*, *Styphelia tameiameiae*, *Vaccinium reticulatum*, weed biological control

Myrica faya Aiton (Myricaceae), commonly called faya, is a small evergreen tree native to Macaronesia (Azores, Madeira, and Canary Islands in the North Atlantic) that was introduced into Hawaii in the late 1800s. In the early 1900s it was used in reforestation programs until its noxious habit became evident. Faya now occupies approximately 32,000 ha throughout Hawaii, mostly in threatened native ecosystems. The largest populations are centered in Hawaii Volcanoes National Park (HAVO) on the island of Hawaii.

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Rapid spread of faya is attributed to prolific fruiting (25) and seed dissemination by native and introduced birds that feed on ripe fruit (15,26). Symbiotic association with *Frankia* sp., a nitrogen-fixing actinomycete, enables faya to outcompete native species in nitrogen-deficient habitats of recent volcanic origin, which are common in Hawaii (21). Once established, faya forms dense canopies and thick litter that limits understory development and interferes with germination of o'hi'a (*Metrosideros polymorpha* Gaud.), the predominant native tree (23,24). Of greatest concern, however, faya increases levels of available soil nitrogen, paving the way for other aggressive weeds to enter otherwise protected sites (21,22).

Biological control has been investigated as a long-term alternative to physical removal and chemical control, which, although effective, are labor-intensive and thus impractical over large areas and in inaccessible terrain (4; cf. 10). Exploratory trips to Macaronesia have yielded few insect pests or pathogens suitable for biological control (cf. 10,11,14,16). Testing under quarantine of those that have been found has been expensive, requiring years of intensive labor. Two lepidopteran enemies of faya have been released (14; G. P. Markin, *unpublished*), but neither currently offers field control.

Legitimate social concerns with nontarget effects of introduced pests has further complicated biocontrol efforts in Hawaii (12). Behavior of released agents in the field is largely unknown, since regulations restrict tests to quarantine facilities. Under these conditions only a small percentage of those species of current aesthetic, ecological, or economic interest can be tested. Numerous significant environmental variables, both abiotic (e.g., daily and seasonal climatic fluctuation, nutrient balances) and biotic (e.g., competition with indigenous organisms occupying targeted niches, host phenological change, hyperparasitism), cannot be considered. Further, possible adverse effects of introduced weed biocontrol agents on other beneficial or native insects or microorganisms in Hawaii have never been properly investigated, although evidence exists that such influence may be significant (12). Exploitation of control agents already established in the local environment would offer the advantages of greatly reducing these risks while circumventing problems associated with establishment of introduced agents. We refer to these insect pests and pathogens as locally established rather than indigenous, as their origins are uncertain.

Recently, fruit rot caused by *Botrytis cinerea* Pers.:Fr. was observed in HAVO. This is the first disease reported on faya in its nonnative habitat (6,17). Infected mature fruits become covered with a mat of gray-brown mycelium and conidia, then prematurely desiccate (Fig. 1). Infected fruit are presumably less attractive to birds, possibly limiting long-range seed dissemination. The objectives of this study were to determine the distribution of the disease, investigate its effects on seed viability, and determine the relationship between *B. cinerea* and larvae of fruit-feeding insects.

MATERIALS AND METHODS

Surveys. To determine the distribution of Botrytis fruit rot throughout the range of faya in Hawaii, flowers and fruit of several hundred pistillate and staminate and functionally monoecious trees (9) representing a range of ages, sizes, and stand densities were observed between the peak reproductive seasons (flowering from March to June and fruiting from July to October) (25) in 1992. Native plants and weeds within the range of faya were also observed for signs of disease. Less frequent surveys were conducted year-round in 1992 and until July 1993. To determine the percentage fruit loss resulting from infection, 100-m transects were established at Upper Byron Ledge (UBL) and Hilina Pali (HP) in HAVO. These sites were selected because they are representative of the moderately wet and dry range of faya; mean annual rainfall is approximately 2,400 mm at UBL and 690 mm at HP. Other characteristics, such as tree size, canopy and stand densities, and species diversity, were similar at both sites. Ten fruit-bearing shoots were randomly collected from each of 11 pistillate trees selected at 10-m intervals. Samples were individually bagged and transported to the laboratory, where the total number of infected mature fruit was determined at 20–45× magnification by observing conidiophores and spores of *B. cinerea*. Infection of flowers and foliage was infrequently observed and therefore not considered in this survey.

Fig. 1. Cluster of mature fruit of *Myrica faya* heavily infected with *Botrytis cinerea* in Hawaii Volcanoes National Park.

B. cinerea may remain quiescent on fruit surfaces as conidia or as latent subepidermal infections causing rot when triggered by fruit maturation and favorable environmental conditions (2). The proportion of infected or infested immature fruit was determined by plating whole green fruit on a *Botrytis* semiselective medium (13) modified by substituting 150 μg/ml of streptomycin sulfate for chloramphenicol and omitting fenarimol. To avoid cross-contamination, fruit were collected with surface-disinfested forceps and transported to the laboratory in individual wells of microtiter plates. Fruit were arranged equidistant approximately 2 mm apart on the medium (20–30 fruit per plate) and incubated at 21–24 C with continuous light for 10–14 days. Infected fruit became covered with gray mycelium, which caused a brown discoloration of the medium after 2–4 days and produced conidia after 7–10 days. Observation of conidiation and the color reaction distinguished *B. cinerea* from other fungi that also grew on the medium, such as *Penicillium* spp., *Pestalotia* spp., and *Trichoderma* spp. In 1992, 897 and 1,951 fruit were collected randomly from 189 and 370 trees selected randomly along the same transects used above at UBL and HP, respectively. Confidence limits ($P = 0.05$) for percentage infection were determined for each site, but no statistical comparisons were performed, since surveys at the two sites were neither synchronous nor of similar sample size.

Effect on seed viability. Naturally infected mature, dried, attached fruit were collected in 1992 from at least 10 trees randomly selected within each of five 10-m^2 blocks along 100-m transects at UBL and HP. Fruit with no sign of *B. cinerea* infection served as the control. Dry fruit pulp was removed from the seeds by hand. Seeds from UBL were stored 2 wk and those from HP 6 wk at 25–28 C prior to planting. Captan was added as a soil drench to half the pots to limit possible postplanting damage. Each treatment consisted of 100 seeds sown on the surface of sterile vermiculite in 12-cm-diameter plastic pots, incubated in Hilo, Hawaii, for 25 wk with 60% shade and watered twice daily to maintain saturation. Germination was recorded weekly, and seedlings were removed to avoid potential autoallelopathy (23,24) or competition. The experiment was arranged as a 2 × 3 factorial in a split-split plot design with a main plot of site, subplot of captan treatment, and sub-subplot of disease status. Each treatment was replicated five times. The main effects of site, captan treatment, and disease status and all interactions were analyzed for significance using an analysis of variance, and means were separated with Fisher's protected least significant difference ($P = 0.05$) procedure (Statgraphics, Statistical Graphics Corp., Rockville, MD).

Vector relations. Larvae of *Cryptoblabes gnidiella* (Milliere) (Lepidoptera: Pyralidae, Phycitinae) and *Amorbia emigratella* (Busck) (Lepidoptera: Tortricidae, Tortricinae) commonly feed on faya fruit in and around HAVO. Their ability to vector *Botrytis* was investigated using an approach similar to that of Fermaud and LeMenn (7) to describe the relationship between *B. cinerea* and larvae of the grape berry moth (*Lobesia botrana* Denis & Schiffermueller) (Lepidoptera: Tortricidae). Naturally infested larvae were collected from infected fruit clusters at UBL and HP in the autumn of 1992. Larvae were carefully removed from the clusters and fixed for at least 24 hr in a solution of 6% glutaraldehyde in 0.05 M phosphate buffer (pH 7.4). They were then dehydrated through graded ethanols and CO_2 critical-point dried. Samples were mounted on aluminum stubs and sputter-coated with gold paladium for observation with a scanning electron microscope (SEM, model WB-6, Topcon). Naturally infested adults were collected after emergence from laboratory-incubated diseased fruit clusters in which larvae were allowed to mature and pupate. Adults were dried in a vacuum bell with desiccant, then prepared for SEM ob-

Table 1. Infection and infestation of *Myrica faya* fruit with *Botrytis cinerea* in Hawaii in 1992

	Immature fruit[a]		Mature fruit[b]	
Site	Number sampled	Percent infected/infested	Number sampled	Percent infected
Upper Byron Ledge	897	48.2 ± 8.84	12,393	41.6 ± 9.07
Hilina Pali	1,951	58.0 ± 14.64	9,705	59.1 ± 8.74

[a]Whole fruit were plated on Kerssie's semiselective medium (13), and brown discoloration of the medium and conidiation of *B. cinerea* were observed.

[b]*B. cinerea* fruiting structures were observed at 20–45×.

servation as above.

To determine the percentage of larvae infested with viable conidia, 100 larvae of *C. gnidiella* were collected from infected fruit clusters, and one fecal pellet from each larva was plated on Difco potato-dextrose agar (PDA). The larvae were then killed with acetone vapors and plated on PDA. Plates with feces and dead larvae were incubated at 24 C and observed daily for 14 days.

RESULTS AND DISCUSSION

Botrytis fruit rot was observed throughout the Hawaiian range of faya on mature fruit of pistillate and functionally monoecious trees. Rot was observed on mature trees of all ages and sizes and with dense or thin canopies. Rot occurred throughout the year but appeared most severe at the end of the peak fruiting season in September and October, when the largest number of fruit matured.

A significant proportion of fruit at the two sites representative of the moderately wet (UBL) and the dry (HP) range of faya in Hawaii was infected by *B. cinerea* (Table 1). The percentages of mature fruit infected were similar to the percentages of immature fruit infested with conidia or infected by mycelia of *B. cinerea* (Table 1). Although these surveys at UBL and HP were conducted in 1992, results appear to be consistent from year to year (*unpublished*). Infection with *B. cinerea* significantly ($P < 0.0001$) reduced viability of seeds collected in 1992 from 66 to 16.8%. Germination was less at HP than at UBL, possibly because seed from HP was stored 4 wk longer prior to planting. Captan seed drench did not have a significant effect on germination ($P = 0.4052$). Foliage of a small number of nontreated seedlings was infected with *B. cinerea*, and one seedling became covered with white mycelium of an unidentified fungus and was killed. All higher order interactions were not significant ($P > 0.10$).

Potential inoculum sources include the small number of fruit produced throughout the year and dried infected fruit from the previous fruiting season that remain attached or fall to the ground. Sclerotia were not observed in the field. Reservoir hosts within the range of faya may serve as a minor inoculum source. Foliage of Japanese anemone (*Anemone hupehensis* Lemoine var. *japonica* (Thunb.) Bowles & W. Stern) and flowers and fruit of banana poka (*Passiflora mollissima* (Kunth) L. H. Bailey), both noxious weeds, were commonly infected. In contrast, *B. cinerea* posed little if any threat to native species found in the range of faya. Although ʻōhelo (*Vaccinium reticulatum* Sm.), pūkiawe (*Styphelia tameiameiae* (Cham. & Schlechtend.) F. v. Muell.), kūkaenēnē (*Coprosma ernodeoides* A. Gray), and ʻakala (*Rubus hawaiiensis* A. Gray) produce abundant fleshy fruit, *B. cinerea* was rarely observed on ʻōhelo and pūkiawe fruit (5) and not at all on the other native species in regular surveys in 1992 and 1993.

Two species of moth larvae that feed on faya fruit, *A. emigratella* and *C. gnidiella*, vector *B. cinerea*. Smooth, elliptical conidia having prominent abscission scars typical of *Botrytis* (2) were observed on all external parts of larvae of both insect species, particularly on the head, around the mouthparts, the base of body setae, and among the hooklike claws of the abdominal legs

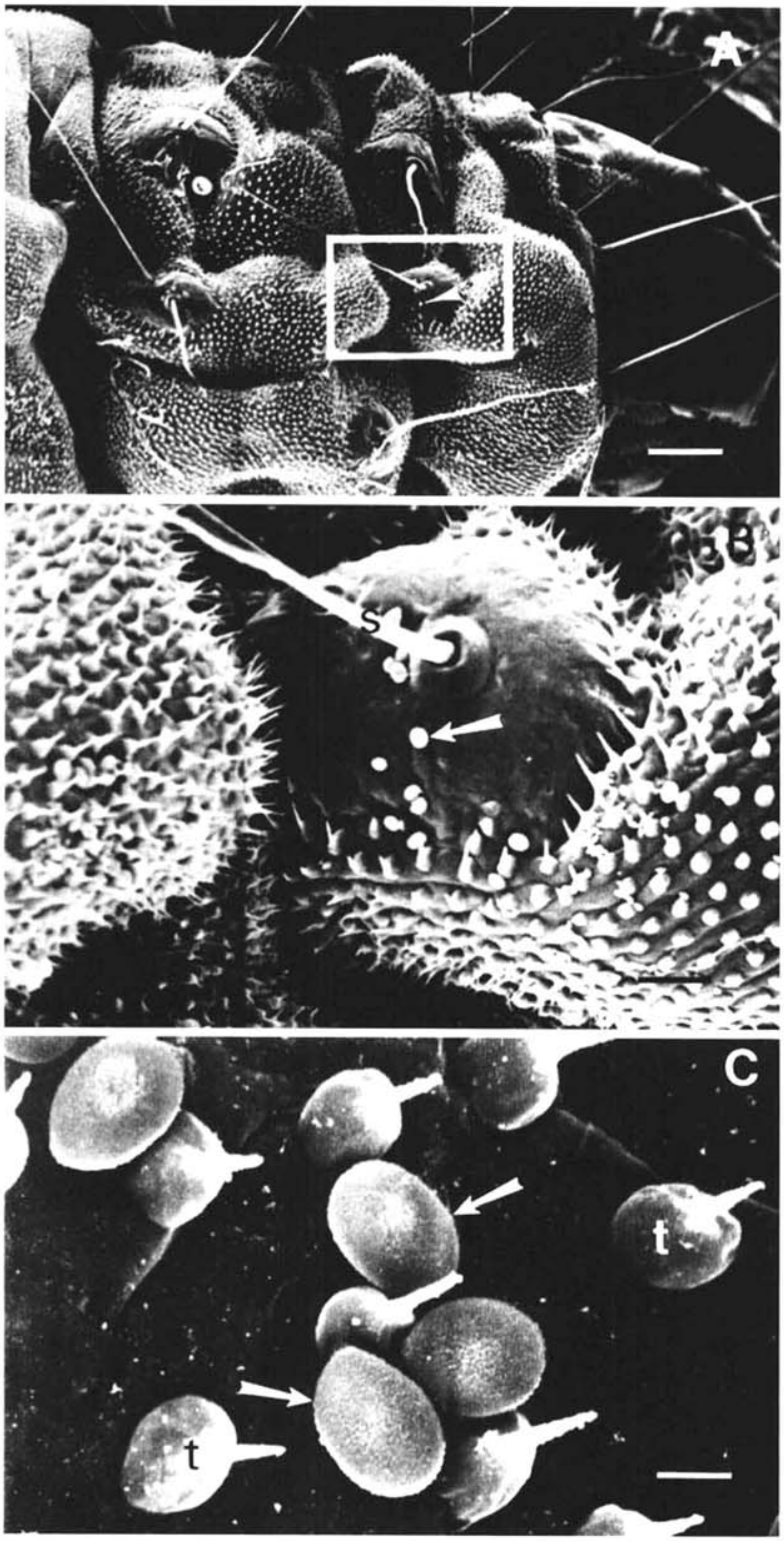

Fig. 2. Scanning electron micrographs showing conidia of *Botrytis cinerea* (arrows) near spinelike setae (s) and thornlike processes (t) of the lower abdomen of *Amorbia emigratella*. B is an enlargement of the inset area of A, and C is an enlargement of the area in B indicated by the arrow. Scale bars: A = 0.30 mm, B = 85 μm, C = 9 μm.

(Figs. 2 and 3). The percentage of field-collected larvae and their feces infested with viable conidia was 16.7 and 12% for each species, respectively. Recovery may have been reduced by *Penicillium* spp. and *Pestalotia* spp., which were commonly recovered, or by cultural conditions. Adults of *C. gnidiella* emerging from infested fruit clusters carried a large number of spores on their heads, wings, and legs (Fig. 4). Since *Botrytis* spores are readily dispersed by wind, adult moths traveling from cluster to cluster depositing eggs may not have an important impact on disease development. Feeding of larvae of *L. botrana* on grape creates wounds and has been shown to increase infection by *B. cinerea* (8). Larvae of *A. emigratella* and *C. gnidiella* damage the fruit they feed on, possibly leading to similar increase in *B. cinerea* on faya. The ability of these insects to transmit the pathogen and their importance in disease development should be further investigated and may prove useful for enhancement of biocontrol.

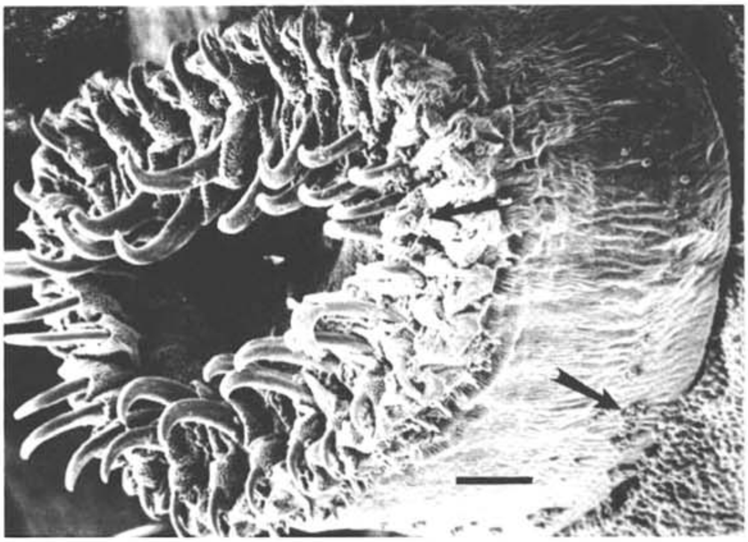

Fig. 3. Scanning electron micrograph showing conidia of *Botrytis cinerea* (arrows) adhering to the abdomen and an abdominal leg of a naturally infested larva of *Cryptoblabes gnidiella.* Numerous conidia are attached to and caught among the hooklike claws of the abdominal leg. Scale bar = 0.175 mm.

Botrytis fruit rot clearly offers a level of faya control in Hawaii beyond that of any introduced agent at present. Without this disease, availability of fruit for long-range dispersal by birds might be greater, possibly resulting in even more widespread invasion. *B. cinerea* is compatible with locally established insect pests. The level of control effected by these agents may be enhanced by introducing laboratory-reared larvae or adults inoculated with *B. cinerea* in large numbers early in the fruiting season. Fermaud and LeMenn (8) reported that introduction of *L. botrana* larvae artificially inoculated with *B. cinerea* increased grape fruit rot in French vineyards. Selection of more aggressive or environment-adapted strains may further enhance control. Combined application with other pathogens or disease-enhancing microorganisms may also enhance control. *Penicillium dendriticum* Pitt was commonly observed on faya fruit; although its pathogenicity is uncertain, it was usually associated with fruit desiccation. *Pestalotia* sp. was observed to cause leaf spots on faya (B. K. Duffy, *unpublished*) and was commonly isolated from fruit surfaces. Schissler et al (18) reported that the foliar disease of *Sesbania exaltata* (Raf.) Rydb. ex. A.W. Hill caused by the biological control fungus *Colletotricum truncatum* (Schwein.) Andrus & W.D. Moore could be enhanced by combination with nonpathogenic epiphytic bacteria isolated from the phyllosphere of this weed.

Expansion of current weed biocontrol efforts in Hawaii to consider locally

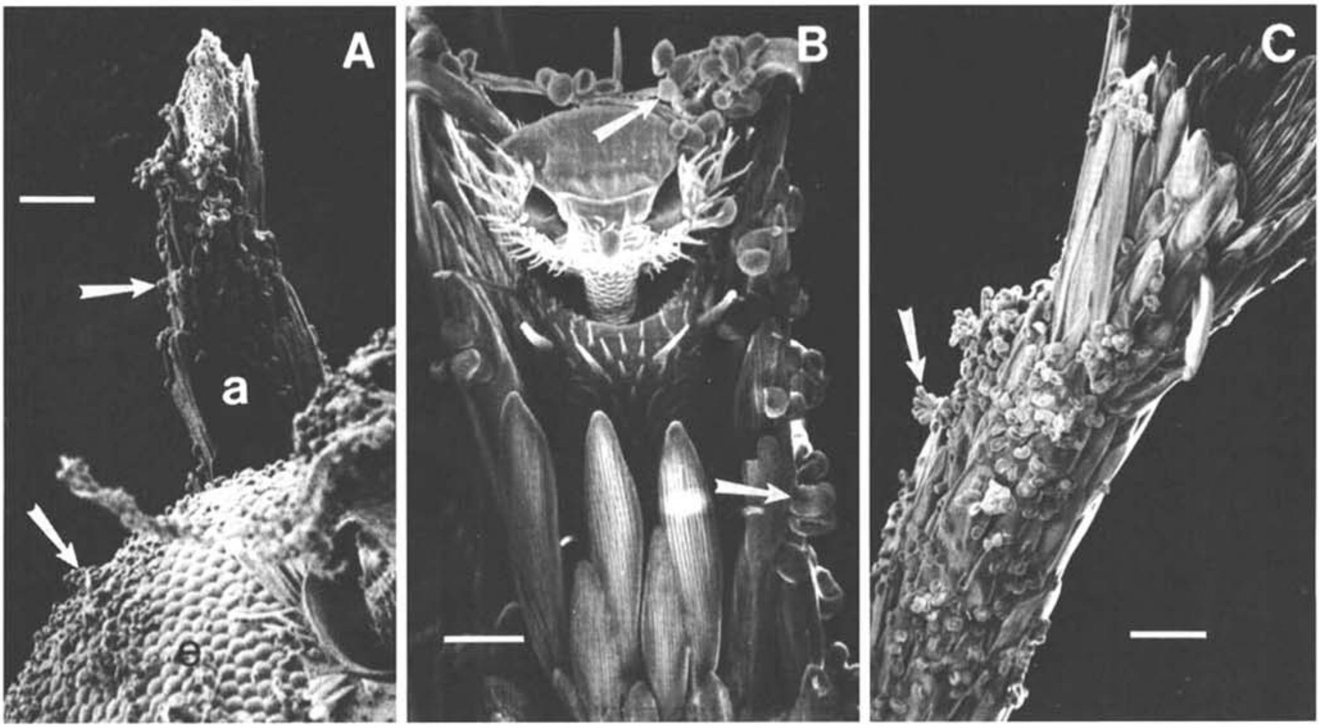

Fig. 4. Scanning electron micrographs showing conidia of *Botrytis cinerea* (arrows) attached to the surfaces of naturally infested *Cryptoblabes gnidiella* adults. **(A)** Conidia on a compound eye (e) and antenna (a). Scale bar = 0.257 mm. **(B)** Conidia on the third leg. Scale bar = 50 μm. **(C)** Conidia on the first leg. Scale bar = 0.130 mm.

established insect pests and pathogens is recommended. Fruit rot and fruit feeding moth larvae on faya are certainly not the only potential control agents associated with weeds in Hawaii (17), and other systems may prove even more promising for exploitation. Consideration of locally established agents is critical prior to introduction of foreign insect pests or pathogens, since they may fail to become established in targeted niches occupied by locally established pests or pathogens or, worse, they may interfere with locally established agents and ultimately result in lower levels of weed control. It may also be advantageous to select foreign pests that are compatible or synergistic with locally established pests. It is important to note that certain effective biocontrol agents may occur only outside the native range of a weed (1,3,19,20). For example, *B. cinerea* was observed on faya fruit only once during the 1993 fruiting season in Macaronesia (B. K. Duffy, *unpublished*). Perhaps most important, the rapidly increasing number of weeds threatening Hawaii's unique ecosystems demands equally rapid implementation of control measures. Locally established pests adapted to the targeted environment are relatively risk-free and could be used in the field literally years before foreign pests exit quarantine.

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