Population Dynamics of Postharvest Decay Antagonists Growing Epiphytically and Within Wounds on Grapefruit

Raymond G. McGuire

U.S. Department of Agriculture, Agricultural Research Service, 13601 Old Cutler Road, Miami, FL 33158.

Accepted for publication 18 July 2000.

ABSTRACT

McGuire, R. G. 2000. Population dynamics of postharvest decay antagonists growing epiphytically and within wounds on grapefruit. *Phytopathology* 90:1217-1223.

Coating formulations of shellac, sucrose ester, and cellulose were tested that support populations of bacterial and yeast antagonists of postharvest *Penicillium* decay of grapefruit. Surface populations of *Pseudomonas syringae*, *P. fluorescens*, and *P. putida* were stable between 10^3 and 10^4 CFU/cm² on shellacked fruit over 4 months at 13°C, but numbers of the yeast *Candida oleophila* rose steadily from 2.35×10^3 to nearly 10^5 . Through the first 15 days of cold storage, surface populations of *P. syringae* and *C. oleophila* were greater when applied to fruit within the pH 7.2 shellac than when applied by dipping fruit into an antagonist suspension and drying the fruit prior to shellacking; numbers were generally equivalent thereafter. Within wounds of shellacked fruit, populations

of *P. syringae* were equal regardless of the method of application and increased from 10^4 to more than 1.2×10^7 CFU within 2 weeks. In contrast, numbers of *C. oleophila* in wounds over the first 2 weeks of storage were greater in fruit dipped and coated with shellac than when the antagonist was incorporated in this material; populations stabilized at $\approx 6 \times 10^6$ CFU after 2 weeks. Incorporation of *P. syringae* and *C. oleophila* into ester and cellulose coatings, however, initially fostered greater numbers of both antagonists in wounds ($\approx 10^6$ CFU) than did the preliminary dip application, and significant population differences persisted for several weeks. In cold storage, although application method did not affect the efficacy of *P. syringae*, *C. oleophila* was more efficacious after 3 and 4 months when applied in the shellac than when applied by a preliminary immersion and subsequent drying of fruit prior to shellacking.

Additional keywords: citrus, green mold, *Penicillium digitatum*.

All citrus are susceptible to infection during postharvest storage by *Penicillium digitatum* Sacc. and *Penicillium italicum* Wehmer, which cause green mold and blue mold, respectively. Typically, these diseases are controlled with fungicides applied in fruit processing (10), but an alternative to chemical control is the use of antagonistic microorganisms that biologically control decay. Strains of the yeast *Candida oleophila* Montrocher and the bacterium *Pseudomonas syringae* pv. *syringae* van Hall are effective antagonists that control *Penicillium* decays on citrus by competition for nutrients (7,22-24). Aspire (Ecogen Inc., Langhorne, PA), a product developed from *C. oleophila*, and Bio-Save (EcoScience, Orlando, FL), developed from *P. syringae*, have been commercialized to control these decays.

Biological control of green mold on citrus is often variable due, in part, to fluctuations in environmental conditions that affect survival of the antagonist in application and postharvest storage (4,8,11,20). Both commercial products recommend application by spraying or dipping fruit prior to a coating process that produces a shine and reduces water loss (11). In this process, the antagonist suspension on the fruit surface must be dried at $\approx 54^\circ\text{C}$ before the fruit coating can be satisfactorily applied, then the coating must be dried at high temperatures. The heat of drying kills a percentage of the antagonist population, and chemicals in many commercial shellac coatings can also be lethal (14).

The coatings most often applied to citrus contain shellac, which is a purified product of the hardened resinous secretion of the

scale insect *Kerria lacca* (Kerr), a plant parasite found in India, Burma, and Thailand (13). These formulations normally consist of a mixture of water, shellac, morpholine, aqueous ammonia, and alcohol, with minor ingredients such as wood resin ester, carnauba wax, oleic acid, polyethylene glycol, antifoam, and fungicide, and their pH is often between 8 and 11 (9,10). Survival of yeast antagonists in the liquid shellac formulation can be significantly improved if the alcohol content is less than 6% and concentrations of morpholine and ammonia are minimized so pH is near neutral (17). Coating formulations based on cellulose or sucrose esters need not require alcohol or bases if shellac is not a component (2). Formulations of methylcellulose or hydroxypropyl cellulose maintain viable yeast indefinitely and, when applied to grapefruit (*Citrus paradisi* Macf.), support surface populations $\approx 10^6$ CFU/cm² (15,18). Coatings based on sucrose esters also support very high surface populations of yeast on grapefruit (16).

With greater numbers of yeast cells surviving the application stage, more are immediately available to colonize the fruit surface and superficial wounds. Generally, the antagonist must reach a surface density of at least 10^4 CFU/cm² for effective biological control (1), and this population must be present during the first 2 weeks of cold storage to effectively control the decay that results from the injuries grapefruit receive in postharvest processing (14). As fruit age in storage, susceptibility to decay increases, and spores from infected fruit can be blown throughout the storage chamber by air circulation, further accelerating decay. Biological control, therefore, should persist throughout the storage period.

Having developed coating formulations previously that support biological control by *C. oleophila*, the objective of this work was to test the suitability of these products for three *Pseudomonas* spp. with a proven or potential ability to antagonize postharvest citrus decay fungi. Additionally, survival of the yeast and bacterial antagonists on the fruit surface and in wounds on grapefruit was compared when biocontrol agents were either incorporated into the

Corresponding author: R. McGuire; E-mail address: miarm@ars-grin.gov

Mention of a trade name does not constitute recommendation by the USDA.

Publication no. P-2000-0814-02R

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 2000.

coatings or applied by dipping fruit before coating applications. In concomitant trials, the effects of these variables on fruit decay were evaluated.

MATERIALS AND METHODS

Citrus coating formulations. An experimental shellac formulation, MBZ4 (Mantrose-Hauser Co., Inc., Attleboro, MA), was the primary coating used in these tests. Although its constituents were typical of those in commercial shellacs used on most fresh citrus fruits in the United States, this shellac was specifically formulated to foster biological control (16). The formulation contained 20% bleached shellac (R-52; Mantrose-Hauser Co.), 2.4% morpholine, 1% oleic acid, and 1% propylene glycol in water sufficient to produce 1 kg; the resulting pH was 7.2. The shellac was compared with two commercial coatings: Citrosline (Agri-Coat Ind. Ltd., Great Shefford, England), a sucrose ester formulation with a pH of 7.3 containing <5% shellac neutralized with NaOH (specific concentrations are proprietary) and Nature Seal 2000 (EcoScience Corp., Worcester, MA), which was cellulose-based and had a pH of 3.2 (the exact formulation is also proprietary).

Culture of the biocontrol antagonists. Of primary interest was a comparison between the yeast *C. oleophila* and the bacterium *P. syringae*, although other bacteria were compared in some trials. A dried formulation of *C. oleophila* strain I-182 (NRRL Y-18846) (Ecogen Inc., Langhorne, PA), originally isolated from tomato by Wilson and Lindow (24), was cultured on malt yeast extract agar (MYEA) containing (per liter of water): 3 g of yeast extract, 3 g of malt extract, 5 g of peptone, 10 g of glucose, and 15 g of agar. This medium was made semiselective for *Candida* spp. by supplementation after autoclaving with streptomycin, cephalixin (Sigma Chemical Co., St. Louis), and chlorothalonil (2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile; Bravo 6F; ISK BioScience, Mentor, OH) at 40, 50, and 20 mg of active ingredient per liter, respectively (MYEA-scb). Strain ESC-10 of *P. syringae*, originally isolated from apple (25), was acquired from EcoScience and was cultured on Bacto Pseudomonas agar F (Difco Laboratories, Detroit) supplemented after autoclaving with cephalixin and cycloheximide (Sigma) at 50 and 100 mg/liter, respectively (PsA-cc). The antibiotics and their specific concentrations in MYEA-scb and PsA-cc were chosen because they had a negligible effect on the recovery of the intended species; recovery of *C. oleophila*

and *P. syringae* was 101.4 and 99.3% from MYEA-scb and PsA-cc, respectively, compared with unamended media. Although contamination was minimal on the two media, other white yeasts grew on MYEA-scb and other fluorescent pseudomonads grew on PsA-cc. The latter medium was also used to culture *P. fluorescens* strain CC-21 and *P. putida* strain K-8 that previously had been isolated in our laboratory from *Litchi chinensis* Sonn. as potential biocontrol agents against *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. Strain B-3 of *Bacillus subtilis*, an antagonist of postharvest decay of stone fruits (19), was provided by P. L. Pusey and cultured on nutrient agar (Difco Laboratories).

To test survival in the liquid formulations, suspensions of each *Pseudomonas* sp. prepared from 18-h cultures on PsA-cc were separately added in 0.1 ml of water at a concentration $\approx 1.2 \times 10^8$ CFU/ml to 5.9 ml of the three coating formulations, and aliquots were removed at 0, 0.5, 1, 2, 4, 9, 17, and 24 h. During this time, cultures were held under ambient conditions ($\approx 24^\circ\text{C}$) and thoroughly mixed prior to sampling. Samples (0.1 ml) of 10-fold serial dilutions were plated on PsA-cc, and colonies were counted after 5 days of incubation at 24°C . All tests were replicated four times.

Survival of yeast on the fruit surface and in surface wounds. 'Marsh' white grapefruit (size 36; 9 to 10 cm in diameter) were obtained in the 1997 to 1998 and 1998 to 1999 growing seasons from a commercial packing house in central Florida. These were received within 24 h of harvest as a mixture from trees in a local orchard and, except for size grading, were unprocessed by the packer. Upon receipt, all fruit were immediately washed in tap water containing 3.5 ppm of chlorine, and, unless otherwise stated, fruit were injured prior to washing with a puncture 4-mm deep by a nail 1.7 mm in diameter embedded in a cork stopper to assure equal depths of penetration. After washing and drying with forced unheated air, fruit were held overnight at 24°C prior to treatment.

During the 1997 to 1998 season, epiphytic populations and decay were evaluated. The three pseudomonads and the yeast were compared directly by incorporating them into the MBZ shellac coating at rates calculated to produce on the fruit surface 2×10^7 and 10^6 CFU/cm², respectively. The antagonists were cultured on the appropriate antibiotic-amended medium and suspended in tap water for incorporation into the fruit coatings. To calculate the coverage rate, ≈ 0.75 ml of shellac containing 4×10^8 CFU/ml of yeast or 8×10^9 CFU/ml of bacteria was applied per fruit with an average surface area of 300 cm². The shellac was spread on the fruit by hand within 30 min of the incorporation of the antagonist and was $\approx 98\%$ of its undiluted strength after addition of the antagonist suspension or water (control fruit).

Two additional treatments compared a second method of antagonist application; commercial formulations of *P. syringae* (Bio-Save 1000) and *C. oleophila* (Aspire) were separately suspended in tap water at label rates designed to produce concentrations approximately 5×10^8 and 1.5×10^8 CFU/ml, respectively. Suspensions were stirred for 15 min, then fruit were dipped in either suspension and agitated for 1 min; fruit were subsequently air dried before the shellac was applied. The three control treatments included uninjured fruit that received no decay control treatment and injured fruit that received either no treatment or were subsequently dipped for 1 min in 1,000 ppm of the fungicide Imazalil (Freshgard 700; FMC Corporation, Orlando, FL); all three were coated with the shellac. Fruit were held at room temperature for 15 h after treatment then placed in storage at 13°C and 95% relative humidity for 4 months. Each of the nine treatments consisted of four boxes of 36 fruit; three boxes were held to evaluate storage decay, and samples of fruit from the fourth box were periodically removed to measure surface populations of antagonists. Harvests in January, February, March, and April constituted four experimental replications.

Fig. 1. Survival of *Pseudomonas* spp. during 24 h at 24°C in shellac (MBZ4; solid lines) and sucrose ester (Citrosline; dashed lines) formulations. Means of three tests; mean LSD_{0.05} for *P. syringae* (○), *P. putida* (□), and *P. fluorescens* (Δ) = 0.09, 0.17, and 0.14, respectively.

Fruit were examined weekly for 2 weeks and then at biweekly intervals, and those showing signs or symptoms of decay were discarded. Decay losses were tallied monthly. On the same weekly then biweekly schedule, antagonist populations were determined over 4 months. From each treatment, two fruit were individually washed in Ziploc freezer bags of 18 × 20 cm for 1 h in 100 ml of 0.1 M phosphate buffer (pH 7.0) containing 0.1% peptone. One-tenth milliliter from 10-fold serial dilutions of the wash was cultured on PsA-cc or MYEA-scb as appropriate. After 5 days at 24°C, antagonist colonies were counted, and epiphytic populations were estimated based on the surface areas of the washed fruits calculated from their diameters.

The experimental design in the 1998 to 1999 season compared three fruit coating materials (MBZ shellac, cellulose of Nature Seal 2000, and sucrose ester of Citroshine), two antagonists (I-182 and ESC-10), and two methods of antagonist application (dipping fruit in a suspension of the antagonist followed by drying at ambient temperature and coating application or mixing the antagonist in the fruit coating prior to application and subsequent drying). Unprocessed fruit were washed and injured as described above prior to dipping and/or hand application of the fruit coating; subsequent handling and storage conditions remained the same as the previous year. In this case, however, each treatment consisted of only two boxes of fruit held in storage at 13°C, because only populations in wounds were monitored, not decay. There were five replications from November 1998 through April 1999.

Antagonist populations in wounds were examined over 2 months, first on a weekly schedule for 4 weeks and then at biweekly intervals. On each sampling day five fruit were selected from each treatment, and the wounded area (encircled at the time of injury) was excised to a depth of ≈6 mm with a brass cork borer 5 mm in diameter that was flame-sterilized between fruit. A piece of the peel that had not been injured was similarly removed from each fruit to allow compensation for antagonists that might be growing epiphytically. Individual sections of peel were placed in small plastic bags with 4.5 ml of 0.1 M phosphate buffer (pH 7.0) containing 0.1% peptone, and these were macerated for 6 min in a Seward Stomacher (Model 80; Thomas Scientific, Swedesboro, NJ) at high speed. Tests showed that this method of extraction of the antagonists did not significantly affect numbers; when yeast or bacteria at 4×10^5 CFU/ml were macerated for 6 min with a 6-mm core of peel (the oils from which were reported to be toxic) (5), recovery was 99.7 and 105.4%, respectively, compared with controls lacking the peel tissue. Samples (0.1 ml) from 10-fold serial dilutions of the macerate were plated on MYEA-scb or PsA-cc as appropriate. After 5 days at 24°C, all colonies of white yeast or fluorescent pseudomonads were counted, and populations in wounds were estimated.

Efficacy of biological control. The efficacy of biological control in cold storage was evaluated in the 1997 to 1998 season by recording the percentage of fruit decayed at monthly intervals.

Concurrently in both seasons, unwounded fruit were washed and set aside to evaluate treatment effects on green mold decay at room temperature. Initially, in the 1997 to 1998 season, only antagonists were compared using uncoated fruit. Twenty-five fruit for each treatment were placed in a single layer on tabletops and injured with the nail as previously described, and 40 µl of a bacterial species or *C. oleophila* (10^8 or 10^7 CFU/ml, respectively, in tap water) was applied to the wound. After 2 h, 20 µl of a suspension of *Penicillium digitatum* [10^4 CFU/ml, washed from cultures grown on potato dextrose agar (Difco)] was pipetted onto the wound. Controls with and without the pathogen were included. The following year, using 36 fruit per treatment, *P. syringae* and *C. oleophila* were compared when applied by either a preliminary dip before drying and coating with the MBZ shellac or incorporation into the shellac. Two injured control groups, either with or without addition of the pathogen, were included. Wounds in all treatments except one of the control groups subsequently

were inoculated 2 h after coating with the pathogen as previously described. In both tests, fruit were monitored at 24°C for 2 weeks to determine decay control. Replication was identical to that of the population studies previously described.

Data analysis. The survival of bacteria in liquid coating formulations was evaluated over 24 h, and data ($\log_{10}$ CFU/ml) were analyzed with SAS Proc Anova (SAS Institute, Cary, NC) as a split plot over time with the hour of sampling as a secondary effect. Data ($\log_{10}$ CFU/cm² or per wound) from population studies of fruit in cold storage were analyzed with SAS Proc Anova as a split plot over time with coating, antagonist, and method of antagonist application as main effects and sampling day as a secondary effect. Harvest dates were treated as experimental replicates because statistical analysis determined equal variances. The efficacy of biological control in cold storage and at room temperature was also analyzed with SAS Proc Anova, and means were separated according to LSD or the *t* test as appropriate.

RESULTS

Survival of antagonists within fruit coatings. The survival of pseudomonads suspended in coating formulations at an initial concentration of $\approx 2 \times 10^6$ CFU/ml was dependent upon the coating and the bacterial species (Fig. 1). Numbers of all three species

Fig. 2. Survival of *Candida* spp. and fluorescent pseudomonads on the surface of grapefruit in storage at 13°C for 4 months (means of four tests). **A.** Native populations on shellacked fruit. **B.** Populations of decay antagonists initially applied in a shellac coating.

increased slightly in the sucrose ester coating. Although numbers of *P. putida* in the shellac rose above 10^6 CFU/ml after an initial drop, populations of *P. syringae* and *P. fluorescens* decreased below 10^4 . In the cellulose-based coating that contained sorbic acid as a preservative, declining bacterial numbers remained above 10^5 CFU/ml at least for the initial 4 h (data not shown); after 24 h however, numbers of *P. putida* and *P. fluorescens* dropped to 10^3 CFU/ml, whereas *P. syringae* could not be recovered from the undiluted coating 17 h after inoculation.

Survival of antagonists on the surface of stored grapefruit.

Native populations of epiphytic fluorescent pseudomonads and white yeasts were low (Fig. 2A). Whereas numbers of pseudomonads on control fruit did not increase much above 10 CFU/cm², the population of yeasts rose steadily to 1,000 CFU/cm² over 4 months. When applied in shellac to grapefruit at 2×10^7 CFU/cm² (calculation based upon volume of coating required for the average-sized fruit of 300 cm² that did not take mortality into consideration), initial recovery of bacterial antagonists was closer to 10^5 CFU/cm² (Fig. 2B). Numbers dropped further by the second day to between 10^3 and 10^4 CFU/cm². Thereafter, populations of *P. syringae* and *P. fluorescens* were fairly stable, whereas *P. putida* decreased in numbers over 4 months. Of the three, *P. fluorescens* maintained the greatest number. Unlike the bacteria, the surface population of *C. oleophila* increased in size over 4 months from 2.35×10^3 (3.02×10^5 when sampled immediately after coating) to nearly 10^5 CFU/cm² (Fig. 2B).

For comparison with application of antagonists within the shellac formulation, *P. syringae* and *C. oleophila* were also applied by dipping fruit into a suspension of the antagonists prior to coating. An analysis of variance indicated significant differences ($P \leq 0.05$) between antagonists and the method of application but no significant interaction between the two. There was, however, an interaction between application method and sampling day. Numbers of both antagonists were initially greater on the fruit surface when these were incorporated in the shellac coating, and these differences persisted through day 15 (Fig. 3). From day 22 there were no differences in epiphytic numbers based on application method. Whereas the population of *P. syringae* remained between 10^3 and 10^4 CFU/cm², that of *C. oleophila* increased to levels more than 10 times greater after 4 months of cold storage.

Survival of antagonists within wounds of stored grapefruit.

Interactions between antagonist and method of application and between antagonist and fruit coating were significant ($P \leq 0.05$);

therefore, analyses of antagonists were separated. Numbers of *P. syringae* within wounds of fruit coated with shellac increased from 10^4 to more than 10^7 CFU per wound through day 22 regardless of the method of antagonist application (Fig. 4A). Populations then declined, but less rapidly in the portion that had been dipped. In the sucrose ester and cellulose treatments, however, populations of *P. syringae* in wounds were significantly greater following application of the antagonist within the fruit coating, at least through the first 3 weeks (Fig. 4B and C). Numbers were initially 10^6 CFU per wound in these cases, rising within a week to 10^7 , whereas numbers in wounds where the bacteria were applied by dipping were initially 0.001 or 0.04 of the corresponding value. Although in two of three cases, application of the bacteria within the fruit coating initially gave higher populations in wounds than when fruit were dipped, these high populations were not sustained past 2 or 3 weeks. In contrast, although wound populations were initially lower in dipped fruit, they steadily increased to numbers approximating those achieved by incorporation of the antagonist in the fruit coating.

Wound populations of *C. oleophila* in shellacked fruit were initially higher if the antagonist was applied by dipping the fruit (Fig. 4D). Thereafter, populations did not increase as quickly as was the case with *P. syringae*, but after the second week and through the remainder of the 2-month storage period the two treatments were equivalent regardless of application method. Although numbers of *C. oleophila* were initially greater than those of *P. syringae* and the population was more stable, the maximum number of yeasts recovered from wounds in this coating treatment never surpassed the number of bacteria. When the sucrose ester and cellulose formulations were used to coat fruit (Fig. 4E and F), greater numbers of *C. oleophila* were recovered from wounds when the antagonist was applied within the coating than by dipping, and this difference persisted up to 2 weeks; thereafter, populations were equivalent regardless of application method.

Efficacy of biological control. In the 1997 to 1998 season, uncoated fruit were used to compare the ability of three pseudomonads, a strain of *B. subtilis*, and the yeast *C. oleophila*, to biologically control green mold at room temperature. Although *P. putida* and *P. fluorescens* provided no control of *Penicillium digitatum* (Fig. 5), the commercial antagonists *P. syringae* and *C. oleophila* and the *Bacillus* spp. significantly reduced decay. Under laboratory conditions, the two commercial biocontrol antagonists provided equal results.

The following year, biocontrol of green mold by *P. syringae* and *C. oleophila* was further compared, again at room temperature when the antagonists were applied either within the shellac coating formulation or by dipping in a suspension prior to coating. Decay was greater the second year. An interaction between the antagonist and the method of application was significant ($P \leq 0.05$) and therefore analyses were done separately on each antagonist. At room temperature there was no difference in the percentage of fruit decayed when *P. syringae* was applied by dipping or applied within the shellac; results were 61 and 62%, respectively. However, with *C. oleophila* 45% decay after dipping in the antagonist was significantly lower than the 74% decay that resulted from the wax treatment. In the uninoculated control and the untreated control, 13 and 87% of the fruit decayed, respectively.

Fruit were also evaluated for decay during 4 months of storage at 13°C. There was a significant interaction ($P \leq 0.05$) between the antagonist and the method of application to shellacked fruit. The method of application of *P. syringae* did not affect decay during any period of time through 4 months (Fig. 6A). However, after 2 months of cold storage, application of *C. oleophila* within the fruit coating was more efficacious than the method of dipping fruit prior to coating (Fig. 6B). In this experiment, 78% of control fruit that were injured but received no type of decay control rotted within 4 months, whereas only 11% of imazalil-treated fruit decayed and 33% of uninjured control fruit rotted.

Fig. 3. Surface populations of *Candida oleophila* and *Pseudomonas syringae* on grapefruit in storage at 13°C when antagonists were applied either by incorporating them into the shellac coating or by dipping fruit in suspensions prior to coating (means of four tests).

DISCUSSION

Biological control of postharvest citrus decay with *C. oleophila* and *P. syringae* has met with limited success when applied in the packing environment (4,11). Superficial wounds must receive a high population of the biocontrol agent for successful competition

with, and control of, the pathogen, but a new report by Brown et al. (5) indicates that peel oil from freshly ruptured oil glands is toxic, at least to *C. oleophila*. Presumably adequate washing can minimize this problem, but harsh chemicals in fruit coatings and high drying temperatures certainly do not favor survival of decay antagonists. Coating a fruit after the application of a decay an-

Fig. 4. Populations in 13°C storage of A through C, *Pseudomonas syringae* and D through F, *Candida oleophila* in puncture wounds in the grapefruit peel as influenced by A and D, fruit coatings of shellac; B and E, sucrose ester; or C and F, cellulose (means of five tests).

tagonist and drying is thought to immobilize the agent beneath the film, but colonization beyond the wound has not been previously investigated. It was unknown whether subsequent wounds or areas of increased susceptibility would be colonized and whether attacks from fungal spores in cold storage might go unchallenged. This work demonstrates that antagonists applied by dipping prior to fruit coating do indeed colonize the fruit surface, but antagonists applied in a hospitable coating have a significant head start.

Although grapefruit are not generally stored beyond 4 or 5 months (21) other citrus such as lemons and some oranges are stored for periods up to 6 months, making storage infection a significant problem. Spores from infected fruit can be blown throughout a storeroom by ventilation systems. The fungicide imazalil controls sporulation of *Penicillium* spp. for a limited time but does not prevent infection in cold storage indefinitely. Currently, the treatment protocol advises that biological control may be enhanced with reduced levels of fungicide application (4,8,11). Using a hospitable coating to cover the entire fruit with a competitive yeast or bacterium might enhance biological control beyond the presently used techniques.

With significant modifications, shellac fruit coatings can be formulated to promote survival of biocontrol yeasts. When applied to grapefruit, some of these biological formulations prolong shelf-life as effectively as fungicide treatment (16,17), although biological control is more variable than chemical control of green mold (8). As demonstrated by this work, bacterial antagonists also survive in the modified formulations, but unlike the yeast they do not appear to increase in numbers on the fruit surface in cold storage. Bull et al. (6) also indicated minimal surface survival of *P. syringae* on citrus with numbers on uncoated fruit dropping from more than 10^6 CFU/cm² to less than 400 within 1 week. Coating fruit increases surface survival nearly 10-fold, and surface numbers approach a level that is minimally necessary for biological control, but it is not certain that these surface bacteria are active combatants in decay control or merely bystanders. Previous work (6) indicates biocontrol activity with these bacteria resides in wounded tissue.

Within wounds, however, *P. syringae* and *C. oleophila* both develop large populations that persist through weeks of cold storage. Numbers are dependent on the method of application of the antagonist and on the type of fruit coating. Although the MBZ4 shellac was made as benign as possible, it is generally less

supportive of both antagonists than the sucrose ester and cellulose formulations in Citrosine and Nature Seal 2000, respectively. An even greater number of antagonists are probably killed by the harsh solvents in commercial shellacs before fruit enter cold storage, regardless of whether the antagonists are applied before coating or in the coating formulation. Once the shellac has dried, however, and the solvents evaporated, growth of the antagonists is unaffected; populations increase to $\approx 10^7$ CFU per wound, although bacterial numbers are consistently higher than those of *C. oleophila*. Thus, there is no advantage in changing the commercial method when using shellac or applying either antagonist prior to coating. In contrast, the sucrose ester and cellulose products initially foster higher populations of *P. syringae* and *C. oleophila* within wounds when the antagonists are incorporated into the coating. Numbers may be higher because the cells are less likely to desiccate during the intervening postharvest treatments or constituents of the coatings may stabilize cell walls and membranes. Previous work with *C. oleophila* has demonstrated the advantages of these coatings for biological control (14–17).

Only one other report has compared the antagonists in Aspire and Bio-Save directly on citrus. On 'Sunburst' tangerines the two products provided similar results at 21°C (4). Results were also

Fig. 5. Green mold decay of uncoated grapefruit at room temperature 2 weeks following application of decay antagonists to puncture wounds in the peel (means of four tests). Bars with the same letter are not significantly different at $P \leq 0.05$ according to LSD.

Fig. 6. Green mold decay of grapefruit in 13°C storage as influenced by method of antagonist application: incorporation into a shellac fruit coating or dipping in a suspension prior to fruit coating (means of four tests). Pairs with the same letter are not significantly different at $P \leq 0.05$ according to the *t* test.

equivalent in trials of this study with uncoated grapefruit at 24°C. When the antagonists were applied by the standard method of dipping prior to drying and shellacking and stored at 13°C, no differences between the two were seen after 4 months. Only when the two were incorporated in a modified shellac during fruit coating and compared after 3 and 4 months was a distinction apparent. The greater efficacy of *C. oleophila* over extended periods of cold storage may result from its ability to reproduce and develop greater surface populations at 13°C than *P. syringae*. Surface populations are greater and biocontrol consistently is better when *C. oleophila* is added to cellulose and sucrose ester formulations (14,16).

These polysaccharide coatings may also better maintain the health of the fruit by promoting a more normal exchange of atmospheric gases. When compared with a Nature Seal formulation of cellulose, oranges coated with a commercial shellac had a lower level of internal O₂ and greater CO₂, with more ethanol and other compounds associated with anaerobiosis and off-flavor (3). A coating of sucrose ester with carboxymethyl cellulose prevented anaerobic respiration in apples and bananas but slowed ripening by preferentially restricting the movement of O₂ (12). The incorporation of biological antagonists in shellac-free coatings, such as those of sucrose ester or cellulose, may be especially useful when fruit such as lemons and Valencia oranges are stored for several months and reprocessed (and recoated) for sale. In storage, the resistance of *Penicillium* spp. to fungicides is becoming a difficult problem (4). When placed in the market, however, the citrus industry generally prefers a shiny coating that can most easily and economically be achieved with shellac (10). For fresh grapefruit and other fruit entering the market, an economic alternative to the current practice of dipping, drying, coating, and redrying for biological control that should be considered, is reformulation of shellac products to incorporate and foster growth of biocontrol antagonists.

ACKNOWLEDGMENTS

This work was supported through a Cooperative Research and Development Agreement (58-3K95-7552) between the Agricultural Research Service and Mantrose-Hauser Co., Inc., Attleboro, MA. I thank E. Schnell for technical assistance with these experiments.

LITERATURE CITED

- Andrews, J. H. 1992. Biological control in the phyllosphere. *Annu. Rev. Phytopathol.* 30:603-635.
- Baldwin, E. A., Nisperos-Carriedo, M. O., and Baker, R. A. 1995. Edible coatings for lightly processed fruits and vegetables. *HortScience* 30:35-38.
- Baldwin, E. A., Nisperos-Carriedo, M., Shaw, P. E., and Burns, J. K. 1995. Effect of coatings and prolonged storage conditions on fresh orange flavor volatiles, degrees brix, and ascorbic acid levels. *J. Agric. Food Chem.* 43:1321-1331.
- Brown, G. E., and Chambers, M. 1996. Evaluation of biological products for the control of postharvest diseases of Florida citrus. *Proc. Fla. State Hortic. Soc.* 109:278-282.
- Brown, G. E., Davis, C., and Chambers, M. 2000. Control of citrus green mold with Aspire is impacted by the type of injury. *Postharv. Biol. Technol.* 18:57-65.
- Bull, C. T., Stack, J. P., and Smilanick, J. L. 1997. *Pseudomonas syringae* strains ESC-10 and ESC-11 survive in wounds on citrus and control green and blue molds of citrus. *Biol. Control* 8:81-88.
- Droby, S., Chalutz, E., Wilson, C. L., and Wisniewski, M. 1989. Characterization of the biocontrol activity of *Debaryomyces hansenii* in the control of *Penicillium digitatum* on grapefruit. *Can. J. Microbiol.* 35:794-800.
- Droby, S., Cohen, L., Daus, A., Weiss, B., Horev, B., Chalutz, E., Katz, H., Keren-Tzur, M., and Shachnai, A. 1998. Commercial testing of Aspire: A yeast preparation for the biological control of postharvest decay of citrus. *Biol. Control* 12:97-101.
- Hagenmaier, R. D., and Baker, R. A. 1994. Wax microemulsions and emulsions as citrus coatings. *J. Agric. Food Chem.* 42:899-902.
- Hall, D. J. 1981. Innovations in citrus waxing: An overview. *Proc. Fla. State Hortic. Soc.* 94:258-263.
- Hofstein, R., Fridlender, B., Chalutz, E., and Droby, S. 1994. Large-scale production and pilot testing of biological control agents for postharvest diseases. Pages 89-100 in: *Biological Control of Postharvest Diseases: Theory and Practice*. M. E. Wisniewski and C. L. Wilson, eds. CRC Press, Boca Raton, FL.
- Kester, J. J., and Fennema, O. R. 1986. Edible films and coatings: A review. *Food Technol.* 40:47-59.
- Martin, J. 1982. Shellac. *Kirk-Othmer Encyclopedia. Chem. Technol.* 20:737-747.
- McGuire, R. G. 1994. Application of *Candida guilliermondii* in commercial citrus coatings for biocontrol of *Penicillium digitatum* on grapefruits. *Biol. Control* 4:1-7.
- McGuire, R. G., and Baldwin, E. A. 1994. Compositions of cellulose coatings affect populations of yeasts in the liquid formulation and on coated grapefruits. *Proc. Fla. State Hortic. Soc.* 107:293-297.
- McGuire, R. G., and Dimitroglou, D. A. 1999. Evaluation of shellac and sucrose ester fruit coating formulations that support biological control of post-harvest grapefruit decay. *Biocon. Sci. Technol.* 9:53-65.
- McGuire, R. G., and Hagenmaier, R. D. 1996. Shellac coatings for grapefruits that favor biological control of *Penicillium digitatum* by *Candida oleophila*. *Biol. Control* 7:100-106.
- Potjewijd, R., Nisperos, M. O., Burns, J. K., Parish, M., and Baldwin, E. A. 1995. Cellulose-based coatings as carriers for *Candida guilliermondii* and *Debaryomyces* sp. in reducing decay of oranges. *HortScience* 30:1417-1421.
- Pusey, P. L., Wilson, C. L., Hotchkiss, M. W., and Franklin, J. D. 1986. Compatibility of *Bacillus subtilis* for postharvest control of peach brown rot with commercial fruit waxes, dicloran, and cold-storage conditions. *Plant Dis.* 70:587-590.
- Roberts, R. G. 1994. Integrating biological control into postharvest disease management strategies. *HortScience* 29:758-762.
- Schiffmann-Nadel, M., Lattar, F. S., and Waks, J. 1971. The response of grapefruit to different storage temperatures. *J. Am. Soc. Hortic. Sci.* 96:87-90.
- Wilson, C. L., and Chalutz, E. 1989. Postharvest biological control of *Penicillium* rots of citrus with antagonistic yeasts and bacteria. *Sci. Hortic.* 40:105-112.
- Wilson, M., and Lindow, S. E. 1994. Coexistence among epiphytic bacterial populations mediated through nutritional resource partitioning. *Appl. Environ. Microbiol.* 60:4468-4477.
- Wilson, C. L., Wisniewski, M. E., Droby, S., and Chalutz, E. 1993. A selection strategy for isolating antagonists to control postharvest diseases of fruits and vegetables. *Sci. Hortic.* 53:183-189.
- Yourman, L. F., and Jeffers, S. N. 1994. Biological control of postharvest diseases of citrus with ESC-10 and ESC-11. (Abstr.) *Phytopathology* 84(suppl.):S1152.