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Application and Mode of Action of Plastic Film to Extend Life of Fruits and Vegetables

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c) Abstract

The mode of action of individual seal-packaging in delaying postharvest deterioration of lemons and bell-peppers was related to the alleviation of water stress. In fact, this technique appears as a practical way of applying water-saturated atmosphere to fruit securing its beneficial aspects and mitigating its disadvantages.

The interaction between decay, fungicides and individual seal-packaging was investigated. Although great variation was found, decay development would not risk utilizing all the possible benefits of this new technique for extending life of fruit. Several means were developed to reduce decay of sealed fruit, such as selecting the effective fungicide, or by incorporating the fungicide onto the film. The basic factors controlling innate and induced resistance to decay were investigated. Seal-packaging was demonstrated as a better substitute to waxing replacing all its functions of reducing weight loss, improving gloss and as a carrier for fungicides.

A combined treatment of ethylene releasing agents and seal-packaging enhances color development of several citrus cultivars at the early season. This treatment may result in less blemishes and better fruit quality as compared with conventional degreening with ethylene gas.

Seal-packaging extended life of bell-peppers and delayed its spoilage. Decay was drastically reduced by the combined application of sealing followed by disinfection with nitrogen trichloride.

d. Body of the report

The research results were summarized into the headings outlined in the research plan of the proposal with some additions due to expansion of the work in certain fields beyond the proposal, as in the case of effects of seal-packaging on gas exchange and decay control.

Mode of action of seal-packaging

The major field investigated was the elucidation of the mode of action by which seal-packaging exerted its effects. Control of deterioration of fruit by placing many fruit in a plastic bag was generally explained on the basis of the 'modified atmosphere', that is, the modification in the concentrations of CO_2 , O_2 , and ethylene (8). These factors also play their role in individual seal-packaging, but to a smaller extent. The increase in resistance to diffusion of these gases, brought about by the film, was counteracted by inhibition of the respiratory activity. This results in a new equilibrium inside the sealed fruit at which the level of these gases was not changed. Endogenous concentrations of O_2 , CO_2 , and ethylene were similar in both sealed and nonsealed citrus fruit, but the rate of their physiological deterioration was altogether different (1,7). The mode of action of delaying deterioration by individual seal-packaging in high density polyethylene film was investigated with lemon (Citrus limon (L.) Burm. f. cv Eureka) and bell pepper (Capsicum annuum L. cv Maor) fruits (1,7). Seal-packaging effects were related to the water saturated atmosphere in the sealed enclosure around the fruit. Softening of fruit was highly correlated with water potential of fruit.

Sealing drastically inhibited softening as well as chemical changes in cell-wall pectins. Sealing also delayed disintegration of membrane as shown by the inhibited leakage of amino acids, in particular, and electrolytes in general. All these effects of sealing were prevented or reduced by introducing hygroscopic CaCl_2 into the sealed enclosure which reduced the ambient humidity. Furthermore, some of these effects of sealing could be achieved also by maintaining nonsealed fruit in water-saturated atmosphere.

These observations conform with the findings of many studies that water stress affects tissue in the same manner as senescence (7,8). The mode of action, as well as the extent of the influence of seal-packaging, varied in different fruits: while lemons and peppers remained practically firm until they rotted, softening of tomatoes was only slightly inhibited (2,8). The ripening process in tomato prevailed over the sealing action. The increase in ethylene production accompanying the climacteric of the tomato raised the concentration in the sealed enclosure up to 1-2 ppm, which probably negated the inhibitory action of sealing. It was suggested accordingly (8) that water stress and its alleviation are more important for pepper and lemon, both nonclimacteric fruits than for tomato, in controlling deterioration. With tomato, and possibly other climacteric fruits, ethylene, O_2 and CO_2 play a more important role than water stress (8).

The involvement of water stress in the physiological deterioration of bell-peppers was studied in greater depth because the pepper was found to be a good model where effects of water stress on senescence could be investigated on fruit ^{of} several stages of physiological maturity without the climacteric intruding (3,11,13). It is hoped that the elucidation of the mode of action of water stress in hastening senescence may be of help in understanding the control of both processes in detached plant organs.

Accordingly, the physiological changes occurring in green and red bell pepper fruit before and after harvest were investigated. (3,11). Red pepper at harvest was less firm and had lower water potential, lower insoluble pectins and higher water-soluble pectins than green bell pepper. However, the changes occurring in both types of peppers after harvest were similar. Postharvest weight loss was accompanied by a decrease in firmness, decrease in water potential, as well as a decrease in insoluble pectin and an increase in soluble pectin in the cell wall. The production of CO_2 showed a slight decrease of ethylene and a significant but small increase after harvest. If the weight loss was prevented by holding pepper in a water-saturated atmosphere, or by seal-packaging, then all of these physiological changes were prevented or markedly slowed. It appears that in the case of bell pepper fruit, water stress hastens and possibly triggers the onset of senescence in harvested fruit.

In a later study by the same authors (13) the effect of water stress was investigated on permeability, microviscosity, and lipid composition of membrane prepared from bell pepper fruit. After harvest membrane permeability increased as measured by leakage of electrolytes and free amino acids. Membrane microviscosity increased between one and two weeks after harvest, and decreased afterwards. In red peppers the increase in microviscosity occurred sooner than in green peppers and the membrane-leakage was higher. Examination of microsomal membrane sterol and phospholipid composition revealed a correlation between sterol level and membrane microviscosity. During postharvest life sterol levels increased and then decreased, while phospholipid levels remained constant. Absciscic acid levels showed an increase after harvest. These changes are related to the fruit water potential and development of water stress in the harvested fruit.

In summary, our work supported the theory that we had proposed that the mode of action of seal-packaging relates to the alleviation of water stress which existed in harvested fruit.

Effects of individual seal-packaging on gas exchange of fruits

The mode of action by which individual seal-packaging was so much more effective in inhibiting transpiration without producing off flavors and dangerous internal atmosphere compared with waxing was investigated (12). The different effects which seal-packaging and waxing have on gas exchange cannot be easily explained by previous morphological studies concerning the distribution of applied waxes or by most current theories of gas mass transport in citrus and other fruits (8). Based on steady state data it was computed that waxing increases only slightly (20%) the resistance of fruit to water vapor, but raises the resistance to CO_2 , O_2 and ethylene by 140, 250 and 100% respectively. Conversely, seal-packaging was found to raise the resistance to water vapor by 830%, but that to CO_2 , O_2 and ethylene by only 20, 50 and 20% respectively. These great differences became more impressive considering that the plastic film is 10-20 μm in thickness and waxing produces a membrane with an average thickness of 1 μm . By utilizing a scanning electron microscope, it was found that waxing partially or completely plugs the stomatal pores (12). A theory was suggested that water vapor moves by different pathways than that of CO_2 , O_2 and ethylene in harvested fruits, this could explain several observations regarding gas exchange of various fruits. According to this theory, the stomata of harvested citrus fruit, though essentially closed, serve as the main pathway for CO_2 , O_2 and ethylene (12). Thus, plugging the residual stomatal opening with wax restricts greatly CO_2 , O_2 and ethylene transport.

Water moves preferentially by a different pathway through the cuticle in a path where water conductance is 60-fold greater than that of CO_2 , O_2 and ethylene. Evaporation of water at the outer cuticular surface creates a suction force which draws liquid water by mass flow from the epidermal cells through the liquid water phase of the cuticular membrane, to the air/water interface (12). Ethylene, O_2 and CO_2 are constrained from using this transport pathway because their diffusivity in water is 10,000-fold less from that in air (12). Waxing has low resistance to water because the new surface layer which is formed has many pits and breaks. The HDPE film is not selectively impermeable to water, but the film reduces water loss by 8.3-fold without substantially inhibiting gas exchange because initially the fruit has far less resistance to water than to O_2 , CO_2 and ethylene. Thus, the added film resistance to water became conspicuous, although the added resistance to the other gases is much less marked. Noteworthy is that waxing affects more significantly the resistance to CO_2 , O_2 and ethylene as the wax formulation liquid was flowing into the stomatal pores, thus plugging the major pathway for their transport, but not so, for the water transport which moves through the cuticle (12).

Reducing decay of sealed fruit

Objective No. 2 of this BARD project of "reducing decay of the sealed produce" was mainly handled by Prof. J.W. Eckert, the collaborating scientist. We also approached this problem (9) with a team reinforced with pathologists. First, the final report of Eckert would be presented.

Final Report

February 1, 1984

APPLICATION AND MODE OF ACTION OF PLASTIC
FILMS TO EXTEND LIFE OF FRUITS AND VEGETABLES

BARD No. F185-80

J. W. ECKERT

I. Effect of Shrink-Wrap Films on Decay of Lemons.

Surface-sterilized lemons were waxed with StaFresh 223 (FMC Corp.) and were wound-inoculated with Penicillium digitatum, P. italicum and Geotrichum candidum at spore dosages estimated (from preliminary experiments) to give 50% infection. One-half of the lemons were individually wrapped with a heat-shrinkable polyolefin film Cryovac D-950, 60 gage (15 μ m) using a Weldtron heat tunnel (Model 7121) set at 350^o F (177^o C). All fruit were incubated at 20^o C and approximately 90% relative humidity (R.H.) for 14 days. In separate experiments with three different isolates of P. digitatum used as inoculum, the level of infection in lots of wrapped fruits was 31, 38 and 43% lower than that in non-wrapped fruits (Table 1). Similar experiments with different isolates of P. italicum and G. candidum did not reveal significant differences in decay between wrapped and non-wrapped lemons. Lemon fruits (1800) were waxed with a water-based formulation containing 3 g/liter thiabendazole, but no 2,4-D. One-half of the fruit (nine replications of 100 lemons each) were wrapped in the shrink-film and all fruit were stored at 20^o C and 90% R.H. for sixteen weeks. Stem-end rot due to Alternaria sp. developed in 56% of the wrapped and 72% of the non-wrapped fruit, but the difference was not statistically significant at the 5% level. Weight loss during the 16-week storage period averaged 5% in the wrapped lemons and 28% in non-wrapped fruit.

II. Factors influencing the development of Sour Rot

The susceptibility of lemons to infection by Geotrichum candidum (Sour Rot) was investigated because this disease is very severe on ripe fruit, especially

those that are produced or stored in a high humidity environment, such as that created by wrapping fruit in a plastic film. Lemons were subjected to several environmental conditions and then inoculated at independent sites with several concentrations of arthrospores of G. candidum. The dosage-infection curve was analyzed by probit analysis and the susceptibility of the fruit expressed as an ED₅₀ value, i.e., the concentration of spores required for the development of active decay lesions at 50% of the inoculation sites.

A. Cultivars. Eureka and Lisbon are the lemon cultivars most extensively planted in California. Fruits of the intravarietal selections of Eureka (Allen and UCLA) and selections of Lisbon (Monroe and Limoneira) were harvested on three dates from a seaside grove and an inland valley grove. No consistent differences were found in susceptibility of the fruit from the cultivars and selections over the three sampling dates. The fruit from different sampling dates showed greater variation in susceptibility than the fruit from different cultivars and selections.

B. Effect of physiological age on susceptibility. Lemons are harvested in California when they attain a marketable size and may then range from green to yellow. They continue to undergo changes in color, texture, and other characteristics during subsequent storage. In this investigation, physiological age was assumed to be correlated with color and postharvest age. Yellow lemons were more susceptible to infection by G. candidum than greener fruit harvested from the same grove at the same time. The incubation period was shorter in yellow fruits (median of 37 hr in yellow fruit and 54 hr in light-green fruit), but the rate of lesion expansion was not significantly different in yellow and green fruits. The rate of lesion expansion differed somewhat among fruits from different groves, but was not correlated with fruit color or with susceptibility to infection. Susceptibility increased with storage time at 25°C; the addition of ethylene (20-50 µ l/L) to

the atmosphere hastened both color change and the increase in susceptibility. Susceptibility increased most rapidly during color change from light-green to yellow and more slowly thereafter. Fruit degreening was more rapid in an atmosphere containing 40-60% oxygen, but this environment slightly inhibited the increase in fruit susceptibility. Treatment of light-green lemons with 2,4-D (400 mg/L) retarded somewhat the rate of color change during storage at 13°C, but this treatment did not significantly delay the increase in susceptibility to infection.

C. Effect of weather and harvest time on susceptibility. Lemons were harvested 12 times from the same eight trees in a grove receiving water only from rainfall during that period (February-April). Fifty lemons were picked on each harvest date. Most lemons on the trees were yellow on the first sampling date and the few light-green fruits were removed and discarded. Lemons were picked at 8 a.m. and 2 p.m. on most dates and placed in polyethylene bags. The fruit were inoculated later the same day, usually with 10^5 , 10^6 , 10^7 and 10^8 spores/ml, each spore concentration at a different site on each fruit. Fruits harvested at 2 p.m. were 2-10 times more resistant than fruits picked at 8 a.m. Differences in susceptibility between lemons picked in the morning and in the afternoon were smaller on overcast than on sunny days. A significant drop in resistance was observed between February 28 and March 2 and between March 14 and March 21, both periods of substantial rainfall. Resistance to sour rot tended to increase during dry, sunny periods.

D. Effect of fruit water status on susceptibility. The xylem pressure potential of lemons picked at 2 p.m. on sunny sampling dates was about two bars lower than that of more susceptible fruit picked at 8 a.m. on the same day. The average water potential of the peel at 8 a.m. and 2 p.m. differed by only 0.7-1.5 bars. The standard deviation of the pressure potential and water potential

measurements was typically 1-1.5 bars. The relationship between the water status of the peel and sour rot susceptibility was studied further in three lots of lemons from a coastal area (Santa Barbara County, CA) and three lots of lemons from an inland area (Riverside County, CA). Sixty lemons from each lot were inoculated at four sites each by means of the inoculation pin. After 3-5 days, five of the most susceptible fruits (3-4 active lesions) and five of the most resistant fruits (0-1 active lesions) were selected from each lot and the water potential and water content of the peel were measured. The experiment was repeated with fruit from the same lots after storage at 12°C for three weeks. The peel of susceptible lemons had a higher water content than the peel of resistant lemons, but the peel water potential of the two groups was not significantly different. Coastal lemons had a significantly ($P < 0.05$) higher peel water potential and water content than inland fruit which was paralleled, in most of our tests, by a greater susceptibility to sour rot in the coastal fruits. The water potential and water content of the lemons decreased significantly during storage at 12°C for three weeks, whereas the average percentage active lesions increased from 45% to 59% during the same period. This indicates that for these fruits physiological age was a more important determinant of susceptibility than water status.

Lemons held at 25°C and 40% R.H. for 2-4 days lost 5-15% of their weight, whereas lemons with an attached stem immersed in water increased weight by 2-5%. A weight change of 1% corresponded to a change in peel water potential varying between 0.29 and 0.35 bars in three tests, and to a change in peel water content of 0.07-0.25%. Susceptibility to infection increased with water uptake by the fruit and decreased with water loss, even when injury to oil glands was avoided by inoculating carefully with a thin needle. This indicates that the difference was not due to a difference in likelihood of rupture of the oil glands. Water loss resulted in a significant ($P = 0.0001$), but small, decrease in the rate of

lesion expansion in one out of two tests and also resulted in a longer incubation period. For example, the median incubation periods after 1.4, 7.6 and 10.5% water loss were 40 hr, 56 hr and 61 hr, respectively. Injection of 1-2 ml of water into the albedo (spongy mesophyll) increased the susceptibility of the flooded area to infection by G. candidum when inoculated an hour later, even though the peel was not visibly water-soaked at the time of inoculation and appeared normal several days after the flooding treatment. Virtually every inoculation site, even on light-green lemons, that was continuously covered with a few drops of water for the first 24-48 hours after inoculation developed an active lesion. Lemons submerged in water for about 20 hr at room temperature became somewhat more susceptible to infection although they gained little weight. Lemons submerged for longer periods developed physiological breakdown of the peel (soft, water-soaked areas) similar to that resulting from storage at low oxygen levels. Areas of the peel so afflicted were extremely susceptible to infection by G. candidum.

E. The influence of preformed characteristics of the peel upon infection by G. candidum. Spore germination, fungal growth, and polygalacturonase liberation in peel wounds in resistant and susceptible lemons were similar for 10 hr after inoculation; the first differences were detected after 15 hr. Addition of nutrients to the inoculum increased its infectivity only slightly, whereas the addition of macerating enzyme boosted infectivity significantly when inoculated in relatively susceptible lemons. No evidence was found that preformed conditions in resistant lemons limited fungal growth and liberation of polygalacturonase. Flooding of intercellular spaces increased susceptibility strongly, probably enhancing the effectiveness of the macerating enzyme of the pathogen. Sterile macerating enzyme extracts injected into the albedo produced more maceration in yellow or turgid fruits, which are relatively susceptible to infection, than

in the more resistant, light-green or subturgid fruits. Albedo tissue strength was higher in light-green fruits and remained higher during the maceration process, even though the rate of tissue softening was slightly faster than in yellow fruits. Patterns of potassium leakage from host cells under the influence of macerating enzyme paralleled patterns of tissue strength.

F. Induced resistance in peel injuries to infection by *G. candidum*.

Injuries in the peel of lemons became progressively more resistant to infection by *Geotrichum candidum* when inoculated 4-20 hours after wounding. No significant accumulation of antifungal compounds was detected in two- or five-day-old arrested infections. The deposition of lignin-like substances around the inoculated tissue paralleled the development of resistance. Deposition was more rapid in the flavedo than in the albedo and was stimulated around inoculated wounds in comparison with sterile wounds. The lignified zones were not resistant to maceration when infiltrated with enzymes of the pathogen, but infiltration with water also overcame the resistance and activated arrested infections. Although the lignified zones may represent a defense mechanism against infection, they did not appear to be responsible for differences in resistance. The more resistant light-green or subturgid lemons actually produced less lignin after inoculation than the more susceptible yellow or turgid fruits. The greater intensity of lignification in susceptible fruit may be a response to greater fungal development.

III. Investigations on postharvest treatments to control fruit decay.

A. Fungicide treatments. The benzimidazole fungicides, thiabendazole and benomyl, have been widely used in commercial packinghouses for the past ten years to control fruit infection by *Penicillium* spp. and to inhibit fungus sporulation on diseased fruits. The full potential of the benzimidazole fungicides has not been realized in practice due to: 1) the proliferation of

benzimidazole-resistant strains of Penicillium following intensive use of these fungicides in commercial packinghouses; 2) the development of the benzimidazole-tolerant fungi, Geotrichum, Alternaria and Phytophthora on fruit treated with thiabendazole or benomyl; and 3) the difficulty in complying with the regulatory requirements of some importing countries with respect to benomyl residues on citrus fruits. The imidazole fungicides, imazalil, prochloraz and phenapronil, were very effective against benzimidazole-resistant isolates of Penicillium and were superior to thiabendazole and benomyl in inhibiting Penicillium sporulation. These compounds show some activity against Alternaria, but are ineffective against Geotrichum and Phytophthora. The triazole fungicides CGA 64250 (propiconazole) and CGA 64251 (etaconazole), were equal to the imidazoles against Penicillium and also controlled Geotrichum (Sour Rot), but not Phytophthora (Brown Rot). Guazatine and fenpropimorph reduced fruit infection by Penicillium and Geotrichum, but did not inhibit sporulation of Penicillium on diseased fruit. Metalaxyl prevented infection by Phytophthora if applied early in the incubation period, but had no effect on the other fruit pathogens. A postharvest treatment combining CGA 64251 and metalaxyl could control the major decays of citrus fruits during storage and marketing.

The effectiveness of imazalil-impregnated shrink-film in controlling P. digitatum was compared with a dip treatment of the same fungicide (500 mg imazalil/liter water). The scale of the test was limited by the small amount of impregnated film received from a European source. Each treatment was applied to three replications of 35 fruit each. The wraps reduced decay of lemons inoculated with P. digitatum from 90% in the untreated control to 53% in the wrapped fruits. Only 6% of the dip-treated fruit decayed. Experimental quantities of imazalil-impregnated film are now being produced in the U.S. and these investigations are continuing.

B. Derivatives of sec-butylamine as fungicides for use with plastic shrink wraps. sec-Butylamine (SBA, 2-aminobutane) is inhibitory to several fungi and controls certain postharvest diseases. This compound is used commercially to control Penicillium decay of citrus fruits and certain storage rots of potatoes. SBA is usually applied to fruits as an aqueous solution of a salt, although a fumigation treatment with the amine base may be advantageous for certain applications. Gaseous SBA is absorbed into the moist acidic injuries on the surface of citrus fruits, and the cationic form that accumulates inhibits the development of Penicillium spp. at these sites. The generation of ammonia gas and volatile aliphatic amines by alkaline hydrolysis of salts within fruit containers has been investigated as a means to control the decay of citrus fruits. The release of a fungicidal gas by hydrolysis of a salt has been difficult to control under practical conditions. We evaluated some azomethines $[R^1R^2C=N-CH(CH_3)C_2H_5]$, derived from sec-butylamine, as generators of SBA within fruit containers. These compounds appeared promising as progenitors of sec-butylamine, particularly as individual compounds of this class vary greatly in oil solubility, volatility and stability and could be applied in conjunction with plastic fruit wraps.

Imines, synthesised by the reaction of sec-butylamine with pentan-2-one, heptan-2-one, octan-2-one, undecan-2-one, benzaldehyde and 2-furaldehyde, were evaluated as vapor-phase treatments to prevent the infection of oranges by Penicillium digitatum. All the imines provided decay control equivalent to SBA when they were introduced into the fruit container at a rate equivalent to 16.4 mg SBA liter⁻¹ air volume. The carbonyl compound component of the imines, tested alone at a five-fold higher rate, did not reduce fruit decay. All the imines were stable in the gaseous state in dry air. The imines of pentan-2-one and undecan-2-one were hydrolyzed rapidly in a humid environment, indicating that their action against P. digitatum was due to the release of SBA into the atmosphere

around the fruit. The instability of the imines of the aliphatic ketones makes them unsuitable as generators of SBA. The imines derived from benzaldehyde and 2-furaldehyde were stable in moist air, but were hydrolyzed to a significant degree on moist filter paper, especially at pH 4.7. These aromatic imines were absorbed from the atmosphere by the fruit and were hydrolyzed rapidly in water, and in a buffer solution that represented the environment in fresh injuries on fruit. Apparently all the imines tested generated SBA in the fruit environment, and this SBA accumulated at potential infection sites, where it prevented the development of decay.

Imines derived from sec-butylamine and selected aromatic carbonyl compounds possess several attributes as candidates for the generation of SBA for decay control in packages: (a) the ease of preparation and chemical stability, especially if formulated in a hydrophobic matrix to prevent hydrolysis before volatilization; (b) the volatility is adjustable within limits by the choice of the carbonyl moiety and formulation ingredients; (c) the GRAS (generally recognised as safe) status of several possible aromatic carbonyl compounds, for instance, benzaldehyde and 2-furaldehyde.

C. Influence of storage temperature upon the effectiveness of sec-butylamine (SBA) against Penicillium decay. Postharvest fungistats such as thiabendazole, sodium o-phenylphenate (SOPP), and sec-butylamine (SBA) are not immediately lethal to fungus pathogens. Thus, the postharvest environment may influence the effectiveness of the treatment, since the pathogen and inhibitor must interact under these conditions. We observed that SBA was significantly more effective against Penicillium decay of lemons stored after treatment at 20°C than at 10°C, although decay in the untreated fruit was less at the lower temperatures. The incidence of decay in fruit stored at 20°C was inversely proportional to the SBA concentration over the range of 0-0.3% w/v; but these SBA treatments did not

influence the level of decay in fruits stored at 10°C (Fig. 1). The adverse effect of 10°C storage upon the effectiveness of SBA, and the decay-reducing effect of the colder storage temperature, was abolished when the treated fruit were held 12 hrs at 20°C before storage for 7 days at 10°C. The reduced effectiveness of SBA against *Penicillium* decay at the lower storage temperature appeared to be due to a lower fungitoxic action at 10°C resulting from a diminished uptake of SBA by the fungus at this temperature.

SBA (2.7 and 4.0 mM) inhibited radial growth of *P. digitatum* on PDA greatly at 20°C but only slightly at 10°C (Fig. 2). SBA (1 mM) inhibited growth of *P. digitatum* over ten hours in orange juice medium at 20, 15 and 10°C by 60%, 35% and 10%, respectively. The growth of *P. digitatum* in a defined liquid medium after 8 hours at 20°C was double that at 10°C. SBA (1 mM) inhibited growth of the fungus 50% at 20°C, but had no significant effect on growth at the lower temperature. The fungus accumulated SBA more rapidly at 20°C than at 10°C (Fig. 3). After 2 hours incubation, the intracellular concentration was 18 µmoles/gram at 20°C and only 4 µmoles/gram at 10°C.

D. Detection and management of fungicide-resistant strains of *P. digitatum* and *P. italicum*. *Penicillium digitatum* and *P. italicum* incite green mold and blue mold of citrus, respectively. Epidemiological studies of these diseases, especially those utilizing culture media exposed to the atmosphere, have been impeded by contaminating microorganisms that over-grow or inhibit the development of *Penicillium* colonies making it difficult to identify and enumerate the latter. This problem is especially troublesome in citrus groves and packinghouses where the number of propagules of contaminating microorganisms may be large relative to the spores of *P. digitatum* and *P. italicum*.

Media varying in nutrients, pH, and inhibitory compounds were evaluated for selective development of colonies of *P. digitatum* and suppression of fungal

contaminants in plates exposed to the atmosphere of citrus groves and packing-houses. Thirteen species of common air-borne fungi were replica-plated to the suppressive media at 25°C. o-Phenylanisole (OPA) and 2,3,4-6 tetrachloroanisole (TCA) were the most selective agents evaluated when added to potato dextrose agar enriched with 2 g/l each of peptone and yeast extract (PDAPYE), while pentachloronitrobenzene (PCNB) and acidification to pH 3.5 were less effective. The PDAPYE medium (pH 5.5) amended with 100 µg/ml OPA and 500 µg/ml PCNB restricted colony size of P. digitatum and greatly reduced contamination for more efficient enumeration of air-borne spores of this pathogen. The medium may be amended with 1 µg/ml carbendazim and 20 µg/ml sec-butylamine for the purpose of enumerating fungicide-resistant spores in packinghouses with minimum interference by non-pathogenic contaminants. These concentrations are well above that found completely inhibitory to sensitive races of P. digitatum, and allow only those spores retaining a relatively high level of fungicide resistance to grow. For the detection of o-phenylphenate resistant spores, no selective amendments are required (other than 100 µg/ml each of penicillin G and chloramphenicol) because the broad-spectrum action of o-phenylphenate effectively suppresses contaminating fungi, thus allowing only spores with a relatively high level of resistance to grow.

Strategies for control of thiabendazole-resistant strains of blue and green mold (Penicillium italicum and P. digitatum) were evaluated in three lemon packinghouses in central California. Packinghouse layout and sanitation significantly influenced the size of the population of thiabendazole-resistant spores and the level of decay in lemons shipped to East Coast markets. Effective strategies involved isolation of sources of fungicide-resistant strains, disinfestation of contaminated equipment and containers, and a program for monitoring the level of fungicide resistance in the packinghouse.

Table 1. Effect of shrink-film wraps on the infection of lemons by Penicillium digitatum.

P. digitatum isolate	% decay at 14 days ^a	
	Non-wrapped	Wrapped ^b
M6R	65	37
YUMA	79	49
CCHI	81	56

^a Each value is the mean of 3 replications of 100 fruit each.

^b The differences between wrapped fruit and non-wrapped fruit are statistically significant (5% level) in all cases.

Figure 1. Effect of sec-butylamine (SBA) treatment and subsequent storage temperature on the decay of lemons by *P. digitatum*. The inoculated fruit were dipped one minute in SBA solutions and decay was evaluated after 7 days storage at the indicated temperatures.

Figure 2. Growth of *Penicillium digitatum* on potato dextrose agar containing 200 $\mu\text{g/ml}$ (2.7 mM) and 300 $\mu\text{g/ml}$ (4.0 mM) sec-butylamine. The plates were incubated at 20°C and 10°C for 4 and 8 days, respectively.

Figure 3. Uptake of sec-butylamine by *P. digitatum* germlings in a sucrose-asparagine-salts medium, pH 4.5, containing 1 mM sec-butylamine.

Interactions between decay control, distribution and persistence of imazalil in Shamouti oranges individually seal-packaged in plastic films.

Seal-packaging presents a practical way of providing water-saturated atmosphere to individual fruit securing its beneficial aspects such as alleviating water stress and mitigating its disadvantages such as almost eliminating the condensation of water droplets. The pathogens, particularly dormant ones, also enjoyed the humid atmosphere, and generally developed faster. Consequently, the balance between host and pathogens may at times be in favor of the pathogen and the percentage of decay rises. For this reason, adequate decay control of sealed fruit is of paramount importance because sealing results in longer fruit life and better conditions for some of the pathogens (5,9). Seal-packaging produces a microatmosphere which can be enriched with a suitable volatile fungicide so that the sealed enclosure forms a fumigation chamber to control decay over a longer period. Recently, several new films were produced that contain fungicides (9). This fungicide-containing film continuously emitted an antifungal material that was able to reduce decay. Thus, imazalil-containing film prevented development of Penicillium digitatum and inhibited markedly that of Diplodia natalensis and Alternaria citri in petri dishes. Imazalil could exert its action through the vapor phase. Imazalil-containing film, $2000 \mu\text{g g}^{-1}$, reduced markedly decay of 'Shamouti' oranges inoculated with P. digitatum if the sealing was applied immediately following the inoculation and only slightly if sealing was delayed 24 h. Disinfection with sodium orthophenyl phenate combined with sealing by imazalil-containing film reduced decay markedly even if applied 24 hours after inoculation. Thus, the basic disinfection of fruit is essential as the first step in the packing house routine (9).

The disinfectant, applied as a liquid, flows all over the surface to bring rapidly into the injured spots the fungicide. The injured spots absorbed higher levels of residues than non-injured tissues. Possible, this advantage of liquid disinfectant of immediate loading of the fungicide onto the sites where it is needed is hard to match with the film that contains the fungicide. It was found that imazalil-containing film, or water application of imazalil followed by seal-packaging, gave better results than the application of imazalil in the wax (9). Thus, seal-packaging may serve as a better substitute to waxing, replacing all its functions of reducing weight loss, improving gloss and as a vector for fungicides. The imazalil-containing film was shown also to be able to reduce decay by continuously releasing the fungicide without loading the fruit with excessive residues. This lower level of toxic residues presents a great advantage considering the aspect of health and the current world trend demanding to lower the toxic residues in food. Possibly the continuous emission of fungicide may enable maintaining effective antifungal atmosphere with lower level of residues. It was found in this work (9) that sealing did not change the efficiency of fungicides in controlling decay of inoculated Shamouti. This observation would be explained by the counteracting effects of the sealing. On the one hand, the water-saturated atmosphere of the sealed enclosure enhanced the development of the pathogen and on the other hand, sealing enhanced the penetration and persistence of the fungicide as compared with waxing and delayed the senescence of the fruit (9). In his section Eckert also showed the marked effect temperature had on host, pathogen and development of decay. The final result on the decay depends on the interaction of all these factors. This explains the great variation observed on the decay response of different cultivars and pathogens to seal-packaging.

New degreening procedure

Seal-packaging might also be utilized advantageously in the degreening process, i.e., the treatment whereby the formation of the desired color in fruit is accelerated, generally by ethylene gas (5,10). Ethylene-releasing agents such as ethephon may be applied prior to seal-packaging with high density polyethylene (HDPE). The sealed enclosure enables the released ethylene to accumulate and reach a level suitable for accelerating the degreening process. This new method could be applied to lemons and other sensitive citrus fruits. Conventional degreening of these fruits by ethylene gas aggravates the development of various blemishes such as oölcellosis and red blotch, resulting at times in the loss of more than half the fruit. Treatment with ethylene-releasing agents by itself accelerates the development of color but also incurs the risk of a high incidence of blemishes. Seal-packaging of fruit in HDPE film markedly inhibited the development of blemishes in both degreened and non-degreened fruit. The combination of seal-packaging with either method of degreening accelerated the color of lemons and other citrus fruits with much fewer blemishes.

A preharvest orchard spray of the ethylene-releasing agents (ERA) (2-chloroethyl) phosphonic acid (ethephon), and 2-chloroethyl-tris (2'-methoxyethoxy) silane (Alsol) was tried. This application has not been successful as a degreening treatment, because of the risk of excessive defoliation with ethephon and phytotoxicity of Alsol, the inhibition of color development of attached fruit by the tree, and by the waxing treatment in the packinghouse. Combined preharvest application of ethephon with various auxins resulted in less defoliation, but many times also inhibited the effect of ethephon in accelerating coloration.

A postharvest application of ERA followed by seal-packaging of the fruit in film of high-density polyethylene, 10 μ m in thickness, gave adequate coloration within 2 weeks to the following cultivars: Shamouti orange, Marsh grapefruit, Michal and Clementine tangerine, Eureka lemon, and Satsuma mandarin. ERA was applied either to the fruit or to the plastic film. Ethylene was released into the micro-atmosphere enclosed with HDPE film to produce an individual microdegreening chamber for each fruit. The major effect of the HDPE was to delay deterioration and to maintain the quality of the fruit. Since sealing could also replace the wax treatment that is applied in the packinghouse, it would also remove the inhibition of coloration exerted by waxing.

Extending shelf-life and reducing spoilage of bell-peppers

Since bell-peppers responded favorably to both individual seal-packaging and to water-saturated atmosphere more efforts were devoted (4,14,15,16) to this relatively important crop, both in Israel, (about 10,000 ton exports annually) and in the USA (about 273 thousand ton production in 1980).

Individual seal-packaging in 10 μ m HDPE film prevented softening and markedly inhibited deterioration of bell-pepper fruit kept for a month at either 17°C or 8°C following by 4 days of shelf-life at 17°C. the non-sealed fruit softened and became unsaleable at 17°C and 85% RH after a week and at 8°C became resilient after 3 weeks; after an additional 3 days it became mostly soft and partly shrunken.

The flavor of sealed pepper was normal throughout the whole month at both conditions, whereas that of nonsealed fruit showed signs of aging. Fumigation with nitrogen trichloride reduced decay by more than half and acted synergistically with seal-packaging; the sealing prevented secondary

infestation and reduced the phytotoxicity of the nitrogen trichloride. Decay percentage was similar under the two temperatures. Sealing by itself somewhat reduced decay of red fruit because of its action in delaying senescence. Thus sealed fruit at 17°C kept better than nonsealed fruit at 8°C with additional shelf life.

During the winter of 1983 sealed peppers were shipped with three different plastic films, with and without cooling (16). Additionally, two different disinfectants were tried. The produce was examined upon arrival at Frankfurt (W. Germany) and after 4 days of shelf-life. All the treatments were duplicated, and there was an imitation shipment which was carried out in the Dept. of Fruit and Vegetable Storage in Rehovot. The fruit was examined weekly for 3 weeks and after shelf-life.

Seal-packaging in high-density polyethylene (HDPE) maintains the quality both with and without cooling. Seal-packaging of the pepper in biaxially oriented polypropylene (BPP) film, 20 µm thick, inhibited gas exchange, respiratory activity and the passage of the volatile disinfectants, and also caused some damage to the fruit upon its shrinkage.

A new automatic horizontal sealing machine of FMC was tested. Due to technical flaws the fruit was damaged, resulting in a higher percentage of decay than with manual sealing.

Disinfection by either fumigation with NCl_3 (Decco) or by a dip in imazalil, reduced the percentage of molds significantly. Fruit brokers in Europe suggested that the film utilized for sealing should be more transparent than HDPE and similar to BPP. Promotion is needed in order to apply this new technique commercially.

The objective of the research plan of this project of a comparative study of the effects of various plastic films was carried out mainly with bell-peppers (2,5,15,16). High density polyethylene gave the best results from both physiological as well as pathological aspects but it has poor gloss and transparency. Polypropylene, biaxially oriented product of E.I. Dupont de Nemours (CLysar EHC50) and of Saifan Saad, Israel had acceptable gloss and transparency but it was not permeable enough to the fungicide NCl_3 . Thus fruit sealed in polypropylene did not give as good decay control after NCl_3 fumigation as did non-sealed fruit or fruit sealed in high density polyethylene. Low density polyethylene was not machinable and so was the plastic film of Esso Chem. Polyvinyl film was not suitable because of being too permeable to water vapor. The P-950 film of W.R. Grace Cryovac was obtained beyond this report and would not be reported here.

Finally, responding to the request of the editor, a feature article of seal-packaging was written for HortScience (8).

e. Description of cooperation

Throughout the time span of this project the participating scientists visited one another twice. (only once, this BARD supported their visit). During the first visit, Prof. Eckert visited me and acquainted himself with the state of the art of this technique. Mr. Boris Shapiro who worked on this project in Israel also visited Eckert with partial support of this BARD. These visits enabled faster exchange of information, better planning of experiments and analysis of data. The research plan was accomplished and even was extended in both laboratories into different but related fields which led into new BARD proposal called: "Application and mode of action of water-saturated atmosphere in extending life of fruits and vegetables".

f. Benefit to Agriculture

This project contributed to agriculture in both advancing our basic knowledge in the fields of water stress of harvested fruits and its interaction with decay and senescence and in establishing a new postharvest technique.

The new packaging method was shown to achieve the following: drastic inhibition of shrinkage and weight loss without any deleterious effect on flavor, doubling and, at times, trebling the life of the fruit as measured by appearance, firmness, shrinkage, weight loss and other keeping qualities. Seal-packaging delayed various parameters of physiological deterioration better than cooling to optimal temperature. Thus, fruit sealed at 20°C lost less weight, shrank less and was firmer than non-sealed fruit at the optimal low temperature. Seal-packaging reduced chilling injuries in various fruits (14). Thus, it could be combined with cooling to extend (maximally) the storage life of fruit. Cooling to a lower temperature reduces decay and, together with seal-packaging, delays deterioration of fruit (8).

Possible contributions of the application of seal-packaging

Seal-packaging may enable the following:

- 1) Substitution of refrigerated by ventilated ships for export of many commodities, such as most citrus fruits.
- 2) Substitution of air transport by marine shipment for some commodities, such as tomatoes, easily peeled citrus cultivars, and others.
- 3) Possible extension of storage life of fruit at a lower cost than by conventional means.

Use of this technique might yield the following specific additional benefits for the citrus industry:

- (a) The conventional practice of waxing could be eliminated, since its function of preventing shrinkage and reducing weight loss would be fulfilled 5 to 10-fold better by the film. Its other function, of imparting gloss, would be hidden anyway by the sealed film, which would provide its own gloss, and finally the film would serve as a better carrier for fungicide.
- (b) Seal-packaging could be utilized also for 'brand' name advertising by preprinting the film as a gift wrap, fancy pack for high-quality premium grade, or for a superior cultivar.
- (c) Far greater latitude in storage temperatures is possible for seal-packaged, as contrasted to nonsealed fruit. Storage periods using optimum refrigeration are greatly extended. Cold night air has been used successfully in South Australia for storage of film-wrapped citrus. Since chilling injury (CI) is reduced, considerable extension of storage periods of CI-susceptible cultivars or the mixing of otherwise incompatible cultivars in storage, is possible.

The automatic sealing machine will mechanize the wrapping process and lead to greater saving in labor and a possible reduction also in costs.

Expected contribution in the underdeveloped nations

More significant gains are expected in the underdeveloped countries. The introduction of such a relatively simple technique of preserving perishables might reduce food losses substantially. The relative lack of modern refrigeration and packinghouse facilities in these countries would increase several-fold the advantages of applying this technique.

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2. Smilanick, J.L. and J.W. Eckert. Cultural and pathogenic characteristics of Penicillium digitatum isolates resistant to sec-butylamine
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