FUMIGANT TOXICITY OF ESSENTIAL OILS AGAINST FRANKLINIELLA OCCIDENTALIS, F. INSULARIS (THYSANOPTERA: THRIPIDAE), AND SOLANUM LYCOPERSICUM (SOLANACEAE) AS AFFECTED BY POLYMER RELEASE AND ADJUVANTS

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ABSTRACT

Some species of thrips have become significant agricultural pests due to their cosmopolitan distribution, feeding damage and vectorial capacity for plant viruses. However, control of this pest is complicated by their life cycle and resistance to conventional insecticides. I sought to explore a novel method of thrips control that addressed these resistance mechanisms by applying essential oils as fumigants. These essential oils included (*R*)-linalool, racemic linalool, or a binary combination of (*R*)-linalool with one of twelve other oils (i.e., peppermint, cedarwood, neem, clove, coconut, jojoba, soybean, olive, α-terpineol, 1,8-cineole, trans-anethole and (*R*)-pulegone) with distilled water as a control. Essential oils were conditioned into hydrogels and exposed to a pesticide resistant species of thrips (*Frankliniella occidentalis*), a pesticide naive species (*Frankliniella insularis*), and a host plant of the former (*Solanum lycopersicum*). Thrips and tomatoes were first exposed separately in *in vitro* trials, and then together in caged-plant trials. Pure (*R*)-linalool and its binary mixture with peppermint oil were the most toxic to both species of thrips and tomatoes in *in vitro* trials. However, caged-plant trials revealed a greater level of resistance to essential oil fumigation than that predicted by *in vitro* trials. This resistance was attributed to behavioral resistance mechanisms precluded by *in vitro* trials. While certain essential oils have demonstrated potential as alternatives to conventional insecticides, glasshouse and field trials are necessary to fully quantify the extent to which the life history traits of thrips contribute to bioinsecticide resistance.

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1.0 Introduction

This review seeks to highlight trends in research pertaining to essential oils as bioinsecticides, focusing on species of thrips that vector plant viruses. The studies referenced in this review were compiled from a bibliographic search in “Web of Science” and “Google Scholar”. The key words “thrips”, “essential oils”, and “fumigation” were queried from 2000 to 2022. Only studies pertaining to members of Thripidae were selected for this review. Of the studies described in Table 1, 43% focus on *Frankliniella* spp. with *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) being the subject of 29%. Filter paper assays were the most common method of essential oil delivery, comprising 50% of these studies. This was followed by leaf disc assays at 36%. Most studies included a host plant as a thrips habitat (60%). Of those, a third used *Phaseolus vulgaris* L. (Fabaceae), *Allium porrum* L. (Amaryllidaceae), or *Cucumis sativus* L. (Cucurbitaceae). Most studies exclusively measured reductions in the frequency of feeding and oviposition behavior (43%) or mortality (43%). The remaining 14% assessed both lethal and sub-lethal effects.

Table 1. Insecticidal effects of various botanicals with varying application methods against Thysanoptera of mixed age.

Authors	Year	Host Plant(s)	Botanicals	Application Method	Target Species	Insecticidal Effects
Woltering et al.	2003	*Phaseolus vulgaris* (Common bean)	1,8-cineole Linalool *p*-Cymene (*S*)-carvone	Leaf disc assay Climate control	*Frankliniella occidentalis*	Mortality
Janmaat et al.	2002	None	*p*-Cymene	Filter paper assay	*F. occidentalis*	Mortality
Picard et al.	2012	*Phaseolus vulgaris* (Common bean)	*Citrus limon* (Lemon) *Gaultheria procumbens* (Wintergreen) *Mentha piperita* (Peppermint) *Origanum compactum* (Oregano) *Satureja montana* (Winter savory) *Thymus serpyllum* (Wild thyme) *Thymus vulgaris* (Common thyme)	Polymer release	*F. occidentalis*	Repellency
Abtew et al.	2015	None	*Anethum graveolens* (Dill) *Cinnamomum cassia* (Chinese cinnamon) *Cinnamomum zeylanicum* (Ceylon cinnamon)	Filter paper assay	*Megalurothrips sjostedti*	Repellency

Table 1 (Continued). Insecticidal effects of various botanicals with varying application methods against Thysanoptera of mixed age.

Authors	Year	Host Plant(s)	Botanicals	Application Method	Target Species	Insecticidal Effects
Abtew et al. (cont.)	2015	None	*Citrus limon* (Lemon) *Commiphora myrrha* (Myrrha) *Conyza newii* (Conyza) *Coriandrum sativum* (Coriander) *Cymbopogon citratus* (Lemongrass) *Cymbopogon nardus* (Citronella) *Eucalyptus globulus* (Eucalyptus) *Litsea cubeba* (May chang) *Mentha pulegium* (Pennyroyal) *Melia azadirachta* (Neem) *Ocimum kilmandscharicum* (African blue basil) *Origanum majorana* (Marjoram) *Pelargonium graveolens* (Geranium) *Piper nigrum* (Black pepper) *Salvia rosemarius* (Rosemary) *Satureja abyssinica* (savory) *Satureja biflora* (Lemon savory) *Solidago canadensis* (Solidago) *Thymus serpyllum* (Wild thyme) *Thymus vulgaris* (Common thyme) *Zingiber officinale* (Ginger)	Filter paper assay	*Megalurothrips sjostedti*	Repellency
Moorthy & Shivaramu	2014	*Capsicum* spp. (Pepper)	*Melia azadirachta* (Neem) *Mentha arvensis* (Mint) *Ocimum tenuiflorum* (Basil)	Leaf disc assay Foliar spray	*Scirtothrips dorsalis*	Reduced feeding
Pumnuan & Insung	2016	*Nelumbo nucifera* (Lotus)	*Alpinia nigra* (Galanga) *Amomum krervanh* (Cardamom) *Cinnamomum bejolghota* (Cinnamon) *Citrus aurantifolia* (Lemon) *Citrus maxima* (Pummelo) *Citrus hystrix* (Kaffir lime) *Citrus reticulata* (Tangerine) *Cymbopogon citratus* (Lemongrass) *Cymbopogon nardus* (Citronella) *Cucurma longa* (Turmeric) *Eucalyptus globulus* (Blue Gum) *Eupatorium odoratum* (Bitter bush) *Ocimum basilicum* (Sweet basil) *Piper betle* (Betel vine)	Filter paper assay	*Frankliniella schultzei* *Pseudococcus jackbeardleyi*	Mortality

Table 1 (Continued). Insecticidal effects of various botanicals with varying application methods against Thysanoptera of mixed age.

Authors	Year	Host Plant(s)	Botanicals	Application Method	Target Species	Insecticidal Effects
Pumnuan & Insung (cont.)	2016	*Nelumbo nucifera* (Lotus)	*Piper nigrum* (Black pepper) *Syzygium aromaticum* (Clove) *Zingiber cassumunar* (Cassumunar ginger) *Zingiber officinale* (Ginger) Eugenol Citral	Filter paper assay	*Frankliniella schultzei* *Pseudococcus jackbeardleyi*	Mortality
Stepanycheva et al.	2019	*Phaseolus vulgaris* (Common bean)	*Litsea cubeba* (May chang) *Melissa officinalis* (Lemon balm) *Mentha arvensis* (Wild mint) *Mentha pulegium* (Pennyroyal) *Mentha spicata* (Spearmint) *Nepeta cataria* (Catmint) *Ocimum basilicum* (Basil) *Origanum compactum* (Oregano) *Origanum majorana* (Marjoram) *Origanum vulgare* (Common Oregano) *Pelargonium roseum* (Rose geranium) *Salvia rosemarius* (Rosemary) *Salvia sclarea* (Clary) *Thuja occidentalis* (Arborvitae) *Thymus mastichina* (Thyme)	Filter paper assay	*F. occidentalis*	Mortality, Reduced oviposition behavior
Riefler and Koschier	2009	*Allium porrum* (Leek) *Cucumis sativus* (Cucumber)	Eugenol	Leaf disc assay	*Thrips tabaci*	Reduced feeding, oviposition behavior
Koschier et al.	2002	*Allium porrum* (Leek)	*Lavandula angustifolia* (Lavender) *Mentha arvensis* (Mint) *Ocimum basilicum* (Basil) *Salvia officinalis* (Sage) *Salvia rosemarius* (Rosemary) *Satureja marjoranum* (Marjoram) 1,8-cineole Eugenol Linalool α-Pinene Terpinen-4-ol	Leaf disc assay	*Thrips tabaci*	Mortality, reduced feeding

Table 1 (Continued). Insecticidal effects of various botanicals with varying application methods against Thysanoptera of mixed age.

Authors	Year	Host Plant(s)	Botanicals	Application Method	Target Species	Insecticidal Effects
Koschier & Sedy	2003	*Allium porrum* (Leek)	*Lavandula angustifolia* (Lavender) *Mentha arvensis* (Mint) *Salvia officinalis* (Sage) *Salvia rosemarius* (Rosemary) *Satureja marjoranum* (Marjoram)	Leaf disc assay Two-choice assay	*Thrips tabaci*	Reduced feeding, oviposition behavior
Reitz et al.	2008	*Solanum lycopersicum* (Tomato)	*Cymbopogon citratus* (Lemongrass) *Melaleuca alternifolia* (Tea tree) Geraniol	Foliar spray Field trials	*Frankliniella* spp.	Reduced TSWV transmission
Lu et al.	2020	None	Acetone Carvacrol Citronellal Cuminaldehyde Diallyl trisulfide Dipentene Eugenol Farnesol Geraniol Isoeugenol L-carvol Linalool L-perillaldehyde Methyl cinnamate Methyl salicylate Terpinolene Thymol Trans-cinnamaldehyde β-citronellol γ-terpinene	Filter paper assay	*Anaphothrips obscurus*	Mortality
Kim et al.	2015	*Cucumis sativus* (Cucumber)	(*R*)-Borneol (*R*)-Bornyl acetate Camphene (*S*)-Camphor 3-Carene α-Caryophyllene β-Caryophyllene Caryophyllene oxide 1,8-cineole Citral Citronellal Citronellic acid β-Citronellol *p*-Cymene	Filter paper assay	*Thrips palmi* *Orius strigicollis*	Mortality

Table 1 (Continued). Insecticidal effects of various botanicals with varying application methods against Thysanoptera of mixed age.

Authors	Year	Host Plant(s)	Botanicals	Application Method	Target Species	Insecticidal Effects
Kim et al. (cont.)	2015	*Cucumis sativus* (Cucumber)	Estragole Geraniol Geranyl acetate Limonene Linalool Linalyl acetate (*R*)-Menthone Myrcene (*S*)-α-Pinene (*S*)-β-Pinene (*R*)-Pulegone α-Terpinene α-Terpineol (*R*)-α-Thujone	Filter paper assay	*Thrips palmi* *Orius strigicollis*	Mortality
Yi et al.	2006	*Cucumis sativus* (Cucumber)	*Abies sibirica* (Pine needle) *Angelica archangelica* (Angelica root) *Artemisia vulgaris* (Armoise) *Chamaemelum nobile* (Chamomile roman) *Cinnamomum camphora* (Howwood) *Citrus aurantium* (Neroli) *Coriandrum sativum* (Coriander) *Cupressus* sempervirens (Cypress) *Eucalyptus globulus* (Eucalyptus) *Gaultheria procumbens* (Wintergreen) *Hysoppus officinalis* (Hyssop) *Mentha pulegium* (Pennyroyal) *Myristica fragrans* (Mace) *Myrtus communis* (Myrtle) *Ocimum basilicum* (Basil) *Salvia officinialis* (Sage, Dalmatian) *Salvia rosemarius* (Rosemary) *Sassafras albidum* (Sassafras) *Thuja occidentalis* (Cedarleaf) *Thymus mastichina* (Marjoram)	Filter paper assay	*Thrips palmi* *Orius strigicollis*	Mortality

1.1 Essential Oils as Fumigants Against Thrips

Certain species of thrips are extremely problematic agricultural pests. They have a wide host range (Yudin et al. 1986), a nearly cosmopolitan distribution (Tommasini et al. 1995), and several species are competent vectors of devastating phytoviruses (Wijkamp et al. 1993). Thrips cause at least $1 billion/year in damages globally to the agricultural industry (Goldbach & Peters 1994), typically requiring control strategies in the form of synthetic insecticides. However, these insecticides damage ecosystems by killing pestiferous and ecologically important arthropods indiscriminately (Malhotra et al. 2021, Mužinić & Želježić 2018), and insects can develop resistance to them in relatively short time scales (Harris et al. 1962, Fu et al. 2018). Furthermore, the hazards to human health posed by conventional insecticides (Parven et al. 2021, Sandal & Yilmaz 2011, Sobti et al. 1982) have created a demand for alternative control methods, such as essential oil[s] (EO).

Essential oils refer to a diverse group of compounds produced by aromatic plants as secondary metabolites, protecting plants from pathogens and pests. These compounds are typically extracted from plants via steam distillation (Bakkali et al. 2007). Many studies have confirmed the insecticidal efficacy of EO, albeit with varied results. Several EO decrease insect feeding or oviposition behavior and increase insect mortality (Koschier 2008). For example, applications of 1% carvacrol and 0.1% thymol deterred the oviposition behavior of *F. occidentalis* and *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) (Sedy & Koschier 2003). However, thrips are varied in their behavioral responses to EO. While carvacrol and thymol act as oviposition deterrents, *F. occidentalis* are attracted to anisaldehyde, creating potential for its application in lures (Kirk 1985, Chermenskaya et al. 2001). However, the extent to which these botanicals influence insect behaviors vary with the type of EO and the target insect.

Researchers have used a wide range of bioassays to assess the insecticidal activity of Labiate botanicals, such as leaf disc and olfactometer bioassays (Koschier et al. 2002, Koschier & Sedy 2003). These studies involve placing thrips in an enclosed area treated with EO of a specified concentration (often on the surface of a leaf), then observing the behavior of the thrips for a specified time. In contrast, olfactometer bioassays involve placing insects into a Y - shaped tube with different treatments at each branch and recording which choice insects move towards. These assays are useful for determining the types and concentrations of EO required to repel thrips.

1.2 Most Effective Essential Oils

Low concentrations of EO from members of the Lamiaceae in leaf disc bioassays have insecticidal properties. Oil of marjoram, *Origanum majorana* Linnaeus (Lamiaceae) applied at concentration of 0.1% - 1% interfered with feeding and oviposition behavior of *T. tabaci* females. Oil of rosemary, *Rosemarinum officinalis* L. (Lamiaceae), applied at a concentration of 1% reduced feeding damage on *A. porrum*

by *T. tabaci* (Koschier et al. 2002). A similar study measured behavioral responses of *F. occidentalis* to volatiles from *Salvia officinalis* L. (Lamiaceae), *Filipendula ulmaria* L. (Rubiaceae) and *Laurel nobilis* L. (Lauraceae) (Chermenskaya et al. 2001). Gas Chromatography - Mass Spectroscopy (GC - MS) was used to identify the active components of these oils as 1,8-cineole and methyl salicylate. In olfactometer bioassays, thrips demonstrated non-preference for both compounds at most concentrations. However, *F. occidentalis* was attracted to 1,8-cineole at low doses. These results accentuate the need to test the behavioral effects of plant volatiles at multiple concentrations; different concentrations of the same EO can cause insect mortality, repellency or attraction.

Another observational study assessed the behavior - modifying activity of eugenol on *T. tabaci* using a video system and software for computing observational data (Riefler & Koschier 2009). Females were placed on treated and untreated leaves of two host plants with dissimilar leaf surfaces, *A*. *porrum* and *C. sativus* . Eugenol applied at concentrations as low as 1% caused thrips to spend more time inactive, compared to thrips on untreated *A. porrum*. The duration of thrips feeding was reduced by 24% on treated leaf discs compared to untreated discs. However, the frequency of leaf probing attempts was 26% greater on treated leaf discs. While reducing the duration of feeding seems promising, an increased number of probing attempts may increase virus transmission (Lu et al. 2019).

Similarly, Stepanycheva et al. (2019) assessed the lethal - and sublethal effects of other members of the Lamiaceae against *F. occidentalis* (Stepanycheva et al. 2019). The most promising potential for control came from *Mentha pulegium* L. (Lamiaceae) and *Thymus mastichina* L. (Lamiaceae), with $LC_{50(90)}$ values of 3.1 (3.8) and 3.6 (4.6) mg L^{-1} air, respectively. *Nepeta cataria* L. (Lamiaceae) demonstrated the greatest inhibition of fertility, with $EC_{50(90)}$ estimated at 0.18 (0.36) mg L^{-1} air.

The work of Pumnuan & Insung (2016) is worth noting because they tested the fumigant activity of multiple EO against a species of thrips, *Frankliniella schultzei* Trybom (Thysanoptera: Thripidae), alongside another greenhouse pest, *Pseudococcus jackbeardsleyi* Gimpel & Miller (Hemiptera: Pseudococcidae). The most effective EO were *Syzygium aromaticum* L. (Myrtaceae), *Cinnamomum bejolghota* Buch. Ham (Lauraceae) and *Cymbopogon citratus* DC (Poaceae), with LC_{50} values of 1.14 - 1.48 and 1.12 - 1.58 µl L^{-1} air for *F. schultzei* and *P. jackbeardsleyi*, respectively (Pumnuan & Insung 2016).

Moorthy & Shivaramu (2014) also conducted a comparative analysis of the toxicity of oils extracted from *Ocimum tenuiflorum* L. (Lamiaceae) and *Mentha arvensis* L. (Lamiaceae) to neem products and systemic insecticides (fipronil and acephate). The efficacy of each treatment was indirectly assessed 10 days post-application by measuring damage to *Capsicum* spp. (Solanaceae) by *Scirothrips dorsalis* Hood

(Thysanoptera: Thripidae). They found that neem products and EO had similar efficacy to the systemic pesticides tested (Moorthy & Shivaramu 2014).

While most of the literature gauges the effects of members of the Lamiaceae on *T. tabaci, F. occidentalis and F. schultzei*, the work of Abtew et al. (2015) is worth noting because it appraises the fumigant activity of oils derived from members of the Lauraceae against thrips in another genus, *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae). Through olfactometer and filter paper bioassays, Abtew et al. (2015) observed the strongest repellency from *Piper nigrum* L. (Solanaceae), *Cinnamomum cassia* Presl (Lauraceae) and *Cinnamomum zeylancium* Blume (Lauraceae) (Abtew et al. 2015). This study highlights the need to test the effects of various EO on different species of thrips; EO that are effective against one species might be less effective against another.

1.3 Multiple Constituent Applications

Numerous published studies have demonstrated that individual EO have significant lethal and sublethal effects on certain insects. Many studies have advanced on this concept by conducting studies of EO mixtures, some uncovering synergistic effects. Lu et al. (2020) assessed the efficacy of binary combinations against *Anaphothrips obscurus* Müller (Thysanoptera: Thripidae). They found that the combination of methyl salicylate and carvacrol had the greatest fumigant toxicity when applied at a ratio of 1:1 (Lu et al. 2020). Isman (2020) also explored the synergistic effects of EO mixtures, discovering that mixtures of terpenoids have the greatest lethality relative to other types of mixtures (Isman 2020).

Pesticides are available in a wide variety of formulations that vary in efficacy with respect to the crop pest and the system in which they are applied (Roy et al. 2014). Ideal pesticide formulations maintain the active ingredient at levels needed for pest control while leaving minimum crop and environmental residues. Encapsulating these active ingredients into the matrices of polymers can achieve these effects. Polymers consist of repeating subunits, and active ingredients encapsulated in polymers must migrate through these matrices to escape and contact target pests (Perlatti et al. 2013). Polymer encapsulated formulations are superior to non-encapsulated commercial formulations in extending insecticidal activity (Rudzinski et al. 2002), as well as reducing losses to evaporation, degradation, and leaching (Garratt & Kennedy 2006). Polymer release formulations also have the potential to enhance the insecticidal properties of EO (Vishwakarma et al. 2016). Kaolin, a naturally occurring mineral, has been integrated into fumigant applications as kaolin - based particle films. Reitz et al. (2008) found that *C. citratus*, *Melaleuca alternifolia* Maiden & Betche (Myrtaceae) and geraniol combined with kaolin did not affect the abundance of *Frankliniella* spp. populations. However, kaolin treatment significantly reduced the incidence of thrips - vectored Tomato spotted wilt virus. In the first year of treatment (2005), kaolin reduced the incidence of the

tospovirus by 32 - 51%, and in the second year (2006) by 6 - 25% compared to the control group (Reitz et al. 2008). Even without EO, kaolin possesses insecticidal properties. Treating *A. porrum* with just kaolin - based particle films and water reduced oviposition, hatch rate and feeding by *T. tabaci*. Kaolin treated thrips also had higher mortality and prolonged nymphal development (Larentzaki et al. 2008).

Picard et al. (2012) also investigated the utility of polymer release but with different polymers. Incorporation of EO from *Thymus vulgaris* L. (Lamiaceae) and *Satureja montana* L. (Lamiaceae) into polymer matrices of alginate or methyl cellulose for a minimum of 3 days resulted in greater repellency of *F. occidentalis* compared to treatments lacking polymer matrices. This effect was measured by counting the number of thrips on leaf surfaces before and after application. Polymer release applications often resulted in 100% of *F. occidentalis* females moving away from leaf disc surfaces within 60 minutes (Picard et al. 2012). Similar to Reitz et al. (2008), Picard et al. (2012) theorized that the increased efficacy of this treatment resulted from the decreased volatility of EO once conditioned into alginate or methyl cellulose polymer matrices.

Climate control shows promise as a method to work synergistically with EO fumigation to increase efficacy. Elevated CO_2 levels (10%) applied in combination with *p* - cymene against *F. occidentalis* resulted in higher mortality of adult and larval thrips compared to the control group (ambient CO_2 with *p* - cymene). The experimental treatment did not affect mortality of eggs (Janmaat et al. 2002). Further manipulation of greenhouse climate conditions determined that while EO applied with elevated CO_2 increased mortality in *F. occidentalis,* increasing CO_2 while decreasing O_2 concentrations further elevated mortality (Woltering et al. 2003).

1.4 Effects on Non - Target Organisms

Biocontrol has potential to be used in conjunction with EO fumigation to increase thrips mortality. Therefore, the effect of EO on biocontrol agents must be assessed alongside their effect on pest species. Yi et al. (2006) assessed the toxicity of several popular EO (pennyroyal, rosemary, basil, marjoram, sage, etc.) against *Thrips palmi* Karny (Thysanoptera: Thripidae) and *Orius strigicollis* Poppius (Hemiptera: Anthocoridae), compared to treatment with four standard synthetic insecticides (spinosad, dichlorvos, emamectin benzoate, and thiamethoxam). Based on the 24 - hour LC_{50}, oil of *M. pulegium* was the most toxic fumigant (2.63 mg L^{-1}air), 23.6 times more toxic than dichlorvos (62.09 mg L^{-1}air) against adult *T. palmi*. Furthermore, dichlorvos was the most toxic fumigant against *O. strigicollis* with an LC_{50} of 6.3×10^{-6} mg L^{-1}air. Against *O. strigicollis*, the LC_{50} of the various EO tested ranged from 17.29 to 158.22 mg L^{-1}air. From the 92 EO tested against both species, *O. strigicollis* was 1.4 - 22.1 less susceptible than *T. palmi* (Yi et al. 2006).

Biocontrol agents are not the only beneficial insects whose susceptibility to EO must be evaluated. Pollinators are essential to increasing yield, especially in crops

vulnerable to thrips such as tomato, *Solanum lycopersicum* L. (Solanaceae) (Bashir et al. 2018). Umpierrez et al. (2017) assessed the toxicity of Asteraceae EO against two pests of tomato, *Tuta absoluta* Geyrick (Lepidoptera: Gelechiidae) and *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae), a pollinator, *Apis mellifera* L. (Hymenoptera: Apidae), and *S. lycopersicum*. They found *T. absoluta* to be less susceptible to these botanicals than *T. vaporariorum*. Application rates required for control of *T. absoluta* reduced germination and resulted in honeybee mortality. However, these effects did not occur at rates required for control of *T. vaporariorum* (Umpierrez et al. 2017). These variations highlight the need to concurrently assess the toxicity of EO against host plants, beneficial and pest arthropods alike. While laboratory trials on a single species have merit, they often provide a myopic view of how these phenomena manifest in cropping systems.

As elucidated by Umpierrez et al. (2017), any treatment applied to a crop pest will also be applied to the crop. Therefore, it is imperative to delineate thresholds for phytotoxicity. Similar to the previous study, several teams have evaluated the toxicity of EO towards tomatoes and their pathogens. Tomazoni et al. (2018) and Lee et al. (2012) conducted studies involving fungal and bacterial pathogens of tomato, respectively. Neither observed phytotoxicity at EO concentrations required to control these pathogens (Tomazoni et al. 2018, Lee et al.2012). Plotto et al. (2003) and Wogiatzi et al. (2009) conducted similar studies assessing the efficacy of EO against fungal and bacterial pathogens of tomatoes, respectively. In contrast, both teams observed phytotoxicity at EO concentrations required for disease suppression (Plotto et al. 2003, Wogiatzi et al. 2009). Other investigations have found that EO phytotoxicity is dose - dependent (Hazrati et al. 2018, Rolli et al. 2014, Ibáñez & Blázquez 2018). The conflicting results of these studies may reflect the different pathogens, EO and methods tested, but they emphasize the need for additional screening of EO phytotoxicity to tomatoes and other host plants of thrips. Furthermore, EO are advantageous to synthetic insecticides in cropping systems because their low human toxicity provides a significantly reduced restricted entry interval (REI) (Regnault-Roger et al. 2012).

1.5 Western Flower Thrips and Insecticide Resistance

Among thrips, *F. occidentalis* is of particular importance due to its superior vector competence with respect to Tomato spotted wilt virus (Wijkamp 1993, Nagata et al. 200)**.** The potential of EO to control *F. occidentalis* is especially promising, considering its resistance to conventional insecticides (Immaraju et al. 1992, Martin & Workman 1994, Cloyd 2009). This resistance is achieved through four mechanisms: detoxification enzymes, reduced penetration of toxicants, alterations of target sites of toxicants and behavioral resistance (Gao et al. 2012). Just as EO can synergize with each other to heighten their insecticidal activity, resistance mechanisms can also synergize with each other to enhance resistance. While laboratory trials are useful in ranking the

effectiveness of various treatments, they often fail to mimic conditions under which thrips exist in cropping systems (Hansen et al. 2003).

1.6 Future Studies

Behavioral studies of adult females are important to determine the effect of fumigants on rates of oviposition behavior, feeding, and mating. Toxicity to thrips eggs and pupa should also be assessed, seeing as Janmaat et al. (2002) found that treatments effective against adults and larvae were less effective against eggs and pupa.

Considering the resistance to conventional insecticides in *F. occidentalis* populations, resistance to EO is also likely to evolve. Long - term studies of thrips treated with EO are warranted to determine the rate of resistance build - up. Additionally, integrated pest management strategies must be encouraged to reduce chemical inputs and delay the onset of resistance. Even if EO fumigants alone cannot achieve the same insecticidal activity as synthetic insecticides, their incorporation into broader IPM programs can likely sustain thrips control.

The present study seeks to compare the insecticidal efficacies of the enantiomers of linalool applied via polymer release against thrips by conditioning these EO into the matrices of polyacrylamide hydrogels. Hydrogels were conditioned in varying ratios of polyacrylamide mass to EO volume to determine how hydrogel saturation affected insecticidal efficacy. After identifying the most effective EO formulation (linalool enantiomer and degree of saturation), binary mixtures of linalool and adjuvants were conditioned into hydrogels and applied to thrips to identify synergists and antagonists. The most potent treatments from binary mixture trials were identified and applied to *S. lycopersicum* seedlings to delineate thresholds for phytotoxicity. Lastly, these same treatments were applied to *F. occidentalis* in caged-plant trials to determine if systems involving host plants affected the capability of EO to control thrips.

Chapter 2: Fumigant toxicity of (*R*)-linalool to *F. insularis* and *F. occidentalis*, *in vitro* trials

2.1 Abstract

Thrips, along with the viruses that they vector, are among the most economically significant pests of greenhouse crops. I assessed the fumigant toxicity of linalool against two species of thrips, *Frankliniella occidentalis* and *Frankliniella insularis*. Both species were exposed to linalool in fumigation chambers (5.8 cm diameter x 6.8 cm height) by

incorporating essential oils into polyacrylamide hydrogels. The tested hydrogels contained either (*R*)-linalool, racemic linalool, or a binary mixture of (*R*)-linalool with one of twelve other essential oils (i.e., peppermint, cedarwood, neem, clove, coconut, jojoba, soybean, olive, α-terpineol, 1,8-cineole, trans-anethole and (*R*)-pulegone). Polyacrylamide conditioned in pure distilled water served as controls, and four replicates were performed for each observation. Linalool conditioned in the least saturated hydrogels was more toxic, regardless of the enantiomer of linalool. The *R* enantiomer, with an LC_{50} of 11.7 mg L^{-1} air, was more toxic to *F. insularis* than the *S* enantiomer, with an LC_{50} of 16.7 mg L^{-1} air. Similarly, for *F. occidentalis*, the *R* enantiomer was more toxic than the *S* enantiomer, with LC_{50} values of 29.0 mg L^{-1} air and 34.9 mg L^{-1} air, respectively. Our results showed that peppermint oil and α-terpineol were the only synergists. The other oils exhibited varying degrees of antagonism. Our study provides a promising alternative for thrips control by incorporating linalool into hydrogels.

2.2 Introduction

Frankliniella occidentalis Pergande (Thysanoptera: Thripidae), the western flower thrips, is one of the most significant agricultural pests globally. This pest can inflict a high degree of damage on a range of crops encompassing more than 500 species spanning 50 plant families (Mau 1993). This includes ornamentals (e.g., orchid, rose, chrysanthemum, etc.) and cultivated crops (e.g., tomato, cucumber, lettuce, etc.) (Yudin et al. 1986). *F. occidentalis* is the most effective vector of tospoviruses such as Tomato spotted wilt virus (TSWV) and Impatiens necrotic spot virus (Wijkamp et al. 1993, Nagata et al. 2002, Robb et al. 1995). TSWV alone causes $1 billion/year in crop damage globally (Goldbach & Peters 1994). This estimate does not include the direct damage caused by the thrips, and it further underestimates present-day losses as *F. occidentalis* has continued to expand its geographical range to become nearly cosmopolitan (Stuart et al. 2011, Kirk & Terry 2003). In contrast, *Frankliniella insularis* Franklin (Thysanoptera: Thripidae) is a relatively minor pest of leguminous crops (Lima et al. 2013). Its inability to vector tospoviruses paired with its narrow host range (Hoddle & Mound 2021) has resulted in a lower level of insecticide exposure and insecticide resistance.

Frankliniella occidentalis has elicited chemical control mainly in the form of organophosphates, carbamates, pyrethroids, and some newer insecticide chemistries. However, the efficacy of these insecticides is unsustainable and fleeting on *F. occidentalis*. Some synthetic insecticides can induce genotoxic, mutagenic, and carcinogenic effects in humans (Sandal and Yilmax 2011, Sobti et al. 1982, Kaushik & Kaushik 2007). Due to their hydrophobic nature, these insecticides are readily absorbed into organic tissue and persist for long periods in soil and water (Lal & Saxena 1982).

Furthermore, 100-fold resistance can develop in as few as 20 generations in members of the Thripidae (Fu et al. 2018, Riley & Pappu 2000).

These issues with conventional insecticides for thrips management have created a demand for alternative control methods against thrips. Linalool, an alcoholic monoterpene, is produced by members of the Lamiaceae, Rutaceae, and Lauraceae. This molecule has long been sought after for its unique odiferous and culinary properties, but recently it has been found to possess insecticidal properties as well (Kamatou & Viljoen 2008). Relatively low concentrations are needed to achieve repellency, mortality (Riefler & Koschier 2009, Stepanycheva et al. 2019), and reduced feeding or oviposition behavior (Koschier et al. 2002). However, direct application of linalool as a spray can be less effective because of its volatility (Moretti et al. 2002).

Insecticides are available in an array of formulations (polymer release, foliar sprays, aerosols, ultra-low volume, etc.) that vary in efficacy with the insecticide and pest in question. In agriculture, fumigation can refer to various pre- and post-harvest pest control protocols (soil fumigation, commodity fumigation, crop fumigation). The present work refers to fumigants as organic, volatile compounds that form vapors above 5°C. Polymer release refers to the permeation-regulated diffusion of essential oils from within the matrices of polymers onto target surfaces (Roy et al. 2014). Hydrogels are highly absorbent polymers, composed of matrices of crosslinked subunits through which essential oils emigrate to reach thrips (Rudzinski et al. 2002). Incorporating essential oils into the matrices of hydrogels allows them to retain their toxicity while slowing their volatilization to varying degrees depending on temperature, polymer pore size, and essential oil constituents (Vishwakarma et al. 2016). This “slow-release” allows the essential oil to persist in plantings so that it can impact existing and incipient populations. Continuous exposure of essential oils to the target pest attainable through release from polymer carriers is necessary considering that thrips thigmotactic behavior and resistance of eggs and pupal stages to pesticides (Janmaat et al. 2002) can increase the likelihood that thrips survive individual applications. Picard et al. (2012) demonstrated that essential oils incorporated into the matrices of alginate and methyl cellulose repelled *F. occidentalis* for longer than treatment solutions lacking these polymers (Picard et al. 2012). Hydrogels have the added benefit of stabilizing linalool against environmental degradation (e.g., light, air, and humidity), reducing mammalian toxicity and human mucous-membrane irritation, reducing phytotoxicity or fish toxicity, reducing evaporation and leaching and reducing environmental pollution and drift (Rudzinski et al. 2002, Regnault-Roger et al. 2012). These polymers are remarkably varied in their applications, also functioning as vehicles to deliver liquid baits to social insects (Tay et al. 2020).

In this study, I assessed the potential of both enantiomers of linalool as a biopesticide against two species of thrips (*F. occidentalis* and *F. insularis*). I compared the insecticidal efficacy of essential oils conditioned in polyacrylamide hydrogels of

varying degrees of saturation to determine if the ratio of essential oil volume to polymer mass impacted insecticidal efficacy. In adjuvant trials, I sought to determine if mixtures of essential oils demonstrated synergistic, additive or antagonistic effects on insecticidal efficacy for both species. For antagonistic adjuvants, I investigated if the decomposition of linalool was responsible for the reduced insecticidal activity. I hypothesized that *F. occidentalis* was more resistant than *F. insularis*. Furthermore, I predicted that (*R*)-linalool would be more toxic than (*S*)-linalool, and that the most saturated hydrogels would be the most effective against both species of thrips.

2.3 Materials and Methods

2.3.1 Test Organisms

2.3.1.1 *Frankliniella occidentalis* Rearing

A laboratory colony of *F. occidentalis* was started from approximately 300 mixed age thrips collected from a *Cucumis sativus* Linnaeus (Cucurbitaceae) greenhouse in Waimea, Hawai'i as previously described (Nicholas & Follett 2018). Rearing chambers for *F. occidentalis* consisted of cylindrical plastic jars (12 cm diameter x 17 cm height) whose caps were fitted with wire mesh to allow ventilation. Each rearing chamber was provided with cabbage, *Brassica oleracea* Linnaeus (Brassicaceae), leaves (8 cm x 8 cm) smeared with 0.1 mL of honey. *F. occidentalis* individuals were deposited into the chambers with a No. 6 paintbrush and allowed to feed and oviposit for 3-day intervals. At each interval, cabbage leaves were transferred to emergence chambers, and rearing chambers were provisioned with new cabbage leaves smeared with honey. Upon eclosion, nymphs were transferred from emergence chambers to rearing chambers. The process was repeated *ad infinitum*. Colonies were maintained at 26 ± 2 °C and 80 ± 5% relative humidity (RH) under a 16:8 L:D photoperiod.

2.3.1.2 *Frankliniella insularis* Rearing

Frankliniella insularis were collected from a hedgerow of *Malvaviscus arboreus* Dill (Malvaceae) in Honolulu, Hawai'i (21.297111, -157.819151) and deposited in jars identical to those used for *F. occidentalis*. Each rearing chamber was provisioned with snow pea pods smeared with 0.1 mL of honey. The transfer of nymphs from emerging chambers and rearing chambers followed the same protocol as *F. occidentalis*. Colonies were maintained at 26 ± 2 °C and 80 ± 5% RH under a 16:8 L:D photoperiod.

2.3.2 Chemical Preparation

Polysorbate 20, both (*S*)- and (*R*)-enantiomers of linalool, 1,8-cineole, α-terpineol, trans-anethole, and (*R*)-pulegone, were purchased from Sigma–Aldrich (St. Louis, MO, USA). Clove, coconut, cedarwood, and peppermint oil were purchased from Carolina Biological Supply Company (Burlington, NC, USA). Neem oil was purchased from Monterey Lawn and Garden Products, Inc. (Fresno, CA, USA). Jojoba, olive, and

soybean oil were purchased from local grocery retailers. The dilutions of (*S*)-linalool (≥97%), (*R*)-linalool (≥95%) and polysorbate 20 included 0, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, and 52 mg L^{-1} air. Binary mixtures of essential oils were prepared by mixing the LC_{50} of (*R*)-linalool for each thrips species (as determined from 2.4.1 Fumigant Toxicity to Thrips, below) with varying concentrations (0, 6, 12, 18, 24, and 30 mg L^{-1} air) of the adjuvant. Pure distilled water (0 mg L^{-1} air essential oil) acted as negative controls for thrips assays. Dilutions were prepared in distilled water, with the volume of (*R*)-linalool + volume of the adjuvant added at a ratio of 1:1 to the volume of polysorbate 20.

2.3.3 Bioassays

2.3.3.1 Fumigation Chamber Assembly

Each fumigation chamber consisted of a glass jar (5.8 cm diameter x 6.8 cm height) provisioned with either an *M. arboreus* petal (for *F. insularis*) or piece of cabbage (for *F. occidentalis*). For ventilation, 2 mm holes were melted into the caps of each jar with 4 mm squares of mesh cloth glued on top. To create a structure from which to suspend the hydrogels, the mesh cloth was cut into 5 cm x 1.5 cm rectangles. The shorter ends of these rectangles were glued to the inside of the plastic cap of each jar 4 cm apart from each other such that they formed “pouches” into which the hydrogels were inserted (Figure 1).

Figure 1. Thrips fumigation chamber with hydrogel “pouch”.

2.3.3.2 Hydrogel Conditioning

Polyacrylamide was purchased as Miracle Gro™ Water Storing Crystals (Miracle-Gro Lawn Products, Marysville, OH). Three series of hydrogels were evaluated for both

enantiomers, each for a different level of hydrogel saturation. Series one represented polyacrylamide saturated in 100-fold its volume (0.1 mL solution/mg polyacrylamide), series two represented 200-fold saturation (0.2 mL/mg), and series three represented 300-fold saturation (0.3 mL/mg). Polyacrylamide crystals (0.01 g per Petri dish) were weighed using Ohaus PR224 PR Series Analytical Balance (Ohaus Corporation, Parsippany, NJ), and the corresponding linalool dilution (from 2.3.2 Chemical Preparation) was pipetted into each. The petri dishes were capped, and the hydrogel crystals were conditioned to allow uptake of the volatile compound by the gel for at least 24 hours prior to bioassays (Figure 2).

Figure 2. (R)-linalool conditioned in 100-fold saturated polyacrylamide.

2.3.3.3 Fumigant Toxicity Assay

For each fumigation treatment described in 2.3.2 Chemical Preparation and 2.3.3.2 Hydrogel Conditioning, 0.3 grams of conditioned hydrogel was inserted into the “pouch”. Ten adult thrips were carefully transferred with a No. 6 paintbrush into each chamber. Each treatment was performed in replicates of four, with each chamber of ten thrips counted as a single observation. Mortality was assessed after 24 hours by counting the number of live and dead thrips. Individuals were determined to be dead if they did not respond to mechanical stimuli or exhibited *rigor mortis*.

2.3.4 Nuclear Magnetic Resonance Sample Preparation

Nuclear Magnetic Resonance (NMR) analysis was performed to determine if the dehydration products of linalool (ocimene and myrcene) were present in the treatment solutions. Five samples were prepared for NMR: 11.7 mg L^{-1} (*R*)-linalool and 24.0 mg L^{-1} neem, 11.7 mg L^{-1} (*R*)-linalool and 30.0 mg L^{-1} neem, 11.7 mg L^{-1} (*R*)-linalool and 18.0 mg L^{-1} cedarwood oil, 11.7 mg L^{-1} (*R*)-linalool and 24.0 mg L^{-1} cedarwood oil, and 11.7 mg L^{-1} (*R*)-linalool and 18.0 mg L^{-1} (*S*)-linalool.

Each sample was prepared directly in the corresponding NMR tube, consisting of 600 µL D_2O and polysorbate 20 added in a 1:1 ratio to the total volume of essential oil. Deuterated chloroform was added to improve the immiscibility of the oils. The 1H NMR experiments were carried out on Agilent 400 DD2 and 600 spectrometers (Agilent Technologies, Palo Alto, CA, USA). Each experiment was performed at room temperature with 256 scans per sample.

2.3.5 Statistical Analysis

Degrees of freedom varied among essential oil treatments because escalating concentrations of each treatment were tested until 100% mortality was achieved for at least 3 successive concentrations. The more lethal oils (e.g. peppermint oil) consistently achieved 100% mortality at lower concentrations, at which point the testing of higher concentrations was superfluous. In contrast, less lethal oils (e.g. cedarwood) required a higher range of concentrations before 100% mortality was achieved.

Data were analyzed using SPSS version 26.0 (IBM Corp 2021). Average mortality data for each treatment from thrips bioassays (hydrogel saturation and binary mixtures with adjuvants) were subjected to probit analysis to estimate the lethal concentration (LC) values. LC values were considered significantly different when their 95% confidence intervals did not overlap.

The binary interactions of essential oils against adult *F. insularis* and *F. occidentalis* were quantified according to the synergism ratio (SR) (Chadwick 1961, Metcalf 1967):

$$SR = LC_{50} \text{ of pure } (R)\text{-linalool} / LC_{50} \text{ of mixture}$$

A synergism ratio of 1.0 indicated that the toxicity of pure (*R*)-linalool was equal to the toxicity of the mixture of (*R*)-linalool and the adjuvant. A value > 1.0 indicated synergism, a value < 0.5 was classified as strong antagonism, and a synergism ratio > 0.5 but < 1.0 was classified as weak antagonism.

Figures 3 and 4 were constructed in SigmaPlot version 14.5 (Systat Software 2021).

2.4 Results

2.4.1 Fumigant Toxicity to Thrips

For *F. insularis*, the median lethal concentrations (LC_{50}) for (*R*)-linalool conditioned in hydrogels with 100-, 200-, and 300-fold saturation were 11.7 (10.0-13.4), 18.1 (14.7-21.4), and 20.6 (19.3-21.8) mg L^{-1} air, respectively, while the LC_{90} values were 18.0 (16.0-21.2), 30.2 (26.2-37.4), and 30.2 (28.4-32.4) mg L^{-1} air, respectively (Figure 3).

For *F. occidentalis*, the LC_{50} values for (*R*)-linalool conditioned in hydrogels with 100-, 200-, and 300-fold saturation were 29.0 (27.1-30.9), 35.1 (33.7-36.5), and 38.9 (37.4-40.4) mg L^{-1} air, respectively, while the LC_{90} values were 36.5 (34.1-40.3), 47.7 (45.6-50.3), and 52.8 (50.4-55.7) mg L^{-1} air, respectively (Figure 3).

No mortality occurred in untreated controls (pure distilled water conditioned in hydrogels). For almost all treatments, (*R*)-linalool conditioned in the least saturated (100-fold) hydrogels of either enantiomer was the most toxic, and the *R* enantiomer was more toxic than the *S* enantiomer. The least saturated polyacrylamide (1 mL solution/mg polyacrylamide) formed the smallest hydrogels with the greatest cumulative surface area, while the most saturated polyacrylamide (3 mL solution/mg polyacrylamide) formed the largest hydrogels with the least cumulative surface area.

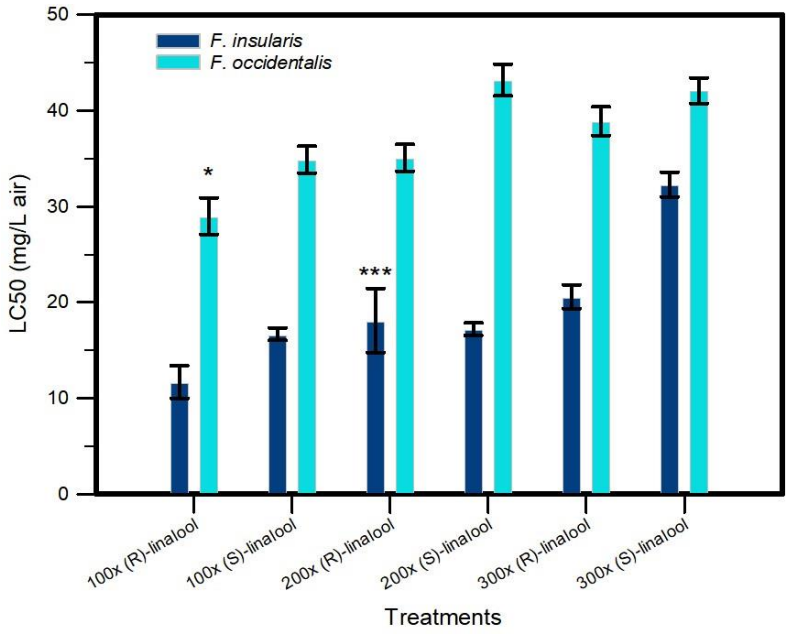

Figure 3. Fumigant toxicity of linalool enantiomers conditioned in hydrogels of varying degrees of saturation against *F. insularis* and *F. occidentalis*. LC_{50} = Concentration to kill 50% of thrips. Error bars indicate upper and lower confidence limits for each LC_{50} value. LC_{50} values that could not be calculated due to insufficient or excessive mortality were not included. p-values obtained using a χ2-test.; LC_{50} values are significantly different at $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

2.4.2 Adjuvant Toxicity to Thrips

For *F. insularis*, peppermint oil was the only synergist, with an LC_{50} of 10.7 (7.3-12.8) and LC_{90} of 16.2 (14.4-18.2) mg L^{-1} air (Figure 4). α-Terpineol induced 100% mortality at all tested concentrations. Several adjuvants (i.e., (*S*)-linalool, cedarwood, neem, and 1,8-cineole) behaved as mild antagonists. The remaining adjuvants (i.e., trans-anethole, (*R*)-pulegone, clove, coconut, jojoba, olive, and soybean) behaved as

severe antagonists (Table 2).

Similar trends were observed in *F. occidentalis* relative to pure (*R*)-linalool with LC_{50} of 29.0 mg L^{-1} air. α-terpineol was the only synergist, with an SR ratio of 1.090 (Table 2). While peppermint oil was not classified as a synergist in *F. occidentalis*, it had the second greatest insecticidal efficacy with an LC_{50} of 30.0 (24.5-33.0) and an LC_{90} of 43.4 (41.0-47.4) mg L^{-1} air (Figure 4). Another four adjuvants (i.e., (S)-linalool, 1,8-cineole, trans-anethole, and (R)-pulegone), behaved as mild antagonists while the remaining adjuvants (i.e., cedarwood, neem, clove, coconut, jojoba, olive, and soybean) behaved as severe antagonists (Table 2).

No mortality occurred in untreated controls (pure distilled water conditioned in hydrogels).

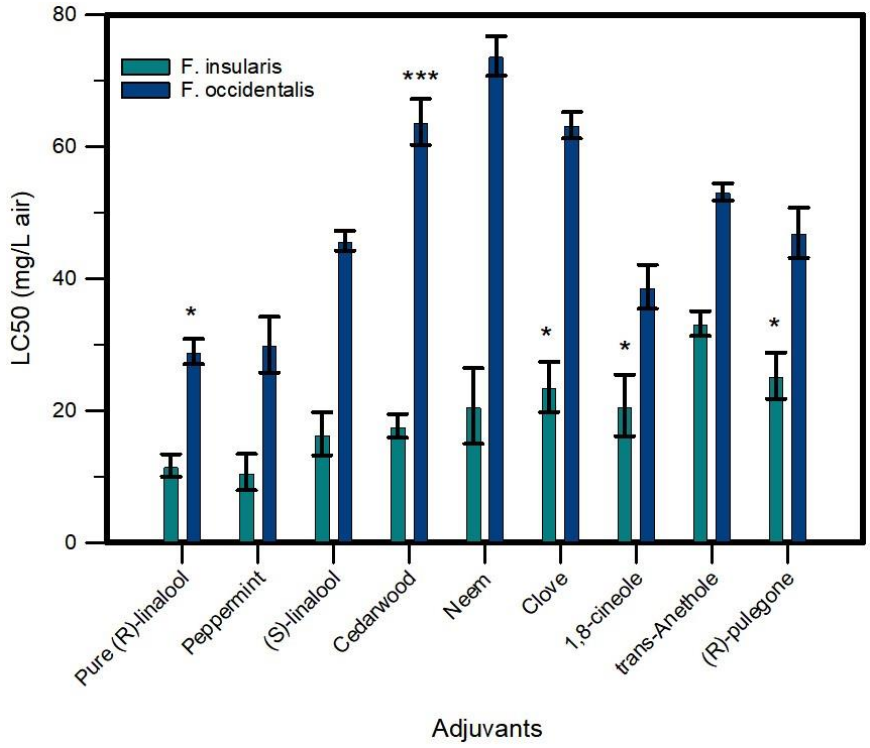

Figure 4. Fumigant toxicity of adjuvants applied with (*R*)-linalool against *F. insularis* and *F. occidentalis*. LC_{50} = Concentration to kill 50% of thrips. Error bars indicate upper and lower confidence limits for each LC_{50} value. LC_{50} values that could not be calculated due to insufficient or excessive mortality were not included. p-values obtained using a χ2-test.; LC_{50} values are significantly different at $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

Table 2. Synergistic ratios (SR) of adjuvants to (*R*)-linalool for *F. insularis* and *F. occidentalis*.

Adjuvant	*F. insularis*	*F. occidentalis*
Peppermint and (*R*)-linalool	1.093	0.967
(*S*)-linalool and (*R*)-linalool	0.709	0.633
Cedarwood and (*R*)-linalool	0.661	0.455
Neem and (*R*)-linalool	0.565	0.393
Clove and (*R*)-linalool	0.496	0.458
1,8-Cineole and (*R*)-linalool	0.563	0.747
trans-Anethole and (*R*)-linalool	0.352	0.545
(*R*)-Pulegone with (*R*)-linalool	0.462	0.617
α-Terpineol with (*R*)-linalool	N/A	1.090

N/A = Not assessed due to 100% mortality at all tested concentrations

2.4.3 Nuclear Magnetic Resonance Analysis

Dehydration products (ocimene and myrcene) were not detected in samples containing (*S*)-linalool or cedarwood oil as adjuvants, but they were detected in samples with neem oil as peaks from 5.80-5.95 ppm. These two samples had nearly identical spectra; therefore, only sample one (Figure 5) is shown because it had peaks of greater intensity in this range. The peaks for ocimene and myrcene had low intensity, indicating that the dehydration products did not constitute a significant portion of the samples (<1%). Linalool formed strong peaks throughout the spectra at 1.27, 1.56, 1.60, 1.68, 1.85, 5.02, 5.11, and 5.20 ppm due to the varying chemical environments of the 1H nuclei along linalool. Polysorbate 20 produced a strong peak at 3.64 ppm.

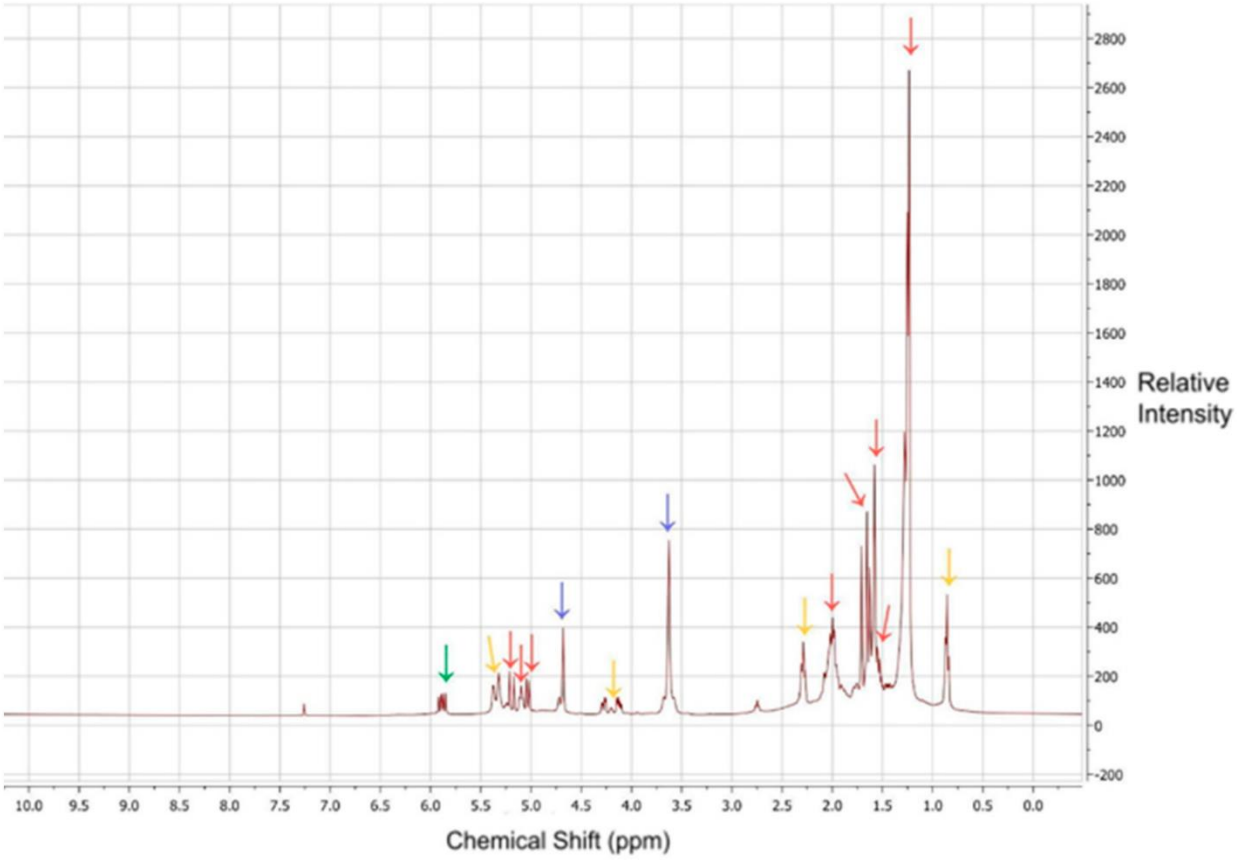

Figure 5. NMR spectra for sample 1. The green arrow indicates peaks for potential dehydration products, red arrows indicate peaks for (*R*)-linalool, blue arrows indicate peaks for polysorbate 20 and yellow arrows indicate peaks for triglycerides and similarly unsaturated fatty acids.

2.5 Discussion

2.5.1 Hydrogel Saturation and Enantioselective Toxicity

Hydrogels conditioned to varying degrees of saturation produced gels of different size, with varying surface areas. The least saturated gels had the greatest cumulative surface area and strongest insecticidal activity. In contrast, polymers conditioned in 3 mL solution/mg polyacrylamide had the least cumulative surface area and weakest insecticidal activity. Considering that surface area is directly proportional to reaction rate, these differences are likely due to varying rates of diffusion from linalool-conditioned hydrogels (Germain et al. 2006). The least saturated hydrogels possessed the greatest cumulative surface area, providing more avenues for linalool to emigrate out of to contact thrips. In contrast, the most saturated hydrogels had the least cumulative surface area, trapping a percentage of linalool to prevent it from contacting the target pest for the duration of the trials.

The enantioselective toxicity of linalool toward thrips was demonstrated in this study; (*R*)-linalool was 35% more toxic than (*S*)-linalool to *F. insularis* and 18.5% more toxic to *F. occidentalis*. Enantioselective toxicity to animals and plants is well documented in many other stereoisomeric compounds (Lordelo et al. 2005, Overmyer et al. 2007, Liu et al. 2005, Sun et al.2018, Wang et al. 2009).

While linalool can inhibit multiple targets in the insect nervous system (acetylcholinesterase, gamma-aminobutyric acid, octopaminergic nervous system), acetylcholinesterase (AChE) appears to be the primary inhibitory target (Shaaya & Rafaeli 2007, Vicenço et al. 2021, López & Pascual-Villalobos 2009). This enantioselective toxicity is thought to be the result of (*R*)-linalool being a more potent AChE inhibitor than (*S*)-linalool. Linalool's ability to bind to AChE is dependent on the alcoholic monoterpenoid's ability to form intermolecular forces (i.e., hydrogen bonds, hydrophobic interactions, Van der Waals) with certain amino acids in AChE (Taktak & Badawy 2019, Farag et al. 2016, Praveena & Sanjayan 2011). It is possible that enantioselective intermolecular forces occur in the (*R*)-linalool-AChE complex but not in the (*S*)-linalool-AChE complex. Since the orientation of the bond of the hydroxyl group to the 3' carbon (Agostini-Costa et al. 2012) was the only difference between these enantiomers, it was likely responsible for the enantioselective formation of intermolecular forces and the consequent steric hindrance (or lack thereof) for the inhibitor-enzyme complexes.

2.5.2 Synergisms

Synergisms resulted when both components of a binary mixture had unique inhibitory targets. The (*R*)-linalool and peppermint oil synergism serves as an example. Linalool inhibits AChE, while peppermint oil inhibits gamma-aminobutyric acid (GABA) (López & Pascual-Villalobos 2009, Heimes et al. 2011). When combined and applied to *F. insularis*, the mortality was higher than that of (*R*)-linalool applied individually

because the simultaneous inhibition of AChE and GABA was more damaging than only AChE inhibition (Lu et al. 2020). Alternatively, these synergisms may reflect the exceptional insecticidal activity of the adjuvants, rather than their interaction with (*R*)-linalool.

2.5.3 Weak Antagonisms

Weakly antagonistic mixtures were those with synergistic ratios less than 1.0 but greater than 0.5. This phenomenon is thought to occur due to overlapping modes of action of (*R*)-linalool and the adjuvant, with the racemate serving as an example in both species (LC_{50} 16.5 mg L^{-1} air for *F. insularis*, LC_{50} 45.8 mg L^{-1} air for *F. occidentalis*). The enantiomers of linalool were expected to have similar modes of action, considering their structural similarity (Agostini-Costa et al. 2012). This overlap in inhibitory targets does not increase mortality because only AChE function was diminished. It is possible that the components of this binary mixture shared not only the same inhibitory target but also the same active site on that target.

2.5.4 Nuclear Magnetic Resonance Analysis

Another possible explanation for the mild antagonisms described above was the decomposition of linalool under low pH, leading to a reduction in its insecticidal efficacy (Chang et al. 2009). Linalool is prone to dehydration because it is not only a tertiary alcohol but also an allylic alcohol. Tertiary alcohols are more reactive because the presence of additional alkyl groups increases the inductive effect. The charge density around the tertiary carbon increases and in the C-O bond consequently, which facilitates the cleavage of that bond (Salvatella 2017). Allylic carbons are more reactive than simple alkanes due to their proximity to adjacent π systems (Hunt 2021). Theoretically, solutions containing neem, cedarwood or *(S*)-linalool as adjuvants caused (*R*)-linalool to dehydrate into a mixture of products (myrcene and ocimene) via unimolecular elimination (Ma 2020). As the proportion of (*R*)-linalool decreased while ocimene and myrcene increased, the insecticidal activity was reduced because these dehydration products had relatively less insecticidal activity (Park et al. 2003, Arena et al. 2018).

However, the absence of strong peaks for dehydration products in the NMR spectra of these solutions revealed that ocimene and myrcene did not occur in samples containing cedarwood oil or (*S*)-linalool. While dehydration products were detected in solutions containing neem oil as an adjuvant, these products did not occur as a significant enough proportion (<1%) to be responsible for the observed reduction in insecticidal efficacy (Figure 5). Nonetheless, their presence highlights the value of detecting biopesticide decomposition. Other adjuvants may influence insecticidal activity by creating conditions conducive to linalool's decomposition. Therefore, identification of the characteristics of these adjuvants can help to exclude potential antagonists.

2.5.5 Strong Antagonisms

In contrast, strong antagonisms arose from the behavior of five oils (clove, coconut, jojoba, soybean, and olive oil) as fixatives. These oils possess synergistic ratios < 0.5, high boiling points and low vapor pressures. Also known as carrier oils, they depress the volatility of other components in the mixture (Songkro et al. 2012, McGovern & Beroza 1967). However, the combined volatility depression of the hydrogel matrix and the fixative rendered (*R*)-linalool so nonvolatile that mortality was significantly reduced for two oils (olive and clove oil) and completely reduced for three oils (coconut, jojoba, and soybean oil). Van der Waals and dipole–dipole interactions between (*R*)-linalool and the fixative potentially contributed to this non-volatility (Hidayatulfathi 2019). Some oils (cedarwood and neem) behaved as weak antagonists for *F. insularis*, but were strong antagonists for *F. occidentalis*, and *vice versa* (trans-anethole and (*R*)-pulegone). The former may be a result of the heightened resistance of *F. occidentalis* to a range of insecticides. The latter phenomenon requires *in vitro* studies of insect nervous systems to clarify the mechanism responsible for this discrepancy.

2.5.6 Bioinsecticide Resistance

The LC_{50} of *F. occidentalis* for both enantiomers of linalool and the adjuvant mixtures was consistently higher than that of *F. insularis*. For example, pure (*R*)-linalool had an LC_{50} of 11.7 mg L^{-1} air in *F. insularis* and an LC_{50} of 29.0 mg L^{-1} air in *F. occidentalis* (Figure 4).

Considering that essential oil fumigation is a relatively novel method, it is improbable that *F. occidentalis* acquired resistance through previous exposure. However, there are records of resistance to synthetic pesticides in *F. occidentalis*, many of which share a mode of action with the essential oils in this study (Immaraju et al. 1992, Gao et al. 2012, Kirk & Terry 2003, Rosello et al. 1996, Reitz & Funderburk 2012, Denholm & Jesperson 1998). The cross-resistance obtained through exposure to other AChE inhibitors (e.g. carbamates and organophosphates) may confer resistance to linalool. Resistance has been documented in closely related species in Hawai'i (Hara et al. 2002, Mau & Gusukuma-Minuto 1998) and in *F. occidentalis* populations in California, China and New Zealand (Kirk & Terry 2003, Gao et al. 2012, Martin & Workman 1994). While resistance may have arisen on the archipelago independently of immigration, it is possible that the trans-Pacific trade of plant material spread *F. occidentalis* populations bearing resistance genes to Hawai'i (Denholm & Jesperson 1998).

Of the 19 synthetic pesticides registered for the management of thrips in the USA, at least five (i.e., abamectin, acephate, chlorpyrifos, methiocarb, and spinosad) have one or both of the same modes of action as the essential oils tested in our study (Cloyd 2009). There are multiple mechanisms potentially responsible for the increased

resistance of *F. occidentalis* compared to *F. insularis*. However, most insecticide-resistant cases result from metabolic detoxification (e.g., cytochrome P450s) (Brattsten et al. 1986). Resistance mechanisms can co-occur and synergize. For example, thigmotaxis may result in a reduced rate of entry of linalool into thrips bodies, enabling metabolic detoxification to occur without P450s being overwhelmed (Gao et al. 2012). While exposure to insecticides undoubtedly plays a role in the development of resistance in *F. occidentalis*, the highly polyphagous nature of this species might also be responsible for its predisposition to metabolic detoxification (Reitz & Funderburk 2012). Due to its widely varied diet, *F. occidentalis* often needs to detoxify allelochemicals produced by some of their host plants. *F. insularis* has a narrower host range; therefore, there is reduced selective pressure for metabolic detoxification.

In terms of the integration of essential oil fumigation with biological control in greenhouses, it is important to consider the impact of essential oils on predators and parasitoids. *Orius strigicollis* Poppius (Hemiptera: Anthocoridae) is a biocontrol agent of thrips which possesses a greater degree of resistance to essential oil fumigation than *Thrips palmi* Karny (Thysanoptera: Thripidae) (Kim et al. 2015, Yi et al. 2006). *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) is another natural enemy of thrips shown to have a degree of resistance to essential oil fumigation (Born et al. 2018, Han et al. 2010). However, whether the resistance of these biocontrol agents is greater than that demonstrated by *F. occidentalis* is yet to be investigated.

2.6 Conclusion

I conducted a series of fumigation assays to assess the vulnerability of *F. occidentalis* and *F. insularis* to fumigation with essential oils via polymer release. Essential oils conditioned in polyacrylamide of the lowest saturation were the most effective, which had the added benefit of requiring the least volume of essential oil. Both species of thrips demonstrated enantioselective toxicity to linalool. However, *F. occidentalis* was significantly more resistant to these treatments than *F. insularis*. These findings underline the need to assess the potential of synthetic synergists (e.g. piperonyl butoxide, diethyl maleate, etc.) applied in tandem with essential oils to disarm insecticide resistance in *F. occidentalis*. Furthermore, the resistance of natural enemies to essential oils should be evaluated to determine if it surpasses the resistance of *F. occidentalis* demonstrated herein.

Chapter 3: Phytotoxicity of essential oils to *S. lycopersicum*

3.1 Abstract

Tomato, *Solanum lycopersicum* L. (Solanaceae) is one of the most economically significant and widely cultivated horticultural crops. It is afflicted by a diverse assemblage of pests and diseases, of which the western flower thrips -Tomato spotted wilt virus (TSWV) complex is particularly damaging. Tomato seedlings were screened for their sensitivity to the most potent fumigants, as determined from *in vitro* bioassays. In phytotoxicity assays, *S. lycopersicum* was exposed to (*R*)-linalool or a binary mixture of (*R*)-linalool and peppermint oil through fumigation (i.e., conditioned hydrogel) or direct foliar spray. Phytotoxicity was assessed by measuring the length of the root and hypocotyl after exposure to the tested concentrations of (*R*)-linalool (21.56 mg L^{-1} air, 29.00 mg L^{-1} air, or 36.89 mg L^{-1} air) and (*R*)-linalool with peppermint oil (29.00 mg L^{-1} air with 3.98 mg L^{-1} air, 11.90 mg L^{-1} air, or 19.90 mg L^{-1} air). All seedling treatments caused root and hypocotyl lengths to be shorter than those of controls. These reductions were most severe for foliar sprays and mixtures containing peppermint oil. Our results indicate that *S. lycopersicum* is sensitive to essential oils applied at concentrations required to control *F. occidentalis*, even when essential oils are applied as fumigants instead of foliar sprays.

3.2 Introduction

Tomato (*Solanum lycopersicum* L., Solanaceae) is one of the most significant horticultural crops, with a global production of 181 million metric tons occupying 5 million hectares, valued at more than $88 billion/year (Food and Agricultural Organization of the United Nations n.d.). Similar to field cultivation of tomatoes, greenhouse cultivation is associated with a high disease pressure (Peet 2012). The western flower thrips - Tomato spotted wilt virus (TSWV) complex is of particular importance to tomatoes. TSWV is one of the most injurious diseases of tomatoes in parts of the world, capable of reducing yield by up to 100% (Roselló et al. 1996, Sevik & Arli-Sokmen 2012). This tospovirus is persistently transmitted by at least 9 species of thrips (Jones 2005) in a propagative-circulative manner (Wijkamp & Peters 1993) to approximately 1,090 plant species spanning 84 families (Parrella et al. 2003). Manifestation of TSWV symptoms depends on plant genotype, developmental stage at time of infection, virus isolate and environmental conditions. Infected tomatoes may not produce fruit at all, or the fruit may be very small and unmarketable (Pataky 1991). Fruit formed after infection may develop small bumps and white or yellowish blotches, while fruit formed prior to infection appear normal. In leaf tissue, TSWV induces characteristic leaf bronzing, leaf distortion, downward leaf curling, the formation of concentric rings, necrotic spots, leaf flecking and plant stunting (Roselló et al. 1996). .

Control of thrips, and consequently of TSWV has been achieved with synthetic insecticides (Todd et al. 1996), but recently this paradigm has shifted towards

bioinsecticides such as essential oils (Koschier 2008). However, the utility of essential oils is constrained by their phytotoxic activity (Tworkoski 2002). Several Labiate essential oils have been found to be phytotoxic to tomatoes in a dose - dependent manner (Wogiatzi et al. 2009, Hazrati et al. 2018). Ibáñez and Blázquez (2018) found that peppermint oil inhibited germination, root, and hypocotyl lengths of tomato seedlings, especially at high concentrations (Ibáñez & Blázquez 2018). Similarly, Rolli et al. (2014) demonstrated that relatively low concentrations of various essential oils (1 mL/L), including those extracted from *Ocimum basilicum* L. (Lamiaceae) and *Mentha pipereta* L. (Lamiaceae), induced dieback and inhibited germination (Rolli et al. 2014). In contrast, other essential oils are capable of controlling pathogens of tomato via spray applications at concentrations that are not phytotoxic (Tomazoni et al. 2018, Lee et al. 2012). However, essential oils can be less effective when applied as sprays due to their volatility (Moretti et al. 2002). Beyond phytotoxicity, essential oils may reduce the marketability of tomatoes by influencing the taste of the crop (Plotto et al. 2003).

Few studies have assessed the fumigant toxicity of essential oils to tomatoes, and even fewer have concurrently assessed their toxicity to a viral vector and its host plant. It is imperative to delineate these thresholds to determine if application rates required to control thrips will reduce yield or marketability of the crop. The present work aims to rectify this deficiency by assessing the phytotoxicity of two application methods (fumigation and foliar sprays) and two essential oil mixtures (pure (*R*)-linalool and peppermint oil with (*R*)-linalool) against tomatoes. These essential oils were applied at concentrations required to achieve control of *F. occidentalis* as determined from *in vitro* trials. Seedlings were used for phytotoxicity bioassays because their physiological responses to stressors correspond with responses at anthesis, indicating that appropriate screening for stressors can be carried out in young plants (Zhou et al. 2017). My objectives were to determine which oil treatments (pure (*R*)-linalool or (*R*)-linalool with peppermint oil) and application method (fumigation or foliar spray) induced a higher degree of phytotoxicity. Additionally, I sought to determine if phytotoxicity occurred in *S. lycopersicum* at the LC_{50} of the two oil treatments to *F. occidentalis* as determined from *in vitro* trials. I hypothesized that the peppermint oil mixture would induce a greater degree of phytotoxicity than pure (*R*)-linalool, and that foliar sprays of these oils would be more damaging as well. Furthermore, it was predicted that phytotoxicity would occur at the LC_{50} of these treatments to *F. occidentalis*.

3.3 Materials and Methods

3.3.1 Plant Material

Solanum lycopersicum seeds (“Beefsteak”) were purchased from Koolua Farmers, Honolulu, Hawai’i. Empty and undeveloped seeds were segregated by floating in tap water and discarded. Seeds were sprouted in potting soil in black plastic pots (5 cm width x 7.5 cm height) in a glasshouse at 26$\pm$2^{o}C and 65$\pm$5% RH.

3.3.2 Phytotoxicity Assays

Solutions of pure (*R*)-linalool and (*R*)-linalool with peppermint oil were prepared according to section 2.4.2 Chemical Preparation. Pure (*R*)-linalool was prepared at 21.56, 29.00 or 36.89 mg L^{-1} air. (*R*)-linalool with peppermint oil mixtures were prepared at 29.00 mg L^{-1} air (*R*)-linalool with peppermint oil at 3.98, 11.90 or 19.90 mg L^{-1} air. Hydrogels were prepared at 0.1 mL solution/mg polyacrylamide according to section 2.4.3.2 Hydrogel Conditioning. For each hydrogel treatment, 2.70 grams of polyacrylamide was placed in each plastic jar (7.6 cm diameter x 10 cm height). For each treatment, 270 mL of solution were prepared and poured into plastic jars containing polyacrylamide crystals. Pure distilled water absorbed into polyacrylamide served as negative controls. A total of 14 treatments were applied with 10 replicates per treatment: three concentrations of pure (*R*)-linalool as a foliar spray, three concentrations of pure (*R*)-linalool as a fumigant, three concentrations of (*R*)-linalool with peppermint oil as a foliar spray, three concentrations of (*R*)-linalool with peppermint oil as a fumigant, the foliar spray negative control (0 mg L^{-1} air) and the fumigant negative control (0 mg L^{-1} air) . The corresponding dilutions were poured into jars and mixed for 5 minutes. The caps were screwed on, and essential oil mixtures were allowed to absorb into the polyacrylamide for at least 24 hours prior to the bioassays.

Upon emergence of the epicotyl, seedlings were randomly divided into groups of 10 and placed into black plastic trays with plastic humidity domes (63 cm length x 33 cm width x 22 cm height) fitted overhead (Figure 6 and 7). Plants were watered before fumigation, as previous experience has shown that plants fumigated under dry conditions are liable to be injured (Hazrati et al. 2018). For hydrogel treatments, a plastic dish containing 64 grams of conditioned hydrogel was placed in the center of the tray. For the foliar spray treatments, groups of 10 seedlings were sprayed with 5 mL of treatment solutions, evenly wetting their cotyledons. Phytotoxicity was assessed after 1 week of incubation by measuring root and hypocotyl lengths. Lengths of 0 cm were recorded for seedlings killed by treatments.

Figure 6. Dorsal view of chamber.

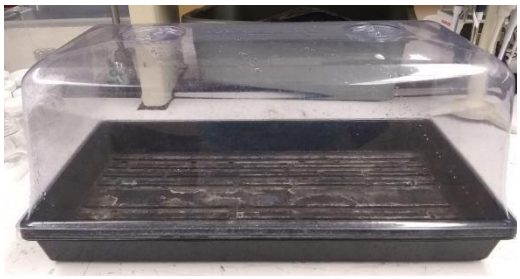

Figure 7. Lateral view of chamber.

3.3.3 Statistical Analysis

Data were analyzed using SPSS version 26.0 (IBM Corp 2021). Average root and hypocotyl lengths were subjected to one-way analysis of variance (ANOVA) to identify significant differences from control lengths due to essential oil concentration, followed by Tukey's honestly significant difference (HSD) test at the $p < 0.05$ level of significance.

A two tailed independent student's t-test was performed to identify significant differences due to the application methods (fumigation and foliar spray). Means were considered significant when $p < 0.05$. Figure 8 was constructed in SigmaPlot version 14.5 (Systat Software 2021).

3.4 Results

The observed symptoms of phytotoxicity included reduced root and hypocotyl lengths, dieback, stunting, depigmentation, and death. The most severe phytotoxicity was recorded for the peppermint oil mixture. Significant differences were found in the hypocotyl lengths of seedlings sprayed with all concentrations of (*R*)-linalool with peppermint oil and the highest concentrations of (*R*)-linalool, as compared with that of the control ($F = 2.88$, d.f. = 26, $p < 0.01$) (Table 3).

Almost all seedlings fumigated with essential oils had significantly higher root and hypocotyl lengths than seedlings sprayed with essential oils. This trend occurred even for control treatments for hypocotyl ($t = 4.95$, d.f. = 18, $p < 0.001$) and root ($t = 2.31$, d.f. = 1, $p < 0.05$) lengths. Even at the lowest concentrations, seedlings treated with peppermint oil (3.98 mg L^{-1} air) had more severe reductions in root ($t = 11.4$, d.f. = 1, $p < 0.001$) and hypocotyl ($t = 28.9$, d.f. = 1, $p < 0.001$) lengths compared to root ($t = 1.96$, d.f. = 1, $p < 0.05$) and hypocotyl ($t = 1.65$, d.f. = 1, $p < 0.01$) lengths of seedlings treated with pure (*R*)-linalool (21.56 mg L^{-1} air). The only exception to this trend were the root lengths of seedlings treated with 29.00 mg L^{-1} air pure (*R*)-linalool ($t = -1.15$, d.f. = 1, $p = 0.265$). Furthermore, these lengths were reduced to a greater extent for applications involving peppermint oil relative to pure (*R*)-linalool (Figure 8).

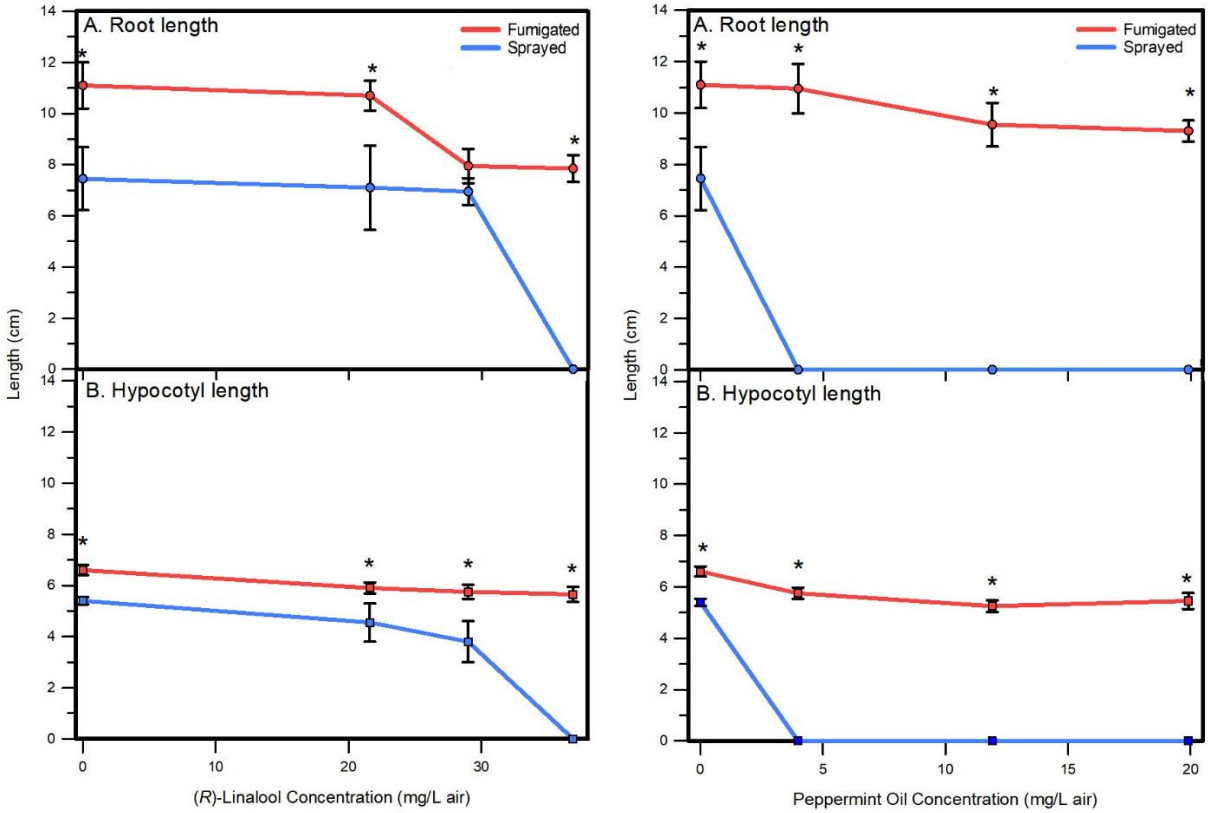

Figure 8. Phytotoxicity of pure (*R*)-linalool (left) and (*R*)-linalool with peppermint oil (right) applied as a fumigant and spray against *S. lycopersicum*. Each data point represents the average of 10 replicates ± standard error. Means accompanied by an asterisk (*) on the same line indicate statistically significant differences from control lengths (one-way ANOVA, followed by Tukey's HSD test; $p < 0.05$).

Table 3. Phytotoxicity of (*R*)-linalool and peppermint oil applied as fumigants or foliar sprays to *S. lycopersicum*.

Treatments		Foliar Spray Lengths ± SE (cm)		Hydrogel Fumigation Lengths ± SE (cm)	
		Hypocotyl	Root	Hypocotyl	Root
Control	0.00	5.40 ± 0.14 a(a)	7.45 ± 1.23 a(a)	6.60 ± 0.19 a(b)	11.1 ± 0.91 a(b)
(*R*)-Linalool Concentration (mg L^{-1} air)	21.56	4.55 ± 0.75 a(a)	7.10 ± 1.65 a(a)	5.90 ± 0.21 ab(b)	10.7 ± 0.59 ab(b)
	29.00	3.80 ± 0.80 a(a)	6.95 ± 0.52 a(a)	5.75 ± 0.28 ab(b)	7.95 ± 0.67 b(a)
	36.89	0.00 ± 0.00 b(a)	0.00 ± 0.00 b(a)	5.65 ± 0.30 ab(b)	7.85 ± 0.52 b(b)
(*R*)-Linalool with Peppermint Oil Concentration (mg L^{-1} air)	3.98	0.00 ± 0.00 b(a)	0.00 ± 0.00 b(a)	5.75 ± 0.21 ab(b)	10.95 ± 0.96 ab(b)
	11.90	0.00 ± 0.00 b(a)	0.00 ± 0.00 b(a)	5.25 ± 0.23 b(b)	9.55 ± 0.85 ab(b)
	19.90	0.00 ± 0.00 b(a)	0.00 ± 0.00 b(a)	5.45 ± 0.31 b(b)	9.30 ± 0.41 ab(b)

SE = Standard Error. Values are averages of ten replicates ± standard error after 1 week of incubation. Means followed by different letters in the same column indicate statistically significant differences from control lengths (one-way ANOVA, followed by Tukey's HSD test; $p < 0.05$). Means followed by different letters in parenthesis in the same row indicate statistically significant differences for each plant tissue lengths (hypocotyl or root) (Two-Tailed Independent Student's T-test; $p < 0.05$).

3.5 Discussion

The presence of significant differences in the root and hypocotyl lengths of seedlings treated with controls compared to escalating concentrations of essential oils indicate that both oil treatments induce a degree of phytotoxicity. While this phytotoxicity is dose-dependent for both treatments, significant reductions in root and hypocotyl lengths occurred at lower concentrations of the peppermint oil mixture, relative to pure (*R*)-linalool. These results suggest that hydrogel fumigation is superior to foliar sprays in achieving suppression of thrips while minimizing phytotoxicity. Furthermore, the exceptional insecticidal activity of peppermint oil demonstrated in *in vitro* trials correlates to increased phytotoxicity. It possesses exceptional insecticidal activity, but its potential to induce phytotoxicity at concentrations required to control *F. occidentalis* may preclude it from being a viable management strategy. Plants vary in their stress responses along ontogenic gradients, and mature plants can be less sensitive than seedlings to certain stressors (e.g. heat stress) (Wahid et al. 2007). Investigations into

the phytotoxicity of essential oils against tomatoes at vegetative, anthesis and fruiting stages are needed to determine application rates sufficient for thrips control without reducing yield. Considering that tomatoes are relatively vulnerable to phytotoxicity following direct exposure to essential oils (Hazrati et al. 2018, Rolli et al. 2014, Ibáñez & Blázquez 2018, 2020), the decreased hypocotyl and root lengths of seedlings treated with the peppermint oil sprays were expected. Several researchers have corroborated the phytotoxicity demonstrated herein of both peppermint oil, and other botanicals of which linalool is a significant component, such as *Ocimum basilicum* L. (Lamiaceae) and *Cannabis sativa* L. (Cannabaceae) (Klimánková et al. 2008, Ibrahim et al. 2019). In agreement with the present work, Rolli et al. (2014) demonstrated that peppermint oil induces a greater degree of phytotoxicity compared to other botanicals containing linalool (Rolli et al. 2014). Ibáñez and Blázquez (2018, 2020) established that linalool botanicals and peppermint oil are phytotoxic in a dose dependent manner (Ibáñez and Blázquez 2018, 2020).

Essential oil mixtures applied as fumigants behave like gasses, filling the volume of their container. This behavior allows the essential oil to contact thrips while minimizing its contact with the plant. In contrast, essential oils applied as sprays are more concentrated on the plant tissue, often resulting in more severe phytotoxicity. This effect was demonstrated in the present study, with seedlings fumigated with both oil treatments almost always possessing significantly greater root and hypocotyl lengths than seedlings sprayed with both treatments. The presence of significant differences between root and hypocotyl lengths of seedlings treated with spray and fumigation controls indicate that without essential oils, foliar sprays are still injurious to tomatoes. However, the mechanism underlying the increased phytotoxicity of peppermint oil compared to pure (*R*)-linalool is less clear.

Essential oils can induce phytotoxicity through disruptions in osmoregulation, membrane potential, mitochondrial respiration, phytohormones, microtubules, genotoxicity and the generation of reactive oxygen species (Werrie et al. 2020). Peppermint oil has been directly implicated in more of these mechanisms than linalool, which may be responsible for its increased phytotoxicity (Schulz et al. 2007, Mucciarelli 2001, Zunino & Zygadlo 2005).

Phytotoxicity has implications on the growth and development of tomatoes beyond those demonstrated in the present study. While seedlings had reduced hypocotyl and root lengths as a result of exposure to essential oils, the effects of these oils on fruit development were not analyzed. However, other studies have found that insecticides applied to tomatoes for the control of phytophagous pests can negatively affect fruiting. Vavrina et al. (1995) treated tomatoes with insecticidal soaps of varying concentrations and frequencies of exposure for control of *Bemisia argentifoli*i Bellows and Perring (Hemiptera: Aleyrodidae). They observed that increased concentrations and frequencies of exposure of insecticidal soaps reduced tomato biomass and yield, as

well as increased time until fruit maturity (Vavrina et al. 1995). Similarly, Hasan et al. (2016) documented significant fruit damage and reductions in marketable fruit yield associated with the application of indoxacarb for control of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) in tomatoes (Hasan et al. 2016).

3.6 Conclusion

Solanum lycopersicum seedlings were screened for their sensitivity to the most potent fumigants, as determined from thrips bioassays. Fumigation of *S. lycopersicum* with the same concentrations of oils required to control thrips caused phytotoxicity. Phytotoxicity was more severe in seedlings exposed to these oils as foliar sprays, affirming the utility of polymer release. The incidence of phytotoxicity at the LC_{50} of both essential oil mixtures against *F. occidentalis* underscores that the success of these biopesticides in managing *F. occidentalis* hinges upon their incorporation into a broader IPM program. By reducing chemical input, phytotoxicity can be commensurately reduced. Additional studies are warranted to assess the toxicity of essential oil fumigants against other non - target species, such as biocontrol agents and pollinators.

Chapter 4: Fumigant toxicity of (*R*)-linalool to *F. occidentalis*, caged - plant trials

4.1 Abstract

Thrips tend to seek out and inhabit confined spaces (i.e.,thigmotaxis), and this behavior can provide them with a degree of behavioral resistance to insecticides. I hypothesized that *F. occidentalis* would exhibit a significantly higher level of resistance to essential oils when fumigated under experimental conditions that better facilitate this behavior. To assess the impact of thigmotaxis, groups of adult female *F. occidentalis* were introduced to semi - mature *S. lycopersicum* and placed in proportionately larger fumigation chambers. *F. occidentalis* - infested tomatoes were fumigated with varying weights of polyacrylamide conditioned in pure (*R*)-linalool or (*R*)-linalool with peppermint oil, and mortality/repellency of *F. occidentalis* were assessed 24- and 48-hours post - fumigation. The LC_{50} of both treatments (46.4 and 34.0 mg L-1 air, respectively) were higher than those estimated by *in vitro* trials. However, considerable repellency was achieved at concentrations (18.3 and 19.8 mg L-1 air, respectively) lower than LC_{50} values estimated from *in vitro* trials. Just as *in vitro* trials provided an overestimation of the sensitivity of *F. occidentalis* to essential oils, caged - plant trials may also provide poor estimates of their true levels. Glasshouse and field trials are needed to quantify the

true extent to which behavioral resistance enhances the ability of *F. occidentalis* to survive essential oil fumigation.

4.2 Introduction

The ability of western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) to utilize a plethora of host plants is matched by its ability to inhabit an abundance of microhabitats. This phenomenon is facilitated by thigmotaxis, the adaptive behavior of many animals where they orient themselves towards a touch stimulus. In thrips, this manifests as individuals seeking out confined spaces to maximize contact between the body and the surfaces of the space (Tommasini & Maini 1995). These spaces can be anthropogenic or naturally occurring, such as those created by plants or soil. Different life stages are unevenly distributed throughout host plants. Adults preferentially occupy flowers (Pickett et al. 1988, Higgins 1992, Shipp & Zariffa 1991), larvae and eggs are found on the leaves and flowers (Tavella et al. 1996), and pupae populate the soil beneath the host plant (Heyler 1995). These distributions may be further influenced by sex ratio (Higgins 1992). Variations in thigmotaxis are integral to thrips development, providing them with protected microclimates which prevent desiccation during larval and pupal stages (Gilbert 2014, Steiner et al. 2011). This behavior creates an additional hurdle to thrips control because these microclimates protect thrips from a variety of stressors, including insecticides (Jensen 2000).

Several studies have assessed the toxicity of essential oils against *F. occidentalis* either in laboratory or field settings. However, these studies often do not accurately replicate the conditions under which thrips exist on their host plants (van Tol et al. 2007), or they do not concurrently assess the toxicity of essential oils to *F. occidentalis* under laboratory and field settings (Koschier & Sedy 2003, Yi et al. 2006, Stepanycheva 2019, Koschier 2000). Studies that have assessed the response *of F. occidentalis* to stressors under laboratory and field conditions have found that this pest is able to survive lower temperatures than laboratory trials have predicted (Brødsgaard 1993, McDonald et al. 1997), most likely because they are able to inhabit protected microclimates in soil or plant material (Kirk & Terry 2003)**.**

The present work seeks to evaluate the extent to which the sensitivity of *F. occidentalis* to essential oils is affected by caged - plant conditions relative to *in vitro* trials. Simultaneously, I sought to determine if thigmotaxis conferred reduced sensitivity to essential oil fumigation. Considering this pest's propensity to thigmotaxis, I hypothesize that the presence of *Solanum lycopersicum* L. (Solanaceae) in fumigation settings will promote this behavior and the decreased sensitivity to insecticides associated with it.

4.3 Materials and Methods

4.3.1 Caged - Plant Trials

Solanum lycopersicum were germinated in black plastic pots (15 cm width x 10 cm height) in potting mix. Plants were maintained in the glasshouse until 20 cm tall. At this stage, individual plants were populated with 10 adult *F. occidentalis* and placed inside cuboid plexiglass chambers (14 cm x 14 cm x 14 cm). One of the panels of these chambers was fitted with a 19 cm diameter hole through which thrips - infested tomatoes were inserted. A similarly sized plastic container served as a stopper for this hole (Figure 9). These chambers were placed into a large standing incubator (VWR #10753-894, VWR International, LLC) maintained at 26 $\pm$ 2^{o}C and 80 $\pm$ 5% RH under 16:8 L:D photoperiod. The chambers were kept in the incubator for 24 hours prior to fumigation to allow thrips and tomatoes to acclimate.

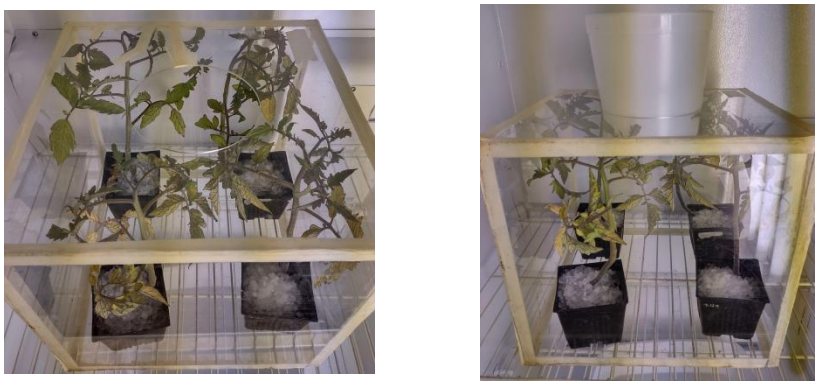

Figure 9. Caged - plant trial fumigation chamber without (left) and with (right) stopper.

Infested tomatoes were fumigated with polyacrylamide conditioned in pure distilled water (negative control), pure (*R*)-linalool, or (*R*)-linalool with peppermint oil. Solutions of pure (*R*)-linalool and (*R*)-linalool with peppermint oil were prepared according to section 2.4.2 Chemical Preparation. Stocks of essential oil hydrogels were prepared at 0.1 mL solution/mg polyacrylamide according to section 2.4.3.2 Hydrogel Conditioning at the LC_{50} of these treatments to *F. occidentalis* determined from *in vitro* trials. For each observation, four plants were exposed simultaneously in the same chamber. Plants and thrips were exposed to 0g, 50g, 100g, 150g, 200g or 250g of conditioned hydrogels applied in equal amounts to each of the four plants. For pure (*R*)-linalool, these masses corresponded to 0, 14.5, 29.0, 43.5, 58.0, and 72.5 mg L^{-1} air essential oil per thrips - infested tomato, respectively. For (*R*)-linalool with peppermint oil, these masses corresponded to 0, 15.0, 30.0, 45.0, 60.0, and 75.0 mg L^{-1} air essential oil per thrips - infested tomato, respectively. Conditioned gels were broadcast from above such that all gels fell on the plant or its soil. Groups of four plants were cumulatively assessed for mortality and repellency. Mortality was defined as the number

of thrips that exhibited *rigor mortis* or did not respond to mechanical stimulation after each observation period. Repellency was defined as the number of live thrips in the fumigation chamber but not on host plants after each observation period. Assessments were performed 24 and 48 hours after exposure to conditioned gels.

4.3.2 Statistical Analysis

Data were analyzed using SPSS version 26.0 (IBM Corp 2021). Contingency table analyses (3x2 tables, with 2 degrees of freedom) were performed to statistically analyze the relationship of mortality and repellency between the two oil treatments (pure (*R*)-linalool versus (*R*)-linalool with peppermint oil) and between observation periods (24 versus 48 hours) at each concentration. Differences were considered significant when $p < 0.05$. The raw data from which cross tabulations were performed are included in the appendices as tables 4 and 5. Figure 10 was constructed in SigmaPlot version 14.5 (Systat Software 2021).

4.4 Results

At the lowest concentrations of both oil treatments (50 grams conditioned hydrogels), there were no significant differences between oil treatments at 24 hours ($\chi^2 = 3.01$, d.f. = 2, $p = 0.222$) or 48 hours ($\chi^2 = 2.51$, d.f. = 2, $p = 0.285$). Similarly, the insecticidal efficacy of the peppermint oil mixture did not vary significantly from 24 to 48 hours ($\chi^2 = 2.30$, d.f. = 2, $p = 0.317$). However, there was a significant difference between the insecticidal efficacies of pure (*R*)-linalool from 24 to 48 hours ($\chi^2 = 8.22$, d.f. = 2, $p < 0.05$). At 100 grams conditioned hydrogels, there were no significant differences between oil treatments at 24 and 48 hours, or between observation periods for each oil treatment.

At 150 grams conditioned hydrogels, the peppermint oil mixture demonstrated significantly higher insecticidal efficacy than pure (*R*)-linalool at 24 hours ($\chi^2 = 17.88$, d.f. = 2, $p < 0.001$). Conversely, there was no significant difference between oil treatments at 48 hours ($\chi^2 = 2.11$, d.f. = 2, $p = 0.348$). Furthermore, the insecticidal efficacy of pure (*R*)-linalool was greater at 48 hours relative to 24 hours post-application ($\chi^2 = 10.27$, d.f. = 2, $p < 0.01$).

At 200 grams conditioned hydrogels, pure (*R*)-linalool ($\chi^2 = 10.10$, d.f. = 2, $p < 0.01$) and (*R*)-linalool with peppermint oil ($\chi^2 = 8.86$, d.f. = 2, $p < 0.05$) demonstrated significantly different insecticidal efficacies from 24 to 48 hours. At this concentration, the insecticidal efficacy between oil treatments varied significantly at 24 hours ($\chi^2 = 11.89$, d.f. = 2, $p < 0.01$), but not at 48 hours ($\chi^2 = 3.70$, d.f. = 2, $p = 0.157$). At 250 grams conditioned hydrogels, there was no significant difference in the insecticidal efficacies of oil treatments from 24 to 48 hours, or between oil treatments at 48 hours. However, the peppermint oil mixture had greater efficacy than pure (*R*)-linalool at 24 hours ($\chi^2 = 7.17$, d.f. = 2, $p < 0.01$).

Most of the hydrogels broadcast from overhead fell onto the soil, rather than the plants. Phytotoxicity of tomato host plants occurred during the 48-hour observation period. Symptoms of phytotoxicity included leaf curling, dieback and depigmentation. These symptoms were more severe for treatments involving peppermint oil, for treatments involving higher concentrations of either essential oil treatment, and for plant parts in direct contact with conditioned hydrogels (e.g. hydrogels falling onto leaves).

Thrips remaining on host plants were most often found inhabiting the apical and axillary buds of *S. lycopersicum*. When leaf curling occurred because of phytotoxicity, thrips inhabited these pseudo - domatia. Thrips that were repelled from *S. lycopersicum* were most often found in the corners of the fumigation chamber.

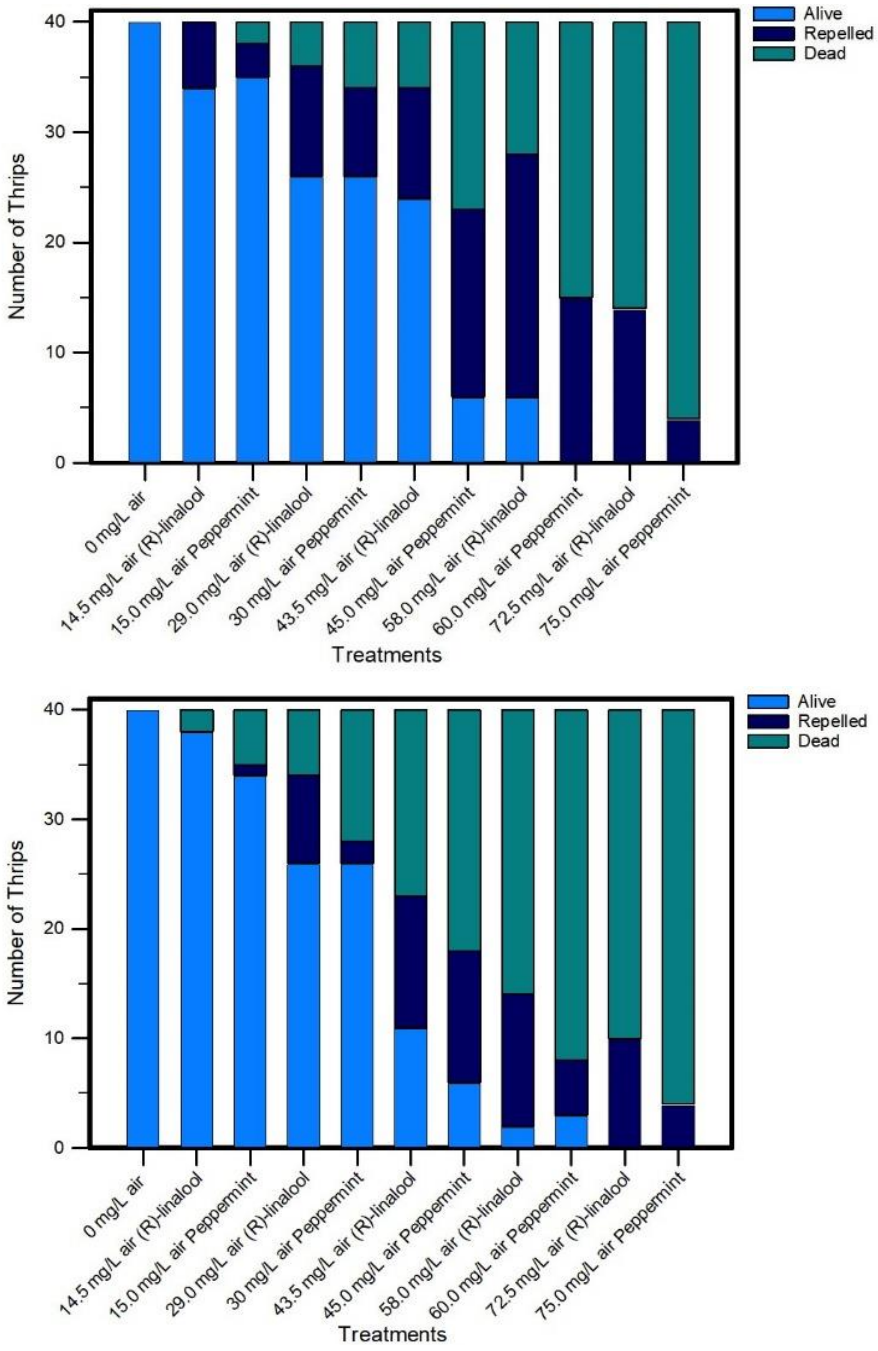

Figure 10. Fumigant toxicity of essential oil treatments against *F. occidentalis* after 24- (top) and 48-hours (bottom).

4.5 Discussion

4.5.1 *In Vitro* Trials vs. Caged - Plant Trials

For applications of 150, 200 and 250 grams of conditioned hydrogels, (*R*)-linalool with peppermint oil had a greater insecticidal activity than pure (*R*)-linalool. This is in agreement with the results of *in vitro* trials which also found the peppermint oil mixture

to be more efficacious than pure (*R*)-linalool (Figure 4). However, higher concentrations of both oils were required to achieve 100% mortality in *F. occidentalis*. In *in vitro* trials, pure (*R*)-linalool and (*R*)-linalool with peppermint oil required at least 40 mg L^{-1} air and 20 mg L^{-1} air, respectively, to achieve complete mortality. In these caged plant trials, pure (*R*)-linalool achieved nearly 100% mortality at 72.5 mg L^{-1} air. Similarly, (*R*)-linalool with peppermint oil achieved nearly 100% mortality at 75.0 mg L^{-1} air. This reduced susceptibility was partially the result of resistance mechanisms that could not be exercised by thrips in *in vitro* trials. In those trials, *F. occidentalis* were provisioned with sections of cabbage leaves that were mostly flat, lacking in the folds and crevices that create protected microclimates. To an extent, this environment prevented thrips from engaging in behavioral resistance (i.e., thigmotaxis). Therefore, the resistance of *F. occidentalis* relative to *F. insularis* demonstrated in *in vitro* trials was most likely a reflection of the other three resistance mechanisms: Detoxification enzymes, reduced penetration of toxicants and alterations of target sites (Jensen 2000).

In caged trial settings with whole plants, the crevices between stems at the axillary buds and between leaves at the apical buds provided such spaces, as exemplified by the tendency of *F. occidentalis* to inhabit these spaces following fumigation. The barrier to thrips control created by this avoidance behavior suggests that systemic and translaminar insecticides may be more effective at repelling and killing thrips (Gao et al. 2012). While some botanicals have proven to have translaminar activity (Campolo et al. 2017), this characteristic has not been found in any Labiate essential oils (Miresmailli & Isman 2006, Bennour et al. 2021). These caged - plant experiments demonstrate that *in vitro* trials often provide an underestimation of resistance. *F. occidentalis* can survive lower temperatures than those predicted by *in vitro* trials by occupying spaces in plant and soil material (Brødsgaard 1993, McDonald et al. 1997). Beyond fostering microclimates protected against low temperatures, these spaces also protect against contact insecticides (Jensen 2020), and potentially against fumigants.

While thrips thigmotactic behavior was likely responsible for their increased resistance to essential oil fumigation relative to *in vitro* trials, the results of these caged-plant trials may still provide an underestimation of resistance. Adult *F. occidentalis* have a flower biased distribution (Pickett et al. 1988, Higgins 1992, Shipp & Zariffa 1991), but the tomato plants in these caged - plant trials were not at anthesis. Additional field studies of host plants at the vegetative stage and at anthesis are required to evaluate whether floral parts create microclimates with a greater degree of protection from insecticides relative to the axillary and apical buds that *F. occidentalis* inhabited during these caged - plant trials. Furthermore, the increased vulnerability of tomatoes at anthesis to stressors (Sato et al. 2000, Abdul-Baki 1991) further complicates control of thrips. The laboratory culture of *F. occidentalis* may also provide an underestimation of resistance due to the duration of their captivity without pesticide exposure. At the time of

this study, this population of *F. occidentalis* has been maintained without insecticide pressure for four years (Nicholas & Follett 2018). The lack of selective pressure for insecticide resistance means that some resistance genes were lost to genetic drift or selected against, influencing assessment of resistance levels derived from this culture (Roush & Miller 1986).

4.5.2 Repellency vs. Mortality

Apart from the application of 200 grams of (*R*)-linalool with peppermint oil conditioned hydrogels, none of the peppermint oil mixture treatments had significantly different activity from 24 to 48 hours. However, pure (*R*)-linalool had significantly different efficacy from 24 to 48 hours at applications of 50, 150 and 200 grams of conditioned hydrogels. This indicates that the active ingredients of conditioned hydrogels exert greater influence than duration of exposure on insecticidal efficacy for the peppermint oil mixture, but not for pure (*R*)-linalool. In partial agreement with the present work, Regnault-Roger et al. (1993) and Kouninki et al. (2005) both found that conventional pesticides have greater insecticidal efficacy at 48 hours compared to 24 hours post treatment. The opposite trend occurs with essential oils applied against thrips (Regnault-Roger et al. 1993, Kouninki et al. 2005). The inverse relationship between duration of exposure and essential oil efficacy is a reflection of their high volatility (Moretti et al. 2002). This efficacy may be further extended by incorporating essential oils into hydrogels with greater crosslinker concentration (Işıklan 2007), or through the use of fixatives to delay the elution of essential oils (Songkro et al. 2012, McGovern & Beroza 1967, Hidayatulfathi et al. 2019).

Pure (*R*)-linalool and (*R*)-linalool with peppermint oil had significantly different insecticidal activities at masses of conditioned hydrogels greater than or equal to 150 grams for 24 hours, but not for 48 hours. It is possible that after this concentration threshold, the insecticidal activity of these oils is constrained by their volatility (Moretti et al. 2002). In the first 24 hours of their exposure, essential oils were migrating out of the gels at a higher rate than during the second 24 hours, at which point the gels may have been depleted of their contents. If thrips were exposed to lower concentrations of these fumigants, then these concentrations would be less likely to achieve significantly different insecticidal efficacies. Similarly, applications of 100 grams or less of conditioned hydrogels failed to achieve significantly different activities between 24 and 48 hours mostly likely because the concentrations were too low to affect *F. occidentalis*.

All treatments achieved repellency at concentrations lower than those required to induce mortality. While repellency is useful in reducing feeding and oviposition behavior (Koschier et al. 2002, Riefler & Koschier 2009), it permits the possibility of resurgence. Furthermore, the repellency of these essential oils can increase virus transmission if thrips migrate to unprotected individuals (Birget & Koella 2015) or if they compensate with an increased frequency of probing attempts (Lu et al. 2019).

4.5.3 Phytotoxicity

The results of phytotoxicity assays demonstrated that concentrations of essential oils (i.e., pure (*R*)-linalool or (*R*)-linalool with peppermint oil) required to control *F. occidentalis* induced phytotoxicity. Although the tomato plants in these caged - plant trials were more mature and therefore less vulnerable to stressors (Wahid et al. 2007), symptoms of phytotoxicity were observed. These symptoms may have created a confounding variable; a portion of the mortality and repellency observed herein may have been a result of a reduction in the quality of the host plant, rather than solely the result of fumigation.

4.6 Conclusion

Caged - plant trials were conducted to determine the extent to which settings that more accurately replicated horticultural conditions would enhance insecticide resistance in *F. occidentalis*. Both oil treatments required significantly higher concentrations to achieve levels of mortality similar to those from *in vitro* trials. However, the LC_{50} values from *in vitro* trials were sufficient to achieve significant repellency. Although *in vitro* trials provided an underestimation of resistance, their ranking of each treatment's efficacy was consistent with caged - plant trials. These findings suggest that glasshouse and field trials may better predict true levels of resistance to essential oil fumigation by *F. occidentalis*.

5.0 Appendices

5.1 Chapter 4 Raw Data

Table 4. Raw data for mortality and repellency of pure (*R*)-linalool to *F. occidentalis*.

	24-hour mortality (number of thrips)					24-hour repellency (number of thrips)				
(*R*)-linalool concentration (mg L^{-1} air)	A	B	C	D	Average	A	B	C	D	Average
0 mg L^{-1} air	0	0	0	0	0	0	0	0	0	0
14.5 mg L^{-1} air	0	0	0	0	0	12	4	0	8	6
29.0 mg L^{-1} air	4	4	8	0	4	12	8	8	12	10
43.5 mg L^{-1} air	0	8	4	12	6	8	12	8	12	10
58.0 mg L^{-1} air	20	4	12	12	12	20	24	24	20	22
72.5 mg L^{-1} air	36	24	24	20	26	14	14	14	14	14
	48-hour mortality (number of thrips)					48-hour repellency (number of thrips)				
(*R*)-linalool concentration (mg L^{-1} air)	A	B	C	D	Average	A	B	C	D	Average
0 mg L^{-1} air	0	0	0	0	0	0	0	0	0	0
14.5 mg L^{-1} air	4	4	0	0	2	0	0	0	0	0
29.0 mg L^{-1} air	12	4	8	0	6	4	8	8	12	8
43.5 mg L^{-1} air	8	20	20	20	17	16	12	0	20	12
58.0 mg L^{-1} air	28	24	24	28	26	12	12	16	8	12
72.5 mg L^{-1} air	40	32	24	24	30	0	8	16	16	10

Letters A, B, C and D refer to each of the four replicates for each treatment.

Table 5. Raw data for mortality and repellency of (*R*)-linalool with peppermint oil to *F. occidentalis*.

	24-hour mortality (number of thrips)					24-hour repellency (number of thrips)				
(*R*)-linalool with peppermint oil concentration (mg L^{-1} air)	A	B	C	D	Average	A	B	C	D	Average
0 mg L^{-1} air	0	0	0	0	0	0	0	0	0	0
15.0 mg L^{-1} air	0	4	4	0	2	0	8	4	0	3
30.0 mg L^{-1} air	16	0	4	4	6	0	8	8	16	8
45 mg L^{-1} air	16	24	12	16	17	8	12	28	20	17
60 mg L^{-1} air	12	28	28	32	25	28	12	12	8	15
75 mg L^{-1} air	36	40	32	36	36	4	0	8	4	4
	48-hour mortality (number of thrips)					48-hour repellency (number of thrips)				
	A	B	C	D	Average	A	B	C	D	Average
0 mg L^{-1} air	0	0	0	0	0	0	0	0	0	0
15.0 mg L^{-1} air	0	12	4	4	5	4	0	0	0	1
30.0 mg L^{-1} air	20	4	16	8	12	4	0	4	0	2
45 mg L^{-1} air	16	32	16	24	22	8	4	24	12	12
60 mg L^{-1} air	20	36	32	40	32	12	4	4	0	5
75 mg L^{-1} air	36	40	32	36	36	4	0	8	4	4

Letters A, B, C and D refer to each of the four replicates for each treatment.

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