Exhibit 1b, #36

Mango Seed Weevil (Coleoptera: Curculionidae) and Premature Fruit Drop in Mangoes

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ABSTRACT The effect of infestations of mango seed weevil, *Sternochetus mangiferae* (F.), on premature fruit drop of mangoes was investigated. Mango fruits ('Haden') of equal size were collected both off the ground and from the tree at four times during the season (June–August). If weevil-infested fruit were more prone to dropping than uninfested fruit, the prediction was that a higher infestation rate would be found in fruit on the ground compared with fruit on the tree. Average fruit weight was used as an indicator of fruit maturity. The seed infestation rate was significantly higher in fruit collected off the ground compared with fruit collected from the tree in 38 g and 79 g (early-season) fruit but not significantly different in 207 g (midseason) and 281 g (late season) fruit. The age distribution of weevils and the number of insects in infested fruits were similar for ground and tree fruits on all dates. Results suggest that mango seed weevil infestation can increase fruit drop during early fruit development.

KEY WORDS *Cryptorhynchus mangiferae*, *Sternochetus mangiferae*, fruit drop, quarantine pest, crop loss

MANGO SEED WEEVIL, *Sternochetus mangiferae* (F.) (syn. mango weevil, *Cryptorhynchus mangiferae*), has been recorded on 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, French Guiana, and Hawaii, where it was first reported in 1905 (Kotinsky 1905). Mango seed weevil is a quarantine pest that prevents the import of mangos into the continental United States from Hawaii and many other mango-producing countries.

Mango seed weevil is univoltine. Females oviposit on immature fruits that are ≈1.9 cm in diameter or larger (Balock and Kozuma 1964, Hansen et al. 1989). The adult female carves out a cavity on the fruit surface and deposits an egg, which is immediately covered by a fruit exudate produced by the wound. Neonates burrow through the pulp to the developing seed. The mango seed is solitary, large, flat, and ovoid-oblong, and is 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 neonate weevils 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 (Balock and Kozuma 1964). 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, the adult weevil emerges within 2 mo after the fruit fall to the ground and deteriorate (Balock and Kozuma 1964), at which time the weevil seeks a protected site (e.g., bark crevices, rock walls) to aestivate. Adult weevils can live for >2 yr when provided fresh mangoes and water (unpublished data). In a survey of mangoes from the five main Hawaiian Islands, Hansen et al. (1989) found no parasitism of mango seed weevil and no other seed-feeding insects.

Mango seed weevil has been elevated to its status as a serious international quarantine pest because of the following three commonly held perceptions relative to its economic impact (Peña et al. 1998): (1) that weevil development in the fruit causes damage to the pulp rendering it unmarketable, or at least unappetizing; (2) that infestation reduces the germination capacity of seeds; and (3) that infestation causes premature fruit drop. Several reports in the literature suggest that pulp-feeding typically is rare (<0.3% incidence) (Hansen et al. 1989, Balock and Kozuma 1964, Follett and Gabbard 2000), and recent studies show that mango seed weevil infestation does not significantly reduce germination rates (Follett 2000, Follett and Gabbard 2000). In this article we present results from studies examining the effects of mango seed weevil infestation on premature fruit drop.

Table 1. Age distribution and infestation rates of mango seed weevil in Haden mangoes in an orchard in Kalapana, Hawaii, during the 2000 growing season

Date	Mean ± SE fruit weight, g	Collection site	No. trees	No. fruit	No. insects	% in-life stage						χ^2	No. infested fruits						χ^2
													No. weevils						
						L1	L2/3	L3/4	L5	Pupae	Adult		1	2	3	4	5	>5	
3 May	40.6 ± 3.8	Ground	29	408	205a	94.1	5.9	—	—	—	—		113	35	6	1	—	—	
	34.6 ± 2.2	Tree	29	408	140b	98.6	1.4	—	—	—	—	2.1	81	20	5	1	—	—	0.5
24 May	75.2 ± 4.1	Ground	29	481	660a	73.0	17.7	8.6	0.6	—	—		249	125	45	4	2	—	
	82.3 ± 4.7	Tree	29	481	577a	75.0	19.6	5.2	0.2	—	—	0.8	218	91	33	11	1	4	0.5
19 June	214.2 ± 6.4	Ground	29	355	263a	—	7.6	4.6	43.4	43.0	1.5		235	14	—	—	—	—	
	200.3 ± 8.1	Tree	29	355	262a	0.8	8.0	5.3	45.4	39.7	0.8	0.1	220	21	—	—	—	—	0.6
12 Aug.	272.5 ± 10.0	Ground	23	317	229a	0.9	—	—	6.6	18.3	74.2		169	27	2	—	—	—	
	288.7 ± 14.1	Tree	23	317	242a	—	—	0.4	10.7	14.9	74.0	0.8	172	25	5	—	1	—	0.1

Means ± SE followed by the same letter for each collection date are not significantly different ($P = 0.05$) by a paired t-test. χ^2 tests were done on the distributions of life stages and the number of weevils in fruits collected off the ground and fruits collected off the tree at each collection date. χ^2 values for each collection date without an asterisk are not significant ($P = 0.05$).

Materials and Methods

Mango seed weevil has not been reared successfully on artificial diet, which precluded doing artificial infestation studies with laboratory-reared insects. Consequently, all observations were made on naturally infested fruit. In 1998, a study was conducted to assess the potential for premature drop as a result of mango seed weevil infestation. Small mango fruits (≈5 cm diameter), collected off the ground and from trees, were cut open to determine the presence or absence of mango seed weevil. In total, 380 small immature fruits from 10 Haden trees in an orchard located at Kalapana, HI, were inspected. Haden mangoes are monoembryonic (single-seeded). If weevil-infested fruit were more prone to dropping than uninfested fruit, the prediction was that a higher infestation rate would be found in fruit on the ground compared with fruit in the tree.

In 2000, the premature-drop study was expanded to include more trees, more fruit per tree, and fruit of different maturities. In total, 3,122 mangoes were collected from the orchard at four times during the season (June–August) from 29 trees. The average size of fruit increased with each successive collection date, but all fruit were immature. On each date, fruit of similar size were first collected off the ground under each tree, and an equal number of similar-sized fruit were harvested from the tree. Mangoes from each position (ground or tree) for each tree were held separately. In the laboratory after fruit were weighed, fruit pulp and seed husks were removed to inspect naked seeds, and all seeds were dissected to determine the number and life stage(s) of any weevils present. Evidence of larval feeding is always apparent on the seed surface of infested seeds. However, larvae tunnel below the seed surface making it difficult to estimate the percentage of seed damage, even with dissection. The orchard used in 1998 and 2000 was selected because it had a very low incidence of disease (e.g., anthracnose) and no internal disorders (e.g., "jelly seed") that could confound results.

Data on percentage infestation were arcsine transformed to normalize the distribution and subjected to analysis of variance (ANOVA), and mean separations were done on the total number of weevils and the fruit infestation rates using a paired t-test (matched pairs being ground fruit and tree fruit for each tree) at $P < 0.05$ (SAS Institute 2000, Sheshkin 2000). Data on the frequency of weevils at each life stage and the number of weevils in infested fruit on each collection date were subjected to a chi-square goodness-of-fit test using the negative log likelihood model (SAS Institute 2000) to detect any differences in distributions for fruit off the ground and fruit from the tree.

Results

In the 1998 test, mangoes collected off the ground weighed 53.8 ± 3.6 g (mean ± SE), and mangoes collected from the tree weighed 54.9 ± 2.5 g ($n = 380$). The incidence of mango seed weevil infestation for fruit on the ground (33.3 ± 7.0%) was higher than for fruit on the tree (22.3 ± 5.8%), but the difference was not significant ($t = 1.7$; df = 1, 8; $P = 0.13$). Mangoes were infested only with first and second instars.

In the 2000 test, weights for mangoes collected off the ground and from trees were similar on all dates (Table 1). Fruit collected on the four dates were assigned average weights of 38 (3 May), 79 (24 May), 207 (19 June), and 281 g (12 August) as a relative index of maturity. Mangoes weighed 300–500 g at harvest maturity. ANOVA on percentage infestation was significant for the effect of date ($F = 12.6$; df = 1, 3; $P > 0.001$), marginally not significant for the effect of on ground versus on tree ($F = 3.4$; df = 1, 28; $P = 0.06$), and not significant for the date by ground-versus-tree interaction. The seed infestation rate was significantly higher in mangoes collected off the ground compared with mangoes collected from the tree for 38 g ($t = 4.2$; df = 1, 28; $P = 0.0002$) and 79 g ($t = 3.0$; df = 1, 28; $P = 0.006$) fruit, but not significantly different for 207 g and 281 g fruit (Fig. 1). The total number of insects was greater in fruits collected off the ground compared with fruits collected from the tree for the first collection date (38 g fruit) ($t = 4.2$; df = 1, 28; $P < 0.0002$), but not significant for any of the later collection dates (79, 207, and 281 g fruit) (Table 1). Chi-square tests on the distribution of life stages were not significant

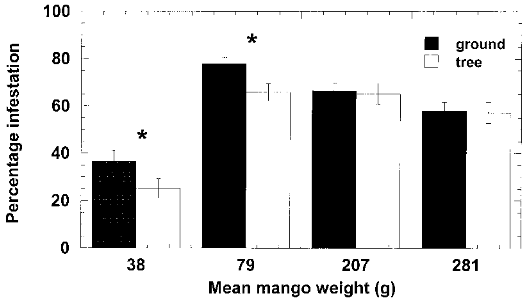

Fig. 1. Percentage infestation by mango seed weevil in mangoes collected off the ground and from the tree at different stages of fruit maturity. *, Denotes significantly different paired t-test ($P = 0.05$).

for any collection date (Table 1), indicating that the age distribution of weevils was the same for fruits on the ground and on the tree. As the season progressed, the age distribution of weevils also progressed from primarily neonates (3 May) to primarily adults (12 August), indicating that most eggs were laid early in the season on small fruit. Chi-square tests on the number of weevils in infested fruits were not significant at any collection date (Table 1), indicating that the frequency of single and multiple infestation was the same for fruits on the ground and on the tree. Multiple infestation was more common in early-season (3 May and 24 May collection dates) mangoes compared with midseason (19 June collection) and late season (12 August collection) mangoes. During early season, 36.8% of infested fruits had >1 weevil, whereas during mid- and late season the rate had decreased to 10.6%. Nine early-instar weevils were found in each of two mangoes on 24 May, which was the maximum number of weevils found on any date. Five adult weevils were found in the seed of a mango from the tree on 12 August, which was the maximum number of adults found on any date.

Discussion

If mangoes infested with seed weevils were more prone to dropping than uninfested fruit at certain times during the growing season, then a higher infestation rate would be found in fruits on the ground compared with fruits on the tree at those times. The 1998 study suggested that mango weevil infestation might cause increased fruit drop in younger fruits, but the power of the experimental design was inadequate to detect this effect. The 2000 study showed that immature mangoes on the ground early in the season had a higher incidence of mango seed weevil infestation than fruit of equal size on the tree, which suggests that mango seed weevil infestation does increase premature drop during early fruit development. The higher infestation rate of fruits on the ground compared with fruits on the tree could have been a result of earlier attack on the tree of the fallen fruit. If this were the case, we would expect a different age distribution in fruit on the ground compared with fruit on the tree. However, results indicated that the age distribution was the same for mangoes on the ground and on the tree at all collection dates, and therefore earlier timing of attack (or, potentially, increased development rate of weevils in the fruit) could be excluded as a possible explanation for the higher incidence of weevils in fruit on the ground. Similarly, the higher infestation rate of fruit on the ground compared with fruit on the tree could have been a result of greater rates of multiple infestation (i.e., fruits with higher numbers of weevils were more prone to dropping). However, the frequency distribution of weevils in infested fruits was the same whether the mangoes were on the ground or on the tree, suggesting that this also could be excluded as a factor contributing to the higher incidence of weevils in fruits on the ground. We can conclude from the results that infested fruits drop at a higher rate than uninfested fruits during early fruit development. Although other factors such as site of feeding in the seed (embryo or cotyledon) by immature weevils, and the position of the fruit both on the panicle and on the tree, were not examined, these factors might have been important. No other insect pests were observed in immature fruits of any size, and there was no evidence that pathogens were introduced into the mangoes by mango seed weevils in this study.

Most mango panicles lose all of the originally set fruitlets, with the greatest losses occurring during the first weeks following anthesis (Nunez-Elisea and Davenport 1983). Abscission of fruitlets is random except for the tendency to retain fruit at the distal end of the panicle. Less than 1% of the flowers setting fruit reach maturity. Therefore, in most years, mango trees naturally drop a large portion of their potential crop. Opinions in the literature on the role of mango seed weevil in increasing fruit drop are mixed and the data are inconclusive. Pope (1924) and Subramanyan (1927) credited mango seed weevil attack with heavy immature fruit drop. In Hawaii, Van Dine (1906) and Swezey (1931, 1943) believed that weevil infestation did not cause premature fruit drop. Steiner and Morashita (1951) found infestation rates of 55–67% in 1,814 kg of ripe mangoes picked at harvest maturity and infestation rates of 33–55% in 544 kg of ground fruit from the same trees, suggesting that weevil-infested fruit were not more prone to dropping than uninfested fruit. Likewise, Balock and Kozuma (1964) found weevils infesting 66% of mangoes on the ground and 76% of mangoes on the tree. Comparisons between these earlier studies and the current study are difficult because often no information is given regarding fruit size, stage of fruit maturity, age distribution of the weevil population, or the numbers of weevils per seed.

Other weevils also are known to cause premature abscission of fruit or fruiting structures, including plum curculio, *Conotrachelus nenuphar* (Herbst) in apples, plums, and peaches; and the boll weevil, *Anthonomus grandis* Boheman, in cotton (Metcalf et al. 1962). As is the case with mango seed weevil, the effect of plum curculio inducing abscission is dependent on the timing of oviposition and larval feeding relative to fruit maturity. Levine and Hall (1977) demonstrated that plum curculio-induced fruit abscission in apples and plums was most likely to take place when fruit were small; apples larger than 28 mm in diameter did not fall unless they were infested with more than one weevil. Abscission was induced by larval feeding, not oviposition. Coakley et al. (1969) showed that second- and third-instar boll weevils (but not eggs or first instars) cause abscission of cotton squares through release of an unknown material rather than by actual feeding damage. The mechanism causing increased early-season drop in mangoes infested by mango seed weevil is unknown.

Mango seed weevil generally does not directly affect marketability because it resides inside the seed within a thick husk in mature mangoes and is rarely encountered. However, it appears that mango weevil can increase premature fruit drop, and, therefore, may be an economic concern. Pest control research for mango seed weevil over the years has tested a number of options, including field sanitation, natural enemies (parasitoids, the fungus *Beauvaria bassiana*), and host plant resistance with little success (CAB and EPPO 1997, Hansen and Armstrong 1990). Insecticides, applied to the trunk to kill adults in the off season, or to the canopy to prevent oviposition, have been only somewhat effective in reducing weevil infestations: Shukla and Tandon (1985) tested eight insecticides against mango seed weevil in mangoes, and the most effective insecticides, fenthion, carbaryl, deltamethrin, and diazinon, reduced incidence from 65–80% to 5–17% when compared with the controls. Economic injury levels relative to premature drop should be developed for mango seed weevil in Haden and other mango cultivars of economic and export importance to determine whether insecticide use is warranted. For now, the greatest significance of mango seed weevil will remain its quarantine importance for exported mangoes because of restrictions imposed by importing countries and states.

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