tection Agency. This fungicide has shown considerable potential as a postharvest fungicide for the control of major decays of Florida citrus fruits, except sour rot. The fungicidal effect of imazalil persists during long term cold storage and a simulated marketing period. The property of imazalil to control strains of green mold resistant to benomyl as well as to arrest mold sporulation makes it a desirable postharvest citrus fungicide.

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PHOSPHINE AS A FUMIGANT FOR GRAPEFRUIT INFESTED BY CARIBBEAN FRUIT FLY LARVAE[1,2]

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Abstract. **Both hatch of eggs and pupation of larvae of the Caribbean fruit fly,** Anastrepha suspensa **(Loew), were reduced or prevented by fumigation with phosphine gas generated from magnesium phosphide containing paper covered flat packets (Fumi-cels®). Fruit flies infesting 'Marsh' seedless grapefruit were controlled when fruit in refrigerated semi-trailer vans was fumigated at 13°C for 96 hr or fruit under a tarpaulin was fumigated at ambient temp for 48 hr. There was no apparent injury to fruit fumigated under these conditions.**

The Caribbean fruit fly, *Anastrepha suspensa* (Loew), a pest of fruit in Florida the past 12 years (Weems, 1966), commonly infests guava, *Psidium guajava* L., tropical almond, *Terminalia catappa*, L., Surinam cherry, *Eugenia uniflora* L., and other tropical and subtropical fruits. Until 1974, citrus had only occasionally been reported as infested. However, in June of that year, Japanese quarantine officials found a total of 14 larvae of the Caribbean fruit fly infesting 11 Florida grapefruit among over 3.5 million kg transported to Japan on 5 ships that had left the United States between April 4 and May 27. All further shipments of Florida grapefruit were then discontinued until schedules for fumigation with ethylene dibromide (EDB) were approved by the Japanese Ministry of Agriculture in consultation with officials of the U. S. Department of Agriculture. Shipments were resumed in February 1975, and that spring over 5 million boxes of grapefruit that were to be shipped to Japan, the equivalent of about 100 million kg, were fumigated with ethylene dibromide in semi-trailer vans or in overseas containers.

Meanwhile, research was initiated at Miami to find other treatments for fruit fly larvae. One possibility is phosphine. Phosphine generated from aluminum phosphide (Phostoxin®) has been commercially available for several years as a fumigant for insect pests of grain and other stored products (Lindgren and Vincent, 1966). However, Phostoxin has not been used to control insect pests of fresh fruit and vegetables because it could be phytotoxic and because such a long fumigation is required. For example, at some conditions (lower temp), phosphine can take as much as 2 days just to evolve to its maximum concn in an enclosed space. However, a new formulation has recently been developed that uses magnesium phosphide (Fumi-cel®) as the material for the generation of phosphine gas. The magnesium phosphide is formulated as a flat, paper-covered packet. Each standard size Fumi-cel (26 x 17.5 x 0.5 cm) contains enough magnesium phosphide to develop 33 g of phosphine (PH_3) for treatment of 28 to 44.8 m^3. Smaller Fumi-cels (7.5 x 6.5 x 0.5 cm) generating 3.3 g of phosphine formulated for use in research can be used to treat 2.8 m^3 spaces.

[1]This paper reports the results of research only. Mention of a pesticide does not constitute a recommendation for use by USDA, nor does it imply registration under FIFRA as amended. Mention of a trade name does not constitute a recommendation for use by the U.S.D.A.

[2]The authors wish to acknowledge assistance in this research from the Florida Citrus Commission, Phostoxin Sales, Inc., Alhambra, California, and Degesch, Frankfort, Germany.

A Fumi-cel is packaged in an air-tight metallic envelope. When this envelope is opened, the chemical reacts with moisture present in the air to generate gaseous hydrogen phosphide (phosphine). The reaction has been slowed by the formulation method so the concn of phosphine builds up over an 8 to 12 hr period if temp and available moisture (as relative humidity) remain sufficiently high. Lack of moisture (R. H. $<$ 50%) and/or low temp ($<$ 10°C) can extend this time, but this can be handled to some extent by using more formulation per unit volume.

Methods

We conducted bioassays in the laboratory to obtain basic data about the comparative effectiveness of phosphine against the 2 immature stages of the fly found in the fruit. In the tests, eggs and larvae of the Caribbean fruit fly were exposed to "pre-generated" concentrations of gaseous phosphine instead of to the gradually built up concn that occurs over a period of hours in a normal fumigation. This technique permitted determination of the relationship between concn and time to mortality with controlled conditions.

The phosphine used in our 1.4 m^3 (50 ft^3) research fumigation chamber was produced by cutting a small Fumi-cel in 2 pieces (to give ca. 1.65 g AI) and placing one portion in the chamber with a damp sponge that provided adequate water vapor for the evolution of the phosphine gas. The reaction was allowed to continue overnight, and the bioassays were conducted after equilibrium was obtained the next day. However, distribution of active ingredient (AI) within the small cell is uneven and the halved cell does not necessarily contain one half the AI, so fumigations with each portion of a cell could result in different concentrations of phosphine.

For a bioassay with eggs, 100 newly oviposited eggs collected from colony cages were placed on a 4 cm^2 piece of dampened blotting paper, and the paper was placed in a high humidity container where the eggs were incubated. After incubation for 24 hr at 25°C, the eggs were placed in a 2 x 4 x 1.5 cm plastic container (similar to the individual containers of jelly used by restaurants), which was inserted into a screw top 0.946 liter (1 qt.) Cubitainer,® a collapsible, thin wall, molded polyethylene sealable container. Phosphine was then pumped from the fumigation chamber through a 3 mm i. d. teflon tube terminating in an 18-gauge hypodermic needle and into the Cubitainer. Once the Cubitainer was inflated with the phosphine-air mixture, the needle was removed, and an adhesive patch was placed over the injection site. Controls were handled the same way except that the Cubitainer was injected with ambient room air rather than with fumigant. After the treatment, the eggs were removed and placed in a covered petri dish to prevent dessication. Four days later they were examined under a microscope to determine mortality caused by the exposure to the phosphine.

For the bioassay with 4-day-old larvae, we first placed ca. 50 eggs from the laboratory colony on 20 ml of agar medium in the 2 x 4 x 1.5 cm plastic container and allowed the eggs to hatch and the larvae to feed and develop until they were four days old. At this time they were placed in the Cubitainer and fumigated as described. After fumigation, the container of larvae was placed in a 235 ml (8 oz) waxed carton containing ca. 25 ml of vermiculite. Larvae surviving treatment left the medium and pupated in the vermiculite. For the bioassay with 6-day-old larvae, we removed 30 four-day-old larvae from the colony stock and placed them in 20 ml of fresh agar in a plastic container. These larvae were then allowed to adjust to the new medium and were not fumigated until they were 6-days-old.

The concn of phosphine in the fumigation chamber was determined at the time the fumigant was pumped into the Cubitainer and at the time the fumigation period ended by aspirating 100 ml of gas through an Auer Phosphine Detector Tube® (PH_3-50). The reduction of a gold complex salt to colloidal gold produced a darkened area proportional to the concn (within a range of 50-2000 ppm with this particular indicator tube).

Other tests were made with 'Marsh' seedless grapefruit that had been processed in a packinghouse. The fruits were infested by placing them on racks in a 20 m^3 outdoor infestation cage containing ca. 200,000 adult laboratory-reared flies of both sexes. The fruit were exposed to gravid females for 1 to 2 weeks, depending on ambient temp (cold weather inhibits oviposition and development of the larvae). The presumably infested fruit were then packed in 4/5-bu fiberboard overseas shipping cartons and usually held as much as one week longer to permit the eggs to hatch and some larvae to reach the third instar. (Some fruit were not held this additional period since we wished to be sure that eggs were present).

Fumigation of the infested fruit was done in a 1.4 m^3 fumigation chamber or in a 22.3 m^3 or 60 m^3 refrigerated semi-trailer van, and under gas-impervious tarpaulins. In some fumigations in the 60 m^3 semi-trailer we used boxes of polystyrene balls to simulate the bulk volume of a full truck load of grapefruit. However, it was then necessary to add additional water (by putting 2 large wet cellulose sponges in the fumigation chamber) to simulate the respiration from a one thousand 4/5-bu box of fruit (estimated to be ca. 142 g water/hr/18,144 kg fruit). In other fumigations, surplus fruit were available to provide a full load of fruit for the small van, the 1.4 m^3 chamber, and the tarpaulin. In the four 96 hr tests in the 22.3 m^3 semi-trailer (Table 1), neither uninfested fruit nor boxes of styrofoam were used in addition to the infested fruit.

Tarpaulin fumigations were conducted by making an eight-layer "chimney" stack of 72 fiberboard cartons of fruit or boxed styrofoam balls and a 20 carton load of infested fruit on a wooden pallet on a concrete slab. This arrangement resulted in a space of 2.8 m^3, so one small Fumi-cel was used to produce a standard dose rate of 33 g/28.3 m^3 (840 ppm). A 7 x 7 m tarpaulin was used to cover the stack and the edges were secured by sand snakes around the perimeter. Three types of material were tested for the tarpaulin covering, a clear 6 mil (0.15 mm) thick nylon-thread reinforced plastic, an 8 mil (0.2 mm) olive drab plastic fumigation tarp from a surplus U. S. Army fumigation kit, and a vinyl-coated 8 oz (227 g) nylon tarpaulin especially made for our use. Efficacy of the fumigation on infested fruit was determined by holding the treated fruit for 5 weeks and comparing the insect yield to that of an untreated control in the same method as described by Burditt and von Windeguth (1975).

Concentrations of phosphine gas were determined in all experiments by using either Drager or Auer detection tubes (Drager® tube phosphine 50/a or Auer phosphine detector tube PH_3-50) as described.

Results and Discussion

Laboratory Bioassay of Phosphine:

The results of the laboratory bioassay with Caribbean fruit fly eggs are summarized in Fig. 1. Mortality of eggs was correlated to some extent with both the *concn of* phosphine gas and the duration of exposure. Probit analysis indicated that ca. 27 hr of exposure to concn of phosphine

Table 1. Recovery of larvae of the Caribbean fruit fly from infested grapefruit after phosphine fumigation at 13°C in a 22.3 m³ semi-trailer van.

Duration of treatment (hr)	Theoretical[z]	Gas concn (ppm) Measured concn. Max.	48 hr	96 hr	Untreated fruit No. of fruit	No. pupae recovered	No. pupae/fruit	Treated fruit No. of fruit	Estimated population[y]	No. of survivors	% Mortality	Probit
48[x]	1060	250	40	—	144	506	3.51	540	1897	9	99.53	
	1060	400	150	—	284	4396	15.5	691	10696	13	99.88	
	1060	500	200	—	218	7394	33.92	712	24149	158	99.35	
	Total								36742	180	99.51	7.6
48[x]	2120	600	365	—	255	703	2.76	507	1398	3	99.79	
	2120	1000	690	—	274	1689	6.16	548	3378	2	99.94	
	2120	700	410	—	281	1500	5.34	560	2989	1	99.97	
	2120	800	480	—	285	3204	11.24	567	6374	0	100.00	
	Total								14139	6	99.96	8.4
96	1060	350	—	75	288	3886	13.49	720	9713	0	100	
	1060	300	150	100	144	1664	11.56	720	8323	0	100	
	1060	350	—	100	288	7723	26.78	612	16424	0	100	
	1060	225	225	100	216	2105	9.7	720	6984	0	100	
	Total								41444	0	100	9.0

[z]Theoretical concn of phosphine produced by 1 Fumi-cel would be 1060 ppm and by 2 Fumi-cels would be 2120 ppm.
[y]Estimated population treated based on mean numbers of larvae/fruit in the control x number of fruit treated.
[x]400 boxes of old grapefruit were used to simulate a load of fruit.

gas > 500 ppm and 39 hr of exposure to concn between 250 and 500 ppm would be required to obtain 95% kill of Caribbean fruit fly eggs.

The results of the Bioassay with Caribbean fruit fly larvae (4 or 6 days old) are summarized in Fig. 2. Ca. 95% of 4-day-old larvae were killed by 16 hr or more exposure to 350-750 ppm of phosphine. An exposure of over 300 hr would be required to kill 95% of 6-day-old larvae.

Fumigation of Infested Fruit:

The results of experimental phosphine fumigation of infested fruit in a 22.3 m³ semi-trailer van are summarized in Table 1. Mortality of Caribbean fruit fly larvae was high when 16 to 20 cartons of infested grapefruit plus ca. 400 cartons (36 grapefruit/carton) of uninfested surplus fruit were fumigated at 10-13°C for 48 hr. Moreover, one Fumi-cel gave 99.5% mortality and 2 Fumi-cels gave 99.96% mortality in 48 hr. When 20 cartons of infested fruit were fumigated for 96 hr (no simulated load of fruit), there were no survivors.

The amount of phosphine present under a tarpaulin varied widely from test to test due to variation in temp, humidity, and wind. However, complete mortality of the fruit fly was usually obtained when infested fruit were fumigated under an olive drab military or a vinyl coated nylon tarpaulin for 48 hr (Table 2). The exceptions were two tests made during January when the ambient outdoor temperature dropped below 10°C. Also, placing fruit under a tarpaulin for 48 hr with no fumigant reduced recovery of fruit fly puparia 82% compared with the yield from the untreated control. In this single test, the fruit temperature reached 32°C.

The data acquired in these current tests in the small (22.3 m³) semi-trailer van are in agreement with other data collected in preliminary tests by von Windeguth et al. (1976). In these tests infested grapefruit were fumigated with phosphine on 6 dates (about one week apart) from July 23 through August 27, 1975, in a 60 m³ van. Fruit were removed after 6, 12, and 24 hr and held for as much as 5 weeks to determine the number of surviving larvae.

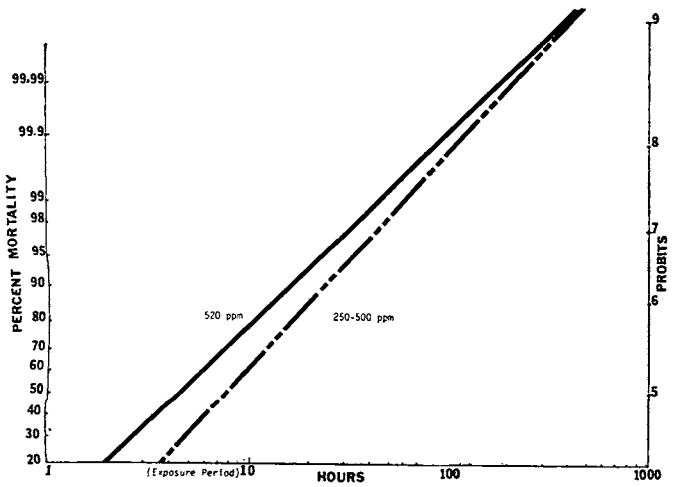

Fig. 1. Mortality of fruit fly eggs following exposure to hydrogen phosphide at various gas concn: Log time-probit lines determined by regression analyses of mortality data corrected for control mortality by Abbott's formula.

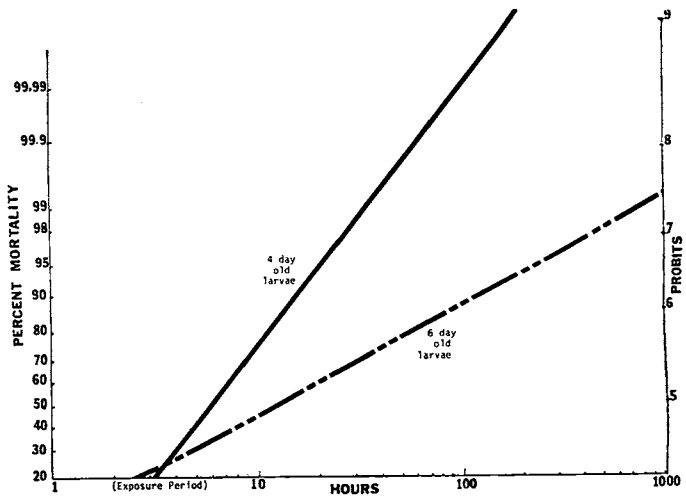

Fig. 2. Mortality of fruit fly larvae following exposure to hydrogen phosphide in concn between 350 and 750 ppm: Log time-probit lines determined by regression analyses of mortality data corrected for control mortality by Abbott's formula.

Table 2. Recovery of larvae of the Caribbean fruit fly from infested grapefruit after phosphine fumigation under a tarpaulin for 48 hr.[z]

Type of tarpaulin	Measured Gas concn (ppm) Max.	Mean	Untreated fruit No. of fruit	No. pupae recovered	No. pupae fruit	Treated fruit No. of fruit	Estimated population[y]	No. of survivors	% Mortality
			Fumigated Fruit						
Clear plastic									
	200	125	255	8071	31.7	511	16173.6	0	100
	500	325	400	884	2.2	977	2159.2	0	100
	500	440	260	6071	23.4	507	11838.5	3	99.9
	Total						30171.3	3	99.99
Olive drab military									
	700	450	256	7231	28.2	380	10733.5	0	100
	600	400	256	777	3.0	508	1541.9	0	100
	Total						12275.4	0	100
Vinyl coated nylon									
	350[x]	250	288	2217	7.7	360	2771.3	23	99.2
	400[x]	250	144	1148	8.0	396	3157.0	243	92.3
	Total						5928.3	266	95.3
	400	375	247	58	0.2	640	150.3	0	100
	600	500	288	98	0.3	576	196.0	0	100
	700	350	216	1288	6.0	612	3649.3	0	100
	Total						3995.6	0	100
			Non-fumigated fruit						
Vinyl coated	0	0	255	2220	8.7	504	4387.8	780	82.2

[z]Theoretical concn of phosphine was 840 ppm.
[y]Estimated population treated based on mean numbers of larvae/fruit in the control x number of fruit treated.
[x]Temp during treatment dropped to below 10°C.

In these tests mortality ranged from 74 to 100% after 6 hr, from 89 to 100% after 12 hr, and from 99 to 100% after 24 hr and was 50% within 2 hr and 95% within 11 hr. They also found that maximum concn of phosphine gas, 300 to 600 ppm, was obtained 6 to 8 hr into the fumigation period and that the concn declined gradually to between 73 and 200 ppm by 24 hr. These relatively low concns were probably the result of opening the side door of the van to remove cartons of fruit for the 6 and 12-hr exposure samples, to low humidity, and to undetected leaks in the van.

The present studies showed that 'Marsh' seedless grapefruit tolerated fumigation with phosphine under the condition of the tests. In contrast, when avocados and mangos were fumigated with phosphine (Spalding, et al. 1977), some varieties of avocados were slightly injured and mangos were injured, had increased decay, and showed retarded ripening. However, in tests in Hawaii (Seo et al., in press), ripening of papayas and avocados was accelerated by fumigation with phosphine at 12.8°C, though bananas, eggplants, bell peppers and tomatoes tolerated phosphine fumigation under certain conditions.

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