

Hawaiian Tephritid Fruit Flies (Diptera): Integrity of the Infestation-Free Quarantine Procedure for 'Sharwil' Avocado

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J. Econ. Entomol. 88(1): 85-96 (1995)

ABSTRACT In 1990, the infestation-free quarantine procedure for 'Sharwil' avocados grown in Kona, HI, was approved based on the assumption that fruits on trees are not hosts of tephritid fruit flies. In February 1992, the infestation-free quarantine procedure was suspended because of discovery of oriental fruit fly, *Bactrocera dorsalis* (Hendel), larval infestation in fruits on trees in certified orchards. Subsequently, an intensive field study was conducted to determine the level of tephritid fruit fly infestations in 'Sharwil' fruits. Results gathered negated two assumptions of the infestation-free quarantine procedure. First, the procedure assumed that only immature and mature green fruits are attached on trees; our data showed that, although most fruits on trees were either immature or mature green, a few ripe fruits occurred during the fruiting season. Second, the procedure assumed that mature green fruits have absolute resistance to tephritid fruit flies occurring in Hawaii; our field data showed that mature green 'Sharwil' avocados are suitable hosts of oriental fruit fly, albeit poor hosts. We present several hypotheses that may explain the failure of the infestation-free quarantine procedure for 'Sharwil' avocados. Morphological, physical, and chemical attributes of maturing 'Sharwil' fruits that may be useful in developing indices of fruit maturity and quality are also presented.

KEY WORDS *Bactrocera dorsalis*, infestation-free quarantine procedure, avocado

CONTRADICTIONARY INFORMATION exists in the literature with regard to the relative resistance of 'Sharwil' avocado (*Persea americana* Mill.) to infestations by tephritid fruit flies occurring in Hawaii. Armstrong et al. (1983) concluded that Hawaii-grown 'Sharwil' avocados are resistant to Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann); melon fly, *Bactrocera cucurbitae* (Coquillett); and oriental fruit fly, *Bactrocera dorsalis* (Hendel), infestations at the mature green stage of maturity. On the contrary, Oi & Mau (1989) concluded that mature green 'Sharwil' fruit is a suitable host of Mediterranean fruit fly and oriental fruit fly based on field experiments that involved caging fruits on trees with sexually mature adults. Armstrong (1991) performed further laboratory and field studies and in a span of 3 yr confirmed his earlier results, concluding that "'Sharwil' avocado is not a host for Mediterranean fruit fly, melon fly, or oriental fruit fly when the fruit is attached to the tree, or when fruits are harvested with stem attached." Consequently, a regulatory procedure based on infestation-free status was developed

with an absolute specification that mature green fruits with the pedicel firmly attached are harvested only from trees that had been certified by the Animal and Plant Health Inspection Service (APHIS) to be truly 'Sharwil' (APHIS 1990). This federally registered quarantine procedure enabled shipment of 'Sharwil' fruits from certified orchards in Hawaii to the contiguous United States without any required conventional quarantine treatment.

While performing routine inspections of orchards and packinghouses in February 1992, quarantine officials in Kona, HI, reported finding 'Sharwil' fruits infested with oriental fruit fly larvae (APHIS 1992a); N.J.L. confirmed this finding. As a result, the 'Sharwil' infestation-free quarantine procedure was suspended, and shipments of 'Sharwil' fruits to the contiguous United States were temporarily banned.

Herein, we report the results of field investigations conducted to determine the level of tephritid fruit fly infestations in 'Sharwil' avocado fruits grown in Kona, HI. We present morphological, physical, and chemical characteristics of 'Sharwil' fruits that may be useful in developing indices of maturity and quality. We also outline our recommendations in developing and implementing infestation-free quarantine procedure.

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or recommendation for its use by USDA.

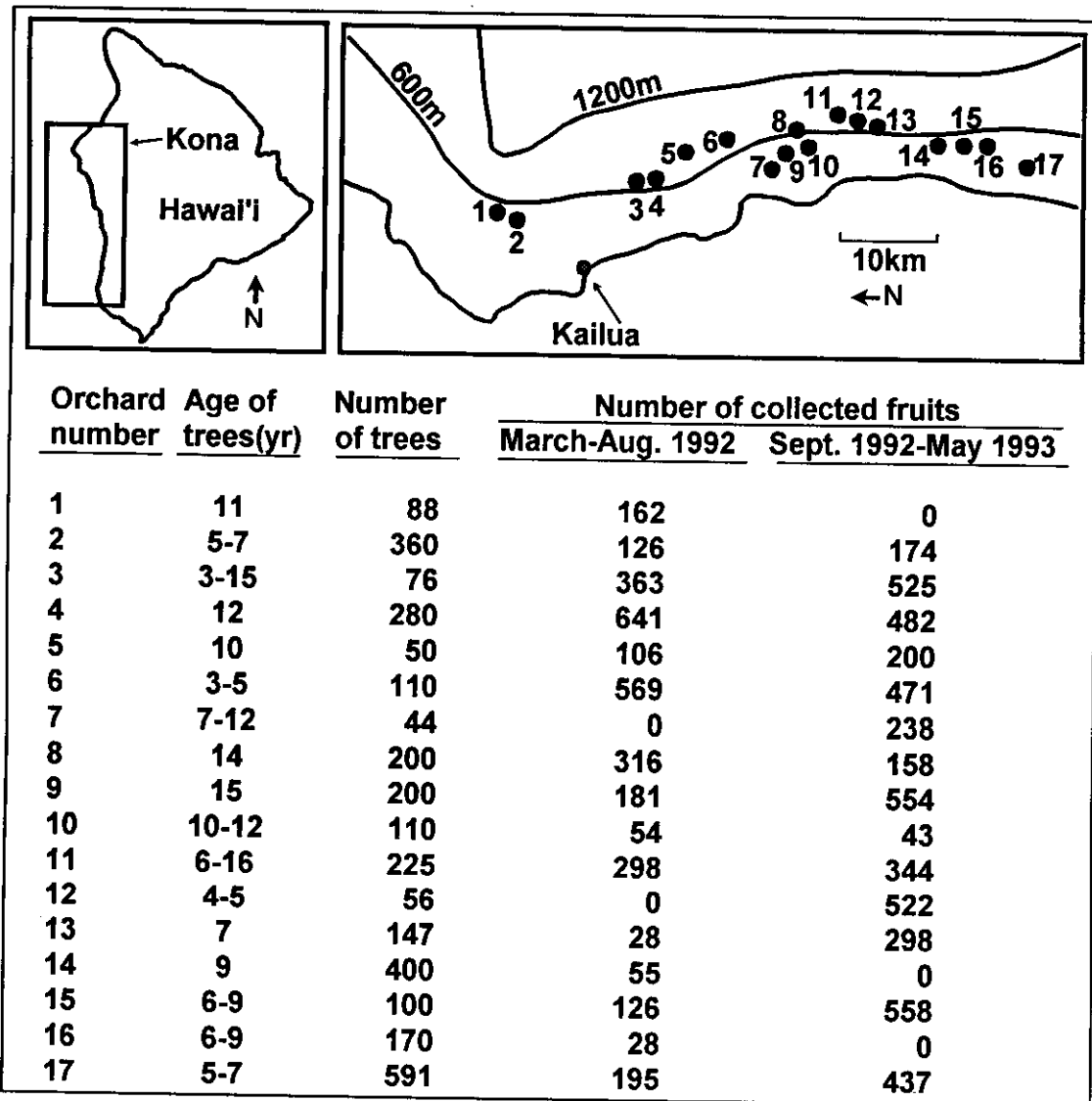


Fig. 1. Location of certified 'Sharwil' avocado orchards in Kona, HI, depicted on elevation (m) contours; included for each orchard are age and number of trees and number of sampled fruits.

Materials and Methods

Field Census. Field investigations were conducted in certified 'Sharwil' orchards (i.e., trees were identified as 'Sharwil' by APHIS officials [APHIS 1990]) located in Kona, HI (Fig. 1) during the following time periods: March–August 1992 and September 1992–May 1993.

March–August 1992. Seventeen certified orchards were in production when the field census was initiated in March 1992; all of these orchards were included in sampling. An initial intense sampling of 1,047 fruits that met the quarantine procedure (i.e., with pedicel attached on the tree) was conducted on 2–10 March. Afterward, ≈ 200 fruits were collected either weekly or biweekly, depend-

ing on available labor. Eight orchards were selected for sampling per sampling occasion, with ≈ 25 fruits sampled from each orchard. In total, 3,273 fruits were sampled; 3,151 of these fruits met the quarantine procedure, but 122 did not because of pedicel detachment during harvesting.

September 1992–May 1993. Sampling was started in September at low-elevation (<500 m) orchards and in January at high-elevation (>500 m) orchards. There were eight productive low-elevation orchards and three productive high-elevation orchards; they were all included in sampling. For the low-elevation orchards, ≈ 300 fruits were collected biweekly. For the high-elevation orchards, ≈ 300 fruits were collected monthly. As in the

March–August 1992 census, fruits were selected based on the requirements of the infestation-free quarantine procedure. In total, 5,004 fruits were sampled; 4,888 of these fruits met the quarantine procedure, but 116 did not because of pedicel detachment during harvesting.

Indices of Fruit Visual Quality and Maturity.

Because of the lack of indices of quality and maturity for 'Sharwil' avocado, each fruit was individually described morphologically and quantified for glossiness and hardness. In addition, percentage dry matter content of sampled fruits was estimated.

Indices of Fruit Visual Quality. All fruits collected from March 1992 to May 1993 were described morphologically with emphasis on the following characteristics: firmness of pedicel's attachment to the fruit; "ringneck" of the pedicel; skin scarring; skin sun scalding; wartlike and ridge-like skin aberrations; corky depression; and abnormality in shape (Fig. 2). Terminology used in describing morphological disorders follows Broadley et al. (1991). Scarring (Fig. 2A, B) was sandpaper-like, rough abrasions on the fruit skin either caused by fruits rubbing against leaves, branch, or other fruits, or probably because of impact of small wind-blown particles. An extreme case of scarring resulted from healing of wounds from rat bites (Fig. 2C). Sunburn (Fig. 2D) was a condition that started as yellowing of the fruit surface facing the sun and then progressed to a severe darkening and sunken, burnt appearance. Ringneck (Fig. 2E) was a brown, corky ring around the pedicel consisting of peeling, dead tissues. Corky, circular depression into the flesh (Fig. 2F) and the hooked-shape fruit (Fig. 2G) were morphological indications of nutrient deficiency. Fruits with loose pedicel attachment had a crack at the base of the pedicel; presence of a crack was verified by using either a hand lens (10 \times) or a dissecting microscope (60 \times).

Indices of Fruit Maturity. All fruits collected from September 1992 to May 1993 were measured for hardness and glossiness. Because the chemical quantification procedure required destruction of fruits, the percentage dry matter content was estimated biweekly on a separate set of fruits.

Determining Fruit Hardness. Subjective determination of hardness was done in the field, immediately after harvesting, by pressing the fruit individually with bare hands. Subjective hardness categories were hard, firm, and soft. Hard fruit was mature green with no soft spot. Firm ripe fruit was relatively hard, ripening fruit with a softer portion. Soft fruit was fully ripe.

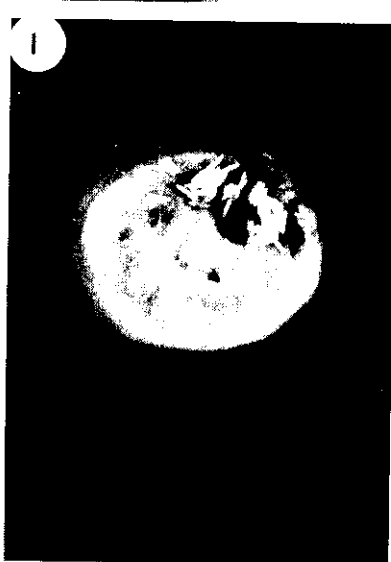
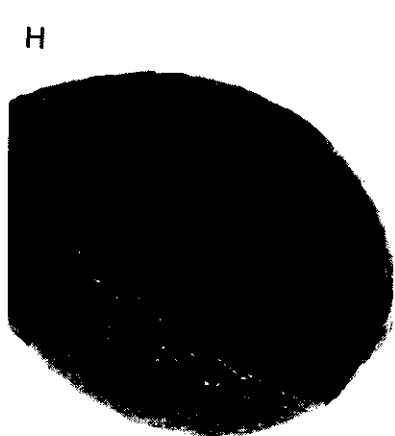
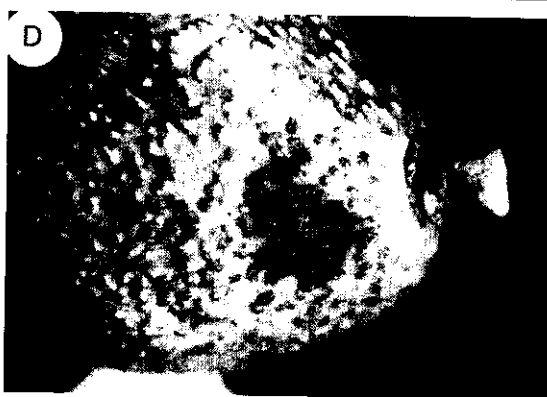
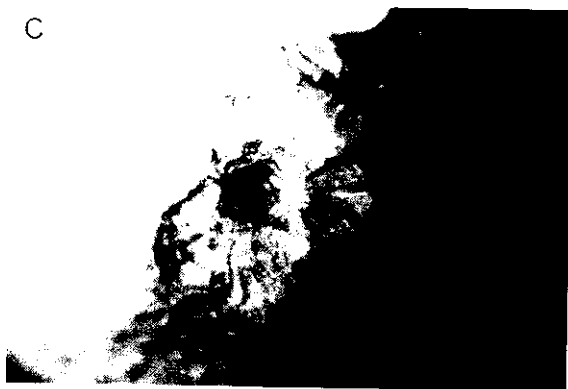
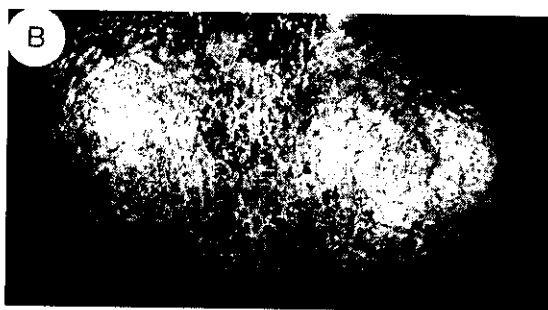
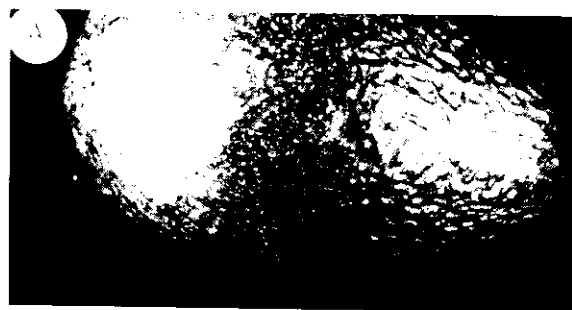
Upon arrival at the laboratory, each fruit hardness was quantified using a force gauge (0–50 kg penetration range [AMETEK AccuForce Cadet, AMETEK Hunter Springs Division, Hatfield, PA]) with an 8-mm-diameter cylindrical probe. The rate of the probe's penetration was \approx 15 cm/min and was controlled automatically by a motorized test stand (AMETEK Model 100). Fruit hardness was

measured at the equatorial region of each fruit; only one probe per fruit was done to minimize fruit damage. For firm ripe fruits, hardness was measured at the soft spot. Hardness readings were done within 8–24 h after harvest.

Determining Fruit Glossiness. Surface gloss was determined using a gloss meter (BYK Gardner Micro-TRI-Gloss Meter [0 to >100 gloss units; BYK Gardner, Silver Spring, MD]), with measurements taken at the 60° angle. Each fruit glossiness value was an average of three readings taken at the equatorial area. To assure absence of light entering between the fruit and the gloss meter, the base of the meter was fitted with a black rubber foam attachment. Fruit glossiness was determined within 8–24 h after harvest.

Determining Percentage Dry Matter Content. A separate set of fruits was used for estimating the percentage dry matter. Over 1,000 visually even-aged immature green fruits were randomly selected and tagged with a flagging tape from four of the eight productive lower-elevation orchards from 14 to 23 September 1992. Fruit selection criteria included surface gloss, pedicel color, and overall fruit size. These characteristics were recorded for at least 30 fruits from each orchard. Additional fruits were tagged based on their similarity to the 30 fruits that had been characterized. Fruit glossiness was determined as described above. Selected fruits had average gloss unit readings of 2–4, with no individual reading >6.0. The pedicel and pedicel base of all selected fruits were green, and fruits were smaller than the mature fruit size. During each subsequent biweekly fruit sampling, 24 tagged fruits and 24 untagged fruits were selected randomly and harvested concurrently. They were brought to the laboratory, immediately described morphologically, and then measured for glossiness, hardness, and percentage dry matter content. Fruits used for estimating percentage dry matter content were all hard, mature green; dry matter estimation was done within 8–24 h after harvest.

Percentage of dry matter content was determined by cutting each fruit in half longitudinally, removing the seed, then cutting thin sections parallel to the exposed tissue. The sections from each half were kept separate to provide two measures of percentage dry matter content per fruit; the average of these two estimates was used as the fruit percentage dry matter content. The flesh sections (averaging \approx 15 g) were cut into small pieces, placed in a tared petri dish, and weighed (fresh weight [FW]). These fruit pieces were dried in a food dehydrator (Harvest Maid, Alternative Pioneering Systems, Chaska, MN) at 60°C until constant weight (dry weight [DW]) was obtained; drying duration was \approx 24 h. Percentage of dry matter was then calculated as follows: $(DW/FW) \times 100$. The above dry matter content quantification procedure is a modification of that described by Rujiraapakorn (1993).



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Determining the Degree of Tephritid Fruit Fly Infestations. To prevent absolutely any possible fruit fly infestation after harvesting fruits off the tree, fruits were placed in screened and sealed plastic bins (66 by 46 by 30 cm) immediately after harvest. Fruits were transported from the field to the laboratory in these plastic bins in air-conditioned vehicles. Within 8–24 h after harvest, each fruit was placed individually in a 1-liter plastic bucket with a thin layer of sand at the bottom. The sand served as an adsorbent of fruit exudate and pupation medium for larvae upon leaving the fruit. Fruits were kept in a tephritid fruit fly-free holding room at 19–24°C, 60–80% RH, and 12:12 (L:D) h photoperiod. After 2 wk, pupating larvae and pupae were separated from the fruit and sand using sieves of increasingly smaller mesh, and then placed in plastic cups (0.25 liter) containing a small amount of sand for pupation. Adult fruit flies were allowed to emerge and then killed and preserved in 70% ethyl alcohol; dead larvae and pupae were sifted from the sand and also preserved in alcohol. Dead larvae and pupae and emerged adults were identified to species (Hardy 1949).

Determining Relative Density of Adults in Orchards. Initial trapping of fruit fly adults with male lures was done in April 1992, but trapping was discontinued because the cooperating farmers did not approve of the presence of traps baited with male lures or any attractant in the field. With the farmers' approval, trapping with protein bait was started in September 1992. Protein bait (9% Nu-Lure [Miller, Hanover, PA], 5% sodium borate, and 86% water by weight) in Nakagawa traps (i.e., 3.8-liter plastic bucket traps) (Nakagawa et al. 1975) was placed in six sites; three in low-elevation orchards, three in high-elevation orchards. Trapping adults using protein bait was done once a month for a duration of 2 wk from September to November 1992.

Because the density of fruit fly adults was low (zero in protein bait traps placed in the middle of the orchards), we convinced the farmers to allow us to replace protein bait traps with those containing the more potent male lures: methyl eugenol (4-allyl-1,2-dimethoxybenzene [Agrisense, Fresno, CA]) for oriental fruit fly, cue-lure (4-[*p*-hydroxyphenyl]-2-butanone acetate [Agrisense, Fresno, CA]) for melon fly, and trimedlure (1,1-dimethyl 4[and 5]-chloro-2-methyl-cyclohexane-1-carboxylate [Agrisense, Fresno, CA]) for Mediterranean fruit fly. Each trap had a 2.5-cm cotton wick impregnated with 1-ml lure and a 2.5-cm piece of dog collar (active ingredient: 15% Naled [1,2-dibromo-2,2-dichloroethyl dimethyl phosphate]

[A. H. Robins, Richmond, VA]). So as not to affect the density of fruit fly adults in the orchards, trapping using male lures was done only once a month for a 4-h trapping duration.

Management and Statistical Analyses of Data. The descriptive statistics of each fruit morphological characteristic were calculated. The differences in percentage occurrences of different morphological characteristics between early-season (September–December) and late-season (January–July) fruits were determined by *G* tests (two rows by two columns) (Sokal & Rohlf 1981). Regression analyses were done to determine the relationship between age of fruits and their glossiness, hardness, and percentage dry matter content. For regression analyses, day of year (DOY) was used as the fruit phenological age, with 1 January 1993 designated as DOY 366. Management and analyses of data were done using SAS version 6.03 (SAS Institute 1988a, b), except for *G* tests, which were performed using BIOM (Rohlf 1988).

Results and Discussion

Fruit Visual Quality. A high proportion of 'Sharwil' fruits exhibited physiological disorders, as manifested by high percentages of fruits with morphological abnormality (Fig. 3). Of the 8,277 sampled fruits, 60.90% had slight scarring, 21.83% had heavy scarring, 18.74% had sun scalding, 25.20% had ridges and warts, 13.44% were hooked shape, and 16.99% had ringneck. Scalding and ringneck were manifestations of water stress; hooked shape indicated boron deficiency (Broadley et al. 1991). Wartlike or ridgelike aberration of the skin surface probably was a manifestation of damage incurred by fruits at an earlier stage of development, probably a result of mite or insect feeding. We also observed occurrences of round fruits and leaves with yellowing between veins, probably indications of zinc deficiency (Broadley et al. 1991). Results presented here attest that 'Sharwil' trees in Kona, HI, at the time of our sampling were water-stressed and suffering from nutrient deficiencies and wound-inflicting mechanical injuries.

Fruits collected at high-elevation orchards from January through August had significantly ($P < 0.05$) higher frequencies of slight scarring ($G_{adj} = 5.69$, $df = 1$), heavy scarring ($G_{adj} = 1.11$, $df = 1$), sun scalding ($G_{adj} = 46.82$, $df = 1$), ringneck ($G_{adj} = 13.45$, $df = 1$), warts and ridges ($G_{adj} = 9.02$, $df = 1$), and hooked shape abnormality ($G_{adj} = 88.78$, $df = 1$) than fruits sampled at low-elevation orchards from September to December. Data collected suggest that the fruit visual quality varied

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Fig. 2. Morphological aberrations of mature green 'Sharwil' fruits: (A) slight scarring; (B) heavy scarring; (C) scarring caused by rat bite; (D) skin scalding caused by sunburn; (E) pedicel with ringneck; (F) corky, circular depression with heavy scarring; (G) abnormal, hooked shape; (H) ridges on skin; (I) fruit with loose pedicel attachment infested with oriental fruit fly eggs.

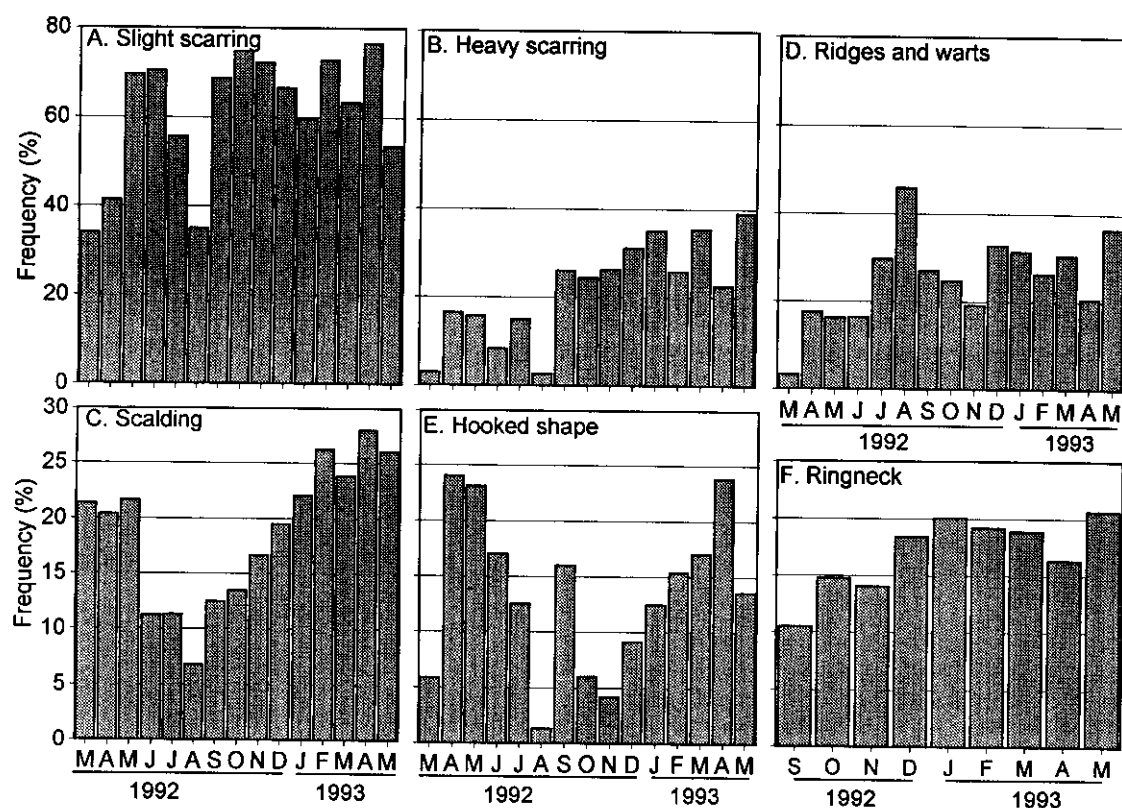


Fig. 3. Frequencies (%) of different morphological aberrations of 'Sharwil' fruits collected from March 1992 to May 1993: (A) slight scarring; (B) heavy scarring; (C) scalding; (D) ridges and warts; (E) hooked shape; (F) ringneck.

with elevation or season and should be considered in assessing fruit quality in the development of conventional (i.e., use of heat, cold, or fumigants) commodity quarantine treatments.

Fruit Maturity. Fig. 4 shows the seasonal changes in fruit glossiness, hardness, and percentage dry matter content with age or degree of maturity. The glossiness of younger fruits appeared higher than that of older fruits: $y = 15.753 - 0.076x + 0.00017x^2$, $r^2 = 0.68$, $df = 9$, $P = 0.006$. We observed that the fruit surface became rougher and darker with age, which probably contributed to a high variation in glossiness readings.

The fruit hardness as indexed by compression value increased with the increase in fruit maturity and then gradually decreased toward ripening. Eighty-four percent of the variation in fruit hardness could be explained by fruit age: $y = -17.319 + 0.219x - 0.0004x^2$, $r^2 = 0.84$, $df = 9$, $P = 0.003$. Other factors such as water stress, deficiency in nutrients, and diseases could have influenced the observed variability in fruit hardness. Individual examination of fruit samples by pressing hands against each fruit and by quantification of hardness using a penetrometer showed that 97.8% of fruits were hard (average compression value of 23.5 [SEM = 0.04, $n = 4,677$] kg), but 1.7% of seemingly hard, mature green fruits had begun to ripen (soften); these fruits, classified as firm ripe, had an

average compression value of 9.3 (SEM = 0.92, $n = 54$) kg. In addition, 0.5% of fruit samples were soft ripe, with average compression value of 2.9 (SEM = 0.61, $n = 10$) kg (Fig. 5). Firm ripe fruits were observed as early as September, when most fruits were visually and phenologically immature.

The fruit percentage dry matter content increased linearly with age: $y = -11.211 + 0.112x$, $r^2 = 0.99$, $df = 10$, $P = 0.0001$. These results indicate that the dry matter content, which is directly proportional with fruit oil content and has been used as an index of maturity in several varieties of avocado (Lee 1981), may be a useful index of maturity for 'Sharwil' fruits.

Infestation of 'Sharwil' Fruits by Tephritid Fruit Flies. *March–August 1992 Field Census.* Of 3,148 fruits that met the requirements of the infestation-free quarantine procedure, 15 were infested with eggs and larvae of oriental fruit fly (Table 1). Of these 15 infested fruits, five were normal fruits without any morphological aberrations or signs of physiological disorder. Ten fruits had morphological aberrations: nine with scarring on the skin surface; two with manifestation of sunburn; two with abnormal, hooked shape; and two had a crack on the skin near the base of the pedicel. Six fruits were hard, mature green; five were firm ripe; and four were soft, tree-ripened fruits. The number of developed third instars and

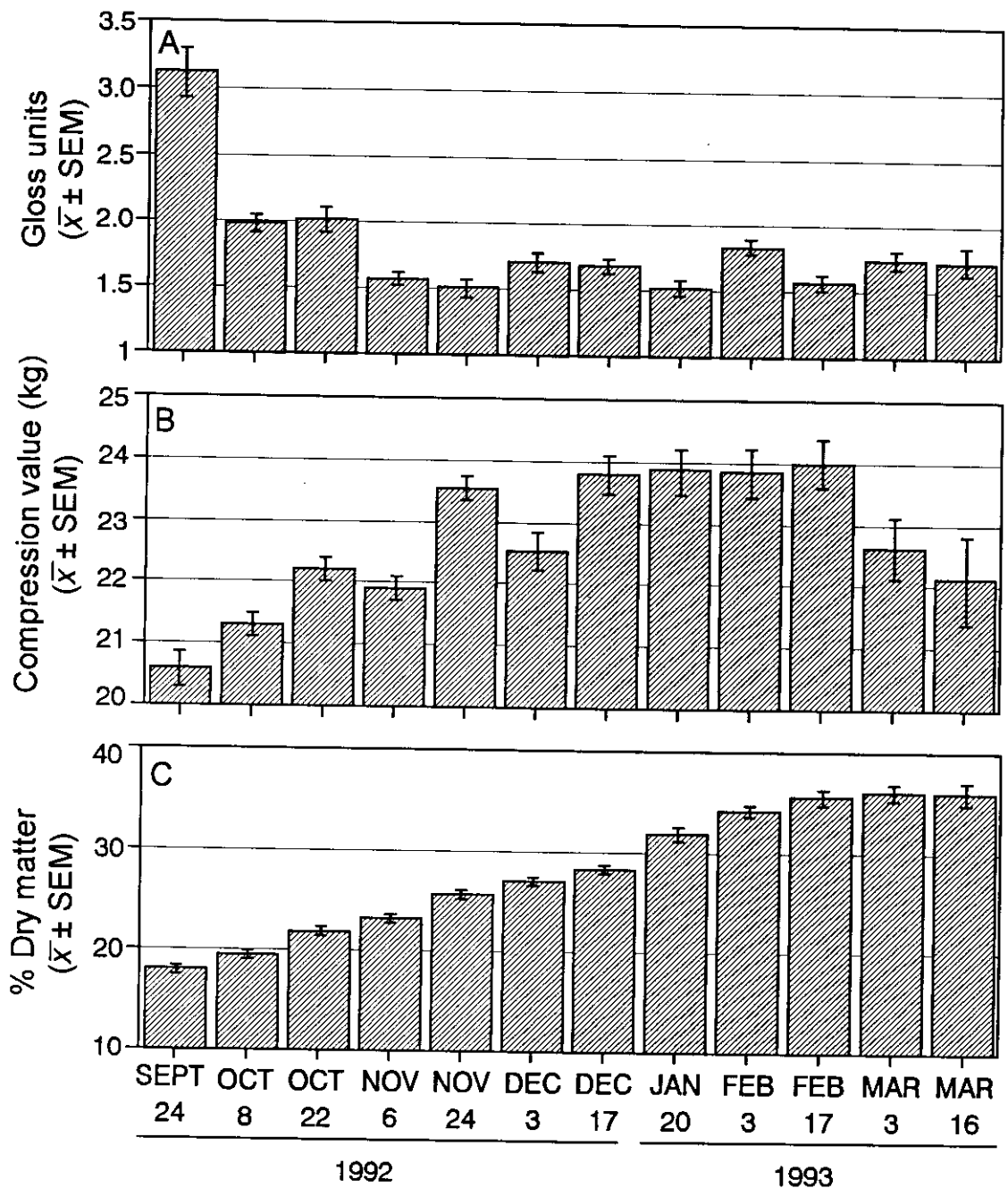


Fig. 4. Relationship between phenological age of 'Sharwil' fruits and their (A) glossiness (gloss units), (B) hardness (compression value [kg]), and (C) percentage dry matter content. Values presented were calculated from 48 samples used for calculating percentage dry matter content.

pupae recovered from these infested fruits ranged from 1 to 41 (mean = 18.3, SEM = 2.9, $n = 15$). Only oriental fruit fly egg and larval infestations were found in 'Sharwil' fruits with pedicel attached on the tree. Infested fruits were from seven orchards with trees ranging from 5 to 15.5 yr old.

In addition, nine fruits that seemingly fit the protocol (i.e., with pedicel attached on tree but the pedicel became detached when harvested with a fruit picker) had infestations of oriental fruit fly eggs and larvae. Eggs were at the junction of the pedicel and the fruit. Numbers of oriental fruit fly third instars and pupae ranging from 1 to

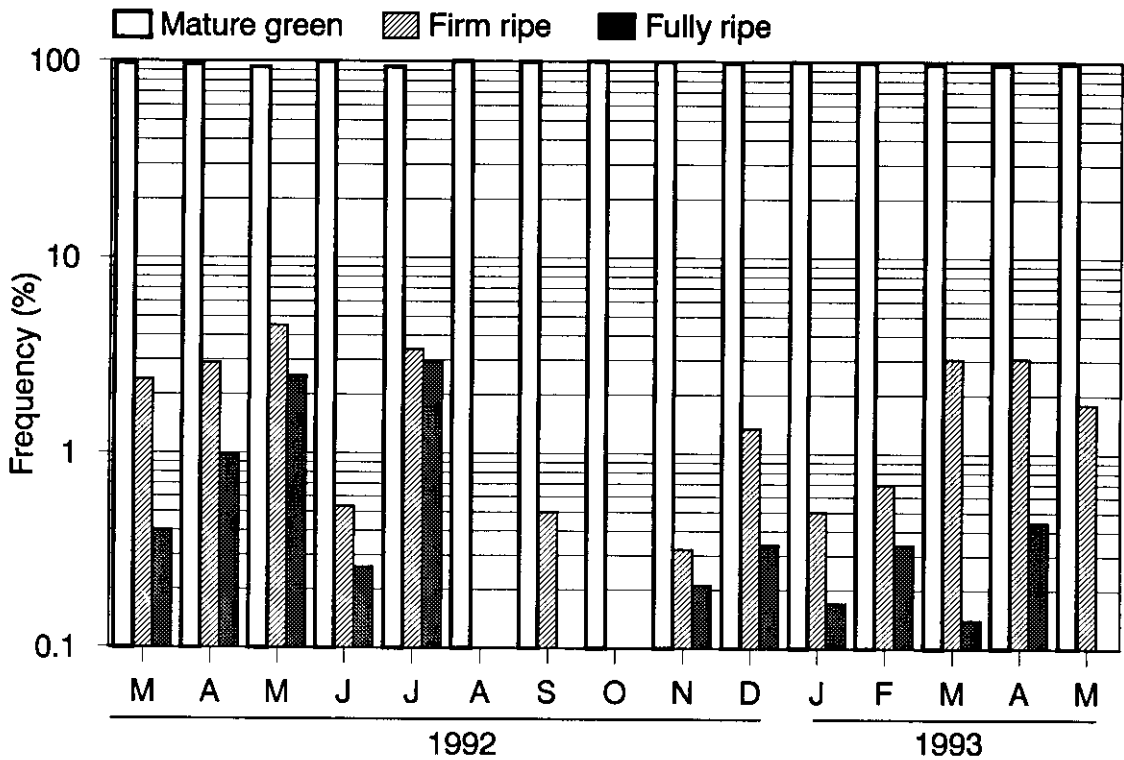


Fig. 5. Frequencies of mature green, firm ripe, and fully ripe fruits collected from March 1992 to May 1993, Kona, HI.

91 (mean = 22.6, SEM = 9.5, $n = 9$) were recovered from these fruits (Table 2).

The low incidence of oriental fruit fly-infested fruit did not allow us statistically to measure the relationships between infestation intensity and the fruits' physical and chemical attributes. However,

our results undoubtedly attest that infestation occurred both in mature green, ripening, and fully ripe fruits.

September 1992–May 1993 Field Census. Not a single fruit sample had oriental fruit fly infestation. The absence of infested fruit during this season

Table 1. Oriental fruit fly infestation in 'Sharwil' avocado fruits collected from the tree with pedicel fully attached

Collection date, 1992	Orchard no. ^a	Wt. g	Maturity level ^b	Morphological aberrations	No. larvae and pupae	No. emerged adults
3 Mar.	10	270	MG	Heavy scarring	1	0
4 Mar.	16	285	F	Slight scarring	21	7
25 Mar.	16	226	MG	Slight scarring, with a crack on the skin basal of pedicel	3	0
25 Mar.	16	209	MG	None	6	0
25 Mar.	16	283	F	Slight scarring	14	0
25 Mar.	16	314	F	None	21	2
25 Mar.	16	278	F	None	24	0
25 Mar.	16	166	R	None	18	3
2 Apr.	11	175	MG	Slight scarring, yellowing	35	19
2 Apr.	16	266	R	Heavy scarring, with a crack on the skin basal of pedicel	41	13
8 Apr.	3	509	MG	None	9	0
8 Apr.	3	350	R	Heavy scarring, scalding, hooked shape	16	1
29 Apr.	4	352	F	Heavy scarring	29	17
21 July	3	354	R ^c	Hooked shape, with a crack on the skin basal of pedicel	22	0
22 July	6	266	MG ^d	Slight scarring, hooked shape, with a crack on the skin basal of pedicel	14	5

^a Refer to Fig. 1 for field locations.

^b MG, mature green; F, firm ripe (mature green with a soft spot); R, fully ripe.

^c 3.9 kg hardness; 1.0 gloss units.

^d 20.4 kg hardness; 1.4 gloss units.

Table 2. Oriental fruit fly infestation in 'Sharwil' avocado fruits with pedicel attached on the tree, but detached in the course of harvesting

Collection date, 1992	Orchard no ^a	Wt, g	Maturity level ^b	Morphological aberrations	No. larvae and pupae	No. emerged adults
4 Mar.	16	361	MG	None	1	0
4 Mar.	16	450	MG	None	9	0
4 Mar.	16	425	MG	None	2	1
4 Mar.	16	209	F	Yellowing	91	55
4 Mar.	16	222	F	Yellowing	42	28
5 Mar.	13	204	R	With a crack on the skin basal of pedicel	17	0
25 Mar.	16	224	F	None	5	0
25 Mar.	16	265	R	Scarring	18	0
28 Apr.	6	265	R	Slight scarring, with a crack on the skin basal of pedicel	18	0

^a Refer to Fig. 1 for field locations.

^b MG, mature green; F, firm ripe (mature green with a soft spot); R, fully ripe.

emphasizes the difficulty of predicting the occurrence and intensity of oriental fruit fly infestation in mature green 'Sharwil' fruits.

Fruit Fly Adults Density in 'Sharwil' Avocado Orchards. The densities of oriental fruit fly, Mediterranean fruit fly, and melon fly adults were consistently low during the trapping period (Fig. 6), suggesting that the pest populations' infestation pressure was similar through the sampling periods.

Integrity of Fruit Fly Infestation-Free Quarantine Procedure for 'Sharwil' Avocado. The in-

festation-free quarantine procedure for 'Sharwil' avocado was developed based on studies that concluded "'Sharwil' fruits are not hosts to trifly if they are picked with pedicel attached and, within 24 h after they were picked . . . packed in cartons impervious to trifly" (Armstrong 1991). The standard operating procedure developed for 'Sharwil' fruits intended for marketing to the contiguous United States requires that fruits are "harvested with stem attached only from certified orchards" (APHIS 1990; Mike Scharf, personal communica-

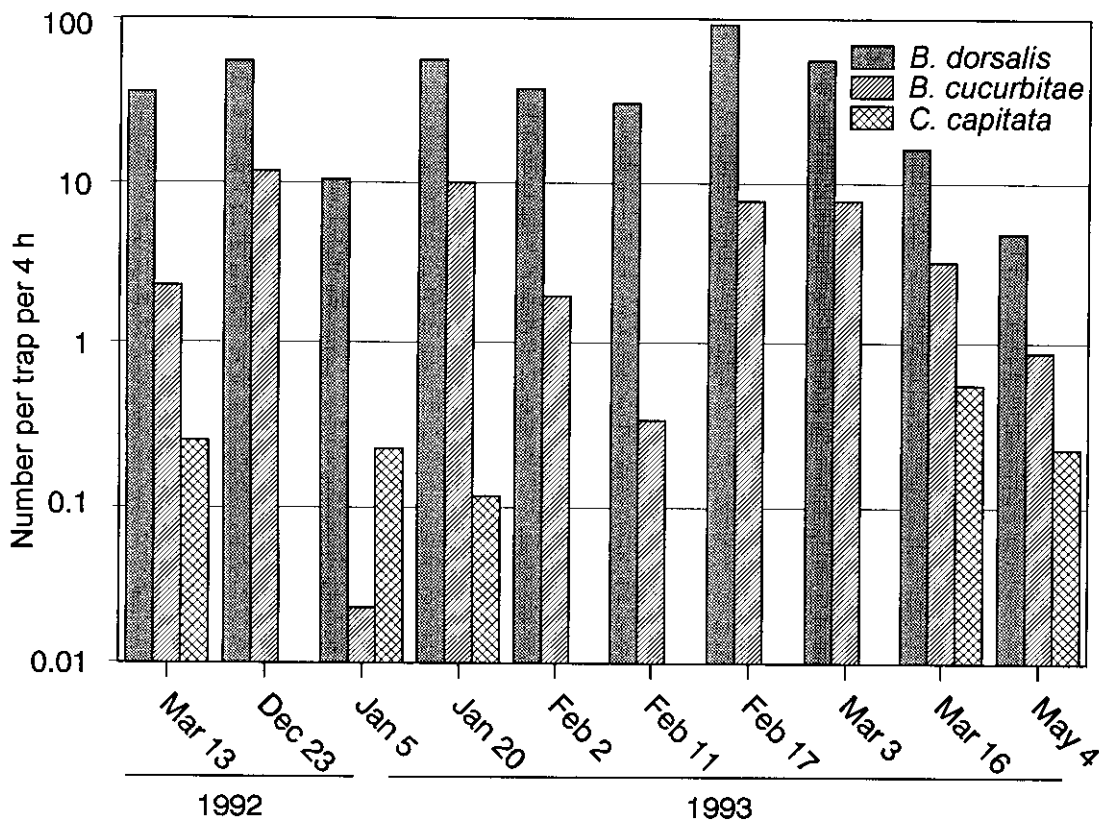


Fig. 6. Relative densities of *B. dorsalis*, *B. cucurbitae*, and *C. capitata* in certified 'Sharwil' avocado orchards.

tion), without any specifications for desired quality and level of maturity. Our data based on the variation in morphology, glossiness, hardness, and percentage dry matter content of 'Sharwil' fruits with age may be used in developing industry standards on fruit market quality and maturity. Based on our observations, 'Sharwil' fruits were harvested initially and marketed when the dry matter content was 20–25%; fruits with 22–28% dry matter content have acceptable eating quality (based on taste, texture, and other parameters) (Rujiraapakorn 1993).

Results presented here negated two assumptions of the 'Sharwil' protocol. First, the protocol assumed that only hard, mature green fruits are fully attached on trees. However, although most of the fruits on trees were hard, mature green (97.8%), a considerable proportion were either firm ripe (at an early stage of ripening [1.7%]) or fully ripe (0.5%). Second, the protocol assumed absolute resistance (Painter 1968) of mature green 'Sharwil' avocado to tephritid fruit flies occurring in Hawaii. Our results, however, showed oriental fruit fly infestations in 0.076% of hard fruits, 3.7% of firm ripe fruits, and 10.5% of soft ripe fruits, harvested from March 1992 to May 1993, that met the infestation-free quarantine procedure.

The conflicting results between this and previous studies (Armstrong et al. 1983, Armstrong 1991) are of academic and regulatory interest. There are several hypotheses that may explain these discrepancies: one, sampling method; two, agronomic and horticultural practices in the experimental orchards; and three, prevailing weather conditions. Initial studies conducted by Armstrong et al. (1983) had either 12 or 25 fruits from a single orchard per sampling occasion over a 3- to 4-mo period (most probably between September and December [J. W. Armstrong, personal communication]), with a total of 821 fruits over an \approx 3-yr study period. Validation studies (Armstrong 1991) were done using fruits that were mostly taken from a single packing house. In contrast, results of our study were based on an initial 1,100 fruit samples collected over a 1-wk period, followed by biweekly or monthly collections of 200–300 fruit samples from all certified, productive orchards over a 15-mo period. Thus, the probability of finding a fruit fly infestation was higher with our study than with the previous studies. In addition, Armstrong et al. (1983) did the field census on early-season fruits; whereas, our study included early-season and late-season fruits. Studies conducted in other host commodities have shown that there is a significant variation in the level of susceptibility to tephritid fruit fly infestations between early-season and late-season fruits (Greany 1989).

Visual inspection of each fruit suggests that trees in the orchards used in the study were suffering from physiological disorders caused by water stress, nutrient deficiencies, and, possibly, mechanical injuries that could have rendered them more

susceptible to fruit fly attack. Thus, our second plausible hypothesis is that the probable differences in the farmers' horticultural practices (i.e., irrigation and fertilization) during the two different time periods may have influenced the data gathered. Studies in other commodities have established that farmers' cultural practices significantly affect intensity of insect pest infestations (National Academy of Sciences 1969).

The third hypothesis that could be presented is variation in weather conditions. The occurrence in summer of 1992 of a prolonged drought may have severely water-stressed 'Sharwil' fruits, possibly making them more suitable for oviposition and for the development of eggs and larvae. Initial field infestation studies by Armstrong et al. (1983) were conducted in a windward area with regular rain as well as in a leeward area that at the time did not experience any prolonged drought (confirmed by examination of historic weather data). All certified orchards from which all fruits with oriental fruit fly infestations in 1992 were collected had drought, as revealed by preponderance of fruits with ringneck and sun scalding.

We contend that the difference in sampling methods, changes in fruit physiology resulting from farmers' horticultural practices, or prevailing weather conditions all contributed to the discrepancies between results of our study and the previous studies (Armstrong et al. 1983, Armstrong 1991) that were the bases of the infestation-free quarantine procedure. We conclude that mature green 'Sharwil' avocado fruits harvested with pedicel attached are infestable by oriental fruit fly, and that 'Sharwil' fruits pose a threat to the agriculture of the southern-situated states of the contiguous United States.

Recommendations for Developing and Implementing Infestation-Free Quarantine Procedure. There are at least three situations when known host plants of one or several quarantined insect pest species may be certified infestation-free and be legally marketed across national and international borders: (1) the host plants are commercially grown in geographic areas where the pest populations have been suppressed (Nilakhe et al. 1991) or eradicated (Anonymous 1990) (i.e., infestation-free zone [pest population may be present but not at a "quarantine risk density"], pest-free zone); (2) the host plants have varieties or stages of maturity that are resistant to pest infestations (i.e., genetic resistance) (APHIS 1992b, c); and (3) the host plants are grown when the pest species are not existing or at extremely low abundance (i.e., ecological resistance—phenological asynchrony or pest-free period) (Yokoyama et al. 1992). It is the second of these situations that had been applied to 'Sharwil' avocado in Hawaii (APHIS 1992a).

Because tephritid fruit fly populations are polyphagous and very adaptive in exploiting host resources, we list several recommendations for de-

veloping and implementing an infestation-free quarantine procedure: (1) a detailed ecological survey, encompassing at least 3 yr of data, should be done with regard to the influence of variety and other horticultural attributes of the plant (i.e., age, agronomic and horticultural practices, and responses to various climatic conditions); (2) a detailed sampling plan should be developed that may be used in estimating or monitoring the pest population; (3) after pest-free or infestation-free status has been established, there should be monitoring of host fruits in certified orchards, as well as monitoring of alternative hosts inside the areas surrounding the orchards, because host-switching (i.e., nonpreferred hosts or hosts not commonly infested are utilized when preferred hosts are scarce) is not a rare phenomenon among tropical and subtropical tephritid fruit flies; (4) after infestation-free certification, orchards must have sanitation strictly maintained, and appropriate pest-suppression measures must be implemented (e.g., border spraying with protein bait and insecticide mixture, augmentative releases of parasites, etc.); and (5) because the acceptability of quarantine procedures is determined by importing countries (or by state and federal agencies in the United States), and the rigor of the quarantine procedures and the required scientific background may consequently vary, biological and ecological criteria for certifying, terminating, and reinstating infestation-free quarantine procedure must be defined.

Current Status in Developing Conventional Quarantine Procedure. Although Armstrong et al. (1983), Oi & Mau (1989), and our study confirmed only oriental fruit fly and Mediterranean fruit fly infestations in 'Sharwil' avocado, the efficacy data for a conventional quarantine procedure may also be required for eggs and larvae of melon fly. APHIS records show interceptions in the mainland United States of contraband avocado fruits from Hawaii that were infested with melon fly larvae (U.S. Department of Agriculture 1983). Decades of research in developing a conventional quarantine procedure for 'Sharwil' avocado has been quite unsuccessful. Heat, cold, fumigation, and irradiation treatments that kill tephritid fruit fly eggs and larvae also severely damage the fruits and render them unsuitable for marketing (Seo et al. 1979; H.T.C., unpublished data). Because various cold and heat quarantine treatments have been certified against Mediterranean fruit fly, oriental fruit fly, and melon fly infestations in many commodities (Fiskaali 1991), our recent approach has been to develop procedures that may alleviate injuries of fruits (darkening of skin and lumpiness of flesh) from these treatments. For instance, H.T.C. (unpublished data) found that preconditioning 'Sharwil' fruits at 38°C for 8–17 h before a cold treatment of 1.1°C for variable number of days prevents the development of greyish, darkened skin, the most common manifestation of chilling injury. The use of warm temperature as a precon-

ditioning procedure before subjecting 'Sharwil' fruits to heat treatments (e.g., vapor heat, high-temperature forced air, and hot-water immersion) is currently being investigated. Once marketable fruit quality is assured, we contend that currently registered efficacious cold and heat treatments for Mediterranean fruit fly, oriental fruit fly, and melon fly infestations may be certified for 'Sharwil' avocado.

Acknowledgments

We thank each cooperating farmer for allowing us to conduct fruit sampling and hang traps in their properties. Special thanks to Glenn Hinsdale and Mike Scharf (USDA-APHIS, Honolulu and Kona, respectively) for their assistance in sampling "certified fruits" from "certified orchards." Paul Barr, Laurel Dekker, Lori Campbell (USDA-ARS, Hilo), James Ragual (University of Hawaii, Honolulu), and Ed McAvoy (USDA-APHIS, Mani) assisted in all aspects of fieldwork. Charmaine Sylva, Marlene Salmo, Chris Matsuo, and Brandie Erbe assisted in processing fruit samples for morphology, physical attributes, and degree of fruit fly infestation. Suzanne Sanxter and Kate Nishijima (USDA-ARS, Hilo) assisted in quantifying fruit dry matter content. Paul Barr assisted in data management. An early version of the manuscript was reviewed by Robert Mangan (USDA-ARS, Weslaco, TX), Victoria Yokoyama (USDA-ARS, Fresno, CA), and James Hansen (USDA-ARS, Miami, FL).

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Received for publication 12 January 1994; accepted 15 September 1994.