Sticky Trap Count Nails Down Thrips Pest Control Dates

Story and Photos By Wayne Cox

"We must forget the calendar way of farming, modify our techniques, and do things scientifically," says pest control consultant Ibrahim Michael, who has devised a citrus thrips control program which is paying big cash benefits to valley citrus producers subscribing to his integrated approach to pest control.

Michael, owner-operator of Michael's Pest Management of Fresno, contends the citrus thrips is one of the most important citrus pests. It damages and reduces both citrus quality and quantity from April through July, causing large amounts of otherwise excellent fruit to be rejected at the packing houses.

For years growers have been using chemical control of citrus thrips on a hit-and-miss basis, said Michael. Often, many of them will spray two or three times a year without achieving effective control. But they cannot be blamed for wasting their time and money when they miss because no one seems to know when is the best time time, precisely, to spray for the most effective control of citrus thrips.

"I don't mean to step on any toes regarding citrus thrips treatment recommendations," said Michael almost apologetically. "But a person can read a whole lot of books, talk with experts in the field and find very little about controlling citrus thrips effectively."

The Cairo-educated toxicologist and plant pathologist believes the answers leading to economic and effective control of citrus thrips lie in understanding the true and complete biology of this injurious pest.

He has devoted 23 years of his life to tracking down clues on citrus thrips control, and now believes he has an economical and effective control technique designed to deal with this citrus pest specifically. His current technique is based on citrus thrips control programs developed over the years by the US Department of Agriculture and the University of California.

Michael freely admits that complete chemical control and total

Cover Story

biological control are not the ways to control thrips in citrus. However, he strongly supports the idea of combining these two practices to achieve an integrated pest control program for the most effective results.

Valley citrus growers employing Michael's citrus thrips control program during the past three years have high praise for his solution to the pest problem. Careful study of the pest's behavior and biology by Michael has led to a monitoring technique which keeps close tabs on the thrips and its development under the citrus groves' leaf canopy.

He explains that citrus thrips larvae, the most dangerous feeding stage of the pest, destroy young leaves and damage tender fruit. When the larvae reach the pugae stage, they drop to the ground to find refuge in the soil. Then they emerge later as adults. These adults fly up into the trees and lay eggs again in the fruit and leaves, causing injury known as "spotting."

This spotting injury is "a cosmetic thing," according to Michael, hut packing houses have to cull citrus affected with this symptom to meet grade standards for fresh-fruit shipment.

Michael, who also studied insect control at the University of California

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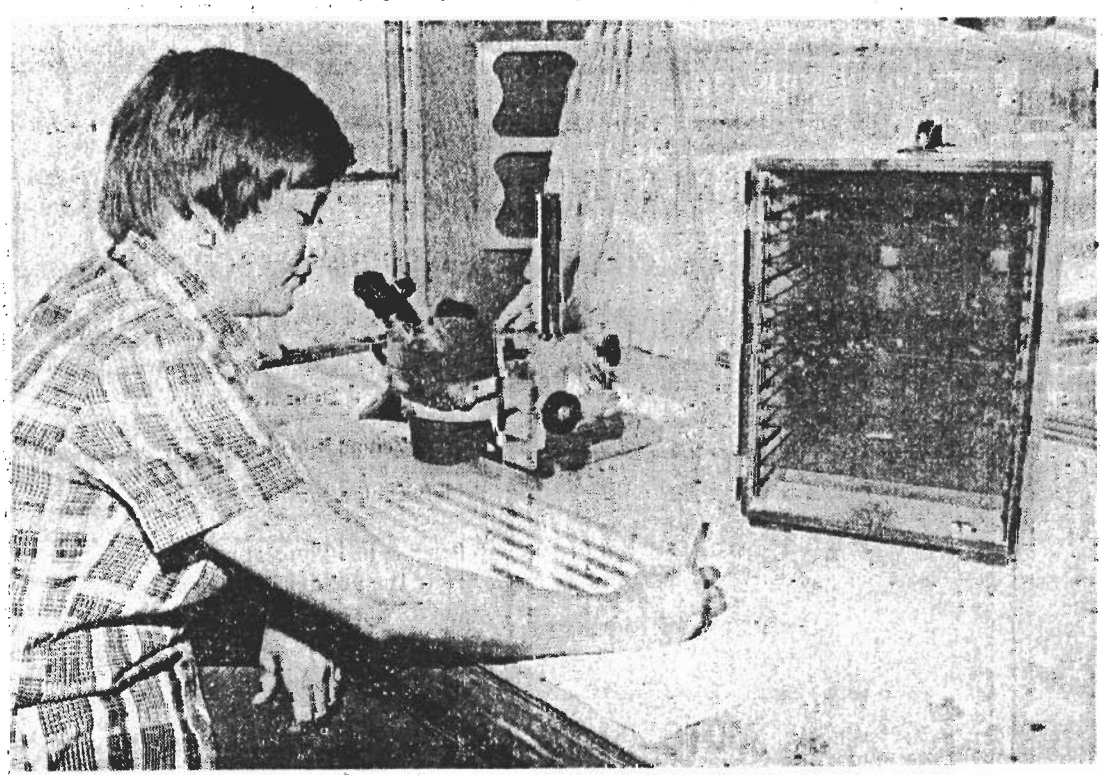
Citrus grower Bob Hines, left, and pest control adviser Ibrahim Michael check a sticky glass plate used for catching citrus thrips in a field monitoring program.



Lab technician Peggy Smith makes thrips counts and graphs them to determine peak buildup. The timing of chemical treatment is based on population levels.

Sunday, August 7, 1977 • THE FRESNO BEE

Thrips: Traps Tattle-Tale On Citrus Bugs

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at Riverside, said, "We found the larvae and the adults have different photostatic behavior in the citrus tree. Therefore, visual inspection is difficult to accomplish. The citrus thrips can also be easily confused with the flower thrips, which is a harmless pest."

The approach used by Michael is a modified technique of control programs applied by the USDA Boyden Laboratory Station at Riverside. "We put this technique into practical use and gain information helpful to our pest control program," he said.

The time of day is a critical factor in determining which thrips growth stage is most dominant in the citrus grove, according to Michael.

He uses traps equipped with plexiglas sheets, which are covered with sticky material on both sides to catch falling larvae and adults trying to wing their way up into the tree. The traps are placed at selected locations (stations) in the grove. They are also rotated around the base of the tree every three to seven days and give Michael an excellent indication of thrips development and activity.

The sticky plates are collected from the grove and checked at Michael's Fresno laboratory. The total number of thrips and their stage of growth are determined at the lab and plotted on a graph.

"With this technique, we have a continuous survey of the insect situation in the citrus grove," Michael explained, "and it enables us to determine the optimum timing for chemical treatment, if it is needed."

The pest control consultant said that thrips are easy to control with chemicals. But timing is the most important factor in effectiveness.

"Our traps will tell us about thrips population buildup and we can warn the grower and tell him when to treat," Michael said.

Bob Hines, an East Side citrus grower located between Centerville and Piedra, started using Michael's thrips control technique nearly three years ago.

Hines said, "I used to spray once, twice and sometimes three times a year for red scale and citrus thrips. Sometimes, my chemical applications were on target and I got good control. Other times, it seemed like I was wasting my money. Timing is the key factor, no doubt about it."

Knowing when to treat and being prepared to do it within a 24 hour period is the way Hines has been able to cope with his thrips problem. He said, "Michael's technique has given me confidence in thrips control, something I didn't have before."

Michael pointed out that spraying is costly and two or three applications a year can amount to a third of a grower's total production costs, excluding harvesting.

"Economics is the name of the game," he said, "and growers are looking for quality fruit with lower production costs. My technique is designed to give this to them."

But Michael's pest control program is more involved than mere trapping, counting and issuing control recommendations. He is continuously collecting field data, which he digests statistically and plugs into his overall agriculture consulting program.

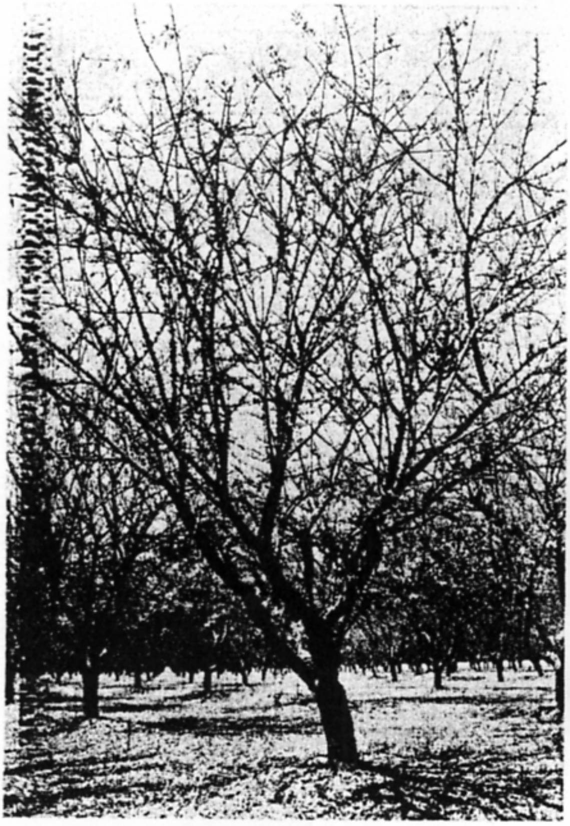
His citrus thrips control program is an off-shoot of an earlier and highly successful technique of controlling citrus red scale with sex pheromone traps.

His pest control techniques are currently being used by grape and almond growers such as Jura Farms of Fresno, the Harris Ranch, and Ivanhoe citrus grower Richard Pelzer.

The thrips control season is over now, but Michael and his crews are prepared for next year with their trapping service. "Thrips like dry weather," Michael said, "and another droughty year could spell trouble."

Michael shows typical thrips injury on a small orange. The spotting and circled area around the base of the fruit will require it to be culled at the packing house.

Mis-shapen leaves from citrus trees are indicative of citrus thrips' damage. Thrips will also attack and scar the surface of maturing fruit.



This defoliated tree was treated with chemical program.

Fresno Bee/Ron Goble

Healthy looking tree was under biological pest management.

Mite

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willing to take a little crop damage in that first transition year," Michael said. "It could cost a grower from $15 to $30 an acre to get the predators established. That all depends on how much chemicals a grower used in the past."

Michael's most recent convert to biological warfare was Superior Farming Co., in Kern County. The firm, one of the leaders in the industry, has some 7,000 acres of almonds and was experiencing problems with navel orangeworm and mite infestations.

A couple years ago Superior turned over 320 acres of almonds to Michael on a trial basis. They identified the parcel as "Block 88." Management wanted to see what he could do there with his biological mite control program.

Today, Bill Duncan, the manager of crop protection for Superior, is encouraged by the results. And he's not alone. There were a lot of outsiders watching Block 88 too. County farm advisers and university researchers were among them. Plus the many employees who work at Superior were aware of what was happening.

Duncan said his management staff and team of employees have worked hard on Block 88 to make it (Michael's program) work.

"We had sustained heavy mite pressure on these acres in the past and decided if program was any good we'd really give it the acid test from the start," he said. "It (Block 88) was tough to irrigate and the water penetration there is poor which only compounds the mite problems."

This year without applying any chemical sprays for mite or NOW the damage was only about 3 per cent.

"We can live with that much damage, he added.

For 1983 Michael will have 2,000 acres of Superior Farming Co. almonds to treat with the six-spotted thrips and predaceous mites — another preditor he uses.

"In my own mind I see a three or four-year plan in getting the (biological control) program established in all our almonds," Duncan said. "But the rate of expansion will depend on how effectively we can keep the navel orangeworm damage down through cultural methods."

Controlling NOW is an important element in the overall program, said a member of Duncan's management team.

"Most of the pesticides for navel orangeworm knock out everything," he said. "We've found that it was a mistake to rely totally on the new synthetic pyrethroids. We could control the NOW, but the mites blew up and defoliated the trees. The new generation of pesticides created a lot of problems for us.

"We found NOW control was possible when we had a timely harvest and initiated a good sanitation program and removed all the 'stick-tights' (nuts) from the trees. That way we don't have to worry about aggravating the mite problem with a NOW spray," he said.

"It is easier to go out and spray than to consistently monitor the pest and preditor activity in the field," said Michael. "Some growers can't take the pressure when the mites begin building up. The first year they have to be willing to take some damage."

A normal spray program in almonds might be to treat twice for NOW and three or four times for mites. The difference between Block 88 (untreated with chemicals) and the adjoining orchard was like night and day. Surrounding acreage that had been under the routine spray program was nearly defoliated.

The biological approach isn't just a matter of getting some good bugs into the field. It takes a concerted effort on the part of management to coordinate the irrigation schedule, plant the proper cover crop and maintain good plant nutrition.

"We believe we can cut costs by at least $100 an acre if we don't have to spray our almonds," said the man from Superior.

"We analyze the whole situation, studying the orchard carefully," Michael explained. "We find that a healthy tree or vine is not so attractive to the mite, so we do petiole analysis yearly to monitor the nutritional needs of an orchard."

Michael and his chief assistant, Gary Smith, make weekly field releases of six-spotted thrips (which have the ability to fly from tree to tree) and predaceous mites (they only crawl) and other mite preditors which they rear at their laboratory facility and greenhouses in Fresno.

The thrips are reared on bean plants in a greenhouse and then those plants are placed throughout the orchard where the preditors are freed to search for prey.

In grapes the leafhopper (Erythroneura elegantula) is a major pest. This is another case where chemical applications to control it aggravate the mites.

"To avoid usuing chemical applications we establish the tiny (almost microscopic) wasp called Anagrus epos," Michael said. "These wasps are particularly valuable because of their amazing ability to locate and attack grape leafhopper eggs. Their extremely short life cycle enables them to reproduce much faster than the hoppers."

"Anagrus can produce nine or 10 generations during the grape season," he said. "So by avoiding spraying for the leafhoppers, farmers can give the mite preditors a chance to get established and maintain good control."

Delano area grower Jim Andreas has been using preditors for hoppers and mites in his Thompson seedless for the last decade. And it means extra money in his pockets. He has been selling his raisins to Bonner Packing Co., Fresno, for $50 a ton higher than the going price because they are organic.

"The organic market is very good and none of my raisins go into the reserve pool," Andreas said. "That means I get paid for the whole crop on delivery."

The normal raisin grower this year will see 57 percent of his crop go into the reserve pool and he is only initially paid on the 43 percent free tonnage.

"Some neighbors think I'm crazy and aren't interested in what I'm doing," the farmer said. "Some do show an interest but change comes about slowly."

Andreas and his two brothers, John and Roger, farm slightly more than 1,200 acres of cotton, table grapes, wine grapes, and grain crops.

"We like to justify what we are doing in the vineyard and must be able to show good results," Andreas said.

"We have to build a relationship with them (growers) and they need to learn that they can trust our judgment," added Smith.

"Sometimes you feel like you're beating your head against a wall because the majority of farmers won't believe that the program works," he recalled. "A lot of growers think we're about three bricks shy of a full load."

"But I like that challenge," interrupted Michael. "It is exciting to me to see them come around to a new way of thinking. And it is good to remember that it is benefiting the entire industry, not just one grower."

Control of Citrus Green Mold by Carbonate and Bicarbonate Salts and the Influence of Commercial Postharvest Practices on Their Efficacy

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ABSTRACT

Smilanick, J. L., Margosan, D. A., Mlikota, F., Usall, J., and Michael, I. F. 1999. Control of citrus green mold by carbonate and bicarbonate salts and the influence of commercial postharvest practices on their efficacy. Plant Dis. 83:139-145.

The toxicity to *Penicillium digitatum* and practical use of carbonate and bicarbonate salts to control green mold were determined. The effective dose (ED_{50}) concentrations to inhibit the germination of *P. digitatum* spores of sodium carbonate (SC), potassium carbonate, sodium bicarbonate (SBC), ammonium bicarbonate, and potassium bicarbonate were 5.0, 6.2, 14.1, 16.4, and 33.4 mM, respectively. All were fungistatic because spores removed from the solutions germinated in potato dextrose broth. SC and SBC were equal and superior to the other salts for control of green mold on lemons and oranges inoculated 24 h before treatment. When sodium content and high pH must be minimized, SBC could replace SC. Furthermore, because a higher proportion of NaOCl would be present in the active hypochlorous acid at the lower pH of SBC compared to SC, sanitation of the SBC solution should be easier to maintain. NaOCl (200 µg/ml) added to SBC at pH 7.5 improved green mold control. Rinse water as high as 50 ml per fruit applied after SC did not reduce its effectiveness; however, high-pressure water cleaning after SC did. Conversely, high-pressure water cleaning of fruit before SC improved control of green mold. The risk of injury to fruit posed by SC treatment was determined by immersing oranges for 1 min in 3% (wt/vol) SC at 28, 33, 44, 50, 56, or 61°C (±1°C) and followed by storage for 3 weeks at 10°C. Rind injuries occurred only after treatment at 56 and 61°C. The risk of injury is low because these temperatures exceed that needed for control of green mold. SC was compatible with subsequent imazalil and biological control treatments.

Bicarbonates and carbonates are common food additives for leavening, pH-control, taste, texture modification, and spoilage control (8). They also control many plant pathogens (12,19,29,33,41). Regulatory barriers to their use are few; most are classified as generally recognized as safe by the US Food and Drug Administration for many applications. In 1997, the US Environmental Protection Agency declared that bicarbonates were exempt from residue tolerances on all agricultural commodities, and the United States Department of Agriculture classified many carbonates and bicarbonates as approved ingredients on products labeled "organic" in proposed regulations to standardize organic practices. Brief immersion of citrus fruit in solutions of sodium bicarbonate ($NaHCO_3$) or sodium carbonate (Na_2CO_3, soda ash) reduces the subsequent incidence of postharvest green mold, caused by *Penicillium digitatum* (2,13,20,23,26,31,35). This practice is inexpensive, poses a minimal risk of injury to the fruit, and can be a useful tool in the management of fungicide resistant isolates, which have become particularly problematic (6,11). Its effectiveness can approach that of the fungicides employed for this purpose (36), and in general is superior to other treatments that are alternatives to fungicides, such as heat (5,7,21,36,37) or biological control (4,34). Sodium carbonate (SC) controls green mold even when applied long after inoculation; the incidence of infections from wounds on lemons inoculated 48 h before treatment was reduced more than 90% (36). Control of green mold after inoculation is important because most infections occur through wounds inflicted during or just after harvest (13,16,32), and often a day or more can elapse before treatments are applied. Previously, we developed a model describing the influence of SC concentration, temperature, and duration of contact on the control of green mold on oranges, where SC had been seldom used (35). Like heat and biological treatments applied to citrus, SC only reduces the incidence of green mold but, unlike fungicides, does not suppress the subsequent production of spores on those fruit that do decay. The deposition of spores on adjacent healthy fruit, termed "soilage," makes cleaning of the remaining fruit necessary (10). SC use has become increasingly popular in California; however, certain aspects of the practical use of SC are incompletely known. Although both SC and sodium bicarbonate (SBC) have been recommended for this purpose, they have not been compared to each other or with ammonium or potassium carbonate salts. This information is of particular interest because the quality of water discharged from SC tanks can exceed pH and sodium content tolerances in some areas; the use of other solutions of lower pH, lower sodium content, or containing salts of less regulatory concern could alleviate this problem.

The inhibitory activity of carbonate or bicarbonate solutions against many microorganisms (28), including *P. digitatum* (22,26), is low and generally fungistatic. Therefore, it is probable that a residue of carbonate or bicarbonate must remain on the fruit, or at least within the wound infection courts occupied by this pathogen, for the treatment to control green mold. Winston (40) showed that the efficacy of the immersion of citrus fruit in heated solutions of borax-boric acid to control postharvest decay, in regimes very similar to those of SC, was greatly reduced by subsequent rinsing of the fruit with large volumes of water, and he stated some optimal level of post-treatment rinsing should be determined empirically where this treatment is used. It is likely that post-treatment rinsing also will influence the effectiveness of carbonate or bicarbonate solutions, because borax-boric acid and carbonate-bicarbonate solutions are both fungistats and of similar effectiveness (22). Rinsing is an important issue because the solutions must be rinsed off the fruit surface after treatment to prevent the deposition of the salts on brushes and belts of packing and sorting equipment, and to prevent phytotoxicity, seen as staining and desiccation of the fruit rind. Another process required after harvest is cleaning of the fruit. Introduced recently for this purpose are high-pressure water washers that blast scale insects, sooty mold, and soil from fruit with water applied at high pressure (150 to 500 psi or 1,000 to 3,500 kPa) in very high volumes. High pressure washing can be applied at any step after harvest before the application of waxes.

Other issues of concern for packinghouse managers contemplating the adop-

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Accepted for publication 15 October 1998.

Publication no. D-1998-1123-01R

tion of a carbonate treatment include the risk of injury to fruit the treatment could pose, particularly for navel orange cultivars. Most of these cultivars were introduced since the original carbonate research was published (2,17,21,23), and managers state these are very susceptible to cosmetic defects of the rind that develop after harvest, such as pitting. Another concern is the influence of carbonate treatment on the effectiveness of subsequent fungicide or biological control treatments.

Objectives in the present work were to (i) compare the fungitoxicity and disease control effectiveness of SC, potassium carbonate, SBC, potassium bicarbonate, and ammonium bicarbonate; (ii) determine the influence of water rinse volume applied after SC treatment on the effectiveness of the treatment to control green mold; (iii) investigate the influence of high-pressure water washing on SC effectiveness; (iv) determine if fruit are susceptible to injury by SC treatment, particularly early season oranges; and (v) determine the influence of prior SC treatment on the effectiveness of subsequent imazalil or biological control treatments.

MATERIALS AND METHODS

Inoculum preparation. *P. digitatum* (Pers.:Fr.) Sacc. isolate M6R (obtained from J. W. Eckert, University of California, Riverside) was cultured for 1 to 2 weeks on potato dextrose agar. Spores were harvested by adding 5 ml of water containing 0.05% Triton X-100 to the Petri dish, rubbing the surface with a sterile glass rod, and passing the suspension through two layers of cheese cloth. The suspension was diluted with water to an absorbance of 0.1 at 425 nm determined with a spectrophotometer; this density contains approximately 10^6 spores/ml (9).

In vitro inhibition of spores of *P. digitatum* by bicarbonate and carbonate solutions. Potato dextrose broth containing 0, 2.5, 5.0, 10.0, 25.0, and 50.0 mM of SC (Na_2CO_3), potassium carbonate (K_2CO_3), SBC ($NaHCO_3$), ammonium bicarbonate (NH_4HCO_3), or potassium bicarbonate ($KHCO_3$) was prepared in 4-ml volumes from anhydrous salts (Sigma Chemical Co., St. Louis). Three replicates of each were prepared, and 10,000 spores of *P. digitatum* were added to each replicate. In the first test, the pH of each solution was not controlled and increased with increase in salt concentration. After 18 h at 22°C (±1°C), 100 µl of acid fuchsin solution (0.2% wt/vol acid fuchsin in a solution containing one part acetic acid and one part 95% ethanol) was added to each well to stop further germination and the percentage of germinated spores determined by observation of 100 to 150 spores within each replicate with an inverted compound microscope (200×). No acid fuchsin was added to a fourth replicate well of each solution; the spores were withdrawn from this well with a Pasteur pipette, suspended in sterile distilled water followed by low-speed centrifugation, and re-suspended in potato dextrose broth for an additional 18 h. In a second test, the bicarbonate salts alone were tested at similar pH; 50 mM phosphate buffer was included and the final pH of the solutions was 7.3 (±2). After 18 h at 22°C (±1°C), acid fuchsin was added to each well to stop further germination and the percentage of germinated spores determined as previously described. The experiments were conducted twice.

Fruit inoculation. Lemons (*Citrus limon* (L.) Burm.) or oranges (*C. sinensis* (L.) Osbeck) that had been commercially harvested no more than 2 days prior to use were randomized and inoculated with *P. digitatum* 24 (±2) h before treatments were applied. This inoculation method simulates infections that occur under commercial conditions and has been recommended for determining the effectiveness of fungicides (9). Fruit were inoculated by immersing a stainless steel rod with a probe tip 1 mm wide and 2 mm in length into the spore suspension and wounding each fruit once. The temperature of the fruit at the time of inoculation and subsequent storage until treatment was 20°C (±1°C). After all treatments were applied, the fruit were placed into plastic cavity trays that prevented contact infections. In every test, controls included fruit that were inoculated and treated with water and fruit that were inoculated and not treated.

Comparison of bicarbonate and carbonate solutions for the control of green mold on lemons. To determine if other bicarbonates or carbonates could effectively control green mold, inoculated lemons were immersed for 1.5 or 2 min in 22-liter volumes of water, SC, SBC, potassium carbonate, ammonium bicarbonate, or potassium bicarbonate. Each solution was applied at 27 or 40°C (±1°C). The temperature of the solutions did not change more than 0.5°C during treatment. After treatment, the fruit were rinsed with 10 ml of deionized water per fruit at low pressure (200 kPa) in a spray 30 cm above the fruit over a period of 5 s, stored for 2 or 3 weeks at 13 or 20°C (±1°C), and the incidence of green mold-infected fruit was determined. The solutions contained equimolar or equal-weight quantities of bicarbonates or carbonates. Each treatment was applied to four or five replicates of 25 fruit each. The test was repeated three times, although not all bicarbonate and carbonate treatments were included in every test.

Comparison of SC and SBC for the control of green mold. SC and SBC solutions were compared on an equal-weight basis on lemons and oranges. Inoculated lemons (*C. limon*) cv. Eureka or oranges (*C. sinensis*) cv. Valencia were immersed for 2 min in 22 liters of water or in equal-weight quantities of SC or SBC at 2 or 3% (wt/vol) at 40 or 45°C (±1°C), rinsed with 10 ml of water per fruit applied as previously described, stored for 2 or 3 weeks at 13 or 20°C (±1°C), and the incidence of green mold infected fruit was determined. Each treatment was applied to four replicates of 25 fruit each. The test was repeated, with minor differences in procedure, three times with Eureka lemons and twice with Valencia oranges.

Influence of chlorination on SBC effectiveness. Solutions containing 0, 0.25, 0.5, 1, and 3% (wt/vol) SBC were prepared, and an identical series of solutions was prepared with the addition of 200 µg/ml of sodium hypochlorite. All were adjusted to pH 7.5 with concentrated HCl. Total chlorine content was periodically measured by combining a diluted sample of the hypochlorite-containing solutions with concentrated HCl and orthotolidine, and the absorbance at 500 nm was compared to a standard curve. The pH was measured repeatedly during the test and did not change. Inoculated lemons were immersed for 2 min in 22 liters of the SBC solutions at 25°C, rinsed with 10 ml of water per fruit applied at low pressure as previously described, and stored 2 weeks at 18°C (±1°C). Each treatment was applied four times to replicates of 25 fruit each. The test was done once.

Influence of post-treatment water rinse volume on SC effectiveness. In a laboratory test, lemons inoculated 24 h earlier were immersed for 1.5 min in 3% (wt/vol) SC solution at 40.6°C (±1°C). After treatment, the fruit were not rinsed or rinsed with 2, 10, or 50 ml of deionized water per fruit applied at 200 kPa in a spray 30 cm above the fruit over a period of 3 to 10 s. Each treatment was applied to four replicates of 25 fruit each. The test was done once. In tests with commercial packing line equipment, fruit inoculated 24 h previously were placed for 60 s into a 2,400-liter-capacity tank containing 3% (wt/vol) SC at 38°C (±1°C). After treatment in the tank, the fruit were immediately rinsed with water (16 to 18°C) from three nozzles placed 20 cm apart and 33 cm above rotating brushes on a 60-cm-wide bed. The 80° solid-cone nozzles were changed to vary water volumes and operated at a water pressure of 300 kPa. Fruit were not rinsed or rinsed after SC treatment with 2, 10, or 50 ml per fruit applied during the approximately 1-s residence period of the fruit under the rinse water nozzles. After rinsing, the fruit were dried for 15 to 20 s through a drier at 32.2°C, a high solids content resin finishing wax (Sealbrite 504, EcoScience Corp., Orlando, CA) was applied, and the fruit were dried again for 15 to 20 s through a drier at 32.2°C. The fruit were placed in fiber board cartons. All fruit were stored for 3 weeks at 10°C before the decayed fruit were counted. Within the test, each treatment was applied to four replicates of 60 to

75 fruit each. The test was done once with cv. Valencia oranges and twice with cv. Eureka lemons.

Influence of high-pressure water washing on SC effectiveness. To determine the influence of high pressure water washing on SC effectiveness, inoculated lemons and oranges were treated with one of the following: (i) immersed in water for 1 min at 44°C; (ii) washed in a high pressure water washer; (iii) immersed in 3% SC for 1 min at 44°C (±1°C) without prior or post-treatment cleaning; (iv) washed in a high-pressure water washer and then immersed in 3% SC; or (v) immersed in 3% SC, then washed in a high-pressure water washer. The residence time in the pressure washer, which operated at 1,380 kPa, was approximately 45 s. The pressure washer was 61 cm wide, 3 m long, and contained 15 rows of three 45° flat-fan nozzles per row. Each nozzle applied 100 ml/s of water. The water in the pressure washer contained 10 μg/ml of sodium hypochorite. The fruit, which were not waxed after treatment, were placed in fiber board cartons and stored 4 for weeks at 10°C before the decayed fruit were counted. Each treatment was applied to four replicates of 60 to 75 fruit each. The test was repeated twice with navel oranges and done once with cv. Eureka lemons.

Influence of SC solution temperature on rind injury of oranges. Navel orange cvs. Atwood, Bonanza, Fisher, New Hall, and Thomson Improved, grown at the University of California Lindcove Citrus Research and Extension Center (Lindcove, CA), were harvested and randomized. Fruit (pulp temperature 12 to 13°C) were immersed in 3% SC maintained at 28, 33, 44, 50, 56, or 61°C (±1°C) in a 2,000-liter-capacity tank for 1 min, rinsed with approximately 10 ml of water per fruit by an overhead spray over rotating brushes, waxed with a high solids content finishing wax containing 2,000 μg/ml imazalil (Fungaflor 500 EC, 44.6% a.i.; Janssen Pharmaceutica, Titusville, NJ), dried in a high-velocity, heated-air dryer, and placed into fiberboard cartons. Each treatment was applied to three replicates of 80 fruit of each cultivar. After storage for 3 weeks at 10°C with 90% relative humidity, fruit were classified into one of four categories, where 1 = no rind blemishes; 2 = slight blemishes present; 3 = moderate blemishes present; and 4 = severe rind injury. Fruit with rind injuries associated with classes 3 and 4 were of sufficient significance that the fruit could not normally be sold commercially without discount. The test was done once with each cultivar.

Influence of SC on the effectiveness of subsequent imazalil or biological control applications. Lemons or oranges were inoculated with *P. digitatum* as previously described, immersed 24 h later in 2 or 3% SBC or SC for 2.5 min at 40.5°C (±1°C), and rinsed briefly with 10 ml of water per fruit. Imazalil at 1,000 μg/ml in water or *Pseudomonas syringae* strain ESC10 (active ingredient of the registered product BioSave 10, a gift of EcoScience Corp.) at 10^9 CFU/ml was applied by an overhead spray until the solutions ran off the surface of the fruit. The fruit were stored for 2 to 4 weeks at 10°C and the incidence of green mold infections was determined. Each treatment was applied to four or five replicates of 25 fruit each. The test was repeated twice.

Statistical analysis. The concentrations of salts that caused 50 and 95% mortality of spores and the upper and lower 95% fiducial limits were estimated by Finney's Probit analysis (15). The incidence of green mold or rind injuries was analyzed by an analysis of variance applied to the square root of the arcsin of the proportion of infected or injured fruit, followed by Fisher's protected least significant difference (LSD) to separate means. Actual values are shown.

RESULTS

In vitro inhibition of spores of *P. digitatum* by bicarbonate and carbonate solutions. The pH of the unbuffered carbonate solutions were similar to each other and higher than the bicarbonate solutions at every concentration (Fig. 1). Fiducial limits of concentrations that inhibited 50% of the spores (ED_{50}) were narrower than those of ED_{95} concentrations (Table 1). The toxicity of SC (ED_{50} = 5.0 mM) to spores of *P. digitatum* was significantly higher than potassium carbonate (ED_{50} = 6.2 mM). The toxicity of SBC (ED_{50} = 14.1 mM) to spores of *P. digitatum* was not significantly different than ammonium bicarbonate (ED_{50} = 16.4 mM), but significantly superior to potassium bicarbonate (ED_{50} = 33.4 mM). Spores removed from these solutions, rinsed in water, and incubated in potato dextrose broth for an additional 18 h had a germination rate of more than 99%. In tests with bicarbonate salts where the pH was 7.2 (±0.2) with phosphate buffer, the ED_{50} concentrations of ammonium bicarbonate, SBC, and potassium bicarbonate were 34.4, 37.2, and 38.1 mM, respectively, and not significantly different from each other.

Comparison of bicarbonate and carbonate solutions for the control of green mold on lemons. The control of green mold was significantly better by SC or SBC solutions compared to potassium or ammonium solutions (Table 2).

Comparison of SC and SBC for the control of green mold. The control of citrus green mold by SBC and SC was not significantly different ($P \geq 0.05$) in repeated tests with lemons and oranges (Fig. 2). Water alone at 40 or 43°C significantly reduced green mold in most tests compared to the untreated, inoculated controls, but the magnitude of the reduction was usually small.

Influence of chlorination on SBC effectiveness. The addition of 200 μg/ml of sodium hypochlorite to water or SBC slightly but significantly (P = 0.002) improved control of green mold on lemons (Fig. 3). The total chlorine content declined slightly from the beginning to the end of the test, from the initial 200 μg/ml to approximately 180 μg/ml after the treatments were applied. The pH and bicarbonate concentrations did not change during the test.

Influence of post-treatment water rinse volume on SC effectiveness. The volume of water sprayed onto fruit after SC treatment did not influence the effectiveness of the treatment to control green mold (Table 3).

Influence of high-pressure water washing on SC effectiveness. Washing fruit with water at high pressure significantly impacted control of green mold by SC treatment (Fig. 4). The incidence of

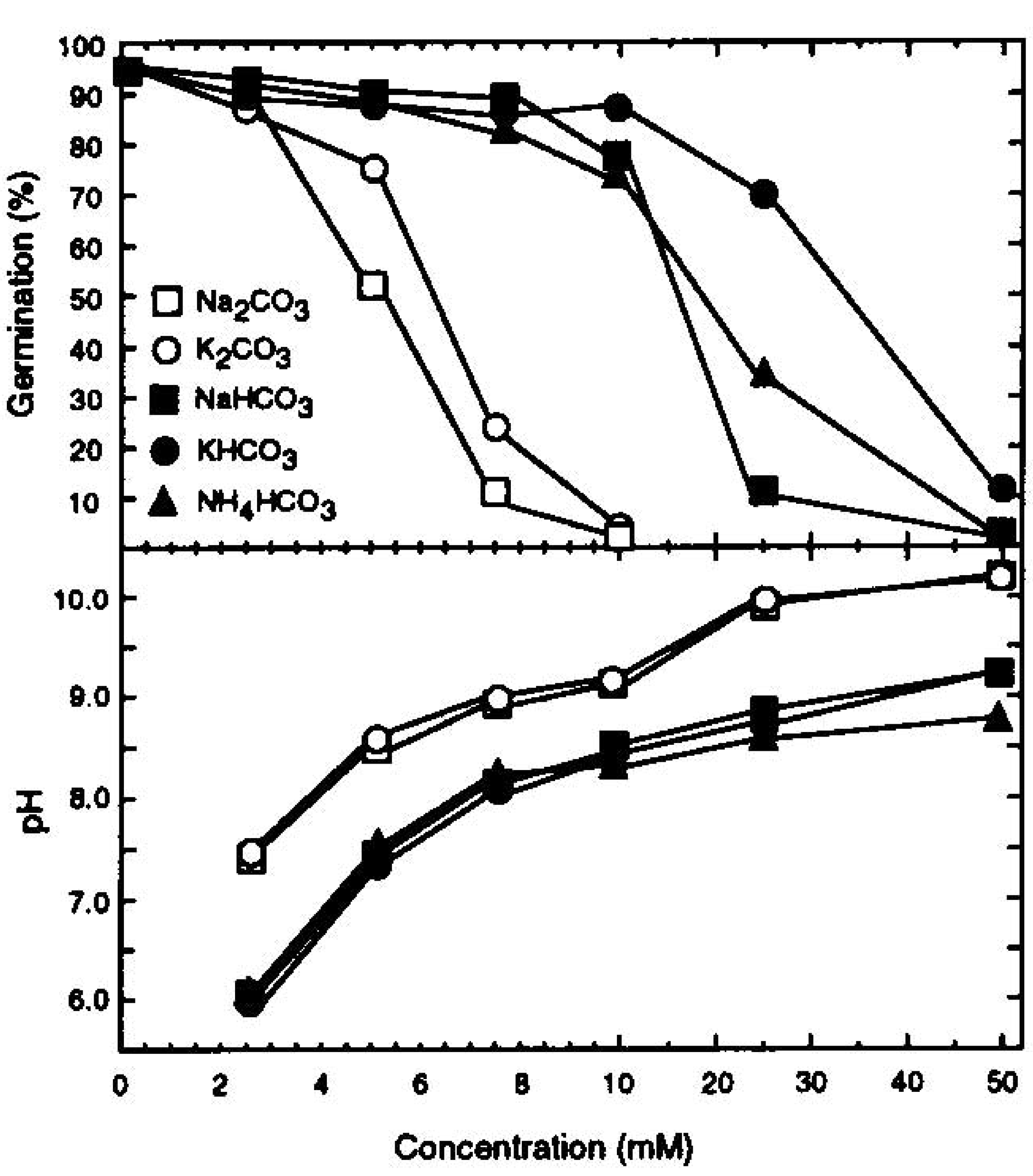

Fig. 1. The germination of spores of *Penicillium digitatum* after 24 h in potato dextrose broth in bicarbonate or carbonate solutions at the pH indicated.

Table 1. Concentrations (mM) of bicarbonate or carbonate salts that inhibited the germination of spores of *Penicillium digitatum*

Salt	ED_{50}[z]	ED_{95}
Na_2CO_3	5.0 (4.8, 5.2)	9.9 (9.4, 10.5)
K_2CO_3	6.2 (5.3, 7.0)	9.6 (8.5, 13.4)
$NaHCO_3$	14.1 (13.4, 14.9)	31.0 (28.4, 34.4)
NH_4HCO_3	16.4 (13.1, 20.4)	43.6 (32.5, 72.1)
$KHCO_3$	33.4 (31.5, 35.4)	70.6 (63.4, 81.4)

[z] ED = effective dose. Each value was calculated from three observations. Values in parenthesis are the upper and lower 95% fiducial limits.

green mold among untreated, inoculated fruit was 98.3%. When SC treatment was applied alone and the fruit were not rinsed after treatment, the green mold incidence was 18.2%. When SC treatment was preceded by washing the inoculated fruit with water at high pressure, a further and significant reduction in green mold incidence to 9.5% occurred on cvs. Frost and Lane Late oranges, while on cv. Eureka lemons a slight but not significant improvement in carbonate effectiveness was observed. When SC treatment was followed by washing the inoculated fruit with water at high pressure, the incidence of green mold was 38.1%. Pressure washing alone did not significantly reduce the incidence of green mold on oranges, but it slightly but significantly reduced green mold on lemons (Fig. 4). The green mold incidence of 97.0% among inoculated control lemons was reduced to 88.9% among those that were washed with water applied at high pressure.

Influence of SC solution temperature on rind injury of oranges. Phytotoxicity increased after SC treatment at 56 and 61°C among navel oranges cvs. Atwood, Bonanza, Fisher, New Hall, and Thomson Improved, but not after treatment at 28, 33, 44, or 50°C (Fig. 5). The cv. Thomson Improved had less injury than the other cultivars. Rind injuries were sunken areas 1 to 2 mm in depth that were light to dark brown in color.

Influence of SC on the effectiveness of subsequent biological control antagonist applications. *P. syringae* strain ESC10 significantly ($P \leq 0.001$) improved the control of green mold when its application followed fruit treatments in heated solutions of water, SC, or SBC treatments (Fig. 6).

Influence of SC on the effectiveness of subsequent imazalil application. The incidence of green mold after the combination of SC treatment followed by treatment by imazalil was significantly lower than after either treatment alone. The incidence of green mold after inoculation alone, SC treatment alone, imazalil (1,000 μg/ml) treatment alone, or SC treatment followed by imazalil treatment was 61, 36, 22, and 6%, respectively. The incidence of green mold after the combined treatment was significantly less ($P \leq 0.001$) than the imazalil or SC treatments alone.

DISCUSSION

The toxicity of SBC (pH 8.4; ED_{50} = 14.1 mM) to spores of *P. digitatum* was much less than that of SC (pH 11.0; ED_{50} = 5.0 mM); however, they were similar in effectiveness for the control of green mold. Therefore, the pH and in vitro toxicity of the solutions did not predict the efficacy of the solutions to control green mold. Although pH values above 8.5 inhibit the germination and growth of *P. digitatum* (10,26) and the higher pH of the carbonates enhanced their inhibitory activity in vivo, the equal performance of SBC and SC to control green mold demonstrates the pH of these solutions did not influence control of green mold significantly. Palmer and coworkers (30) and Homma and coworkers (10) showed that pH alone cannot explain the inhibitory action of these compounds. The pH of the solution deposited within the inoculation site was probably influenced by the albedo tissue of the rind, which is usually approximately pH 5.5, and the pH of the residual of both bicarbonate and carbonate solutions within the infection sites may have been similar. Furthermore, sodium apparently had some role in the control of the disease because the sodium salts were superior to the ammonium and potassium carbonate and bicarbonate solutions. The superior performance of the sodium salts was not anticipated. We and

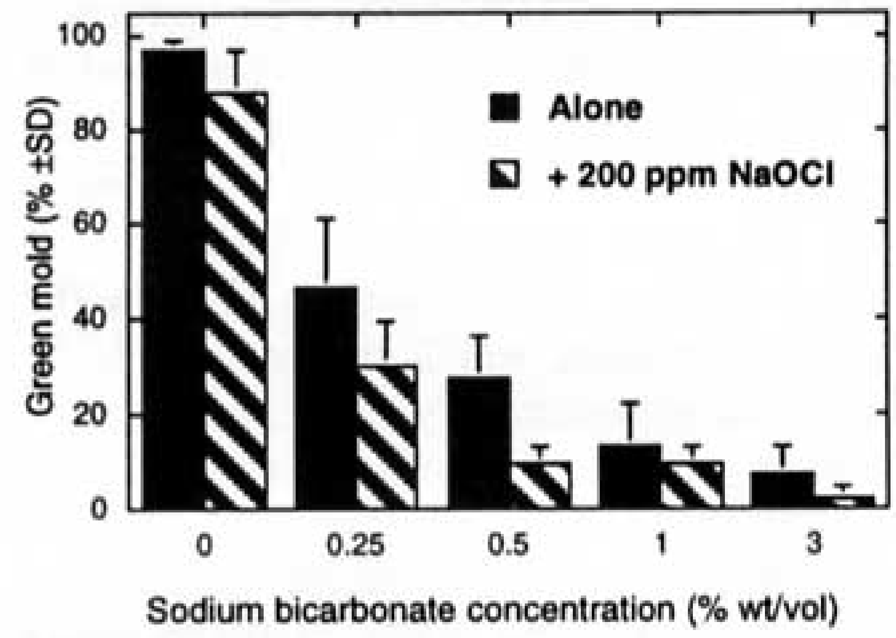

Fig. 3. The incidence of green mold on lemons after 2 min of treatment at 25°C in pH 7.5 solutions of water alone or sodium bicarbonate with or without 200 μg/ml of sodium hypochlorite. Inoculated fruit were immersed in each solution for 2 min and stored for 2 weeks at 18°C before the incidence of green mold was determined.

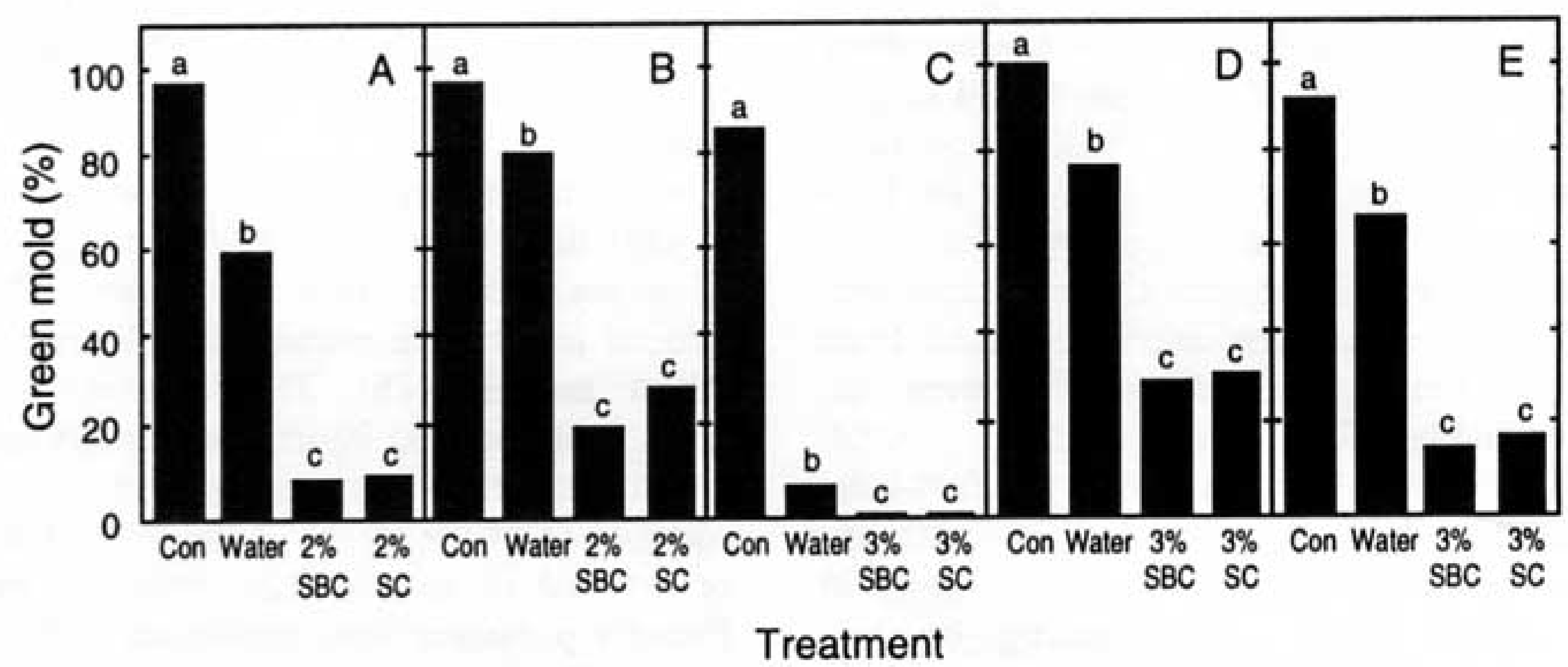

Fig. 2. The incidence of green mold on lemons or oranges after treatment in solutions of water alone or sodium carbonate (SC) or sodium bicarbonate (SBC) at 2 or 3% (wt/vol). Inoculated fruit were immersed in each solution for 2 min, rinsed with 10 ml of water per fruit, and stored 3 weeks at 13°C before the incidence of green mold was determined. **(A)** Treatment temperature 45°C; in all other tests, temperature was 40°C. **(A) (B)** and **(C)** Results with lemons; **(D)** and **(E),** results with cv. Valencia orange. Columns with unlike letters are significantly different by Fisher's least significant difference ($P \leq 0.05$).

Table 2. The incidence of green mold on lemons after treatment in solutions of carbonate or bicarbonate salts

Treatment	pH	Test no. 1[x] M[y]	Test no. 1[x] %	Test no. 1[x] Green mold (%)[z]	Test no. 2 M	Test no. 2 %	Test no. 2 Green mold (%)	Test no. 3 M	Test no. 3 %	Test no. 3 Green mold (%)
Inoculated, not treated	NA	NA	NA	87.5 a	NA	NA	99.0 a	NA	NA	99.7 a
Water	7.2	NA	NA	67.5 b	NA	NA	91.1 b	NA	NA	97.6 a
Na_2CO_3	11.0	0.50	5.3	9.9 d	0.28	3.0	20.0 d	0.47	5.0	13.6 d
$NaHCO_3$	8.5	0.50	4.2	14.5 d	...	...	...	...	...	...
K_2CO_3	11.0	...	...	...	0.30	3.0	33.0 c	0.50	5.0	27.2 c
$KHCO_3$	8.4	0.50	5.0	23.4 c	...	...	...	0.50	5.0	41.6 b
NH_4HCO_3	7.6	0.50	4.0	24.4 c	0.38	3.0	39.0 c	...	...	...

[x] Values in test 1 are the means of 10 replicates of 25 lemons each, immersed for 2 min at 26°C and stored for 3 weeks at 13°C; values in test 2 are the means of 4 replicates of 25 each, immersed for 2 min at 40°C and stored for 2 weeks at 20°C; values in test 3 are the means of 5 replicates of 25 each, immersed for 1.5 min at 40°C and stored for 2 weeks at 20°C.

[y] M = concentration in molarity; % = concentration in wt/vol; NA = not applicable; ... = treatment not applied.

[z] Values within columns followed by unlike letters are significantly different ($P \leq 0.05$) by Fisher's least significant difference applied after an analysis of variance of arcsin transformed values. Actual data are shown.

other workers have shown that sodium, potassium, and ammonium bicarbonates have similar inhibitory activity against spores (Fig. 1; 29,30), and they were equal to or superior to SBC for the control of other diseases (18,29). Ammonium bicarbonate in particular would be anticipated to be effective, because ammonia and ammonium salts have been used to control green mold (39).

This work offers partial solutions for the disposal of the process water from packinghouses, where the discharge to ponds or sewers of various solutions used to treat citrus, namely borax-boric acid, sodium ortho-phenyl phenate, and SC, is under regulatory control. An equivalent-weight solution of SBC has a lower pH and less sodium than a similar solution of SC. Equimolar amounts of SBC contain 27.4% sodium compared to 43.4% sodium in SC. Another very significant advantage of bicarbonate salts is that they can be effectively chlorinated, because at the pH of these solutions a substantial portion of the hypochlorite is protonated and active. The addition of 200 µg/ml of sodium hypochlorite to SBC at pH 7.5 slightly but significantly improved control of green mold on lemons, and the hypochlorite will kill spores and other microbes contaminating the solution. A disadvantage of SBC is that heating the solution will cause carbon dioxide evolution into air with a concomitant increase in solution pH. At ambient temperatures, carbon dioxide evolution from bicarbonate solution is not significant and the pH of the solution does not change. Unfortunately, heating sometimes significantly improved carbonate and bicarbonate solution effectiveness in practical tests. Heating of the solutions to 60°C when fruit are not present, a practice used to sanitize SC solutions of contaminating microbes and spores, cannot be done with SBC, but the addition of hypochlorite to SBC should accomplish this task. Other carbonate or bicarbonate salts could be adopted but they are less effective than SBC and SC. In other work, we that showed ethanol and sulfur dioxide could replace SC, although they were less effective (36). Other solutions that are suitable for discharge to sewers or ponds, applied to land as soil amendments or fertilizers, or recycled, should be examined for this application.

The solutions we tested must be rinsed off the surface of the fruit to prevent the deposition of the salts on brushes and belts of packing and sorting equipment, and to prevent staining and desiccation of the fruit rind. Marloth (26) and Hwang and Klotz (22) reported the effect of carbonate or bicarbonate solutions on spores of *P. digitatum* was primarily fungistatic; many spores remained germinable after exposures of 5 min or longer in 10% (wt/vol) Na_2CO_3 or $NaHCO_3$. Spores survive the treatments of 1 or 2 min in duration that control green mold on fruit (34); therefore, it is probable that a residue of carbonate or bicarbonate must remain on the fruit, or at least within the wound infection courts occupied by this pathogen, for the treatment to inhibit infection. Water rinsing by

Table 3. Incidence of green mold after sodium carbonate treatment followed by a water rinse and 3 weeks of storage at 10°C

	Green mold incidence (%)[z]			
Rinse volume per fruit (ml)	Test 1	Test 2	Test 3	Mean
Inoculated, not treated	96.8 a	96.7 a	84.6 a	92.7 a
0	9.6 b	22.7 b	11.7 b	14.7 b
2	20.0 b	12.0 b	13.3 b	15.1 b
10	13.6 b	16.1 b	5.8 c	11.8 b
50	19.2 b	18.0 b	8.8 bc	15.3 b

[z] Test 1 = lemons, $n = 5$ replicates of 25; test 2 = lemons, 4 replicates of 75; test 3 = oranges, 4 replicates of 60. Test 1 was a laboratory test; tests 2 and 3 were done with commercial packing equipment.

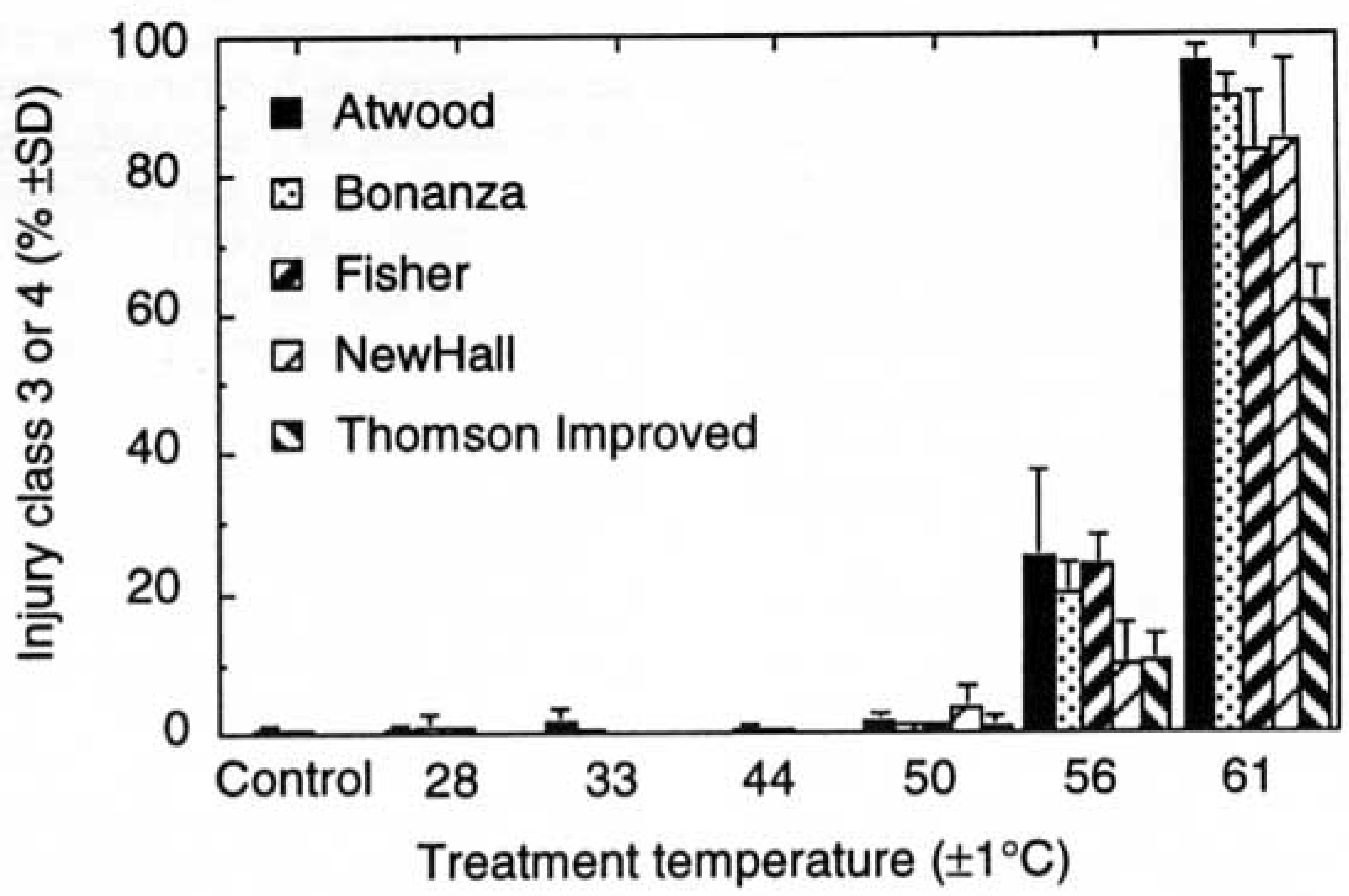

Fig. 5. Influence of the temperature of sodium carbonate treatment (3% wt/vol for 1 min) on the appearance of oranges after treatment and storage at 10°C for 3 weeks. Injury classes: 1 = no rind blemishes; 2 = slight blemishes present; 3 = moderate blemishes present; and 4 = severe rind injury.

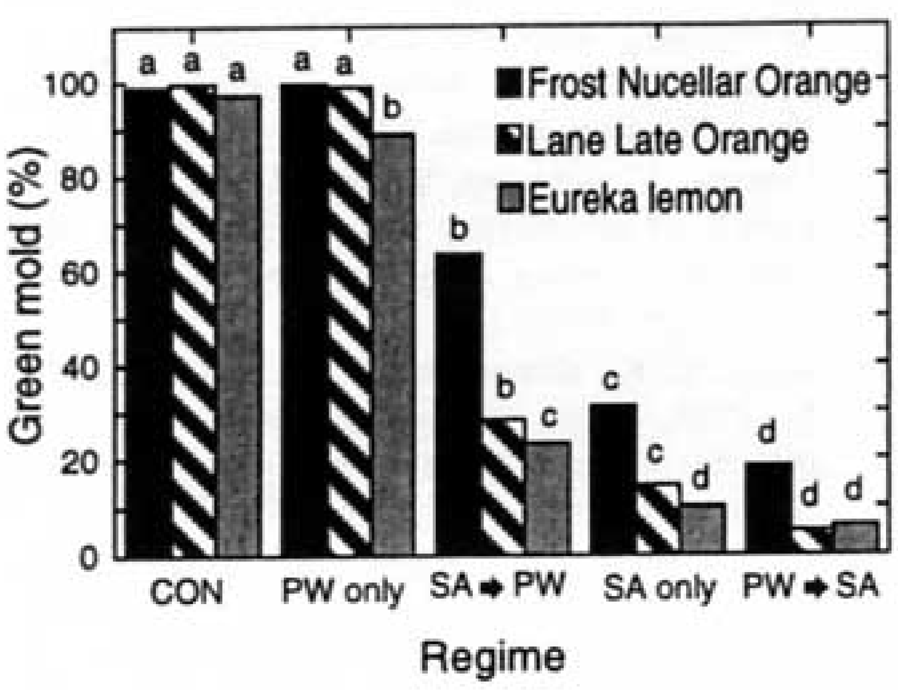

Fig. 4. The incidence of green mold on lemons or oranges after treatment in regimes where the fruit were inoculated and not treated (CON); inoculated and washed with water at high pressure (PW only); treated with sodium carbonate, then washed with water at high pressure (SA → PW); treated with sodium carbonate alone (SA only); or washed with water at high pressure, then treated with sodium carbonate (PW → SA).

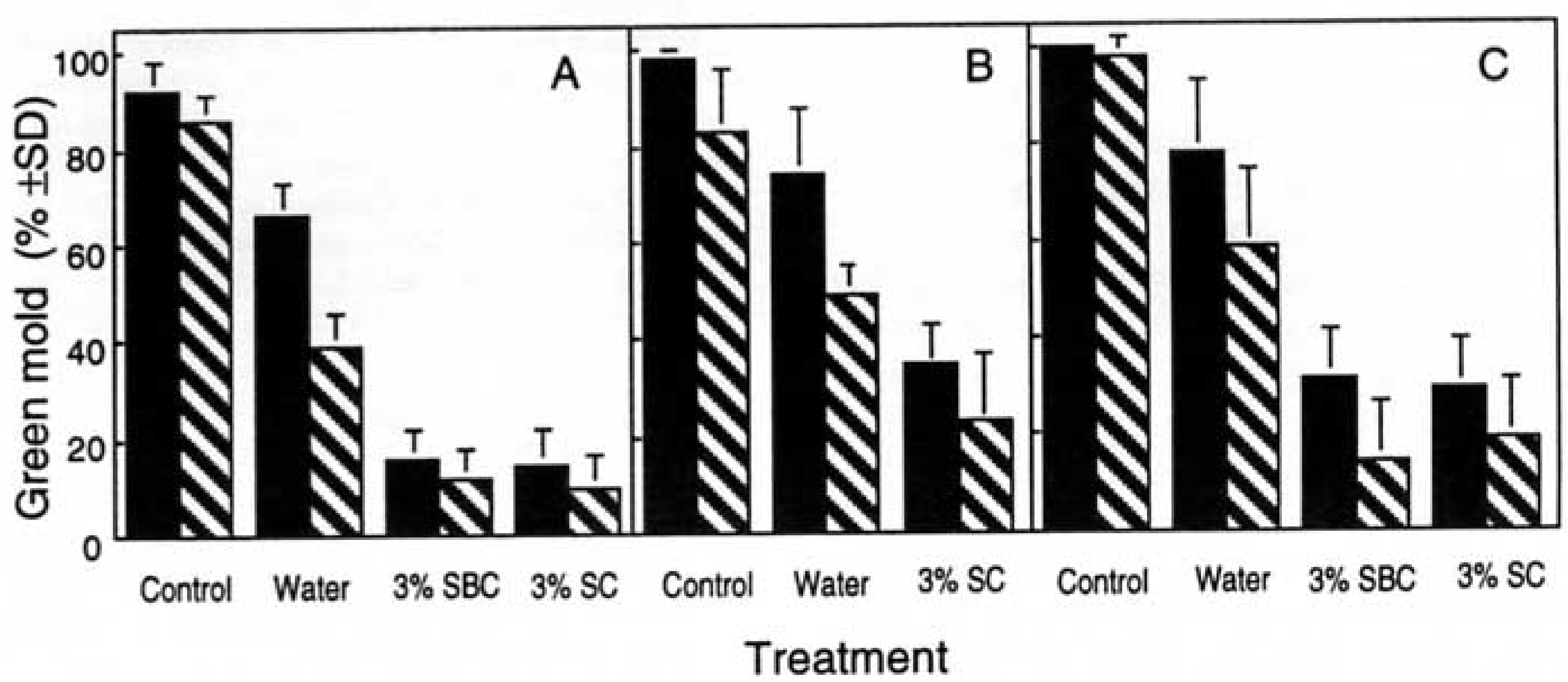

Fig. 6. The incidence of green mold on cv. Valencia oranges after immersion in water or in solutions of 3% sodium bicarbonate (SBC) or 3% sodium carbonate (SC) alone (solid bar) or each followed by the application of *Pseudomonas syringae* strain ESC10 (striped bar). **(A)** Treatment solution temperature = 45°C; **(B)** and **(C)** treatment solution temperature = 40°C.

low-pressure overhead sprays, typically used for this purpose, did not influence SC effectiveness significantly, while high-pressure water washing significantly diminished its efficacy. High-pressure water washing, and probably other fruit-washing procedures that employ extensive brushing, soaps, and high water volumes, reduce SC effectiveness because this residue is removed. Presumably, the residue persists through low-pressure water rinses but it is substantially removed by high-pressure water washing of fruit. We were unable to correlate rind sodium content and SC effectiveness because the sodium content of the rind of lemons and oranges was naturally high (about 130 μg/g fresh wt) and variable.

Citrus fruit should be cleaned before SC treatment because the effectiveness of SC treatment will be diminished if they are washed rigorously afterward. Two other benefits of washing the fruit before SC treatment were considered. First, residual SC remaining on treated fruit would not contaminate the water of the pressure washer. SC would raise the pH of the water and diminish the efficacy of the sodium hypochlorite used to sanitize the pressure washer water. Second, if the fruit are cleaned before they enter the SC tank, the solution will remain cleaner and require less frequent replacement. A disadvantage of this sequence is that cleaning of fruit may require higher water pressure that may increase the risk of mechanical injury to the rind. Some managers report SC treatment prior to pressure washing partially cleans and conditions soiled fruit so cleaning can be accomplished with lower water pressure.

Washing inoculated fruit with water applied at high pressure alone did not reduce the incidence of green mold on oranges, and slightly but significantly reduced it on lemons. Pressure washing also may have affected a mechanical removal of some of the germlings. Other workers reported that green mold incidence is reduced when wounds on fruit are rigorously rinsed and then inoculated, because washing removes nutrients that stimulate *P. digitatum* spore germination (1) or removes rind oils that retard the lignification of wounds (3). The inoculum may have been reduced by mechanical removal of the germlings by high-pressure water washing in our tests.

The risk of SC injury to citrus fruit has been discussed previously (13,23,35,36). In the present work, rind injuries were only associated with SC treatments at 56°C or higher. Therefore, the risk of injury is low because these temperatures are above that needed for the control of green mold (35). In addition to visible scalding injury to the rind, harsh chemical (16) or hot-water treatments exceeding 48°C (17,27) can render the fruit susceptible to infection without obvious wounds, although *P. digitatum* typically requires wounds for infection to occur. Compared to hot water alone, SC is effective for the control of green mold at much lower temperatures (35,37), so the risk of injury to the fruit is reduced (21,24). A reason to use SC at higher temperatures (50°C) is to control brown rot, caused by *Phytophthora* spp. (14). Its control requires temperatures (25) that occasionally injure fruit (24). Brown rot is usually of less concern than green mold (10) and is associated with high rainfall and poor cultural practices (14). Carbonate and bicarbonate treatments inhibit the growth of the pathogen, but do not injure the fruit or its defensive responses. SC treatment at 40°C did not influence the synthesis of phloroglucinol-positive compounds within wounds associated with inhibition of infection by *P. digitatum* by lemon fruit (37; R. R. Stang, personal communication).

The effectiveness of SBC and SC was significantly improved when these treatments were followed by the fungicide imazalil or *P. syringae* strain ESC10. The combination of bicarbonate or carbonate followed by the biological control antagonist *P. syringae* overcomes significant shortcomings of either of these treatments alone. Biological control antagonists are poor eradicants that are usually incapable of controlling green mold when the fruit are inoculated 24 h before treatment (34), while imazalil, SC, and SBC can control these infections. SC and SBC do not provide persistent protection of the fruit from re-infection after treatment, while residues of imazalil or biological control antagonists persist for long periods after treatment and protect fruit from re-infection. Apparently, the residual of the bicarbonate or carbonate solutions that remained in inoculated wounds on the fruit was tolerated by the antagonist but inhibitory to the pathogen. For this combination to be used reliably under commercial conditions, a study of the tolerance of the antagonists to the bicarbonate and carbonate solutions and the influence of their residues on the populations of the antagonists in wounds on fruit is needed.

ACKNOWLEDGMENTS

We thank the California Citrus Research Board for assistance; D. Sorenson of Sunkist Growers, Lindsay, California; A. El-Gaouth of USDA-ARS, Kearneysville, West Virginia; J. Stack of Eco-Science Corp., Orlando, California; J. W. Eckert of the University of California, Riverside; and J. Maze, W. Stutzman, and L. Whitendale of the University of California, Lindcove.

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The influence of pasteurization on microbe populations in valencia orange juice.

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Juice from valencia oranges was extracted, pasteurized at various temperatures, and the population sizes of microbes in the juice determined immediately and weekly during cold storage (0.5°C) for three weeks on semi-selective agar media. Three media were employed: 1) Dichloran Rose Bengal Chloramphenicol Agar (DRBC) incubated at 20°C, which selects for yeasts and molds; 2) Plate Count Agar (PCA) incubated at 37°C, which selects for aerobic mesophilic bacteria; and 3) Orange Serum Agar (OSA) incubated at 20°C, which favors the development of citrus juice colonizing microbes. Duplicate juice samples were collected in 1 L volumes in sterile containers. Each container was sampled during storage, a dilution series in sterile water was prepared, and each dilution was distributed to triplicate agar plates of each medium. Colonies on PCA were counted after three days. Colonies on DRBC and OSA were counted after five days.

When the juice of unwashed fruit immediately after harvest was extracted,populations of microbes in the unpasteurized juice was about 200 colonies per milliter of juice, most of which were yeasts. Pre-washing fruit before extraction by passing the fruit through 3% (wt/vol) soda ash for one minute reduced populations by about one half in tests one and two (Figures 1 and 2) increased bacterial populations compared to fruit that had not been washed in a third test (Figure 3a, 3b, 3c). Soda ash can support the growth of some bacteria, probably the solution had a significant bacterial population in it during the third test. The solution itself should be periodically heated when the fruit are absent to prevent bacterial growth in the solution.

Tests one and two were preliminary tests that employed fewer media and only one sampling time, after three weeks of juice storage. Populations of microbes in the juice after pasteurization at all temperatures tested (71°C to 83°C) were low (<10 colony forming units per milliter of juice) or zero in the juice three weeks after it was extracted (Figures 1 and 2).

Test three employed all three media and was the most comprehensive. Populations of microbes in the juice after pasteurization at all temperatures tested (71°C to 83°C) were low (<10 colony forming units per milliter of juice) or zero in the juice immediately after the juice was extracted and for two weeks afterward. After three weeks, populations, particularly of yeasts on DRBC, increased significantly in most of the samples.

In conclusion, pasteurization temperatures employed in all three tests adequately reduced microbe populations for between two and three weeks in chilled juice.

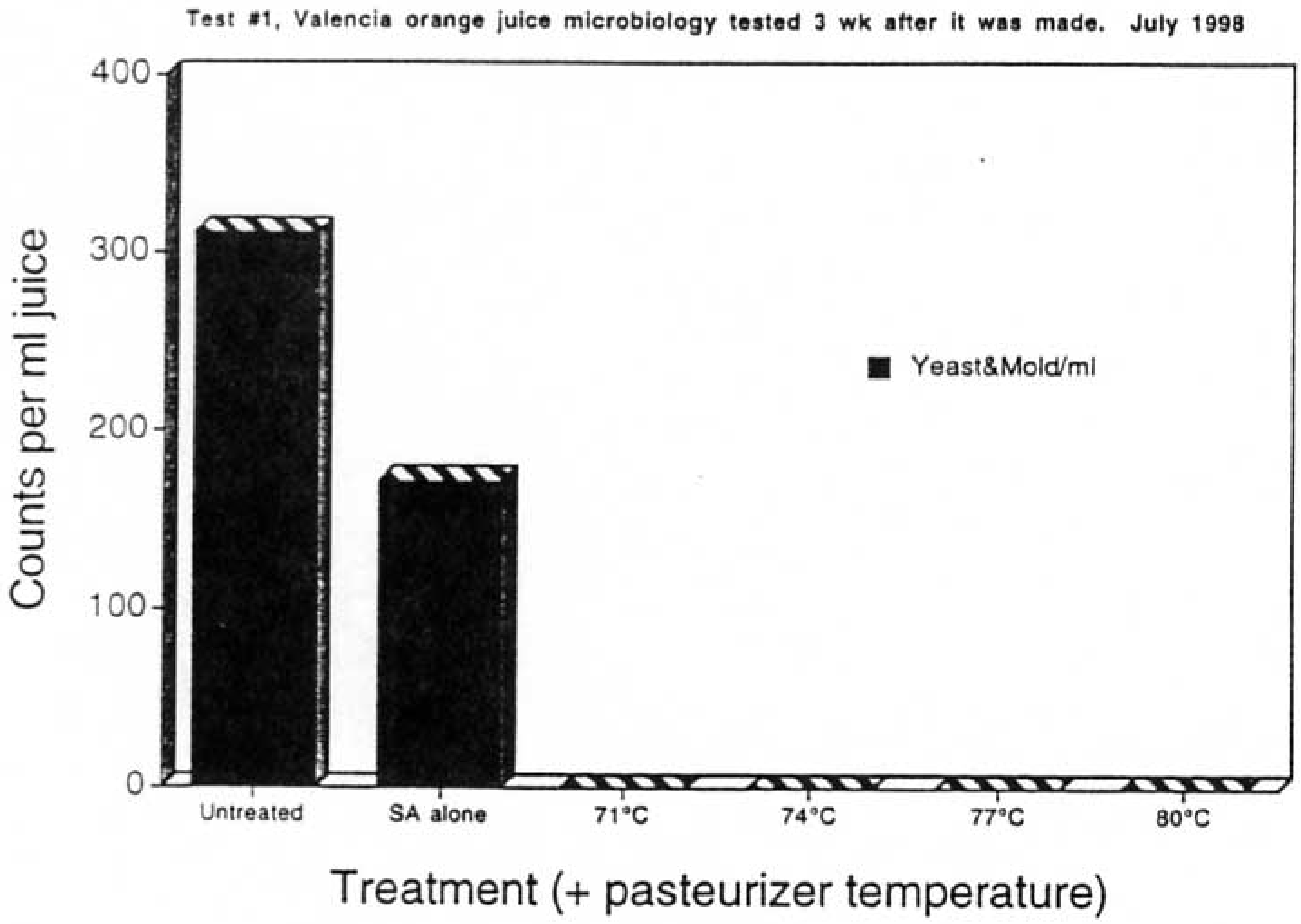


Figure 1

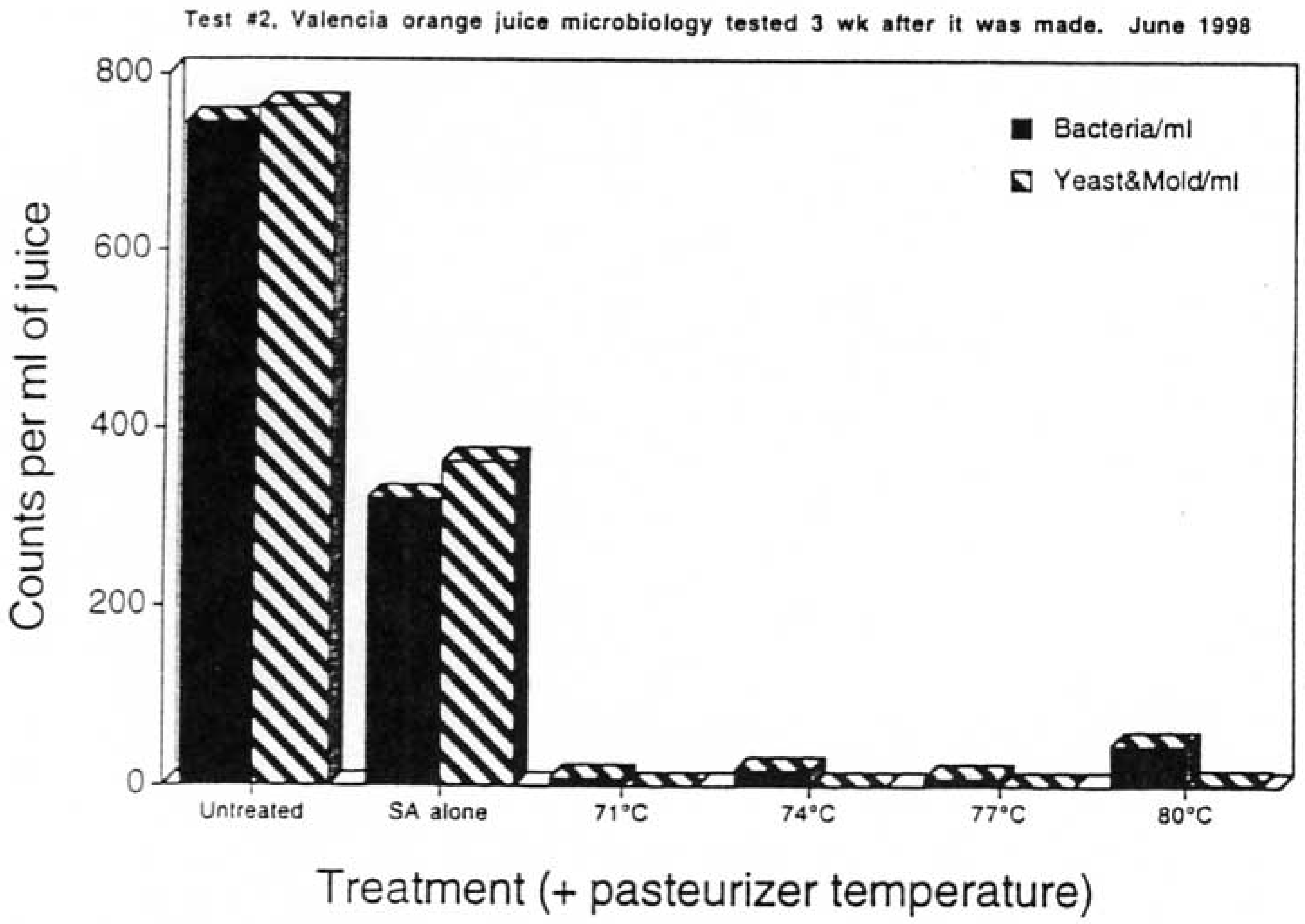
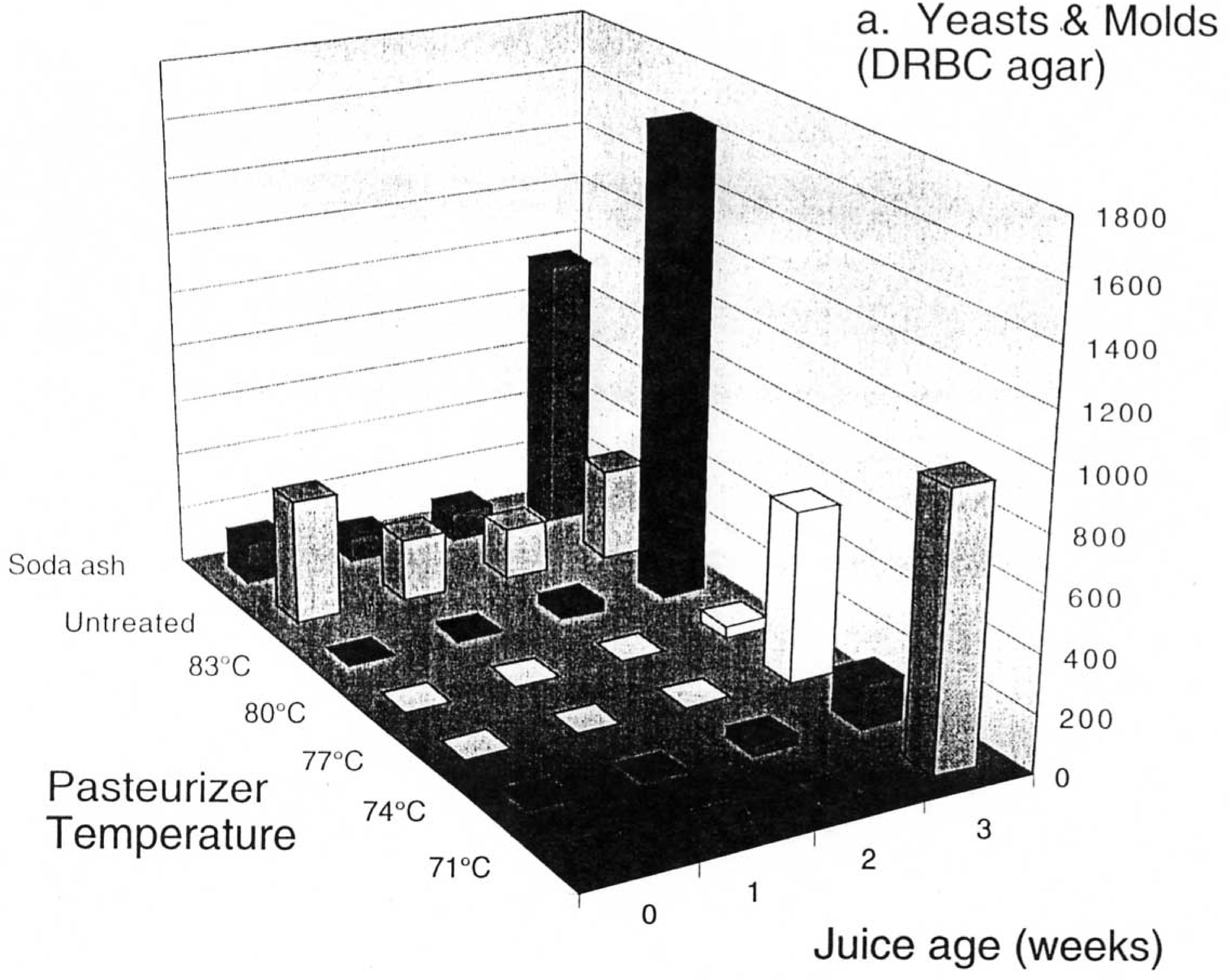
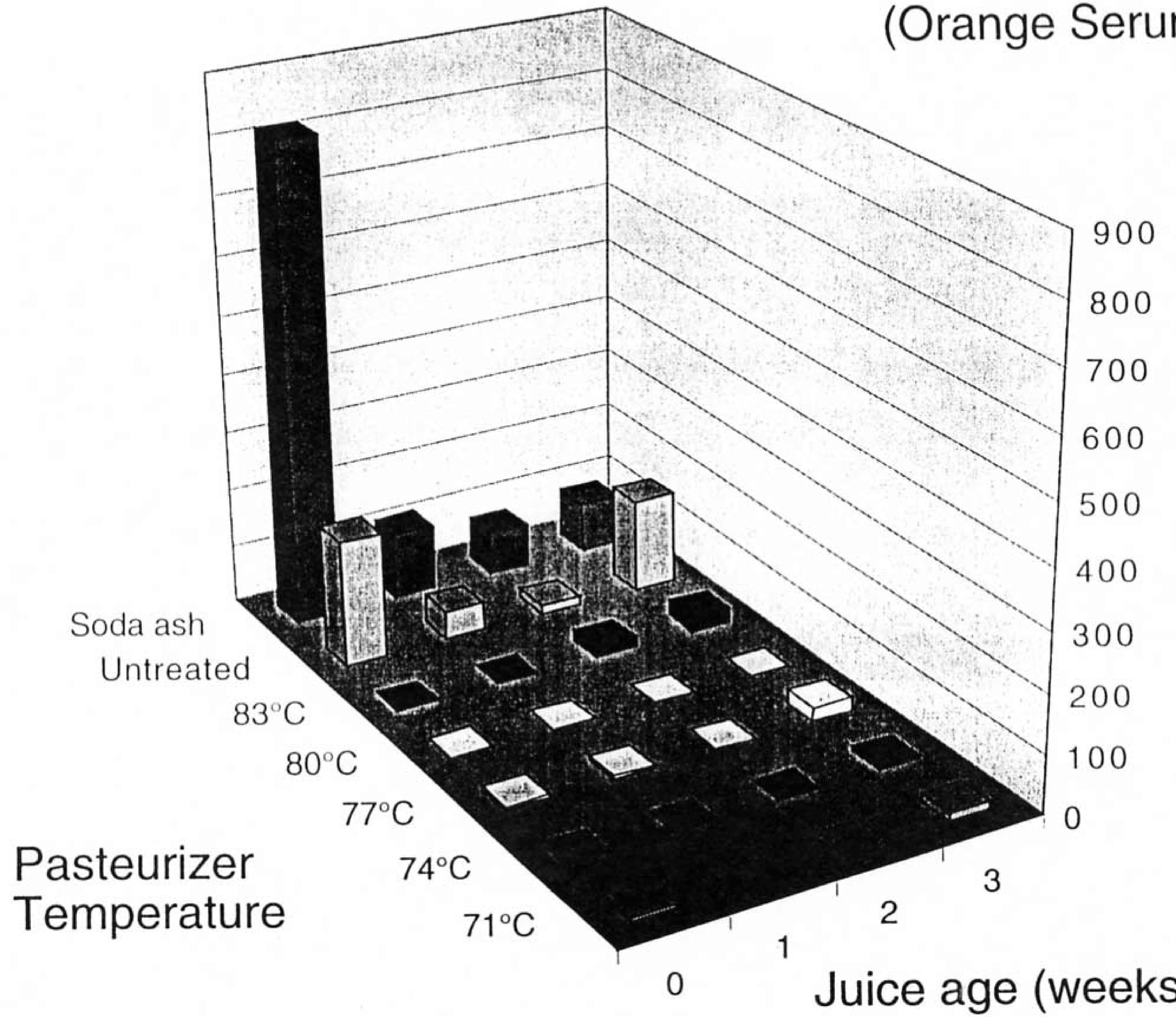
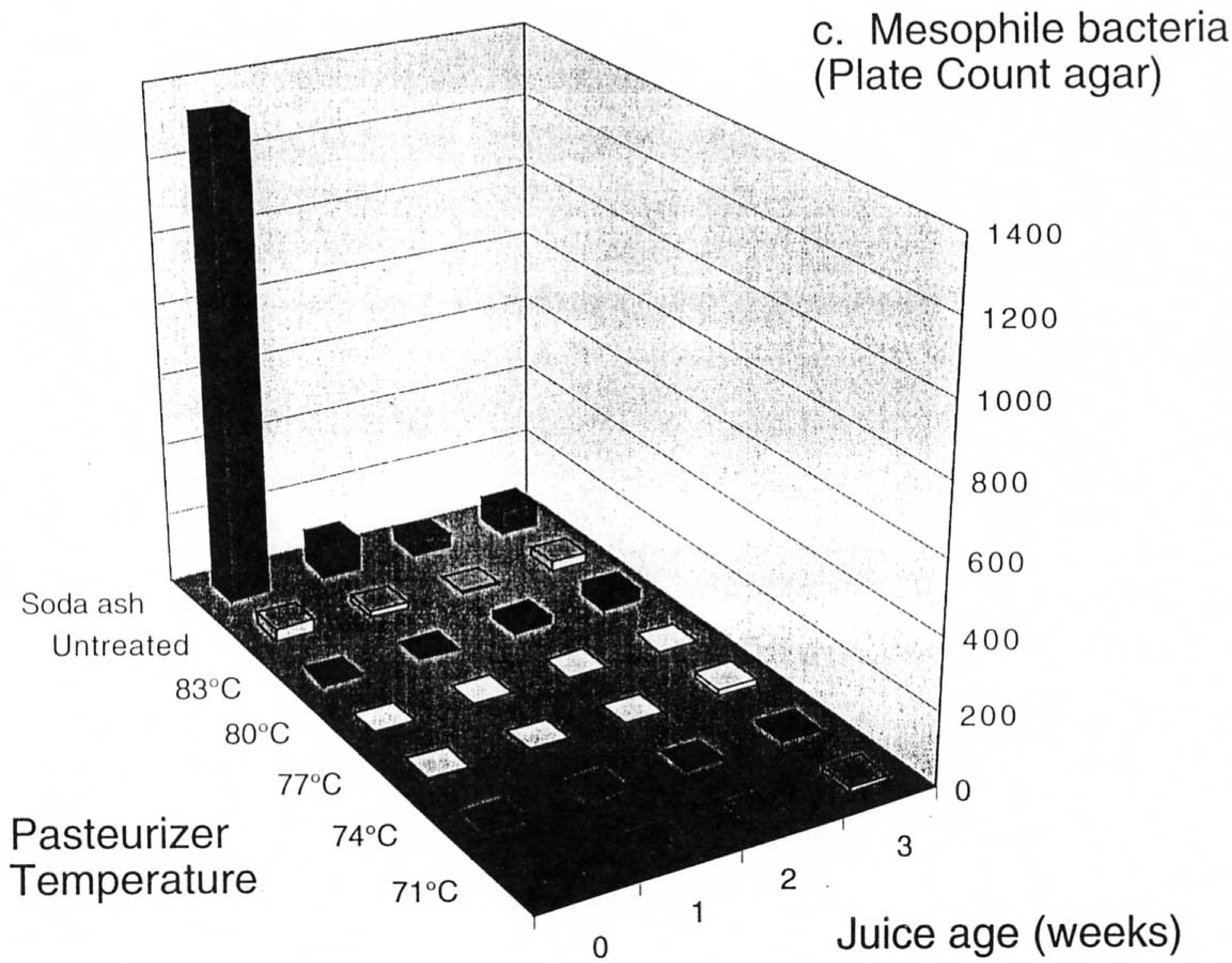


Figure 2


a. Yeasts & Molds
(DRBC agar)
Soda ash
Untreated
83°C
80°C
77°C
74°C
71°C
Pasteurizer
Temperature
0
1
2
3
Juice age (weeks)
0
200
400
600
800
1000
1200
1400
1600
1800

b. Bacteria
(Orange Serum agar)




c. Mesophile bacteria
(Plate Count agar)
1400
1200
1000
800
600
400
200
0
Soda ash
Untreated
83°C
80°C
77°C
74°C
71°C
Pasteurizer
Temperature
0
1
2
3
Juice age (weeks)

Improved Control of Green Mold of Citrus with Imazalil in Warm Water Compared with Its Use in Wax

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ABSTRACT

Smilanick, J. L., Michael, I. F., Mansour, M. F., Mackey, B. E., Margosan, D. A., Flores, D., and Weist, C. F. 1997. Improved control of green mold of citrus with imazalil in warm water compared with its use in wax. Plant Dis. 81:1299-1304.

The effectiveness of imazalil for the control of citrus green mold (caused by *Penicillium digitatum*) improved significantly when fruit were treated with heated aqueous solutions of the fungicide as compared with the current commercial practice of spraying wax containing imazalil on fruit. When applied at less than 500 μg·ml^{-1} in solutions heated to 37.8°C, control of postharvest green mold of citrus was significantly superior to applications of 4,200 μg·ml^{-1} imazalil in wax sprayed on fruit at ambient temperatures. The improvement in imazalil efficacy was obtained with a decrease in fungicide residues on the fruit. Residues of about 3.5 μg·g^{-1} imazalil deposited by the application of imazalil in wax reduced the incidence of green mold on lemons from 94.4% among untreated controls to 15.1%, whereas an equal residue deposited by passing fruit through heated aqueous imazalil reduced green mold incidence to 1.3%. Similar differences were found in tests with oranges. Residues of 2 and 3.5 μg·g^{-1} imazalil were needed to control the sporulation of *P. digitatum* on oranges and lemons, respectively. The mode of application of imazalil did not influence control of sporulation. The influence of immersion time, imazalil concentration, and solution temperature on imazalil residues on oranges and lemons was determined in tests using commercial packing equipment, and a model that describes residue deposition was developed. Residues after a 30- or 60-s treatment in heated aqueous imazalil were sufficient to control sporulation, but residues after 15-s treatments were too low and required an additional application of 1,070 μg·ml^{-1} imazalil in wax to deposit an amount of imazalil sufficient to control sporulation. An imazalil-resistant isolate of *P. digitatum* was significantly controlled by heated aqueous imazalil. The incidence of green mold of navel oranges was reduced from 98.8 to 17.4% by treatment in 410 μg·ml^{-1} imazalil at 40.6°C for 90 s. However, control of the resistant isolate required imazalil residues on the fruit of 7.9 μg·g^{-1}, which is within the U.S. tolerance of 10 μg·g^{-1} but above the 5 μg·g^{-1} tolerance of some countries that import citrus fruit from the United States.

Imazalil is a fungicide registered for postharvest application to citrus fruit to reduce both the incidence of decay and sporulation of *Penicillium digitatum* (Pers.:Fr.) Sacc., cause of citrus green mold. In Arizona and California, imazalil is usually added to fruit waxes and applied to fruit as a non-recovery spray over rotating brushes. This method is used because it is convenient and effective, although Brown and coworkers (2,4) showed that imazalil, unlike other fungicides used for this purpose, controlled green mold significantly better when applied in water than when applied in wax. Similarly, other researchers (8,15,16) reported superior control of green mold by the application of aqueous as compared with wax imazalil formulations. Imazalil loses effectiveness in wax because a substantial portion of the residue remains immobilized in the wax, and because waxes are more viscous than water, they less effectively penetrate the small wound infection courts that are exploited by *P. digitatum* on the rind of citrus fruit (2).

One reason the application of imazalil in wax has continued, in addition to its convenience, is its reliable control of *P. digitatum* sporulation. Anti-sporulant activity is important because it controls a condition termed soilage, a cosmetic defect that occurs when healthy fruit within cartons are soiled by spores from adjacent decayed fruit (7). Reducing sporulation also reduces the airborne inoculum within citrus packinghouses. This is particularly important because the inoculum within packinghouses is often composed of fungicide-resistant isolates (8). Brown and Dezman (3) reported that control of sporulation on Valencia oranges with aqueous, non-recovery sprays of imazalil or imazalil in wax both required a residue of about 2 μg·g^{-1} fresh fruit weight. However, with a non-recovery aqueous spray, coverage sufficient for sporulation control required better contact between brushes and fruit than was achieved in many commercial packinghouses. Therefore, in commercial packinghouses in Florida where imazalil is applied in aqueous sprays, this is followed by a second application of 1,000 to 2,000 μg·g^{-1} imazalil in wax. Fruit treated in this manner have residues of about 4 μg·g^{-1}, which is within the tolerance of 10 μg·g^{-1} in the United States and the 5 μg·g^{-1} tolerance of most importing countries (6).

An alternative to optimize the efficacy of imazalil would be to apply the fungicide by immersing fruit in heated aqueous solutions of the fungicide. Rapid treatment, an important attribute when very large numbers of fruit are processed, would be facilitated because imazalil residues accumulate on oranges about three times faster when the fruit are dipped in the fungicide rather than sprayed with it (3). Heating fungicide solutions also accelerates the accumulation of fungicide residues in fruit (19). Sufficient residues from a heated aqueous imazalil solution could control sporulation of *P. digitatum* and make a second application of imazalil in wax, such as is needed after aqueous sprays, unnecessary. Efficacy is also improved by heating. The control of postharvest decay by imazalil on mangos (22), grapefruit (14), and lemons (18,19) was enhanced when the fungicide was heated. Furthermore, a synergistic interaction between heat and imazalil treatment might provide better control of imazalil-resistant isolates than imazalil applied alone.

The purpose of this work was to determine with commercial-scale packing equipment the influence of immersion time, imazalil concentration, and temperature on concentration of imazalil residues in citrus fruit; to compare the efficacy of optimal regimes of imazalil applied in

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Accepted for publication 1 August 1997.

Publication no. D-1997-0829-01R

heated solutions to the conventional industry practice of spraying imazalil in wax on fruit for the control of green mold; and to determine if an imazalil-resistant isolate of *P. digitatum* could be adequately controlled by applying heated imazalil solutions.

MATERIALS AND METHODS

Fruit and packing line configuration. Sweet orange (*Citrus sinensis* [L.] Osbeck) cultivars Valencia and Washington and lemon (*C. lemon* [L.] N. L. Burm.) cultivar Eureka grown in the San Joaquin Valley of California were commercially harvested 1 to 2 days before they were selected by hand from field bins and randomized. When inoculated, they were inoculated once each about 24 (±2) h before treatment. The internal temperature of the fruit was 18–20°C. All treatments were applied on a commercial-scale packing line to four replicates of 60 oranges or 75 lemons each. A fifth replicate of 30 to 50 uninoculated fruit was included in some tests for residue analysis and later inoculation in sporulation tests. The temperatures of fruit were monitored with 1-mm-diameter, copper-constantan probes (Gaffney Engineering, Gainesville, FL) attached to a thermocouple thermometer (model HH11, Omega Engineering, Stamford, CT). The temperatures of air or water were monitored with a 3-mm-diameter by 30-cm-long corrosion-resistant, copper-constantan probe (model TJ48, Omega Engineering) attached to the same thermocouple thermometer. The temperatures of water or imazalil solutions reported are ± 0.5°C.

Each replicate was placed on the packing line just before a high-pressure washer. The residence time in the high-pressure washer, operating at 1,500 Newtons pressure, was 12 s. The water in the pressure washer contained 10 to 50 $\mu g \cdot ml^{-1}$ sodium hypochlorite at pH 7.2. After the high-pressure washer, the fruit passed for 15 to 20 s over rotating brushes through a dryer operating at 32.2°C to a moving belt sorting table, where decayed or damaged fruit were removed, and passed into a 2,400-L capacity tank containing water or imazalil. The concentration of imazalil in the tank was determined periodically. The pH of water and imazalil solutions was 7.2. The residence time of the fruit in the tank was 15, 30, 60, or 90 s. After treatment in the tank, the fruit were dried for 15–20 s through a dryer operating at 32.2°C (first stage of drying), wax was applied, and the fruit were dried again for 15–20 s through a dryer operating at 32.2°C (second stage of drying). Two waxes were used—a high solids content, shellac-based organic finishing wax was applied in all tests with fruit that were dried in the first and second dryers and a water-emulsion lemon storage wax with a 2% solids content was applied on lemons that were dried in the first stage dryer and air-dried after wax treatment.

Green mold control. An imazalil-sensitive isolate M6R of *P. digitatum* (a gift of J. W. Eckert, University of California-Riverside) and an imazalil-resistant isolate (151, a gift G. R Holmes, University of California-Riverside) were used in tests where fruit were inoculated. To determine the minimum imazalil concentration that was inhibitory to these isolates, 2-mm-diameter mycelial plugs from 1-week-old potato dextrose agar (PDA) cultures of each isolate were transferred to imazalil-amended PDA followed by 3 days incubation at 25°C. To inoculate the fruit, a steel rod with a 2-mm-long by 1-mm-wide tip was dipped into a water suspension of 10^6 $spores \cdot ml^{-1}$, then the tip of the rod was used to puncture the fruit. Inoculated fruit were stored at 10°C in cartons lined with perforated plastic liners and the incidence of green mold was recorded after 3 weeks.

Sporulation tests. To determine the influence of treatments on sporulation, the method of Eckert and Brown (7) was used. Fruit were inoculated by the injection of 0.10 ml volume of water containing of 10^6 $spores \cdot ml^{-1}$ of imazalil-sensitive isolate M6R about 1 cm below the rind surface, then the abundance of sporulation was recorded after incubation for 21 days at 20°C. A sporulation index that described the percentage of the fruit surface covered with green mold spores was used where 5 = 100%; 4 = 90–99%; 3 = 50–89%; 2 = 11–49; 1 = 1–10%; 0 = 0%. Each value represented the mean of sporulation ratings for 12 to15 fruit per treatment.

Imazalil analyses. Imazalil residues on fruit are expressed as $\mu g \cdot g^{-1}$ fresh weight. Because the surface to mass ratio will change with fruit size, and presumably influence the quantity of residue per gram of fruit weight, the orange and lemons selected for analysis were of uniform size and represented common commercial size classes. The oranges were 7 cm in diameter. The lemons were 7.5 cm in length and 5.5 cm wide. Imazalil residues in fruit were determined from two replicates of eight fruit each. Each fruit was sliced into eight portions, and one portion from each of the eight fruit was combined before analysis to comprise one replicate. To the combined fruit portions, a water volume one-half the weight of the fruit sample was added, blended at high speed in a blender, and an aliquot extracted with 10% (w/v) sodium hydroxide saturated with sodium chloride, followed by the addition of ethyl acetate. The suspension was briefly macerated in a homogenizer, mixed by rotation for 30 min, centrifuged, an aliquot of the supernatant (ethyl acetate layer) was then removed. After 0.05 N H_2SO_4 was added to this aliquot, it was shaken vigorously for 2 min, the ethyl acetate layer was discarded, and the aqueous layer was adjusted to pH 9 to 10 with sodium hydroxide and trisodium phosphate buffer. An aliquot of ethyl acetate was added and shaken vigorously. The ethyl acetate layer was removed, dried with anhydrous sodium sulfate, and analyzed by gas chromatography with an electron capture detector. Recovery of imazalil from fruit portions spiked with 1 to 5 $\mu g \cdot g^{-1}$ imazalil was about 95%. The imazalil content of water or wax solutions was determined by analysis. Water or wax solutions of imazalil were extracted with ethyl acetate, dried with sodium sulfate, and analyzed by gas chromatography.

Influence of fungicide concentration, temperature, and immersion period on imazalil residues. To determine the influence of imazalil (Fungaflor 500EC, 44.6% a.i.; Janssen Pharmaceutica, Titusville, NJ) concentration, tank temperature, and immersion time on imazalil residues on Valencia oranges, the fruit were pressure washed with water and immersed in 0, 153, 244, or 335 $\mu g \cdot ml^{-1}$ imazalil at 32.2, 37.8, or 43.3°C for 15, 30, or 60 s. After treatment, the oranges were dried in the first stage of the packing line dryer as previously described; waxed with a high solids content, shellac-based organic finishing wax on the packing line; and dried in the second stage of packing line dryer as previously described. To determine the influence of imazalil concentration, tank temperature, and immersion time on residues

Table 1. Influence of imazalil concentration, temperature, and immersion period on on imazalil residues ($\mu g \cdot g^{-1}$) on Valencia oranges

Imazalil ($\mu g \cdot ml^{-1}$)	Temperature(°C)	Immersion time (s) 15	30	60
153	32.2	1.04 (1.18)[a]	1.21 (1.30)	1.29 (1.55)
153	37.8	1.09 (0.90)	1.45 (1.36)	2.41 (2.29)
153	43.3	1.26 (1.66)	2.85 (2.46)	4.20 (4.06)
244	32.2	1.24 (1.38)	2.02 (1.59)	2.95 (2.03)
244	37.8	1.39 (1.12)	1.54 (1.87)	2.24 (3.38)
244	43.3	2.17 (1.90)	2.82 (3.19)	5.85 (5.76)
335	32.2	2.05 (2.57)	2.94 (2.88)	3.26 (3.50)
335	37.8	2.88 (2.33)	3.78 (3.38)	5.35 (5.47)
335	43.3	2.84 (3.14)	4.71 (4.91)	8.84 (8.45)

[a] Each value ($\mu g \cdot g^{-1}$) is the mean of two analyses. Values in parentheses are predicted by a second order surface model. Imazalil residues = 25.74 – 0.0117 (*IC*) – 1.25 (*T*) – 0.000345 (*IC* * *T*) – 0.00662 (*t*) – 0.000761 (*IC* * *t*) + 0.000134 (*T* * *t*) + 0.0000258 (*IC* * *T* * *t*) + 0.0000604 $(IC)^2$ + 0.0170 $(T)^2$. *IC* = aqueous imazalil concentration ($\mu g \cdot ml^{-1}$); *T* = aqueous imazalil solution temperature (°C); and *t* = immersion time (s) in aqueous imazalil.

on lemons, the fruit were pressure washed with water and immersed in 0, 93, 160, or 405 μg·ml^{-1} imazalil heated to 32.2, 37.8, or 43.3°C for 15, 30, or 60 s. After treatment, the lemons were dried in the first stage of the packing line dryer as previously described, waxed with a water-emulsion lemon storage wax, and dried in air on wire screens. Fruit were stored at 10°C and the imazalil content of the fruit analyzed within 7 days as previously described.

Comparison of imazalil efficacy when applied in wax or in water heated to 37.8°C. To compare the influence of imazalil (Fungaflor 500EC) applied in wax or in water heated to 37.8°C on control of the incidence and sporulation of *P. digitatum* using oranges and lemons, all fruit were inoculated with isolate M6R 24 h before treatment. Fruit were pressure washed with water, then treated with imazalil by: 1) immersion in 136, 240, 350, or 490 μg·ml^{-1} imazalil at 37.8°C for 15 s, dried in the first stage of the packing line dryer, waxed, and dried in the second stage of the packing line dryer as previously described; or 2) after drying in the first stage of the packing line dryer, they were sprayed with a high solids content, shellac-based organic finishing wax containing 1,100, 2,200, 3,400, or 4,200 μg·ml^{-1} imazalil, and dried in the second stage of the packing line dryer as previously described. Fruit were stored at 10°C and the imazalil content of the fruit analyzed within 7 days as previously described. Green mold incidence and sporulation of *P. digitatum* was determined as previously described.

Preliminary tests showed fruit immersed in heated aqueous solutions of imazalil for 15 s or less often had imazalil residues too low to control sporulation of *P. digitatum*, therefore, the heated imazalil treatments were followed by a spray of imazalil in wax to increase imazalil residues. Lemons were treated by immersing in 0, 93, 160, or 405 μg·ml^{-1} imazalil heated to 32.2, 37.8, or 43.3°C for 15 s, dried in the first stage packing line dryer as previously described, waxed with water-emulsion lemon storage wax alone or storage wax containing 1,070 or 1,910 μg·ml^{-1} imazalil, and dried in air on wire screens. Fruit were stored at 10°C and the imazalil content of the fruit analyzed within 7 days as previously described.

Control of imazalil-sensitive or imazalil-resistant *P. digitatum* isolates. Navel oranges were inoculated using two *P. digitatum* isolates, imazalil-sensitive isolate M6R or imazalil-resistant isolate 151, incubated 24 h at 20°C, and immersed in 247 or 410 μg·ml^{-1} imazalil (Magnate Sulfate 750 WP, 75% a.i.; Makhteshim Agan, Republic of South Africa) heated to 21.1 or 40.6°C for 90 s; dried in the first stage of the packing line dryer as previously described, waxed with a high solids content, shellac-based organic finishing wax; and dried in the last stage of the packing line dryer as previously described. Fruit were stored at 10°C and the imazalil content of the fruit analyzed within 7 days as previously described. Green mold incidence and sporulation of *P. digitatum* were determined as previously described.

Statistical analyses. Imazalil residues, the incidence of green mold, and sporulation of *P. digitatum* were analyzed by two- or three-way analysis of variance. An arcsin transformation of the square root of the proportion of decayed fruit was applied before analysis of the incidence of green mold. Duncan's new multiple range test ($P = 0.05$) to separate means or orthogonal contrasts were applied to compare treatments. A second order response surface model was fitted using SAS PROC GLM (SAS/STAT, Vers. 6, 4th Ed., Cary, NC) to predict the residues and 95% confidence intervals (CI). Regression expressions describing the relationship between sporulation indices and imazalil residues were fitted using Cricket Graph III (1).

RESULTS

Influence of fungicide concentration, temperature, and immersion period on imazalil residues. Increasing the temperature, immersion time, or imazalil concentration increased imazalil residues on Valencia oranges (Table 1) and Eureka lemons (Table 2). Doubling the imazalil concentration, doubling the immersion time, or increasing the solution temperature by 5.6°C increased imazalil residues approximately one and one-half to two times on the fruit. Separate second order models describing imazalil residues for oranges and lemons were prepared that can be used to predict residues. Residues predicted by both models were similar to those measured (Tables 1 and 2).

Table 2. Influence of imazalil concentration, temperature, and immersion period on imazalil residues (μg·g^{-1}) on Eureka lemons

Imazalil (μg·ml^{-1})	Temperature (°C)	Immersion time (s) 15	30	60
93	32.2	0.22 (0.25)[a]	0.33 (0.36)	0.46 (0.43)
93	37.8	0.34 (0.36)	0.57 (0.47)	0.60 (0.64)
93	43.3	0.50 (0.44)	0.61 (0.62)	0.94 (0.96)
160	32.2	0.54 (0.50)	0.58 (0.64)	0.84 (0.84)
160	37.8	0.68 (0.67)	1.07 (0.92)	1.58 (1.37)
160	43.3	0.79 (0.88)	1.18 (1.30)	2.11 (2.21)
405	32.2	0.92 (0.91)	1.42 (1.27)	1.75 (1.94)
405	37.8	1.42 (1.39)	1.77 (2.07)	3.88 (3.62)
405	43.3	2.06 (2.09)	3.70 (3.34)	6.65 (6.67)

[a] Each value (μg·g^{-1}) is the mean of two analyses. Values in parentheses are predicted by a second order surface model. The natural logarithm of imazalil residues in lemons = $-7.87 + 2.27*\text{Log}_e IC - 0.918*(T) + 0.0257*(\text{Log}_e IC*T) - 0.0307*t + 0.00579\ (\text{Log}_e IC*t) + 0.000807*(T*t) - 0.229*\text{Log}_e IC^2 - 0.00178*t^2$. IC = aqueous imazalil concentration (μg·ml^{-1}); T = aqueous imazalil solution temperature (°C); and t = immersion time (s) in aqueous imazalil.

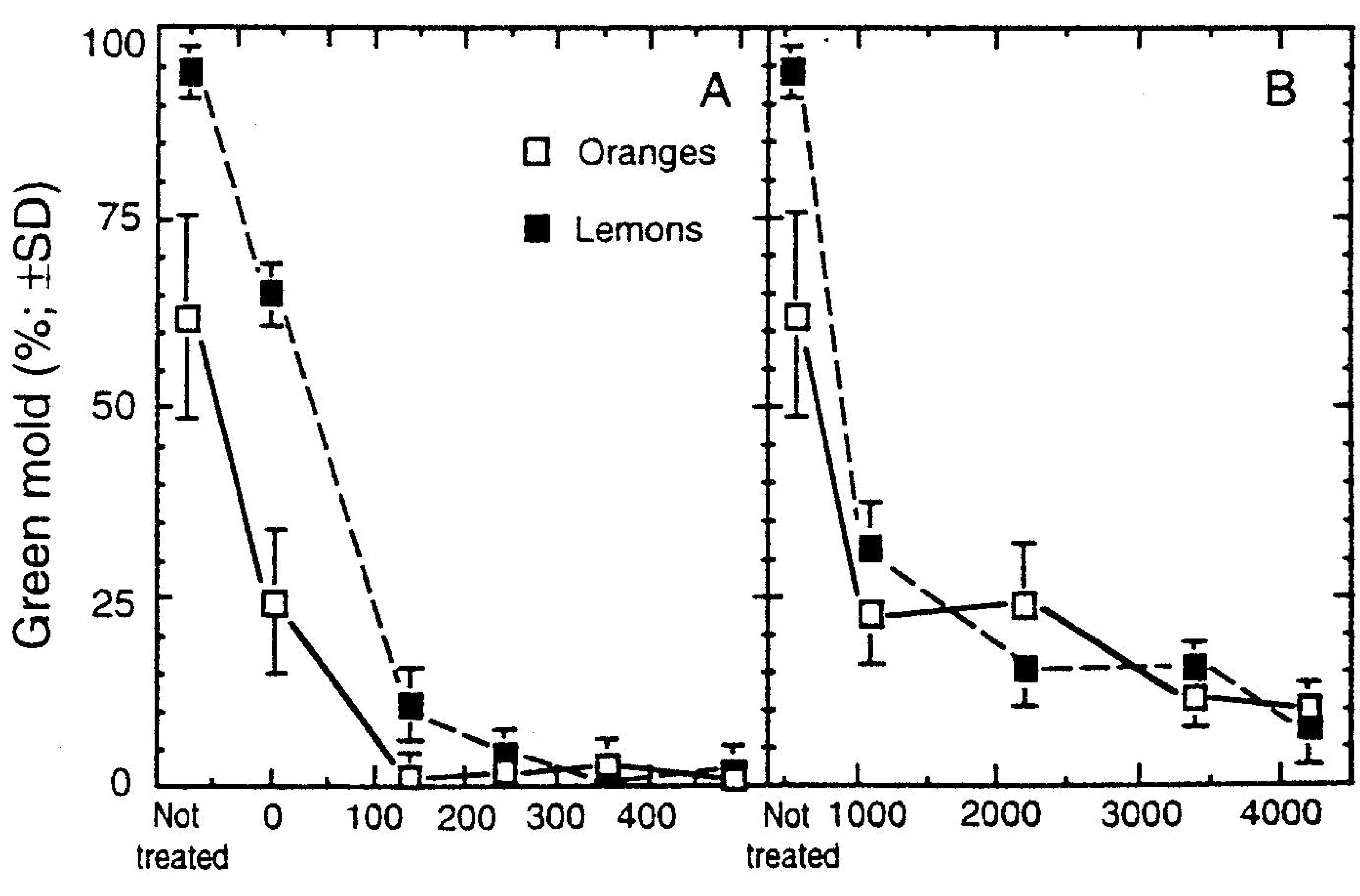

Fig. 1. Influence of imazalil concentration (μg/ml) and method of application on the incidence of postharvest green mold of oranges and lemons. The fruit were (A) immersed for 15 s in imazalil in water at 37°C or (B) sprayed with imazalil in wax over rotating brushes at 23°C. All fruit were inoculated 24 h before treatment with spores of *Penicillium digitatum*.

Comparison of imazalil efficacy when applied in wax or in water heated to 37.8°C. The control of green mold on oranges and lemons was significantly superior ($P < 0.0001$, orthogonal contrast) by imazalil applied in heated water to that in wax (Fig. 1), although the imazalil residues deposited on the fruit significantly exceeded ($P < 0.0001$, orthogonal contrast) those of imazalil applied in heated water (Fig. 2). Residues of about 3.5 $\mu g \cdot g^{-1}$ imazalil deposited by the application of imazalil in wax reduced the incidence of green mold on lemons from 94.4% among untreated controls to about 11.0%, whereas an equal residue deposited by passing fruit through heated aqueous imazalil reduced green mold incidence to less than 1.0% (Fig. 2). Similar differences occurred with oranges. Treatment with water at 37.8°C reduced green mold incidence of oranges and lemons by about 30 and 55%, respectively. Residues of 2 and 3.5 $\mu g \cdot g^{-1}$ imazalil were needed to control sporulation on oranges and lemons, respectively (Fig. 3). Because control of sporulation by imazalil applied in water or wax sporulation was not significantly different, all rating of control of sporulation were pooled.

Augmentation of imazalil residues by a second application of imazalil in wax, in order to obtain residue levels sufficient to control *P. digitatum* sporulation, were most effectively achieved by a concentration of 1,070 $\mu g \cdot ml^{-1}$ imazalil in the wax (Table 3). When the second application of wax with 1,910 $\mu g \cdot ml^{-1}$ imazalil was applied, residues were higher than needed to control sporulation and above the 5 $\mu g \cdot g^{-1}$ imazalil residue tolerance for citrus fruit of many importing countries.

Control of imazalil-sensitive or imazalil-resistant *P. digitatum* isolates. Growth of mycelial plugs of isolates M6R and 151 was stopped in PDA with imazalil concentrations of 0.1 $\mu g \cdot ml^{-1}$ and more than 2 $\mu g \cdot ml^{-1}$, respectively. Heating the imazalil solution increased residues in navel oranges and improved control of both *P. digitatum* isolates (Fig. 4). Control of imazalil-sensitive isolate M6R was superior to control of imazalil-resistant isolate 151. Heating 410 $\mu g \cdot ml^{-1}$ imazalil from 21.1 to 40.6°C further reduced green mold incidence from 16.2 to 5.3% among oranges inoculated with isolate M6R, and from 71.2 to 17.4% among oranges inoculated with isolate 151. Heating 410 $\mu g \cdot ml^{-1}$ imazalil from 21.1 to 40.6°C also increased imazalil residues on oranges three- to fourfold. Sporulation of the sensitive isolate was controlled by all imazalil treatments, while sporulation of the imazalil-resistant isolate was not controlled (data not shown). Treatment with water alone at 40.6°C had no significant influence on the incidence of green mold in this test.

DISCUSSION

The higher effectiveness of imazalil shown in this study was due to three factors: i) application of the imazalil in water instead of wax; ii) heating of the aqueous imazalil solution; and iii) immersing the fruit in a tank of the solution instead of spraying the fungicide on fruit to make the treatment brief enough to be practical. In our tests, fruit immersed briefly in heated aqueous imazalil, leaving residues of 1 to 3 $\mu g \cdot g^{-1}$, had about 95% less green mold than control fruit, while similar residues deposited by spraying fruit with imazalil in wax reduced green mold incidence only about 60%. Several aspects of this work were established in prior studies. Eckert et al. (8) showed imazalil reduced green mold more effectively when applied in water instead of wax. Imazalil applied at 2000 $\mu g \cdot ml^{-1}$ in wax or in water reduced the incidence of green mold by 48 and 98%, respectively. Brown and Dezman (3) reported that application of aqueous solutions of imazalil by immersion in a tank deposited imazalil residues approximately three times faster than spraying the same solution on citrus fruit. Heating imazalil improved its effectiveness on mangos (22), grapefruit (14), and lemons (18,19). Temperatures we used are too low to reliably control green mold by heat (20), although partial control of green mold by heated water alone did occasionally occur in our work.

Imazalil residues required to control green mold sporulation we report are slightly higher those reported by others. Most researchers reported residues of about 1 to 2 $\mu g \cdot g^{-1}$ were needed to control sporulation on oranges and lemons (3,13), whereas we found 2 and 3.5 $\mu g \cdot g^{-1}$ were needed, probably because of differences in incubation temperatures of the fruit or the subjectivity of sporulation indices. Brown and Dezman (3) reported that control of sporulation of the green mold fungus on Valencia oranges with aqueous, nonrecovery sprays of imazalil required a residue of about 2 $\mu g \cdot g^{-1}$, the same level required when imazalil is applied in wax (13). They (3) showed control of sporulation on Valencia oranges with aqueous

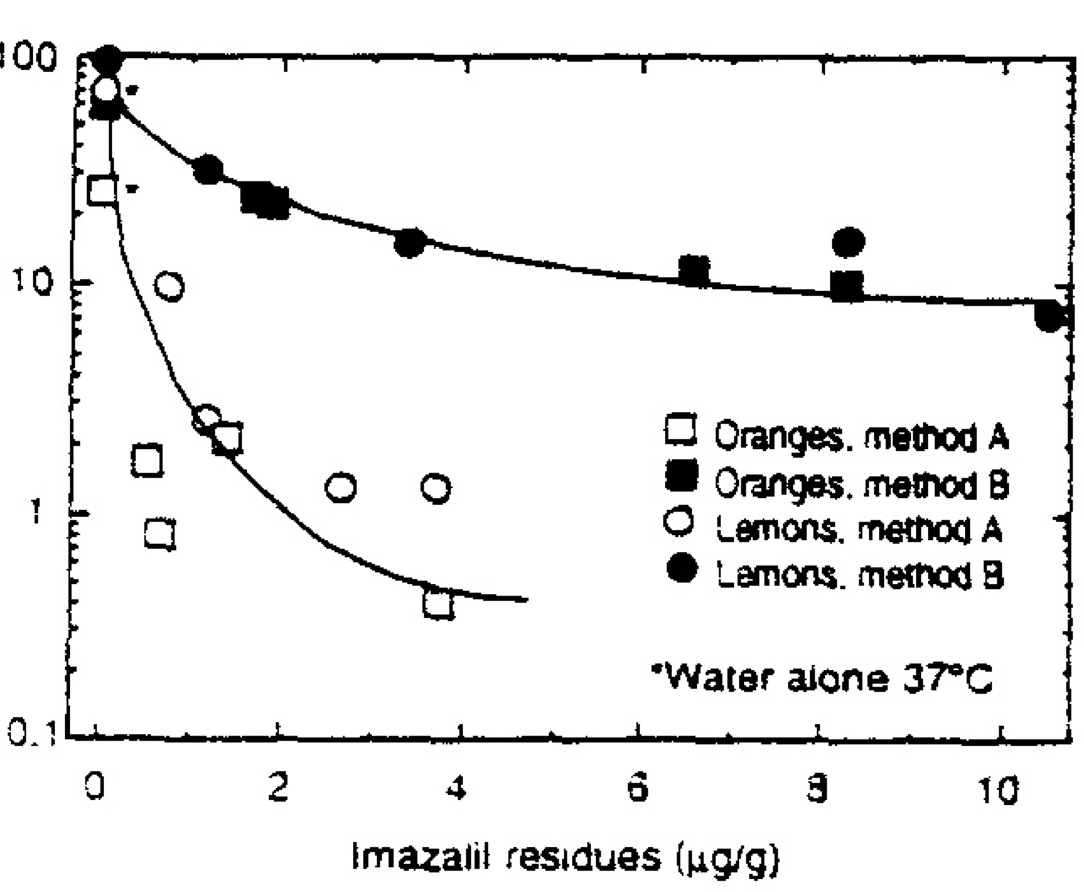

Fig. 2. The relationship between imazalil residues on fruit and the method of application of imazalil on the incidence of postharvest green mold of oranges and lemons. In method A, the fruit were immersed for 15 s in 136–490 $\mu g \cdot ml^{-1}$ imazalil in water at 37°C. The incidence of green mold = 1.995 (imazalil residue)$^{-0.576}$, $r^2 = 0.62$. In method B, the fruit were sprayed with 1,100 to 4,200 $\mu g \cdot ml^{-1}$ imazalil in wax over rotating brushes at 23°C. The incidence of green mold = −20.421 * log(imazalil residue) + 29.23, $r^2 = 0.89$. All fruit were inoculated 24 h before treatment with spores of *Penicillium digitatum*.

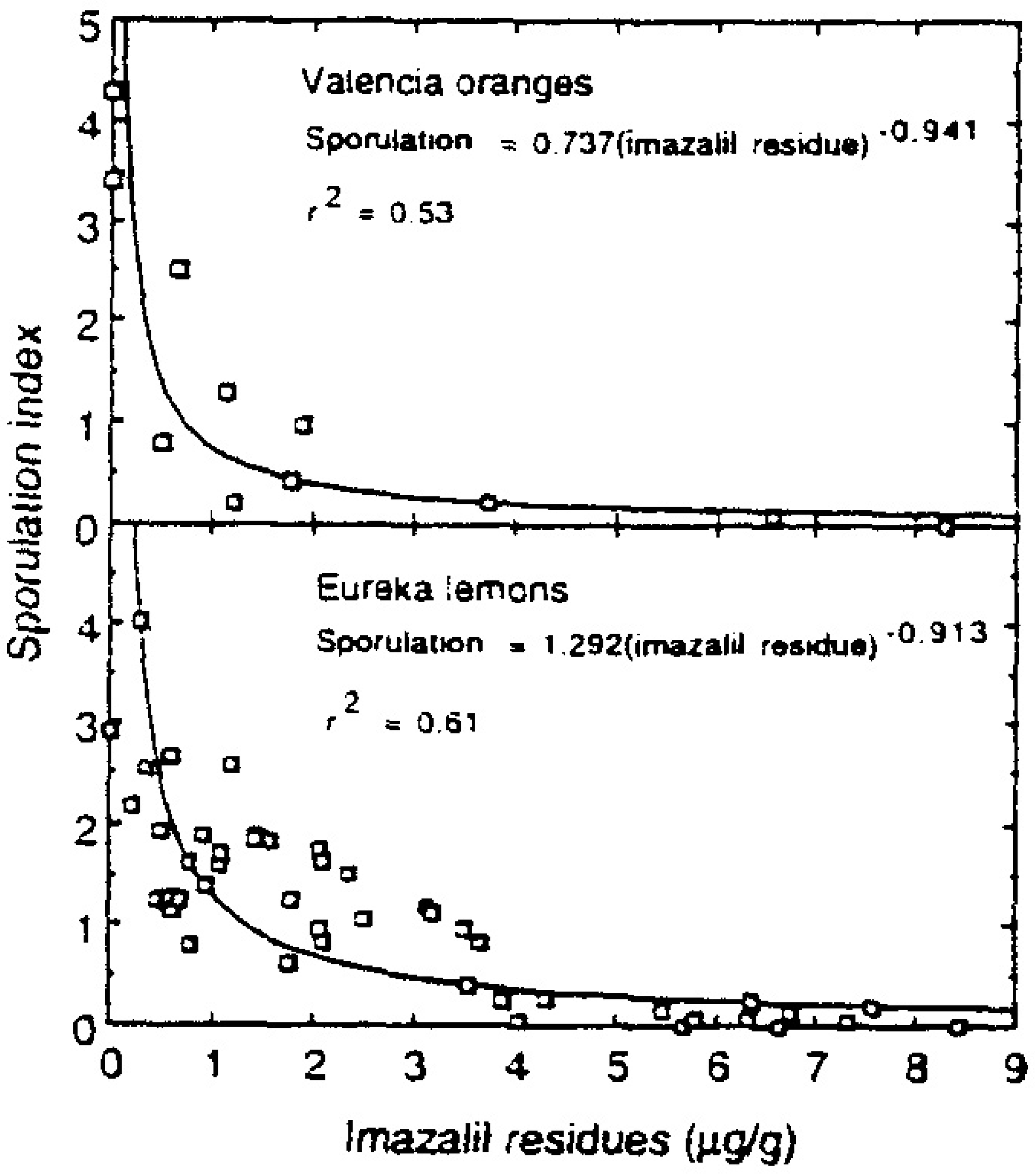

Fig. 3. Influence of imazalil residues on the sporulation of *Penicillium digitatum* on green mold infected oranges and lemons stored 3 wk at 10°C after imazalil treatment. The sporulation index describes the percentage of the fruit surface covered with green mold spores where 5 = 100%; 4 = 90–99%; 3 = 50–89%; 2 = 11–49; 1 = 1–10%; and 0 = no sporulation on the surface of the fruit.

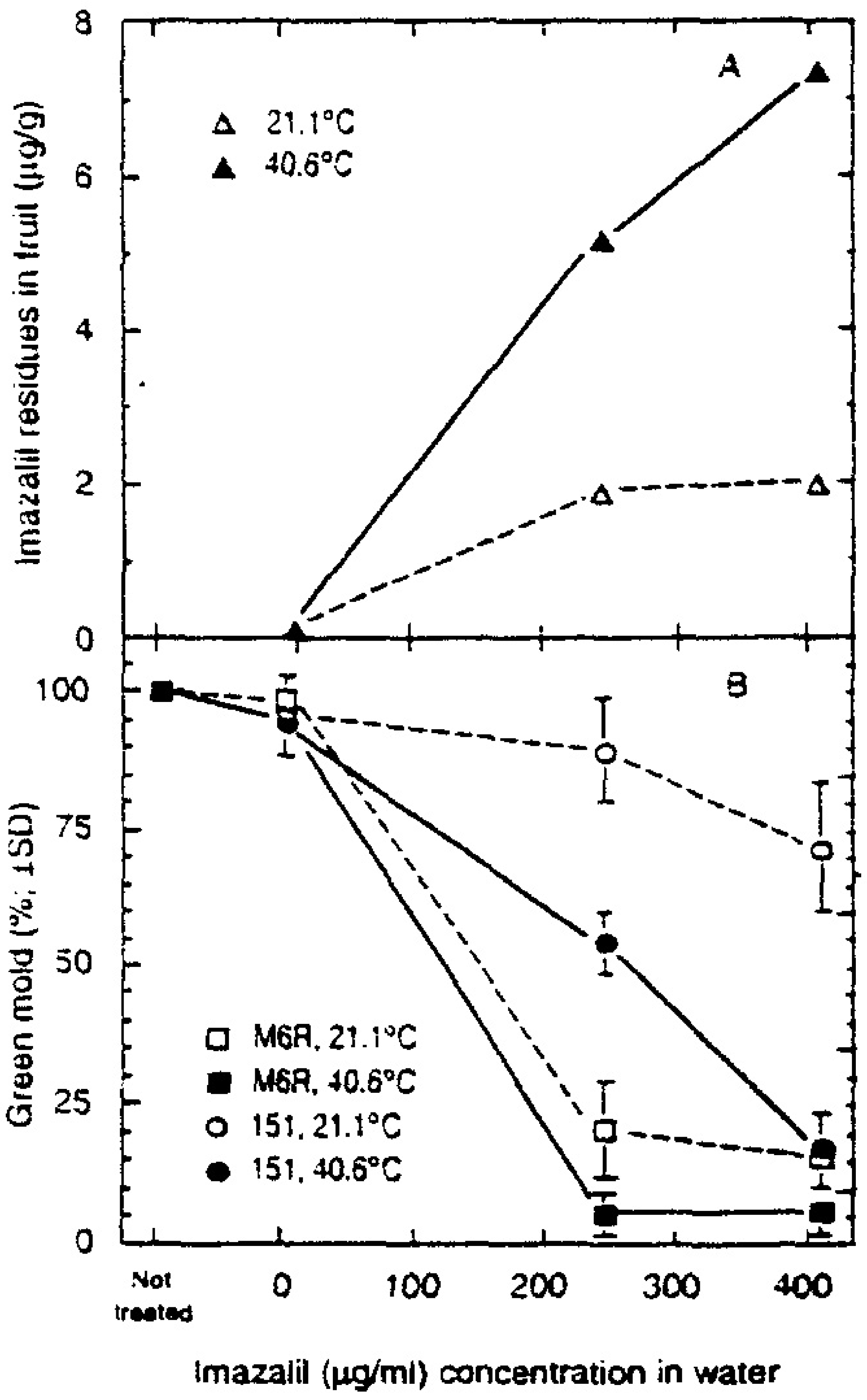

Fig. 4. Imazalil residues (A) and the incidence of green mold (B) of navel oranges immersed in imazalil in water at 21.1 or 40.6°C for 90 s. All fruit were inoculated 24 h before treatment with spores of imazalil-sensitive isolate M6R or imazalil-resistant isolate 151 of *Penicillium digitatum*.

imazalil depended on the deposition of imazalil in natural epicuticular wax, when the wax was removed, residues of 2 μg·g^{-1} did not control sporulation. Brown and Dezman (3) stated sufficient coverage to get control of sporulation required better contact between brushes and fruit than was achieved in many commercial packinghouses when a non-recovery aqueous imazalil spray was used; as a consequence, commercial packinghouses in Florida where imazalil is applied in aqueous sprays follow this application with imazalil in wax at 1,000 to 2,000 μg·g^{-1} to ensure adequate control of sporulation. Our results indicate that if imazalil is applied in heated solutions for 30 s or longer, adequate residue deposition can occur to make a second application of imazalil in wax for sporulation control unnecessary. However, treatments using heated imazalil for 15 s required an additional application of 1,070 μg·ml^{-1} imazalil in wax to leave residues sufficient to control sporulation.

Partial control of an imazalil-resistant *P. digitatum* isolate was achieved with aqueous imazalil, but relatively high residue levels were associated with its control. We reduced the incidence of green mold on oranges after inoculation with a resistant isolate by 78% by treatment for 1 min in 410 μg·ml^{-1} imazalil at 40.6°C. However, imazalil residues in the fruit where the resistant strain was partially controlled were 7.9 μg·g^{-1}; much higher than the 1 to 2 μg·g^{-1} required to control an imazalil-sensitive isolate. Higher rates must be used with care because established tolerances can be exceeded. The U.S. imazalil tolerance is 10 μg·g^{-1} and that of many citrus-importing countries is 5 μg·g^{-1} (6). Although the incidence of imazalil-resistant isolates of *P. digitatum* in California is high (8,10), imazalil remains a useful fungicide to manage green mold. In addition to partial control of resistant isolates with higher rates of imazalil that we and others (8) reported, other aspects of imazalil-resistant isolates suggest they can be managed: i) resistant isolates are less fit than imazalil-sensitive isolates and decline in competition with sensitive strains in the absence of the fungicide (5,11,23); ii) imazalil-resistant isolates primarily occur in packinghouses and not in groves, therefore imazalil effectively controls the sensitive isolates from the groves; iii) because imazalil-resistant isolates primarily occur only in packinghouses, sanitation measures can be successfully applied; and iv) although the proportion of resistant isolates collected in 26 California packinghouses was high (77.2 and 74.2% in 1990 and 1994, respectively) and it did not increase in this period (10).

No rind injury was observed in any of the our tests, and the risk of injury from heated aqueous imazalil treatments we evaluated is probably low. Rind injury from hot water alone usually does not occur below 50°C on lemons (12,20) or oranges (21,25), unless the fruit are cool and turgid at the time of treatment (9). A benefit of immersion in heated water or imazalil solutions is a reduction in the susceptibility of the fruit to subsequent chilling injury (14,17,24). We did not evaluate temperatures as high as those employed in those studies, however, because good control was obtained without using higher temperatures and prior reports that the addition of imazalil to water lowers the temperature at which rind injury and internal quality changes occur in lemons (18,19). Shirra et al. (19) immersed lemons in water or 250 to 1,500 μg·ml^{-1} imazalil for 3 min at 50°C and stored them for 14 weeks at 9°C. Imazalil-treated lemons did not differ in titratable acidity or soluble solids from untreated or water-treated fruit, but imazalil concentrations of 500 μg·ml^{-1} or higher applied at 50°C caused rind injuries and increased ethanol and acetaldehyde contents. These injuries did not occur when imazalil was applied at 20°C. To reduce the risk of injury, regimes we evaluated employed cooler (43.3°C or less), briefer (60 s or less), and lower aqueous imazalil concentrations (less than 500 μg·ml^{-1}) than those Schirra and coworkers (18,19) showed to injure lemons.

Imazalil effectiveness on citrus can be substantially improved when the fruit are passed through heated aqueous solutions of the fungicide compared to the current commercial practice of spraying the fungicide at ambient temperatures in waxes. The improvement is probably due to more effective infiltration of the fungicide into the wound infection courts that are exploited by *P. digitatum* on the rind of citrus fruit (2). From our work, 30 s immersion of lemons or oranges in 350 to 400 μg·ml^{-1} imazalil heated to 37.8°C should deposit residues of 2 to 4 μg·g^{-1}. This regime enhanced imazalil efficacy, deposited sufficient residues to control sporulation, facilitated rapid treatment, partially controlled an imazalil-resistant isolate of *P. digitatum*, and posed minimal risk of injury to the fruit. The treatment could be shortened to 15 s if it is followed by the application of about 1,000 μg·ml^{-1} imazalil in wax, or if higher imazalil concentrations or temperatures were used in tanks, although the risk of injury to the fruit may be increased.

ACKNOWLEDGMENTS

We gratefully acknowledge useful ideas and suggestions of G. E. Brown, J. W. Eckert, and William Goodwine, and the assistance of Micro-bac Laboratories, Inc. and Pent-A-Vate, Inc. Biological Testing and Research Laboratory with imazalil analysis, the donation of supplies by of Janssen Pharmaceutica and Makhteshim Agan, and the financial support of the California Citrus Research Board.

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Table 3. Imazalil residues (μg·g^{-1}) on lemons immersed for 15 s in water or imazalil followed by application of a storage wax containing imazalil

		Imazalil concentration in wax (μg·ml^{-1})						
Imazalil (μg·ml^{-1})	**Temperature (°C)**	**0**	**1,070**	**1,910**	**Slope**	**Intercept**	R^2	***P***
0	0	0.00[a]	1.08	2.34	0.001	–0.067	0.99	0.072
93	32.2	0.22	2.10	5.47	0.003	–0.090	0.95	0.147
93	37.8	0.34	2.49	6.32	0.003	–0.010	0.95	0.147
93	43.3	0.50	2.07	5.79	0.003	0.007	0.90	0.210
160	32.2	0.54	3.52	6.37	0.003	0.457	0.99	0.036
160	37.8	0.68	3.14	7.55	0.004	0.275	0.95	0.148
160	43.3	0.79	3.17	5.67	0.003	0.687	0.99	0.053
405	32.2	0.92	3.65	6.76	0.003	0.762	0.99	0.068
405	37.8	1.42	4.03	7.33	0.003	1.216	0.98	0.087
405	43.3	2.06	4.31	8.44	0.003	1.674	0.94	0.152

[a] Each value (μg·g^{-1}) is the mean of two analyses.

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Sunday, August 3, 1975

CALIFORNIA COUNTRY Life

The Fresno Bee

Researching Herbicides' Tree Impact

Insect Breeder's Sweep

'Bugging' Job Goes 2 Ways In Pest Control

Story and Photos By Leo Dollar

Integration. It's a word we hear more every day. It is even becoming the name-of-the-game in fighting off the bugs trying to do us out of our food and fiber crops.

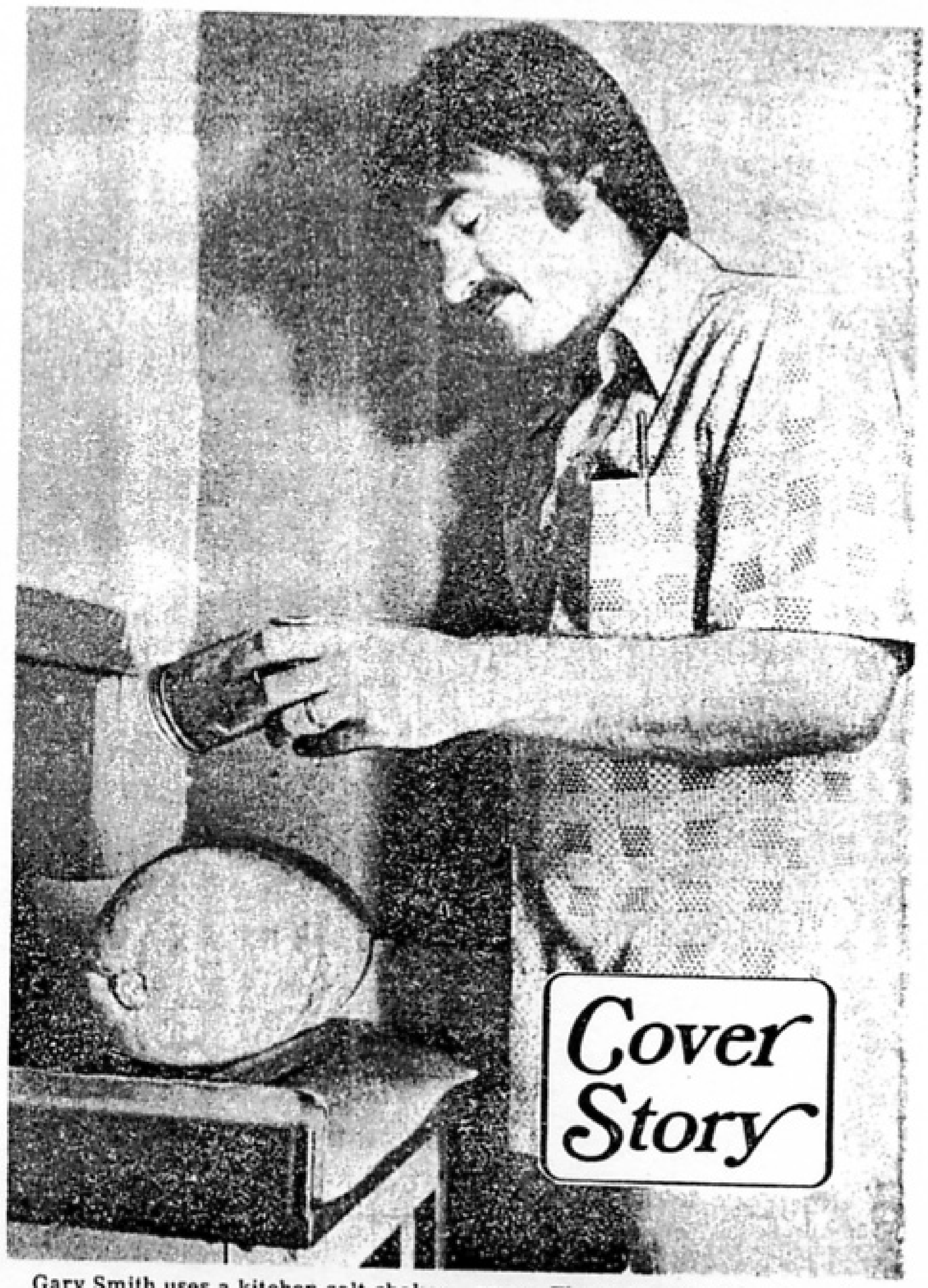
Environmentalists' pressures and new laws squeezing down on our potent modern pesticides are forcing today's up-to-date farmer to join the mopement...like it or not. The simple fact of staying alive economically is one of the strongest arguments for "integrated insect control" that teams hordes of hand-raised beneficial predatory or parasitic insects with carefully timed spray or dust programs to create the highest possible "kill factor" against our common cropland enemies. With land, labor, water, fertilizers, pesticides and tools becoming more costly day by day, any way to make any one of them work better must be followed...and integration appears to be one of the best.

One of the latest additions to this ultra modern approach is the compact laboratory and insectary (a special nursery for raising the armies of beneficial biological warriors) operated by Ibrahim F. Michael and Gary Smith on the Clovis Avenue edge of Fresno's air terminal property. Michael Pest Manage-

See Bugging Page F-12

A microscopic census of male red scale insects trapped on the sticky cards of an orchard survey trap is tallied by Darllynne Michael to keep tab on any increase in the hatch of the pest in citrus. A clear plastic grid, like the one standing liue a teepee at left, is marked for the random squares from which she takes the counts with the mechanical counter in her left hand.

Ibrahim F. Michael, who set up his Michael Pest Management in 1973 as both a supplier of beneficial insects for pest control and traps for determining pest infestations, shows potatoes carrying hatches of tuber moth to be used as sex lure in field traps to determine timing for sprays.



Gary Smith uses a kitchen salt shaker to dust a new colony of Aphytis melinus parasitic wasps on the rind of a banana squash in the insectary where he and Michael breed beneficial insects to quell pests that "bug" our crops. They also operate other chambers nearby (though in isolation) to breed the pests themselves, red scale and tuber moth, to provide bait for their survey traps.

'Bugging': Fresno Lab Makes Good, Bad Pay

From Page F-3

ment, as the barely two-year- old consulting service is known, is designed to provide bug bedeviled San Joaquin Valley farmers in several crops with life-saving scientific advice and coaching on bug control

Their principal targets are California red scale in citrus and worms afflicting cotton and tomatoes as well as the tuber moth in potatoes They also have advisory plans they offer growers of grapes figs almonds and alfalfa, most employing integration of chemical with biological control methods

The hottest item right now is the red scale because the summer flight of the males (delayed two to three weeks this year by our cold spring weather) is just winding up its first peak period. And this is one of the most important parts of their program for arriving at the proper control procedures.

They provide a survey, employing sex attractant traps much like those used in tracking the pink bollworm in cotton, which pinpoints the hot spots of the infestations as populations rise. They start in March with a pilot trap set every 100 to 150 acres, changing them weekly until the fall rebound occurs in November. This way they can plot the hatches week by week and track them geographically in the orchards to zero-in on the destructive pests.

"This is our newest and most exciting venture," the tall intense Michael says. "As soon as a population increase is seen, we move in with a concentration of survey traps, setting out one trap for every three to four acres for the next week ortwo. This is where we can prove our value to the orchardist. The old way of surveyibg orchrds by random samples of leaves and branches is expensive because of the man-hours involved. It can also be expensive because it can easilymiss a hot spot that will turn out swarms of males for breeding right after the sampler has passed through the orchard.

"The trap system is immensely more accurate. The sticky cards on top of each trap catch the flying males (they only last 24 hours as breeders but they can fly against the wind several hundred yards and spread the infestation amazingly in the single day they last to mate and die) and provide solid mathematical evidence of the hatch.

Michael, who was trained in toxicology and plant pathology at Cairo University in the '60s (that's right Cairo, Egypt), points out the tricky part of this work is the fact there is no synthetic pheromone to imitate the female red scale's alluring sexual perfume. The pink bollworm's heady scent was successfully synthesized several years ago.

Consequently, a large part of Michael's and Smith's time is spent in supervising the raising of colonies of virgin red scale females. You got that, didn't you? The word was "virgin" and it is vital to their success.

Little Mr. Red Scale, limited to just one day of romancing in his life cycle, is picky, picky. No fallen maids for him. He's not like Harold Hill, The Music Man who said he preferred the "girl too late to save." No, sir, he is hot for the girl just like the girl who married dear old Dad and purity is everything to him.

So, they operate meticulously guarded hatcheries using thousands of green lemons to raise the necessary virgins (which necessitates killing off each generation's accompanying males in the brief span between their attainment of sexual maturity). Then the lemons go into the pint size ice cream cartons rigged as traps for their critical measuring job in the citrus groves. It is a tricky process. Timing is honed as fine as mosquito's beak. And quarantinelike safety precautions must be followed to avoid escapes or contamination by other insects getting inside.

But the job is only half done once they show a grower where his scale problem lies. Next they coach him on his approach to control. And this brings in another clan of insects along with the spray crews, with timing planned to get the most out of each.

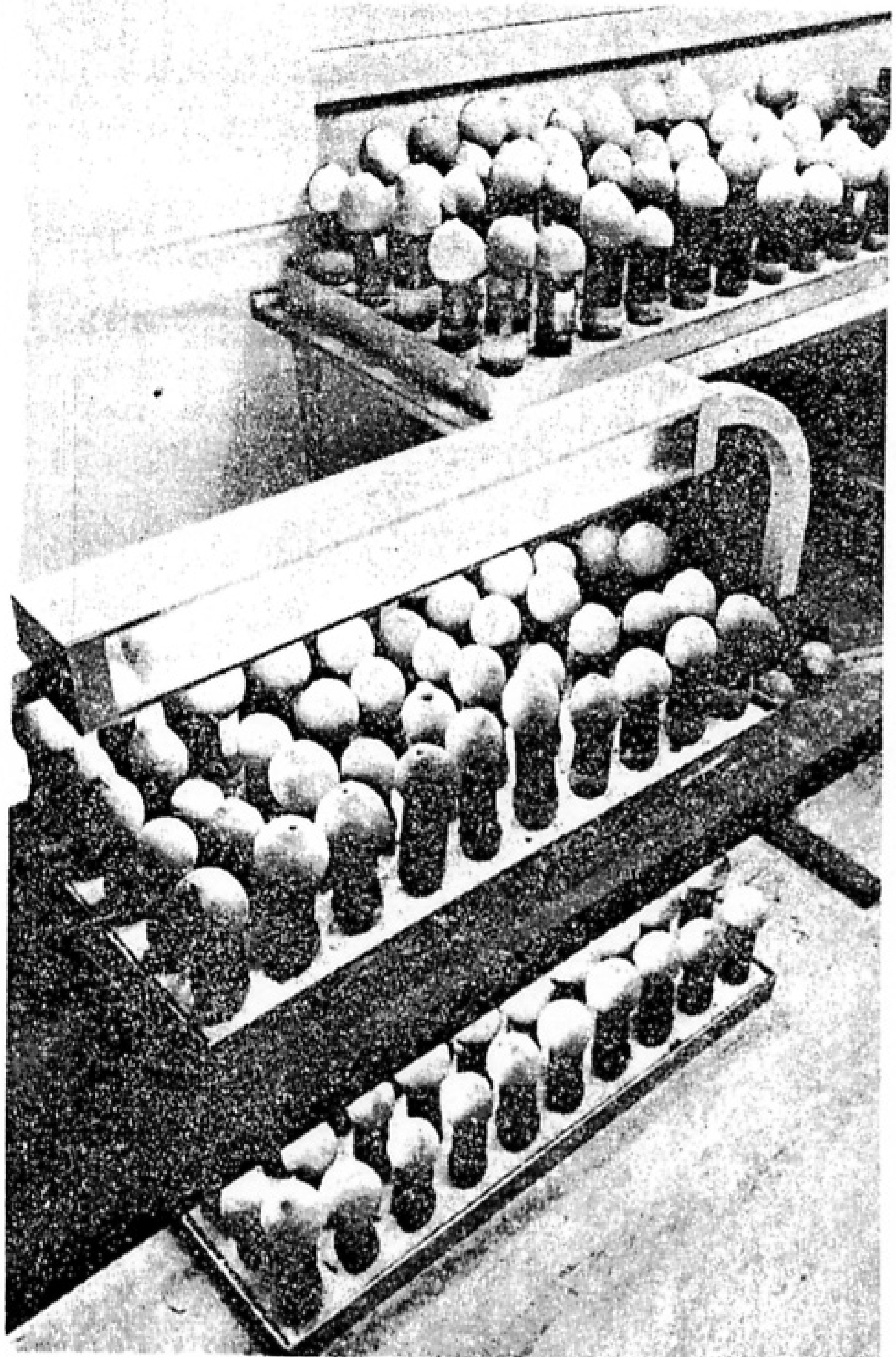
And here is where they are into the "livestock" business again, only they are growing the enemies of the red scale for this. Two tiny parasitic benefactors of mankind are hatched by the millions on stacks of banana squash in their lab.

One is Aphytis melinus, a tiny wasp that can land on a pinhead with space left over, and the other is Comperiella bifascista. Both do their destructive job on red scale by depositing their eggs inside the waxy shell of the pest as it clings to the citrus like a microscopic abalone. Their young finish the job as they hatch and feed on their unwilling hosts.

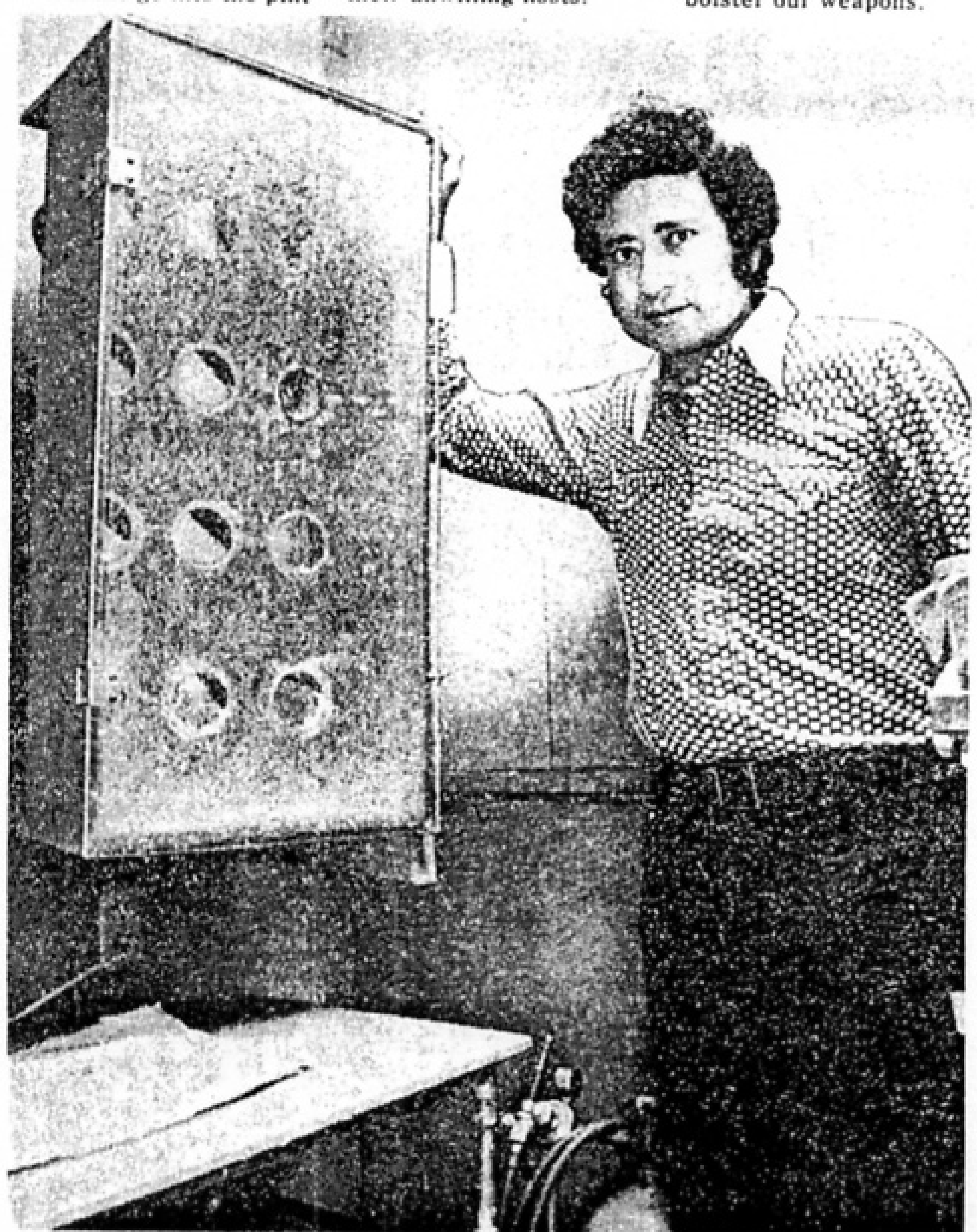
Both are released by thousands when the sticky cards on the white cylinder traps show heavy flights of brood ready males. These tiny destroyers can do a far better job of control than spray booms because they can get inside the tightest foliage to hunt out their prey. But Michael and Smith do not say the little hunters can entirely replace spray crews, although they do have one subscriber to their service who has gone two years without spraying.

They point out that just as no spray can do the control job alone, neither can their helpful little flying killers go it alone. Integration of the two, along with learning to live with the pest where it can be brought down to the point the trees' vigor can withstand moderate infestations, is the most satisfactory goal, say the combination bug breeders and killers.

They point out that their traps are beginning to level off and soon will show a decline of scale activity for a few months until the pre-Thanusgiving rebound occurs. Then they and their tiny helpers will be on the go again, proving how integrating chemicals with living helpers from the insect world can bolster our weapons.

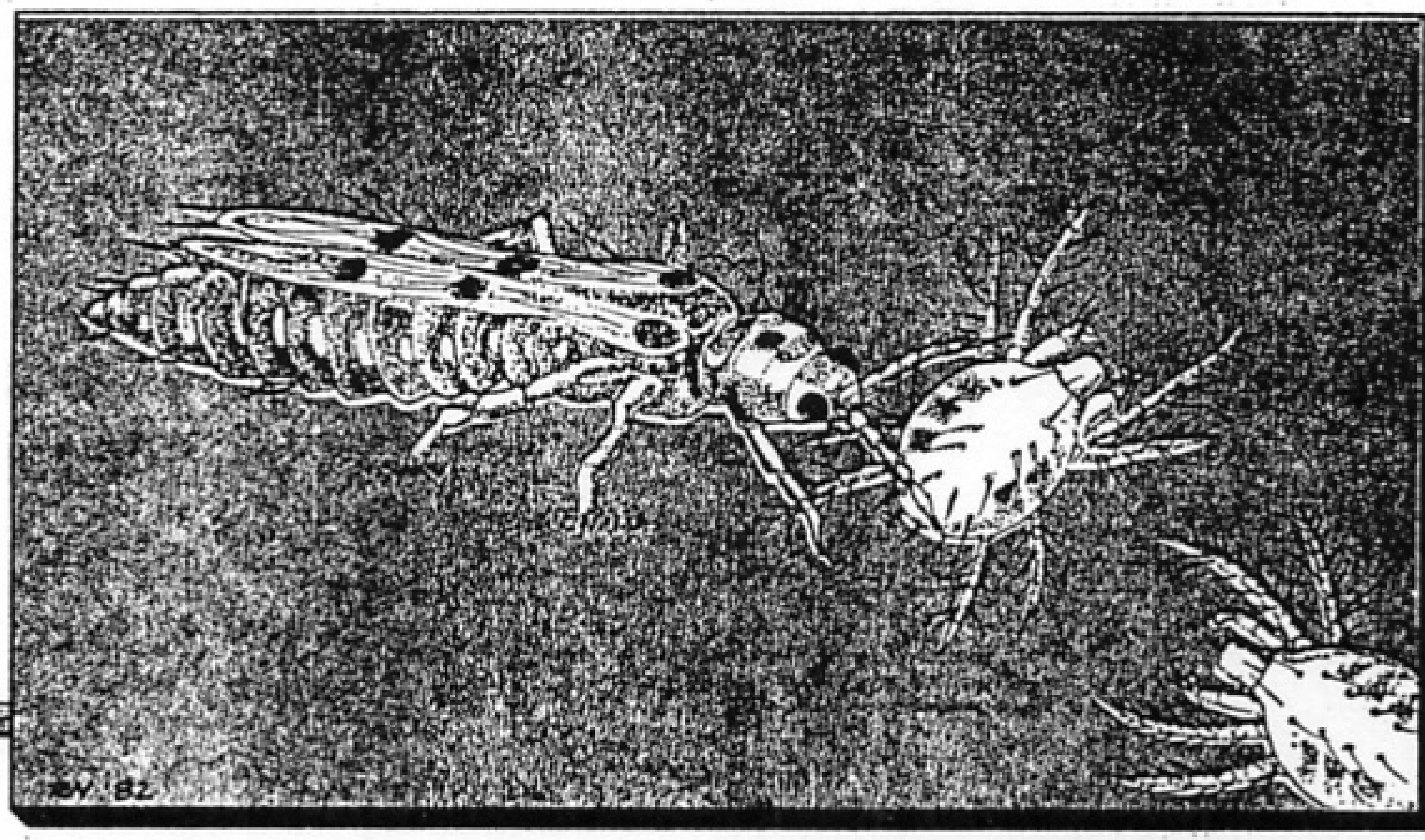


Green lemons bearing new colonies of red scale being grown for later use as lures in the sex pheromone traps are seen under special lights in the Michaels' insectary. A special wax is used to coat a large portion of the lemon to preserve it for the period needed to raise each generation of red scales. An antiseptic dip just before they are put in the traps kills the males, leaving behind only the virgin female red scale to attract the "wild" males in the grove and show the population level.



Ibrahim F. Michael (his name preserves the Egyptian form in the first name and uses the Anglicized form in his surname) checks out a cabinet used in growing new supplies of tuber moths.

Mites take beating in bio warfare



Six-spotted thrips are tigers when it comes to battling spider mites.

ı Andreas, a Delano-area
mer, takes a look at how
ıe of his "organic" raisins
drying. He had about 50
cent of his crop rolled when
rains hit early Friday
·ning.

Story and Photos
By RON GOBLE

For many years farmers have been caught-up in the seemingly endless chemical battle to control the prolific spider mite — a tiny pest which thrives in the hot, dry and dusty grape vineyards and almond orchards of the San Joaquin Valley.

The major weapon in the grower's arsenal has been a handful of potent and expensive chemicals. Now, however, there is an alternative.

With the costs of doing business getting higher and higher, farmers are looking for ways of saving on expenses. That is why the rules of the insect battle are changing. There is a different kind of war going on today.

This fight is between the pesky mites and a *natural* enemy, commonly known as the six-spotted thrips (Scolothrips sexmaculatus).

A number of almond growers are turning away from what they considered a routine chemical spray program, and are choosing to call in an army of six-spotted thrips instead.

Farmers spend thousands of dollars every year on chemicals in what has been a in many cases a losing battle against Pacific mite (Tetranychus pacificus) and two-spotted mite, (Tetranychus urticae). Each year the costs climb higher and the resistance of the pest increases.

These insect pests can cause extensive damage to trees and vines.

"Even with increased applications and costs, the mite has not been combated effectively. Mite populations are aggravated by sprays for other pests like navel orangeworms (NOW) in the almonds and leafhopper in the grapes," said Ibrahim F. Michael, an agricultural consultant who operates his own pest management firm based in Fresno.

Michael has developed special programs to control the mites by using cultural and biological means, leaving the chemicals as a "last resort" and then only on a spot treatment basis.

"I haven't sprayed my almond trees in 10 years," declared longtime Clovis almond grower, Frank Sorrenti. "We ran a test block with Dr. Michael way back then, and to be honest, I thought at the time it would be a big flop. But it wasn't. We did what he said and it worked.

"I haven't had to fire up a spray rig since then and that has saved us a lot in production costs over the years," Sorrenti declared.

Sorrenti is no newcomer to almond growing. His father, Robert, started in the business back in 1926. Today Frank farms 150 acres of almonds and some acreage of other crops too. In addition, Sorrenti has an almond hulling operation.

"Growers always give me their worst trees or vines to work on," Michael said with a grin. "But that doesn't matter as long as they are committed to the program."

Michael explained that it is tough on a grower to change his way of thinking. They see some mite activity in their orchard and begin to "get nervous" and want to go back to the old comfortable chemical approach.

"We start from ground zero and a grower must be

See Mite, Page F2

Ibrahim Michael is surrounded by bean plants for rearing beneficial six-spotted thrips used to fight mite.

gain foothold in San Joaquin Valley