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Enhancement of Citrus Resistance to Tephritid Fruit Flies

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C. ABSTRACT

The plant growth regulator gibberellic acid (GA_3) was successfully employed to reduce senescence-related susceptibility of citrus fruit to fruit fly attack. Larval survival was affected to a greater degree than ovipositional suitability. Oranges were more easily protected than grapefruit in tests against the Mediterranean fruit fly (Ceratitis capitata) in Israel. Oranges treated with 50 ppm GA_3 retained a green cast to some degree throughout the season, but internal ripening was not affected. Treatment effects were dose-dependent (50 ppm $\gg$ 20 ppm $>$ 5 ppm), and were most effective when GA_3 was applied prior to colorbreak.

Tests in Florida against the Caribbean fruit fly (Anastrepha suspensa) showed that grapefruit could be protected to a significant degree by early-season treatments with GA_3 . Benefits were dose-dependent (100 ppm $\gg$ 50 ppm $>$ 20 ppm). Some increase in susceptibility occurred despite treatment; this coincided with decreased peel oil content of treated and control fruit. Treated fruit exhibited greater puncture resistance throughout the season, however. Benefits associated with GA_3 treatment were therefore correlated with retained physical toughness of the peel rather than peel oil content.

Comparative tests on the resistance of lemons and grapefruit to fruit flies suggested that lemon immunity is due to the abundance of peel oil rather than relative oil toxicity differences; lemons possess ca. 2x more oil per cm^2 than grapefruit, but lemon oil was no more toxic than grapefruit oil to A. suspensa eggs or larvae.

D. OBJECTIVES OF RESEARCH

The specific objectives of the research were as follows:

- (1) To determine whether preharvest treatment of citrus fruit by the senescence-retarding growth regulator gibberellic acid (GA_3) could be used to reduce preharvest susceptibility of citrus fruit to attack by the Mediterranean and Caribbean fruit flies.
- (2) To define the chemical and physical bases for increased susceptibility of senescent citrus fruit
- (3) To elucidate the mechanisms accounting for virtual immunity of lemons to fruit fly attack.

E. INTRODUCTION, METHODS, RESULTS, AND CONCLUSIONS

The true fruit flies (Diptera: Tephritidae) are perhaps the single greatest threat to citrus production worldwide, not only because of the direct damage they cause, but also because of the need to fumigate exported fruit to meet the quarantine requirements of many importing states/nations. Various methods are used to deal with chronic fruit fly problems in citrus, including use of bait sprays directed against the adult fly population, but no preharvest method currently exists to reduce damage to the fruit should infestation occur. Instead, reliance is placed upon a variety of postharvest treatments including fumigation, exposure to heat or cold, or gamma irradiation.

A potential alternative to use of conventional means of dealing with fruit flies in citrus became apparent during the course of earlier work (Greany et al. 1983, 1985) on the innate resistance of citrus fruit to fruit fly attack, and on the decline in resistance of senescent fruit. We recognized that tephritid fruit flies are similar to plant pathogens in that they are also best able to attack citrus fruit when senescent. We took note of the fact that the resistance of citrus to plant pathogens had been successfully manipulated by treating the fruit early in the season, prior to colorbreak, with the plant growth regulator gibberellic acid (GA_3) (Coggins 1973), and we determined to test its use for fruit fly prophylaxis.

The principal focus of this project, then, was to determine whether GA_3 could be used to reduce the susceptibility of citrus

fruit to two tephritid fruit flies, the Mediterranean fruit fly (Ceratitis capitata) and the Caribbean fruit fly (Anastrepha suspensa) in Israel and Florida, respectively. In addition, we sought to define the chemical and physical bases for senescence-related susceptibility of citrus fruit, and to identify the mechanism(s) accounting for the virtual immunity of lemons to fruit fly attack, in contrast with grapefruit, which are highly resistant only early in the season.

In keeping with these plans, we performed two seasons' research on gibberellic acid use for fruit fly prophylaxis in both locations. We also performed numerous tests on the chemical and physical bases for resistance of citrus fruit to the Caribbean fruit fly, and measured senescence-related changes in selected parameters. The results of these studies are presented below, beginning with the work in Israel.

MEDFLY STUDIES - ISRAEL

MATERIALS AND METHODS

PRELIMINARY TESTS 1982/3 - DETERMINATION OF FLY DENSITY FOR CITRUS FRUIT EXPOSURE.

The tests were carried out in the laboratory in plastic cages (38 x 32 x 16 cm) with glass covers. Each cage was provided with water and adult diet (3 parts yeast hydrolyzate and 1 part sucrose). Flies used for these tests derived from the regular laboratory colony (as were the flies used in all subsequent tests).

Four fully mature grapefruits were placed in each cage and flies were added to each cage at a predetermined density, and an oviposition period of 48 hours. Each fly density was tested in 4 replicates (except for the highest density, which had only 2 replicates). Fly densities tested were 1, 5, 10, 25 and 50 females per fruit; thus, a total of 4 females and 4 males were used in the lowest density, 20 females and 20 males at the next density, etc. At the end of the oviposition period the individual fruits were inspected for oviposition punctures and dissected to count eggs. This test and all the subsequent tests were carried out at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

We analyzed the effect of fly density on the following parameters (with the Duncan multiple range test):

1. No. of oviposition punctures per fruit,
2. No. of eggs per fruit,
3. No. of eggs per oviposition puncture,
4. No. of eggs and oviposition punctures per female.

TESTS WITH GIBBERELLIC ACID IN 1982/83.

The application of GA_3 on citrus.

The tests were carried out on the following citrus varieties: Marsh grapefruit, Shamouti and Valencia oranges. All 3 varieties were located in the "Gan-Haim" grove, situated in the coastal region of Israel. The grove was maintained under a biological control regime and received no pesticide applications during the experiment period or in the previous years. The native Mediterranean fruit fly population was controlled by the grove owners through ground applications of malathion bait sprays; the experimental trees were avoided.

A commercial preparation of GA_3 (Pro-Gibb^R 10% soluble powder) was used for the gibberellic acid treatments.

The following GA_3 treatments were applied:

1. Controls (0 ppm),
2. 5 ppm GA_3 ,
3. 50 ppm GA_3 .

For each citrus variety, 3 trees were selected and each was treated with 40 litres of the appropriate GA_3 mixture. GA_3 was applied at 2 separate dates, an early application on September 23, 1982 and on October 18, 1982.

Exposure of citrus fruits to medflies in the laboratory (1982/83).

Fruit was exposed in the laboratory to medflies at predetermined intervals. A total of 12 fruits from each treatment and variety were brought to the laboratory and placed in plastic cages with 5 pairs of, 5

days old, medflies per fruit. The flies remained in the cages for an oviposition period of 3 days. Two cages with 6 fruits were used for each testys and treatment. Of these, 2 fruits were dissected and the number of oviposition punctures and eggs were counted. (Except for the first 3 tests which were "choice tests were carried out as will be described in the RESULTS section.) The remaining 4 fruits were held for emergence and pupation of larvae. Thus, a total of 4 fruits were dissected and 8 fruits were held for pupation for each treatment in each test, and for each citrus variety tested.

Exposure of citrus fruit to medflies in the field (1982/83).

Fruit was exposed to medflies in the field at predetermined dates (concomitant with the lab. exposure). Small, cylindrical net cages (15 x 15 cm) were prepared and placed around individual fruits on the trees for a period of 3 days. Five pairs of medfly males and females were placed in each cage; a single sugar cube was added (no water was provided). The flies were from the regular laboratory colony and 5 days old when placed in the cages. The temperature was measured inside the tree canopy with a RYAN^R temperature recorder during the exposure period.

Fruit from each treatment were brought, at the end of the oviposition period, to the laboratory for the following inspection:

1. Twenty fruits were dissected and oviposition punctures as well as eggs were recorded,
2. Thirty fruits were held together for fly development and emergence.

Internal maturation.

Fruits were checked for internal maturation and color change during the test period. The following maturation parameters were checked: Percent juice, & T.S.S. (total soluble solids), % acid and the maturation ratio (%T.S.S. / % acid).

TESTS WITH GIBBERELIC ACID IN 1983/84.

The application of GA_3 on citrus.

The tests in 1983/84 were carried out in two groves near Rehovot (coastal plain of Israel): A Marsh grapefruit and Shamouti orange grove inside the "Weizmann Institute" in Rehovot and a Valencia orange grove in a backyard in the village of Gibton (east of Rehovot).

GA_3 was applied at rates of 20 and 50 ppm using 40 litres spray mixture per tree. The Marsh grapefruits were treated on September 20, 1983, the Shamouti oranges on October 18, 1983 and the Valencia oranges on November 20, 1983. All the trees were treated prior to colorbreak of the respective fruit variety. Fifteen Trimedlure traps were placed in the "Weizmann" citrus grove. Of these, 9 were located inside the experimental plot and 6 in the surrounding plots. The traps were checked weekly and the fly population recorded. Medfly control was directed by the the inspections of these traps, and malathion bait mixture was applied from the ground to the surrounding plots only, thus maintaining a natural fly population in the experimental plots. Medfly control in the Valencia grove was carried out regularly by the regional control

unit, avoiding the experimental trees. No additional traps were placed in that grove and the fly population was not monitored by us.

Infestation of citrus fruit by natural medfly population.

The natural infestation of citrus by the medfly was monitored on the Marsh grapefruit and Shamouti oranges in the Weizmann grove. A total of 24 fruits were picked from each variety on 3 different dates during the season. The fruits were picked randomly from different trees but so as to represent the entire treated plot. Each fruit was inspected for oviposition punctures and held individually for emergence and pupation of larvae. At the end of the season, 500 additional fruits were commercially harvested and were carefully inspected in the field for oviposition punctures. Unfortunately the grapefruits were picked by the grove owner without notifying us and hence only the Shamouti oranges were inspected for natural medfly infestation at the end of the season.

Exposure of citrus fruits to medflies in the laboratory (1983/84)

Fifty fruits of each variety and treatment (controls, 20 and 50 ppm GA₃) were brought to the laboratory at predetermined intervals. The fruits from each treatment were placed together in a large screen cage (130 x 70 x 30 cm) in a "no-choice" test with 250 pairs of 5-6 days old laboratory-reared medfly males and females. Each cage was provided with water and adult food and placed in a room with continuous light at 25°C ± 1°C. The flies were kept in the cage for an oviposition period of 2 days and any dead flies were replaced with fresh flies. At the end of the oviposition period the fruits were divided into 2 lots: 20 fruits

were individually inspected for oviposition punctures and dissected to count the eggs in the punctures. The remaining 30 fruits were inspected individually for oviposition punctures and then placed in cages for larval emergence and pupation. The color of the Valencia oranges was also individually assessed prior to their placement in the cages.

RESULTS

PRELIMINARY TESTS 1982/3 - DETERMINATION OF FLY DENSITY FOR CITRUS FRUIT EXPOSURE.

The number of oviposition punctures per fruit increased with the fly density from up to the density of 25 females per fruit. A further increase to 50 females per fruit reduced the number of the oviposition punctures (Table 1). The number of eggs per fruit increased with the number of females, although no significant difference could be observed between the densities of 5 and 10 females per fruit or 25 and 50 females per fruit. The presence of numerous females seemed to trigger repeated oviposition in the same oviposition puncture, and the average number of eggs increased from 7.3 eggs per puncture for a single female to 20.6 - 25.8 per puncture for 5 - 25 females. We can not know whether more than one female oviposited in a single oviposition puncture.

The number of oviposition punctures per female (Table 2) dropped significantly (from 6.94 to 2.51) when 5 females were available for each fruit. This could be due to repeated oviposition in the same puncture by the same or different females. There was no difference in the number of eggs per female when 1 or 5 females were present per fruit (Table 2). It

dropped however significantly when the density of females reached 10 per fruit or more . Thus a density of more than 5 females caused interference among females and prevented them from exerting their full ovipositional potential.

We chose the density of 5 females per fruit could be the best compromising choice for our further tests with GA_3 .

TESTS WITH GIBBERELIC ACID IN 1982/83.

Exposure of citrus fruits to medflies in the laboratory (1982/83).

Choice tests. In the first 2 exposures of grapefruits and the first exposure of the Shamouti oranges to medflies in the laboratory, we carried out small choice tests in the following manner: Two fruits of each of the 2 GA_3 treatments and the "controls" were placed in an oviposition plastic cage (6 fruits per cage). A total of 3 cages were arranged in that manner. Five males and females per fruit, were placed for an oviposition period of 3 days. The fruit was then dissected and checked for oviposition punctures and eggs. These tests were carried out concomitantly with the "no-choice" tests where each oviposition cage contained 6 fruits of the same treatment. The results did not lend themselves to statistical analysis. They did however reveal the effect of the September GA_3 treatment on grapefruit and Shamouti (Table 3). Less eggs and less oviposition punctures were found in GA_3 treated fruit compared to non-treated fruit. The 50 ppm GA_3 treated fruit was the least infested. The treatment of grapefruits with GA_3 on October 18, 1982 had no effect on the oviposition of the medfly (Table 3).

The rest of the exposure tests were carried out in a "no-choice" manner.

When discussing the results of all the tests which were performed in the course of the study, we will distinguish between the "acceptance" of the fruit by the medfly females (as expressed by the number of oviposition punctures and eggs per fruit, and the number of eggs per oviposition puncture) and "suitability" of the fruit which denotes the ability of the eggs and larvae of the fly to complete its development and emerge from the fruit. The latter is expressed by the number of pupae produced.

The results of the "no-choice" laboratory exposures of fruits to medflies are summarized in Tables 4 - 9.

Marsh grapefruits (Tables 4 & 5). The treatment of GA_3 on October 18, had no effect on the acceptance or suitability of the fruit to medfly infestation (Table 5). It seemed that the date was too late to exert any effect on the treated fruit. Fruit treated on September 20 did however show GA_3 related differences although statistically not significant (Table 4). Thus, the number of eggs per fruit as well as the number of the oviposition punctures per fruit decreased with the increase in the treatment level. The highest treatment level (50 ppm) produced the least eggs and punctures per fruit. The differences for the individual exposure dates were not statistically significant but the overall treatment averages showed significant differences. The number of eggs per punctures was similar for all 3 treatments except for the early exposure date (Nov. 14, 1982), where the poor oviposition in the GA_3 treated fruit resulted (obviously) in poor density of eggs per puncture. The controls and the 5 ppm treatment showed also an increase

in the susceptibility when the season progressed. Not so with the 50 ppm treatment where the fruit susceptibility remained more or less stable throughout the season. The most prominent effect of the GA_3 treatment on September 20 was on the suitability of the treated fruit as expressed by the number of emerging larvae and pupae produced. Here, the 50 ppm GA_3 treatment undoubtedly had a strong negative effect. Very few pupae (or none) were produced by the 50 ppm treated fruit throughout the season, regardless of the number of eggs oviposited in the treated fruit.

Shamouti oranges (Tables 6 & 7). The most striking result in the tests of laboratory exposure of the Shamouti oranges to medflies was the very low emergence of larvae from fruits with heavy oviposition, regardless of treatment level or application date. These results changed very little during the season and it must be concluded that the Shamouti oranges (at least in our tests) were not suitable for the development of the pre-imaginal stages of the medflies. The GA_3 treatment on September 23 affected oviposition (Table 6) until December 3, 1982. Fruits treated with 5 and 50 ppm GA_3 had significantly less eggs and oviposition punctures than the "controls" fruit. The difference between the 5 and 50 ppm treatments was not statistically significant, although much less eggs and punctures were found in the 50 ppm treatment. The significant difference among the non-treated and treated fruit on December 3, and March 9, and the trend towards reduced oviposition on March 4, contributed to the overall significant difference among treated and non-treated fruit, as expressed in the treatment averages in Table 6. There seemed to be no clear trend towards susceptibility of fruit

in the various treatments during the season (Table 6 - horizontal comparisons and Averages/dates). We saw no difference in the number of eggs per puncture among treatments. The low suitability of the fruit prevented from drawing any conclusion as to the effect of GA_3 on same. There was no effect of the October 18 treatment on the fruit's susceptibility (Table 7).

Valencia oranges (Tables 8 & 9). As with the Shamouti oranges, very few pupae were produced by the fruits exposed to the medfly in the laboratory. Oviposition seemed also to be lower than in the Shamouti oranges. There was no significant difference among treatments nor among different exposure periods in the season. There even seemed to be a trend towards reduced susceptibility of the Valencia oranges to oviposition by the medflies during the season.

Exposure of citrus fruits to medflies in the field (1982/83).

Marsh grapefruit (Tables 10 & 11). The GA_3 treatments did not affect the acceptance of the grapefruits to medflies in the field exposure tests. In each of the exposures dates there was no differences in the number of eggs and the oviposition punctures per fruit in the various treatments. There seemed also to be no increase in the susceptibility of the fruit in the season. The Weather seemed to be the governing factor in the oviposition behaviour and success of the flies. Thus, in February 11, 1983 temperatures were rather high and resulted in a high infestation level of grapefruits (Table 10). Six days later (February 17, 1983) the temperatures dropped to $10^{\circ}C$ and below and hence no oviposition or larval development occurred (Table 11) regardless

of treatments. The suitability of the fruit was very low early in the season, and very few pupae emerged from the heavily infested fruit. At a later date, (during the month of December) when the fruit became more suitable for medfly development there seemed to be a certain effect of the GA_3 treatment (in both application dates) on the survival of larvae in the fruit, and few pupae were produced by the GA_3 treated fruit. The effect of the GA_3 seemed also to be dose related (Tables 10 & 11). These apparent differences did however disappear completely in February (Table 10) and numerous pupae emerged from all the exposed fruits. Thus it seems as if the GA_3 treatment had no effect on oviposition but did reduce the pupation until the end of December for both application dates.

Shamouti oranges (Tables 12 & 13). The weather until March 11th, was the dominant factor preventing oviposition and survival of medflies in the Shamouti oranges. Thus, the data pertaining to the early application date of GA_3 were completely unrelated to the treatment or treatment dose. Mid-march is usually the end of the picking season of Shamouti. The data for the first two exposures of the later application date (October 18, Table 13) were also completely overshadowed by the rough weather and low temperatures. Only in March 11 were the temperatures high enough to permit oviposition and subsequently the development of eggs and larvae to the pupal stage (Table 13). The last exposure of fruits to medflies was carried out in April 22, well beyond the end of the picking season. The acceptability of the Shamouti fruit could therefore be assessed only from the March 11th exposure date (Table 13), and showed no correlation to the GA_3 treatments (Table 13). There

was however a marked effect of the 50 ppm GA_3 treatment on the suitability of the fruit where no pupae emerged, in contrast with the "5 ppm" and "control" fruit, where relatively high numbers of pupae were produced. It seemed therefore that the 50 ppm had an effect on pupation. However, that effect subsided completely in April 22nd, and pupae were produced in all treatments.

Valencia oranges (Tables 14 & 15). The effects of the cold and rainy season during the test period, overshadowed here too the effects of the GS_3 treatments during the month of January. March and April, on the other hand, were relatively warm, and did not mask the effects of the treatments. Little or no effect of the treatment on the acceptability of the fruits (in terms of oviposition parameters) was evident for both application dates (Tables 14 & 15). In two cases (Table 14, March 18 and Table 15, April 29) there were significantly more eggs in the GA_3 treated fruit. There was however no increase in the number of oviposition punctures in March 18 and some increase in the number of oviposition punctures in the 5 ppm treatment only in April 29. The increased egg number could be explained by the increase in the number of eggs per puncture. It might suggest that repeated oviposition took place in those fruits, where convenient spots for oviposition were detected by the females. The fruit as a whole was not necessarily more susceptible than the untreated fruit. Except for the April 29th exposure (Table 15), the treated fruit produced no pupae regardless of the oviposition rate, repeating the results obtained with the Shamouti and grapefruits in the previously described tests.

Internal maturation of citrus fruit treated with GA₃ (1982/93).

The internal maturity tests showed no effect of the GA₃ treatments on the parameters checked. We therefore did not present the individual data nor have we analyzed them statistically. Grapefruits were checked on 4 different dates in the season beginning on November 11 and ending on February 14. Fruit in both GA₃ application dates (including controls) reached the final maturity level already in November and that did not change to the end of the season. The Shamouti oranges were checked on December 5 and January 31. There was a slight increase in the % T.S.S. which obviously increased the ratio T.S.S./acid from December to January for both treated and untreated fruit. The Valencia oranges were checked on 3 dates, beginning on January 17 and ending on May 2. Here too, the ratio T.S.S./acid increased in the season, but no effect of the GA₃ treatment was evident. Thus, although GA₃ had a retarding effect on colorbreak (which was not measured, but only observed) it had no effect on the internal quality of the fruit.

Tests with gibberrellic acid 1982/83 - general conclusions.

1. Field vs. Lab exposures of fruit to medflies. The unpredictability of the weather during the test period, interfered with the field exposures tests and rendered the results uninterpretable. Thus, although field exposure would obviously be expected to be closer to the natural situation, we decided not to use them in the second year, and concentrate instead on laboratory exposures. The exposure period was however reduced in the second year to 2 days, to minimize the effects of

post-harvest fruit maturation on the susceptibility of the fruit to medfly attack.

2. The effect of the application date. The early treatment date (September 23, 1982) seemed to be more effective than the later date (October 18, 1982) in grapefruits. In the Sahmouti oranges the later application date reduced oviposition (in the lab. exposure) up to February, whereas the early application date seemed to lose its effect in January (Tables 6 & 7). The susceptibility of the Valencia oranges was not affected by the application date.

3. Overall effect of GA_3 on the susceptibility of citrus fruit to medfly attack. The results suggested that the effect of GA_3 on the acceptability of the fruitg (oviposition) was very low or non-existent. On the other hand the GA_3 application diud affect the suitability of the fruit, reducing the survival of the pre-imaginal stages of the medfly in the infested fruit. The effect of the lower treatment level (5 ppm) was much less evident than the higher level (50 ppm). The effect on grapefruits subsided rather early in the season, whereas for Shamouti and Valencia oranges it seemed to be present to a late date in the season.

4. Effect on internal maturation. GA_3 , in both application dates, had no apparent effect on the internal maturation parameters.

TESTS WITH GIBBERELIC ACID IN 1983/84.

Exposure of citrus fruits to medflies in the laboratory (1983/84).

Marsh grapefruit (Table 16 & 19). The acceptability of the grapefruits was not affected by the treatment with GA_3 throughout the season. There seemed however to be a difference (although statistically not significant) in the % fertile punctures among treatments in November and December. We could also see a significant increase in the % fertile punctures during the season in the GA_3 treated fruit. It might indicate that females attempted to oviposit, but declined from laying eggs in some of the oviposition attempts in the treated fruit early in the season. Could that be interpreted as avoidance of laying eggs in an unsuitable fruit? This question remains to be answered.

The suitability of the grapefruits did improve during the season, and significantly more pupae were produced per fruit in January and February than in November and December. In the earlier dates, GA_3 treated fruit produced less pupae than untreated fruit (statistically not significant) and the production of pupae seemed to be negatively dose related.

Shamouti oranges (Table 17 & 19). The acceptability of the Shamouti oranges was not affected by the GA_3 treatment. There was also no change in the acceptability of the fruit during the season. The treatment did however affect the suitability of the fruit and less pupae emerged from the treated fruit (Table 19). The differences were not significant in the exposures of December, and were significant in the

exposures of February and March (Table 17). The overall emergence of pupae from the infested fruit was very low (as was the case in the previous year), but it did increase during the season and the emergence of pupae in March was somewhat and significantly higher than in previous dates (Table 17).

Valencia oranges (Table 18 & 19). The results with GA_3 treatment on Valencia oranges were most interesting. GA_3 affected both the acceptability and suitability of the Valencia oranges to medfly. Both GA_3 treatments resulted in significantly less eggs and less oviposition punctures per fruit. There was also a reduction in the number of pupae produced by the treated fruit, but as the total number of pupae was very low and highly variable, the results were not significantly different. The non-treated fruit became more susceptible to medfly attack as the season progressed. Not so the treated fruit, which remained more or less resistant to the medfly attack (expressed in the number of eggs per fruit).

The most striking effect of GA_3 was on colorbreak. We have recorded the color of each of the 50 Valencia fruits that was brought to the laboratory for the various tests. The grading was according to the Colour Guide for Citrus Standard (Citrus Marketing Board, 1969). Grade 1 = full orange color, grade 3 = orange with green shadows, 6 = green with orange shadows, 8 = totally green. The results were analysed separately for the fruits used for dissection (20 fruits - Table 20) and those which were kept for the emergence of pupae (30 fruits - Table 21). Colorbreak was clearly retarded in the GA_3 treated fruit to the end of the season, with little difference between both GA_3 treatments. Fruit

color was significantly and positively correlated with the dose of GA_3 applied (Table 22). We checked the correlation between the fruit color (as affected by the GA_3 treatment) and various parameters of infestation by the medfly (Table 23), and found good correlations between color and eggs and oviposition punctures per fruit. There was also a correlation between the fruit color and the eggs per puncture which might indicate that in the less ripe fruit, repeated oviposition becomes more frequent.

The results of the 1983-84 laboratory exposures are depicted in Figure 1; it should be noted that while an effort was made to show the color effect of GA_3 on the fruit, it is difficult to represent this faithfully. Still, the figures approximate differences in color of treated vs. control fruit as well as differential susceptibility to fly attack as a result of treatment.

Infestation of citrus fruit by natural medfly population.

Regardless of the very large medfly population that prevailed in the grove throughout the citrus season, the natural infestation of the oranges and grapefruits was negligible and could not be related to the treatments in the test. The frequencies of infested Shamouti and Valencia oranges (punctures/fruit) is presented in Table 24. The results indicated non-randomness of infestation in all treatments on Shamouti oranges and in the non-treated Valencia oranges. There was, however, no trend that could be related to the GA_3 treatment level.

Tests with gibberellic acid - 1983/84: general conclusions.

The reliance on natural infestation by field populations of the medfly proved to be hazardous. The results of the laboratory exposure of fruits to medflies produced interesting results:

1. Grapefruit responded little to the GA_3 treatments.
2. Shamouti oranges became less suitable for the development of medfly eggs and larvae (and emergence of pupae) as a result of the GA_3 treatments.

3. Valencia oranges were the most responsive to GA_3 treatments, both in acceptability (oviposition) and suitability (development of the pre-imaginal stages) of the medfly. The response was correlated with the colorbreak retardation caused by the GA_3 treatment.

REFERENCE.

- 1969. Colour Guide for Citrus Standard, (Coloured Plates).
Citrus Marketing Board of Israel.

Table 1. Preliminary test - effect of fly density on fruit infestation parameters.

Pairs/ fruit	No. of fruits	Total punct.	Punctures / fruit		Eggs / fruit		Eggs / puncture	
			average + S. E.		average + S.E.		average + S. E.	
1	16	111	6.94+ 0.75 d		50.63+ 5.56 c		7.30+ 0.59 d	
5	16	201	12.56+ 0.79 c		258.31+ 19.40 b		20.56+ 1.19 bc	
10	16	296	18.50+ 1.36 b		330.56+ 24.87 b		17.87+ 0.91 c	
25	16	382	23.88+ 0.92 a		517.38+ 49.84 a		21.67+ 1.00 b	
50	8	165	20.63+ 2.29 ab		532.50+ 64.26 a		25.82+ 1.73 a	

DF (4,67)	DF (4,67)	DF (4,1150)
F=37.84	F=34.43	F=20.58
PR>F=0.0001	PR>F=0.0001	PR>F=0.0001

Table 2. Preliminary test. Interference among medfly females
ovipositing on citrus fruit. (Effect of flies density).

Pairs / fruit	Total No. of females	Eggs / female		Punctures / female	
1	16	50.63	a	6.94	a
5	80	51.66	a	2.51	b
10	160	33.06	b	1.85	bc
25	400	20.70	c	0.96	bc
50	400	10.65	c	0.41	c

DF (4,13)

F=24.66

PR>F=0.0001

DF (4,13)

F=22.10

PR>F=0.0001

Table 3. Infestation of citrus fruits treated with GA₃. Medflies given choice of treated and untreated fruits.

Item	Treatment		
	Controls	5 ppm GA ₃	50 ppm GA ₃
Grapefruit treated with GA ₃ on September 23, 1982. Exposed on November 14.			
Eggs/ fruit	55.50 _± 38.15	1.50 _± 1.50	4.83 _± 4.83
Punc./ fruit	2.83 _± 1.66	0.17 _± 0.17	0.17 _± 0.17
Eggs/ punc.	19.59 _± 3.05	9.00 _± 0	29.00 _± 0
Grapefruit treated with GA ₃ on October 18, 1982. Exposed on November 26.			
Eggs/ fruit	32.83 _± 15.18	36.17 _± 11.13	18.50 _± 8.78
Punc./fruit	3.00 _± 0.52	4.67 _± 0.92	1.83 _± 0.48
Eggs/ punc.	10.94 _± 3.79	7.75 _± 1.31	10.09 _± 2.77
Shamouti treated with GA ₃ on September 23, 1982. Exposed on December 3.			
Eggs/ fruit	26.33 _± 10.69	7.00 _± 4.61	2.83 _± 2.29
Punc./fruit	6.33 _± 2.33	3.17 _± 1.25	1.33 _± 0.61
Eggs/ punc.	4.16 _± 0.94	2.21 _± 1.13	2.13 _± 1.74

Table 4 . Infestation of GA₃ treated grapefruit with the Mediterranean fruit fly, following GA₃ treatment in September 23, 1982. Fruits exposed to the flies in the lab.^{1,2/}

Item	Treat.		Date checked				Treatment average ^{3/}
	GA ₃ ppm		Nov. 14, 1982	Dec. 17, 1982	Feb. 11, 1983		
Eggs/	0		42.67 $\pm$ 35.75 a B	81.00 $\pm$ 6.15 a AB	142.00 $\pm$ 54.86 a A		81.79 $\pm$ 23.30 a
fruit	5		1.67 $\pm$ 1.67 a B	80.25 $\pm$ 16.31 a A	92.75 $\pm$ 22.86 a A		50.14 $\pm$ 13.77 ab
	50		0 a A	63.50 $\pm$ 28.09 a A	61.75 $\pm$ 6.99 a A		35.79 $\pm$ 11.36 b
Average/date ^{4/}			14.61 $\pm$ 12.16 B	74.92 $\pm$ 10.26 A	98.83 $\pm$ 20.61 A		
Punctures/	0		1.33 $\pm$ 0.95 a C	8.25 $\pm$ 0.48 ab B	13.00 $\pm$ 2.94 a A		6.64 $\pm$ 1.62 a
fruit	5		0.17 $\pm$ 1.67 a B	10.50 $\pm$ 1.55 a A	11.00 $\pm$ 2.55 a A		6.21 $\pm$ 1.64 a
	50		0 a B	5.25 $\pm$ 1.31 b A	6.00 $\pm$ 0.41 b A		3.21 $\pm$ 0.85 b
Average/date ^{4/}			0.50 $\pm$ 0.33 B	8.00 $\pm$ 0.90 A	10.00 $\pm$ 1.48 A		
Eggs/	0		31.63 $\pm$ 7.33 a A	9.82 $\pm$ 1.45 a B	10.92 $\pm$ 1.02 a B		12.31 $\pm$ 1.15 a
puncture	5		10.00 $\pm$ 0.00 b A	7.64 $\pm$ 1.14 a A	8.43 $\pm$ 0.93 a A		8.07 $\pm$ 0.72 b
	50		0	12.09 $\pm$ 2.37 a A	10.29 $\pm$ 1.76 a A		11.13 $\pm$ 1.44 a
Average/date ^{4/}			29.22 $\pm$ 6.89 A	9.37 $\pm$ 0.88 B	9.88 $\pm$ 0.66 B		
Puparia/	0	-		5.38 $\pm$ 1.18	16.50 $\pm$ 1.32		10.94 $\pm$ 1.67
fruit	5	-		3.75 $\pm$ 0.76	41.37 $\pm$ 2.22		22.56 $\pm$ 4.99
	50	-		0	1.00 $\pm$ 0.19		0.50 $\pm$ 0.16
Average/date ^{4/}				3.04 $\pm$ 0.65	19.62 $\pm$ 3.56		

1/ Duncan multiple range test $\alpha = 0.05$

2/ Lower case letters - vertical comparison, capital letters - horizontal comparison

3/ All dates pooled.

4/ All treatments pooled.

Table 5 . Infestation of GA₃ treated grapefruit with the Mediterranean fruit fly, following GA₃ treatment in October 18, 1982. Fruits exposed to the flies in the lab. ^{1,2/}

Item	Treat.		Date checked				Treatment Average ^{3/}
	GA ₃	ppm	Nov. 26, 1982	Dec. 24, 1982	Feb. 18, 1983		
Eggs/ fruit	0		53.33±18.28 a B	133.50± 9.13 a A	94.50±31.96 a AB		88.00±14.63 a
	5		17.67± 5.26 a B	160.00±22.54 a A	106.00±14.57 a A		83.57±18.29 a
	50		18.50± 5.20 a B	208.50±71.51 a A	31.25± 1.25 a B		76.43±29.67 a
	Average/date ^{4/}		29.83± 7.37 C	167.33±24.62 A	77.25±14.51 B		
Punctures/ fruit	0		6.33± 0.84 a A	11.00± 1.78 a A	10.50± 2.40 a A		8.86± 1.04 a
	5		5.17± 0.91 a B	12.50± 0.96 a A	10.25± 1.44 a A		8.71± 1.06 a
	50		4.00± 0.63 a B	14.50± 3.62 a A	3.75± 0.48 b B		6.93± 1.65 a
	Average/date ^{4/}		5.17± 0.49 C	12.67± 1.32 A	8.17± 1.27 B		
Eggs/ puncture	0		8.42± 1.42 a A	12.14± 1.46 a A	9.00± 1.09 a A		9.94± 0.78 a
	5		3.42± 0.58 b B	12.80± 1.60 a A	10.34± 0.92 a A		9.59± 0.18 a
	50		4.63± 0.98 ab B	14.38± 1.33 a A	8.33± 1.05 a B		11.03± 0.95 a
	Average/date ^{4/}		5.77± 0.70 C	13.21± 0.84 A	9.46± 0.63 B		
Puparia/ fruit	0		-	0.25± 0.10	16.75± 0.66		8.50± 2.15
	5		-	0.25± 0	32.75± 2.17		16.50± 4.33
	50		-	1.50± 0.57	18.75± 2.17		10.13± 2.48
	Average/date ^{4/}			0.67± 0.22	22.75± 1.79		

1/ Duncan multiple range test, $\alpha = 0.05$

2/ Lower case letters - vertical comparison. Capital letters - horizontal comparison.

3/ All dates pooled.

4/ All treatments pooled.

Table 6 . Infestation of GA₃ treated shamouti oranges with the Mediterranean fruit fly, following GA₃ treatment in September 23, 1982. Fruits exposed to the flies in the lab. ^{1,2/}

Item	Treat.	Date checked				Mar. 9, 1933		Treatment Average ^{3/}	
	GA ₃ ppm	Dec. 3, 1982	Jan. 28, 1983	Mar. 4, 1983	Mar. 9, 1933				
Eggs/	0	36.83± 6.50 a AB	15.75± 8.25 a B	39.00±24.12 a AB	72.75±32.52 a A	40.61± 9.64 a			
fruit	5	9.83± 6.94 b A	23.25±12.19 a A	14.25± 5.92 a A	5.50± 2.22 b A	12.83± 3.80 b			
	50	0.67± 0.67 b A	27.50±15.16 a A	11.25± 6.52 a A	1.00± 1.00 b A	9.05± 4.18 b			
Average/date ^{4/}		15.78± 4.77 A	22.17± 6.54 A	21.50± 8.60 A	26.41±13.95 A				
Punctures/	0	5.67± 0.95 a B	5.25± 0.85 a B	5.25± 1.44 a B	9.75± 1.97 a A	6.17± 0.68 a			
fruit	5	1.50± 0.43 b B	5.25± 0.75 a A	2.25± 0.63 a B	2.50± 0.29 b AB	2.72± 0.42 b			
	50	0.33± 0.21 b B	4.50± 1.19 a A	1.75± 1.44 a AB	1.50± 0.87 b B	1.83± 0.56 b			
Average/date ^{4/}		2.50± 0.65 C	5.00± 0.51 A	3.08± 0.79 BC	4.25± 1.17 AB				
Eggs/	0	6.50± 1.25 a A	3.00± 1.37 a A	7.43± 2.66 a A	8.31± 2.23 a A	6.59± 0.98 a			
puncture	5	6.56± 3.06 a A	4.43± 1.61 a A	6.33± 0.89 a A	2.20± 0.89 a A	4.71± 0.96 a			
	50	2.00± 2.00 a A	6.11± 1.95 a A	6.43± 3.11 a A	0.67± 0.67 a A	4.94± 1.29 a			
Average/date ^{4/}		6.31± 1.12 A	4.43± 0.94 A	6.97± 1.65 A	6.22± 1.60 A				
Puparia/	0	-	0	1.88	2.63				
fruit	5	-	0	0	0.13				
	50	-	0	0	0				
Average/date ^{4/}									

1/ Duncan multiple range test, $\alpha = 0.05$

2/ Lower case letters - vertical comparison. Capital letters - horizontal comparison.

Table 7 . Infestation of GA₃ treated shamouti oranges with the Mediterranean fruit fly, following GA₃ treatment in October 18, 1982. Fruits exposed to the flies in the lab. ^{1,2/}

Item	Treat.	Date checked				Treatment average ^{3/}
		GA ₃ ppm	Feb. 4, 1983	Mar. 11, 1983	Apr. 22, 1983	
Eggs/ fruit	0		115.25 _± 30.96 a A	10.00 _± 4.34 a B	67.75 _± 32.36 a A	64.33 _± 18.77 a
	5		83.25 _± 10.57 ab A	25.75 _± 4.31 a B	48.75 _± 12.97 a AB	52.58 _± 8.83 a
	50		36.75 _± 10.14 b A	21.00 _± 12.02 a A	44.00 _± 22.08 a A	33.92 _± 8.67 a
Average/date ^{4/}			78.42 _± 14.18 A	18.92 _± 4.53 B	53.50 _± 12.82 A	
Punctures/ fruit	0		6.50 _± 1.32 a A	2.00 _± 0.71 a A	3.75 _± 0.95 a A	4.08 _± 0.77 a
	5		7.50 _± 1.44 a A	3.25 _± 0.48 a AB	2.50 _± 0.87 a B	4.42 _± 0.85 a
	50		6.50 _± 0.87 a A	4.75 _± 1.49 a A	5.50 _± 3.52 a A	5.58 _± 1.20 a
Average/date ^{4/}			6.83 _± 0.66 A	3.33 _± 0.62 B	3.92 _± 1.19 B	
Eggs/ puncture	0		17.73 _± 3.07 a A	5.00 _± 1.47 a B	18.07 _± 5.30 a A	15.76 _± 2.38 a
	5		11.10 _± 2.47 ab AB	7.92 _± 1.79 a B	19.50 _± 2.98 a A	11.91 _± 1.64 a
	50		5.65 _± 1.78 b A	4.42 _± 0.96 a A	8.00 _± 3.07 b A	6.07 _± 1.25 b
Average/date ^{4/}			11.48 _± 1.52 B	5.68 _± 0.81 B	13.66 _± 2.39 A	
Puparia/ fruit	0		0	0	3.38 _± 0.52	
	5		0	1.63 _± 0.24	5.75 _± 2.17	
	50		1.63 _± 0.61	0	2.38 _± 0.90	
Average/date ^{4/}						

1/ Duncan multiple range test, $\alpha = 0.05$

2/ Lower case letters - vertical comparison. Capital letters - horizontal comparison.

3/ All dates pooled.

4/ All treatments pooled.

Table 8 . Infestation of GA₃ treated valencia oranges with the Mediterranean fruit fly, following GA₃ treatment in September 23, 1982. Fruits exposed to the flies in the lab. ^{1,2/}

Item	Treat.	Date checked					
		GA ₃ ppm	Jan. 14, 1983	Mar. 18, 1983	Apr. 18, 1983	Treatment average ^{3/}	
Eggs/ fruit	0		26.25 _± 4.17 a AB	51.00 _± 27.47 a A	7.25 _± 2.93 a B	28.17 _± 10.00 a	
	5		34.75 _± 15.44 a A	21.52 _± 10.09 ab A	14.50 _± 12.88 a A	23.50 _± 7.24 a	
	50		31.00 _± 11.32 a A	5.50 _± 3.77 b A	1.50 _± 1.50 a A	12.67 _± 5.35 a	
	Average/date ^{4/}		30.67 _± 6.00 A	25.91 _± 10.56 AB	7.75 _± 4.32 B		
Punctures/ fruit	0		5.75 _± 1.75 a A	4.25 _± 0.25 a A	2.50 _± 0.87 a A	4.17 _± 0.72 a	
	5		4.00 _± 0.02 a A	4.00 _± 1.35 a A	2.50 _± 1.04 a A	3.50 _± 0.61 a	
	50		5.25 _± 1.60 a A	2.75 _± 0.85 a AB	0.75 _± 0.75 a B	2.92 _± 0.81 a	
	Average/date ^{4/}		5.00 _± 0.79 A	3.67 _± 0.53 AB	1.92 _± 0.53 B		
Eggs/ puncture	0		4565 _± 1.32 a B	12.00 _± 3.97 a A	2.90 _± 1.16 a B	6.76 _± 1.57 a	
	5		8.69 _± 3.19 a A	5.31 _± 1.23 ab A	5.80 _± 4.49 a A	6.71 _± 1.66 a	
	50		5.90 _± 1.55 a A	2.00 _± 1.18 b A	2.00 _± 1.15 a A	4.34 _± 1.05 a	
	Average/date ^{4/}		6.13 _± 1.13 A	7.07 _± 1.71 A	4.04 _± 1.99 A		
Puparia/ fruit	0		0	0	0		
	5		0	2.50 _± 0.95	0		
	50		0	0	0		
	Average/date ^{4/}						

1/ Duncan multiple range test, $\alpha = 0.05$

2/ Lower case letters - vertical comparison. Capital letters - horizontal comparison.

3/ All dates pooled.

4/ All treatments pooled.

Table 9 . Infestation of GA₃ treated valencia oranges the Mediterranean fruit fly, following GA₃ treatment in October 18, 1982. Fruits exposed to the flies in the lab. ^{1,2/}

Item	Treat. GA ₃ ppm	Date checked				Treatment average ^{3/}
		Jan. 21, 1983	Mar. 27, 1983	Apr. 29, 1983		
Eggs/ fruit	0	22.00 _± 4.06 a A	20.50 _± 3.52 b A	21.75 _± 11.58 a A		21.42 _± 3.85 b
	5	27.00 _± 10.16 a B	51.25 _± 4.07 a A	21.50 _± 9.12 a B		33.25 _± 5.79 a
	50	18.75 _± 3.09 a A	15.75 _± 1.93 b A	9.50 _± 6.06 a A		14.67 _± 2.43 b
	Average/date ^{4/}	22.58 _± 3.58 A	29.17 _± 5.05 A	17.58 _± 5.10 A		
Punctures/ fruit	0	6.25 _± 1.31 a A	4.50 _± 1.71 a A	5.75 _± 1.38 a A		5.50 _± 0.80 a
	5	2.50 _± 0.50 b A	3.50 _± 0.50 a A	4.50 _± 0.87 ab A		3.50 _± 0.42 b
	50	3.00 _± 0.71 b A	4.25 _± 0.63 a A	2.25 _± 0.75 b A		3.17 _± 0.44 b
	Average/date ^{4/}	3.92 _± 0.69 A	4.08 _± 0.58 A	4.17 _± 0.69 A		
Eggs/ puncture	0	3.52 _± 0.87 b A	4.56 _± 1.62 b A	3.78 _± 1.08 a A		3.89 _± 0.66 b
	5	10.80 _± 2.26 a A	14.64 _± 2.50 a A	4.78 _± 1.36 a B		9.50 _± 1.31 a
	50	6.25 _± 1.90 ab A	3.71 _± 1.39 b A	4.22 _± 2.24 a A		4.63 _± 1.00 b
	Average/date ^{4/}	5.77 _± 0.90 AB	7.14 _± 1.23 A	4.22 _± 0.79 B		
Puparia/ fruit	0	0	0	0.25 _± 0.09		
	5	0	2.13 _± 0.80	5.13 _± 1.94		
	50	0	0	0		
	Average/date ^{4/}					

1/ Duncan multiple range test, $\alpha = 0.05$

2/ Lower case letters - vertical comparison. Capital letters - horizontal comparison.

3/ All dates pooled.

4/ All treatments pooled.

Table 10. Infestation of GA₃ treated grapefruit with the Mediterranean fruit fly, following GA₃ treatment in September 23, 1982. Fruits exposed to the flies in the field.^{1,2/}

Item	Treat. GA ₃ ppm	Date checked				Treatment average ^{3/}
		Nov. 8, 1982	Dec. 17, 1982	Feb. 11, 1983		
Eggs/ fruit	0	17.50 _± 7.79 b B	53.35 _± 11.56 b A	76.85 _± 10.59 a A		49.23 _± 6.56 b
	5	14.70 _± 3.98 b B	71.85 _± 7.73 ab A	35.50 _± 7.52 b B		40.68 _± 4.86 b
	50	86.05 _± 11.92 a A	93.40 _± 9.44 a A	60.15 _± 7.15 ab B		79.87 _± 5.81 a
	Average/date ^{4/}	39.42 _± 6.47 C	72.87 _± 5.91 A	57.50 _± 5.34 B		
Punctures/ fruit	0	0.65 _± 0.18 b C	1.80 _± 0.35 b B	3.55 _± 0.37 a A		2.00 _± 0.24 b
	5	1.60 _± 0.55 b B	2.80 _± 0.30 b A	2.30 _± 0.45 b AB		2.23 _± 0.26 b
	50	3.50 _± 0.41 a B	4.85 _± 0.44 a A	3.90 _± 0.39 a AB		4.08 _± 0.25 a
	Average/date ^{4/}	1.92 _± 0.28 B	3.15 _± 0.27 A	3.25 _± 0.25 A		
Eggs/ puncture	0	13.35 _± 5.87 b B	23.83 _± 5.22 a A	22.41 _± 2.40 a AB		19.87 _± 2.76 a
	5	7.80 _± 2.07 b B	27.23 _± 2.64 a A	12.37 _± 1.72 b B		15.81 _± 1.64 a
	50	22.98 _± 2.32 a A	21.67 _± 2.83 a A	16.00 _± 1.50 ab A		22.22 _± 1.35 a
	Average/date ^{4/}	14.71 _± 2.32 B	24.25 _± 2.15 A	16.93 _± 1.21 B		
Puparia in all fruits	0	0	185	698		
	5	0	54	408		
	50	0	2	377		
Temperature range		not checked	3.9 - 19.9 °C	3.9 - 23.9 °C		

1/ Duncan multiple range test, $\alpha = 0.05$

2/ Lower case letters - vertical comparison. Capital letters - horizontal comparison.

3/ All dates pooled.

4/ All treatments pooled.

Table 11. Infestation of GA₃ treated grapefruit with the Mediterranean fruit fly, following GA₃ treatment in October 23, 1982. Fruits exposed to the flies in the field.^{1,2/}

Item	Treat. GA ₃ ppm	Date checked			Treatment average ^{3/}
		Nov. 29, 1982	Dec. 24, 1982	Feb. 17, 1983	
Eggs/	0	1.05± 0.60 c A	6.35± 2.32 b A	0 a A	2.47± 0.86 b
fruit	5	18.40± 5.21 b A	8.25± 2.92 b AB	0 a B	8.88± 2.19 b
	50	63.85± 11.78 a A	27.55± 8.13 a B	0 a C	30.46± 5.79 a
Average/date ^{4/}		27.77± 5.45 A	14.05± 3.18 B	0 C	
Punctures/	0	0.50± 0.18 c A	0.80± 0.26 b A	0 a A	0.43± 0.11 b
fruit	5	1.50± 0.30 b A	1.15± 0.30 b A	0 a B	0.88± 0.16 b
	50	3.60± 0.52 a A	2.35± 0.47 a B	0 a C	1.98± 0.30 a
Average/date ^{4/}		1.87± 0.27 A	1.43± 0.22 A	0 B	
Eggs/	0	0.56± 0.31 b A	3.43± 1.08 a A	0 a A	1.33± 0.42 b
puncture	5	10.53± 2.61 a A	3.90± 1.16 a B	0 a B	4.81± 1.09 a
	50	14.08± 2.50 a A	7.11± 1.42 a B	0 a C	7.06± 1.20 a
Average/date ^{4/}		8.39± 1.40 A	4.81± 0.73 B	0 C	
Puparia/	0	23	137	4	
in all	5	4	41	10	
fruit	50	0	5	6	
Temperature range		not checked	2.7 - 17.2°C	5.0 - 10.0°C	

1/ Duncan multiple range test, $\alpha = 0.05$

2/ Lower case letters - vertical comparison. Capital letters - horizontal comparison.

3/ All dates pooled.

4/ All treatments pooled.

Table 12 . Infestation of GA₃ treated shamouti oranges with the Mediterranean fruit fly, following GA₃ treatment in September 23, 1982. Fruits exposed to the flies in the field.^{1,2/}

Item	Treat.		Date checked						
	GA ₃ ppm	Dec. 3, 1982		Jan. 28, 1983		Mar. 4, 1983		Treatment average ^{3/}	
Eggs/	0	0	b A	0	a A	0	a A	0	a
fruit	5	0	b A	0	a A	0.20± 0.20	a A	0.07± 0.07	a
	50	0.25± 0.16	a A	0	a B	0	a B	0.08± 0.05	a
Average/date ^{4/}		0.08± 0.05	A	0	A	0.07± 0.07	A		
Punctures/	0	0	a A	0.10± 0.10	a A	0.45± 0.20	a A	0.18± 0.07	a
fruit	5	0.05± 0.05	a B	0.25± 0.20	a AB	0.75± 0.26	a A	0.35± 0.12	ab
	50	0.25± 0.10	a B	0.35± 0.18	a B	1.10± 0.49	a A	0.57± 0.18	a
Average/date ^{4/}		0.10± 0.04	B	0.23± 0.10	B	0.77± 0.20	A		
Eggs/	0	0	a A	0	a A	0	a A	0	a
puncture	5	0	a A	0	a A	0.07± 0.07	a A	0.02± 0.02	a
	50	0.25± 0.16	a A	0	a A	0	a A	0.08± 0.05	a
Average/date ^{4/}		0.08± 0.05	A	0	A	0.02± 0.02	A		
Puparia/	0	0		0		0			
in all	5	0		7		0			
fruits	50	0		0		0			
Temperature range	4.4 - 13.3°C		1.1 - 17.7°C		3.3 - 11.7°C				

1/ Duncan multiple range test, $\alpha = 0.05$

2/ Lower case letters - vertical comparison. Capital letters - horizontal comparison.

3/ All dates pooled.

4/ All treatments pooled.

Table 13. Infestation of GA₃ treated shamouti oranges with the Mediterranean fruit fly, following GA₃ treatment in October 18, 1982. Fruits exposed to the flies in the field.^{1,2/}

Item	Treat.	Date checked			Apr. 22, 1983	Treatment Average ^{3/}
		GA ₃ ppm	Dec. 12, 1982	Feb. 4, 1983	Mar. 11, 1983	
Eggs/	0		0.10± 0.10 a B	0.75± 0.41 a B	5.10± 2.34 b B	23.55± 6.71 b A
fruit	5		0.70± 0.49 a B	0.60± 0.42 a B	29.55± 7.49 a A	30.30± 4.48 ab A
	50		1.00± 0.75 a B	1.85± 1.52 a B	37.00± 8.05 a A	30.70± 4.48 a A
Average/date ^{4/}			0.60± 0.30 C	1.07± 0.54 C	21.80± 3.39 B	30.28± 3.80 A
Punctures/	0		0.10± 0.07 a B	0.20± 0.09 a B	0.80± 0.28 b B	2.70± 0.68 b A
fruit	5		0.10± 0.07 a C	0.15± 0.08 a C	2.50± 0.46 a B	4.50± 0.74 a A
	50		0.25± 0.12 a C	0.95± 0.40 a C	3.55± 0.44 a A	2.15± 0.40 b B
Average/date ^{4/}			0.15± 0.05 C	0.43± 0.15 C	2.28± 0.27 B	3.12± 0.38 A
Eggs/	0		0.10± 0.10 a A	0.75± 0.41 a A	4.84± 1.20 b A	2.03± 4.84 b A
puncture	5		0.70± 0.49 a B	0.60± 0.42 a B	9.51± 2.24 a A	8.71± 2.20 b A
	50		0.85± 0.71 a C	0.43± 0.30 a C	7.34± 0.97 a B	16.90± 3.89 a A
Average/date ^{4/}			0.56± 0.29 C	0.59± 0.22 C	6.29± 0.95 B	10.15± 1.65 A
Puparia	0		0	0	52	98
fruit	5		0	0	38	71
	50		0	0	0	116
Temperature			0 - 19.4°C	rain	5.0 - 20.6°C	6.1 - 22.8°C

Table 14. Infestation of GA₃ treated valencia oranges with the Mediterranean fruit fly, following GA₃ treatment in September 23, 1982. Fruits exposed to the flies in the field.^{1,2/}

Item	Treat.		Date checked				Treatment average ^{3/}
	GA ₃ ppm		Jan. 14, 1983	Mar. 18, 1983	Apr. 11, 1983		
Eggs/	0	0	a B	11.35 _± 4.38 b A	0 a B	3.78 _± 1.59 b	
fruit	5	0	a B	27.45 _± 5.23 a A	10.05 _± 3.95 a B	12.50 _± 2.61 a	
	50	0	a B	31.25 _± 7.32 a A	3.85 _± 1.79 a B	11.70 _± 3.06 a	
Average/date ^{4/}	0		B	23.35 _± 3.47 A	4.63 _± 1.52 B		
Punctures/	0	0.65 _± 0.21 a AB		1.20 _± 0.30 a A	0.05 _± 0.65 b B	0.63 _± 0.13 b	
fruit	5	1.35 _± 0.38 a A		1.80 _± 0.24 a A	0.95 _± 0.30 ab A	1.37 _± 0.18 a	
	50	1.10 _± 0.46 a A		1.85 _± 0.29 a A	1.30 _± 0.39 a A	1.42 _± 0.22 a	
Average/date ^{4/}		1.03 _± 0.21 B		1.62 _± 0.16 A	0.77 _± 0.18 B		
Eggs/	0	0	a A	5.42 _± 2.14 b A	0 a A	1.81 _± 0.78 b	
puncture	5	0	a B	12.50 _± 2.26 a A	4.09 _± 1.59 a B	5.53 _± 1.13 ab	
	50	0	a B	18.49 _± 6.24 a A	3.08 _± 1.70 a B	7.19 _± 2.37 a	
Average/date ^{4/}	0		B	12.14 _± 2.39 A	2.39 _± 0.79 B		
Puparia	0	0		14	36		
in all	5	0		0	0		
fruits	50	0		0	0		
Temperature range	5.6 - 15.6°C		3.3 - 29.4°C		6.7 - 20.0°C		

1/ Duncan multiple range test, $\alpha = 0.05$

2/ Lower case letters - vertical comparison. Capital letters - horizontal comparison.

3/ All dates pooled.

4/ All treatments pooled.

Table 15. Infestation of GA₃ treated valencia oranges with the Mediterranean fruit fly, following GA₃ treatment in October 18, 1982. Fruits exposed to the flies in the field.^{1,2/}

Item	Treat.		Date checked				Treatment average ^{3/}
	GA ₃	ppm	Jan. 21, 1983	Mar. 27, 1983	Apr. 29, 1983		
Eggs/	0	0	a B	8.75 _± 3.71 a B	43.10 _± 5.32 b A	17.28 _± 3.22 b	
fruit	5	0	a C	19.55 _± 6.19 a B	74.20 _± 10.34 a A	31.25 _± 5.68 a	
	50	0	a B	16.70 _± 6.68 a B	91.65 _± 11.25 a A	36.12 _± 6.73 a	
Average/date ^{4/}	0		C	15.00 _± 3.28 B	69.65 _± 5.91 B		
Punctures/	0	0.20 _± 0.09	a B	1.10 _± 0.38 a B	2.95 _± 0.29 b A	1.42 _± 0.22 b	
fruit	5	0.45 _± 0.22	a B	1.40 _± 0.31 a B	5.55 _± 0.51 a A	2.47 _± 0.36 a	
	50	0.65 _± 0.24	a C	1.65 _± 0.35 a B	4.65 _± 0.55 a A	2.32 _± 0.32 a	
Average/date ^{4/}		0.43 _± 0.15	C	1.38 _± 0.20 B	4.38 _± 0.30 A		
Eggs/	0	0	a B	3.00 _± 1.25 b B	14.35 _± 1.40 b A	5.81 _± 1.01 b	
puncture	5	0	a B	12.33 _± 4.85 a A	12.77 _± 1.81 b A	8.37 _± 1.86 ab	
	50	0	a B	5.93 _± 2.29 ab B	25.34 _± 4.04 a A	10.42 _± 2.07 a	
Average/date ^{4/}	0		C	7.11 _± 1.87 B	17.49 _± 1.69 a A		
Puparia	0	0		29	8		
in all	5	0		5	243		
fruits	50	0		0	18		
Temperature		1.0 - 18.3°C		1.7 - 19.4°C	15.6 - 19.4°C		

1/ Duncan multiple range test, $\alpha = 0.05$

2/ Lower case letters - vertical comparison. Capital letters - horizontal comparison.

3/ All dates pooled.

4/ All treatments pooled.

Table 16. Infestation of GA₃ treated grapefruit with the Mediterranean fruit fly, following GA₃ treatment in September 20, 1983. Fruits exposed to the flies in the lab^{1,2/}. (20 fruits dissected per treatment.)

Item	Treat.	Date checked															
		GA ₃ ppm		Nov. 11, 1983				Dec. 14, 1983				Jan. 10, 1984				Feb. 14, 1984	
Eggs/ fruit	0	10.25±	2.25	a	A	5.25±	1.29	a	A	8.05±	2.39	a	A	16.60±	3.67	b	A
	20	17.40±	5.54	a	B	9.45±	1.93	a	B	13.40±	3.09	a	B	30.45±	5.95	a	A
	50	20.15±	10.94	a	A	1.05±	0.47	a	B	18.25±	3.16	a	A	21.30±	4.80	ab	A
Average/date ^{4/}		15.93±	4.12		AB	5.25±	0.89		C	13.23±	2.71		B	22.78±	2.88		A
Punctures/ fruit ^{3/}	0	2.70±	0.41	a	A	2.75±	0.33	a	A	2.30±	0.61	a	A	2.85±	0.45	a	A
	20	3.60±	0.59	a	A	3.05±	0.40	a	A	2.65±	0.39	a	A	3.85±	0.60	a	A
	50	2.85±	0.77	a	A	0.65±	0.26	b	B	2.45±	0.38	a	A	3.35±	0.59	a	A
Average/date ^{4/}		3.05±	0.35		AB	2.15±	0.24		C	2.47±	0.27		BC	3.35±	0.32		A
Fertile punctures/ fruit	0	1.95±	0.41	a	AB	0.90±	0.22	ab	B	1.45±	0.37	a	AB	2.25±	0.38	b	A
	20	2.35±	0.60	a	B	1.65±	0.29	a	B	2.15±	0.36	a	B	3.65±	0.56	a	A
	50	1.70±	0.51	a	A	0.40±	0.15	b	B	2.25±	0.38	a	A	2.90±	0.55	ab	A
Average/date ^{4/}		2.00±	0.29		B	0.98±	0.15		C	1.95±	0.21		B	2.93±	0.30		A
% fertile punctures/ fruit	0	62.50±	9.43	a	AB	33.58±	8.00	a	B	42.65±	9.49	a	AB	72.98±	8.21	a	A
	20	48.28±	8.32	a	B	48.33±	7.71	a	B	76.08±	7.95	a	AB	86.87±	6.90	a	A
	50	57.37±	8.34	a	B	21.67±	8.39	a	C	81.25±	8.29	a	AB	95.06±	22.66	a	A
Average/date ^{4/}		56.05±	5.00		B	34.53±	4.78		C	66.66±	5.37		B	84.97±	8.30		A

Table 16 (continued). Infestation of GA₃ treated grapefruit with the Mediterranean fruit fly, following GA₃ treatment in September 20, 1983. Fruits exposed to the flies in the lab^{1,2/}. (20 fruits dissected per treatment.)

Item	Treat. GA ₃ ppm	Date checked			
		Nov. 11, 1983	Dec. 14, 1983	Jan. 10, 1984	Feb. 14, 1984
Eggs/ puncture ^{3/}	0	3.72 ₊ 0.83	1.95 ₊ 0.47	2.16 ₊ 0.50	5.64 ₊ 1.13
	20	3.23 ₊ 0.71	2.91 ₊ 0.55	5.10 ₊ 0.88	6.92 ₊ 0.86
	50	4.88 ₊ 0.94	0.47 ₊ 0.20	7.46 ₊ 1.35	6.56 ₊ 1.64
Average/date ^{4/}		3.94 ₊ 0.48 B	1.78 ₊ 0.25 C	4.91 ₊ 0.62 AB	6.37 ₊ 0.71 A
Punctures/ fruit ^{5/}	0	2.83 ₊ 0.38 a AB	1.90 ₊ 0.24 a B	3.10 ₊ 0.38 a A	3.93 ₊ 0.68 a A
	20	2.10 ₊ 0.27 a BC	1.67 ₊ 0.30 a C	3.07 ₊ 0.50 a AB	3.30 ₊ 0.52 a A
	50	0.90 ₊ 0.29 b B	0.93 ₊ 0.22 a B	1.60 ₊ 0.16 b B	4.03 ₊ 0.39 a A
Average/date ^{4/}		1.94 ₊ 0.20 C	1.50 ₊ 0.15 C	2.59 ₊ 0.23 B	3.76 ₊ 0.31 A
Puparia/ fruit	0	1.47 ₊ 0.91 a B	4.20 ₊ 1.08 a B	14.63 ₊ 2.84 b A	11.00 ₊ 1.59 a A
	20	0.47 ₊ 0.25 a B	1.47 ₊ 0.68 a B	10.87 ₊ 1.83 b A	10.20 ₊ 2.15 a A
	50	0.13 ₊ 0.13 a C	0.83 ₊ 0.37 a C	20.33 ₊ 2.06 a A	13.33 ₊ 1.99 a B
Average/date ^{4/}		0.69 ₊ 0.32 C	2.17 ₊ 0.46 C	15.28 ₊ 1.37 A	11.51 ₊ 1.11 B

1/ Duncan multiple range test, $\alpha = 0.05$

2/ Lower case letters - vertical comparison. Capital letters - horizontal comparison.

3/ In the dissected fruit

4/ All treatments pooled.

5/ In fruits left for pupation.

Table 17. Infestation of GA₃ treated shamouti oranges with the Mediterranean fruit fly, following GA₃ treatment in October, 18, 1983. Fruits exposed to the flies in the lab^{1,2/}. (20 fruits dissected per treatment.)

Item	Treat. GA ₃ ppm	Date checked							
		Dec. 9, 1983		Dec. 30, 1983		Feb. 7, 1984		Mar. 11, 1984	
Eggs/ fruit	0	17.10±	5.54 a A	26.55±	7.42 a A	13.20±	6.05 a A	15.15±	3.93 a A
	20	16.30±	3.54 a AB	26.45±	6.64 a A	16.90±	3.90 a AB	10.75±	3.20 a B
	50	15.40±	3.93 a A	10.90±	3.16 b A	12.00±	3.01 a A	12.80±	2.25 a A
Average/date ^{4/}		16.27±	2.51 AB	21.30±	3.55 A	14.03±	2.57 AB	12.90±	1.83 B
Punctures/ fruit ³	0	3.20±	0.44 b AB	4.40±	0.55 a A	2.45±	0.39 b B	3.10±	0.41 a AB
	20	4.95±	0.57 a A	3.50±	0.40 a B	2.80±	0.37 ab B	2.30±	0.38 a B
	50	3.60±	0.52 b A	1.70±	0.45 a A	3.80±	0.44 a A	2.35±	0.30 a D
Average/date ^{4/}		3.92±	0.31 A	4.20±	0.28 A	3.02±	0.24 B	2.58±	0.21 B
Fertile punctures/ fruit	0	1.70±	0.33 a AB	2.50±	0.39 a A	0.90±	0.19 b B	1.40±	0.21 a B
	20	1.90±	0.25 a A	2.25±	0.36 a A	2.10±	0.35 a A	1.40±	0.31 a A
	50	1.55±	0.37 a A	1.65±	0.37 a A	1.75±	0.32 ab A	1.85±	0.27 a A
Average/date ^{4/}		1.72±	0.18 AB	2.13±	0.22 A	1.58±	0.18 B	1.55±	0.15 B
% fertile punctures/ fruit	0	47.88±	8.14 a AB	58.14±	6.97 a A	33.08±	7.35 b B	41.98±	6.23 b AB
	20	42.59±	7.09 a A	58.11±	7.75 a A	65.00±	9.16 a A	51.08±	9.88 b A
	50	32.40±	7.06 a B	28.80±	5.93 b B	45.92±	8.01 ab B	76.17±	7.32 a A
Average/date ^{4/}		40.96±	4.31 B	48.35±	4.32 AB	48.00±	4.96 AB	56.41±	4.89 A

Table 17 (continued). Infestation of GA₃ treated shamouti oranges with the Mediterranean fruit fly, following GA₃ treatment in October 18, 1983. Fruits exposed to the flies in the lab^{1,2/}. (20 fruits dissected per treatment.)

Item	Treat.	Date checked															
	GA ₃ ppm	Dec. 9, 19833		Dec. 30, 1983		Feb. 7, 19844		Mar. 11, 1984									
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Eggs/	0	4.74 _±	1.42	6.08 _±	1.50	3.37 _±	1.21	5.49 _±	1.92								
puncture ^{3/}	20	4.51 _±	1.49	6.38 _±	1.33	5.12 _±	1.11	3.95 _±	1.00								
	50	3.42 _±	0.89	1.95 _±	0.58	3.32 _±	0.86	5.97 _±	1.29								
Average/date ^{4/}		4.22 _±	0.74	A	4.80 _±	0.73	A	3.94 _±	0.62	A	5.14 _±	0.83	A				
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Punctures/	0	2.90 _±	0.39	a	B	3.97 _±	0.48	a	A	2.07 _±	0.30	a	B	2.70 _±	0.37	a	B
fruit ^{5/}	20	3.10 _±	0.49	a	A	2.20 _±	0.29	b	AB	1.40 _±	0.28	a	B	2.63 _±	0.29	a	A
	50	.30 _±	0.22	b	B	3.57 _±	0.34	a	A	1.63 _±	0.24	a	B	3.57 _±	0.57	a	A
Average/date ^{4/}		2.43 _±	0.24		B	3.24 _±	0.23		A	1.70 _±	0.16		C	2.97 _±	0.25		AB
<hr/>																	
Puparia/	0	0		a	B	0.60 _±	0.54	a	B	2.27 _±	1.13	a	B	6.47 _±	2.03	a	A
fruit	20	0		a	B	0.83 _±	0.83	a	AB	0		b	B	2.33 _±	1.01	b	A
	50	0		a	A	0		a	A	0		b	A	0.33 _±	0.19	b	A
Average/date ^{4/}		0			B	0.48 _±	0.33		B	0.76 _±	0.39		B	3.04 _±	0.80		A
<hr/>																	

1/ Duncan multiple range test, $\alpha = 0.05$

2/ Lower case letters - vertical comparison. Capital letters - horizontal comparison.

3/ In the dissected fruit

4/ All treatments pooled.

5/ In fruits left for pupation.

Table 18. Infestation of GA₃ treated valencia oranges with the Mediterranean fruit fly, following GA₃ treatment in November 20, 1983. Fruits exposed to the flies in the lab^{1,2/}. (20 fruits dissected per treatment.)

Item	Treat.	Date checked							
	GA ₃ ppm	Jan.24, 1984		Feb. 21, 1984		Mar. 21, 1984		Apr. 10, 1984	
Eggs/	0	16.25 _±	4.81 a B	4.40 _±	2.17 a C	17.75 _±	7.01 a AB	27.15 _±	8.56 a A
fruit	20	0.20 _±	0.20 b A	0.35 _±	0.25 a A	0.25 _±	0.20 b A	0.65 _±	0.32 b A
	50	2.20 _±	1.23 b A	0.85 _±	0.71 a A	1.45 _±	0.92 b A	3.20 _±	1.47 b A
Average/date ^{4/}		6.22 _±	1.87 AB	1.87 _±	0.79 B	6.48 _±	2.54 AB	10.33 _±	3.24 A
Punctures/	0	2.40 _±	0.38 a A	2.30 _±	0.41 a A	2.15 _±	0.31 a A	1.40 _±	0.33 a B
fruit	20	0.25 _±	0.12 b A	0.50 _±	0.15 b A	0.35 _±	0.13 b A	0.20 _±	0.09 b A
	50	0.25 _±	0.12 b A	0.40 _±	0.17 b A	0.60 _±	0.23 b A	0.40 _±	0.15 b A
Average/date ^{4/}		0.97 _±	0.19 A	1.07 _±	0.19 A	1.03 _±	0.17 A	0.67 _±	0.14 A
Fertile	0	1.50 _±	0.34 a A	0.50 _±	0.15 a B	0.80 _±	0.19 a b	1.30 _±	0.32 a A
punctures/	20	0.05 _±	0.05 b A	0.15 _±	0.08 a A	0.10 _±	0.07 b A	0.20 _±	0.09 b A
fruit	50	0.25 _±	0.12 b A	0.15 _±	0.11 a A	0.20 _±	0.09 b A	0.35 _±	0.15 b A
Average/date ^{4/}		0.60 _±	0.15 A	0.27 _±	0.07 B	0.37 _±	0.08 AB	0.62 _±	0.13 A
% fertile	0	48.92 _±	9.12 a AB	18.25 _±	6.23 a C	30.83 _±	7.08 a BC	55.83 _±	10.83 a A
punctures/	20	5.00 _±	5.00 b A	12.50 _±	7.14 a A	7.50 _±	5.47 b A	20.00 _±	9.18 b A
fruit	50	20.00 _±	9.18 b A	7.50 _±	5.47 a A	8.33 _±	3.92 b A	25.00 _±	9.93 b A
Average/date ^{4/}		24.64 _±	5.13 AB	12.75 _±	3.63 B	15.56 _±	3.50 B	33.61 _±	6.04 A

Table 18 (continued). Infestation of GA₃ treated valencia oranges with the Mediterranean fruit fly, following GA₃ treatment in November 20, 1983. Fruits exposed to the flies in the lab^{1,2/}. (20 fruits dissected per treatment.)

Item	Treat. GA ₃ ppm	Date checked			
		Jan. 24, 1983	Feb. 21, 1984	Mar. 21, 1984	Apr. 10, 1984
Eggs/	0	5.12 _± 1.23	1.45 _± 0.64	5.76 _± 2.12	11.38 _± 2.88
puncture ^{3/}	20	0.20 _± 0.20	0.22 _± 0.14	0.15 _± 0.15	0.65 _± 0.32
	50	1.65 _± 0.82	0.42 _± 0.35	0.51 _± 0.31	2.35 _± 1.12
Average/date ^{4/}		2.32 _± 0.56 B	0.70 _± 0.25 B	2.14 _± 0.78 B	4.79 _± 1.19 A
Punctures/	0	2.31 _± 0.27 a A	1.70 _± 0.25 a B	1.73 _± 0.26 a B	1.23 _± 0.23 a B
fruit ^{5/}	20	0.66 _± 0.15 b B	1.33 _± 0.19 a A	0.80 _± 0.14 b AB	0.73 _± 0.19 ab B
	50	0.39 _± 0.10 b A	0.53 _± 0.13 b A	0.57 _± 0.13 b A	0.57 _± 0.14 b A
Average/date ^{4/}		1.12 _± 0.14 AB	1.19 _± 0.12 A	1.03 _± 0.12 AB	0.84 _± 0.11 B
Puparia/	0	3.72 _± 2.97 a AB	2.57 _± 0.86 a B	1.33 _± 0.63 a B	6.97 _± 2.64 a A
fruit	20	0.41 _± 0.40 a A	0 a A	0 a A	2.23 _± 1.59 b A
	50	0.18 _± 0.17 a A	0.30 _± 0.21 a A	0 a A	0.33 _± 0.23 b A
Average/date ^{4/}		1.44 _± 1.00 AB	0.96 _± 0.32 B	0.44 _± 0.22 B	3.18 _± 1.06 A

1/ Duncan multiple range test, $\alpha = 0.05$

2/ Lower case letters - vertical comparison. Capital letters - horizontal comparison.

3/ In the dissected fruit

4/ All treatments pooled.

5/ In fruits left for pupation.

Table 19. Infestation of GA₃ treated citrus fruits with the Mediterranean fruit fly, following GA₃ treatment. Fruits exposed to the flies in the lab.^{2/} (per treatment - all dates pooled).

Item	Treat. GA ₃ ppm	Citrus variety		
		Grapefruit	Shamouti oranges	Valencia oranges
Eggs/ fruit	0	10.04 _± 1.33 b	18.00 _± 2.94 a	16.39 _± 3.14 a
	20	17.67 _± 2.36 a	17.60 _± 2.31 a	0.36 _± 0.12 b
	50	15.19 _± 3.17 ab	12.77 _± 1.55 a	1.92 _± 0.56 b
Punctures/ fruit	0	2.65 _± 0.23 ab	3.29 _± 0.24 a	2.06 _± 0.18 a
	20	3.29 _± 0.25 a	3.39 _± 0.24 a	0.32 _± 0.06 b
	50	2.32 _± 0.29 b	3.61 _± 0.23 a	0.41 _± 0.09 b
Fertile punctures/ fruit	0	1.64 _± 0.18 b	1.63 _± 0.16 a	1.02 _± 0.14 a
	20	2.45 _± 0.25 a	1.91 _± 0.16 a	0.13 _± 0.04 b
	50	1.81 _± 0.23 b	1.70 _± 0.16 a	0.24 _± 0.06 b
% fertile punctures/ fruit	0	52.93 _± 4.66 a	45.27 _± 3.68 a	38.46 _± 4.49 a
	20	64.89 _± 4.25 a	54.20 _± 4.29 a	11.25 _± 3.44 b
	50	63.84 _± 7.29 a	45.82 _± 4.07 a	15.21 _± 3.80 b
Eggs/ puncture	0	3.37 _± 0.42 b	4.92 _± 0.76 a	5.93 _± 1.02 a
	20	4.54 _± 0.42 ab	4.99 _± 0.62 a	0.31 _± 0.10 b
	50	4.84 _± 0.65 a	3.67 _± 0.49 a	1.23 _± 0.37 b

1/ Duncan multiple range test, $\alpha = 0.05$

Table 19 (continued). Infestation of GA_3 treated citrus fruits with the Mediterranean fruit fly, following GA_3 treatment. Fruits exposed to the flies in the lab.^{1/} (per treatment - all dates pooled).

Item	Treat.		Citrus variety		
	GA_3 ppm	Grapefruit	Shamouti oranges	Valencia oranges	
Punctures/ fruit ^{2/}	0	2.94 $\pm$ 0.23 a	2.91 $\pm$ 0.20 a	1.74 $\pm$ 0.13 a	
	20	2.53 $\pm$ 0.21 a	2.33 $\pm$ 0.18 b	0.88 $\pm$ 0.09 b	
	50	1.87 $\pm$ 0.18 b	2.52 $\pm$ 0.21 ab	0.51 $\pm$ 0.06 c	
Puparia/ fruit ^{2/}	0	7.83 $\pm$ 1.00 ab	2.33 $\pm$ 0.63 a	3.65 $\pm$ 1.03 a	
	20	5.75 $\pm$ 0.84 b	0.79 $\pm$ 0.33 b	0.66 $\pm$ 0.41 b	
	50	8.66 $\pm$ 1.06 a	0.08 $\pm$ 0.05 b	0.20 $\pm$ 0.09 b	

1/ Duncan multiple range test, $\alpha = 0.05$

2/ In the 30 fruits kept for pupation.

AVERAGE NUMBER
MEDFLIES / FRUIT
GRAPEFRUIT

Figure 1a. Effect of GA_3 treatment upon susceptibility of Marsh grapefruit to attack by *C. capitata* in the laboratory (1983-84 season).

AVERAGE NUMBER MEDFLIES / FRUIT
SHAMOUTI

Figure 1b. Effect of GA_3 treatment upon susceptibility of Shamouti oranges to attack by *C. capitata* in the laboratory (1983-84 season).

AVERAGE NUMBER MEDFLIES / FRUIT
VALENCIA

Figure 1c. Effect of GA_3 treatment upon susceptibility of Valencia oranges to attack by *C. capitata* in the laboratory (1983-84 season).

Table 20. Effect of GA₃ treatment (appl. Nov. 20, 1983) on color break in valencia oranges (average of 20 fruits checked for oviposition in Lab. exposure 1983/4).¹

Treat. GA ₃ ppm	Jan. 24, 1984	Feb. 21, 1984	Mar. 21, 1984	Apr. 10, 1984	Average/treat.
0	1.90± 0.14 c A	1.00± 0 b A	1.25± 0.14 b B	1.10± 0.07 b B	1.31± 0.07 B
20	5.15± 0.27 a A	3.35± 0.15 a B	2.60± 0.18 a C	2.85± 0.08 a BC	3.49± 0.14 A
50	4.20± 0.30 b A	3.85± 0.40 a AB	2.85± 0.20 a BC	3.25± 0.35 a BC	3.54± 0.17 A BC
Average/date	3.75± 0.23 A	2.73± 0.21 B	2.23± 0.14 C	2.40± 0.17 BC	

1/ Duncan multiple range test = 0.05.

Table 21. Effect of GA₃ treatment (appl. Nov. 20, 1983) on color break in valencia oranges (average of 30 fruits checked for pupation in Lab. exposure 1983/4).¹

Treat. GA ₃ ppm	Jan. 24, 1984	Feb. 21, 1984	Mar. 21, 1984	Apr. 10, 1984	Average/treat.
0	1.62± 0.12 c A	1.00± 0 c B	1.23± 0.10 b AB	1.23± 0.06 b AB	1.27 c
20	3.98± 0.18 b A	3.10± 0.19 b A	2.10± 0.15 b C	3.70± 0.22 a A	3.22 b
50	4.53± 0.22 a A	3.93± 0.14 a B	3.30± 0.20 a C	3.33± 0.26 a B	3.93 a
Average/date	3.38 A	3.68 C	2.21 D	2.95 b	

1/ Duncan multiple range test $\alpha = 0.05$.

Table 22. Effect of GA₃ dose on colorbreak in valencia oranges (correlation coefficients).

Exposure date	<u>OVIPOSITION</u> (n=60)		<u>PUPATION</u> (n=90)	
	Cor. coeff.	P<	Cor. coeff.	P<
Jan. 24, 84	0.4713	0.0001	0.7134	0.0001
Feb. 21, 84	0.6733	0.0001	0.8044	0.0001
Mar. 21, 84	0.5961	0.0001	0.7132	0.0001
Apr. 10, 84	0.6334	0.0001	0.6338	0.0001

Table 23. Correlation between fruit color in valencia oranges and infestation by the medfly.

Exposure date		Eggs/fruit	punctures/fruit	eggs/puncture	pupae/fruit
Jan. 24, 84	r	-0.3776	-0.5876	-0.3625	-0.2099
	P	0.0029***	0.0001****	0.0044***	0.0470*
Feb. 21, 84	r	-0.2313	-0.4621	-0.2221	-0.3178
	P	0.0754*	0.0002*	0.0881*	0.0023***
Mar. 21, 84	r	-0.2908	-0.5228	-0.3036	-0.2199
	P	0.0242**	0.0001****	0.0184**	0.0373**
Apr. 10, 84	r	-0.2465	-0.2468	-0.2299	-0.2324
	P	0.0576*	0.0573*	0.0773*	0.0275**

*, **, ***, **** = significance levels.

Table 24. Infestation of Shamouti and Valencia oranges by natural populations of the weevil in the "Weizmann" grove in 1984.

No of punctures per fruit.	Frequency of fruit in each class					
	Shamouti			Valencia		
	0 ppm	20 ppm	50 ppm	* 0 ppm	20 ppm	50 ppm
0	379	417	419	*	485	483
1	81	51	56	*	10	17
2	27	18	22	*	3	0
3	10	7	2	*	1	0
4	2	3	0	*	1	0
5	0	1	0			
6	0	0	1			
7	1	1	0			

$$\chi^2 = 223.75 \quad 116.94 \quad 33.90 \quad 81.15 \quad 0.33 \quad 0.39$$

$$\chi^2_{0.001} = 9.210 \quad \chi^2_{0.005} = 7.739$$

Certain of the proposed studies concerning physical and chemical determinants of citrus resistance to the Caribbean fruit fly were performed during the season prior to initiation of the BARD-sponsored research and the results have been published (Greany et al. 1983, 1985; Styer and Greany 1983 - reprints attached). Since these tests were already completed, we were able to incorporate additional studies (on use of GA_3 for caribfly control in Florida) that were not originally planned, but seemed appropriate in light of the encouraging results from the Israeli studies on use of GA_3 against the medfly. These tests were accompanied by complementary biochemical and biophysical measurements on the effects of GA_3 treatments on the fruit peel.

Thus, all the research outlined in the original proposal was completed, and in addition, tests on GA_3 effects on caribfly susceptibility and upon chemical and physical properties of the fruit peel also were performed under the auspices of this grant.

FLORIDA STUDIES ON THE CARIBBEAN FRUIT FLY

EXPERIMENTS AND RESULTS

Certain of the following studies are fully described in the accompanying reprints, as indicated. For convenience, the abstracts from each paper are excerpted and are included here.

- I. Relationship between peel morphology and fly survival^a
- II. Toxicity of peel oils to fly eggs and larvae
 - Acute toxicity to eggs^{a,b}
 - Toxicity to larvae
 - Acute^{a,b}
 - Chronic (see below)
- III. Studies on relationship between grapefruit senescence and susceptibility to the caribfly
 - Observed increased in susceptibility of fruit to the caribfly throughout the season
 - Effects on ovipositional acceptability^c
 - Effects on developmental suitability^c
 - Relationship of fruit senescence to biochemical and biophysical properties of the peel
 - Peel oil composition^c
 - Peel sugar content^c
- IV. Manipulation of fruit susceptibility with GA₃ (see below)
 - Results of 1983-84 season
 - Fly susceptibility
 - Peel softness/penetrability
 - Results of 1984-85 season
 - Fly susceptibility
 - Peel softness/penetrability
 - Peel oil abundance
 - Pectin methylesterase activity
 - Peel color
 - Effects of GA₃ treatment on degreening propensity

^asee Greany et al. 1983

^bsee Styer and Greany 1983

^csee Greany et al. 1985

BIOCHEMICAL RESISTANCE OF CITRUS TO FRUIT FLIES. DEMONSTRATION AND ELUCIDATION OF RESISTANCE TO THE CARIBBEAN FRUIT FLY, *ANASTREPHA SUSPENS*

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Resistance of citrus fruit to attack by the Caribbean fruit fly, *Anastrepha suspensa* (Loew), was attributed to allelopathic essential oils in the flavedo region of the peel. Hatchability of eggs laid between peel oil glands was significantly greater than that of eggs laid into glands. Mortality occurred principally in the 1st larval instar, and most larvae died before reaching the albedo (the non-oily region of the peel). Grapefruit were more susceptible to larval development than oranges, and lemons were virtually immune to successful attack by this fly. Fruit that were allowed to remain on the tree until over-ripe were somewhat more susceptible than early-season fruit. Fruit resistance was correlated with (1) flavedo thickness, (2) a high concentration of linalool in relation to limonene in the peel oil, and (3) the absolute amount of oil per unit area of peel. Volatile components of the peel oil rather than high boiling fractions appear to account for oil toxicity. Possible applications of these findings are discussed.

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Increased Susceptibility of Laboratory-Reared vs. Wild Caribbean Fruit Fly, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae), Larvae to Toxic Citrus Allelochemicals¹

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ABSTRACT Selected citrus peel oils and oil constituents were tested for toxicity to eggs and larvae of laboratory-reared and wild Caribbean fruit flies, *Anastrepha suspensa* (Loew). In general, the responses of both laboratory-reared and wild fly eggs to these agents were quite similar. In contrast, laboratory-reared larvae were more susceptible than wild larvae to all test agents. Possible explanations for the observed difference in larval susceptibility are presented.

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SENESCENCE-RELATED SUSCEPTIBILITY OF MARSH GRAPEFRUIT TO LABORATORY INFESTATION BY *ANASTREPHA SUSPENS* (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Susceptibility of Marsh grapefruit to laboratory infestation by *Anastrepha suspensa* was determined each month between October 1982 and May 1983. Criteria for susceptibility included comparisons of oviposition rates and fly developmental success. Five times as many flies were produced per fruit in May as in October. Fruit exposed in May received ca. 2.5X as many eggs as in October, and egg-adult success was ca. 2X greater in fruit exposed in May (8.0% vs. 3.9% in October). Selected chemical parameters (peel oil linalool concentration and peel sugar concentration) monitored throughout the season did not correlate with the observed increase in susceptibility of senescent fruit.

RESULTS OF EXPERIMENTS NOT ALREADY PUBLISHED

Studies on chronic exposure of caribfly larvae to lemon and grapefruit peel oils.

Earlier tests involving acute toxicity assays of peel oils revealed no difference among lemon, orange, and grapefruit oils in their effects on Caribbean fruit fly eggs and larvae (Greany et al. 1983, Styer and Greany 1983). These tests involved exposing the larvae to peel oils in vitro, then placing them on a hospitable artificial medium so that survival could be assessed. It was felt, however, that chronic exposure of the larvae to low concentrations of the peel oils might reveal differences in the relative toxicity of citrus oils, and that such differences might correlate with observed differences in susceptibility of various fruit cultivars. Because lemons are virtually immune, and grapefruit are somewhat susceptible, we tested these two types of oil under chronic exposure conditions.

We chose conditions that allowed high survival of the larvae despite the presence of mineral oil in the artificial diet. We found that 2.7% mineral oil (v/v) in the medium was about the maximum allowable amount. For these tests, we used the same medium formulation, inoculation methods, and holding conditions used in the former tests (Greany et al. 1983). However, mineral oil was added to the dry dietary components before hydration; this yielded a homogeneous medium with the oil uniformly dispersed after gelation. Diets also were prepared using graded mixtures of citrus peel oil and mineral oil, holding the total

oil concentration to 2.7% (v/v) in the diet.

These tests showed, like earlier acute exposure test, that lemon oil was not any more toxic than grapefruit oil to caribfly larvae, even under chronic exposure conditions (Fig. 2). In the absence of another explanation, we now feel that a factor other than differences in relative peel oil toxicity must account for the difference between susceptibility of lemons and grapefruit. One likely explanation relates to the fact that lemons possess ca. 2x more peel oil, per cm², than grapefruit (Greany et al. 1983). This observation correlates with findings reported below on increased susceptibility of grapefruit commensurate with senescence-related loss of peel oil.

Manipulation of fruit susceptibility with GA₃

The encouraging results obtained from the 1982-83 GA₃ studies in Israel prompted us to resume testing of this agent in Florida. We applied this growth regulator to Marsh grapefruit trees in mid-September, 1983 (prior to colorbreak), using 20 or 50 ppm. Abbott Laboratories ProGibb^R was used for these treatments. Experimental details are included in the caption for Figure 3. Control and treated fruit all were quite resistant in October, but by November it was clear that the treated fruit were significantly less susceptible, and that the response was dose-dependent. This trend was even more marked in December. Dr. Roy McDonald of the ARS Horticulture Research Laboratory (Orlando, FL) collected data on the puncture resistance of the peel of treated vs. control fruit; these data (Fig. 3) clearly show that

there was a marked difference in puncture resistance of treated vs. control fruit. Unfortunately, the fruit were destroyed by an extremely hard freeze on Dec. 25th, so that no further data could be collected, and plans were made to repeat the tests the following year.

A grove was located in a more frost-proof setting for the 1984-85 tests. This time, a treatment of 100 ppm was included since it appeared that 50 ppm was advantageous over 20 ppm in the preceeding year, and we wanted to determine whether any further advantage could be gained by an even higher dose. The results from the 1984-85 season (Fig. 4) again showed a definite benefit from use of GA₃ treatments. It should be noted that fewer flies were produced per fruit through December than in the preceeding year because fewer flies were placed into the exposure cages (32 vs. 200) to avoid overwhelming the fruit and masking treatment benefits. Even the untreated fruit were quite resistant through November, but in December a clear difference was apparent. This trend persisted throughout the season, with consistent dose-dependency.

A high degree of resistance was maintained through January or February, but even the 100 ppm fruit became relatively susceptible by March or April. The puncture resistance of these fruit is indicated in Fig. 5 (data provided by R. McDonald); it is again clear that treatment effects were dose-dependent, with the controls being considerably softer than any treated fruit soon after treatment.

With the aid of Dr. Phil Shaw of the ARS Citrus & Subtropical

Products Research Laboratory (Winter Haven, FL), the oil content of the peel of the treated and control fruit was measured each month. The data from these tests showed no difference in oil content among the treatments, but it was evident that all the fruit lost a significant amount of oil during the season (Fig. 6). Plotting oil content vs. fly susceptibility, we saw a very good linear relationship, with a correlation coefficient of 0.79 (Fig. 7). Thus, it appeared that the benefits associated with GA₃ treatment were attributable to its effects on peel softness rather than an effect on peel oil content. In addition, there may have been a degree of ovipositional preference by the caged flies for the control fruit, especially early in the season when there were obvious color differences among the fruit depending upon treatment dose. No data were collected on oviposition rates in the respective treatments in this study, however, due to limited availability of technical help.

Color differences due to treatment are indicated along with data on the number of days of ethylene treatment required to achieve a commercially acceptable color standard in Fig. 8. Through December, the 100 ppm fruit were generally refractory to degreening, but by January it was possible to degreen them in only 1-2 days; no ethylene was required thereafter.

Studies on activity of the enzyme pectinmethylesterase (PME) were conducted by Dr. Shaw in an effort to account for softening of the peel. Two eight-fruit composite samples of each treatment were prepared by randomly selecting 2 fruit from each of the 4 samples for each treatment. Albedo from each eight-fruit sample

was removed and two 40-gram composite samples were each treated with 400 ml of 10% sodium chloride solution for the September and October samples, and 200 ml of the same solution for the remaining samples. Two 10-ml portions of each extract were titrated at 30°C by the procedure of Vas et al. (1967) for pectinmethylesterase activity (a total of 4 measurements for each treatment). Pectinmethylesterase activity in the albedo of the grapefruit samples is shown in Table 25.

These values are comparable to those reported earlier for PME activity in grapefruit (Rouse, 1977; Vas et al. 1969). The only dramatic change was in the activity of all samples between September and October. Some of the treatments with gibberellic acid resulted in significant differences in activity when compared to control fruit. However, certain treatments resulted in lower PME activity and others in greater PME activity than found in the untreated fruit. Also, the highest level of treatment (100 ppm) did not show the greatest differences when compared to untreated control fruit. These results do not correlate with peel softness, and do not show trends that would be meaningful in correlating with decreased susceptibility of early season treated fruit to attack by the Caribbean fruit fly.

Figure 2. Relative toxicity of lemon vs. grapefruit oil to Caribbean fruit fly larvae under chronic exposure conditions. For these tests, 10 newly-hatched larvae were placed on the surface of ca. 6 ml test media in 10 mm deep x 35 mm diam. petri dishes, and the no. of puparia produced was recorded. Tests on lemon oil were replicated 10 times on each of 8 occasions; grapefruit oil tests were replicated 10 times on each of 5 occasions. Test media were prepared using an agar-based diet for the caribfly to which either no oil was added or peel oil-mineral oil mixtures were added to the dry dietary components before hydration, with a final oil concentration of 2.7% (v/v). Oil concentrations tested included: (1) no oil, (2) plain mineral oil; (3) 25% cold pressed peel oil diluted with mineral oil; (4) 50% peel oil; (5) 75% peel oil, and (6) 100% peel oil. Survival rates were corrected using Abbott's formula, using the "no oil" survival rates as the control.

Figure 3. Results from 1983-84 season on use of gibberellic acid to reduce susceptibility of Marsh grapefruit to attack by the Caribbean fruit fly. The fruit were sprayed to runoff with solutions of 0, 20, or 50 ppm gibberellic acid prior to colorbreak (in September 1983), using 3 trees for each treatment type. Eight fruit were sampled from each tree each month thereafter and were exposed in the laboratory to gravid female caribflies in 14x14x18 cm screen cages for 3 days, then were held for larval development. Twenty four fruit of each type were exposed each month, using 3 fruit of each treatment in each of 8 cages (9 fruit per cage total) inoculated with 200 female caribflies. Frost damage precluded tests being performed after December. Vertical lines represent std. errors of the means; bars for a given month annotated with the same letter are not significantly different (5% level) by Duncan's new multiple range test.

Peel softness also was measured during the season (plotted above bar graphs). The force (in Newton's) required for a 1 mm diam. probe to penetrate the peel was recorded from a sample of 24 fruit of each treatment type each month (std. errors accompany average values).

Figure 4. Results from 1984-85 season on use of gibberellic acid to reduce susceptibility of Marsh grapefruit to attack by the Caribbean fruit fly. The fruit were sprayed to runoff with solutions of 0, 20, 50 or 100 ppm gibberellic acid prior to colorbreak (in September 1984), using 4 trees for each treatment type. Eight fruit were sampled from each tree each month thereafter and were exposed in the laboratory to gravid female caribflies in 14x14x18 cm screen cages for 3 days, then were held for larval development. Thirty two fruit of each type were exposed each month, using 2 fruit of each treatment in each of 16 cages (8 fruit per cage total) inoculated with 32 female caribflies. Vertical lines represent std. errors of the means; bars for a given month annotated with the same letter are not significantly different (5% level) by Duncan's new multiple range test.

Figure 5. Puncture resistance of gibberellic acid treated vs. control fruit throughout the 1984-1985 season. An Instron^R Food Testing System (Model 1132) was used to measure firmness of the grapefruit. A constant weight of 10 kg was applied at a constant rate of speed (5 cm/min) to a 1.02 mm diameter cylindrical tip the amount of force required to puncture the rind was recorded. Each month, 8 fruit were collected from each of 4 trees of each treatment type (32 fruit per treatment per month). Four punctures were made on each test fruit at evenly spaced locations in the equatorial region of the fruit and these measurements were averaged. These average values were used to determine an overall average for each treatment each month.

BLOCK CHART FOR OIL-CONTENT: MONTH * GA-LEVEL

BLOCK CHART OF OIL_CONT

Figure 6. Relationship between GA_3 treatment and peel oil content of Marsh grapefruit throughout the 1984-85 season. Oil content (as mg per gram flavedo) was determined each month by removing and weighing the flavedo from duplicate 8-fruit samples of each treatment (2 fruit from each of the 4 trees of each treatment type were combined). These fruit were extracted in hexane, the hexane was removed by distillation, and the remaining oil was measured.

Figure 7. Relationship between peel oil content and susceptibility of Marsh grapefruit to Caribbean fruit fly attack. Since gibberellic acid treatment had no observable effect on oil content (Fig. 6), all the data for all treatments were combined and plotted vs. the average no. flies produced from corresponding fruit samples exposed to fly attack in the laboratory (eye-fitted regression).

Figure 8. Days required to degreen gibberellic acid treated vs. control fruit (using 5 ppm ethylene gas at 88-92% RH, 28°C) to a commercially acceptable color index. Color is expressed as an a/b ratio (ratio of green-red to blue-yellow), measured by a Hunterlab D25 Color Difference Meter (all treatments spontaneously achieved the required ratio of -0.1 in the field after January). Average values were obtained for each treatment each month using a sample of 10 washed fruit which were each measured at 3 equidistant points around the equator; these fruit were measured each 1-2 days thereafter until an acceptable a/b ratio was obtained.

Table 25. Pectinmethylesterase activity in grapefruit albedo treated with gibberellic acid.^a

Date harvested	PME units $\times 10^3$			
	Control	20 ppm	50 ppm	100 ppm
09/15/84 ^b	6.2	6.3	6.1	5.8
10/15/84 ^b	3.4	3.4	3.6	3.3
11/15/84	3.4	3.3	3.8	3.3
12/15/84	3.3	3.9	4.2	3.5
01/15/85	4.0	3.7	3.4	4.0
02/15/85	3.0	3.1	3.3	2.9
03/15/85	3.3	3.0	3.2	3.1

^aPectinmethylesterase (PME) activity in $\text{mmoles min}^{-1}\text{g}^{-1}$ of albedo -
 - each value is an average of 4 determinations except 09/15/84
 and 10/15/84 values.

^bAverage of 2 determinations.

CONCLUSIONS

The results we obtained strongly support the original premise that preharvest treatment of citrus fruit with the plant growth regulator gibberellic acid (GA_3) may significantly reduce the susceptibility of the fruit to fruit fly attack. We noted that the degree to which this was the case was dependent upon a variety of parameters: (1) treatment dose (100 ppm > 50 ppm > 20 ppm > 5 ppm); treatment timing (must be applied prior to colorbreak); (3) fruit type (oranges are more easily protected than grapefruit); and (4) species of fly (Mediterranean fruit flies are more difficult to guard against than Caribbean fruit flies, especially in the case of grapefruit).

An important outcome of these studies is the recognition that there are two independently operating primary factors -peel softness and peel oil content- that together govern senescence-related susceptibility of citrus to fruit fly attack. Peel softness, but not peel oil content, was affected by GA_3 treatment. Lemon oil, orange oil, and grapefruit oil all were about equivalent in toxicity to Caribbean fruit fly eggs and larvae, suggesting that the virtual immunity of lemons is associated with oil abundance rather than absolute toxicity (lemons possess ca. 2x more oil per cm^2 than grapefruit).

Susceptibility of the fruit may occur once a critical combination of oil loss and peel softening occurs. Since lemons (and oranges) have more peel oil than grapefruit to begin with, they are less likely to reach a susceptible state, especially if

GA₃ is used to reduce peel softening.

The Mediterranean fruit fly may be more successful than the Caribbean fruit fly in attacking citrus because of differences in oviposition behavior, rather than differences in ability to tolerate the fruit oils (preliminary tests on toxicity of citrus peel oils to medfly eggs and larvae showed no difference in the susceptibility of this species vs. Caribbean fruit flies to these agents - Greany et al., unpublished). Medfly females typically deposit a clutch of many eggs at a given site, and this site may be repeatedly visited by the same or other females, so that ≥ 100 eggs may be placed together. In contrast, Caribbean fruit fly females typically deposit only 1 or 2 eggs at a given site, then move to other areas of the fruit. This difference in behavior could be significant as each caribfly larva must overcome the fruit's resistance factors essentially alone, while there is a potential for cooperativeness among the medfly larvae.

Much of the oil from oil glands into which the medfly eggs are deposited is displaced by the large number of eggs placed there. Hatching medfly larvae probably encounter less oil in their immediate surroundings, enabling them to penetrate the flavedo and reach the hospitable albedo and pulp regions of the fruit. Furthermore, should all the larvae from the first clutch of eggs die, larvae hatching from a subsequent clutch of eggs could be benefitted from the partial entry of the first group of larvae. In addition, microorganisms (molds and bacteria) coincidentally present could assist the fly larvae in penetrating the peel. Thus, with no physiological difference in ability of the

Caribbean and Mediterranean fruit flies to tolerate citrus peel oils, medfly larvae would likely be much more successful because of their grouped attack behavior.

These studies indicate that GA_3 applications have the potential to significantly reduce the susceptibility of citrus fruit to fruit fly attack. In the case of oranges, the degree of benefit was marked, perhaps because of the innately greater resistance of oranges. Grapefruit, however, were more difficult to protect, especially against the Mediterranean fruit fly. Additional field studies should be conducted to verify the efficacy of GA_3 treatments against wild populations. Since the infestation rate in commercial citrus groves in both Israel and in Florida typically is very low, even in the absence of control measures, we have initiated tests on GA_3 use in a Peruvian orange grove that is habitually infested by wild medflies. It is hoped that these tests will confirm the positive results obtained in these BARD-sponsored study.

Our tests also point up the potential for manipulating the resistance of citrus through the use of growth regulators that sustain initially high peel oil levels or perhaps even increase the levels beyond those found early in the season. Such agents are already being tested for other reasons, and we hope to evaluate their use for fruit fly control as well. By sustaining both the mechanical and oil-based resistance properties of the peel, we may be able to routinely prevent damage to citrus by fruit flies without the need to use conventional hard pesticides or post-harvest fumigants. This could help prevent inadvertent

pesticide damage to biological control agents of value against pests such as scales, etc., and it could provide an answer to the problems associated with fumigant residues.

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F. DESCRIPTION OF COOPERATION

Planning of the initial and subsequent years' studies was performed cooperatively, with both principal investigators participating in the design of research to be conducted at both locations. This was facilitated by travel to Israel by Dr. Greany and to Florida by Dr. Rössler. The results obtained during the first year at each location were taken into account in the design of additional experiments that were conducted thereafter at each location. In addition to research performed by Drs. Greany and Rössler, the following USDA-ARS investigators contributed important ancillary information on the effects of GA₃ upon fruit peel softness, fruit color, degreening propensity, and chemical changes: Drs. Roy McDonald & Tim Hatton (ARS - Orlando, FL), and Dr. Phil Shaw (ARS - Winter Haven, FL).

G. BENEFIT TO AGRICULTURE

The studies conducted under the auspices of this grant support the idea of using gibberellic acid to reduce the susceptibility of citrus fruit to fruit fly attack. Pilot field tests still are needed to establish the credibility of these tests, most of which were conducted in the laboratory because of the low incidence of wild flies in citrus both in Israel and in Florida. Toward this end, we are initiating tests in Peru in an orange grove habitually infested with wild Mediterranean fruit flies. The tests we have already conducted were helpful in establishing treatment timing and optimal dosages that will be of value in the Peruvian tests.

Should the Peruvian tests further confirm the benefits observed in Florida and Israel, growers may be well advised to use prophylactic treatments of gibberellic acid to avert fruit fly attack. This might preclude the need for any further postharvest disinfestation procedure, at least in oranges, and in grapefruit harvested prior to a specific cutoff date judged to be safe.

The revelation that gibberellic acid affects peel softening but not oil loss is significant; we now plan to use plant growth regulators that are claimed to increase or sustain peel oil content in conjunction with GA₃ treatments to maintain the fruit in an even more resistant state.

This approach to dealing with citrus-infesting fruit flies has many potential benefits as contrasted with conventional

techniques: (1) it is biorational (it should not adversely affect non-target organisms including biological control agents important in the control of pests such as scales); (2) it could benefit growers by increasing the yield of peel oil (a valuable byproduct); (3) by eliminating the need for postharvest disinfestation, it could help solve the problems associated with fumigants (e.g., EDB residue problems); and (4) in areas (such as Peru) that often sustain yield losses due to fruit fly infestation, yields may be improved, with commensurate economic benefits.

H. LIST OF PUBLICATIONS

Published

Greany, P. D., P. E. Shaw, P. L. Davis, and T. T. Hatton. 1985. Senescence-related susceptibility of Marsh grapefruit to laboratory infestation by Anastrepha suspensa (Diptera: Tephritidae). The Fla. Entomol. 68:144-150.

In preparation

Greany, P. D., and Rössler, Y. Use of the plant growth regulator gibberellic acid to reduce susceptibility of citrus fruit to attack by the Mediterranean fruit fly: a novel, biorational approach. To be submitted to Science.

Greany, P. D., McDonald, R., Shaw, P. E., Hatton, T. T., Jr., Schroeder, W. T., and Rasmussen, G. K. Use of gibberellic acid to reduce susceptibility of citrus fruit to the Caribbean fruit fly by delaying peel senescence. To be submitted to Tropical Science.