12-2004

Evaluation of Botanical Oil Formulations for Management of Powdery Mildew and Mites

Adriane Lorraine Cannon
University of Tennessee, Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Adriane Lorraine Cannon entitled "Evaluation of Botanical Oil Formulations for Management of Powdery Mildew and Mites." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

Dennis Deyton, Major Professor

We have read this thesis and recommend its acceptance:

Carl Sams, William Klingeman, Donna Fare

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Evaluation of Botanical Oil Formulations for Management of Powdery Mildew and Mites

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Adriane Lorraine Cannon
December 2004

Dedication

This thesis is dedicated to my parents, Denise Cannon and Jesse N. Cannon III, whose love and support made this all possible. Their constant encouragement kept me going when I felt that I had nothing left to give. I am grateful to have them in my life and will never be able to repay them to the full extent they deserve.

Acknowledgements

I am grateful to my major professor, Dr. Dennis E. Deyton, for giving me the opportunity to work on this research project. I value the friendship that we have developed over these two years and will never forget the support and encouragement that he provided. I also appreciate the support of my Thesis Committee: Dr. Carl Sams, Dr. William Klingeman, and Dr. Donna Fare. I would also like to thank the UTK Department of Plant Sciences for their contributions to my education.

I would like to thank John Cummins for his assistance in carrying out this project. He always provided help whenever I asked for it.

Many thanks go out to the coworkers in the lab and in the office. I appreciate having you to talk to and joke with even on those not so good days. I will greatly miss our time spent together and will never forget the friendships that have developed.

Abstract

Experiments were conducted to evaluate the effects of newly created soybean oil formulations on powdery mildew (*Erysiphe pulphra*), photosynthesis, phytotoxicity, and broad mites (*Polyphagotarsonemus latus* (Banks)) on dogwood (*Cornus florida* (L.) 'Cloud Nine') trees, and to evaluate the amounts of formulations that are deposited and washed off of peach (*Prunus persica*, (L.)) leaves, dormant oak (*Quercus phellos*, (L.)), and viburnum (*Viburnum x juddii*) twigs after simulated rainfall. Oil treatments were applied using a back pack mist blower sprayed pre- and post- inoculation of powdery mildew to evaluate powdery mildew, photosynthesis, and phytotoxicity. Oil treatments were also applied to the dogwood trees after infestation of broad mites. Powdery mildew and phytotoxicity were rating using an eight and five-point rating scale, respectively. Photosynthesis was measured using an ADC-3 model portable infrared gas analyzer. Trees and shrubs were arranged in randomized complete block designs around a 2.7 (9 ft) high spray nozzle in order to evaluate the effects different soybean oil formulations had on oil deposited on leaves and dormant twigs and amounts washed off by simulated rainfall. The amounts of oil present on the leaves and twigs after spraying and simulated rainfall were determined by chloroform extraction. In both pre- and post-inoculation evaluations of oil formulations, less powdery mildew was present on the oil treated leaves than the water treated leaves. Sprays of oil formulations caused some yellowing on the foliage whether sprayed pre- or post-inoculation. None of the oil formulations significantly controlled broad mites. Oil formulations differed in the amount of oil residue left on the leaves and dormant twigs before and after different simulated rainfall amounts.

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Part I

Introduction

Many pesticides have been eliminated by the United States (U.S.) Environmental Protection Agency (EPA), leading to the need to develop substitutes for synthetic pesticides (U.S. Congress, 1996). There has been a rise in interest to use vegetable oils as pesticides due to the EPA issuing a rule that established an exemption from normal pesticide registration for several botanical oils, including soybean oil (*Glycine max* (L.)) (U.S. Congress, 1996; Quarles, 1996). Of the approximate 100 million tons of oils and fats produced worldwide in 1999, 85% was of botanical origin (USDA-NASS, 2001), with soybean oil being the most abundant botanical oil in the world. In 2000, the U.S. produced 13,749 million pounds of soybean oil that was used for consumption, accounting for 45% of the total world production (United States Soybean Board, 2001).

In Alabama, powdery mildew (*Microsphaera penicillata* (Wallr.)) is the most common foliar disease of flowering dogwood (*Cornus florida* (L.)) (Hagan et al., 1997). To chemically control powdery mildew, synthetic fungicides are applied throughout the season (Hansen et al., 2000). Season long applications could lead to the development of resistance (Pasini et al., 1997). A reduction in the occurrence of powdery mildew on dogwoods has been seen using 1% or 2% soybean oil sprays (Deyton et al., 2000).

Miller and Uetz (1998) stated that the risk of phytotoxicity is the greatest obstacle to the increased use of horticultural mineral oils on ornamentals. Plant tissue damage is characteristic of oil-induced stresses and is normally associated with membrane disruption due to the physical presence of the oils (Hodgkinson et al., 2002). Acute symptoms of phytotoxicity include lesions, increased incidence of sunburn damage on fruit, damage to meristematic tissues, significantly increased premature fruit drop, and

twig dieback (Hodgkinson et al., 2002). Chronic symptoms include growth suppression, transient inhibition of metabolic processes such as photosynthesis, alternate cropping, and degradation of juice sugar levels (Hodgkinson et al., 2002). It is difficult to know the specific cause of spray-oil-induced phytotoxicity due to the many factors that can culminate in symptoms (Hodgkinson et al., 2002). Butler and Henneberry (1990) reported no phytotoxicity on vegetables sprayed with 1%-2% oil sprays.

Haustellate (sap-feeding) arthropods, which include spider mites (*Tetranychus urticae* (Say)), broad mites (*Polyphagotarsonemus latus* (Banks)), and citrus red mites (*Panonychus citri* (McGregor)), are the most common pests of landscape ornamentals. These arthropods remove leaf cell contents by inserting needle-like mouthparts (stylets) into the mesophyll cells (Barrett, 1996). If damage is severe enough, significant leaf drop can occur. However, these pests usually cause cosmetic damage to their host ornamentals (Barrett, 1996). Most miticides have become ineffective because of their intensive use (Gough 1990). Resistance to pesticides have also evolved due to heavy dependence on them (Raupp et al.,1992). These sap-feeding pests are vulnerable to oil due to their small, soft bodies. Oils are the only class of insecticides that few, if any, insects have developed resistance to (Pless, 1995). European red mite (*Panonychus ulmi* (Koch)), San Jose scale (*Quadraspidiotus perniciosus* (Comstock)), white peach scale (*Psuedaulacaspis pentagona* (Targioni-Tosswtt)), and terrapin scale (*Mesolecanium nigrofasciatum* (Pergande)) have been controlled with winter-time sprays of soybean oil (Deyton et al., 2002).

The efficacy of foliar-applied insecticides is affected by rain. A reduction in pest mortality can be caused by rain removing the deposits of insecticides from the surface of

the foliage (McDowell et al., 1984). Fifty percent or more of initial deposits of insecticides have been shown to be washed off by 2 to 5mm of simulated rain (Pick et al., 1984). Decisions on whether to respray an insecticide after rainfall is dependent on that insecticide and its formulation (Mashaya 1993). The reason why some insecticides are more resistant to rainfall is due to the inherent qualities of the formulation (Mashaya 1993).

This thesis research prepared new soybean oil formulations, with botanical or food grade emulsifiers, to be used as pesticides. Different adjuvants were tested for their ability to stay in emulsion with soybean oil and water. Selected formulations were then evaluated for efficacies on powdery mildew, broad mites (*Polyphagotarsonemus latus* (Banks)), phytotoxicities and effects on photosynthesis of dogwood trees. The formulations were then evaluated for their potential residue deposit and rain-fastness as either summer or dormant season sprays.

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Part II

Literature Review

Types of Oils

Oils are complex hydrocarbons that differ depending on the source of the oil and the refining processes used (Anonymous, 2000). There are several classes of petroleum oils used as pesticide sprays on plants. A Cornell home grounds fact sheet (Anonymous, 2000) defined mineral oil as oil located in the rock strata of the earth. Kuhlmann et al. (2002) further described mineral oil as oil that can be safely used as a part of non-food products intended for use in contact with food and that complies with the Food and Drug Agency (FDA) rulings. Based on the FDA compliances, not all mineral oils are suitable for agriculture uses. Kuhlmann et al. (2002) also proposed that agricultural mineral oils include all mineral oils made for use as adjuvants on growing plants that can tolerate many different products. These oils are used in vegetation management of forestry, rangelands, and rights-of-way of industrial sites and on row crops (Kuhlmann et al., 2002). Herbicidal oils are aromatic petroleum oils of high plant toxicity and potential mammalian toxicity (Anonymous, 2000). Napthenic and asphaltic oils are used for motor fuels, fuel oil and solvents and are aromatic, highly unsaturated, and highly toxic to plants (Anonymous, 2000). The colorless, tasteless oils that are derived from petroleum oils and used for pharmaceutical or medicinal purposes are termed white mineral oils (Anonymous, 2000). Paraffinic oils are the bases from which horticultural oils are refined. These paraffinic oils are highly saturated and are used as lubricating oils (Anonymous, 2000). Horticultural mineral oils (HMO) are the most highly refined oil products that pose minimum risk of acute and chronic damage to trees, vines, ornamentals or vegetables and have optimum pesticidal efficacy (Kuhlman, 2002).

Horticultural mineral oils can also be used as adjuvants for use with chemical pesticides on crops or when the greatest possible enhancement of chemical pesticides is desired (Kuhlman, 2002). The most crucial differentiation between agricultural mineral oils and horticultural mineral oils (HMO) is the narrow distillation range characteristics of HMOs (412-468 °F) (Kuhlman, 2002). Dormant and summer oils are terms referring to the timing of application and not the type of oil (Anonymous, 2000). Dormant oils are limited to use on woody plants before buds open and summer oils can be used on green plants (Anonymous, 2000). Summer oils are safer to use on green plants due to their 92 to 96% unsulfonated residues, while dormant oils cause damage to green plants due to their 50 to 90% unsulfonated residues (Baker, 1994).

Petroleum Oils

Petroleum-derived oils have been used for pest control for more than 200 years (Lawson and Weires, 1991). The development of fast-breaking oil-water emulsions (oil and water separate quickly after landing on the plant) in the 1920s improved coverage and increased pest control (Davidson et al., 1991). Rae et al. (2002) found that two narrow-range and one broad-range petroleum oil formulations reduced pest numbers present on sweet orange (*Citrus sinensis* (L.)) and pummelo (*C. grandis* (L.)) as compared to untreated trees. Today, petroleum-derived oils are considered to be among the best available pesticides to control scale insects and mites on dormant plants (Davidson et al., 1991; Johnson, 1980). However, there is an increasing interest in using vegetable and animal oils for crop protection needs (Agnello, 2002). Hare et al. (1999)

stated that the restriction of the use of petroleum oils in the future must be taken into consideration.

Botanical Oils

It was reported as early as the nineteenth century that botanical oil sprays could control scale insects (deOng et al., 1927). deOng et al. (1927) reported that cottonseed (*Gossypium hirsutum* (L.)), linseed (*Linum usitatissimum* (L.)), and castor oils (*Ricinus communis* (L.)) controlled insects, but were more phytotoxic to plants than petroleum-derived oils. Petroleum oils were also cheaper and botanical oils received little attention until recently. An advantage to using horticultural oils, botanical oils, and soaps as pesticides is that they cause little or no mammalian toxicity, have a wide range of pest control, and fit easily into integrated pest management (IPM) programs (Miller, 1997). The EPA (U.S. Congress, 1994; Quarles, 1996) issued a rule that established an exemption from normal pesticide registration for several botanical oils, including soybean (*Glycine max* (L.)), maize (*Zea mays* (L.)) and garlic oils (*Allium sativum* (L.)), because they had no significant adverse effects on the environment, are non-persistent in the environment and are relatively non-toxic to humans. Of the approximate 100 million tons of oils and fats produced worldwide in 1999, 85% was of botanical origin (USDA-NASS, 2001). The use of botanical oils instead of petroleum-based oils has many benefits. One advantage of plant oils is that they are derived from renewable resources as opposed to petroleum oils that are produced from somewhat non-renewable fossil fuels (Quarles, 1996). Butler et al. (1989) found that cottonseed oil (*Gossypium hirsutum* (L.)) repelled sweetpotato whitefly (*Bemisia tabac* (Gennadius)) adults for up to nine days

when applied to cotton (*Gossypium spp.*), squash (*Cucurbita spp.*), lettuce (*Lactuca spp.*), and carrot (*Daucus spp.*) seedlings. Cottonseed oil has also been shown to reduce egg viability of sweetpotato whiteflies by 84% (Butler et al., 1988). Beattie et al. (2002) showed that two oils developed from rapeseed (*Brassica napus* (L.)) reduced the mean number of citrus leafminer (*Phyllocnistis citrella* (Stainton)) per leaf compared to the water sprayed control.

Soybean Oil

The research in this laboratory has focused on using soybean oil because it is abundant and readily available in the U.S. It is also the most abundant botanical oil in the world. In 2000 the U.S. produced 13,749 million pounds of soybean oil that was used for consumption, accounting for 45% of the total world production (United States Soybean Board, 2001). Research at the University of Tennessee has shown that 1% or 2% soybean oil sprays reduced the occurrence of powdery mildew on dogwoods (*Cornus florida* (L.)) (Sams et al., 2000). Soybean oil has also been shown to delay peach tree flowering, to thin fruit and kill key arthropod pests of deciduous fruit trees (Deyton et al., 2002). Winter-time sprays of soybean oil has been shown to control European red mite (*Panonychus ulmi* (Koch)), San Jose scale (*Quadraspidiotus perniciosus* (Comstock)), white peach scale (*Pseudaulacaspis pentagona* (Targioni-Tozzetti)), and terrapin scale (*Mesolecanium nigrofasciatum* (Pergande)) on peach (*Prunus persica* (L.)) and apple (*Malus domestica* (Borkh)) (Deyton et al., 2000). Summer-time sprays of soybean oil have also effectively controlled populations of two-spotted spider mites with minimum phytotoxicity to burning bush plants (*Euonymous compactus* (L.)) (Lancaster et al.,

1998). Butler et al. (1988) found that a 5% soybean oil treatment caused adult sweetpotato whiteflies to avoid cotton seedling for as long as seven days.

Adjuvants

Texturant-systems (Anonymous, 2001) defined an emulsion as "a dispersion of droplets of a non-miscible liquid into another." A film is formed between both products when the emulsifier is absorbed in the interface. The polar part of the emulsifier has an affinity with water and the non-polar part (fatty chain) adheres to the oil phase. Webster's Revised Unabridged Dictionary (Merriam-Webster, 2004) defines an adjuvant as an ingredient that aids or modifies the action of the principal ingredient. Herbicide performance can be altered by adjuvants because they affect the spread of spray droplets on the leaf surface, retention of spray on the leaf, and penetration of the herbicide through the plant cuticle (Young, 2003). Adjuvants, however, cannot directly affect the inherent active ingredient toxicity (Zabkiewicz, 2002). Research has shown that spray droplets of water plus methylated seed oil spread more on the leaf surface than droplets of water plus petroleum oil (McWhorter et al., 1993). Appropriate formulation technology to aid droplet spread, spray redistribution and surface wetting is essential (Zebkiewicz, 2002). Numerous plant-oil-derived adjuvants are used commercially today (Harvey, 1993). Many natural occurring emulsifiers were exempted by the EPA ruling (U.S. Congress, 1996). Sams and Deyton (2002) reported that "the ideal plant oil formulation should form a strong emulsion, be fast breaking (oil and water separate quickly after landing on the plant surface), form a strong oil film on the plant surface, have a wide temperature stability, have a consistent persistency, be low foaming, and have no phytotoxicity."

Phytotoxicity

Miller and Uetz (1998) stated that the risk of phytotoxicity is the greatest obstacle to the increased use of HMOs on ornamentals. They also stated that if an HMO does not cause yellowing, scorching or leaf drop, then oil soaking alone will not affect the marketability of plants. Hodgkinson et al. (2002) stated that spray oil induced phytotoxic symptoms usually occur as part of the plants response to its current physical, chemical, or biological stresses. Plant tissue damage normally associated with membrane disruption, or suppression of plant function due to the physical presence of the oil are characteristics of oil-induced stresses. Hodgkinson et al. (2002) separated the symptoms of phytotoxicity into two categories: acute and chronic. Acute symptoms of phytotoxicity were defined as burns on plant tissue in the form of lesions, increased incidence of sunburn damage on fruit, damage to meristematic tissues, significantly increased premature fruit drop, and twig dieback. Growth suppression, transient inhibition of metabolic processes such as photosynthesis, alternate cropping, and reduction of juice sugar levels were described as chronic symptoms. As stated before, both acute and chronic symptoms can be attributed to stress responses by the sprayed plants. Many factors can culminate in the symptoms observed often making it very difficult to determine the specific cause of spray-oil-induced phytotoxicity (Hodgkinson et al., 2002).

Multiple applications of 2% summer oil sprays at 2-week intervals for a year did not cause phytotoxicity on azalea (*Rhododendron* sp.), boxwood (*Boxus* sp.), camellia (*Camellia* sp.), and holly (*Ilex* sp.) (Tippins, 1974). An application of 4-6% horticultural

oil sprayed during the summer on a wide range of woody ornamentals caused no phytotoxic damage (Johnson, 1985). Davidson et al., (1990) applied multiple sprays of 2% Sunspray 6E Plus (Sunoco Inc., Philadelphia, Pa.) to 52 species of woody nursery plants under drought stress and only six cultivars showed objectionable levels of discoloration. Russell and Mizell (1991) found that five applications of 2% Sunspray Ultra-fine Spray Oil can be safely used on 30 different species of trees and shrubs during the summer season in Florida and Southern Georgia without general problems of phytotoxicity. Zheng et al. (2002b) reported that four weekly dips of 0.5% and 1.0% HMO during dormancy did affect the number of new shoots, but did not affect the early bloom date of azalea. Butler and Henneberry reported no phytotoxicity on vegetables sprayed with 1-2% oil sprays (1990b, 1991a). Zheng et al. (2002a) reported that severe levels of leaf scorch were reached after only one application of 2% HMO in several species of ferns.

Gas Exchange Disruption

Oils may also physically inhibit plant gas exchange. In 1923, Burroughs stated that the oil film remaining on the plant surface might interfere with plant gas exchange and reduce transpiration rates, possibly causing abscission. Johnson (1982) stated that dilute oil sprays cover the stomates of leaves and bark with a deposit of oil that disrupts gas exchange. Symptoms of injury depend on the number of stomates that are closed, amount of oil deposited, how fast the oil is evaporating, and the clearing capacity of the stoma guard cells (Johnson, 1982). Hesler and Plapp (1986) reported that mineral and crop oils counteracted volatilization, photo-degradation, and biodegradation of synthetic

pesticides when both chemicals were mixed and applied together. Northover and Schneider (1996) stated that after an oil application to grape vines (*Vitis vinifera* (L.)), respiration increased and that photosynthesis and transpiration decreased. Zheng et al. (2002b) reported that when azalea was dipped in a 5% HMO solution there was a significant reduction in transpiration rates immediately after each application during summer. The effect was short-lived, with rates returning to normal within seven days.

Powdery Mildew

Powdery mildew, caused by the fungus *Microsphaera penicillata* (Wallr.) (renamed *Erysiphe pulphra*), has replaced spot anthracnose (*Discula distructiva* (Redlin)) as the most common disease of flowering dogwood in residential landscapes across Alabama (Hagan et al., 1999). Symptoms of powdery mildew include cottony growth of the causal fungus covering the leaf surface and often the leaves are twisted, curled, and may be smaller than normal (Hagan et al. 1997). In addition, shoot tips may be killed and buds may fail to open (Hansen et al., 2000). The causal fungus survives as hyphae in buds and fruiting bodies on fallen leaves. In areas with mild winters, the fungus over-winters as conidia or mycelium in infected buds, leaves, stems or other plant parts (Hansen et al., 2000). Powdery mildew fungi are usually most active when the days are warm and nights are cool. On dry days conidia can be blown to healthy tissue. As air cools at night and humidity rises, the spores absorb moisture, germinate, and infect (Hansen et al., 2000). Frequent rainfall will suppress the spread and development of the fungi. Powdery mildew is best controlled by planting disease resistant cultivars and by

spraying fungicides that are registered for the control of powdery mildew (Hagan et al., 1997).

Nicetic et al., (2002a) reported that tomato powdery mildew (*Leveillula taurina* (L.)) in greenhouse hydroponic tomatoes (*Lycopersicon esculentum* (Mill.)) was prevented and suppressed when a horticultural mineral oil (HMO) was used. They also showed that HMOs provided significant protection to sepals against powdery mildew infections and suggested this as a major economic benefit to growers. Grove et al. (2002) reported that HMO-based management programs may suppress cleisothecia formation and prevent transport of wild and demethylation–inhibiting fungicide resistant isolates of *Podosphaera clandestine* (Wallr.) to other areas, thus reducing powdery mildew in nurseries. Entire cherry crops destined for fresh-market sale have been rejected due to fruit infection by *Podosphaera clandestine*. Nicetic et al. (2002b) also reported that 0.3%-0.5% v/v HMO sprays applied prophylactically prevented the occurrence of the disease and had a curative effect on rose powdery mildew. Cooper et al. (2002) reported excellent control of powdery mildew with 0.3% horticultural mineral oil treatments on greenhouse roses (*Rosa spp.)*. Kallianpur et al. (2002) found that two HMOs inhibited mycelial growth and sporulation of *Podosphaera leucotricha* (Ell. & Ev.) on leaves of apple (*Malus domestica* (Borkh)). They also found a reduction of the carryover of inoculums to the next season. HMOs provide excellent means of disease control in the nursery and offer an alternative to the demethylation-inhibiting fungicides and other fungicides that have a moderate or high resistance risk (Grove et al., 2002).

Insects and Mites

Haustellate (sap-feeding) arthropods are the most common pests of landscape ornamentals. Sap-feeding arthropods insert needle-like mouthparts (stylets) into the mesophyll cells within leaves, and remove the cell contents (Barrett, 1996). Most piercing-sucking pests are small and soft-bodied, making them vulnerable to oil treatments. These pests often cause cosmetic damage to their host ornamentals as well as inducing significant leaf drop when damage is severe (Barrett, 1996). Some sap-sucking insects exude a carbohydrate-water complex (honeydew) during feeding that is harmless to plants, but provides growth medium for a variety of the sooty mold fungi (Pierce et al., 1998). There are several theories regarding how oils kill arthropods, depending on the physical characteristics of the oil. The best-known theory is that they act physically, by blocking the spiracles (Traverner, 2002). There is a lot of evidence to support this theory, but it does not account for all situations. Shepard (1939) presented three theories of how petroleum derived spray oils kill arthropods: "the saturated components of the oil block the spiracles, resulting in suffocation; the liquid unsaturated components penetrate the tissue, 'corroding' them; and volatile components act as fumigants." Taverner (2002) stated that oil fractions that are heavily saturated travel short distances into the tracheae and block gas exchange with a physical barrier. He then stated that oil fractions that are light and unsaturated pass into the body cavity, eventually dissolving the internal cellular structure. It has been suggested that oils probably solubilize membrane lipids disrupting the cellular membranes (van Overbeek and Blondeau, 1954). Freeborn and Atsatt (1918) theorized that the vapors of kerosene, used in the early 20th century, penetrated the tracheae and produced the lethal results. Taverner (2002) however, stated that the

lubricating oils and spray oils used today are not volatile enough to show fumigant activity. Taverner (2002) concluded that the primary cause of arthropod death can be anoxia (suffocation) but cannot be presumed as the only mode of action.

Hare et al. (1999) found that differing rates of narrow-range oil sprays effectively suppressed citrus bud mite populations. HMO treatments have been shown to reduce crapemyrtle aphid (*Tinocallis kahawaluokalani* (Kirkaldy.)) populations (Pierce et al., 1998). Baxendale and Johnson (1990) reported that Sunspray 6E at 2% oil provided good ovicidal activity against wooly larch adelgid (*Adelges laricis* (Vallot)), appeared to give complete control of the crawler stage of the cottony maple scale (*Pulvinaria innumerabilis* (Rathvon)) within a twenty-four hour period, and provided good control of honeylocust plant bugs (*Diaphanocoris chlorionis* (Say)) and leafhoppers (*Macropsis fumipennis* (Gillette and Baker)). They also stated that Sunspray 6E is effective against many pests found on ornamentals of the northeastern United States (Baxendale and Johnson, 1988). Butler and Henneberry (1989) showed that fewer sweet potato whiteflies were found on cottonseed oil treated plants for 7-9 days after treatment. They also showed that when sweet potato whiteflies encountered plants with oil residue on them, within 24 hours after spraying, that they were entrapped in the residue and died. Butler and Henneberry (1990) discovered that cottonseed oil had insecticidal activity to several pest insect species and acaricidal activity to spider mites.

Gough (1990) reported that intensive use of miticides has caused widespread mite resistance, causing most of the chemicals to become ineffective. Also, many of the miticides were shown to be phytotoxic. Currently, researchers are investigating new, non-synthetic mechanisms to control spider mites, including releasing predatory mites

(Zhang and Sanderson, 1992). Walsh and Zalom (2002) showed that applications of winter HMOs and agricultural mineral oils suppressed two-spotted spider mite populations, increased marketable yields and economic returns of California strawberries. Nicetic et al. (2002b) also showed that HMO sprays maintained populations of two-spotted mite below the economic threshold. They also stated that replacing synthetic pesticides with HMOs with or without predatory mites has significant cost benefits. Lancaster et al. (1998), found that soybean oil sprayed in the summer could control two-spotted spider mites.

The broad mite (*Polyphagotarsonemus latus* (Banks)) has a worldwide distribution. It is found in Africa, Asia, Australia, North America, South America, and the Pacific Islands (Kessing and Mau, 1993). The broad mite is considered a sub-major pest at lower elevations in Hawaii during the summer months. This mite feeds by piercing plant cells and removing the sap (Waterhouse and Norris, 1987), causing an instability of water balance and reduction in photosynthesis (Kessing and Mau, 1993). Infected flowers may not open properly or at all (Baker, 1997). Infested leaves become puckered, crinkled, curled and brittle (Baker, 1997), with corky brown areas appearing between the main veins on the underside of the leaf (Kessing and Mau, 1993). The mites are small in size (0.3 mm long) and prefer to feed on the underside of the leaves, usually near eggs, making them hard to see with the naked eye (Kessing and Mau, 1993). Male broad mites live 5 to 9 days and females live 8 to 13 days, laying 30 to 76 eggs during that time (Baker, 1997).

From the 1930s to 1960s, the citrus red mite (*Panonychus citri* (McGregor)) was reported as a serious economic pest of Florida citrus. The citrus red mite feeds on leaves,

fruit, and green twigs resulting in light colored stippling that gives a grayish or silvery appearance to the leaves and fruit. It has been shown that a citrus red mite population can increase 8.5 fold in ten days (Childers and Fasulo, 1995). Cen et al. (2000) found that oil deposits repelled female adult citrus red mites for at least 3 days. They also found that when red mite eggs were sprayed with increasing rates of oil, hatching rates decreased significantly. They also showed that a low concentration HMO could control the citrus red mite by toxicity and behavioral effects.

Residue Wash Off

Rain is a major environmental factor affecting the efficacy of foliar-applied insecticides. The deposits of insecticides are removed from the surface of the foliage by rain, causing a reduction in pest mortality (McDowell et al., 1984). Simulated rain of 2 to 5 mm has been shown to wash off 50% or more of the initial deposit of insecticide (Pick et al., 1984). This large removal of insecticides increases the reapplication of sprays causing increased chemical, fuel, labor, and machinery expenses (Mashaya, 1993). Cotton farmers follow a rule that if 9 mm of rain falls within 4 hours of spraying they should respray (Pick et al., 1984). Mashaya (1993), however, found that recommendations on whether to respray after rain falls is dependent on the insecticide and its formulation. Mashaya (1993) stated that the inherent qualities of a formulation is the reason why some pesticides seem to be more resistant to rainfall than others. The speed that the pesticide penetrates the leaf surface helps to determine its resistance to wash off. This speed is possibly influenced by the make up of the active ingredient and the agents added to the formulation, which may aid in the transport of the active

ingredient into the plant tissue (Pick et al., 1984). Mashaya (1993) stated that the total amount of rainfall is possibly more important than the rainfall intensity in determining the amount of insecticide that will be washed off. He reported that all of the insecticides he tested had reduced levels of insect control due to rain affecting the biological activity of the insecticides. "Anything that may increase rain-fastness would thus improve control of the pest and reduce the cost of pest control" (Pick et al., 1984). Bondada et al. (2000) found a negative linear relationship between oil retention on peach and apple stems and rainfall. They further stated that research on the relationship between rain and soybean oil deposits will aid in the decision process of whether to respray.

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Part III

Effects of Soybean Oil Formulations on Powdery Mildew, Broad Mites and Photosynthesis of Dogwoods

Abstract

Three experiments were conducted in greenhouses, to evaluate the effects of newly created soybean oil formulations on powdery mildew (*Erysiphe pulphra*), photosynthesis (Pn), and phytotoxicity of dogwood (*Cornus florida,* L., 'Cloud Nine') trees. In experiments one and two, oil treatments applied pre- and post-inoculation, respectively, of powdery mildew were evaluated. In the first experiment, treatments were sprayed one day before exposure to powdery mildew inoculum, while in the second experiment trees were sprayed four days after initial exposure to the inoculum. The third experiment also evaluated the efficacy of the formulations for control of broad mites (*Polyphagotarsonemus latus* (Banks)). In the third experiment, trees were sprayed with the treatments after infestation with broad mites and thirty-four days after initial exposure to powdery mildew inoculum. In each experiment, two-year-old potted trees were sprayed with 1.5% soybean oil (v/v in water) in the different formulations. The newly developed formulations (with adjuvants) were TNSOY20 (teric/termul), TNSOY21 (lauriciden), TNSOY22 (lecithin), TNSOY23 (lecithin/ MD), TNSOY24 (lecithin/ MD 2), and TNSOY25 (Latron B-1956®). Treatments also included Golden Natur'l®, a commercial formulation, and water (control). In the first experiment, trees sprayed pre-inoculation with Golden Natur'l, TNSOY22, TNSOY23, or the TNSOY25 had less powdery mildew than water-treated trees at 19 and 24 days after spraying (DAS). Leaves sprayed with TNSOY22 or TNSOY25 formulations had 68% and 40% lower Pn rates, respectively, than water-treated leaves one DAS. Sprays of the oil formulations tended to cause some yellowing of foliage. Leaves sprayed with oil four days after exposure to powdery mildew inoculum had less powdery mildew than the water-treated

leaves at 16 DAS. Leaves sprayed with TNSOY 20, TNSOY21, TNSOY22, or TNSOY24 continued to have less powdery mildew than the water-treated trees at 28 DAS. Oil formulations reduced Pn rates at six DAS, with only Golden Natur'l treated leaves recovering to rates similar to the water-treated leaves by 15 DAS. The TNSOY20, TNSOY21, TNSOY22, and TNSOY24 formulations caused more phytotoxicity at 42 DAS than the water treatment. In experiment 3, none of the oil formulations significantly controlled broad mites on dogwoods. The oil treated leaves had less powdery mildew 7 DAS than the water-treated leaves. All oil treated leaves had significantly lower photosynthetic rates the day after treatment than the water-treated leaves.

Introduction

Powdery mildew, caused by the fungus *Microsphaera pulchra,* was seen on a single flowering dogwood in Alabama in 1993. By the spring of 1994, the disease was commonly found on flowering dogwood in landscapes statewide. Since 1994, powdery mildew has remained the most common foliar disease of flowering dogwood in Alabama (Hagan et al., 1997). The prolific growth and sporulation of the fungus give the affected leaf surfaces and plant parts a white powder-like appearance. New growth of infected plants may be completely covered with powdery mildew, often reducing leaf and shoot growth and causing new shoots to curl. Older leaves and plant parts can also have severe powdery mildew infections. Heavily infected leaves may become chlorotic and senesce early. The unattractive appearance produced by powdery mildew infection on nursery plants may make them unsuitable for sale (Hansen et al., 2000). Season-long applications of synthetic fungicides are often involved in the chemical control of powdery mildew

(Hansen et al., 2000). Such long-term use of these chemicals may be unsustainable because of the possible development of resistance (Pasini et al., 1997). Horticultural (HMO) and agricultural mineral oils have been reported to control the disease with low risk of resistance and phytotoxicity (Nicetic et al., 2002). Oils may also physically inhibit plant gas exchange. In 1923, Burroughs stated that the oil film remaining on the plant surface might interfere with plant gas exchange and reduce transpiration rates, possibly causing abscission.

The broad mite (*Polyphagotarsonemus latus* (Banks)) has a worldwide distribution. It is found in Africa, Asia, Australia, North America, South America, and the Pacific Islands (Kessing and Mau, 1993). At lower elevations during the summer months the broad mite is considered a sub-major pest. Baxendale and Johnson (1990) found that Sunspray 6E at 2% oil provided good ovicidal activity against wooly larch adelgid (*Adelges laricis* (Vallot)), appeared to give complete control of the crawler stage of the cottony maple scale (*Pulvinaria inumerabilis* (Rathvon)) within a twenty-four hour period, and provided good control of honeylocust plant bugs (*Diaphanocoris chlorionis* (Say)) and leafhoppers (*Macropsis fumipennis* (Gillette and Baker)).

The purposes of this research are to develop new soybean oil formulations, evaluate their efficacies against powdery mildew and broad mites, and evaluate their phytotoxicities and effects on photosynthesis of dogwood trees.

Materials and Methods

Formulation Development: Mixtures of emulsifier (adjuvants), water and refined soybean oil were prepared in 150 ml volume capped nalgene bottles based on a percent volume ratio of emulsifier to oil. The formulations contained 1%, 5%, and 10% emulsifier in oil (v/v). Each spray treatment had 5% oil mixtures (95 ml of water and 5 ml of oil). Thus, the 1% formulations had 0.10 ml of emulsifier, the 5% formulations had 0.25 ml of emulsifier, and the 10% formulation had 0.5 ml of emulsifier. The emulsifiers tested were calcium stearate, calcium stearoyl lacylate, calcium dodecylbeneze sulfontae, sodium lauryl sulfate, glycerol monostearate, triglycerol monostearate, sorbitan monostearate, Latron B-1956 Spreader Sticker® (Rohm and Haas, Philadelphia, Pa.), Lecithin, lauriciden, Teric, Termul, Michigan emulsifiers A and B (experimental), and Yucca Aide 10 and 20. The nalgene bottles containing the mixtures were then placed in a shaker and shaken for one minute at a rate of 12 rpm. Pictures were taken prior to shaking and at 30 seconds, one minute, two minutes and five minutes after being shaken. Visual measurements of the time it took for the oil to separate from the water were taken using the photographs. The mixtures that stayed in emulsion the longest (two or more minutes) were then placed in cold storage at 2 °C (36 °F) overnight to see if the emulsions were stable at that temperature. Emulsifiers that were stable were chosen for further evaluation in trials.

TNSOY20 was formulated by emulsifying the 1.5% soybean oil in water by adding 0.015% Teric (a surfactant) and 0.165% Termul (an emulsifier) (Table 1). The adjuvants are expressed as a percentage of the volume of oil used. Soybean oil

Table 1. Concentrations of active and inactive ingredients in the soybean oil formulations used on dogwood trees.

		Adjuvants (%)				
Formulations	**Soybean oil (%)**	**Latron[z]**	**Lauriciden[y]**	**Lecithin[x]**	**Teric/ Termul[w]**	**MD[v]**
TNSOY20	99.82				0.015/ 0.165	
TNSOY21	99.97		0.03			
TNSOY22	99.85			0.15		
TNSOY23	99.78			0.15		0.075
TNSOY24	99.85			0.075		0.075
TNSOY25	99.85	0.15				

[z] Latron B-1956 Spreader Sticker.
[y] Soap.
[x] By product of the de-gumming process of refining soybean oil.
[w] A surfactant and emulsifier.
[v] Experimental emulsifier developed at the University of Tennessee, Knoxville.

was emulsified with 0.03% lauriciden (soap) to form TNSOY21. TNSOY22 was developed by emulsifying soybean oil with 0.15% Lecithin, a by-product of the de-gumming process of soybean oil. TNSOY23 was composed by emulsifying soybean oil with 0.15% Lecithin and 0.075% MD (an experimental adjuvant developed at the University of Tennessee (UT). TNSOY24 also has 0.075% MD, but a lesser amount of Lecithin (0.075%). TNSOY25 used 0.15% Latron B-1956 Spreader Sticker® (Rohm and Haas, Philadelphia, Pa.) to emulsify soybean oil in water, though at a lower concentration than previously reported (Lancaster et al., 1998).

Experiment 1: The first experiment (Expt.1) was conducted to evaluate the effect of the soybean oil formulations on powdery mildew on dogwood, when sprayed pre-exposure to the inoculum; as well as effects on photosynthesis (Pn) and phytotoxicity. Forty-eight

two-year old 'Cloud Nine' dogwood (*Cornus florida* L.) trees in 19 L (five-gallon) containers were placed in a greenhouse at UT, Knoxville. Trees were sprayed until runoff with 1.5% refined soybean oil (in water, v/v) in the formulations of TNSOY20 (Teric/Termul), TNSOY21 (lauriciden), TNSOY22 (Lecithin), TNSOY23 (Lecithin/MD), TNSOY24 (Lecithin/MD), or TNSOY25 (Latron B-1956). Treatments also included 1.5% Golden Natur'l® (Stoller Enterprises, Inc., Houston, Texas) and water (control). The treatments were sprayed until runoff using a backpack mist blower on 3 Oct. 2002. The trees were arranged in a randomized complete block (RCB) design with six replications.

The trees were exposed to powdery mildew the following day (4 Oct.) by placing severely infested, one-year old dogwood trees among the experimental units. Powdery mildew ratings were taken on 24 Oct. and 7 Nov. using the following modified eight-point rating scale (Azam et al., 1998): 1 = 0%, 2 = 1-3%, 3 = 4-6%, 4 = 7-12%, 5 = 13-25%, 6 = 26-50%, 7 = 51-87%, 8 = 88-100% of the foliage visually displaying powdery mildew.

One recently, fully expanded leaf was randomly selected per plant and net photosynthetic rates (Pn) were measured using an ADC-3 model portable infrared gas analyzer (ADC Inc., Hoddenson, UK) on 2, 4, 14, and 23 Oct., and 8 Nov. Photosynthesis measurements were made between 10:00 AM and 3:00 PM when photosynthetic active radiation (PAR) was >800 $\mu mol \cdot m^{-2} s^{-1}$. The same leaf was used throughout the Pn sampling dates by marking to leaf to the left of the sampled leaf.

Phytotoxicity was evaluated by rating symptoms on all leaves. Ratings were made on 19 Nov. using the following five-point rating scale (Davidson et al., 1990): 1=

no visible damage, 2 = slight yellowing on some leaves, 3 = moderate yellowing on most leaves, 4 = burn without dieback, and 5 = burn with dieback. All data were analyzed with the General Linear Models (GLM) procedure and Duncan's Multiple Range test (Statistical Analysis Systems software, SAS 9.0, SAS Institute, Cary, N.C.).

Experiment 2: A similar experiment in the same greenhouse was conducted to evaluate the same soybean oil treatments for the effects on powdery mildew, photosynthesis, and phytotoxicity when sprayed post-exposure to powdery mildew inoculum. Two-year-old 'Cloud Nine' dogwood trees in 19 L (five-gallon) containers were arranged in a RCB design with six replications. The trees were exposed to powdery mildew by placing severely infested one-year old dogwood trees among the experimental units on 4 Oct. The same oil treatments used in Expt.1 were sprayed until runoff using a backpack mist blower on 8 Oct. 2002, four days after initial exposure to powdery mildew.

Powdery mildew ratings were taken on 24 Oct. and 5 Nov. using the same eight-point rating scale used in Expt.1. Net photosynthetic rates (Pn) were measured on 14 and 23 Oct. and on 8 Nov. using the ADC-3 model analyzer in the same manner described in Expt.1.

Phytotoxicity ratings were taken on 19 Nov. using the same five-point rating scale as described above. All data were analyzed in the same manner as described above.

Experiment 3: Research was conducted to evaluate the soybean oil formulations for efficacy of broad mites (*Polyphagotarsonemus latus* (Banks)), eradicative control of powdery mildew when sprayed 34 days after initial exposure to powdery mildew inoculum, and for the effects on photosynthesis of dogwood trees. Forty-eight two-year-old 'Cloud Nine' dogwood trees in 19 L (five-gallon) containers were placed in a

different greenhouse at UT, Knoxville than were Expts.1 and 2. The trees were arranged in a RCB design with six replications. Trees were sprayed until runoff with the same treatments as described above. The trees were initially exposed to powdery mildew on 4 Oct., by placing heavily infested, one-year-old dogwood trees in the same greenhouse as the experimental units. The treatments were sprayed until runoff using a backpack mist blower on 7 Nov. 2002.

An infestation of broad mites was established by placing an infested tree among the experimental units (5 Oct.). Five newly formed terminal leaves were removed (14 Nov.) at the petiole from each experimental unit and placed into *Nasco*-Whirl Packs® and stored in cold storage at 2 °C (36 °F), for approximately 3 weeks. Each of the five leaves were placed, one at a time, into 10 ml of ethyl alcohol that was in a 50 ml plastic capped centrifuge tube (8 Jan.). The tube was shaken vigorously for ten seconds. The leaf was then washed with 1 ml of ethyl alcohol allowing the wash to collect in the same tube. This was done again with the remaining four leaves such that the five leaves of each tree were washed and mites collected in the same 10 ml of ethyl alcohol. The ethyl alcohol was then pored through Whatman 45 mesh filter paper. The mites and the filter paper were then placed on a grid and the numbers of broad mites present in pre-determined areas were counted using a dissecting microscope at a power of 40x. The area of each leaf was then measured using an area meter (Delta-T LTD, Cambridge CB5 OEJ, England) allowing for the calculation of the number of broad mites/cm^2 of leaf on one side of the leaf.

Powdery mildew ratings were taken on 7 and 14 Nov. and 2 Dec. using the same modified eight-point rating scale as used above. Leaf (Pn) rates were measured 7,8 and

14 Nov. using the ADC-3 gas analyzer as described in Expt.1. Data were analyzed using the GLM procedure (Statistical Analysis Systems software, SAS 9.0, SAS Institute, Cary, N.C.).

Results

Experiment 1: Trees sprayed with 1.5% soybean oil in Golden Natur'l, TNSOY20 (Teric and Termul used as adjuvants), TNSOY22 (Lecithin), TNSOY23 (Lecithin/MD), or TNSOY25 (Latron B-1956) formulations, one day prior to exposure to powdery mildew, had less powdery mildew than the water-treated trees at 21 DAS (23 Oct.) (Table 2). Foliage sprayed with Golden Natur'l, TNSOY22, TNSOY23, and TNSOY25 formulations continued to have significantly less powdery mildew than the water-treated plants at 35 DAS. The TNSOY25 treated trees had powdery mildew ratings 46% of the water-treated trees at 21 DAS. TNSOY22 treated trees had 50% as high of ratings at 35 DAS as the water-treated trees. Although the treatments had significant effects, the occurrence of powdery mildew on control plants was relatively low with less than 10% of foliage showing symptoms.

Net photosynthetic rates of leaves were similar among treatments when measured one day before spraying. The 1.5% TNSOY25 (Latron B 1956) was the only formulation that significantly reduced Pn at 1 DAS (Table 3). By 1 DAS, Pn rates of leaves sprayed with TNSOY22 (Lecithin) or TNSOY25 (Latron) formulations were 68% and 40% of the control leaves. By 11 DAS, no treatments significantly reduced Pn compared to the

Table 2. The effects of 1.5% soybean oil formulations on powdery mildew ratings when applied the day before exposure to powdery mildew.

		Powdery mildew [y]	
Formulations[z]	**Adjuvants**	**23 Oct.**	**11 Nov.**
Water (control)	- -	3.5 a[x]	2.8 a
Golden Natur'l	- -	2.0 bc	1.8 bc
TNSOY20	Teric/Termul	2.2 bc	2.1 abc
TNSOY21	Lauriciden	3.0 a	2.2 ab
TNSOY22	Lecithin	2.2 bc	1.4 c
TNSOY23	Lecithin/MD	2.0 bc	1.8 bc
TNSOY24	Lecithin/MD	2.7 ab	2.5 ab
TNSOY25	Latron B-1956	1.6 c	1.9 bc

[z] Trees were sprayed on 3 Oct. and exposed to powdery mildew inoculum on 4 Oct.

[y] Powdery mildew rating scale: 1 = 0%, 2 = 1-3%, 3 = 4-6%, 4 = 7-12%, 5 = 13-25%, 6 = 26-50%, 7 = 51-87%, 8 = 88-100% of the foliage visually displaying powdery mildew.

[x] Means within a column followed by the same letter are not significantly different, by Duncan's Multiple Range Test, $P < 0.05$.

Table 3. The effects of 1.5% soybean oil formulations on net photosynthetic rates when applied the day before exposure to powdery mildew.

		Net photosynthetic rates ($\mu mol \cdot m^{-2}s^{-1}$)				
Formulations[z]	**Adjuvants**	**2 Oct.**	**4 Oct.**	**14 Oct.**	**23 Oct.**	**8 Nov.**
Water (control)	- -	9.7 a[y]	8.7 a	7.2 a	7.8 a	5.6 ab
Golden Natur'l	- -	9.7 a	8.1 a	8.6 a	7.5 a	5.5 ab
TNSOY20	Teric/Termul	9.7 a	7.4 a	5.9 a	6.6 a	4.9 ab
TNSOY21	Lauriciden	10.1 a	7.4 a	8.3 a	7.5 a	6.4 ab
TNSOY22	Lecithin	10.0 a	5.9 ab	6.0 a	5.8 a	5.6 ab
TNSOY23	Lecithin/MD	9.7 a	7.3 a	7.4 a	7.3 a	5.5 ab
TNSOY24	Lecithin/MD	9.7 a	7.0 a	5.7 a	6.8 a	7.0 a
TNSOY25	Latron B1956	7.8 a	3.5 b	6.0 a	5.8 a	4.5 b

[z] Trees were sprayed on 3 Oct. and exposed to powdery mildew inoculum on 4 Oct.
[y] Means within a column followed by the same letter are not significantly different, by Duncan's Multiple Range Test, $P < 0.05$.

water sprayed plants. The Pn rates of Golden Natur'l treated leaves remained very similar to those of the controls for a month after spraying.

Most of the 1.5% oil formulations caused little or no yellowing of dogwood foliage. Leaves sprayed with TNSOY21 (lauriciden) or TNSOY22 (Lecithin) had more yellowing than the water treated leaves (Table 4). The other formulations did not cause significant phytotoxicity compared to the water treatment.

Experiment 2: Sprays of the 1.5% oil formulations, four days after exposure to powdery mildew, reduced powdery mildew ratings to less than 60% as high as the control at 16 DAS (Table 5). Foliage treated with the TNSOY20 (Teric/Termul), TNSOY21

Table 4. The effects of 1.5% soybean oil formulations on phytotoxicity when applied the day before exposure to powdery mildew.

Formulations[z]	Adjuvants	Phytotoxicity[y] 19 Nov.
Water (control)	- -	1.0 b[x]
Golden Natur'l	- -	1.2 ab
TNSOY20	Teric/Termul	1.2 ab
TNSOY21	Lauriciden	2.3 a
TNSOY22	Lecithin	2.3 a
TNSOY23	Lecithin/MD	2.0 ab
TNSOY24	Lecithin/MD	1.8 ab
TNSOY25	Latron B-1956	1.5 ab

[z] Trees were sprayed on 3 Oct. and exposed to powdery mildew inoculum on 4 Oct.

[y] Phytotoxicity rating scale: 1= no visible damage, 2 = slight yellowing on some leaves, 3 = moderate yellowing on most leaves, 4 = burn without dieback, and 5 = burn with dieback.

[x] Means followed by the same letter are not significantly different, by Duncan's Multiple Range Test, $P< 0.05$.

Table 5. The effects of 1.5% soybean oil formulations on powdery mildew ratings when applied four days after exposure to powdery mildew.

		Powdery mildew [y]	
Formulations[z]	**Adjuvants**	**24 Oct.**	**5 Nov.**
Water (control)	- -	3.3 a[x]	2.3 a
Golden Natur'l	- -	2.0 b	2.0 ab
TNSOY20	Teric/Termul	1.4 bc	1.2 b
TNSOY21	Lauriciden	1.7 bc	1.4 b
TNSOY22	Lecithin	1.2 c	1.2 b
TNSOY23	Lecithin/MD	1.6 bc	1.5 ab
TNSOY24	Lecithin/MD	1.3 bc	1.4 b
TNSOY25	Latron B-1956	1.7 bc	1.6 ab

[z] Trees were exposed to powdery mildew inoculum on 4 Oct. and sprayed on 8 Oct.
[y] Powdery mildew rating scale: 1 = 0%, 2 = 1-3%, 3 = 4-6%, 4 = 7-12%, 5 = 13-25%, 6 = 26-50%, 7 = 51-87%, 8 = 88-100% of the foliage visually displaying powdery mildew.
[x] Means within a column followed by the same letters are not significantly different, by Duncan's Multiple Range Test, $P < 0.05$.

(lauriciden), TNSOY22 (Lecithin), and TNSOY24 (Lecithin/MD) formulations still had significantly less powdery mildew than the water-treated foliage at 23 DAS. The TNSOY20 (Teric/Termul) and TNSOY22 (Lecithin) formulations reduced powdery mildew ratings to less than 52% of the control at 23 DAS.

Net photosynthetic rates of all oil treated leaves were significantly lower than control leaves, only 70% at 6 DAS (Table 6). TNSOY22 (Lecithin) reduced Pn rates to 28% of the control leaves at 6 DAS. All oil formulations, except Golden Natur'l, still reduced Pn at 15 DAS compared to the water-treated leaves. By 31 DAS none of the formulations significantly reduced Pn rates compared to the water treatment. However, most oil treatments still tended to reduce Pn rates. The TNSOY22 (Lecithin)

Table 6. The effects of 1.5% soybean oil formulations on net photosynthetic rates when applied four days after exposure to powdery mildew.

Formulations[z]	Adjuvants	Net photosynthetic rates ($\mu mol \cdot m^{-2}s^{-1}$)		
		14 Oct.	23 Oct.	8 Nov.
Water (control)	- -	9.8 a[y]	9.4 a	4.3 ab
Golden Natur'l	- -	6.8 b	8.2 ab	5.2 a
TNSOY20	Teric/Termul	4.3 bc	4.5 cd	2.8 ab
TNSOY21	Lauriciden	5.2 bc	5.8 bcd	4.4 ab
TNSOY22	Lecithin	2.7 c	3.3 d	2.2 b
TNSOY23	Lecithin/MD	5.0 bc	5.4 cd	2.7 b
TNSOY24	Lecithin/MD	4.0 bc	4.8 cd	2.9 ab
TNSOY25	Latron B-1956	6.4 b	6.7 bc	3.9 ab

[z] Trees were exposed to powdery mildew inoculum on 4 Oct. and sprayed on 8 Oct.
[y] Means within a column followed by the same letter are not significantly different, by Duncan's Multiple Range Test, $P< 0.05$.

had Pn rates that were 50% of the control leaves at 31 DAS. Treatments containing Lecithin tended to have among the lowest foliar Pn rates during the month after spraying. There was a trend for oil treated leaves to have slightly more yellowing than water-treated foliage at 42 DAS (19 Nov.) (Table 7).

Experiment 3: None of the oil formulations significantly controlled broad mites on dogwood (data not shown). Trees sprayed with TNSOY23 (Lecithin/MD) and TNSOY20 (Teric/Termul) both had 22% and >300 more broad mites than the water treated leaves.

Plants exposed to powdery mildew 34 days before spraying oil treatments had similar ratings of infection the day before spraying treatments (Table 8). Oil treated plants had significantly lower powdery mildew ratings than the water treatment at 7 DAS.

Table 7. The effects of 1.5% soybean oil formulations on phytotoxicity when applied four days after exposure to powdery mildew.

Formulations[z]	Adjuvants	Phytotoxicity[y] 19 Nov.
Water (control)	- -	1.0 b[x]
Golden Natur'l	- -	1.7 ab
TNSOY20	Teric/Termul	2.7 a
TNSOY21	Lauriciden	2.7 a
TNSOY22	Lecithin	2.7 a
TNSOY23	Lecithin/MD	1.8 ab
TNSOY24	Lecithin/MD	2.2 a
TNSOY25	Latron B-1956	1.8 ab

[z] Trees were exposed to powdery mildew inoculum on 4 Oct. and sprayed on 8 Oct.

[y] Phytotoxicity rating scale: 1= no visible damage, 2 = slight yellowing on some leaves, 3 = moderate yellowing on most leaves, 4 = burn without dieback, and 5 = burn with dieback.

[x] Means followed by the same letter are not significantly different, by Duncan's Multiple Range Test, $P< 0.05$.

Table 8. The effects of 1.5% soybean oil formulations on powdery mildew ratings when applied thirty four days after initial exposure to powdery mildew.

		Powdery mildew [y]		
Formulations[z]	**Adjuvants**	**7 Nov.**	**14 Nov.**	**2 Dec.**
Water (control)	- -	2.8 a[x]	4.5 a	6.3 a
Golden Natur'l	- -	2.0 a	2.3 b	4.5 ab
TNSOY20	Teric/Termul	2.3 a	1.3 c	2.4 b
TNSOY21	Lauriciden	2.2 a	1.3 c	3.5 b
TNSOY22	Lecithin	2.3 a	1.3 c	2.7 b
TNSOY23	Lecithin/MD	3.0 a	1.6 bc	4.5 ab
TNSOY24	Lecithin/MD	2.0 a	1.8 bc	3.5 b
TNSOY25	Latron B-1956	2.5 a	1.7 bc	4.3 ab

[z] Trees were exposed to powdery mildew inoculum on 4 Oct. and sprayed on 7 Nov.

[y] Powdery mildew rating scale: 1 = 0%, 2 = 1-3%, 3 = 4-6%, 4 = 7-12%, 5 = 13-25%, 6 = 26-50%, 7 = 51-87%, 8 = 88-100% of the foliage visually displaying powdery mildew.

[x] Means within a column followed by the same letters are not significantly different, by Duncan's Multiple Range Test, $P< 0.05$.

The control plants had ratings of 4.5 (approximately 10% infected foliage) while plants sprayed with TNSOY20 (Teric/Termul), TNSOY21 (lauriciden), and TNSOY22 (Lecithin) had ratings of 1.3 (< 3% infected foliage). Plants sprayed with the oil still tended to have lower powdery mildew ratings by 26 DAS. Leaves sprayed with TNSOY20 (Teric/Termul), TNSOY21 (lauriciden), TNSOY22 (Lecithin), and TNSOY24 (Lecithin/MD) formulations still had significantly less powdery mildew than the water treatment by 26 DAS. The TNSOY20 (Teric/Termul) and TNSOY22 (Lecithin) treated leaves had approximately 2% infected leaves while control plants had >25% leaf infection.

The Pn rates of plants were similar among plants the day before spraying (Table 9). All oil treated leaves had significantly lower Pn rates than the water-treated leaves at 1 DAS. TNSOY22 (Lecithin) greatly reduced the Pn rates to approximately only 5% of the Pn rates of water sprayed leaves at 1 DAS and 6 DAS. TNSOY24 (Lecithin/MD) reduced Pn rates to 25% and 33% of the control treatment at 1 and 6 DAS, respectively. By 7 DAS, only the foliage treated with Golden Natur'l or TNSOY25 (Latron) had Pn rates that were not significantly different from the control and had recovered to 80% of control leaves Pn rates.

Table 9. The effects of 1.5% soybean oil formulations on net photosynthetic rates when applied thirty four days after initial exposure to powdery mildew.

Formulations[z]	Adjuvants	Net photosynthetic rates ($\mu mol \cdot m^{-2} s^{-1}$)		
		7 Nov.	8 Nov.	14 Nov.
Water (control)	- -	9.5 a[y]	11.1 a	10.3 a
Golden Natur'l	- -	9.1 a	6.3 b	8.4 ab
TNSOY20	Teric/Termul	9.1 a	7.3 b	7.1 bc
TNSOY21	Lauriciden	8.8 a	6.3 b	5.6 bcd
TNSOY22	Lecithin	9.4 a	0.5 d	0.5 e
TNSOY23	Lecithin/MD	9.7 a	5.8 b	5.4 cd
TNSOY24	Lecithin/MD	9.1 a	2.8 c	3.3 d
TNSOY25	Latron B-1956	9.8 a	5.7 b	8.3 ab

[z] Trees were exposed to powdery mildew inoculum on 4 Oct. and sprayed on 7 Nov.
[y] Means within a column followed by the same letter are not significantly different, by Duncan's Multiple Range Test, $P < 0.05$.

Discussion

Our research indicates that all soybean oil formulations, whether sprayed pre- or post-exposure to powdery mildew, reduced powdery mildew compared to the water-treated plants. The leaves treated with the TNSOY22 formulation generally had less powdery mildew, but also had lower Pn rates during the duration of the experiment. The reduction of powdery mildew may be due to the oil effectively covering and smothering the powdery mildew. The coverage of oil may also cover or penetrate too much of the leaf surface and suppress leaf photosynthesis.

Application of the soybean formulations temporarily decreased Pn rates of the dogwoods. The oil sprayed trees in the first experiment were able to recover from the reduction in Pn rates by 11 DAS. The third experiment had only the Golden Natur'l and TNSOY25 treated trees recovering from reduced Pn rates by 7 DAS.

Chapman (1967) stated that phytotoxicity is one of the principal limitations of spray oils. Hodgkinson et al. (2000) stated that spray-oil-induced phytotoxic symptoms usually occur as part of the plants response to its current physical, chemical, or biological stresses. There were some symptoms of phytotoxicity present in the first experiment, but were not significant compared to the control treatment. However, in the second experiment, three of the formulations caused significant phytotoxicity compared to the water-treated trees. The difference between the first and second experiments could be due to stress of the plants in the presence of powdery mildew.

The oil formulations had no effect on the presence of broad mites. This may be due to the fact that broad mites are small and tend to be next to the mid vein of the leaf.

This would possibly make it difficult for the oil formulations to actually contact the broad mites.

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Part IV

The Spray Residues of Soybean Oil Formulations Deposited on Peach Foliage and Removed by Rainfall

Abstract

Research was conducted to evaluate the amounts of different soybean oil formulations that were deposited on peach (*Prunus persica* (L.)) tree leaves and washed off by 1.3 cm (0.5 in) and 2.6 cm (1.0 in) of simulated rainfall. Ninety-six, two-year-old peach trees in 10.8 L (three-gallon) containers were placed outdoors at the University of Tennessee, Knoxville and arranged in a randomized complete block design in a circle around a 2.7 meter (9 ft) high spray nozzle in three blocks, eight treatments and 12 replications. The trees were sprayed until runoff with 2% refined soybean oil (v/v of water), in formulation of TNSOY26, TNSOY27, TNSOY28, TNSOY29, TNSOY25, and TNSOY30 of the total volume of oil. Treatments of 2% Golden Natur'l and water (control) were also used in the experiment. Ten leaves were sampled from each tree after oil treatment and simulated rainfall of 1.3 cm (0.5 in) and 2.6 cm (1.0 in). Chloroform extraction was performed on the leaves to determine how much oil remained on the leaf surface after the different rainfall simulations. The TNSOY25 treatment left more spray residue on leaves after spraying and each rainfall regimen. TNSOY26 left the least amount of spray deposits after spraying and each rainfall regimen.

Introduction

Rain is a major environmental factor affecting the efficacy of foliar-applied pesticides. McDowell et al. (1984) reported that a reduction in pest mortality was caused by removal of the deposits of insecticide from the leaf surfaces of the foliage by rain. Simulated rain of 2 to 5 mm has been shown to wash off 50% or more of the initial deposit of insecticides (Pick et al., 1984). In turn, insecticide loss leads to repeated

sprays that causes increased chemical, fuel, labor, and machinery expenses (Mashaya, 1993).

Bondada et al. (2000) found that peach leaves sprayed with 1% soybean oil plus Latron B-1956 retained 82%, 38%, or 18% of the applied oil after 0.25, 1.25, or 2.54 cm of rainfall, respectively. They also found that there was an accumulation of wax around the stomates of peach leaves, but not apple leaves. However the epicuticular wax was not affected by the oil formulations.

The objectives of this research were to develop new botanical formulations using botanical or food grade emulsifiers, evaluate their spray residues on leaves, and the potential wash-off (rain-fastness) of the residue. The emulsifiers chosen were Latron B-1956, Alginate, Lecithin, Guar Gum, and MD.

Materials and Methods

Ninety-six, one-year-old 'Contender' peach trees (*Prunus persica* (L.)) were potted in 10.8 L (three-gallon) containers and sprayed until runoff, on 4 June, with different formulations of 2% refined soybean oil (v/v) in water. The formulations with their adjuvants were, TNSOY25 (Latron B-1956 Spreader Sticker) (Rohm and Hass, Philadelphia, Pa.), TNSOY26 (Alginate/ MD), TNSOY27 (Guar Gum/ MD), TNSOY28 (Lecithin/ MD/ Guar Gum), TNSOY29 (Guar Gum/ MD), and TNSOY30 (Lecithin/ MD/ Guar Gum). The percentages of adjuvants (percentage of the total volume of oil) are shown in Table 1. Latron B-1956 is sold as a spreader-sticker. Alginate is used in the food industry as a thickener. Lecithin is a by-product of the de-gumming process of

Table 1. Concentrations of active and inactive ingredients in the soybean oil formulations used on peach leaves.

		Adjuvants (%)				
Formulations	**Soybean oil (%)**	**Latron[z]**	**Alginate[y]**	**Lecithin[x]**	**Guar Gum[w]**	**MD[v]**
TNSOY25	99.85	0.15				
TNSOY26	99.4		0.10			0.50
TNSOY27	99.4				0.10	0.50
TNSOY28	99.3			0.10	0.10	0.50
TNSOY29	99.45				0.05	0.50
TNSOY30	99.35			0.10	0.05	0.50

[z] Latron B-1956.
[y] Used in the food industry as a thickener.
[x] By product of the degumming process of refining soybean oil.
[w] Used in the food industry as a thickener and stabilizer.
[v] Experimental emulsifier developed at the University of Tennessee, Knoxville.

refining soybean oil. Guar Gum is used in the food industry as a thickner and stabilizer. MD is an experimental emulsifier developed at the University of Tennessee, Knoxville. Treatments of 2% Golden Natur'l (Stoller Enterprises, Inc., Houston, Texas) and water (control) were also used in the experiment. Treatments were applied until runoff using a backpack mist blower in the morning of 4 June. The trees were then placed outdoors at the University of Tennessee, Knoxville. Trees were arranged in a randomized complete block design in a circle around a nine-foot high spray nozzle in three blocks, eight treatments and 12 replications.

After treatments dried for one hour, ten leaves were randomly selected from each tree for measurement of spray oil residues prior to rainfall treatment. Leaves were stored in Nasco whirl packs in a cold storage unit at 2.2 °C (36 °F) for five to ten days until the

oil deposits could be extracted. Average temperature during spraying and collection time was approximately 18 °C (65 °F). Trees in the first four replications were then exposed to 1.3 cm (0.5 in) of simulated rainfall on 5 June. Treated leaves air dried for one hour and ten leaves were collected from each tree and stored in the same manner as above. The same trees were subsequently exposed to 1.3 cm (0.5 in) more of simulated rainfall on 5 June, for a total of 2.6 cm (1.0 in), left to dry for an hour and ten leaves were collected and stored. Trees in the remaining 8 replications were exposed to the same rainfall treatments on 6 June (two days after oil treatments were applied) for a total of 2.6 cm (1.0 in) and stored in the same manner.

Starting 9 June, the ten leaves from each tree for each rainfall regimen were then submerged, one at a time, into 20 ml of chloroform in a small porcelain dish for 30 seconds. After all ten leaves had been dipped, the solution was poured through an 11.0 cm Whatman filter paper and collected in a pre-weighed test tube. The porcelain dish was then rinsed with 10 ml of chloroform and poured through the same filter paper and collected in the same test tube. The tube was placed in a chemical hood until the chloroform had evaporated. Tubes were reweighed after chloroform evaporation. The amount of oil deposited (plus leaf wax) was determined by subtracting the post-evaporation weights from the pre-evaporation weights. The surface area of the ten leaves for each tree was then measured using a Delta-T area meter (Delta T, Cambridge CB5 OEJ, England). The leaf area measurement was doubled to obtain the total area of the axial and abaxial surface of the leaves. A mean wax weight ($\mu g/cm^2$) of water-treated (control) leaves was then used as the standard amount of wax present on the leaves for each rain regimen. The mean value of wax on control leaves was subtracted from the

oil/wax values of other treatments to estimate the amount of oil residue on leaves of each treated tree. Data were analyzed with the General Linear Models (GLM) procedure and Duncan's multiple range test (Statistical Analysis Systems software SAS 9.0, SAS Institute, Cary, N.C.).

Results

The TNSOY25 soybean oil formulation left significantly more oil residue on the foliage before rainfall than did any other formulation (Table 2). Trees sprayed with other formulations had only 36% (Golden Natur'l) to 55% (TNSOY28) as much oil residue left on leaves as those sprayed with TNSOY25. The other treatments were not significantly different from each other at 0 cm of simulated rainfall. The leaf wax on control leaves was 66.0 $\mu g/cm^2$.

Trees sprayed with TNSOY25 continued to have more oil residue on leaves after 1.3 cm (0.5 in) rainfall than did the other formulations (Table 3). Leaves treated with other formulations had 44% to 67% as much oil residue after the 1.3 cm (0.5 in) rainfall as those treated with TNSOY25. The other formulations were not significantly different from each other. The control leaves had 44.1 $\mu g/cm^2$ after 1.3 cm (0.5 in) rainfall. The TNSOY25 treated leaves still tended to have the most oil residue after 2.6 cm (1.0 in), though not significantly different from several other formulations (Table 4). Leaves treated with TNSOY26, TNSOY27 and TNSOY29 had 9%, 25%, and 21% as much oil residue, respectively, as the leaves sprayed with TNSOY25. Leaves treated with Golden Natur'l had only 40% as much oil residue after rain as leaves treated with TNSOY25. The water-treated (control) leaves had 45.9 $\mu g/cm^2$of wax.

Table 2. The effect of soybean oil formulations on the amount of oil residue present on peach tree leaves before rainfall.

Formulations[z]	Adjuvants	Leaf area[y] (cm^2/leaf)	Oil residue ($\mu g/cm^2$)
Water (control)	- -	32.5	- -
Golden Natur'l	- -	32.5	42.3 b[w]
TNSOY25	Latron B-1956	28.9	118.3 a
TNSOY26	Alginate/MD	30.0	62.0 b
TNSOY27	Guar Gum/MD	30.6	44.9 b
TNSOY28	Lecithin/MD/Guar Gum	29.8	65.5 b
TNSOY29	Guar Gum/MD	32.6	59.9 b
TNSOY30	Lecithin/MD/Guar Gum	31.0	60.2 b

[z] Trees were sprayed on 4 June and leaves collected on 4 June.
[y] Leaf area is total of axial and abaxial surfaces.
[x] Leaf wax on control leaves = 66.0 $\mu g/cm^2$.
[w] Data was analyzed by Duncan's Multiple Range Test. Means followed by the same letter are not significantly different at $P< 0.05$.

Table 3. The effect of soybean oil formulations on the amount of oil residue present on peach tree leaves after a simulated 1.3 cm rainfall.

Formulations[z]	Adjuvants	Leaf area[y] (cm^2/leaf)	Oil residue ($\mu g/cm^2$)
Water (control)	- -	35.1	- -
Golden Natur'l	- -	34.4	64.1 b[w]
TNSOY25	Latron B-1956	33.8	98.9 a
TNSOY26	Alginate/MD	33.4	43.8 b
TNSOY27	Guar Gum/MD	31.9	45.0 b
TNSOY28	Lecithin/MD/Guar Gum	31.3	66.6 b
TNSOY29	Guar Gum/MD	35.3	56.4 b
TNSOY30	Lecithin/MD/Guar Gum	32.3	56.1 b

[z] Trees were sprayed and exposed to rain on 4 June. Leaves were sampled from replications 1-4 on 5 June and from replication 5-12 on 6 June.
[y] Leaf area is total of axial and abaxial surfaces.
[x] Leaf wax on control leaves = 45.9 $\mu g/cm^2$.
[w] Data was analyzed by Duncan's Multiple Range Test. Means followed by the same letter are not significantly different at $P< 0.05$.

Table 4. The effect of soybean oil formulations on the amount of oil residue present on peach tree leaves after a simulated 2.5 cm rainfall.

Formulations[z]	Adjuvants	Leaf area[y] (cm^2/leaf)	Oil residue ($\mu g/cm^2$)
Water (control)	- -	35.0	- -
Golden Natur'l	- -	34.9	26.80 ab[w]
TNSOY25	Latron B-1956	33.4	68.30 a
TNSOY26	Alginate/MD	35.2	6.18 b
TNSOY27	Guar Gum/MD	34.0	17.06 ab
TNSOY28	Lecithin/MD/Guar Gum	32.0	47.60 ab
TNSOY29	Guar Gum/MD	36.7	14.10 ab
TNSOY30	Lecithin/MD/Guar Gum	32.8	65.50 a

[z] Trees were sprayed and exposed to rain on 4 June. Leaves were sampled from replications 1-4 on 5 June and from replications 5-12 on 6 June.

[y] Leaf area is total of axial and abaxial surfaces.

[x] Leaf wax on control leaves is 46.0 $\mu g/cm^2$.

[w] Data was analyzed by Duncan's Multiple Range Test. Means followed by the same letter are not significantly different at $P< 0.05$.

Discussion

Our research indicates that TNSOY25 treated leaves had the most spray residue on peach tree leaves prior to and after each rainfall. Latron B-1956, the adjuvant in TNSOY25, is advertised as a spreader sticker, thus perhaps explaining the greater residue. However, our other research on dogwoods has shown that TNSOY25 tends to increase phytotoxicity and to reduce net photosynthetic rates compared to other soybean oil formulations. Perhaps the formulation remains on the leaf surface for too long a time period in some cases, disrupting gas exchange. The emulsifier may aid penetration of the leaf surface tissue by the formulation causing physical cell or disrupting metabolic processes.

TNSOY26 and TNSOY29 treated leaves had similar amounts of oil residues as other treatments (other than TNSOY25), but much less residues after 1.3 cm of rainfall. Alginate, MD, or the combination of the two in TNSOY26 apparently resulted in less sticking of the soybean oil to leaves. Since MD is an adjuvant in each of the other formulations, it is probable that Alginate is associated with the greater wash off of spray residue. Similarly, the Guar Gum may be responsible for greater wash off of spray residue from TNSOY26 and TNSOY29. Enough of the oil must remain present on the leaf surface to provide pest control but not so much that it disrupts photosynthesis or causes phytotoxicity. More research is needed on the formulations that did not have as much residue present as the TNSOY25 formulation, but had more present than the TNSOY26 formulation.

The formulations (TNSOY28 and (TNSOY30) with the adjuvant of Lecithin, Guar Gum and MD tended to leave more oil residue as compared to the formulations with just the adjuvants Guar Gum and MD. The addition of Lecithin appears to make the formulations more rain-fast after 1.3 cm (1.0 in) of rain. More research is needed to determine the benefit of increasing the percentage of Guar Gum in combination with Lecithin and MD.

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Part V

The Spray Residues of Soybean Oil Formulations Deposited on Dormant Oak Twigs and Removed by Rainfall

Abstract

Research evaluated residue volumes from soybean oil formulations remainig on dormant oak (*Quercus phellos* (L.)) twigs after exposure to 1.3 cm (0.5 in) and 2.6 cm (1.0 in) of simulated rainfall. Thirty-six, two-year-old oak trees in 10.8 L (three-gallon) containers were sprayed until runoff with 10% refined soybean oil (v/v of water), with TNSOY26, TNSOY31, TNSOY32 and TNSOY36 of the total volume of water. Treatments of 10% Golden Natur'l and water (control) were also used in the experiment. The treatments were sprayed until runoff on 11 February 2003. Three twigs were removed from each tree before treatments to establish a baseline of wax present on the twigs. Three twigs were removed from each tree after spray treatments and after each rainfall treatment. Spray residues were removed from twigs by chloroform extraction. There were no significant differences in oil residues on the twigs after spray applications. After the 1.3 cm (0.5 in) of simulated rainfall the Golden Natur'l and TNSOY36 formulations were significantly different from the other formulations, but not from each other. The Golden Natur'l formulation and TNSOY36 had 33% and 50%, respectively, of the oil residue compared to TNSOY32.

Introduction

Summer-time applications of 0.5% to 2.0% soybean oil can be used to control insects and mites (Lancaster et **al.**, 1998). However, higher rates of 3% or 4% soybean oil should be used during the winter (dormant sprays) to control insect or mite populations (Pless et **al.**, 1995). Dormant plant twigs can tolerate higher rates of oil without phytotoxicity than can foliage in the spring and summer. Respiration rates of

insect and mite eggs are lower in cooler weather, because oils kill by smothering the eggs, longer exposure to the oil is needed. Soybean oil formulations for dormant sprays must be able to remain in stable spray emulsion in cool temperatures, leave more oil deposits on the target surface, and remain on the target for longer periods of time than summer-time sprays. However, relatively high spray rates of 5% to 10% soybean oil applied in winter can delay fruit tree bloom and thin fruit (Myers et al., 1996). The application of high rates Tnsoy1 (soybean emulsified with Latron B-1956) left more oil deposits on twig/buds (Moran et al., 2000). Further, spray oils for bloom delay are expected to have to stay on the tree for longer periods of time and have been observed by members of this project to be on trees for over a month in the winter.

The objectives of this research were to develop new botanical formulations with botanical or food grade emulsifiers, evaluate their spray residue on twigs in wintertime, and the potential wash-off (rain-fastness) of the residue.

Materials and Methods

After evaluating data from the previous trials (reported Part II and Part III), several of the formulations were modified by changing adjuvant amounts or combinations. New formulations, named TNSOY31, TNSOY32, and TNSOY36, were prepared using refined soybean oil as the active ingredient.

Research was conducted in Feb. 2003 to evaluate the effects of rainfall volumes on different soybean oil formulations sprayed during the wintertime (dormant sprays) on oak (*Quercus phellos* (L.)) plants (Table 1). TNSOY31 contained Lecithin and MD; TNSOY32 contained Lecithin, Alginate and MD (combinations not previously used); and

Table 1. Concentrations of active and inactive ingredients in the soybean oil formulations used as dormant spray on oak trees.

		Adjuvants (%)			
Formulations	**Soybean oil (%)**	**Latron[z]**	**Alginate[y]**	**Lecithin[x]**	**MD[w]**
TNSOY26	99.4		0.10		0.50
TNSOY31	99.4			0.10	0.50
TNSOY32	99.39		0.10	0.01	0.50
TNSOY36	98.0	1.0			

[z] Latron B-1956.
[y] Used in the food industry as a thickener.
[x] By product of the de-gumming process of refining soybean oil.
[w] Experimental emulsifier developed at the University of Tennessee, Knoxville.

TNSOY36 used a lower rate of Latron B-1956 Spreader Sticker (Rohm and Haas, Philadelphia, Pa.). The percentages of adjuvants in the formulation are of total volume of spray in this trial rather than percentage of the soybean oil. Latron B-1956 is a commercial spreader-sticker, Lecithin is a by-product of the de-gumming process of refining soybean oil, Alginate is used in the food industry as a thickener and stabilizer, and MD is an experimental emulsifier developed at the University of Tennessee, Knoxville. Guar Gum was not included in this trial due to the limited number of experimental units.

Thirty-six, two-year old oak (*Quercus phellos* (L.)) trees in 10.8 L (three-gallon) containers were placed outdoors at the University of Tennessee, Knoxville. The trees were sprayed until runoff on 11 Feb. 2003 using a backpack mist blower with 10% TNSOY26, TNSOY31, TNSOY32, TNSOY36, or Golden Natur'l (Stoller Enterprises, Inc., Houston Texas), or with water. The trees were arranged in a randomized complete

block design in a circle around a nine-foot high spray nozzle in two blocks, six replications, and six treatments for exposure to rainfall.

Three twigs were removed from each tree before oil applications to determine the amount of wax present on the twigs. The twigs were placed in Nasco whirlpacks and stored in a cold storage unit at 2.2 °C (36 °F) until the chloroform extraction process could be completed, approximately 4 days. The average temperature for the week of spraying and collection was 35 °F (1.7 °C). After the trees were sprayed, the oil treatments were allowed to dry for one hour and three twigs were removed from each tree and stored to represent oil/wax deposits prior to rainfall. On 13 Feb. 2003, the trees were exposed to 1.3 cm (0.5 in) of simulated rainfall and allowed to dry for an hour. Three twigs were then sampled from each tree and stored as previously described.

Approximately four days later, three twigs from each tree for each rain regimen were individually dipped for 30 seconds into 15 ml of chloroform in a pre-weighed test tube. All three twigs from a single tree were dipped into the same test tube. The test tubes were then place in a chemical hood to allow for the evaporation of the chloroform. The test tubes were then re-weighed. The original tube weight was subtracted from tube plus oil/wax to obtain an amount of oil and wax present on the twigs. The length and circumference of the twigs were then measured and the total surface area was estimated using the equation for the surface area of a cylinder (surface area = 2*3.14 (shoot diameter)(shoot length)). The amount of oil plus wax/surface area ($\mu g/cm^2$) of a twig was calculated by dividing the amount of oil plus wax extracted by the surface area of a twig. Twigs were collected from control plants prior to and after 1.3 cm (0.5 in) of rainfall. The mean wax/surface area of the control twigs was used as the standard amount

(baseline) for oil treated twigs in each rainfall. This wax amount was subtracted from the oil plus wax/surface area of the other treatments to obtain the amount of oil/surface area that was present on the twigs. Data were analyzed with the General Linear Models (GLM) procedure and Duncan's multiple range test (Statistical Analysis Systems (SAS 9.0, SAS Institute, Cary, N.C.).

Results

The twigs from the potted, dormant oak trees had similar amounts of wax before oil treatments were applied, ranging from 0.04 $\mu g/cm^2$ to 2.65 $\mu g/cm^2$. The sampled twigs averaged 8.07 cm in length and 0.271 cm in diameter. The control twigs (water treated) sampled after the oil treatments were applied, but before the rainfall, had 16.8 $\mu g/cm^2$ of wax. These twigs had been selected from those remaining after the prior selection and tended to be slightly thicker (0.283 cm), with lengths cut to an average of 8.16 cm. The small potted trees only had a limited number of twigs available for sampling. Twigs used in the second sampling were generally taken from slightly lower on the plants and were probably slightly older. Thus, selection of slightly older twigs may account for more wax on the twigs in the second sampling. The control twigs (water treated) sampled after the rainfall were generally taken from even lower on the trees and tended to be slightly thicker (0.333 cm, mean length of 8.20 cm) and have slightly more wax (20.3 $\mu g/cm^2$).

Sprays of soybean oil formulations left residues (presumably >98% soybean oil) on the control twigs (after spraying but before rainfall) that varied from 92.8 $\mu g/cm^2$ to 222.5 $\mu g/cm^2$ (Table 2). However, due to the variability in data, the differences were not

Table 2. Oil residue from different soybean oil formulations left on oak twigs prior to rainfall.

Formulations[z]	Adjuvants	Surface area (cm^2)	Oil residue ($\mu g/cm^2$)
Water (control)	- -		—[y]
Golden Natur'l	- -	76.9	118.0 a[x]
TNSOY26	Alginate/MD	71.9	222.5 a
TNSOY31	Lecithin/MD	65.1	136.7 a
TNSOY32	Lecithin/MD/Alginate	60.3	180.0 a
TNSOY36	Latron	57.9	92.8 a

[z] Trees were sprayed and twigs collected on 11 Feb. 03.
[y] Standard wax = 16.8 $\mu g/cm^2$.
[x] Means within a column followed by the same letter are not significantly different, by Duncan's Multiple Range Test, $P< 0.05$.

significant. Surprisingly, the formulation with Latron B-1956 (TNSOY36) tended to leave less oil residue than formulations with Alginate/MD or Lecithin/MD. Formulations containing Alginate tended to leave more oil residue on the twigs than the other formulations.

Treatments varied in spray deposits on twigs after the 1.3 cm (0.5 in) rainfall (Table 3). After the rain, 10% Golden Natur'l and TNSOY36 treated twigs had significantly less oil residue than twigs treated with other formulations. Golden Natur'l and TNSOY26 treated twigs had only 33% and 50%, respectively, as much oil residue as twigs treated with TNSOY32. Trees treated with formulations (TNSOY26 and TNSOY32) containing Alginate and MD adjuvants tended to have more spray residues after the rain. Twigs treated with TNSOY36 had as much oil residue in 1.3 cm (0.5 in) after rainfall (Table 3) as before (Table 2), thus perhaps the adjuvant Latron B-1956

Table 3. The effect of soybean oil formulations on the amount of oil residue left on oak twigs after 1.3cm (0.5 in) of rainfall.

Formulations[z]	Adjuvants	Surface area (cm^2)[y]	Oil residue ($\mu g/cm^2$)
Water (control)	- -		--[y]
Golden Natur'l	- -	69.70	64.4 b[x]
TNSOY26	Alginate/MD	80.79	183.4 a
TNSOY31	Lecithin/MD	91.19	163.3 a
TNSOY32	Lecithin/MD/Alginate	72.55	194.6 a
TNSOY36	Latron	74.03	97.7 b

[z] Trees were sprayed on 11 Feb. 2003, rain simulated on 13 Feb. 2003, and twigs collected 13 Feb. 2003.

[y] Standard wax = 20.3 $\mu g/cm^2$.

[x] Means within a column followed by the same letter are not significantly different, by Duncan's multiple range test, $P< 0.05$.

(spreader-sticker) made the soybean oil more rain-fast. However, twigs treated with the formulations (TNSOY26 and TNSOY32) containing Alginate and MD had more spray residue before and after the rain. Twigs treated with 10% Golden Natur'l had 83% more oil residue than those treated and then receiving 1.3 cm (0.5 in) rainfall

Discussion

This research showed that adjuvants tended to influence the soybean oil residues deposited on twigs during cool temperature. For a winter season oil spray, the formulations will need to remain on the twigs longer so that there can be a reduction in the number of applications needed.

The Golden Natur'l and TNSOY36 formulations had the least amounts of oil residue present on the twigs after 1.3 cm (0.5 in) of simulated rainfall. Although Latron

B-1956 (in TNSOY36) is a spreader sticker, it appeared to not work as such in this study. However, perhaps the percentage of Latron was enough that the formulation might have adhered to the water molecules causing it to be washed off instead of adhering to the twig surface. Golden Natur'l formulation is sold as a summertime application and was probably not formulated to adhere to the plant surface for an extended period of time. This could explain why the Golden Natur'l formulation was washed off quickly. Further research is needed to confirm the trends in spray deposits and wash-off found in this trial.

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Part VI

The Spray Residues of Soybean Oil Formulations Deposited on Dormant Viburnum Twigs and Removed by Rainfall

Abstract

Research was conducted in March 2004 to evaluate the amounts of different soybean oil formulations that were deposited on "Juddii" viburnum (*Viburnum* x *juddii)* twigs and that remained after 1.3 cm (0.5 in) of simulated rainfall. Forty-two, one-year-old viburnum shrubs in 10.8 L (three-gallon) containers were placed outdoors at the University of Tennessee, Knoxville. The trees were arranged in a randomized complete block design in a circle around a nine-foot high spray nozzle with six replications, and seven treatments. The trees were sprayed until runoff with 10% refined soybean oil (v/v in water) in the formulations TNSOY28 (0.1% Lecithin/ 0.5%MD/ 0.1% Guar Gum), TNSOY30 (0.1% Lecithin/ 0.5% MD/ 0.05% Guar Gum), TNSOY33 (0.01% Lecithin/ 0.5% MD/ 0.5% Guar Gum), TNSOY34 (0.1% Lecithin/ 0.5% MD/ 1.0% Latron B-1956, and TNSOY35 (0.1% Lecithin/ 0.5% MD/0.5% Latron B-1956) of the total volume of oil. Treatments of 10% Golden Natur'l and water (control) were also used in the experiment. Three twigs from each tree were removed after the trees had been sprayed and before and after rain simulations. Wax and oil was removed from the twigs with a chloroform extraction. There were no significant differences in the amount of oil present on the twigs before rainfall. After the 1.3 cm (0.5 in) of rainfall the Golden Natur'l treated twigs had significantly more oil residue than twigs from other treatments.

Introduction

Reduction of pest mortality following foliar application of soybean oil may occur due to environmental factors such as rainfall (Bondada et al., 2000). They found that there was a negative linear relationship between oil retention on stems and rainfall on

species of apple (*Malus sylvestris* (L.)) and peach (*Prunus persica* (L.)). They also showed that a major portion of applied oil was washed off by 2.54 cm of rainfall. Determining the effect of rain on soybean oil deposits will aid in the decision of whether respraying is needed. Sprays of (3-4%) dormant-season petroleum oil sprays are typically recommended, however sprays of 10% soybean oil can delay peach flower anthesis and thin fruit (Deyton et al., 2002).

The objectives of this research were to develop new botanical formulations with botanical or food grade emulsifiers, evaluate their spray residue on twigs, and the potential washoff of the residue.

Materials and Methods

New combinations of adjuvants with soybean oil were prepared in 2004 and tested as wintertime sprays on viburnum (*Viburnum x juddii*) plants (Table 1). The adjuvants in Table 1 are expressed as a percentage of the soybean oil (not the total spray volume). Guar Gum was included more frequently as an adjuvant than in previous trial. This laboratory developed new formulations of TNSOY28, TNSOY30, TNSOY33, TNSOY34 and TNSOY35 with refined soybean oil as the active ingredient. The adjuvants of the formulations are as follows (percentage of adjuvants in the formulation are of the soybean oil not the total spray volume): TNSOY28 (0.1% lecithin/ 0.5% MD/ 0.1% guar gum), TNSOY30 (0.1% lecithin/ 0.5% MD/ 0.05% guar gum), TNSOY33 (0.01% lecithin/ 0.5% MD/ 0.5% guar gum), TNSOY34 (0.1% lecithin/ 0.5% MD/ 1.0% Latron B-1956 Spreader Sticker) (Rohm and Haas, Philidelphia, Pa.) and TNSOY35 (0.1% lecithin/ 0.5% MD/ 0.5% Latron B-1956) (Table 1). Lecithin is a by-product of

Table 1. Concentrations of active and inactive ingredients in the soybean oil formulations used on viburnum.

		Adjuvants (%)			
Formulations	**Soybean oil (%)**	**Latron[z]**	**Lecithin[y]**	**Guar Gum[x]**	**MD[w]**
TNSOY28	99.3		0.10	0.10	0.50
TNSOY30	99.35		0.10	0.05	0.50
TNSOY33	98.9		0.10	0.50	0.50
TNSOY34	98.4	1.00	0.10		0.50
TNSOY35	98.9	0.50	0.10		0.50

[z] Latron B-1956.
[y] By product of the de-gumming process of refining soybean oil.
[x] Used in the food industry as a thickener and stabilizer.
[w] Experimental emulsifier developed at the University of Tennessee, Knoxville.

the degumming process of soybean oil, MD is an experimental adjuvant developed at the University of Tennessee, and Guar Gum is used in the food industry as a thickner and stabilizer.

Forty-two, one-year old viburnum shrubs in 10.8 L (three-gallon) containers were placed outdoors at the University of Tennessee, Knoxville. The trees were sprayed to runoff on 23 Mar. 2004 at approximately 4:30 PM EST with 10% refined soybean oil (v/v in water) in formulations of TNSOY28, TNSOY30, TNSOY33, TNSOY34 and TNSOY35. Treatments of 10% Golden Natur'l (Stoller Enterprises, Inc., Houston, Texas) and water (control) were also used. The trees were arranged in a randomized complete block design in a circle around a nine-foot high spray nozzle with six replications, and seven treatments.

After the trees were sprayed on the morning of 23 Mar., the oil treatments were allowed to dry for one hour and three twigs were removed from each tree to determine the amount of oil/wax on the twigs prior to rainfall. The twigs were placed in Nasco whirlpacks and stored in a cooler at 2.2 °C (36 °F) until the oil/wax could be extracted, approximately 5 days after spraying. The mean temperature of the treatment date was 3.5 °C (38 °F). After the initial sampling of twigs, the trees were exposed to 1.3 cm (0.5 in) of simulated rainfall. The trees were allowed to dry for an hour and then three more twigs were sampled per tree and stored as previously described.

The oil and wax on twigs from each treatment were measured by removing the twigs from the cooler and dipping each twig individually for thirty seconds in 10 ml of chloroform in a pre-weighed test tube. Each of the three twigs from a treatment were dipped into the same test tube, thus combining the oil/wax from the three twigs into a single sample. The test tubes were then place in a chemical hood and the chloroform evaporated over a couple of days. The test tubes were re-weighed after chloroform evaporation. The weight of oil and wax per three twigs was determined by subtracting the original weight of the dry test tube from the weight of the test tube with oil and wax. The length and circumference of each twig was then measured and the total surface area estimated using the equation for the surface area of a cylinder (surface area = 2*3.14(shoot diameter)(shoot length)). The amount of oil plus wax present on the surface of each twig was calculated ($\mu g/cm^2$). The mean amount of wax ($\mu g/cm^2$) on twigs collected from control plants prior to the rainfall treatments was used as the standard (baseline) amount for twigs of other treatments. This baseline amount of oil plus wax on untreated twigs was subtracted from of twigs of other treatments to obtain

the amount of oil on the twigs. Data were analyzed with the General Linear Models (GLM) procedure and Duncan's Multiple Range test (Statistical Analysis Systems software, SAS 9.0, SAS Institute, Cary, N.C.).

Results

Unsprayed viburnum had 0.232 $\mu g/cm^2$ of wax on the surface of twigs prior to being subjected to rain (Table 2). After the application of 10% oil treatments, there were oil residues left on the twigs by each formulation, but less than was expected. The Golden Natur'l left more than six-fold more oil residue on twigs than did TNSOY30, TNSOY34, or TNSOY35. TNSOY28 left two-fold more residue than the other TN formulations. The addition of more Guar Gum as an adjuvant (TNSOY33) did not increase oil deposits compared to TNSOY28 or TNSOY30. Likewise, the increased amount of Latron B-1956 in TNSOY34 did not increase oil residues compare to TNSOY35.

Unsprayed viburnum had 1.905 $\mu g/cm^2$ of wax on the surface of twigs after the rainfall of 1.3 cm (0.5 in) and tended to have slightly less wax than before the rain. This may have been due to differences is twigs sampled before and after rain simulation. Golden Natur'l treated twigs tended to have more oil residue after the rain than those treated with the TN formulations (Table 3). Twigs treated with TNSOY33 (the formulation having the highest amount of Guar Gum, 0.5%) had more than twice as much oil residue as TNSOY28 (0.1% Guar Gum) and almost four-fold more residue than twigs sprayed with TNSOY30. Twigs sprayed with the formulation (TNSOY36) containing the higher rate of Latron B-1956 (1.0%) tended to have less residue than TNSOY34. The

Table 2. The effect of soybean oil formulations on the amount of oil residue present on viburnum twigs prior to rainfall.

Formulations[z]	Adjuvants	Surface area (cm^2)	Oil residue ($\mu g/cm^2$)
Water (control)	- -	126.88	
Golden Natur'l	- -	112.12	0.030 a[y]
TNSOY28	Lecithin/MD/Guar Gum	95.21	0.010 ab
TNSOY30	Lecithin/MDGuar Gum	161.56	0.004 b
TNSOY33	Lecithin/MD/Guar Gum	131.78	0.003 b
TNSOY34	Lecithin/MD/Latron	141.57	0.001 b
TNSOY35	Lecithin/MD/Latron	185.42	0.003 b

[z] Trees were sprayed, rain simulated, and twigs collected on 23 March.
[y] Letters indicate mean separation by Duncan's Multiple Range Test, $P< 0.05$. Standard wax = 0.00232 $\mu g/cm^2$.

Table 3. The effect of soybean oil formulations on the amount of oil residue left on viburnum twigs after 1.3 cm (0.5 in) of rainfall.

Formulations[z]	Adjuvants	Surface area (cm^2)	Oil residue ($\mu g/cm^2$)
Water (control)	- -	189.42	
Golden Natur'l	- -	164.39	0.045 a[y]
TNSOY28	Lecithin/MD/Guar Gum	137.81	0.014 a
TNSOY30	Lecithin/MDGuar Gum	178.83	0.007 a
TNSOY33	Lecithin/MD/Guar Gum	216.74	0.028 a
TNSOY34	Lecithin/MD/Latron	170.96	0.003 a
TNSOY35	Lecithin/MD/Latron	211.53	0.017 a

[z] Trees were sprayed, rain simulated, and twigs collected on 23 March.
[y] Letters indicate mean separation by Duncan's Multiple Range Test, $P< 0.05$. Standard wax = 0.00191 $\mu g/cm^2$.

treatment had only 34% as much oil residue present on the twigs as the trees sprayed with the Golden Natur'l treatment.

Discussion

Golden Natur'l left more soybean oil residue on viburnum twigs before rainfall and more residue persisted after 1.3 cm of rain. Golden Natur'l is a commercially available soybean oil formulation that is recommended for use in the summer. The Golden Natur'l formulation can adhere to twigs as a dormant spray. Research is needed to determine if dormant sprays of Golden Natur'l affect fruit thining, bloom delay, and efficacy of dormant mites. The twigs sprayed with formulations containing concentrations of 0.5% Guar Gum, tended to have more oil residue after the rainfall than those with lower concentrations. Thus, Guar Gum may have potential as a sticker to reduce wash-off of soybean oil.

The TNSOY34 (with Latron B-1956) treated twigs had the least amount of oil residue present before and after rainfall. Latron B-1956 is a spreader sticker, but the addition of Lecithin and MD may have caused the oil to adhere more efficiently to the water molecules. As a result, the soybean oil in this formulation may have washed off the twigs easier. The TNSOY35 formulation with the lesser amount of Latron B-1956 in the formulation left more oil residue on the twigs. More research needs to be done to evaluate the effects of Latron B-1956 concentration in soybean oil on rain-fastness of the oil. Also, more research is needed to evaluate the effects of combinations of emulsifiers on oil residues, deposits, and wash-off potential. A study is also needed to determine the effect of timing of rainfall after spray application and resulting wash-off.

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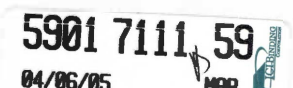
Vita

Adriane Lorraine Cannon was born to Denise and Jesse N. Cannon III on 4 April 1979 in Tallahassee, Florida. She has an older sister, Gwendolyn and brother-in-law Tim. At the age of two she moved to Columbia, South Carolina where her love for the outdoors began. At the age of eight she fell in love with idea of becoming a veterinarian. During the summer before fifth grade the family moved for the last time to Kingsport, Tennessee. Her love of science continued to grow throughout middle and high school. Soccer was a major part of her life in Tennessee. In high school she was awarded numerous awards for her performance on the field. Winning the U-19 club state championship helped to solidify her acceptance to Carson-Newman College in Jefferson City to play soccer. She entered Carson-Newman in the fall of 1997 with all intentions of graduating from the pre-vet program. During her freshman year she was awarded the Golden Boot award (leading scorer), most dedicated award her junior year, and earned a captains position her senior year for her performance on the soccer field. Her academic success was awarded by receiving a Special Achievement Scholarship and Carson-Newman Grant all four years and being named to the Deans List in the Spring of 2001. A botany class her junior year led her to change her biology degree emphasis from pre-vet to environmental sciences. She earned her Bachelor of Arts with an emphasis in environmental sciences from Carson-Newman College in May 2001. A year later she found herself entering the Master's program in Plant Sciences at the University of Tennessee, Knoxville.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