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Phosphine as a Post-Harvest Treatment of 'Ruby-Red' Grapefruit Against Eggs and Larvae of the Mexican Fruit Fly, *Anastrepha ludens* (Diptera:Tephritidae)

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ABSTRACT

Phosphine killed all Mexican fruit fly, *Anastrepha ludens* (Loew), larvae and pupae in 'Ruby Red' grapefruit, *Citrus paradisi* Macfadyen after 2 to 4 days of exposure at 0.125 and 0.25 g/m³. Environmental conditions during exposure ranged from 8 to 48°C and 50% or greater RH. After 2 to 4 days of exposure, 95.2051% to 99.8846% kill of eggs and larvae and pupae were determined, at 0.031 and 0.062 g/m³. Utilizing probit analysis 0.125 to 0.5 g/m³ fumigant required 4 to 7 days of fumigation to kill 99.9968% of eggs, larvae and pupae. Equal doses killed all stages of this insect. There was no significant difference in fruit quality of 'Ruby Red' grapefruit fumigated for 4 days at 0.5 g/m³ and nonfumigated grapefruit up to 34 days post-fumigation.

RESUMEN

El tratamiento con fosfina mató a todas las larvas y las pupas de la mosca mexicana de la fruta, *Anasthrepha ludens* (Loew), en toronja "Ruby Red", *Citrus paradisi* Macfadyen, después de dos a cuatro días de exposición a las dosis de 0.125 y 0.25 g/m³. Las condiciones ambientales durante la exposición variaron entre 8 a 48 °C y se presentó una humedad relativa del 50% o mayor. Después de 2 a 4 dias de exposición a 0.031 y 0.062 g/m³, se deteminó de un 95.2051% a un 99.8846% de mortalidad de huevos, larvas y pupas. Utilizando el análisis probit se determinó que con los tratamientos de 0.125 a 0.5 g/m³ de fumigante se requirió de 4 a 7 dias de exposición para matar al 99.9968% de los huevos, larvas y pupas. Dosis indénticas mataron a todos los estadíos de este insecto. No hubo diferencia significativa entre la calidad de la fruta de toronjo "Ruby Red fumigada pro cuatro días a 0.5 g/m³ y la fruta no fumigada por un período hasta de 34 días después de la fumigación.

Key Words: Diptera, Tephritidae, *Anastrepha ludens* (Loew), Phosphine, Grapefruit, Fruit Quality

Methyl bromide was approved in 1984 for use as a postharvest treatment of grapefruit, *Citrus paradisi* Macfadyen, against the Mexican fruit fly, *Anastrepha ludens* (Loew), Williamson et al. (1986). However, it will be banned from use after the year 2000.

Phosphine was found to be effective against a related species, *A. suspensa* (Loew), (Von Windeguth et al. 1976 and 1977 and Hatton et al. 1982). Hatton et al. (1982) showed minimal rind injury of 'Marsh Seedless' Florida grapefruit when fumigated at 1.48 g/m³ for 4 days and held at 21°C for 4 weeks. However, after 4 weeks at 10°C, excessive rind injury was indicated at the same rate. In addition, Moshonas and Shaw (1982) found that there were significant differences in peel oil aroma from 'Marsh Seedless' grapefruit from Florida treated with the 1.48 g/m³ phosphine, but there was no difference in concentrations of ascorbic acid, % oil, % acid, and brix.

Since phosphine has been reported to cause fruit damage in Florida, experiments were conducted to determine if it would cause the same injury at lower rates to 'Ruby Red' grapefruit produced near Weslaco, Texas, in the Lower Rio Grande Valley and treated during October to May 1987-88 and 1988-89. In addition, tests were conducted to determine efficacy of phosphine against the Mexican fruit fly in grapefruit during the same time. Results are reported here.

MATERIALS AND METHODS

The laboratory strain of Mexican fruit fly used in these tests was obtained from an existing culture at the USDA, ARS laboratory at Weslaco, Texas. Its origin and rearing methods were described by Williamson et al. (1986). Technical, as 100% magnesium phosphide (unformulated), was used (Daegish America, Inc., Weyers Cave, VA). Doses were determined from percentage phosphide of the magnesium phosphide. The walls of the 3.96 m³ chamber were constructed of plywood with plastic sealant. Sealant was applied to reduce loss of the fumigant. The chamber was outdoors and not covered.

During these experiments, ambient temperatures ranged from 8 to 28°C and relative humidities ranged from 50 to 75% outside the fumigation chamber; relative humidity levels inside chamber was not determined. When each experiment was initiated ambient temperatures inside chamber ranged from 17°C to 28°C. Also, we placed 50 ml water inside the chamber when each test was initiated. According to current registration, phosphine cannot be used when the temperature of the commodity to be treated is 4.44°C or lower. Fruit temperatures were determined before treatment by thermometer and were always >21°C when placed in the chamber, but fruit

temperatures were not recorded during the fumigation.

Ripe (greenish to yellow) 'Ruby-Red' grapefruit, 200-300/test were exposed to ovipositing adults within 24 h after harvest for infestation as described by Williamson et al. (1986). Eggs (4 days or less following oviposition) and 6-9, 10-11, and 12-17 day old larvae in fruit were fumigated for 1, 2, 3, or 4 days at 0.031, 0.062, 0.125, 0.25 or 0.5 g/m^3 for 80 combinations. Fruit for each age larvae were placed in the chamber with proper dose of magnesium phosphine. We aereated chamber 1-3 hrs between tests. Load factor (area occupied by fruit vs area in chamber) was 5 or 10% in all tests.

Larval ages were determined from the first day of exposing the fruit to the gravid females in the oviposition chamber to the time the experiment was initiated. Fruit with three day old larvae were exposed to ovipositing adults seven days earlier. This is because the eggs hatch in 3-5 days.

Draeger tubes, both the high (50/a) and low (0.1/a) ranges, were used to measure gas concentrations of phosphine in ppm within the chamber after 24 h. This was the time of maximum concentrations inside the chamber according to von Windeguth et al. (1977) and Hatton et al. (1982). The line from the exit port was placed in the middle of the area occupied by the fruit.

Fumigated and control fruit were held on wire racks at 25$\pm$2°C for larvae to emerge and pupate in pans containing fine vermiculite. Control fruit were 10 to 15% of total fruit infested for each test; a total of 1615 fruit were used for the control. Pupae were removed and held for adult emergence. Doses exposure time and immature stages were conducted with each of the 16 groups of fruit that were infested. Three to 5 replicates were conducted for each of the 80 combinations. Estimated larvae present in the fruit at the time of treatment were determined from number of pupae per fruit in the control and number of fumigated fruit used for each age of larvae, time of treatment, and dose tested. Ninety-five to 190 fruit/dose, age of insect and per treatment time in all the replicates were tested. Larval mortality was percentage fewer pupae of the estimated larvae determined for each treatment. Pupal mortality was percentage fewer adults of the same estimated larvae for each treatment.

All possible combinations of each dose (analyzed over days) and days (analyzed over doses) were analyzed by linear regression (SAS Institute 1988). When no significant difference (by F for 2, 17 df) was determined at $P<0.05$ between each dose (over days) or day (over doses) they were analyzed until we determined only the groups of doses or days which showed significant differences. Then average percentage mortality of the doses and days in the grouping were analyzed by probit (SAS Institute 1988) and dose or days required to kill 99% and 99.9968% of eggs and larvae and pupae and their 95% confidence interval were calculated. Data were not transformed for analysis.

Fruit Quality Test. 'Ruby Red' grapefruit (30/test) were harvested from the tree and fumigated on the same day at 0.5 g/m^3 for 4 days to determine if the fumigant caused rindburn to Texas grapefruit. Fruit were harvested on days 123, 130, 166, and 171, 1989; each day was considered a replicate. The same visual rating classes as described by Anonymous (1979) and Hatton et al. (1982) were used for treatment and control fruit: Class 1 was no damage, class 2 was visible damage on 0.1 to 3.0% of the total surface area, class 3 was visible damage on 4 to 25% of the total surface area, while fruit were considered class 4 and 5 when rindburn and pitting were visible on $\geq$26% and $\geq$50%, respectively, of the total surface of each fruit. When any tissue appeared to be rotten at the stem-end of the fruit or 26% or more of the surface area had oil spots, the fruit was classed as unacceptable. After the four days of treatment the fruit were held at 26$\pm$3°C and visually rated on 1 to 5, 11 to 15, 18 to 22, and 27 to 34 days posttreatment. Rindburn, pitting, stem-end breakdown, and oil spotted fruit graded as 4 and 5 were considered unacceptable.

Thirty fruit per four harvest dates (or replicate) of both fumigated and control fruit were used for the visual rating. Percentage acceptable fumigated and unfumigated fruit were calculated for each harvest date and compared using a t-test at $P = \leq 0.05$. Data were not transformed for analysis.

RESULTS AND DISCUSSION

Insect Mortality Test. Mortalities of Mexican fruit fly eggs and all ages of larvae ranged from 88.3993% to 100% (Table 1) following treatment of grapefruit with phosphine at 0.031 to 0.5 g/m^3 for 1 to 4 days. This is about 12% difference in dose and time for phophine; the results show that all doses and times tested were toxic to this insect. The difference is variation in response by this insect. When fruit was fumigated for 2 to 4 days at 0.125 to 0.5 g/m^3 egg and larval mortalities ranged from 99.7333 to 100% and pupal mortalities ranged from 99.777 to 100%.

Untreated fruit had 12.67$\pm$11.67 (standard deviation) pupae/fruit (range = 1-27) and 11.6$\pm$13.51 adults/fruit (range = 1-26) and 92% adult emergence. A total of 40493 estimated larvae (based on pupal populations) and 37416 estimated pupae (based on adult populations) were determined to be in fumigated fruit.

Regression of larvae and pupae for day 1 were significantly different from days 2-4 by $F = 6.612$, $> P = 0.0075$ and $F= 6.068$, $P > 0.0103$, respectively. (Degrees of freedom of all regressions were 2,17). Mean for 1 day kills were significantly greater. Regression also showed that mortalities of larvae following 0.031 and 0.062 g/m^3 were significantly different from 0.125 to 0.5 g/m^3 by $F = 4.45$, $> P = 0.0279$. Regression analysis showed the mortalities of pupae following 0.031 and 0.062 g/m^3 fumigation were significantly different from 0.125 to 0.5 g/m^3 by $F = 4.316$, $> P = 0.0305$. These were the only significant differences determined. Intercept and slope values were similar which would be expected with only 12% difference in high and low mortalities; thus, they are not presented.

At the lowest 2 doses tested we could not kill 99.9968% of the larvae or pupae in less than about 6 days of exposure (Table 2). At the greatest 3 doses about 4 days were required to kill 99.9968% of the insects tested. At the greatest 3 doses less than 1 day was required to kill 99% of the insects tested

while 3-4 days were required at the lower two dosages. The 95% confidence limits overlapped all calculated doses and all calculated days.

Hatton et al (1982) evaluated 0.53 g/m³ (lowest dose tested) against the Caribbean fruit fly and I compared 0.5 g/m³ (greatest dose tested) against the Mexican fruit fly and mortalities were similar, i.e. 98% to 100%. However, the same percentage of immatures of the Mexican fruit fly was killed at a dose about 16X lower than the 0.53 g/m³ evaluated against the Caribbean fruit fly.

Draeger tube readings of phosphine, taken after 24 h, averaged 0.2, 15, 44.6, 90, and 225 ppm for 0.031, 0.062, 0.125, 0.25, and 0.5 g/m³, respectively. Other readings were taken at the various concentrations after 4 and 48h but they were not determined consistently to show the rise in concentrations to 24h and the fall in concentrations after 48 h. These readings showed the same trends of Von Windeguth et al. (1977) and Hatton et al. (1982). Von Windeguth et al. (1977) and Hatton et al. (1982) determined maximum ppm values after 24 hrs of 127, 217, 447, 735, 794, 770, 1425, and 775 for 0.53, 0.71, 1.06, 1.24, 1.41, 1.77, 2.12, and 2.96 g/m3, respectively. When similar dosages were compared (0.5 g/m³ and 0.53 g/m³) we determined greater concentrations of phosphine in the chamber after 24 h. For comparison, theoretical concentrations of phosphine/g of magnesium phosphide would be 17, 33, 66, 132, and 262 ppm for our concentrations of 0.031, 0.062, 0.125, 0.25, and 0.5 g/m³ according to von Windeguth et al. (1977). Actual concentrations were always less than the theoretical concentrations probably because the fumigant sorbed onto the surface of the chamber, fruit and plastic field box.

Fruit Quality Test. Percentage acceptable grapefruit ±SE for the marketplace was determined to be 70±8, 59±7, 63±10, and 22±7% when fumigated for 4 days at 0.5 g/m³ (maximum time and dosage tested) and 83±6, 52±10, 41±9, and 6±4% for unfumigated fruit after 1-5, 11-15, 19-22, and 27-34 days post-fumigation, respectively. There was no significant difference in percentage acceptable fumigated and unfumigated fruit for any group of days post-fumigation. Based on these evaluations phosphine does not cause rindburn of 'Ruby Red' grapefruit at 0.5 g/m³ held at 26.3+3°C to 34 days post-fumigation.

The major disadvantage of phosphine as a postharvest treatment of grapefruit against the Mexican fruit fly is the time required to kill the insect. The greatest three doses could be used to provide quarantine security of grapefruit provided that the fruit are fumigated for 3 or 4 days.

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Table 1. Toxicity of phosphine against eggs and larvae of the Mexican fruit fly in "Ruby Red" grapefruit. Weslaco, TX. 1987-89.

	Dose (g/m³)									
	0.031		0.062		0.125		0.25		0.5	
Treatment Duration (days)	Estimated number[a/]	Mortality (%)	Estimated number[a/]	Mortality (%)	Estimated number[a/]	Mortality (%)	Estimated number[a/]	Mortality (%)	Estimated number[a/]	Mortality (%)
	Estimated Larval Mortality (%) From Fumigated Eggs and Larvae									
1	2029	97.1417	1709	95.0839	1677	99.2189	1973	99.5945	1578	88.3993
2	1095	99.2692	2340	99.6153	2679	99.9626	1111	100.0	3000	99.9000
3	1734	99.8846	1877	89.8774	2869	100.0	2758	100.0	3240	100.0
4	1836	99.1829	2191	98.9957	1999	99.9499	748	100.0	2250	99.7333
	Estimated Pupal Mortality (%) From Fumigated Eggs and Larvae									
1		98.1766		98.6539		99.5826		99.6959		95.2456
2		99.5432		99.6581		100.0		100.0		99.9333
3		99.8846		95.2051		100.0		100.0		100.0
4		99.4008		99.5435		100.0		100.0		99.7777

[a/] Larvae (as pupae) in fumigated fruit.

Table 2. Estimated exposure time and dosages of phosphine required to obtain Mexican fruit fly egg, larval and pupal mortalities[a]

Evaluation	Mortality (%)			
	Of Eggs and Larvae		Of Pupae	
	99	99.9968	99	99.9968
Duration (days)	Estimated Doses (g/m^3)			
1	0.46 (0.098 - 9.23)	9.48 (0.15 - 76,398.0)	0.25 (0.069 - 2.17)	6.24 (0.096 - 60,806.5)
2, 3, & 4	0.023 (0.0058 - 0.14)	0.48 (0.099 - 10.62)	0.011 (0.00004 - 0.17)	0.29 (0.073 - 3.27)
Doses (g/m^3)	Estimated Time (days)			
0.0031 and 0.062	4.03 (1.78 - 9.26)	7.69 (1.4 - 24.1)	3.22 (1.6 - 6.4)	6.91 (1.5 - 21.7)
0.125, 0.25 and 0.5	0.71 (0 - 3.2)	4.37 (1.8 - 10.6)	0.0028 (0 - 3.3)	3.7 (1.7 - 8.2)

[a]Mortalities for multiple linear regression taken from Table 1.

Table 3. Toxicity of phosphine at 0.031, 0.062, 0.125, 0.25 and 0.5 g/m^3 doses against the egg stage and 3 ages of larvae in "Ruby Red" grapefruit. Weslaco, TX. 1987-89.

	Larval Age at Time Treatment (days)							
	Eggs		6-9		10-11		12-17	
Duration Treatment (days)	Estimated number	Mortality (%)	Estimated number	Mortality (%)	Estimated number	Mortality (%)	Estimated number	Mortality (%)
	Estimated Larvae and Egg Larval Mortality From Fumigated Eggs and Larvae							
1-2	4808	99.9792	2200	99.8636	1024	96.2872	3986	95.8356
3-4	6893	99.9854	680	100.0	668	100.0	2865	99.7905
	Estimated Larvae and Pupal Mortality From Fumigated Eggs and Larvae							
1-2		100.0		99.9090		97.5574		98.4195
3-4		100.0		100.0		100.0		99.8255

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