

## Using Food-Safe Ingredients to Optimize the Efficacy of Oil-in-Water Emulsions of Essential Oils for Control of Waxy Insects

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### Abstract

Waxy insects such as mealybugs and scale insects are difficult to kill using contact insecticides because the waxes produced by these insects form a physical barrier preventing chemical penetration. Exported horticultural commodities can be rejected or destroyed if found infested at the destination. Post-harvest dips of soaps and paraffinic oils can be used to control these pests, but these remedies are relatively ineffective and/or phytotoxic in comparison to properly formulated oil-in-water emulsions of terpene oils such as limonene or essential oils such as peppermint oil or spearmint oil. In bioassays with longtailed mealybugs [*Pseudococcus longispinus* (Targioni Tozzetti)] we found that insecticidal soap containing 49.5% potassium salts of fatty acids or Tween<sup>®</sup> containing 100% polysorbate 80 can be used to create aqueous, plant-safe emulsions of these oils that are effective in controlling waxy insects. When sodium lauryl sulfate and citric acid are included in the formulation, efficacy increases dramatically. It is expected that many types of ornamental plants will tolerate these enhanced mixtures, which penetrate and kill mealybugs within seconds.

### INTRODUCTION

Waxy insects such as scale insects and mealybugs are frequently intercepted by quarantine inspectors on horticultural commodities. For example, over a one-year period ending June 2009, there were at least 34 quarantine rejections because of pests on potted plants shipped from Hawaii to California, and 69 rejections for pests on cut flowers. Scale insects and mealybugs were responsible for 47 and 59% of these rejections, respectively (Hawaii Department of Agriculture, unpublished data compiled from rejection notices, used with permission). One reason these pests are present on exported plant material is because they are difficult to kill using contact insecticides. In some cases, tolerance is related to feeding site. For example, mealybugs are commonly found at plant nodes where they are protected from attack by overlapping leaves, bracts and bark crevices. An even more important factor for both scale insects and mealybugs is the protective covering of wax that these insects produce over their flattened bodies. The waxy coverings repel aqueous solutions, allowing the solution to drain away before the active ingredient of the pesticide penetrates to the living insect beneath.

Essential oils have potential as post-harvest insecticides for control of waxy, hard-to-wet pests such as scale insects and mealybugs on fruits, vegetables and potted plants.

An essential oil is a hydrophobic liquid containing volatile aroma compounds from plants, usually extracted by distillation. Essential oils penetrate the wax of insects, soaking into their bodies and killing them within seconds. Many essential oils are classified as G.R.A.S. (Generally Recognized As Safe) compounds by the US Food and Drug Administration and approved as food additives. While some would be too expensive to use in a pesticide mixture (e.g. sandalwood oil, at US\$3,538.00 per kg), others are inexpensive, such as orange oil (US\$11.32 per kilo) (The Essential Oil Company website, <http://www.essentialoil.com>, accessed August 20, 2009). The challenge is to create aqueous formulations of essential oils that readily penetrate and kill waxy insects without

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harming the leaves or roots of potted plants, or without removing protective waxes of fruits and vegetables.

Researchers at the Agricultural Research Service, Hilo laboratory, have been working since 2003 to develop plant-safe aqueous emulsions of limonene for control of waxy insects. Limonene is a monoterpene and the major chemical component in citrus peel oil. It is inexpensive and readily available and has the pleasant smell of oranges. Hollingsworth (2005) demonstrated that aqueous emulsions of 1% limonene, created using two different surfactants, were safe for most plants and provided good control of mealybugs, scale insects, whiteflies and aphids when sprayed or used in 1-min dips. In side-by-side greenhouse tests, sprays of this mixture provided superior control of mealybugs compared with 2% solutions of either insecticidal soap or horticultural spray oil (Hollingsworth, 2005). Later it was discovered that addition of citric acid (CA) and sodium lauryl sulfate (SLS) to the limonene mixture dramatically improved the ability of the solutions to remove the wax of mealybugs, yet did not increase phytotoxicity. This discovery was the basis for a patent application submitted in 2008 (Hollingsworth, 2009) and this is the first manuscript describing results using the improved formulations. Our objectives for this paper are to demonstrate the following: (1) efficacy of 1% limonene emulsions against mealybugs is enhanced when CA and SLS are also present in the mixture; (2) when these mixtures used polysorbate 80 (Tween) instead of insecticidal soap (IS) containing potassium salts of fatty acids as the emulsifier, emulsions were more stable and efficacy increased over time; and (3) these formulation methods yield effective mixtures not only using limonene but also peppermint and spearmint oil.

## MATERIALS AND METHODS

### Mixing Methods for Bioassay Solutions

Chemicals used in bioassay mixtures are shown in Table 1. Solutions were mixed using the following method: the oil (limonene, peppermint or spearmint essential oil) and the emulsifier (Tween or IS) were first mixed together for 30 s using a 2.5 cm magnetic stir bar at 130 rpm in the bottom of a glass container (140-ml beaker, 125-ml Erlenmeyer flask, or 250-ml Erlenmeyer flask, depending on the test). Water was added in a quantity sufficient to achieve a 1% concentration of the oil, and this was mixed at 300 rpm for 3 minutes. Then powdered ingredients were added, first the sodium lauryl sulfate (SLS), followed by anhydrous citric acid (CA) crystals (if the test called for these ingredients). The mixture was stirred for at least 6 minutes after powdered ingredients were added. Mixtures held for more than one day were kept in tightly-capped Erlenmeyer flasks to prevent evaporation of water and to minimize loss of volatiles from the emulsified oil.

### Laboratory Bioassays Using Insects and Plant Material

Longtailed mealybugs, *Pseudococcus longispinus* (Targioni-Tozzetti), were reared on green beans in ventilated containers in the laboratory. Longtailed mealybugs were exposed to solutions in 5-second or 1-minute dips. For each dip, a portion of a heavily infested bean was completely submerged while being held vertically within a 140-ml beaker containing a test solution or water. Afterwards, the bean piece was placed horizontally in the bottom of a 1-liter plastic container covered with a lid ventilated with silk-screen material. Insects were examined 24-48 h later (depending on the test) and 3<sup>rd</sup> and 4<sup>th</sup> instars were counted as dead or alive. Only 3<sup>rd</sup> and 4<sup>th</sup> instars were considered as these represented the most waxy life stages (late nymph and female adult) and therefore potentially the most challenging for oil-in-water emulsions to penetrate. Insects affected by bioassay solutions usually died in less than 24 h (in some cases, within minutes). Therefore data associated with different holding periods (24 versus 48 h) were combined before analysis for data presented in Table 2. Dipping bean pieces infested with longtailed mealybugs into distilled water or treatment solutions for up to one minute generally dislodged few mealybugs (<5%). Therefore, mealybugs removed during dipping were not considered in mortality assessments. The number of 3<sup>rd</sup> and 4<sup>th</sup> instar mealybugs used in

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each dip (replicate) varied from 58 to 372 for data presented in Table 2 and between 12 and 99 for data displayed in figures. The number of replicates used in each test is indicated in table and figure captions.

To determine the phytotoxicity of a solution, gardenia branch tips (5-10 leaves each) were dipped into treatment solutions for 1 minute. Relative to other types of ornamental foliage and flowers, gardenia is a moderately sensitive indicator plant for phytotoxicity symptoms associated with limonene exposure (Hollingsworth, 2005). After treatment, branch tips were placed in florists' tubes holding a commercial hydrating solution (Floralife Flower Food, Floralife, Inc., Walterboro, SC, USA) and held on the lab bench for 24 h before phytotoxicity assessments. Bioassay-related damage was evidenced by oil-soaked areas on the leaves. Phytotoxicity was scored on a 10-point scale, with a rating of "0" indicating no oil soaking, and a rating of "1" corresponding to 1 or 2 damaged areas, each less than 3 mm in maximum diameter. Ratings from 2-9 were assigned subjectively, with a rating of "2" representing minor cosmetic damage and a rating of "9" representing oil-soaking of one-half or more of the leaf area.

## RESULTS AND DISCUSSION

Sixty-second dips into distilled water were associated with an average mortality of 2% (Table 2). This mortality was probably due to handling, as beans were infested on all sides and mealybugs were occasionally crushed when bean pieces were placed in the bottoms of the plastic containers used as holding arenas. Aqueous solutions containing 1% limonene emulsified with IS (0.5-1.0%) but containing no other ingredients killed from 3 to 12% of mealybugs exposed in one minute dips (Table 2). If the mixture also included 0.5-1.0% SLS, mortality increased dramatically, and ranged from 93 to 98% (Table 2). Lewthwaite et al. (1999) found that SLS enhanced the efficacy of sodium bicarbonate dips and was also effective on its own. To better study the effect of including SLS and CA together in bioassay mixtures, dip duration was reduced from 60 to 5 s. Insects dipped for 5 s in solutions containing 1% limonene emulsified with IS, which also included 1% SLS, killed 38% of mealybugs (Table 2). When CA was added to this mixture at a rate of 1, 2 and 4%, mealybug mortality increased to 65, 84 and 94%, respectively (Table 2). It wasn't possible with this emulsifier to test CA in the absence of SLS, as this combination resulted in an oily slick. Using 5-second dip data from 1% limonene solutions shown in Table 2 after arc-sine transformation of mortality data, multiple regression analysis indicated significant effects associated with using different levels of SLS (0, 0.25, 0.5 and 1%;  $F=17$ ,  $P=0.0002$ ,  $df=3$ ) and CA (0, 1, 2 and 4%;  $F=23$ ,  $P=0.0001$ ,  $df=3$ ) in bioassay solutions. However, there was no effect associated with using different levels of IS (0.5, 0.75, and 1%;  $F=1.8$ ,  $P=0.2$ ,  $df=2$ ) (SAS Institute, 2008). In general, there was no phytotoxicity (oil soaking) associated with any of the treatment combinations shown in Table 2 ( $N=41$ ). Minor leaf burn or oil soaking occasionally occurred at leaf margins if leaves had already been damaged by spider mites, thrips or leaf-feeding bugs. Informal observations indicated that SLS and CA sometimes caused phytotoxicity when used in concentrations greater than 1 and 4%, respectively (data not shown).

Although IS produced good emulsions of limonene in combination with SLS and CA, these were not stable. Immediately after mixing, mixtures were "milky" (whitish in color and opaque, indicating a macro-emulsion). Over a period of weeks, mixtures became translucent (a micro-emulsion) and lost much of their ability to remove the wax of mealybugs, even if mixtures were stored in tightly-capped glass containers with no air space. We suspected this change occurred because the sizes of limonene micelles became smaller over time. Therefore, to increase the stability of solutions, we began using the non-ionic surfactant Tween as our emulsifier instead of IS. Tween is commonly used in laboratory settings, and is also used as an emulsifier in food products, including ice-cream. Mixtures containing 1% of limonene or an essential oil emulsified with Tween were initially somewhat translucent, but became increasingly opaque over a period of 1-2 weeks. Coincident with this change was the appearance of a large number of micelles less

than 0.005 mm in diameter, as viewed through a phase contrast microscope.

Insecticidal mixtures containing 1% limonene, 1% SLS, 4% CA and emulsified with 2% Tween became more effective as they aged. Using 5 s dips, an average of 29% of mealybugs were killed if dipping occurred immediately after mixing. However, mortality averaged 72% if the mixture was used 2 days after mixing, and increased to 89% by day 16 (Fig. 1A). There was no phytotoxicity to gardenia leaves dipped for one minute in any of these mixtures (N=27 gardenia branch tips).

When we substituted 1% peppermint oil or 1% spearmint oil for limonene in mixtures containing 2% Tween, 1% SLS and 4% CA, mortality associated with 5 s dips was high (81-95%) even when mixtures were used immediately after mixing (while they were still translucent). Over a nine-day period, these solutions gradually became more opaque, and mortality results trended upwards, reaching 100% (Fig. 1B). Peppermint oil was somewhat more effective against insects than spearmint oil, but also caused more phytotoxicity, particularly as the mixtures aged. About one-fourth of the 33 gardenia samples dipped in the peppermint oil mixtures that were held up to 9 days showed a minor degree of phytotoxicity (ratings either 1 or 2). Mixtures of peppermint oil were not tested after nine days, because phytotoxic oil droplets collected on the surface of the solution at about this time. For this oil, it appears that a different emulsifier, or an additional, more lipophilic surfactant, may be required to obtain a stable emulsion (Hargreaves, 2003).

For mixtures containing limonene emulsified with Tween described above, efficacy against mealybugs depended on the inclusion of both the SLS and CA. Using an independent data set, when all four ingredients were present, the percentage of mealybugs killed was 42% if the mixture was used immediately, increasing to 88% if treatment was delayed until 2 days after mixing (Fig. 2). However, if the mixture lacked either SLS or CA (or both, data not shown), mortality never exceeded 20% (Fig. 2). When CA was included in the mixture, dead insects lost moisture and became flattened on the surface of the bean over the 24-hour holding period. This observation suggests that CA enhances moisture loss in insects that had lost their waxy protection due to other ingredients in the bioassay mixture. When both SLS and CA were present in bioassay mixtures, wax removal from mealybugs was rapid, with significant loss occurring within seconds after dipping. This suggests that SLS and CA are interacting synergistically to remove the wax from the mealybugs. The mechanism for this synergy is not known, but may be due to ionic attraction between the two compounds.

## CONCLUSIONS

Mixtures containing 1% limonene or essential oils, which also contain SLS and CA, are effective against mealybugs even when these are exposed in dips as short as 5 s in duration. These results and related unpublished findings are the basis of a patent application filed in 2008 (Hollingsworth, 2009). Gardenia foliage, used as a phytotoxicity indicator, dipped for 60 s in these mixtures was generally undamaged. Extrapolating from previously published results that tested 1% limonene mixtures lacking SLS and CA, we believe these improved bioassay solutions will control not only scales and mealybugs, but also aphids and whiteflies, as these latter two types of insects were controlled using sprays of one-half and one-quarter rates of the old mixture. The potential for economic use of essential oil emulsions for control of waxy insects on high value commodities appears to be high. However, cost of ingredients used in these bioassays is also high. Based on bulk costs for limonene (~US\$2.00 per kg; see Hollingsworth, 2005) and the cost of other ingredients if purchased in relatively large quantities (as priced via a web search), we estimate the cost of ingredients for a liter of diluted treatment solution containing 1% limonene, 2% Tween, 1% SLS and 4% CA at US\$2.20. The bulk of this cost is related to the surfactants used (\$1.17 for 10 g of SLS and \$0.63 for 20 ml of Tween). Possible solutions to the high cost include using less expensive surfactants, and reducing the concentration of active ingredients. Previous experience using 1% limonene mixtures indicated that a 60-second dip was roughly equivalent in efficacy to a targeted



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low-pressure spray using the same mixture. Given that we achieved good control of a difficult-to-wet insect using 5-second dips, it is likely that many insects would be adequately controlled using spray applications having much lower concentrations of active ingredients.

#### ACKNOWLEDGEMENTS

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#### Literature Cited

- Hargreaves, A.E. 2003. Chemical Formulation: An Overview of Surfactant-based Preparations Used in Everyday Life. A.E. Hargreaves. Royal Society of Chemistry, Science Park, Cambridge, United Kingdom, p.180.  
Hollingsworth, R.G. 2005. Limonene, a citrus extract, for control of mealybugs and scale insects. Journal of Economic Entomology 98:772-779.  
Hollingsworth, R.G. 2009. Essential oil compositions for control of pests. Non-provisional patent filed Sept. 16, 2009 for prior (2008) application no. 61097263. US Patent and Trademark Office, Alexandria, VA.  
Lewthwaite, S.E., Dentener, P.R. and Connolly, P.G. 1999. Mortality of *Epiphyas postvittana* (Lepidoptera: Tortricidae) after exposure to sodium bicarbonate at elevated temperatures or combined with emulsifiers. New Zealand Journal of Crop and Horticultural Science 27:83-90.  
SAS Institute. 2008. Software version 9.2 for Windows, Cary, NC, USA.

#### Tables

Table 1. Chemicals used in bioassay mixtures.

| Product name (abbreviation)                        | Active ingredient/purity                            | Function in mixture                                 | Manufacturer/supplier                              |
|--|---|---|--|
| Citric acid, anhydrous (CA)                        | 100%  | Dehydrates insects, increases viscosity of solution | Fisher Scientific, Pittsburgh, PA, USA             |
| D-limonene, technical grade terpene oil (limonene) | 96% purity, steam extracted from citrus peels       | Removes wax of insects                              | Florida Chemical Co., Winter Haven, FL, USA        |
| Peppermint essential oil                           | 100% pure distilled oil of <i>Mentha piperita</i>   | Removes wax of insects                              | Essential Oil Co., Portland, OR, USA               |
| Safer Insecticidal Soap (IS)                       | 49.5% potassium salts of fatty acids (ethanol base) | Emulsifies oil                                      | Woodstream Corp., Lititz, PA, USA                  |
| Sodium lauryl sulfate (SLS)                        | 85%+ purity   | Surfactant  | Acros Organics, Geel, Belgium                      |
| Spearmint essential oil                            | 100% pure extract of <i>Mentha spicata</i>          | Removes wax of insects                              | Wholesalesuppliesplus.com, Broadview Hts., OH, USA |
| Tween <sup>®</sup> 80 (Tween)                      | 100% polysorbate 80                                 | Emulsifies oil                                      | Fisher Scientific, Pittsburgh, PA, USA             |

Table 2. Percentage mortality of 3<sup>rd</sup> and 4<sup>th</sup> instar longtailed mealybugs counted 24-48 h following 5 or 60 s dips in aqueous mixtures containing 1% limonene.

| Dip duration (s) | % Limonene | % IS | % SLS | % CA | Average % mortality | No. replicates |
|------------------|------------|------|-------|------|---------------------|----------------|
| 5                | 0          | 0    | 0     | 0    | 1.5                 | 3              |
| 5                | 1          | 0.5  | 1     | 2    | 91.3                | 1              |
| 5                | 1          | 0.75 | 0     | 0    | 2.7                 | 1              |
| 5                | 1          | 0.75 | 0.25  | 4    | 38.4                | 1              |
| 5                | 1          | 0.75 | 0.5   | 2    | 76.5                | 2              |
| 5                | 1          | 0.75 | 0.5   | 4    | 83.5                | 1              |
| 5                | 1          | 0.75 | 1     | 0    | 38.0                | 2              |
| 5                | 1          | 0.75 | 1     | 1    | 64.6                | 1              |
| 5                | 1          | 0.75 | 1     | 2    | 83.6                | 2              |
| 5                | 1          | 0.75 | 1     | 4    | 94.4                | 5              |
| 60               | 0          | 0    | 0     | 0    | 2.0                 | 5              |
| 60               | 1          | 0.5  | 0     | 0    | 12.4                | 1              |
| 60               | 1          | 0.75 | 0     | 0    | 8.7                 | 3              |
| 60               | 1          | 0.75 | 0.5   | 0    | 95.3                | 1              |
| 60               | 1          | 0.75 | 0.5   | 2    | 97.9                | 1              |
| 60               | 1          | 0.75 | 1     | 0    | 99.7                | 2              |
| 60               | 1          | 0.75 | 1     | 4    | 99.8                | 3              |
| 60               | 1          | 1.0  | 0     | 0    | 3.0                 | 2              |
| 60               | 1          | 1.5  | 0.5   | 0    | 93.3                | 1              |
| 60               | 1          | 1.5  | 1     | 0    | 98.1                | 1              |

**Figures**

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| % | No. replicates |
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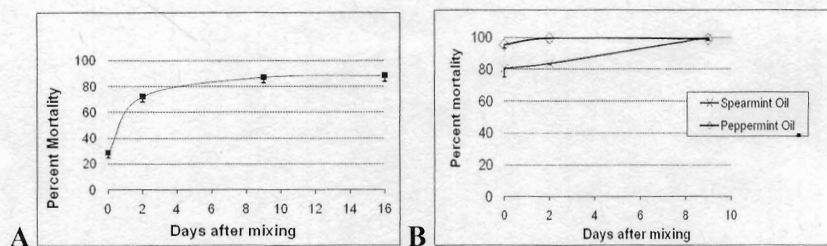


Fig. 1. Percentage mortality of 3<sup>rd</sup> and 4<sup>th</sup> instar mealybugs counted 24 h following 5 s dips in solutions containing (A) 1% limonene or (B) 1% peppermint oil or spearmint oil, as a function of the number of days after mixing before mealybugs were dipped. Each mixture also contained 2% Tween (polysorbate 80), 1% sodium lauryl sulfate and 4% citric acid. Error bars show one SEM (N=3-9 replicates).

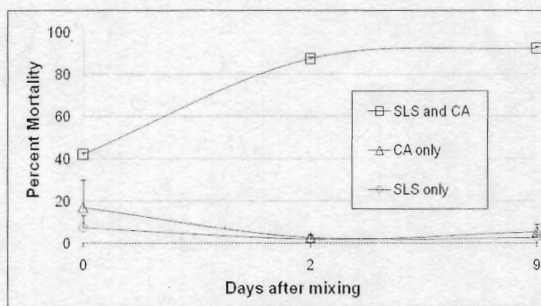


Fig. 2. Percentage mortality of 3<sup>rd</sup> and 4<sup>th</sup> instar mealybugs exposed to 1% solutions of limonene emulsified 2% Tween (polysorbate 80), and with 1% sodium lauryl sulfate (SLS) and 4% citric acid (CA) or lacking either of these ingredients. Each treatment/day combination used 3 replicates.