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EFFICACY OF METHYL ANTHRANILATE AS A BIRD REPELLENT ON CHERRIES, BLUEBERRIES AND GRAPES

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ABSTRACT: Anthranilitic acid derivatives, used as common food additives, have been explored as bird repellent agents for a number of years. Research in this study show that methyl anthranilate, when exposed to the UV spectrum of sunlight, readily dissipates within 64 hours. The addition of surfactants and extenders did not appreciably alter the degradation curve, nor did they lessen the phytotoxic properties of the chemical.

Field trials under IR-4 guidance and support indicate that methyl anthranilate (MA) is an effective, biodegradable, nontoxic bird repellent. In formulation with a lipid molecular binding compound degradation of methyl anthranilate was extended from four to ten days. Phytotoxicity, at effective application rates, was eliminated. Damage to cherries was reduced 43% to 98% depending on cultivar, number of birds present, and crop loads when the treated crops were compared with untreated crops. Depredation of blueberries was reduced 65% and 99% for two varieties. Feeding on wine grapes was diminished 58% to 88%, depending on the affected vinifera. Tasters could not distinguish between treated and untreated fruit nor could certified graders find any reduction in fruit quality.

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INTRODUCTION

Dimethyl and methyl anthranilate have been used as food additives for a number of years. The orange-flower odor and slightly bitter, pungent taste have added their characteristic qualities to alcoholic and nonalcoholic beverages, ice creams, candy, baked goods, gelatins, drugs and chewing gum (Furia and Bellanca 1989) as well as perfumes (Bedoukian 1951). Research has also found that certain anthranilitic acid derivatives may be effective as bird repellents (Kare 1961). Most of the work has centered on incorporating derivatives into starch matrices for cattle feed (Mason et al. 1983, 85, 88, 91; Mason and Clark 1987; Bean and Mason 1987) and solvents (Askham and Fellman 1989; Avery 1989, 91). Extending these formulations to fruit production applications has produced limited results. The major problems with dimethyl anthranilate (DMA) and methyl anthranilate (MA) are that they are volatile nonsoluble phytotoxic compounds (Thomas 1984). Further that incorporation with solvents increases solubility and volatility while encapsulation reduces volatility and phytotoxicity. Starches, however, dissolve in aqueous solutions leaving anthranilates to precipitate in the medium thus limiting their use on agricultural crops.

These findings indicate that an alternative formulation was required to produce a viable repellent compound to control bird depredation on agricultural commodities. The following documents the rationale for and the results of a series of experiments used to develop an alternative anthranilate derivative formulation as an effective bird repellent.

PHOTODETERIORIOZATION

The examination of research indicated that anthranilates may be photosensitive. Since no work had been conducted on this aspect of these compounds a simple test was devised to 1) assess the effect of incandescent, fluorescent and ultraviolet light on MA, and 2) assess the effect of a light inhibitor when combined with MA on the photodegradation process.

Two formulations were prepared. Formula 1. = 90 ml of technical grade (99.9%) MA : 10 ml ethanol (ETOH) 95%. Formula 2. = 76.5 ml of MA: l0 ml ETOH: 13.5g P-Aminobenzoic Acid (PABA). One-hundred µ1 of formulas 1 and 2 were pipetted onto 27 labeled glass slides and placed approximately 1 m beneath 8, 100-watt incandescent bulbs, 4 high-output cool white fluorescent tubes and 1,30-watt ultraviolet (UV) tube (200-320 nanometers) in a light cabinet maintained at 29.5°C. Three slides from each formulation were removed from the chamber at 0.5, 1, 2, 4, 8, 16, 32 and 64 hours (h), placed into glass petri dishes and washed in 5 ml ETOH for 10 min. A 1-ml subsample was extracted from each petri dish and placed into a 2-ml glass gas chromatograph (GC) sampling vial, capped, enclosed in a light-proof box, and refrigerated at -2°C until injected into a Hewlett-Packard 5890 GC equipped with a flame-ionization detector and a model 3396A digital integrator (Askham and Fellman 1989).

Decomposition appears to begin 8-h after exposure with all light sources; roughly two-thirds of a day of sunlight. Approximately 50% remains after 16-h, 10% after 32-h and less than 1% after 64-h or 1-1/3, 2-2/3, and 5 days (d) of normal light exposure (Figure 1).

These data indicate that MA rapidly decomposes when exposed to incandescent, fluorescent and ultraviolet light sources, and that the PABA does little to inhibit MA's degradation under these artificial conditions. These data, however, do not identify which of the three light sources are responsible for the degradation process nor indicate the compound's be-

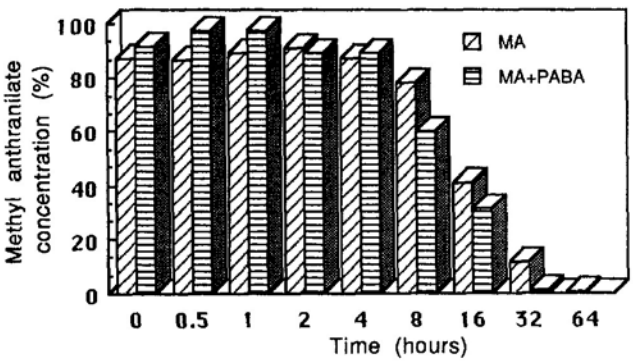

Figure 1. Effect of incandescent, fluorescent and ultraviolet light on methyl anthranilate (MA) and methyl anthranilate combined with P-Aminobenzoic Acid (MA+PABA).

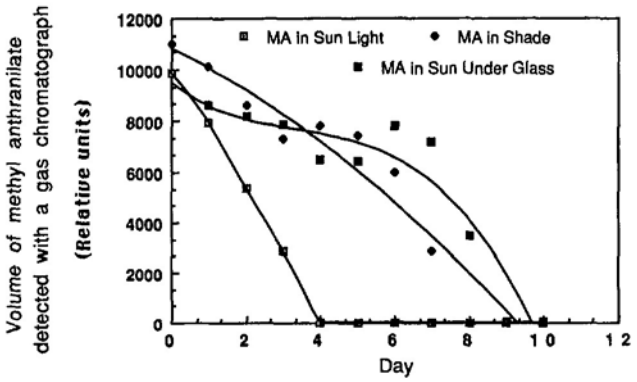

Figure 2. Amount of methyl anthranilate (MA) remaining at 24 hour intervals after being exposed to direct sunlight, placed in the shade and under glass.

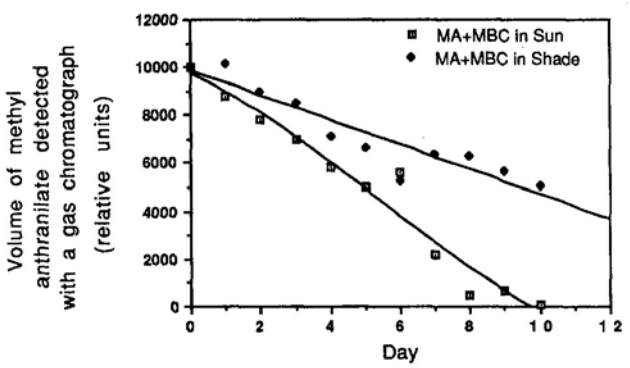

Figure 3. Amount of methyl anthranilate (MA) remaining at 24 hour intervals after being combined with a molecular binding compound (MBC) and exposed to direct sunlight and placed in the shade.

havior under ambient light and air conditions that might be encountered in an orchard, field or vineyard.

A second simple test was devised to assess the effect of natural sunlight on MA. Formula = 90 ml MA: 10 ml of methanol (METH) 95%. Ten ml of the formula was pipetted into 93 glass petri dishes. Three dishes were retained as controls (time = 0). The remainder were evenly divided and placed in direct sunlight, direct sunlight with a glass cover, and shade. Three dishes were removed from each treatment at 24-h intervals, processed and analyzed using the same procedure as used in the first series of trials.

Approximately 20% of the MA was lost during the first day, 50% by the second and 75% by the third. None was detected after the fourth day. When placed in the shade, approximately 50% of the methyl anthranilate was still detectable on the seventh day. When placed under glass approximately 25% of the materials was still detectable after the tenth day (Figure 2).

These results indicate that MA readily dissipates within four days when exposed to the full spectrum of sunlight. Filtering the sunlight with clear glass and placing the compound in the shade while leaving it exposed to ambient air temperatures and currents suggests that MA is sensitive to UV light. If MA had been sensitive to the remainder of the spectrum the differences found between the glass covered and shade covered samples would have been greater. These data also suggest that ambient air temperatures are sufficient to increase the compound's volatility. It is hypothesized that adding solvents to the compound only increase this volatility. The work is yet to be undertaken.

Further tests were devised to determine if the addition of a lipid molecular binding compound (MBC), a proprietary compound with a pending patent, affected the photodegradation and volatility of MA. Ten ml of the formulation was pipetted into 63 glass petri dishes. Three were retained as controls and the remainder placed in equal numbers either in direct sunlight and or shade. Samples were withdrawn at 24-h intervals, processed and evaluated as in the previous tests.

The Results show that approximately 50% of the MA combined with the MBC was detected after 5 d of exposure (Figure 3). Two days later this was reduced to 25%. On day 8, approximately 2% was detected. None was found by day ten. Over 50% was found when the combined compounds were placed in the shade after the tenth day.

Incorporating the MBC into the formulation appears to protect the MA from the UV light spectrum as well as reduce volatility. The MBC was also found to have several important effects on the MA. First, the MA did not return to its original solid state when temperatures were reduced below 82°F (28°C) or stored at -10°F (-23°C). Second, MA readily dispersed in water, did not precipitate in 24-h and formed an even film on any surface to which it was applied. Tangential to the experiment was the discovery that the MBC may have insecticidal properties. In early field trials more flying insects were noted on the treated trees than the untreated trees. In the studies using formula 1 (MA+ETOH), 42 fruit flies (*Chloropidae thaumatomya glabra*) were found adhering to the petri dishes. In the studies using formula 2 (MA+MBC) only 3 were found even though both experiments had been conducted under the same conditions and at the same location during the same time period.

PHYTOTOXICITY

Another simple test to evaluate the effects of MA+alcohol and MA+MBC on plant tissue phytotoxicity was developed to establish the relative concentrations at which foliar burn is produced. Cherries, blueberries, grapes and raspberries were selected for the study because past experience indicated each had a different MA tolerance level. Formula 1. = 90 ml MA: 10 ml ETOH. Formula 2. = MBC. Formula 3. = MA + MBC. Three sets of 8 treatments each were prepared by diluting each formula with water until 0.063, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0 and 8.0% (v/v) or 660, 1320, 2640, 5260, 10560, 21120, 43240 and 84489 ppm respectively of the active ingredient (MA) was obtained. The seven distal leaves of four branches from five plants of the four species were then immersed for 10 sec and allowed to drip dry. Observations, noting color change from "0" (no change) to "5" (severe discoloration or foliar burning) or other symptoms of morbidity were made at 10, 20 and 30 minutes and at 1, 6 and 24-h after treatment.

Discoloration and foliar burning (phytotoxicity) of raspberry and grape leaves with Formulation 1 was immediately evident with the 0.063% and 0.125% (v/v) concentrations. Phytotoxicity began to appear with the formulation to cherries and blueberries within 20 min after application. Higher concentrations appeared to accelerate the foliar burning process.

No adverse effects were noted with Formula 2. Phytotoxicity began to appear with Formulation 3 when greater than 2.0% concentration rates were applied to raspberries and 8.0% concen-

tration rates were applied to cherries, blueberries and grapes.

EFFICACY

Flight Pen Trials

Feeding trials with starlings (*Sturnus vulgaris*) following the study reported earlier (Askham and Fellman 1989) were repeated to validate earlier observations. Two choices, each containing treated and untreated cherries and blueberries were presented on trays or suspended on branches from wires in a large aviary. In each of the trials the birds stopped feeding on the samples treated with 0.25% ai (v/v) within 5 min of exposure. All of the untreated fruit was consumed within 24-h.

The data indicate that MA+MBC had the potential to be an effective repellent compound for agricultural crops. The questions that remained were 1) was MA+MBC an effective bird repellent under field conditions? and 2) what are the optimal time and treatment intervals at which the compound should be applied?

Field Evaluations

Early Ripening Cherries—A one-ac orchard of early ripening experimental varietals with a history of extensive bird depredation was selected for the experiment at the Washington State University Irrigated Agriculture Research and Extension Center (IAREC) in Prosser, Washington. Ten trees of experimental variety PC 7174-3 were selected. Four were randomly designated as the controls (untreated). Three were randomly assigned and treated once, 15-d prior to harvest. The remainder were treated twice, at 7-d intervals, prior to harvest. Pretreatment samples were collected from both sides of each tree between the rows at approximately 6 feet above ground level (AGL). Posttreatment samples were collected from mid-range (6 ft AGL) and from the tops of each tree. All of the whole and damaged fruit as well as stems were counted 12 inches inward along the branch from the most distal ripening cherry. Trees were individually treated with a hand held Solo, backpack, gasoline-powered sprayer with a 0.25% v/v (2640 ppm) solution of the bird repellent compound (MA+MBC). The number and species of birds feeding within the block were recorded for 1-h immediately prior to collecting both pre- and post-treatment samples.

Minor damage (1.1% to 1.5%) was noted on the early ripening variety research cherries two weeks prior to harvest and before the first application of the bird repellent compound. By harvest 19% of the crop had been removed or damaged in the untreated (control) trees (range = 7.6 - 41.5). In **a** single, 15-d treatment depredation was reduced 13% (range = 6 - 20.3). With two, 7-d treatments overall damage was reduced 54% (range = 2.8-15.4). Depredation in the tops of the trees was more than twice that found at mid-range (6 ft. AGL). The number of robins (*Turdus migratorius*) remained relatively constant throughout the treatment period (range = 12-36).

These data indicate that the compound was somewhat effective when applied once or twice, two weeks preharvest. When the trials began the birds had already habituated to the crop. Moreover, most of the trees in the block were left untreated (the bird's primary food sources) which continued to draw the birds to the site.

Bing Cherries—It was then theorized that introducing the treatments earlier and increasing the application frequency might improve efficacy. Ten mature Bing cherry trees were randomly selected from **a** 25 year old, 1-ac block at the IAREC. Three were randomly designated as controls, 4 for two 10-d treatments and 3 for four, 5-d treatments prior to harvest. Pre- and post-treatment samples, as well as bird counts, were collected and repellent applications made as described for the Early Ripening Varietal treatment.

No damage was recorded on the Bing cherries prior to the first application of the bird repellent. At harvest, 22-d later, depredation of the trees treated twice, at 10-d intervals, was reduced from 24.5% (controls) to 5.73% (74% reduction) and 0.56% for those treated at 5-d intervals (98% reduction). Depredation in the tops of the control trees was about 38% greater than found at mid-range. Damage in the tops of the trees treated at 10-d intervals was approximately 58% greater than at mid-range. Little significant difference in damage by location was recorded for the trees treated at 5-d intervals. The number of robins in the one acre orchard ranged between 12 and 36 throughout the trials.

These data indicate that the timing, as well as starting treatments when the crop begins to mature, are important in reducing bird damage to cherries. The crop must be protected with the repellent compound early to establish a negative sensory correlation between fruit quality and palatability and at regular intervals to maintain a protective cover.

Commercial Treatment—Tests were then designed to explore the use of commercial applications, as well as harvest techniques, in using and evaluating the repellent compound in orchards. Two 1-ac blocks of randomly planted Bing, Sam, Rainier, Chinook and Lambert cherries at the Washington State University Tree Fruit Research and Extension Center (TFRC) at Wenatchee, Washington were subdivided into two halves. One half was designated as the control (untreated). The remaining half was treated, at 7-d intervals, with a 0.25% ai (v/v) solution (2640 ppm) of MA+MBC and water with a 1964 Parker speed sprayer delivering 400 gallons per acre (gal/ac) at 200 pounds/square inch (psi). Four random samples of ripening fruit were collected, as described above, at 7-d intervals throughout the treatment period; two from 6 ft. AGL and two from the tops of each tree. Approximately 25 to 30 robins, starlings and Cedar Waxwings (*Bombycilla cedrorwri*) foraged throughout each block during the trials All of the Bing trees were hand-harvested from each treatment and block 7-d after the last bird repellent application. The remainder of trees were not harvested because of extensive winter fruit injury.

No damage was noted on the Bing cherries prior to or 7-d after the first application of the bird repellent compound. By the 14th day that damage had increased to 5.34%. At harvest 15-d later, the damage estimate had increased to 7.67%. Estimated damage for the treated trees ranged from 0.52%, at 14-d, to 4.45% by harvest Total harvested weight of the treated trees, however, was 43% greater than the untreated trees.

Several factors had an important bearing on the final outcome; weather, bird numbers and sampling procedure. Almost all of the cherry crop was destroyed in the state during the preceding December by a 58°F (32°C) drop in temperature in 36-h. The number of birds changed dramatically during the study. Around 200 were counted in the orchards prior to the first repellent treatment. Only one was seen two weeks later. At harvest about two dozen were feeding on the crop. No reason could be established for these changes.

Highbush Blueberries—The efficacy trials were then

shifted to a crop that had survived the winter well and had a long history of bird damage. In these trials six rows of Pemberton, Rubel and Jersey Highbush blueberries within a quarter-acre planting at the Washington State University Western Washington Research and Extension Center (WWREC) in Puyallup were selected for study. Each variety was divided into 2 equal units, control and treated, of 3 rows with 20 plants. Within each variety, eight plants were selected as controls; four netted and four unnetted. Four unnetted plants were left in the treated plot. Plant selection was made on the basis of uniform size (height and diameter), vigor, density and crop load. Black woven plastic ground cover (8 ft. x 8 ft.) were placed under each plant and secured with soil to receive any fallen berries.

Bird repellent treatments were made at 7-d intervals, starting when the fruit began to ripen, with a 0.25% ai (v/v) solution (2640 ppm) of MA+MBC and water with a tractor-drawn gasoline-driven four-nozzle shrouded sprayer that simultaneously treated both sides and the top of each row, delivering 60 gal/ac at 80 psi. The number and species of birds foraging within each block were observed and recorded for approximately 1-h prior to the application of each treatment and at each harvest. All of the fruit from each plant was hand harvested at 7-d intervals after the proceeding bird repellent application.

Total Pemberton crop production was reduced about 7 lbs (3200 g) per plant or 25% of the crop in the untreated plot. Crop production in the treated plot was reduced about 2.6 lbs (1200 g) per plant or about 9% of the total crop; a 63% reduction in damage. Total Jersey crop production was reduced about 3.3 lbs (1500 g) or 19% of the total crop for the untreated plants. A slight production gain of about 0.4 lbs (175 g) or 2.5% was recorded for the treated plants. Between 0.6% and 1.8% of the total crop was lost from ripe fruit falling (drop) from the plants. These data confirm that the MA+MBC compound is an effective bird repellent agent on blueberries when treatments start as the fruit begins to mature and is applied at regular 7-d intervals through the final harvest.

Wine Grapes—Six rows of Gewurtztraminer, Semillon and Limberger wine grapes, within a quarter-acre planting at the IAREC in Puyallup, were divided into 2 equal units, control and treated, of 3 rows with 40 vines. Pre- and post-treatment samples were collected by removing eight clusters of grapes, at predetermined intervals, from the middle row of each treatment and recording the number of undamaged, damaged and missing fruit.

One repellent treatment was made 12-d prior to harvest, with a 0.50% ai (v/v) solution (5280 ppm) of MA+MBC and water with a hand-held Solo, back-pack gasoline-powered sprayer that forced the repellent under the leaf canopy to coat the fruit. The number and species of birds foraging within each block were observed and recorded for approximately 1-h prior to the application of the first treatment and harvest.

The data indicate that the application of the bird repellent significantly reduced bird damage to the wine grapes. Bird damage in the untreated Gewurtztraminer grapes averaged about 21% (range = 8-35). Damage to the treated fruit was reduced to about 9% (range = 2-19) or a reduction of approximately 57%. Damage to Semillon grapes was decreased from a little less than 10% in the controls (range = 4-14) to about 4% in the treated rows (range = 0.5-5); a 62% reduction. Damage in the Limberger plot was decreased from 30% in the controls (range = 23-37) to about 5% in the treated vines (range = 4-12); a 80% reduction.

Application of a 0.5% ai MA+MBC formulation, twice the concentration of that used in previous trials, appears to be an effective bird repellent compound for grapes. An underlying concern for growers, however, is the potential for unwanted residues. Even though GC/MS data indicate that no residue is detected at harvest, there is still a potential that it can be detected by the wine consumer. The use of repellents on grapes may not be practical, however, until a mechanical application procedure can be developed. With current technology, sprays are applied to the outer foliage with tractor-drawn air blast sprayers. As the air reaches the leaves they flatten against the stems to form a protective layer over the fruit that pesticides can not penetrate. Bird repellents must be applied directly to the fruit to be effective.

RESIDUE

Earlier data indicate that little or no residues are detectable at harvest (Askham and Fellman 1989). Additional residue analysis for cherries was conducted in two stages. In the first stage 10 mature Bing cherries were removed from random locations within each tree immediately after the first treatment and at 24-h intervals for 10-d in the early maturing varietal research block. Each sample was placed in 10 ml of methanol and agitated for 1 min. A 1-ml sample of the extract was removed, sealed in a glass GC vial and stored under refrigeration at -1°C in a light-proof container. In stage 2, one-half Kg samples of fruit was removed from random locations within each tree immediately prior to harvest in the Bing orchard, sealed in polyethylene bags and stored at -1°C. Stems and pits were removed and l00g of the fruit was blended with 100 ml of methanol for 5 min, and extracted with a Buchner suction funnel. A 1-ml sample was extracted and stored in sealed glass GC vials as above.

Residue measurements were made with a Hewlett-Packard 5890/5970 GC/MSD using a 30-meter, DB5, open tubular capillary (250 micron I.D.) (J&W Scientific). Splitless injections of 1µl samples were made with an autosampler into an He carrier gas flowing at 1 ml/min. After 5 min. at 40°C, the oven temperature was ramped at 20°C/min to 250°C where it was held for 5 min.

Standards were prepared from MA stock supplied by the distributor (Bell Flavors and Fragrances). Serial dilutions of 25, 2.5, 0.25, 0.025 and 0.0025 ppm were prepared from a 250 ppm stock solution in MeOH. Blanks were inserted after each standard during the analysis to check for potential sample carryover. The lowest level of detection was 125 ppb.

In the second part of the residue analysis 500 g of Pemberton blueberries were removed from random locations from each plant immediately before the final harvest. Samples were sealed in polyethylene bags and stored at -1°C until 100 g of the fruit was blended with 100 ml of METH for 5 min, and extracted with a Buchner suction funnel. A 1 ml sample was extracted and stored as above until evaluated with the Hewlett-Packard GC/MSD.

In stage 1, in which the treated fruit was evaluated every day, residues gradually decreased in the cherries until none could be detected 7-d posttreatment. In Stage 2, when the cherries were evaluated at harvest, no residues were detected. No residues were detected in the blueberries.

FRUIT QUALITY

Grade

While no residues could be detected with scientific equipment there were still questions about the effect of the MA+MBC on fruit quality and taste. To find out if the physical quality of the fruit had been adversely affected by the formulation 2.25 Kg (5 lbs) samples of Bing cherries were removed from each harvested tree at the TFRC. All of the samples from each treatment were commingled (pooled) by variety, 100 individual fruits removed, and graded for defects, size, firmness, brightness and color by four Washington State Department of Agriculture (WSDA) inspectors.

No discoloration, desiccation or abnormalities were noted. Overall fruit quality was improved approximately 67%. Skin breaks and decay, found by the graders, was reduced 87% and 88%, respectively. Bruising, a function of handling procedures, was more serious in the treated crop. No consequential differences in the amount of fruit pitting was found between treated or untreated fruit

These results indicate that the MA+MBC treatment does not adversely affect cherry quality. It may actually improve overall quality by reducing the number of skin breaks and decay found in the fruit.

Taste

Taste tests were designed to find out if consumers could distinguish the difference between treated and untreated fruit. In this trial 2.25 Kg of fruit were randomly selected from each cherry and blueberry harvest, placed in polyethylene bags, and stored at -1°C for 24-h. Immediately before the taste panel trials, the fruit was removed from refrigeration, washed with cold tap water and the damaged, deformed and infected fruit removed. Twenty volunteer subjects were each presented with triangularly placed sets of six equal combinations of treated and untreated fruit selected from two varieties (Triangle Test; Meilgaard et al. 1987). Samples of each fruit were placed before each volunteer under a blue incandescent light. Each participant was told that two of the fruit were identical and one was different, asked to circle the number on the score sheet which corresponded with the odd sample on the plate, and record any observations.

The detection of MA residues in the harvested crops by volunteer tasters was not significantly consistent. One-half of the volunteers correctly identified the "odd" sample during the cherry taste trial; a 50/50 probability of detection. Less than half of the volunteers correctly identified the "odd" sample for either of the blueberry trials.

SUMMARY

Prior research has shown that anthranilitic acid derivatives, particularly dimethyl and methyl anthranilate, possess repellent properties that when incorporated in starches or combined with solvents can reduce bird depredation. The research reported here describes the results of added compounds that circumvent the compound's phytotoxic properties, volatility, and immiscibility while providing effective repellent coverage, without reducing fruit quality, within potential economic boundaries.

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