Exhibit 1a, #28

Effect of Mango Weevil (Coleoptera: Curculionidae) Damage on Mango Seed Viability in Hawaii

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ABSTRACT The mango weevil, *Cryptorhynchus* (=*Sternochetus*) *mangiferae* (F.), is a federally quarantined pest that prevents shipment of mangos from Hawaii into the continental United States. Although this monophagous weevil allegedly causes reduced seed germination, damage to the fruit pulp, and premature fruit drop in mangos, there are few studies examining these potential sources of crop loss. We conducted studies to assess the effect of mango weevil infestation on seed viability while making observations on the frequency of pulp feeding. Naturally infested seeds from mature fruit were planted in pots and scored for successful germination. Germination rates for infested seeds were equal to those of uninfested control seeds in a polyembryonic cultivar ('Common'), whereas germination was significantly reduced for infested seeds of a monoembryonic cultivar ('Haden') compared with uninfested control seeds but germination of infested seeds was still >70%. To assess seed tolerance of damage, seeds were artificially damaged by cutting away 25, 50, or 75% of the cotyledon before planting and scored for germination. None of the damage treatments was significantly different from the undamaged controls, indicating that mango seeds can withstand substantial damage and still germinate successfully. Over the 2-yr period we conducted experiments, only four of 3,602 mango fruits (0.11%) showed evidence of direct feeding damage to the pulp. Results suggest that *C. mangiferae* is a less serious pest of mangos than previously thought.

KEY WORDS *Cryptorhynchus mangiferae, Sternochetus mangiferae,* mango, quarantine pest, crop loss

MANGO WEEVIL (SYN. mango seed weevil), *Cryptorhynchus* (=*Sternochetus*) *mangiferae* (F.), has been recorded from mango, *Mangifera indica* L., in Asia, Africa, and Oceania (including Australia) (CAB and EPPO 1997). The weevil is strictly monophagous and, therefore, probably native to the Himalayan foothills of the India-Myanmar region, the origin of the mango (Jagatiani et al. 1988). In the Western Hemisphere its distribution is limited to several islands in the Caribbean (Barbados, Dominica, Guadeloupe, Martinique, St. Lucia, Trinidad and Tobago, and the U.S. Virgin Islands), French Guiana, and Hawaii, where it was first reported in 1905 (Van Dine 1906). Mango weevil is a federally quarantined pest that prevents the import of mangos into the United States from many producing countries. The presence of this weevil also prevents the shipment of mangos from Hawaii to the continental United States. Mangos are grown commercially in the United States primarily in southern Florida, and it is this industry that the federal quarantine serves to protect.

Mango weevil is univoltine. Females oviposit on immature fruits that are ≈1.9 cm in diameter or larger. The adult female carves out a cavity on the fruit surface and deposits an egg, which is immediately covered by a fruit exudate caused by the wound. The neonate burrows through the pulp to the developing seed (Balock and Kozuma 1964, Hansen et al. 1989). The mango seed is solitary, large, flat, and ovoid oblong, and surrounded by a fibrous endocarp (or husk) at maturity (Mukherjee 1997). As the fruit matures and increases in size, the endocarp thickens and becomes difficult for first instars to penetrate. Larvae feed within the seed and pupate in the seed cavity. Larval development within the seed takes 20–30 d under field conditions in Hawaii. The majority of infested seeds have one or two weevils, but seeds containing five or more weevils have been reported (Balock and Kozuma 1964, Hansen et al. 1989). In Hawaii, within 2 mo after the fruits fall to the ground and deteriorate the adult weevil emerges (Balock and Kozuma 1964) and seeks a protected site (e.g., bark crevices, rock walls) where is can overseason. Adult weevils can live for 2 yr or more when provided food and water (Balock and Kozuma 1964; P.A.F., unpublished data). In a survey of mangoes from the five main Hawaiian Islands, Hansen et al. (1989) found no parasitism of mango weevil and no other seed-feeding insects. Pest control research for mango weevil over the years has looked at a number of options, including field sanitation, chemical sprays (trunk and foliar), natural enemies (parasitoids, the fungus *Beauvaria bassiana*), host plant resistance, and x-ray fruit culling technology with little success (CAB and EPPO 1997).

Mango weevil has been elevated to the status of quarantine pest because of three commonly held perceptions relative to its economic impact (for review, see Pena et al. 1998): (1) that weevil development in the fruit causes damage to the pulp rendering it unmarketable, or at least unappetizing; (2) infestation reduces the germination capacity of seeds; and (3) that infestation can cause premature fruit drop. However, data to support these different types of crop loss are scarce. We conducted studies to specifically address the effects of mango weevil infestation on seed germination in Hawaii. During these studies, observational data also were gathered on the frequency of mango weevil feeding on, or damage to, the mango fruit pulp.

Materials and Methods

Natural infestation and artificial damage studies were conducted to test the assumption that weevil-damaged seeds have a lower germination rate than undamaged seeds. For the natural infestation study, 443 harvest-mature 'Haden' (monoembryonic) and 'Common' (polyembryonic) mango fruits were collected from the Yamada orchard in Kalapana, HI, in 1998. Fruit were collected from four trees (replicates) for each cultivar. Monoembryonic (one seedling per seed) and polyembryonic (multiple seedlings per seed) cultivars were included because they potentially respond to weevil injury differently. The fruit pulp and seed husks were removed and naked seeds were assigned to the infested or uninfested treatments by inspecting the dehusked seeds for evidence of larval feeding (tunneling, frass). All infested seeds showed extensive damage consistent with feeding by one or more late instars. For infested seeds, evidence of larval feeding is always apparent on the seed surface, but most tunneling is subsurface and therefore estimating the percentage of damage to the seed is difficult without dissection. Seeds were planted individually in 15.2-cm pots containing a 4:1 mix of Sunshine potting Soil no. 4 and Perlite no. 4. Seeds in pots were germinated in a black screen shade house. Percentage germination data for each cultivar were arcsine transformed to normalize the distribution and subjected to analysis of variance (ANOVA) (SAS Institute 1997). Mean separations for each cultivar were done using a Student *t*-test at $P \leq 0.05$.

Artificial damage studies were conducted in 1998 and 1999 to examine the effect of different levels of damage on germination rates. Harvest mature Haden mango fruits were collected from the Yamada orchard in 1998 and the Greenwell farm in 1999. Fruits were weighed and cut open, and only uninfested and undamaged seeds were used in the experiment. The experimental design consisted of four damage treatments created by cutting away 25, 50, or 75% of the seed, or leaving the seed whole as a control. In all treatments the seed cotyledon was the portion cut away and the germ or embryo was left undamaged. The cotyledon constitutes the vast majority of the seed bulk and, therefore, provides the primary source of food for the developing weevil. Seeds were planted in pots in a shade house for germination as described above. Twenty fruits were used in each treatment in 1998 and 40 fruits in 1999. Data on percentage of germination were arcsine transformed to normalize the distribution and subjected to ANOVA (SAS Institute 1997), and mean separations were done using the Tukey–Kramer method at $P \leq 0.05$.

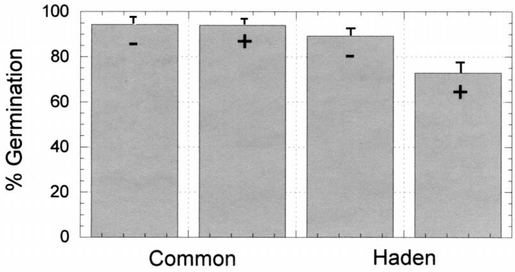

Fig. 1. Percentage of seeds germinating in mango weevil infested (+) and uninfested (−) fruits of a polyembryonic cultivar (Common) and a monoembryonic cultivar (Haden) of mangos.

Results

The average weight of mango fruits used in the germination study with naturally infested fruits was 243.2 g (149.5–433.6 g) and 376.3 g (217.0–601.5 g) for Common and Haden mangos, respectively. Germination rates of infested (+) and uninfested (−) Common mangos were not significantly different ($t = 0.32$; df = 1, 6; $P = 0.76$) (Fig. 1). Germination rates for infested and uninfested Haden mangos were significantly different ($t = 3.41$; df = 1, 5; $P = 0.02$), but germination was relatively high in both groups. Germination rates averaged 89% in uninfested Haden seeds and 73% in infested Haden seeds.

In the artificially damaged seeds experiment, the average weight of Haden mango fruits was 243.2 g (149.5–433.6 g) and 376.3 g (217.0–601.5 g) in 1998 and 1999, respectively. Actual percentage of seed removal by weight for the 25, 50, and 75% treatments was 19 (12.8–27.7%), 46.8 (37.6–57%), and 76.9% (65.7–85.2%), respectively. Germination in all treatments was high but there were significant treatment effects ($F = 12.8$; df = 3, 1; $P = 0.02$) (Fig. 2). The 75% cotyledon removal treatment was not significantly dif-

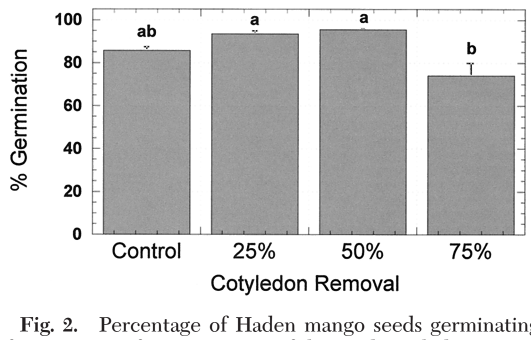

Fig. 2. Percentage of Haden mango seeds germinating after excision of 25, 50, or 75% of the seed cotyledon. Treatments with the same letter are not significantly different from each other using a Tukey–Kramer test at $P \leq 0.05$.

ferent from the control, but had a significantly lower germination rate than the 25 and 50% treatments. The 25 and 50% treatments were not significantly different from the control treatment.

Discussion

The high-risk quarantine pest status given to mango weevil is mainly in response to concerns from the mango industry in southern Florida that *C. mangiferae* infestation will reduce seed germination and therefore limit seed production in nurseries and orchards (Balock and Kozuma 1964). Our study indicates that a mango seed can withstand substantial damage and still germinate successfully. Natural infestation by mango weevils did not reduce germination in the Common mangos with multiple seeds. In single-seeded Haden mangos, germination of infested seeds was significantly reduced compared with uninfested seeds, but germination in infested seeds was still >70%. In the artificial damage study, removal of 75% of the cotyledon of Haden seeds resulted in a lower germination rate compared with controls and 25 and 50% damaged seeds, but the germination rate was still >65% and not significantly different from uncut control seeds. Seeds in the 25 and 50% cotyledon removal treatments actually had a higher germination rate than seeds in the undamaged control group. We estimate that a mango seed weevil typically consumes <25% of the seed, so our artificial damage treatments were exaggerated.

Various reports occur in the literature regarding pulp feeding by mango weevil. In Hawaii, the neonate exits through the bottom of the egg and burrows into the fruit to the seed. The path may appear as a black thread running through the fruit flesh from just under the skin to the seed surface, or may not be visible at all, when it first occurs, and later disappears completely. Larval development occurs entirely in the mango seed. Larvae are rarely found feeding on the pulp close to the seed. Our studies with the mango weevil from 1998 to 1999, where we cut 3,602 mango fruits, showed that pulp feeding was rare. Observations of pulp feeding or pulp damage at the Kalapana orchard was 0.04% (one adult in 2,502 fruits) and at the Kona orchard was 0.27% (three larvae in 1,100 fruits). Other mango weevil studies in Hawaii concur. Hansen et al. (1989) found pulp feeding in three out of 230 fruits, and Balock et al. (1964) found pulp feeding in only one fruit during several seasons of studying mango weevil in the late 1950s and early 1960s. Because the mango weevil egg is deposited essentially inside the fruit, the initial tunneling of the neonate to the seed does not introduce pathogens for secondary infection.

Therefore, mango weevil attack usually goes unnoticed and does not reduce fruit marketability. The confusion on this point in the mango weevil literature (Pena et al. 1998) may be the result of mistaken identity. There are several other closely related weevil species with the colloquial name "mango weevil" that feed primarily on the pulp (e.g., *Sternochetus frigidus* F., the mango pulp weevil; and *S. gravis* (F.), the Javanese mango weevil) (Balock and Kozuma 1964, Heather and Corcoran 1992). The behaviors of pulp-feeding species reported in the literature may have been inadvertently attributed to seed-feeding species through broad use of the name "mango weevil." For this reason the common name "mango seed weevil" is often used for *Cryptorynchus mangiferae* (Hansen et al. 1989, Heather and Corcoran 1992, CAB and EPPO 1997, Pena et al. 1998). However, it is important to note that *C. mangiferae* is reported to frequently cause pulp damage when it emerges from fruit on the tree (causing exit holes) in late-season cultivars in South Africa (Milne et al. 1977).

The other potentially important source of crop loss from mango weevil infestation is premature fruit drop. A preliminary premature drop study was conducted in 1997 by simply collecting immature mango fruits of equal size from the ground and from the tree (P.A.F., unpublished data). If weevil-infested fruit were more prone to dropping than uninfested fruit we would predict a higher infestation rate in fruit on the ground than fruit on the tree. Mango fruits on the ground had a numerically higher incidence of mango weevil than fruit on the tree, but the difference was small (mean ± SE, 33. 3 ± 7.0% for fruit collected from the ground and 22.3 ± 5.8 for fruit harvested from the tree), and not significant ($t = 1.35$; df = 1, 8; $P = 0.19$). The seed infestation rate in the trees used in our study ranged from 2 to 50%. In trees with seed infestation rates in the 50–100% range, premature drop may be more pronounced. Additional studies on premature drop are needed that more closely examine the relationship between time of infestation and fruit drop.

Our field studies to date suggest mango weevil does not seriously affect mango yields or marketability. The greatest significance of mango weevil as a pest is its interference with fruit exports because of quarantine restrictions imposed by importing countries and states. Postharvest researchers have attempted to kill mango weevil in mangos (while maintaining fruit quality) by using heat, cold, and fumigation treatments without success (Balock and Kozuma 1964). Irradiation may be a viable alternative to disinfest mangos of weevils (Seo et al. 1974, Heather and Corcoran 1992; P.A.F., unpublished data) while maintaining high fruit quality.

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