Study of Biopolymers and Paraffin as Potential Controlled-Release Carriers for Insect Pheromones

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Starch, whey, and soy proteins and paraffin wax were tested as potential controlled-release carriers for insect sex pheromones used for mating disruption of orchard pests. Geranyl propionate was used as a model pheromone in preliminary experiments. Geranyl propionate and Oriental fruit moth pheromone release-rates from various carrier materials were measured in laboratory experiments by extraction of residual pheromone from biopolymer films and by trapping pheromone released from carrier materials into an air stream using Super Q traps. The carrier materials could be applied to tree bark or foliage at ambient temperature as aqueous emulsions. After drying, the film or coating would release the entrapped pheromone, then slowly erode from the bark, and degrade in soil. Paraffin performed better than starch or protein as a controlled-release carrier for the compounds studied because of better initial entrapment and a more constant release-rate.

Keywords: *Pheromone; biopolymers; paraffin; controlled-release*

INTRODUCTION

There has been a heavy reliance on synthetic pesticides for insect pest control in the United States since World War II. For many years, these chemicals seemed to provide the solution to crop damage by insects. However, a number of problems associated with the use of synthetic pesticides have emerged over the past few decades. This has led to an interest in alternative methods of insect control (Farrell et al., 1992).

One method of insect control that can be used as part of an Integrated Pest Management (IPM) program is mating disruption with synthetic insect sex pheromones. Sex pheromones represent an important class of semiochemicals (behavior-modifying chemicals) and are used by insects to communicate the location and sexual availability to a prospective mate. Mating disruption is a control technique which prevents mate location by interfering with the in-flight maneuvers that bring the sexes together (Cardé, 1990). Once the components of the pheromone have been identified, synthetic sex pheromones can be used to modify insect behavior. When synthetic pheromones are released at the appropriate time, location, and rate, males are prevented from locating fertile females, thus controlling future generations (Kydonieus and Beroza, 1982). Because pheromones are species-specific, they can be used to control a specific pest without affecting beneficial insects.

The use of pheromones for mating disruption of insect pests can potentially lead to a large decrease in the amount of synthetic pesticides that are used by farmers for insect pest control. Because many synthetic pesticides create problems related to insecticide-resistant strains, effects on nontarget organisms, worker exposure, food safety, and contamination of soil, water, and air, the use of pheromones has a tremendous advantage. Pheromones are relatively nontoxic, are used in small quantities, and are biodegradable. Pheromones are also easy to handle, have fewer regulatory restrictions, and result in minimal disruption of other orchard or crop operations.

Presently, most pheromone chemicals used for mating disruption are encased in plastic dispensers which are manually attached to trees or plants. The pheromone slowly diffuses through the plastic walls of the dispenser or the polymeric material over a period of 2−3 months. While effective control of certain insect pests has been achieved with plastic pheromone dispensers, the application is labor-intensive, and the dispensers typically have to be applied several times per season. Also, if the insects are located in the top of a tall tree, it is difficult to manually place the pheromone dispenser in this portion of the canopy.

The difficulty in developing controlled-release formulations that could be sprayed on field or orchard crops and release pheromone at a constant rate over an extended period of time (six weeks or more) has been a factor limiting the use of mating disruption for pest control. Spray applications usually require the active agent to be dispersed in the carrier, resulting in release-rates that decrease over time. However, a constant (zero-order) release-rate is necessary for controlled-release of pheromones to be most economical. While the concept of insect control by mating disruption has been proven effective, a means of dispensing pheromone at a constant rate for an extended time period is necessary to gain widespread acceptance and use.

The overall goal of this research was the development of a sprayable and biodegradable carrier material for insect sex pheromones used for mating disruption of

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orchard pests. The concept involves direct application of the pheromone carrier to trees, with the carrier then slowly releasing the pheromone over an extended period of time. Biopolymers and paraffin were investigated as potential pheromone carrier materials, and the inherent release characteristics were compared. Paraffin can be applied as an aqueous emulsion, but initial release-rates were measured from solid paraffin (Atterholt, 1996; Delwiche et al., 1998). Pheromone release-rates were measured in the laboratory. Additionally, field studies were conducted to determine the feasibility of applying each of the above materials to orchard trees and the efficacy of the method as measured by trap shut-down and damage to fruit at harvest (Atterholt, 1996; Rice et al., 1997).

MATERIALS AND METHODS

Environmental Chambers and Flow Cells. Two different environmental chambers were built to determine the effects of temperature, airflow, pheromone concentration, formulation, and surface area on the pheromone release-rate. The first environmental chamber (E−1) with temperature and airflow control contained six flow cells. All of the biopolymer materials were tested in E−1. Initial experiments with paraffin were conducted in E−1, but a second environmental chamber was built with additional capacity for factorial experiments. The second environmental chamber (E−2) contained 27 flow cells with temperature and airflow control.

The E−1 flow cells were machined from aluminum with a 15-cm diameter and a 5-cm depth. An aluminum lid was bolted to the top of each flow cell and sealed with a Teflon gasket. Compressed air from a supply line ran to a manifold to which six flow cells were connected, each equipped with a flow meter (Dwyer Instruments, Anaheim, CA). The air stream was distributed over the surface of the carrier material after passing through two metal diffusing screens.

The E−2 flow cells were also machined from aluminum with an 8.3-cm diameter and a 5.4-cm depth (Figure 1). The flow cells were placed in E−2 maintained at a constant temperature during the experiment. A compressed air line was connected to a manifold where air was distributed to flow meters before entering the flow cells. There was one flow meter for each of the 27 flow cells. The air stream entered the flow cells and was distributed over the surface of the carrier material after passing through a 10 μ sintered metal diffuser (Pacific Sintered Metals, Los Angeles, CA).

Geranyl propionate (GP) (Pfaltz and Bauer, Waterbury, CT) is structurally similar to many pheromones and was used as a model compound in many lab tests because of its lower cost.

Oriental fruit moth (OFM) (Bedoukian Research, Inc., Danbury, CT) pheromone was used for many lab tests and is a mixture of Z(8)-dodecenyl acetate, E(8)-dodecenyl acetate, and Z(8)-dodecenol in a 93:6:1 ratio.

For the protein films, whey protein isolate (Bi−Pro, Le Seur Isolates, Le Seur, MN), Alacen-878 whey protein concentrate (New Zealand Milk Products, Santa Rosa, CA), and Supro 670 soy isolate (Protein Technologies International, St. Louis, MO) were used. For the starch films, Crisp-Tex starch (American Maize-Products Co., Hammond, IN) was used. For the biopolymer solutions, propylene glycol alginate (Kelco, San Diego, CA) was used as an emulsifier, and glycerin (Fisher Scientific, Pittsburgh, PA) was used as a film plasticizer.

General Procedure for Preparing Biopolymer Films. The first step in preparing a biopolymer film matrix for the controlled release of pheromone was to make an aqueous biopolymer solution. The powdered starch or protein was dispersed in water and then heated. This heat process denatured the protein so that it could make a network capable of forming a film upon drying. For starch, the heat process caused a rupturing of the starch granules so that a biopolymer film formed upon drying. After heating, the biopolymer solution was cooled to room temperature. At that time, the pheromone, emulsifier, and plasticizer were added to the biopolymer solution. Propylene glycol alginate was used as an emulsifier to disperse the pheromone, which was insoluble in water. Glycerin was used as a plasticizer to provide flexible films and prevent the dried films from cracking.

A high-speed mixer (Ultra-turrax T25, with a KG probe and a 25F head, IKA-Works, Cincinnati, OH) was used to disperse the GP in the biopolymer solution. After a well-emulsified biopolymer/GP mixture was prepared, a series of films was cast on plates. These round casting plates were made from aluminum or poly(methyl methacrylate), with a lip to contain the aqueous solution. Under ambient laboratory conditions, the water evaporated, forming flexible, intact films containing the dispersed pheromone.

Whey Protein Concentrate Films. A 10% (w/w) whey protein concentrate solution was used to prepare films. Whey protein concentrate powder (10 g) was dispersed in 80 g of water and then heated for 15 min at 75−80 °C. GP (0.1 g), 0.1 g of propylene glycol alginate emulsifier, and 10 g of glycerin were added to the biopolymer solution. After a well-emulsified biopolymer/GP mixture was prepared, a series of films was cast from a batch of solution by placing 15 g samples on the film casting plates with a surface area of 170 cm^2. After the water evaporated, the resulting films had an average thickness of 0.125 mm.

Soy Protein Isolate Films. A 10% (w/w) soy protein isolate solution was used to prepare soy protein films. Soy protein isolate powder (10 g) was dispersed in 85 g of water and then heated for 15 min at 75−80 °C. After heating, 0.1 g of GP, 0.1 g of propylene glycol alginate emulsifier, and 5 g of glycerin were added to the biopolymer solution. After a well-emulsified biopolymer/GP mixture was prepared, a series of films was cast from a batch of solution by placing 15 g samples on film casting plates with a surface area of 170 cm^2. After the water evaporated, the resulting films had an average thickness of 0.125 mm. The dried films were exposed to air under ambient laboratory conditions (24−27 °C and approximately 35% relative humidity) for a period of several weeks.

In one experiment, the effect of soy protein concentration on pheromone release-rate was measured. In addition to the 10% (w/w) solution, 7.5 and 5% solutions were prepared and tested in the same manner.

Starch Films. A 10% (w/w) starch solution was used to prepare starch films. Starch powder (10 g) was dispersed in 85 g of water and then heated for 15 min at 100 °C. GP (0.1 g), 0.5 g of propylene glycol alginate emulsifier, and 5 g of glycerin were added to the starch solution. A series of films was cast from a batch of solution by placing 15 g samples on film casting plates with a surface area of 170 cm^2. After the water evaporated, the resulting films had an average thickness of 0.125 mm. The dried films were exposed to air under ambient laboratory conditions (24−27 °C and approximately 35% relative humidity) for a period of weeks.

Paraffin Disks. To prepare paraffin disks for testing pheromone release-rates, paraffin wax (13.1 g) was first heated to 65 °C to melt the wax. OFM pheromone (0.65 g) and vitamin E (1.3 g) were then mixed into the molten paraffin while stirring rapidly, and the molten mixture was poured into molds to form disks of uniform surface area and thickness (7.6 cm diameter × 0.4 cm thickness). Vitamin E was initially added as an antioxidant but was also found to decrease the pheromone release-rate. The pheromone-containing paraffin disks were tested in lab flow cells in an environmental chamber to measure the pheromone release-rate.

Release-Rate Determination. The films were exposed to air under ambient laboratory conditions (24−27 °C and approximately 35% relative humidity) for a period of weeks. Each film in a series was held in the laboratory for a different time period and then removed from the casting plate, and the GP was extracted by placing the film in 100 mL of ethanol. The solvent and film were shaken, allowed to stand overnight, and then shaken vigorously before sampling. The amount of GP was quantified by gas chromatography. By extracting the GP from films held for different time periods, the release-rate could be determined by measuring the residual pheromone and

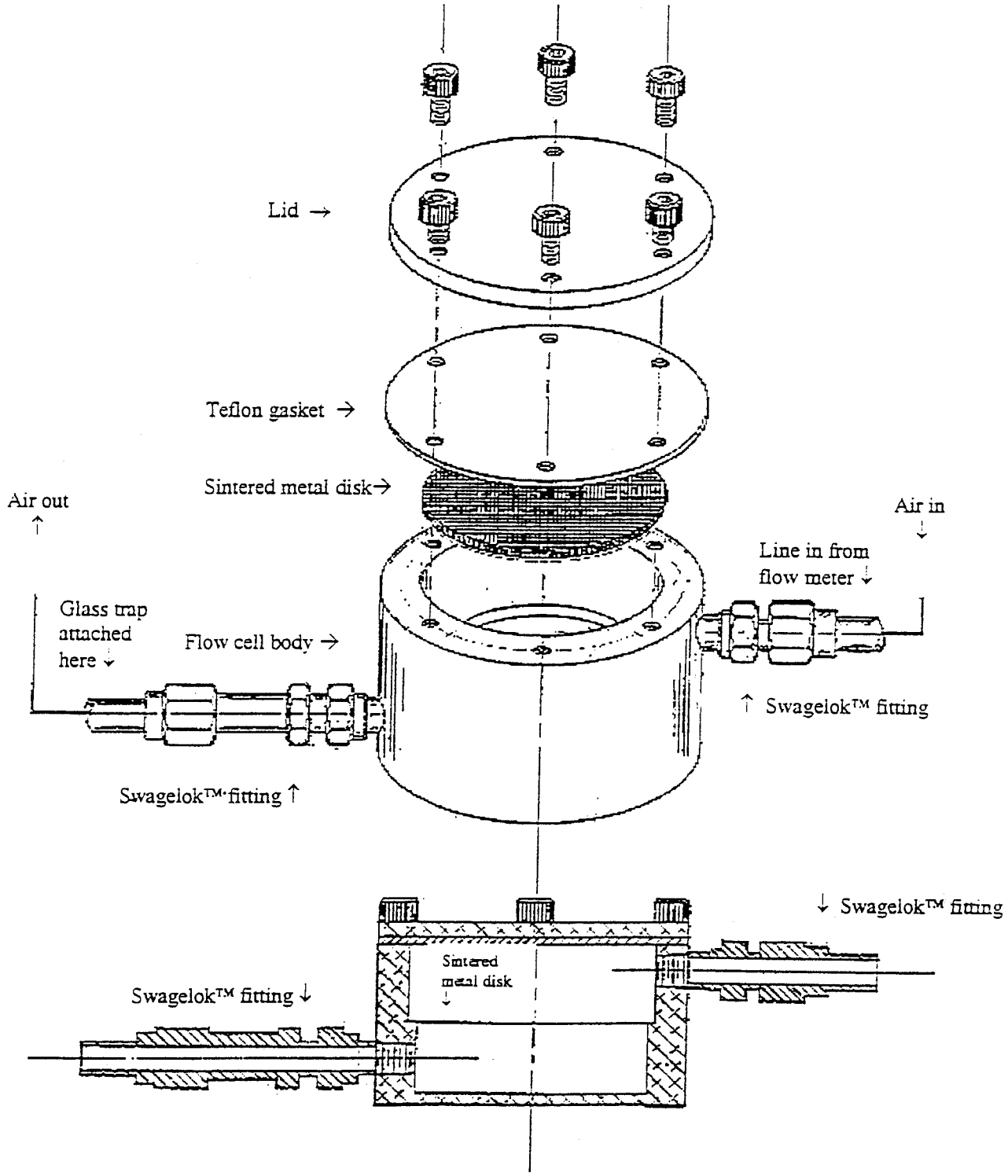

Figure 1. Diagram of flow cell used in laboratory studies of release-rates of geranyl propionate and Oriental fruit moth pheromone.

calculating the mass released. Also, several of the films were placed in a flow cell environmental chamber (E−1) to measure the GP release-rate.

For samples placed in an environmental chamber, released pheromone was trapped from the air stream at the flow cell exit ports on Super Q (Alltech, Deerfield, IL) packed in glass tubes (15 cm × 0.6 cm OD). Silane-treated glass wool (Alltech, Deerfield, IL) was used to hold a 5 cm column of Super Q adsorbent in the traps. Released GP or pheromone was periodically eluted from the traps with 2 mL of ethanol and measured by gas chromatography.

A gas chromatograph (GC) (Hewlett-Packard Model 5890, Palo Alto, CA) equipped with a flame-ionization detector and a capillary column (30 m × 0.25 mm, DB-1, J&W Scientific, Folsom, CA) was used for analysis of samples. Methyl tridecanoate (MTD) (Aldrich Chemical Co., Milwaukee, WI) was used as an internal standard to quantify the amount of pheromone present. The injector port and the detector temperatures were 250 and 280 °C, respectively. The oven temperature program was 135 °C for 2 min and then 8 °C/min to 200 °C, held for 2 min. Helium was the carrier gas.

RESULTS AND DISCUSSION

Whey Protein Films. Figure 2 shows GP release from a representative set of whey protein films, as

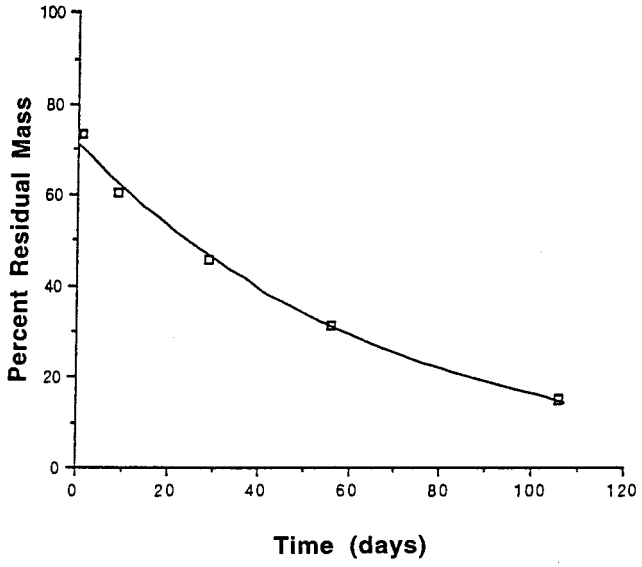

Figure 2. Geranyl propionate release from whey protein films measured by extraction (15 mg of geranyl propionate, 15 g of whey solution, 170 cm^2 surface area).

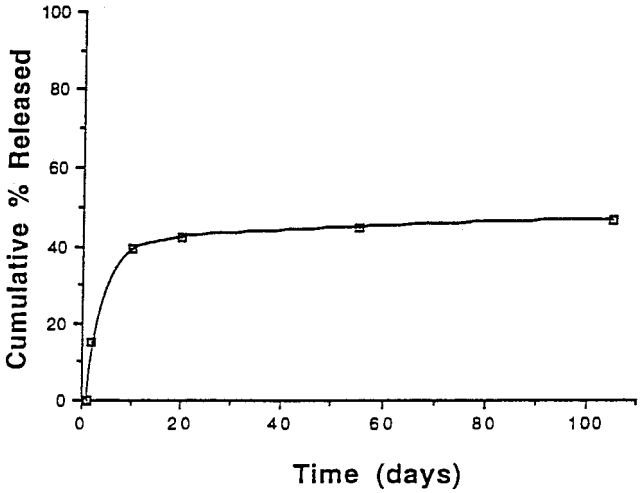

Figure 3. Geranyl propionate cumulative release from whey protein films measured by flow cell (15 mg of geranyl propionate, 15 g of whey solution, 0.5 L/min air flow, 27 °C).

measured by extraction. The graph shows typical results obtained from whey protein films. Approximately 27% of the GP was lost overnight as the films dried, and another 60% was released over the next 3−4 months. The average release-rate for GP was 0.49 μg/(cm^{2}day), calculated over a 106 day time period. Replicate experiments yielded similar results.

According to the Higuchi model (Higuchi, 1961), a linear relationship is obtained for matrix-controlled release when the cumulative mass released is plotted against the square-root of time. When the cumulative release of GP from whey protein films was graphed vs the square root of time, a linear relationship was obtained ($R^2 = 0.996$), indicating that the release of GP from whey protein films was matrix-controlled.

The release of GP from whey protein films was also determined using a lab flow cell in environmental chamber E−1. The emulsion was placed in a flow cell, and the released GP was measured for over 100 days. The results are shown in Figure 3. While the water was evaporating to form the film, 40% of the GP was lost. Because the humidity of the compressed air flowing through the flow cell was very low, the release of entrapped GP from the dried film was extremely slow. Under conditions of very low humidity, the hydrophilic whey protein film would be a tight polymer matrix that would be a good barrier to hydrophobic molecules such as pheromones. Thus the pheromone diffused from the whey protein slowly, with only an additional 5% released over the next 3 months.

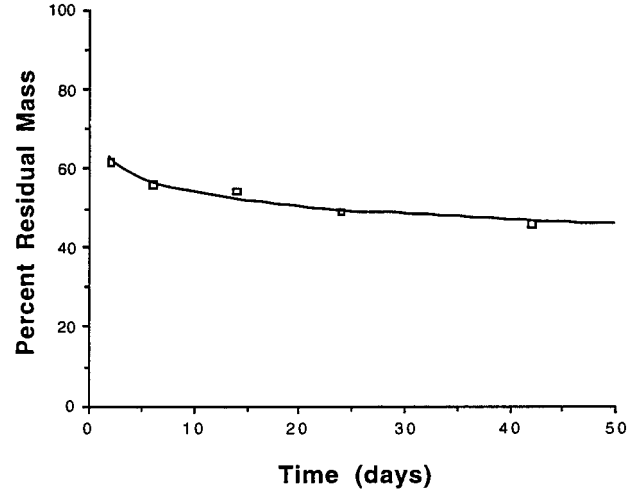

Figure 4. Geranyl propionate release from soy protein isolate films (15 mg of geranyl propionate, 15 g of soy solution, 170 cm^2 surface area).

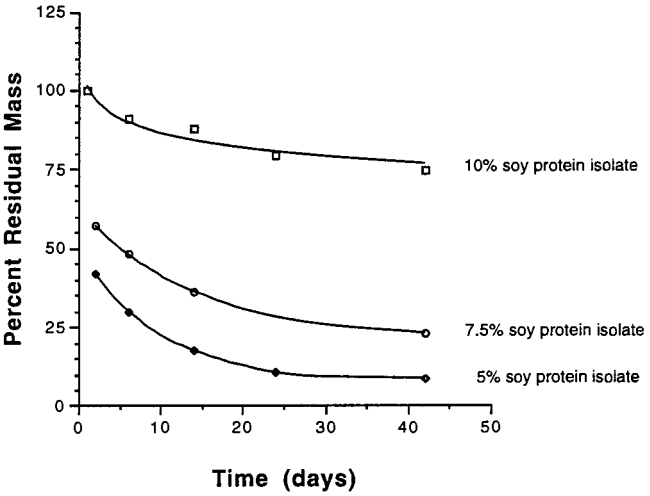

Figure 5. Geranyl propionate release from soy protein isolate films (15 mg of geranyl propionate, 15 g of soy solution, 170 cm^2 surface area).

Soy Protein Isolate Films. Figure 4 shows release of GP from a representative set of soy protein films, as measured by extraction. An average release of 0.33 μg/(cm^{2}day) was calculated over a 42 day period. The release of GP from soy protein films was similar to that from whey protein films. When the cumulative mass released was plotted vs the square-root of time, a linear relationship was obtained ($R^2 = 0.983$), suggesting matrix-controlled release. Additionally, the results of an experiment measuring the effect of the percent of soy protein (w/w) in the film on GP release are shown in Figure 5. A higher percent soy protein resulted in a greater initial entrapment of GP and a slower release-rate.

Starch Films. Figure 6 shows the GP release from a representative set of starch films as measured by extraction. In contrast to the protein films, the amount

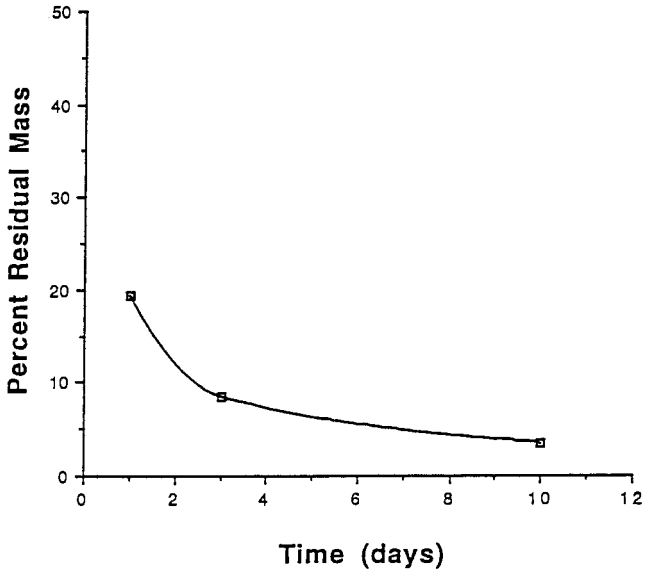

Figure 6. Geranyl propionate release from starch films (15 mg of geranyl propionate, 15 g of starch solution, 170 cm^2 surface area).

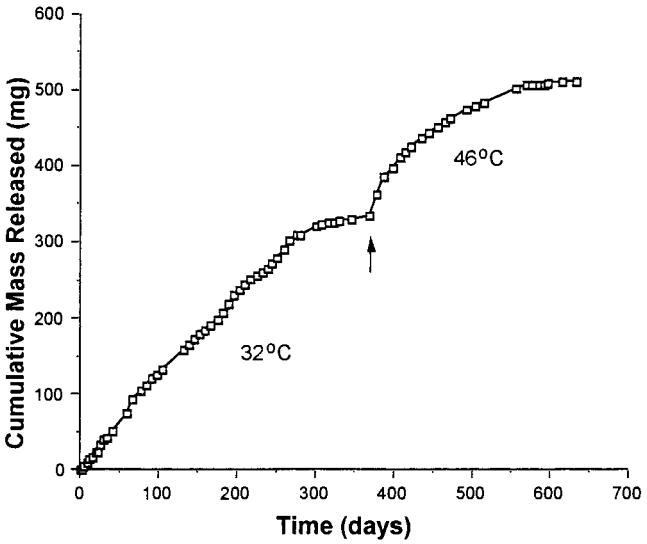

Figure 7. OFM pheromone release from a paraffin disk (650 mg of pheromone, 15 g of paraffin, 1.3 g of vitamin E, 45.6 cm^2 surface area, 0.5 L/min air flow). The temperature was increased from 32 to 46 °C on day 375, indicated by the arrow.

of GP initially entrapped in the starch films was only about 20%, and the starch films released the GP much more quickly than the protein films. An average GP release-rate from dried starch films was calculated to be 0.14 $\mu g/(cm^2 day)$ over a 10 day period of time. As with the other biopolymer films, the graph of the cumulative release of GP from starch films vs the square-root of time was linear ($R^2 = 0.995$), indicating matrix-controlled release.

Whey and soy protein films entrapped a larger amount of GP and released it more slowly than starch films. Proteins are better emulsifiers for nonpolar molecules than starch because of their hydrophobic regions. Thus, proteins complemented the emulsifying ability of propylene glycol alginate and entrapped more GP, which was released over a longer time period.

The effects of lipids and lipid-based emulsifiers were not evaluated in this study of biopolymer films measuring the release-rate by extraction of residual GP. Lipids included in the formulation were extracted into the ethanol with the GP, accumulated on the GC column, and interfered with analytical results. However, lipids such as acetylated monoglycerides and modified lecithins would most likely have a beneficial effect on GP release from biopolymer films. The flowcell method could be used to measure GP release when these materials are included in the formulation.

Paraffin Disks. The results of a long term experiment measuring cumulative OFM pheromone release from a solid paraffin disk are shown in Figure 7. This pheromone was chosen because synthetic OFM pheromone is available at a relatively low cost, OFM is a common pest of peach, and pheromone dispensers for OFM are now in commercial use. An average release of 24 $\mu g/(cm^2 day)$ was obtained for about the first 280 days, or until approximately 50% of the pheromone had been released. Because the release-rate leveled off at this point, the temperature was then increased to 46 °C at 375 days to determine if any additional pheromone would be released at a higher temperature. As shown in the graph, the release-rate increased when the temperature was increased, but first-order release was obtained instead of zero-order. Approximately 80% of the pheromone was released over a 2 year period, showing that long-term pheromone release can be obtained with paraffin.

The constant release-rate obtained for the first 9 months of this experiment suggests that the pheromone is soluble in paraffin wax, with a small air/wax partition coefficient controlling the release-rate. Differential scanning calorimetry experiments were conducted to confirm the solubility of the pheromone in the paraffin (Atterholt, 1996). Furthermore, field tests conducted in conjunction with the laboratory experiments indicated that the release-rates obtained in this experiment were sufficient to affect the mating behavior of the OFM (Atterholt, 1996).

Paraffin does have the characteristics necessary for use as a controlled-release carrier for pheromones, and paraffin emulsions could be used as a controlled-release matrix applied directly to tree bark (Atterholt, 1996; Delwiche et al., 1998; Rice et al., 1997). Biopolymer films did not provide the slow, constant pheromone release necessary for insect control by mating disruption, but the effects of lipids in the biopolymer films have not been thoroughly examined and will be the subject of a future investigation.

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LITERATURE CITED

Atterholt, C. Controlled Release of Insect Pheromones from Sprayable, Biodegradable Materials for Mating Disruption. Ph.D. Dissertation, University of California, Davis, 1996.

Cardé, R. Principles of mating disruption. In *Behavior-Modifying Chemicals For Insect Management, Applications of Pheromones and Other Attractants*; Ridgway, R. L., Silverstein, R. M., Inscoe, M. N., Eds; Dekker: New York, 1990.

Delwiche, M.; Atterholt, C.; Rice, R. Spray application of paraffin emulsions containing insect pheromones for mating disruption. *Trans. ASAE* **1998**, *41* (2), 475−479.

Farrell, K.; Flint, M.; Lyons, J.; Madden, J.; Schroth, M.; Weinhold, A.; White, J.; Zalom, F.; Jaley, M. *Beyond Pesticides: Biological Approaches to Pest Management in California;* ANR Publications: University of California, Oakland, CA, 1992.

Higuchi, T. Rate of release of medicaments from ointment bases containing drugs in suspension. *J. Pharm. Sci.* **1961**, *50* (10), 874−875.

Kydonieus, A.; Beroza, M. Pheromones and their use. In *Insect Suppression with Controlled Release Pheromone Systems*; Kydonieus, A., Beroza, M., Ed.; CRC Press: Boca Raton, FL, 1982; Vol. I.

Rice, R.; Atterholt, C.; Delwiche, M.; Jones, R. Efficacy of mating disruption pheromones in paraffin emulsion dispensers. *IOBC wprs Bull.* **1997**, *20* (1), 151−161.

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