THE EFFECTS OF SEED-STORAGE LIPID EMULSION SPRAYS ON THE INTERACTIONS BETWEEN PLANTS AND THEIR FUNGAL PATHOGENS

by

RODERICK JAMES YOUNG

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Department of BOTANY

The University of British Columbia
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I. ABSTRACT

Emulsions of the seed-storage lipids jojoba wax and canola oil were tested for phytoprotective activity against two powdery mildews and two rot-causing fungi, and for phytotoxicity on two host plants, grape (*Vitis vinifera*) and cucumber (*Cucumis sativus*). Against the powdery mildews tested (*Erysiphe cichoracearum* and *Uncinula necator*), greenhouse cucumber plants and field grape plants treated with jojoba wax emulsions showed 75-100% reductions in powdery mildew disease severity, as compared to water sprayed plants. The canola oil emulsion reduced cucumber powdery mildew disease severity by 63-69%. Scanning electron microscope analyses of *E. cichoracearum* conidia on cucumber leaves sprayed with the seed-storage lipid emulsions showed normal germination structures with inhibited appressorium formation. The jojoba wax emulsions did have slight phytotoxic effects observable on both the cucumber and grape plants, especially during periods of higher temperature. However, these effects were not observed to reduce the yield or survival of the grape plants sprayed in field trials.

Jojoba wax and canola oil emulsions did not seem to have an effect against the infection processes of rot-causing fungi (*Botrytis cinerea* and *Didymella bryoniae*). Instead, the lack of differences between the growth of *B. cinerea* colonies on potato dextrose agar (PDA) treated with the seed-storage lipid emulsions or the emulsifying surfactant Triton X-100 alone indicated that the presence of the surfactant was correlated with reduced colony growth. Colony growth on Triton X-100-treated PDA was reduced by 87-92% as compared to the water control-treated PDA. In addition, field grape plants treated with emulsions containing Triton X-100 showed a 68-87% reduction

in *B. cinerea*-caused disease incidence. While similar reductions in *D. bryoniae* growth were observed *in vitro* for PDA treated with emulsions containing Triton X-100, the surfactant or the lipid emulsions did not reduce *D. bryoniae*-caused disease incidence in the *in vivo* trials on greenhouse cucumbers plants. Applied treatments may have been washed away by exuding sap.

II. TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

III. LIST OF TABLES

LIST OF TABLES (Continued)

IV. LIST OF FIGURES

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1. INTRODUCTION

Every year, diseases caused by fungal plant pathogens destroy approximately 12% of crops produced worldwide, resulting in an estimated loss of $9.1 billion in the United States alone [Agrios 1988]. While many chemical and biological controls of fungal pathogens exist, recent research has shown the potential for using antitranspirant lipid emulsions as plant prophylactics against fungal pathogens [Quarles 1991]. However, commercially available antitranspirants like Folicote, WiltPruf, Sunspray and others with known prophylactic effects are too costly for application in situations larger than small-scale nurseries [Das and Raghavendra 1979]. Alternatively, the emulsified storage lipids of some seeds, such as canola oil and jojoba wax, are inexpensive and present a low hazard to labourers and consumers [Ackman 1990, National Research Council (US) 1985]. In this study, the effects of jojoba wax and canola oil emulsions on the infection processes and diseases caused by certain powdery mildews and rot-causing fungal plant pathogens were studied in comparison with the effects of Folicote and WiltPruf. These comparisons were done (1) to determine whether seed-storage lipid emulsions have potential as plant prophylactic substances and (2) to explore how lipid emulsions applied to plant epicuticles affect fungal plant pathogen infection processes.

1.1. Fungal Infection Processes

There are four general processes which the fungal spore must undergo before successfully entering into plant tissues: activation from the dormant state in which it arrives on the plant [Sussman and Douthit 1973], germination with the production of the germ tube [Van Etten *et al* 1983], recognition during which information regarding a penetration site is received from the epicuticle [Kunoh 1984, Kunoh *et al* 1991], and

penetration during which a specialized hypha called a penetration peg is forced through the epicuticle and the underlying cuticle into plant tissues [Emmett and Parberry 1975]. While some fungal pathogens can (and do) enter into the plant via injuries and natural openings such as stomates [Agrios 1988], many fungal pathogens gain access to their hosts by penetration of unwounded tissue [Koller 1991]. The simplest manner of penetration is via stomates, but stomates take up on average only 1% of the entire leaf area on most plants [Gay and Pearce 1984]. Therefore, most pathogenic fungi must overcome the epicuticle-cuticle-cell wall barrier before entering plant tissues. Any or all of the processes summarized above may be affected by applications of exogenous lipid emulsions. The following describes in greater depth background information on the fungal infection process.

1.1.1. Activation

Fungal spores are in a quiescent state before the activation of physiological processes necessary for germination [Van Etten *et al* 1983, Sussman and Douthit 1973]. The major reason spores remain dormant is their state of desiccation, which maintains the endoplasmic reticulum in vesicle form, thus compartmentalizing enzymes and separating mRNA from translational compounds [Sussman and Douthit 1973]. In addition, compartmentalization of respiratory substrates lowers respiration and anabolic processes [Sussman and Douthit 1973]. Desiccation is an exogenous constraint; particular species may be additionally constrained by constitutive constraints, such as the slow decay or leaching of some inhibitory cellular component feeding back to the genome which prevents activation until the concentration of the component has an insignificant inhibitory effect [Macko 1981]. Activation occurs when sufficient amounts of water and heat activate the spore's metabolism to allow for growth [Van Etten *et al* 1983, Sussman 1966]. Activation of spores from a particular species may also depend

on the elimination of constitutive constraints [Sussman 1966], or the presence of exuded sugars on the epicuticular surface as a result of diffusion through underlying anticlinal cell walls, such as at leaf veins [Carvers and Thomas 1990, O'Connell *et al* 1985].

1.1.2. Germination

Activation starts the process of germination, an irreversible process of morphological change from the dormant form of the organism to the active form [Van Etten *et al* 1983, Kennedy 1990, Sussman and Douthit 1973]. During germination the spore develops a germ tube which breaks through the spore cell wall using combined physical pressure and enzymatic degradation; the developing germ tube forms a protective chitin cell wall after extension [Gooday and Trinci 1980]. During the growth of the germ tube and the penetration process, a sheath of mucilage is produced by the spore which surrounds the entire organism [Nicholson 1984]. This mucilage acts to attach the growing spore to the plant surface, to seal the penetration site, and to protect the growing fungus against limiting environmental conditions - functions which are vital for successful germination [Kunoh *et al* 1991, Nicholson and Epstein 1991, Nicholson 1990, Koller *et al* 1982].

1.1.3. Recognition

Appropriate penetration sites are recognized on the basis of the local chemical and topographical cues from the epicuticle, although particular species may use one more than the other in site location [Emmett and Parberry 1975]. Chemical, or chemotropic, recognition may refer to the recognition of particular epicuticular components and component chain lengths [Hoch and Staples 1991]. This type of recognition was observed in *Colletotrichum gloeosporioides* Penz., where appressorium

formation on avocado (*Persea americana* Miller var Haas) fruit was shown to occur preferentially in the presence of very long chain primary alcohols isolated from the avocado fruit epicuticle [Podila *et al.* 1993]. Chemical recognition also may refer to the recognition of sites where a greater rate of exosmosis occurs relative to other local areas of the plant surface, as a result of diffusion through underlying anticlinal cell walls. This has been suggested due to the high correlation of fungal penetration sites with cell junctions, such as leaf veins [Carvers and Thomas 1990, Emmett and Parberry 1975, O'Connell *et al* 1985, Hoch and Staples 1991]. The other type of recognition which uses cues from the topography is referred to as the tactile, or thigmotropic recognition of the distribution of particular wax bodies or structures in the epicuticle [Emmett and Parberry 1975, Hoch and Staples 1991, Staples and Hoch 1987]. Upon successful recognition of a penetration site, the fungal germ tube begins to form the structures needed for penetration.

1.1.4. Penetration

After recognition, the fungal germ tubes produce appressoria, which are lobate structures at the ends of the germ tubes. These adhere to the plant surface and produce a penetration peg that moves through the epicuticle, the actual surface of the plant, down through the cuticle and cell wall and eventually into the plant epidermis [Kunoh 1984]. Growth of the peg is similar to the germ tube, except the peg stays narrow in diameter while passing through the epicuticle-cuticle-cell wall barrier [Mims *et al* 1989]. This barrier consists of the epicuticle, a biopolyester of unsubstituted and monoesteric long-chain hydrocarbons, 22-50 carbons long; the cuticle, an underlying supporting structure of interesterified hydroxy- and epoxy- fatty acids and tri- and di-acylglycerides, 16-18 carbons long; and the cell wall, composed mainly of cellulose [Gay and Pearce

1984, Kollatukudy 1976,1984]. The appressorium provides both mechanical anchoring and barrier-weakening enzymes which enable growth of the penetration peg.

Cuticle-weakening enzymes are provided in the same mucilage which surrounded the growing germ tube. This mucilage, which also surrounds the appressorium and penetration peg, contains in some species positively-charged glycoproteins [Tunlid *et al* 1991, Epstein *et al* 1987]. These glycoproteins have been shown to be cutinases and serine-esterases, proteins with high specificity for hydrolysis of cuticle, and particularly ester bonds [Koller 1991, Deising *et al* 1992, Pascholati *et al* 1992] which the fungal spore produces in response to the presence of cuticular monomers [Kollatukudy 1985]. Hence, in addition to protecting the spore during growth, mucilage initiates the breakdown of the cuticle by stimulating the production of cutinases, which degrade the cuticle in advance of the penetration peg. However, the longer the chain length of the compound, the greater the resistance to degradation [Kollatukudy 1985]. As a result, the mucilage-borne enzymes alone are not usually sufficient to breach the epicuticle [Kollatukudy 1985, Kollatukudy *et al*. 1987]. Therefore, in addition to the degradation of the cuticle by the mucilage enzymes, mechanical pressure exerted on the growing peg by the appressorium anchored to the epicuticle by water tension and the mucilage [Howard *et al* 1991] can force the penetration peg through the epicuticle, and expose the cuticle to further chemical degradation. Exogenous lipid emulsions applied to plant epicuticles may alter infection processes by changing conditions needed for activation and/or germination, by interfering with recognition, and/or by inhibiting or promoting penetration.

1.2. Previous Studies of Lipid Emulsion Use on Plants and Their Fungal Pathogens

Lipids were studied and used as antitranspirants before their consideration as plant prophylactic substances against fungal pathogens. In general, antitranspirants reduce drought-induced water stress and improve water use efficiency [Quarles 1991]. For instance, when water-stressed peach trees were sprayed with WiltPruf (10% β–pinene (monoterpene) polymer emulsion, Nursery Specialty Products, Greenwich, NT), the trees showed a 30% reduction in water use over 90 days with no loss of growth [Steinburg *et al* 1990]. However, polymer antitranspirants provide no advantage in irrigated crops, and phytotoxic effects can retard plant growth and reduce plant yields [Quarles 1991]. Tomato and cotton plants sprayed with antitranspirants have shown as much as a 4°C increase in leaf temperature, as transpiration was diminished [Gale and Hagan 1966]. Also, photosynthesis decreases, as gas exchange and water vapour movement across the cuticle are inhibited [Quarles 1991, Kastori *et al* 1991]. Surfactants (such as the Triton X-series) used to emulsify lipids can induce the formation of ethylene and other symptoms associated with a wounding response [Lownds and Bukovac 1989]. Triton X-100 increased cuticular permeability up to 15 times when applied at 0.2% on pear and orange leaves [Riederer and Schonherr 1990]. Only in areas which have little water or poor soil does the increased water use efficiency outweigh the detrimental effects of the antitranspirant sprays [Kamp 1985]. However, lipid emulsions applied to plants as antitranspirants have been shown in several cases to reduce fungal disease [Quarles 1991]. In these cases, the protection against fungi and subsequent increases in yields overcome the detrimental physiological effects of the antitranspirant substances.

Folicote, an paraffin wax emulsion (Crystal Soap and Chemical Co., Langsdale, PA), has shown some use as a plant prophylactic against fungal pathogens. Ziv and Fredericksen [1983, 1987] demonstrated that Folicote (10% active ingredient) reduced disease incidence by 66-78% for *Erysiphe graminis* DC. ex Mercat (wheat powdery mildew), *Puccinia recondita* Rob. ex Desm. (wheat rust), *Puccinia polyspora* Underw. (corn rust), or *Excerohilum turcium* Pass. Leonard and Suggs. (sorghum leaf blight), as compared to plants sprayed with the fungicide benomyl (Benlate, du Pont Chemical, Wilmington, Delaware). Also, the incidence of disease caused by *Botrytis cinerea* Pers. ex Fr. on bean, tomato, pepper and cucumber leaves treated with a 30g/L solution of Folicote was reduced 60% 8 days after inoculation [Elad *et al*, 1990]. Formation of appressoria by *E. graminis* was shown in another study [Zekaria-Oren *et al* 1991] to be reduced 20% after application of Folicote (2% active ingredient). At this concentration Folicote did not form a continuous coat on the leaf surface when observed by SEM. Thus, previous work has shown that Folicote forms a concentration-dependent film over the plant epicuticle which apparently interfered with fungal recognition processes. Germination was not prevented by the paraffin wax emulsion, but appressoria formation and subsequent penetration were inhibited.

While the mechanism of action of the triacylglyceridic oils from canola, sunflower, safflower and soybean seeds is not yet known, applied emulsions of these oils have been reported to have plant prophylactic activity [Northover *et al* 1993]. Applied at 0.5% as emulsions in 0.012% Agral surfactant, they reduced *U. necator* (grape powdery mildew)-caused disease incidence on grape plants by at least 86% [Northover *et al*. 1993]. In situations where a large amount of inoculum was present, the efficacy of the oil emulsions was reduced, however. This may be due to insufficient coverage on the host plant, an effect similar to the concentration-dependent coverage afforded by Folicote.

WiltPruf is also a useful plant prophylactic against fungal pathogens. WiltPruf-treated wheat plants showed a 99% reduction in the incidence of *Erysiphe graminis* f. sp. *tritici* DC. (powdery mildew)-caused disease as compared to an untreated control [Ziv and Fredericksen 1983]. On cucumber plants a 55% reduction in disease incidence caused by *Erysiphe cichoracearum* DC. (powdery mildew) was observed when 20g/L was used; WiltPruf was observed to inhibit germination [Elad *et al*, 1989]. Against *B. cinerea*, WiltPruf at 20g/L reduced disease incidence by ~50% on bean, tomato, pepper, and cucumber leaves and fruits [Elad *et al*, 1990]. The high degree of control afforded by these lipid emulsions indicates possible practical usage for these substances as plant prophylactics.

1.3. Systems Chosen for Study

1.3.1. Lipids Emulsions to be Applied to the Plants

The four lipids that were selected for testing in this study included two commercial preparations, Folicote and WiltPruf, and two seed-storage lipids, canola oil and jojoba wax. Both Folicote (paraffin wax) and WiltPruf (β–pinene) consist of lipids that are similar to compounds which are abundant in the cuticle, but not the epicuticle. The first seed-storage lipid, canola oil, also has similarities to cuticular components. It is the storage lipid of rapeseed (*Brassica campestris* L.), which consists 93% of triacylglycerides with oleic fatty acids [Ackman 1990]. The second seed-storage lipid, jojoba wax, was selected for its similarity to epicuticular lipids. It is a product of the fruit of the jojoba bush (*Simmondsia chinensis* L.) composed 98% of esters of cis-monounsaturated ω9 C_{18} to C_{26} alcohols and cis-monounsaturated ω9 C_{16} to C_{24} fatty acids [National Research Council (US) 1985, Desert King Jojoba Corp. 1990]. The

majority (~90%) of the waxes are between 38 and 44 carbons long [Johnson and Hinman 1980], and thus have similarities to the esters in plant epicuticles. The two seed-storage lipids were emulsified using either Triton X-100, which is 1-(*p*-tert-octylphenyl)-ω-hydroxy-poly(oxyethylene) nonionic surfactant (Union Carbide, Montreal, Que.), or ISOL-RB, a polyglycerol nonionic surfactant (Leo-Chem Inc, Oakville, Ont.). The fact that the exogenous lipids have at least some similarity to epicuticle/cuticle lipids may be useful in suggesting the role that the epicuticle/cuticle lipids have in interfering with fungal infection processes.

1.3.2. Plant-Fungal Systems Chosen for Study

In order to compare the effects of jojoba wax and canola oil with Folicote and WiltPruf on interactions during infection processes, four plant-fungal systems were chosen. Cucumber plants (*Cucumis sativus* L.) and two pathogens, *Erysiphe cichoracearum* DC. (powdery mildew) and *Didymella bryoniae* Auersw. (black rot fungus) were selected. Grape plants (*Vitis vinfera* L.) and two pathogens, *Uncinula necator* Schw. (powdery mildew) and *Botrytis cinerea* Pers. ex Fr. (bunch rot fungus) were also selected. The obligate powdery mildews and the nonobligate rot-causing fungi were chosen for their dissimilar interactions with the epicuticle-cuticle, so as to determine whether the exogenous lipid emulsions would have an effect on particular fungal infection processes.

1.3.2.1. The Powdery Mildews

Both the powdery mildews (*U. necator* and *E. cichoracearum*) are common and conspicuous obligate pathogens of their respective hosts forming spots or patches of white to grayish powdery growth on the upper surface of leaves and on shoots, and

covering young plant organs [Sitterly 1978, Pearson and Gadoury 1987, Jarvis and Nuttall 1979, Bulit and Lafon 1978]. Both types of fungi penetrate only green, living epidermal cells, invariably forming an appressorium over and entering through anticlinal cell walls and forming haustoria in the cell lumen [Bulit and Lafon 1978, Sitterly 1978, Aist and Bushnell 1991, Pearson 1988]. The resultant infection rarely kills the host, but, in utilizing photosynthate and nutrients via endohaustoria in epidermal cells, they can reduce the yield of the host by 20-40%.

Research on infection mechanisms exists for at least two species of powdery mildew. *U. necator* (powdery mildew of grape) has been observed to germinate and to produce functional appressoria on any surface that presents a relative humidity of 99.8% in the absence of free moisture [Blaich *et al* 1989, Heintz 1986]. Appressoria formed regardless of whether these conditions were maintained by the use of different osmotica in or an amyl acetate polymer over an artificial agar substrate, or by the use of dried grape epidermal cell calluses in appropriate conditions of high humidity [Blaich *et al* 1989]. Germination did occur at lower relative humidities and in the presence of free water, but no appressoria were developed. Therefore, it seems unlikely that the epicuticle-cuticle layer (or a exogenous lipid emulsion layer) might interfere with *U. necator* spore activation or germination. Also, the presence or absence of cuticle or flat surface or even normal tissue topography (thigmotropic recognition cues) were not important for appressorium formation. Moreover, under the strict environmental conditions needed for appressorium formation, Heintz [1986] showed that penetration pegs were able to penetrate artificial membranes impervious to enzymatic degradation. This result indicates the importance of the strict environmental conditions needed for appressoria formation, and the unimportance of chemotropic recognition cues. Waard [1971] suggested that a condition of high relative humidity in the absence of free moisture necessary for conidial infection processes could be provided by the

transpirational gradient of grape leaves. Thus, the epicuticle-cuticle barrier (and therefore perhaps the lipid emulsions) may act to modulate the transpirational gradient and thus interfere with the formation of appressoria by *U. necator* on the epicuticle surface. The epicuticle-cuticle layer may provide resistance to penetration, but the layer's first action would seem to be interference with appressorial formation, since appressoria need to form before penetration is possible.

Erysiphe graminis f. sp. *avenae* (powdery mildew of rye) also requires particular conditions for appressorium formation. Appressorium formation and penetration occurs naturally on *E. graminis*-inoculated ryegrass (*Lolium* spp.), but not *in vitro* as seen in a microscopic survey of *E. graminis* on agar [Carver and Ingerson 1987]. This indicates the requirement for some particular surface or condition modulated by the surface to initiate appressorium formation. However, appressorium development has been observed on the upper surface of ryegrass leaves with or without the epicuticle-cuticle removed [Carver *et al* 1990, Carver and Thomas 1990]. Since the cell wall morphology of the upper surface observed with epicuticle removed was different from the epicuticular wax morphology [Carver and Thomas 1990], recognition by the spore on the surface was not by thigmotropic or chemotropic cues, as these would have been altered by removal of the epicuticle-cuticle from the surface. The main effect of the epicuticle may have been interference with environmental conditions necessary for fungal infection by some factor intrinsic to the wax [Carver *et al* 1990]. Again, the epicuticle-cuticle layer may provide resistance to penetration, but the layer's first action would seem to be interference with appressorium formation, since appressoria need to form before penetration.

For both species of powdery mildew, it appears that the epicuticle-cuticle barrier primarily interferes with particular environmental conditions needed for appressorium

formation. If all powdery mildew fungi behave similarly to the two described, then the epicuticle-cuticle barrier (and perhaps the applied exogenous lipids) of other host plants may interfere with appressorium formation in a similar manner.

1.3.2.2. The Rot-Causing Fungi

B. cinerea is a nonobligate pathogen which establishes appressoria at the junction of the berry and the pedicel, and then grows to cover grape berries with a gray fruiting layer [Jarvis 1977]. These infections damage the grape epidermis, leading to uncontrolled evaporation and eventual desiccation of the berry [Bulit and Dubois 1988]. *B. cinerea* can penetrate intact epicuticle-cuticle barriers by the use of appressoria like the powdery mildews [McKeen 1974], even penetrating nonliving substances like paraffin and gold film [Brown and Harvey 1927]. Serine-esterases have been detected during the penetration process [Shishiyama *et al* 1970] and they seem to aid the penetration peg, as penetration sites in the epicuticle-cuticle have sharp edges, rather than the indented appearance expected of a purely physical penetration [McKeen 1974]. However, the fungus has been observed only rarely to form appressoria over living, turgid cells, with penetration most often occurring into senescent tissues [Brown and Harvey 1927, Bulit and Dubois 1988, Dugan and Blake 1989]. Direct penetration into healthy tissues also seems unlikely because of the observation that cutinases produced by *B. cinerea* on tomato with no external sugar source degraded only 3-5% of the epicuticle-cuticle thickness in 18 hours [Salinas *et al* 1986]. Most penetration attempts have been observed in areas of the epicuticle which are thin due to injury and necrosis [Marois *et al* 1986] or to microfissures particularly near the pedicel [Blaich *et al* 1984] or in peristomatal areas of grape berries where grape berry sugars are in greatest abundance [Jarvis 1977]. The fungus is often observed microscopically to move towards an abundance of exuded sugars [Jarvis 1977, Dugan and Blake 1989, Padgett and

Morrison 1990]. Therefore, the epicuticle-cuticle probably interferes with access to exudates required for both the germination and growth of *B. cinerea* spores.

Black rot of cucumber, caused by *D. bryoniae*, is a nonobligate pathogen of cucumber [Jarvis and Nuttall 1979]. After penetration, *D. bryoniae* occupies cucumber tissues, causing wilting, desiccation, and canker formation [Jarvis and Nuttall 1979, Leski 1984]. Entry into plant tissues has been shown to be primarily via injured material [Svedelius 1990, de Neergaard 1989, Bergstrom *et al* 1982, Van Steekelenburg 1985, Svedelius and Unestam 1978], as opposed to direct penetration using appressoria. *D. bryoniae* pycnospores germinated in distilled water, tap water, or a supernatant of macerated cucumber or squash; the degree of germination differed only in that the germ tubes in the macerated cucurbit extract exhibited greater branching and more vigorous growth [Chiu and Walker 1949, Svedelius and Unestam 1978]. This stimulating effect of cucurbit extracts on the infection process of *D. bryoniae* pycnospores has been shown in areas of hydathode guttation [Svedelius 1990], and at necrotic areas and near veins [Svedelius and Unestam 1978]. However, while artificial nutrient supplements increased the number of lesions produced by a conidial suspension as opposed to the number of lesions produced by an unsupplemented conidial suspension, mycelial suspensions produced an equal number of lesions regardless of the presence or absence of additional nutrients [Svedelius 1990]. This observation indicates that the exudates at damaged or necrotic sites on the plant likely promote germination, and have only a small effect in chemotropic recognition of these damaged sites by the germ tube; if chemotropic recognition had been important, then one would expect the supplemented mycelia to cause more lesions. Therefore, similar to the proposed exudate barrier of the epicuticle-cuticle for *B. cinerea*, the epicuticle-cuticle barrier may interfere with the germination of *D. bryoniae* by limiting access of the pycnospores to the germination-promoting plant exudates.

1.4. Study Outline

The literature survey suggests that epicuticle-cuticle barriers interfere with the environmental conditions required for the induction of appressorium formation needed for infection by powdery mildews, and with the exudation of exuded sugars needed for infection by rot-causing fungi. Seed-storage lipid emulsions may have a similar effect, as the emulsions are lipidic like the epicuticle-cuticle. The objectives of this study were the following: (1) to determine whether seed-storage lipid emulsions have potential as plant prophylactic substances and (2) to explore how lipid emulsions applied to plant epicuticles affect fungal plant pathogen infection processes. Seed-storage lipids were compared with commercial lipids with known modes of action in *in vitro*, greenhouse and field trials.

2. MATERIALS AND METHODS

For all experiments (except where indicated), data were analyzed for significant differences between treated groups by ANOVA, followed by Duncan's multiple range tests where applicable, except where other tests are indicated. For all tests, α=0.05. ANOVA and Duncan's multiple range tests were completed using the computer program PROC GLM provided in the SAS statistics package [SAS Institute 1985].

2.1. Preliminary Studies

2.1.1. Determination of Phytotoxic Lipid Emulsion Concentrations

In order to determine the lipid and surfactant concentrations that were likely to provide the most interference with fungal processes and the least damage to the subject plants, a greenhouse study was done at Agriculture Canada Research Station (ACRS) at Summerland, B.C. One hundred 'Auxerrois' grape cuttings from one parent plant were rooted and potted in 10 cm pots in topsoil and peat. At the start of the experiment, each four-week-old plant was pruned for the strongest shoot, which was tied to a 1 m stake pushed into the pot. The plants were hedged to the top of this stake when they were seven weeks old and every two weeks thereafter; adventitious shoots were removed as they became apparent. All plants were watered regularly, receiving approximately 100 mL of water per weekday, and were maintained at 25°C and ~80% R.H. throughout the experiment.

Four blocks of plants consisted of six treatment representing six concentrations of jojoba wax - 0%, 0.1%, 1.0%, 2.0%, 5.0% and 10.0% - in water with 1.0% Triton X-207 (Rohm and Haas, Philadelphia, PA) surfactant. Four plants were unsprayed and were used as a control for the effects of the surfactant. All plants were sprayed at seven weeks with 1.0 L of treatment sprays using a 12 L Sanex backpack sprayer. Both sprays were applied to run-off, which means the entire aerial surface of the plants was covered, and the spray was dripping off leaf margins. While Triton X-207 was used in this test, it was due to its availability that it was used in lieu of Triton X-100, which was used in all other experiments. Triton X-100 is generally more available, and is extensively used in the agricultural industry as a spreader/sticker [Vielvoye 1984]. A preliminary experiment also showed that it was able to emulsify jojoba wax to the same degree (subjectively determined) as Triton X-207.

The effect of the sprays on the plants was determined once at 10 weeks (21 days after spraying) by measuring the area of the primary leaf from the sixth node of each treated plant with a Licor 3000 Aerometer (Li-Cor Inc., Lincoln, Nebraska). Damage to the leaf was estimated by cutting necrotic material out of the leaf, and determining leaf area remaining; the difference was the leaf damage. In addition to these measurements, subjective observations were made throughout the experiment, and photographs were taken six weeks after spray application. This experiment was done once.

2.1.2. Effect of Exogenous Lipid Emulsions on the Growth of *Didymella bryoniae* and *Botrytis cinerea In Vitro*

This experiment was designed to determine the effect of jojoba wax and canola oil when the lipids were placed in direct contact with *B. cinerea* and *D. bryoniae*. It also tested the effects of the surfactants Triton X-100 and ISOL-RB on the fungi, so that this

effect could be accounted for in subsequent studies of the lipids' effects. The trials consisted of 520 agar petri dishes separated into 13 treatments, as listed in Table 1.

Table 1. Experimental seed-storage lipid emulsions applied to 1% w/v sucrose-enriched potato dextrose agar to evaluate the relative efficacy of the emulsions on the growth of *D. bryoniae* and *B. cinerea*

Control Treatments	Lipid-Containing Treatments
Untreated	1.0% jojoba wax + 0.5% Triton X-100
Water only	2.0% jojoba wax + 0.5% Triton X-100
0.5% benomyl/1.0% Captan mixture	1.0% canola oil + 0.5% Triton X-100
(*B. cinerea* commercial control)	2.0% canola oil + 0.5% Triton X-100
1.0% iprodione	1.0% jojoba wax + 0.5% ISOL-RB
(*D. bryoniae* commercial control)	2.0% jojoba wax + 0.5% ISOL-RB
0.5% Triton X-100	WiltPruf (10% active ingredient)
0.5% ISOL-RB	Folicote (4% active ingredient)

The sucrose-enriched potato dextrose agar petri dishes were made using a mixture containing 40 g of potato dextrose agar (Difco Laboratories, Detroit, Mich.), 10 g of sucrose and 1.0 L of tap water, which was sterilized at 1500 kPa in a Barnstead Automatic Autoclave (Barnstead Still and Autoclave, Co, Boston, Mass.). The resulting solution was poured into 80, 10 cm-diameter petri dishes per litre. The treatments were prepared under sterile conditions in an Ultraclean laminar flow hood (Agnew-Higgins Inc., Garden Grove, CA) using sterile water. For each treatment a 300 μL aliquot of the treatment solution was spread onto each petri dish allocated to the treatment using a LabLine petri dish rotary autoplater (LabLine Instruments Inc., Melrose Park, Ill.) and a bent glass rod. The 300 μL treatment volume was used because a preliminary experiment showed that 300 μL aliquots allowed differentiation between the surfactant and the lipid effect.

After a 24 hour drying period, the petri dishes for each treatment were separated into four blocks of 130 petri dishes, one for each of the three *B. cinerea* isolates (from

Interior Spruce, from Western Hemlock, and from Lodgepole Pine, each supplied by B. Dennis, Pacific Forestry Centre, Victoria, B.C.) and one for a *D. bryoniae* isolate isolated from a black rot-diseased greenhouse cucumber plant. The petri dishes for each isolate were inoculated in three equidistant spots with 10 μl of the respective spore suspension (10×10^5 spore mL^{-1}), and then were incubated at approximately 20°C. The inoculation spot boundaries were marked with a fine black pen after 24 hours, and the diameters of the original spot and the final colony were measured after 4 days. As there was no independence between spots on the individual petri dishes, means for the spot diameters for each plate were used in the subsequent analysis. Also, data on colony diameters from the petri dishes for the separate *B. cinerea* isolates were pooled, since there was no significant difference for the treatment effects on the isolates. This experiment was completed twice.

2.2. Comparison of Exogenous Lipid Emulsions as Phytoprotectants and as Antitranspirants of Cucumber Plants

All plants for the cucumber plant trials were "Chicago Pickling" cucumbers started from seeds (Sunset Seed Co, Ltd., Armstrong, B.C.) and grown in a greenhouse during October and November in 10 cm pots using a medium of 20 kg topsoil, 1 kg peat, and 170g of 14-14-14 Osmocote fertilizer (Grace Sierra Horticultural Products, Malipatus, Calif.). The plants were staked and tied as necessary, watered daily, and maintained between 15 and 30°C at ~60% R.H. If the plants were on a mist bench, the temperature was maintained at ~22°C and >95% R.H. with free moisture.

2.2.1. Effects of Exogenous Lipid Emulsions on Powdery Mildew of Cucumber Plants

Trials were done on cucumber plants in greenhouse situations at UBC in 1993 in order to determine the effects of exogenous lipids on *E. cichoracearum* . Eleven, 6-week-old plants were used per treatment for both trials (Table 2). Treatments were applied using a 12 L Solo backpack sprayer.

Table 2. Experimental seed-storage lipid emulsions applied to "Chicago Pickling" cucumber plants in greenhouse trials for determining the effect of the emulsions on powdery mildew disease severity

Control treatments	Experimental treatments
0.5% Triton X-100	1.0% Jojoba wax + 0.5% Triton X-100
0.5% ISOL-RB	1.0% Jojoba wax + 0.5% ISOL-RB
1.0% w/v sulfur[1]	1.0% Canola oil + 0.5% Triton X-100
Water only	WiltPruf (10% active ingredient)
	Folicote (4% active ingredient)

1. Kumulus D (sulfur) manufactured by BASF, Germany

The cucumber plants were inoculated by taking sporulating powdery mildew-diseased leaves from seed-grown cucumbers and various squash, and shaking these leaves over the cucumbers plants, one day after they were sprayed. The number of spores was determined for the second trial by placing 20 microscope slides with 4 cm of double-sided Scotch tape (3M Canada, London, Ont.) on the greenhouse table next to and inside the block of cucumber plants. Spores adhering to the tape were counted under a microscope at 10 microscope fields per slide using a 40x objective. Approximately 2.0×10^4 spores cm^{-2} were being placed on the cucumber leaves. Estimates varied from 2.4×10^4 spores cm^{-2} on the outer leaves to 1.7×10^4 spores cm^{-2} on the inner leaves. Since powdery mildew requires humidity for germination, the

plants were kept on an operating mist bench for 4 days with precautions to ensure that no free moisture could accumulate on the foliage.

The powdery mildew disease severity on the cucumber plants was measured 17 days after inoculation by counting the number of colonies per third, fourth and fifth leaves, then measuring leaf area (Licor 3000 Aerometer). These measurements were averaged per plant for subsequent analyses. Estimations of the colony sizes on the leaves were noted. A preliminary trial done in 1992 with the same design was done, but is not discussed, since the results were not as clear as the 1993 trials. This experiment was done twice in 1993.

2.2.1.1. Scanning Electron Microscope Studies of the Effects of Exogenous Lipid Emulsions on *Erysiphe cichoracearum* on Cucumber Leaves

In order to more closely visualize the effects of the applied lipids on the *E. cichoracearum* infection signs, nine 6-week-old cucumber plants, one for each of the treatments listed in Table 2, were separated from the other cucumber plants. These plants were then inoculated by the same method as the other cucumber plants, and two leaves were removed from the plants after 48 hours. For each leaf from each treated plant, a 1 cm diameter cork borer was used to remove a leaf disk immediately adjacent to the main leaf vein disk from the centre of the leaf. These disks were immediately affixed using paper glue (UHU Stick, Eberhard Faber, Oakville, Ont.) to 1 cm-diameter scanning electron micrograph (SEM) plugs. The leaf-covered disks were left to air dry for 10 days.

After the drying period, the dried disks were gold coated in an argon environment using a Nanotech II SEMPrep Sputter Coater. The disks were then observed under a

Cambridge 250 Scanning Electron Microscope (Cambridge Scientific Instruments, Inc.). Micrographs were taken using Polaroid Landmark 55 film. Spores observed on the dried cucumber leaf disks were similar in proportion and shape compared to micrographs of *E. cichoracearum* spores on disks of air-dried cucumber leaves displayed by Samuels *et al.* [1991].

The micrographs were studied to determine what differences, if any, might exist in spore germination on leaves treated with different exogenous lipid emulsions. The number of germinated spores and the number of spores with appressoria in the each of the 38 SEM sample fields (300 μm wide) observed per leaf disk were counted. This experiment was done once.

2.2.2. Effects of Exogenous Lipid Emulsions on Black Rot and Growth of Cucumber Plants

In 1993 in the same greenhouse as the trials testing the effects of exogenous lipids on powdery mildew, trials testing the effect of exogenous lipids on black rot disease incidence on cucumber plants were done. Eleven, 6-week-old plants were used per treatment for both trials (Table 2). The same treatment sprays that were used in the powdery mildew trial were used, except that 1.0% w/v iprodione (Rovral, May and Baker Ltd., Essex, England) replaced sulfur as the commercial control. The same treatments were used because a preliminary trial had shown Folicote and Triton X-100 might be effective in reducing black rot disease incidence. Since *D. bryoniae* is a wound pathogen, cucumber plants were prepared by slicing the petioles on the third, fourth, and fifth nodes in half. After a 3 hour drying period, the treatments were applied using a 12 L Solo backpack sprayer; the sprays were allowed to dry for 3 hours. Then 10 μL of a *D. bryoniae* spore suspension (5 x 10^5 spores mL^{-1}) was dropped onto the spray-

covered injured petioles. The plants were then placed on a mist bench for 4 days, then removed to the greenhouse. The effects of the treatments were determined by counting the number of diseased inoculation points per plant 17 days after inoculation. This experiment was done twice.

The leaf area measurements taken for all the leaves that were sampled for powdery mildew colony presence gave an indication for growth. In order to determine the effect of the lipid emulsion applications on growth, the random effect of the number of powdery mildew colonies was accounted for in the subsequent ANOVA.

2.3. Effect of Seed-storage Lipid Emulsions as Phytoprotectants and as Antitranspirants of Grape Plants

In order to test how jojoba wax and canola oil would perform as prophylactics in field situations, three trials were completed at two sites - Torrs Vineyard (TV) in Kelowna, B.C., and the Agriculture Canada Research Station (ACRS) at Summerland, B.C. Trials were done at TV for two years (1991 and 1992) and at ACRS during 1992 only.

2.3.1. Plant Material, Husbandry, and Spraying

The trial at ACRS used 'Riesling' grape plants 3 years old spaced at 3 m x 2 m (row x vine) in five blocks. At TV, in Kelowna, B.C., 4-year-old 'Auxerrois' plants spaced at 2.4 m x 1.5 m were used in six blocks. Vines were trained to 0.6 m-high bilateral cordons and pruned to 16 shoots m^{-1} as 2-node upward-oriented spurs. Shoots were trained upward to form a vertical canopy. Each block was divided into treatments, with each treatment represented by a "post-length" of grapes, meaning the grape plants

between the two posts which supported the trellis wires for those plants. Treatments were randomized with respect to their position within the blocks to reduce the in-block variation. Treatments and spray application dates for the trials are listed in Table 3. The treatment names "bloom", "pre-veraison", and "post-veraison" refer to general period of grape plant growth as adapted from Eichhorn-Lorenz growth stages [Pearson and Goheen 1988]. In both field sites, rows of plants were oriented north-south.

Table 3. Experimental seed-storage lipid emulsions and schedule of their application to 'Auxerrois' grape plants at TV and 'Riesling' plants at ACRS, applied to determine the effects of the various treatments in reducing powdery mildew disease severity and/or incidence

TV 1991 Treatment [1]	May 22	Jun 4	Jun 22	Jul 3	Jul 14	Jul 28	Aug 10	Aug 25
Water control	+	+	+	+	+	+	+	+
Commercial control[2]	+	+	+	+	+	+	+	+
1.0% Jojoba "All-season"[3]	+	+	+	+	+	+	+	+
1.0% Jojoba "Bloom"	+	+	+					
1.0% Jojoba "Pre-veraison"			+	+	+			
1.0% Jojoba "Post-veraison"					+	+	+	+

TV 1992 Treatment	May 12	May 23	Jun 6	Jun 23	Jul 6	Jul 21	Aug 5 [4]
Water control	+	+	+	+	+	+	+
0.2% Triton X-100 control	+	+	+	+	+	+	+
1.0% Jojoba "All-season"	+	+		+	+	+	+
0.2% Jojoba "All-season"	+	+	+	+	+	+	+
1.0% Jojoba "Pre-veraison"			+	+	+		
0.2% Jojoba "Pre-veraison"			+	+	+		

ACRS 1992 Treatment	[5]	Jun 18	Jul 3	Jul 17	Jul 31	Aug 12 [6]	Aug 21
Water control		+	+	+	+	+	+
Commercial control		+	+	+	+		+
1.0% Jojoba		+	+	+	+		+
1.0% Canola		+	+	+	+		+
0.5% Canola		+	+	+	+		+

[1] Treatment names refer to types of controls (ACRS and TV), concentrations of sprays (ACRS and TV), or the general period of spray application (TV). '+' indicates a particular treatment was sprayed on a certain day.

[2] Commercial spray was 5% sulfur (Kumulus S, BASF, Germany) sprayed biweekly from 10 to 15 cm new growth to 30 days prior to harvest, and at TV, 1% Captan (Standard Oil Development Co.) or 1% iprodione (Rovral, May and Baker Ltd., Essex, England) (i) at bloom, (ii) at 80% capfall, (iii) at bunch closure, (iv) at veraison and (v) 10 to 14 days later if needed. Captan/iprodione combinations were not sprayed at ACRS.

[3] Sprays included the lipid at the concentration indicated in water with 0.2% Triton X-100 surfactant for emulsification.

[4] Due to overabundance of powdery mildew, the commercial control was used after August 5 for all plants.

[5] All plants were sprayed with 5% sulfur until two weeks before June 18.

[6] Due to farmer error, plants were sprayed with 5% sulfur on August 12.

With respect to husbandry, all plants were watered, pruned, suckered, and tied according to grape industry standards [Vielvoye 1984]. ACRS grapes were watered by a microjet system, whereas grape plants at TV were watered by overhead sprinklers. Both vineyards were hedged in midsummer, and both were cluster-thinned to two clusters per shoot. Treatments were applied in the 1992 field trial at ACRS by a 12 L Sanex backpack sprayer. All other sprays were applied using a tractor mounted 220 L sprayer. Sprays were applied to run-off; that is, the entire aerial surface of the plants were covered, and the spray was dripping off leaf margins.

2.3.2. Effects of Exogenous Lipid Emulsions on Powdery Mildew and Bunch Rot of Grape Plants

In 1991, grape plants at TV were inoculated with *U. necator* on August 11 by hanging powdery mildew-diseased grape clusters collected from ACRS from the 1.5 m wire of the grape trellis and irrigating the vineyard for two hours, and then removing the diseased grape clusters 24 hours later. Sufficient overwintering inoculum was present in 1992 at TV to provide adequate natural inoculation. In 1992, infected clusters from TV were used in two attempts in late July to inoculate vines at ACRS, but this failed to initiate disease, and further attempts were not made. At TV in 1991 and at ACRS in 1992, plants were inoculated with *B. cinerea* two and three weeks before harvest respectively. Berry clusters were sprayed to run-off with a conidia suspension of approximately 2.5×10^4 spores mL^{-1} in a 2% sucrose solution. Inoculation of TV in 1992 was not attempted due to an *U. necator* epiphytotic, which had already resulted in the desiccation of the berry clusters.

To assess the degree of powdery mildew disease incidence, *U. necator* colonies for two leaves per vine at the 1.5 m level were counted at TV in 1991 and 1992. In

addition, the number of mildewed grape berry clusters was counted at harvest in 1991. The number of clusters with bunch rot-diseased areas greater than the size of a single grape were counted at TV in 1991 and at ACRS in 1992.

2.3.3. Effects of Exogenous Lipid Emulsions on Grape Plant Growth, Yield and Survival

In 1991 at TV, transpiration was measured in the centre of leaves on the south and outside of the grape plant canopies at the 1.5 m level 4-6 days after each spray using a Licor 1600 porometer (Li-Cor Inc., Lincoln, Nebraska). At TV in 1992, stomatal resistance was measured at 9:30am and at 12:30 am for leaves selected as in 1991 on three dates in mid-season (June 7, 17, July 3). This was done to determine different stomatal aperture sizes at the beginning of the day, so that stomatal resistance differences in mid-day, when the plants normally experienced some water stress (Dr. A Reynolds, pers. comm.), could be determined controlling for the original aperture sizes. No transpiration measurements were taken at ACRS.

Growth was estimated at TV in 1991 by comparing the grape plants to an adapted Eichhorn-Lorenz growth stage outline [Pearson and Goheen 1988]. At TV and ACRS in 1992, measurements were made throughout the growing season from two shoots on each plant, one nearest to and one furthest from the trunk on the south side. Fifth and tenth internodes were marked with spray paint, and every tenth leaf was identified with a twist tie. On these shoots, weekly measurements were made of the number of leaves and internodes per shoot, of the length of the fifth and tenth internodes, the tenth leaf width, basal cluster length, and the average berry width. Leaf area was estimated by assuming the leaf width represented the diameter of a circle, the area of which was then calculated. Berry volume was estimated by calculating the

volume of a sphere with berry width as the diameter. Also, leaves were sampled weekly for chlorophyll determination. Chlorophyll was extracted in 5 mL of N-N-dimethylformamide from 2 cm^2 leaf portions next to the main vein, using the method of Inskeep and Bloom [1985].

In order to isolate the effects of treatment on each measurement, without the complicating effects of other interdependent measurements on the same plant, a type of path analysis (described by Li [1975]) was done. In the first of two steps, the effects on a particular variable of other measured variables were removed, and then, in the second step, the effects of the treatments alone on the particular variable were studied. In the first step, each of the dependent measured variables (number of leaves, number of internodes, fifth and tenth internode length, tenth leaf area, leaf chlorophyll, rachis length, berry volume, and the amount of powdery mildew where applicable) was isolated as the single dependent variable, with the remaining dependent variables held as independent. Using PROC REG [SAS Institute 1985], the subset of the variables considered independent for the analysis which made a contribution to the correlation coefficient was selected by forward stepwise selection. The residuals from this model were then taken into the second step of the analysis, where they were subjected to an ANOVA to determine whether the various treatments had effects on the subject variable with respect to its predicted value. The steps in the analysis are diagrammed in Figure 1. This kind of analysis may be considered to create a regression representing the amount of variation in a given dependent (e.g. berry volume) contributed by each of the other variables (e.g. leaf chlorophyll, leaf number, rachis length, etc.). The regression is thus a calculated mean for the dependent variable which takes into account the contributions of the variables held as independent variables; that is, the other dependent variables. The regression does not take into account variation caused by treatment or block effects. Therefore, a subsequent ANOVA of the residuals of the regression, the

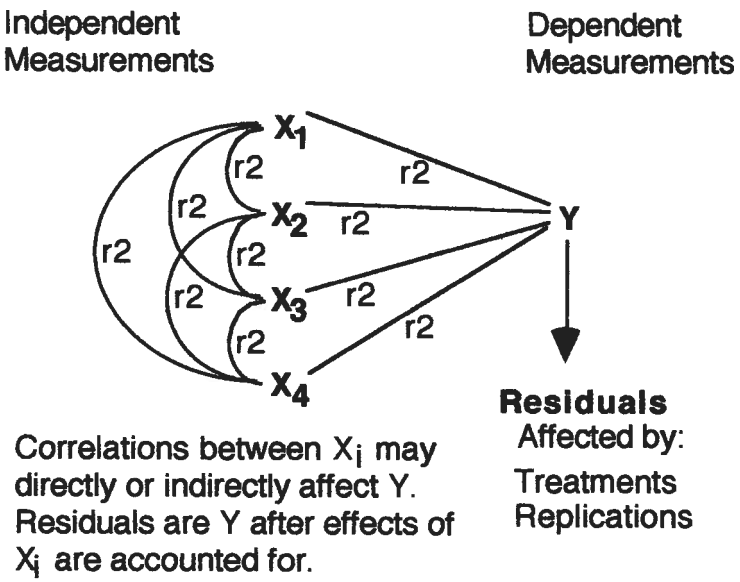

Figure 1. Diagram of interrelationships between data and the method used to determine the effects of the interrelationships on a single variable, so that those effects may be accounted for in subsequent ANOVA. Adapted from Li [1975].

"distance" between the observations and the calculated mean for the dependent variable, allows study of the variation in the dependent variable not complicated by the effects of the other dependent variables held as independent variables in the regression. This variation will be due to the treatment and block effects. This two-step process permits the separation of true treatment effects.

Grape clusters were harvested October 11 at TV in 1991, September 20 at TV in 1992, and October 16 at ACRS in 1992. During harvest, the number and weight of grape berry clusters were determined for each plant. Except for the TV 1992 trial, from which the grapes were too desiccated by the *U. necator* epiphytotic to analyze further, random berry samples (100 berries per treatment) from each treatment were immediately taken to ACRS and stored at -40°C. At a later time, the berries were

weighed (per hundred berries), juiced, and the concentration of soluble solids (in °Brix) for the berries was determined using an Abbé refractometer (AO Instruments, Buffalo, NY). Then, pH was determined for the berries with a Fisher 825MP pH meter (Fisher Instruments, Vancouver, B.C.). The total titratable acids were determined for 50g extracts of whole berries using the extraction and measurement method described by Mattick [1983].

Both titratable acidity and pH were measured because they are not necessarily correlated quantities. The main berry acids (tartaric acid and malic acid) exist partially as potassium salts at normal berry pH [Coombe 1992]. After veraison, catabolism of these acids, primarily malate, occurs as respiration increases, but tartarate remains mainly in salt form, degrading at a minimal rate in comparison to the malate [Winkler *et al* 1974, Coombe 1992, Gutierrez-Granda and Morrison 1992]. Malate degradation contributes directly to increased pH, while the titratable acidity measurement done which includes tartarate does not necessarily.

The effect of the lipid sprays on plant survival was determined in all trials before and after the growing season by weighing cane prunings (the winter before, and then after) on site using a dairy scale accurate to 10 g. The difference between the before and after prunings indicated changes in the degree of survival to the next growing year.

3. RESULTS

3.1. Effects of Exogenous Lipid Emulsions as Phytoprotectants of Grape and Cucumber Plants

3.1.1. Effects of Exogenous Lipid Emulsions on Powdery Mildew of Cucumber Plants

When the seed-storage lipid emulsions were tested in comparison to commercial lipid emulsions against *E. cichoraceaum*, only plants sprayed with jojoba wax/Triton X-100 consistently showed as few *E. cichoracearum* colonies per leaf area as those treated with the commercial control in 1993 (Table 4). However, the trend was that the jojoba wax/ISOL-RB, WiltPruf, and canola oil/Triton X-100-treated leaves had fewer colonies per leaf area than leaves sprayed with other treatments. Fewer colonies were observed on the leaves treated with the Triton X-100 control than the water control-, ISOL-RB control- or the Folicote-treated leaves. These latter three treatments showed no significant difference in the number of *E. cichoracearum* colonies per leaf area. While the separation between plants sprayed with the various treatments was not as clear as the 1993 trials, similar trends were observed for trials with an identical design done in 1992 (Appendix 1).

Table 4. Number of *E. cichoracearum* colonies per leaf area 17 days after inoculation on greenhouse-grown "Chicago Pickling" cucumber plants treated with experimental exogenous lipid emulsions

	Number of *E. cichoracearum* colonies per cm^2 leaf	
Treatment	Trial 1	Trial 2
Water control	0.8 A	1.3 A
0.5% ISOL-RB control	0.7 A	1.3 A
4.0% Folicote	0.7 A	1.2 A
0.5% Triton X-100 control	0.4 B	0.7 B
1.0%Canola/0.5% Triton X-100	0.3 BC	0.4 C
10% WiltPruf	0.2 CD	0.1 D
1.0% Jojoba/0.5% ISOL-RB	0.1 D	0.3 CD
1.0% Jojoba/0.5% Triton X-100	0.1 D	0.1 D
Commercial control	0.0 D	0.0 D

[1] For both trials, n=11. Means represent averages of all plants of a particular treatment, with counts of three leaves (3^{rd}, 4^{th}, and 5^{th} from base) per plant. Column means sharing the same letter are not significantly different according to Duncan's multiple range test, $p<0.05$.

3.1.1.1. Scanning Electron Microscope Studies of the Effects of Exogenous Lipid Emulsions on Powdery Mildew of Cucumber Plants

The scanning electron micrographs taken of cucumber leaves 48 hours after inoculation revealed different infection structure morphology on leaves treated with different exogenous lipid emulsions. Germination occurred on all leaves except those treated with WiltPruf (Table 5). Only the water and surfactant control-treated leaves had germ tubes which actually produced appressoria (Table 5). After 48 hours, while the commercial control- and Folicote-treated leaves had germinated spores with a few appressoria formed, all leaves sprayed with the jojoba wax or canola oil emulsions showed germinated spores without appressorium formation.

Table 5. Number of *E. cichoracearum* infection structures observed by SEM on "Chicago Pickling" cucumber leaves air-dried 48 hours after spraying with experimental exogenous lipid emulsions, and subsequent inoculation with *E. cichoracearum*

Treatment	% Germinated [1]	% Germinated with appressoria
Water control	50.0	100.0
0.5% Triton X-100 control	60.5	60.9
0.5% ISOL-RB control	44.7	100.0
Commercial control	55.3	4.8
Folicote (4% active ingredient)	42.1	6.3
1.0% Canola oil/ 0.5% Triton X-100	60.5	0.0
1.0% Jojoba wax/0.5% Triton X-100	39.5	0.0
1.0% Jojoba wax/0.5% ISOL-RB	39.5	0.0
WiltPruf (10% active ingredient)	0.0	0.0

1. n=38.

When observed with the SEM, the leaves sprayed with ISOL-RB were similar to the water control-sprayed leaves; therefore, in the micrographs, only a sample from the water control-treated leaves is shown (Figure 2A) Similarly, Folicote-, canola oil/Triton X-100- and jojoba wax/Triton X-100 emulsion-treated leaves were not different from one another, so only a sample from the jojoba wax emulsion-treated leaves is shown (Figure 2D). Spores germinated on jojoba wax/ISOL-RB leaves were similar to the water control-sprayed leaves, except that no appressoria were formed. Spores on WiltPruf-treated leaves did not germinate or produce any germination structures. The water- and ISOL-RB-treated leaves had spores with long, branched germ tubes with an appressorium at the end of each germ tube branch. However, the Triton X-100 control-treated leaves showed extremely short germ tube growth with large, elaborate appressorial structures (Figure 2C). Like the spores on the Triton X-100 control-treated leaves, the leaves treated with the commercial control (Figure 2B) or any of the lipid-Triton X-100 combinations except WiltPruf showed extremely short germ tubes; however, there was no appressorium development at the end of the germ tubes.

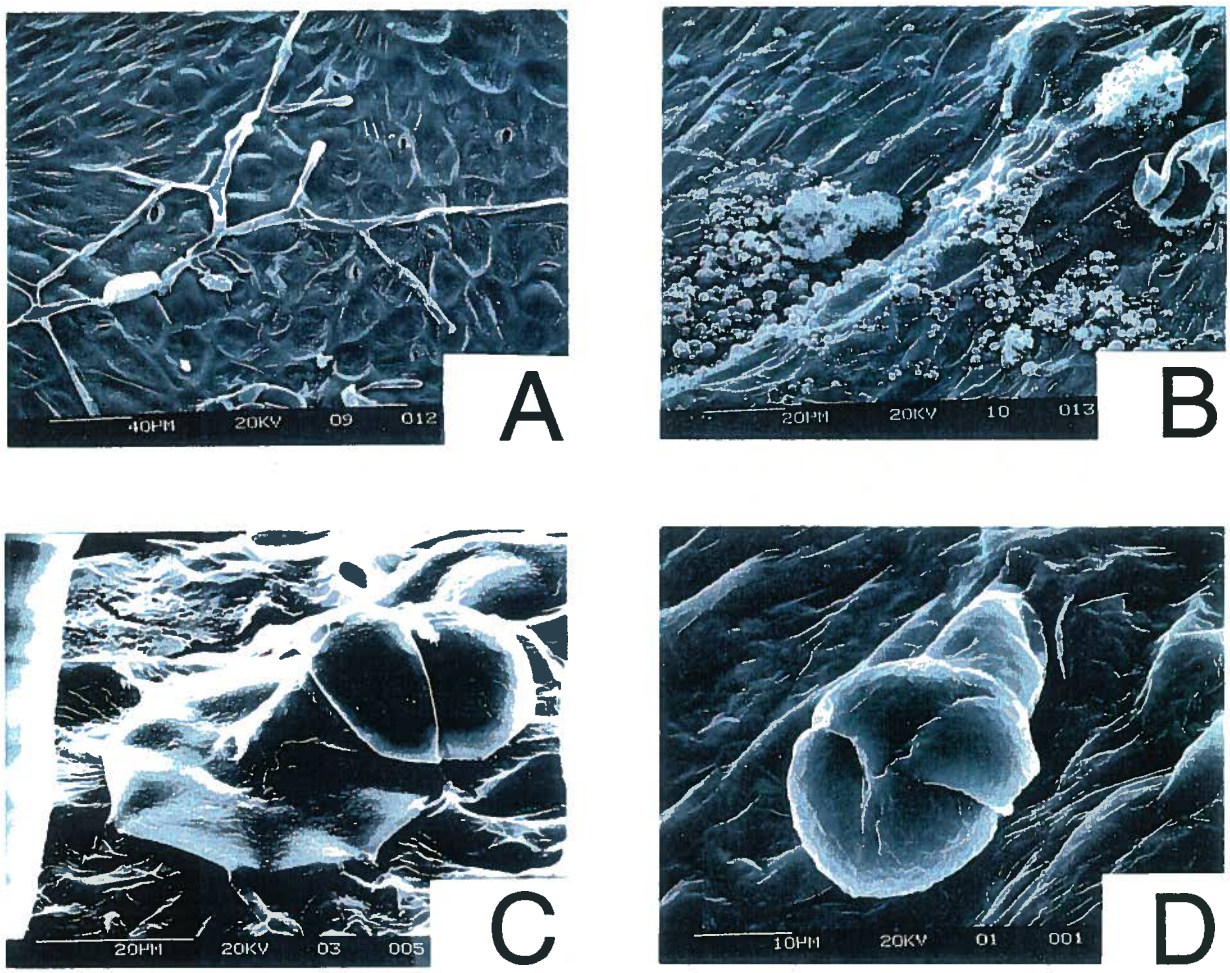

Figure 2. Examples of *E. cichoracearum* infection structures observed by SEM on "Chicago Pickling" cucumber leaves air-dried 48 hours after spraying with experimental exogenous lipid emulsions, and subsequent inoculation with *E. cichoracearum*. (A) Water control-treated leaves. (B) Commercial control-treated leaf. (C) Triton X-100 control-treated leaf. (D) Jojoba wax emulsion-treated leaves.

3.1.2. Effects of Exogenous Lipid Emulsions on Powdery Mildew of Grape Plants

On 'Auxerrois' grape plants at TV, the pre-harvest measurement of September 27, 1991 showed no *U. necator* colonies on the 1.0% "all-season" and the commercial control-treated leaves (Table 6). The 1.0% "pre-veraison"- and 1.0% "post-veraison-treated leaves were also not significantly different from the commercial control. In 1992, a September 7 pre-harvest colony count showed fewer colonies on the 1.0% "all-season"-sprayed leaves as compared to all other treatments. In addition, the Triton X-100 control, both the 1.0% and 0.2% "pre-veraison", and the 0.2% "all-season"-treated leaves showed fewer *U. necator* colonies than those treated with the water control (Table 6). At harvest in 1991, there was no difference in the number of powdery mildew-diseased clusters in the 1.0% "all-season", 1.0% "pre-veraison", 1.0% "post-veraison", and commercial control-treated plants.

Harvest data for 1992 is not displayed for TV because overwintering *E. cichoracearum* inoculum from 1991 initiated a substantial epiphytotic before the jojoba wax emulsion sprays were applied. By the harvest time, the berries on the grape clusters were diseased with powdery mildew to the point where all the berries were desiccated, and many had fallen off the clusters. Because of the *U. necator* epiphytotic, no colony counts after August 7 were collected either (Table 6). However, leaves which were observed to be healthy (uninfected) early in the season which received at least one spray of the jojoba wax emulsion remained healthy throughout the season.

Table 6. Mean number of *U. necator* colonies per experimental seed-storage lipid emulsion-treated 'Auxerrois' grape leaf in the preharvest, and mean number of experimental jojoba wax emulsion-treated 'Auxerrois' grape clusters at harvest showing symptoms of powdery mildew disease

	Number of colonies/leaf		Number of diseased clusters
Treatment	Sept. 27 1991	Aug. 7 1992	Oct. 11 1991
Triton X-100 control	-	26.9 B	-
1.0% "Bloom"	69.2 A	-	4.8 A
Water control	63.3 A	65.2 A	4.3 AB
1.0% "Pre-veraison"	40.8 AB	25.8 B	1.8 BC
0.2% "Pre-veraison"	-	27.4 B	-
0.2% "All-season"	-	18.9 B	-
1.0% "All-season"	0.0 B	3.2 C	0.4 C
1.0% "Post-veraison"	20.0 B	-	1.2 C
Commercial	0.0 B	-	0.4 C

[1] For 1991 samples, n=24; for 1992, n=16 for the Triton X-100 control, and both "Pre-veraison" treatments, n=24 for both "All-season" treatments, and n=64 for the water control. Leaf means represent averages of all plants of a particular treatment, with counts of two leaves per plant. Column means sharing the same letter are not significantly different according to Duncan's multiple range test, $p<0.05$.

3.1.3. Effect of Exogenous Lipid Emulsions on the Growth of *Didymella bryoniae* and *Botrytis cinerea In Vitro*

The petri dishes spread with the commercial control, WiltPruf, and treatments which contained Triton X-100 showed retarded *B. cinerea* and *D. bryoniae* colony growth (Table 7). The untreated petri dishes and those spread with the water control, all treatments containing ISOL-RB, and Folicote (with the exception of the first *D. bryoniae* trial) showed the greatest colony growth. The trend was that the petri dishes spread with the treatments which contained Triton X-100 showed the least colony growth for both species of fungi, consistently second only to that presented in the commercial control-treated petri dishes (Table 7). In addition, the lipid components of the Triton X-100-

containing emulsions did not seem to make a consistent difference amongst the petri dishes in which Triton X-100-containing treatments were spread. The trend, however, was that the petri dishes which were spread with an emulsion which included a lipid component showed more growth than the petri dishes spread with Triton X-100 alone.

Table 7. Difference in *B. cinerea* and *D. bryoniae* colony area between 1 and 4 days after inoculation on experimental exogenous lipid emulsion treated 1% sucrose-enriched PDA

Treatment	Difference in *B. cinerea* colony area (mm^2)		Difference in *D.. bryoniae* colony area (mm^2)	
	Trial 1	Trial 2	Trial 1	Trial 2
Untreated control	1500.0[1] A[2]	1500.0 A	1500.0 A	1500.0 A
Water control	1500.0 A	1500.0 A	1397.2 A	1500.0 A
0.5% ISOL-RB control	1500.0 A	1500.0 A	1460.0 A	1500.0 A
1.0% Jojoba/0.5% ISOL-RB	1500.0 A	1500.0 A	1500.0 A	1500.0 A
1.0%Canola/0.5% ISOL-RB	1500.0 A	1500.0 A	1500.0 A	1500.0 A
4.0% Folicote	1500.0 A	1500.0 A	1120.0 B	1500.0 A
10% WiltPruf	1000.7 B	865.7 B	719.8 D	583.6 B
1.0%Canola/0.5% Triton X-100	525.7 C	119.6 D	846.9 C	246.8 D
2.0%Canola/0.5% Triton X-100	222.1 D	201.3 C	489.8 E	506.4 B
1.0% Jojoba/0.5% Triton X-100	118.3 E	159.3 CD	156.6 F	358.4 C
2.0% Jojoba/0.5% Triton X-100	233.0 D	100.6 D	445.0 E	236.0 D
0.5% Triton X-100 control	195.1 DE	125.5 D	226.1 F	386.6 C
Commercial control	0.0 F	0.0 E	20.0 G	21.0 E

1. Maximum colony diameter before observable colony interference was 44 mm; that is, 1500 mm^2.

2. For *B. cinerea* trials, n=30; for *D. bryoniae* trials, n=10. Each means represents averages of all petri dishes of a particular treatment with three colonies per dish. Column means sharing the same letter are not significantly different according to Duncan's multiple range test, $p<0.05$.

3.1.4. Effects of Exogenous Lipid Emulsions on Black Rot of Cucumber Plants

When the exogenous lipids were tested against *D. bryoniae*, plants sprayed with any treatment except the Folicote spray in the first trial and the commercial control in both trials did not differ in the percentage of diseased, inoculated, injured petioles as compared to the water control (Table 8).

Table 8. Percentage of black rot-diseased inoculated petioles on six-week-old "Chicago Pickling" cucumber plants 17 days after application of experimental exogenous lipid emulsion treatments, and inoculation with *D. bryoniae*

	Percentage of black rot-diseased petioles per plant	
Treatment	Trial 1	Trial 2
1.0% Jojoba/0.5% Triton X-100	67 A[1]	48 A
1.0%Canola/0.5% Triton X-100	68 A	50 A
10% WiltPruf	57 AB	47 A
0.5% ISOL-RB control	57 AB	50 A
1.0% Jojoba/0.5% ISOL-RB	50 AB	50 A
Water control	47 AB	50 A
0.5% Triton X-100 control	33 BC	43 A
4.0% Folicote	17 CD	33 A
Commercial control	0 D	3 B

[1]. For both trials, n=11. Means represent averages of all plants of a particular treatment, with three petioles per plant. Column means sharing the same letter are not significantly different according to Duncan's multiple range test, $p<0.05$.

3.1.5. Effects of Exogenous Lipid Emulsions on Bunch Rot of Grape Plants

At TV in 1991, there was no difference in the number of bunch rot-diseased 'Auxerrois' grape clusters between treated plants sampled 14 days after inoculation, except that the 1.0% "all-season" and "post-veraison"-treated plants were significantly different from the 1.0% "pre-veraison"-treated plants (Table 9). However, there were

fewer bunch rot-diseased 'Riesling' grape clusters from plants treated with any of the three lipid sprays at ACRS in 1992 than were observed on plants treated with either the commercial or water control (Table 9). No assays of bunch rot disease incidence for the 'Auxerrois' plants at TV in 1992 are indicated because the *U. necator* epiphytotic damaged the grape berries beyond the point where such damage could be accounted for in subsequent analyses.

Table 9. Mean number of experimental seed-storage lipid emulsion treated-'Auxerrois' and 'Riesling' grape clusters harvested per plant showing bunch rot disease incidence

Treatment	Number of 'Auxerrois' grape clusters with bunch rot 1991	Treatment	Number of 'Riesling' grape clusters with bunch rot 1992
Water control	1.7 AB[1]	Water control	19.1 A
Commercial control	1.2 AB	Commercial control	11.9 A
1.0% "All-season"	0.8 B	1.0% jojoba wax	3.3 B
1.0% "Bloom"	1.2 AB	1.0% canola oil	4.2 B
1.0% "Pre-veraison"	3.1 A	0.5% canola oil	6.0 B
1.0% "Post-veraison"	0.4 B		

1. For 'Auxerrois' grape clusters, n=24; for 'Riesling' grape clusters, n=12. Column means sharing the same letter, or where letter is not indicated, are not significantly different according to Duncan's multiple range test, $p<0.05$.

3.2. Effect of Exogenous Lipid Emulsions as Antitranspirants on Cucumber and Grape Plants

3.2.1. Determination of Phytotoxic Lipid Emulsion Concentrations

The primary goal of the greenhouse study was to determine the maximum concentration of jojoba wax that could be applied to grape plants without observable detrimental effect. The area of leaves showing necrosis increased for plants sprayed

with concentrations higher than 1.0% (Table 10, Figure 3D, E, F). In fact, plants sprayed with 10% jojoba wax had no leaves when the treatments were photographed; no picture is included in Figure 3. The plants sprayed with the 1.0% concentration were not different from the water controls. As a result, 1.0% was used as an upper limit to concentrations of both jojoba wax and canola oil in subsequent trials.

Table 10. Amount of experimental jojoba wax emulsion-treated 'Auxerrois' grape leaf damage calculated using greenhouse plants which were sprayed at 7 weeks and sampled at 10 weeks

Treatments	Leaf area (cm^2)		
	Total (Necrotic + Undamaged)	Undamaged	Damaged (Necrotic)
Water control	-[1]	-	0.0
Triton X-207 control	96.4	94.6	1.8
0.1% Jojoba wax/1.0% Triton X-207	88.8	87.2	1.7
1.0% Jojoba wax/1.0% Triton X-207	84.8	79.4	5.4
2.0% Jojoba wax/1.0% Triton X-207	83.0	65.6	17.4
5.0% Jojoba wax/1.0% Triton X-207	65.7	43.3	22.5
10.0% Jojoba wax/1.0% Triton X-207	64.2	40.1	24.2

[1]. For all plants of a particular treatment, n=24.

Figure 3. Photographs of experimental jojoba wax emulsion- and Triton X-100 surfactant treated-'Auxerrois' leaf damage using greenhouse plants which were sprayed at 7 weeks and sampled at 10 weeks. (A) Water control-treated plant. Remainder of plants treated with 1.0% Triton X-207 and jojoba wax at (B) 0.0%, (C) 0.1%, (D) 1.0%, (E) 2.0%, (F) 5.0%.

In addition to information about appropriate concentrations of jojoba wax, this portion of the study also provided data on the effect of the Triton X-series surfactants. Jojoba wax could not be dispersed into solution without the aid of a surfactant. However, all plants sprayed, even those receiving only the Triton X-207 surfactant (Figure 3B, C, D, E), appeared a lighter green than those receiving the water controls (Figure 3A), and developed translucent spots in leaf depressions. In order to reduce these effects, 0.2% Triton X-100 surfactant, the lowest concentration observed which could emulsify jojoba wax, was utilized in subsequent *in vivo* trials.

3.2.2. Effects of Exogenous Lipid Emulsions on Cucumber Growth

There were no significant differences in cucumber leaf size between plants sprayed with different treatment (Table 11). However, there were other signs of damage present. While the canola oil/Triton X-100 and water control-treated plants showed no leaf damage, all other treated plants showed some leaf margin necrosis, with the greatest damage observed in the commercial control and WiltPruf treated plants. This was similar to the damage seen in the grape greenhouse study (Figure 3D, E, F) for emulsions containing jojoba wax concentrations over 1.0%.

Table 11. Leaf area of six-week-old greenhouse-grown "Chicago Pickling" cucumber plants, treated 17 days previously with experimental exogenous lipid emulsions

	Leaf area (cm^2)	
Treatment	Trial 1	Trial 2
0.5% Jojoba wax/0.05% Triton X-100	97.6[1]	114.7
0.5% Canola oil/0.05% Triton X-100	95.4	132.5
0.5% Jojoba wax/0.5% ISOL-RB	95.5	141.9
4% Folicote	96.9	101.9
0.5% ISOL-RB	108.4	123.1
0.05% Triton X-100	107.4	105.3
Commercial	103.3	95.8
Water	119.6	123.6
10% WiltPruf	100.4	100.9

1. For both trials, n=11. Means represent averages for all plants of a particular treatment, with three leaf areas (3rd, 4th, and 5th leaves from base) per plant.

3.2.3. Effects of Exogenous Lipid Emulsions on Grape Plant Growth, Yield and Survival

3.2.3.1. Growth and Survival

All grape plants sprayed with lipid emulsions (those containing jojoba wax in all three trials and canola oil at ACRS in 1992) exhibited some lightening of leaf colour and slight chlorosis. This seemed to be correlated with the number of spray applications. Plants sprayed with the "all-season" (TV 1991), 1.0% "all-season" and 0.2% Triton X-100 control (TV 1992), and 1.0% jojoba wax and 1.0% canola oil (ACRS 1992) treatments showed the largest effects. Figure 4 shows increasing chlorosis towards the base of the canopy of "all-season" treated 'Auxerrois' grape plants from TV in 1991 (Figure 4B) as compared to the commercial control-treated plants (Figure 4A). In addition, all lipid emulsion-treated grape berries exhibited a darker green, slightly

bronzed appearance, which can be seen in a comparison of the "all-season" treated plants in Figure 4 to those whitish-green berries of the commercial control-treated plants. The lipid emulsion-treated grape berries in all trials became bronzed particularly in August, when ambient temperatures reached their highest in all three trials (Figure 5). The bronzing was observed at TV in 1991 at approximately the same time (around August 10) that the "all-season" treated plants had a higher stomatal resistance, which was followed by a slowed growth stage progression between August 10 and September 7 (Table 12). Earlier in the growing season of 1992, there were no significant differences observed between treated plants for stomatal resistance (Table 13).

Figure 4. Comparison of the phytotoxic effects of (A) the commercial control and (B) the 1.0% "all-season" jojoba wax sprays on 'Auxerrois' grape plants. Photographs taken August 24, 1991 at TV.

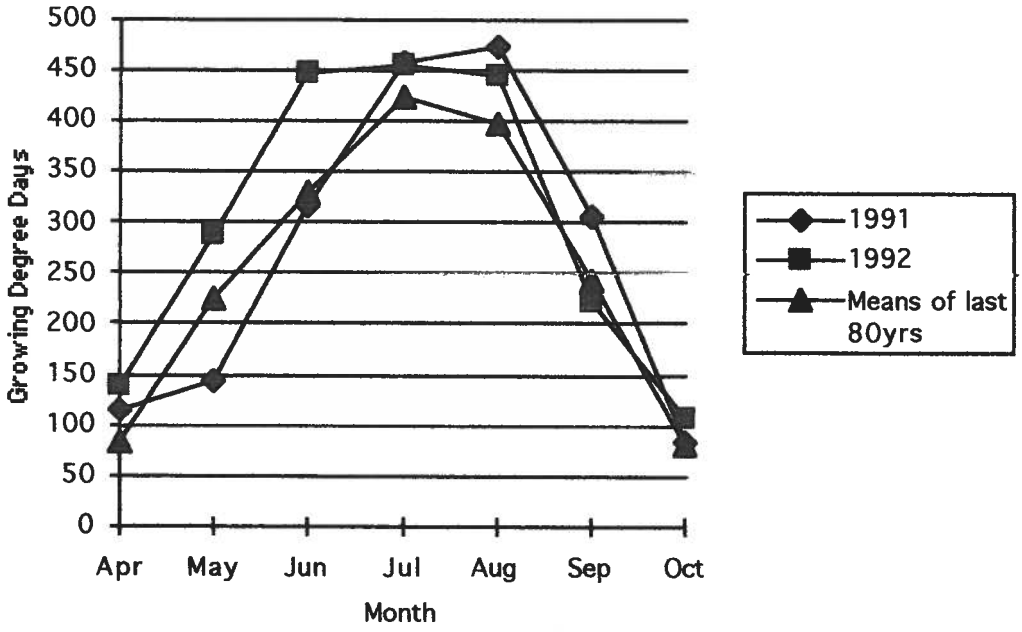

Figure 5. Growing degree days for 1991, 1992 and previous 80-year mean, for the Central Okanagan Region, in which are located both TV and ACRS

Table 12. Percentage of experimental jojoba wax emulsion-treated field-grown 'Auxerrois' grape plants observed in a growth stage, and stomatal resistance measurements of grape leaves

Treatment	Growth stage		Stomatal resistance on August 10 ($s\ mol^{-1}\ m^{-2}$)
	August 10 - colour change	September 7 - berry ripening	
Water control	39% A[1]	51% A	2.4 B
Commercial control	36% A	49% A	2.1 B
"All-season"	26% B	34% B	2.9 A
"Bloom"	37% A	46% AB	2.4 B
"Pre-veraison"	37% A	51% A	2.6 AB
"Post-veraison"	38% A	38% AB	2.4 B

1. n=24. Data with significant differences displayed only; remainder of data set is displayed in Appendices 2 and 3. Column means sharing the same letter are not significantly different according to Duncan's multiple range test, $p<0.05$.

Table 13. Stomatal resistance at mid-season of field-grown 'Auxerrois' grape leaves treated with experimental jojoba wax emulsions

	Stomatal resistance (s mol-1 m-2)					
	July 4		July 11		July 25	
Treatment	0930	1200	0930	1200	0930	1200
Water control	1.5[1]	2.1	0.8	1.7	3.9	3.0
0.2% Triton X-100 control	2.3	2.3	1.3	2.1	3.6	4.0
1.0% jojoba wax "all-season"	2.2	2.2	1.1	1.7	3.1	3.4
1.0% jojoba wax "pre-veraison"	2.0	2.1	1.1	2.0	2.9	3.6
0.2% jojoba wax "all-season"	2.3	2.2	1.2	1.9	3.4	5.5
0.2% jojoba wax "pre-veraison"	2.1	2.2	1.1	1.9	2.4	2.0

1. n=16 for the Triton X-100 control, and both "Pre-veraison" treatments, n=24 for both "All-season" treatments, and n=64 for the water control.

The raw growth data collected during the growing season from plants at TV in 1992 are displayed in Appendix 4. No data were collected after August 8 because the *U. necator* epiphytotic was observed to be a complicating factor with respect to the accuracy of the measurements. The regression analysis followed by an ANOVA of the residuals revealed that no significant effect on the number of leaves per shoot, number of internodes per shoot, fifth and tenth internode length, tenth leaf area, berry volume, and rachis length could be attributed to any particular treatment. However, the 1.0% "pre-veraison", 0.2% "all-season", and 1.0% "all-season" treatments had significantly greater effects on the amount of chlorophyll in the leaves as compared to other treatment effects on July 10 (Figure 6). The effect of these three treatments on chlorophyll amount decreased over the three remaining sampling dates, with the 0.2% "all-season" treatment effect decreasing fastest, then the 1.0% "pre-veraison" treatment effect, and the 1.0% "all-season" treatment effect decreased slowest. The Triton X-100 treatment had an intermediate effect on chlorophyll as compared to the other treatments. The water control and 0.2% "pre-veraison" treatments had the least effect

on chlorophyll throughout the sampling period; it was to their level of effect to which the 0.2% "all-season"- and 1.0% "pre-veraison" treatments decreased.

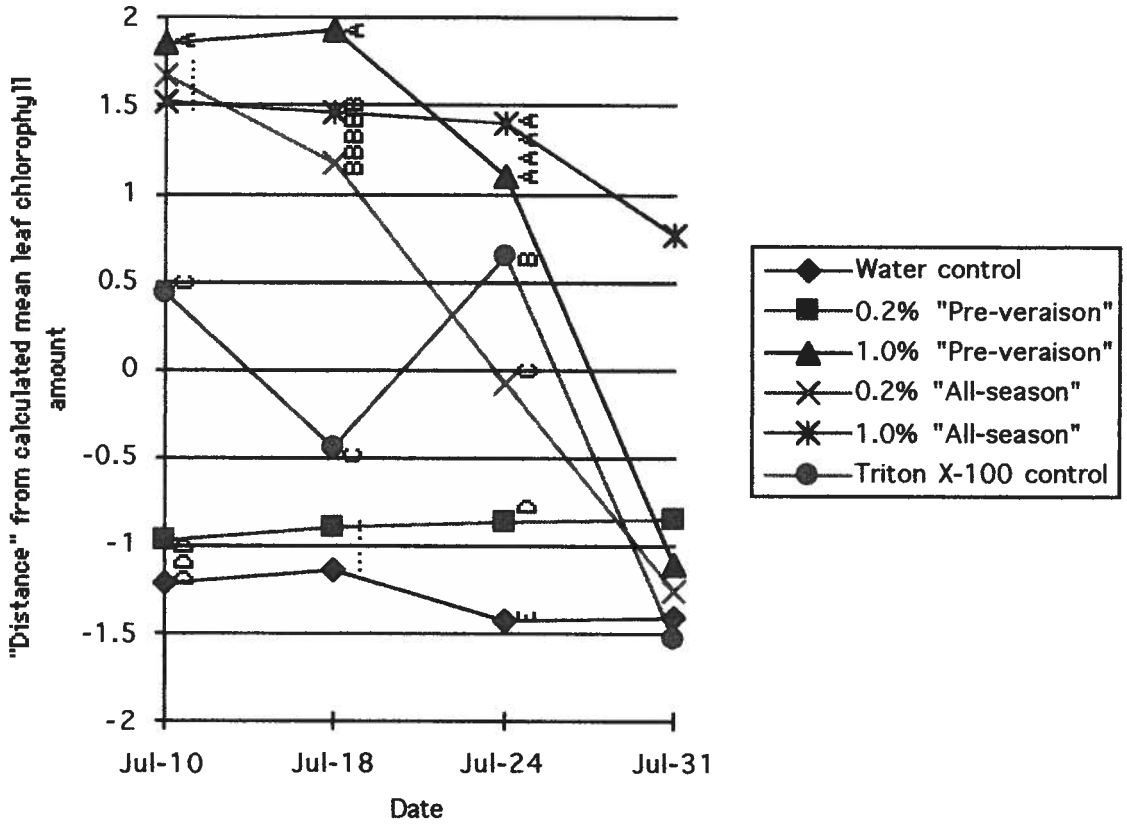

Figure 6. Residuals from stepwise multiple regression analysis showing jojoba wax emulsion treatment effect on 'Auxerrois' grape leaf chlorophyll at TV in 1992. Zero line is the regression line representing leaf chlorophyll amount after the effects of the amount of powdery mildew, and number of leaves/number of internode ratio have been removed. Data points with the same letter are not significantly different according to Duncan's multiple range test, $p<0.05$. Where "...." is displayed, this indicates overlapping of Duncan's letter categories; no significance between treatments indicated can be discerned.

The detailed measurement of 'Riesling' grape plant modules made throughout the growing season at ACRS in 1992 showed little difference for growth between treated plants for the per shoot number of leaves or number of internodes, fifth and tenth internode length, tenth leaf area, rachis length, and berry volume measured (Appendix

5). In addition, with respect to survival, the treated plants showed no statistically significant differences in cane pruning weights in all three trials (Table 14).

Table 14. Wet weight of experimental seed-storage lipid emulsion-treated 'Auxerrois' and 'Riesling' grape plant cane prunings per plant before and after the growing season for field trials

	Cane pruning weight (kg)					
	1991 'Auxerrois'		1992 'Auxerrois'		1992 'Riesling'	
Treatments	Before	After	Before	After	Before	After
Water control	0.8	2.2	0.7	1.6	-	1.0
Commercial control	0.8	2.6	-	-	-	0.9
1.0% jojoba "all-season"	0.6	1.6	0.3	0.7	-	-
1.0% jojoba "bloom"	1.1	2.2	-	-	-	-
1.0% jojoba "pre-veraison"	0.9	2.0	0.7	1.9	-	-
1.0% jojoba "post-veraison"	0.6	2.1	-	-	-	-
0.2% Triton X-100 control	-	-	1.1	1.5	-	-
0.2% jojoba "all-season"	-	-	0.5	1.2	-	-
0.2% jojoba "pre-veraison"	-	-	0.6	2.0	-	-
1.0% jojoba (ACRS only)	-	-	-	-	-	1.0
1.0% canola (ACRS only)	-	-	-	-	-	0.9
0.5% canola (ACRS only)	-	-	-	-	-	1.0

[1]. For 1991 samples, n=24. For 1992, the 'Auxerrois' grapes had n=16 for the Triton X-100 control, and both "Pre-veraison" treatments, n=24 for both "All-season" treatments, and n=64 for the water control; the 'Riesling' grapes had n=12.

3.2.3.2. Yield

There were no significant differences with respect to 'Auxerrois' grape berry or cluster weight, soluble solids concentration, titratable acidity or pH of whole berry samples among treated plants at TV in 1991 (Table 15). There was a trend, however, towards slightly lower cluster weights for all lipid emulsion-treated plants compared to those sprayed with the commercial control. 'Auxerrois' grape cluster weights for TV in

1992 were complicated by the high degree of powdery mildew disease severity; thus, the data is not discussed here (Appendix 6).

Table 15. Cluster weight and berry weight and composition measurements sampled from grapes harvested from experimental jojoba wax emulsions-treated field-grown 'Auxerrois' plants in 1991

Treatment	Cluster weight (g)	Berry weight (g)	Soluble solids (°Brix)	Titratable acidity (g/L)	pH
Water	120 B[1]	1.5	18.1	8.9	3.3
Commercial	160 A	1.6	17.7	7.8	3.3
"All-season"	114 B	1.5	18.2	9.3	3.3
"Bloom"	112 B	1.5	18.1	9.6	3.4
"Pre-veraison"	141 AB	1.5	18.1	8.6	3.4
"Post-veraison"	123 B	1.6	18.2	8.5	3.4

1. n=24. Column means sharing the same letter (or where not indicated) are not significantly different according to Duncan's multiple range test, $p<0.05$.

At ACRS in 1992 (Table 16), there was no significant difference between treated 'Riesling' grape plants with respect to grape berry or cluster weight, soluble solids concentration, titratable acidity or pH of whole berry samples (Table 16). However, there was a trend to slightly lower berry weights for the lipid emulsion and commercial control-sprayed plants.

Table 16. Yield, and berry composition of grapes harvested from experimental seed-storage lipid emulsions-treated field-grown 'Riesling' grape plants

Treatment	Cluster Weight (g)	Berry Weight (g)	Soluble solids (°Brix)	Titratable Acidity (g/L)	pH (-log[H^+])
1.0% Jojoba	231	1.1 B[1]	20.8	9.3	3.4
1.0% Canola	235	1.1 B	18.3	9.6	3.3
0.5% Canola	222	1.1 B	20.4	9.5	3.4
Water control	299	1.3 A	19.5	10.3	3.3
Commercial	258	1.3 AB	19.7	10.0	3.3

1. n=12. Column means sharing the same letter (or where not indicated) are not significantly different according to Duncan's multiple range test, $p<0.05$.

4. DISCUSSION

The overall objectives of this project were (1) to determine whether seed-storage lipid emulsions have potential as plant prophylactic substances and (2) to explore how lipid emulsions applied to plant epicuticles affect fungal plant pathogen infection processes. The different emulsions had different effects on the various fungal pathogens studied; these differences indicated not only the degree but the nature of the interference provided by the lipid emulsions against the fungal plant pathogens.

4.1. Effects of Seed-Storage Lipid Emulsions as Phytoprotectants against Powdery Mildews

4.1.1. Efficacy of Lipid Emulsions against Powdery Mildews

Plants sprayed with seed-storage lipid emulsions showed reduced powdery mildew disease severity as compared to water control-treated plants. Cucumber plants sprayed with jojoba wax-containing emulsions showed a 77-92% reduction in powdery mildew disease severity; plants sprayed with canola oil emulsions showed a 62-69% reduction (Table 4). Also, the 1.0% "all-season" jojoba wax emulsion-treated grape plants showed a 92-100% reduction in powdery mildew disease severity on the leaves during the growing season and reduced disease incidence on the fruit at harvest at TV (Table 6). Thus, emulsions of the two seed-storage lipids utilized are effective prophylactic mixtures against the powdery mildews studied. However, the lipid emulsions seemed to have prophylactic protective effects only, with no capacity to eradicate an existing infection. The 1.0% "pre-veraison" and "post-veraison" jojoba wax emulsion-treated grape plants at TV in 1991 were not significantly different from the

1.0% "all-season"-treated plants with respect to powdery mildew disease severity of leaves or disease incidence of clusters, probably because the artificial inoculation took place after at least one spray had been applied to all of these plants (Table 6). However, the "bloom"-treated plants at TV in 1991 became diseased, probably because the sprays stopped before the majority of the plant canopy had grown; leaves which grew later in the season were unprotected (Table 6). Also, the disease progression on the grape clusters and leaves which existed previous to sprays of the jojoba wax emulsions at TV in 1992 was unimpeded by subsequent sprays; only on plant material not already diseased were the subsequent sprays able to provide any protection against the fungal pathogen. This solely prophylactic protection has also been observed for triacylglyceridic lipid emulsion sprays on grape plants by Northover *et al.* [1993], where the emulsions also provided no eradicant type of control for *Uncinula necator*.

Slight physiological damage consistent with increases in leaf and fruit temperature as a result of decreased transpiration [Quarles 1991] were concurrent with the beneficial prophylactic effects of the lipid emulsion sprays. Damage to grape leaves occurred at controlled greenhouse temperatures when emulsions containing >1.0% jojoba wax were applied (Table 10). These concentrations (higher than the concentrations used for testing the prophylactic effects of the lipids against fungal pathogens) probably inhibited transpiration which raised leaf temperature sufficiently to cause observable damage, as opposed to the transpiration inhibition caused by lower lipid concentrations. However, even the lower concentrations of lipids (≤1.0%) seemed to cause some damage where ambient temperatures were already higher, as higher stomatal resistances and berry bronzing were observed on lipid emulsion-sprayed grape plants (Table 12, Figure 4) with the onset of hotter late-summer temperatures in field trials (Figure 5). This did not occur earlier in the season, when it was cooler (Table 13), nor did it occur on water control-treated plants (Table 12). Berry bronzing and

subsequent desiccation might also account for the slightly lower cluster and berry weights of lipid emulsion-treated plants (Table 15 and 16).

Treatment effects on vegetative growth related to the slight physiological damage observed were subtle (Table 12, Figure 6). Growth stage progression was slightly diminished for the 1.0% "all-season" treated-plants at TV in 1991 (Table 12). At TV in 1992, the effect of treatments with higher concentrations of jojoba wax or more sprays of the jojoba wax emulsion on the amount of leaf chlorophyll was greater than other treatments with lower concentrations or fewer sprays (Figure 7). These subtle effects may have been stimulated by hormone production in damaged leaves [Quarles 1991, Kastori *et al.* 1991]. Increased leaf temperature due to decreased transpiration would have resulted in damage to the photosynthetic apparatus [Nover 1989]. However, there was no long term effect on survival, as measured using field-grown grape plants (Table 14).

Both grape (Figure 3) and cucumber leaves were observed to have a slight chlorosis which was not correlated with lipid emulsion treatment, but rather the application of Triton X-series surfactants. Surfactants of the Triton X-series type are known to induce ethylene formation, producing symptoms of senescence [Lownds and Bukovac 1989]. A surfactant effect may account for the small amount of chlorosis observed.

Slight damage was observed on the plants treated with the seed-storage lipid emulsions; there were no large, general effects on growth or yield observed. Therefore, the advantages of the prophylactic usage of seed-storage lipid emulsions outweigh the small damage caused by the sprays.

4.1.2. Mechanism of Lipid Emulsions against Powdery Mildews

The two seed-storage lipids may have theoretically affected any of the four infection processes of the powdery mildew fungi. For both seed-storage lipids, germination interference can be ruled out as a mechanism, as SEM analyses showed that germination of *E. cichoracearum* spores on leaves treated with these emulsions was as frequent as those on leaves treated with water alone (Figure 2A, D, Table 5). In contrast, spores observed on WiltPruf-treated leaves did not germinate, a phenomenon which has been observed previously [Ziv and Fredericksen 1983, Elad *et al.* 1989, 1990]. However, while 60-100% of the samples of the water and surfactant control-treated leaves showed appressorium formation at 48 hours, samples of cucumber leaves treated with the jojoba wax- or canola oil-containing emulsions showed germinated spores with no appressorium formation. This observation indicates that the surfactants had no effect on the incidence of appressorium formation, a fact which implicates the action of the seed-storage lipids alone on incidence of appressorium formation at 48 hours. However, the lack of appressoria on leaves treated with the lipid emulsions could be explained by inhibition of germ tube growth, differentiation, or the recognition process.

If the seed-storage lipids interfered with the growth process, meaning that appressorium development had simply not yet occurred at 48 hours because of a direct action of the lipids on the germ tube, then one would expect the difference in germ tube length to vary with the presence or absence of the lipids. However, the *E. cichoracearum* germ tubes of spores on Triton X-100 control- and seed-storage lipid/Triton X-100-treated leaves showed diminished growth as compared to water-sprayed leaves (Figure 2C), whereas germ tubes on leaves treated with ISOL-RB control- and jojoba wax/ISOL-RB-treated leaves showed no diminished growth (Figure

2A). This seems to indicate that Triton X-100, both alone and in the lipid emulsions, had the effect on germ tube growth. A retardation of growth can be mitigated by time, and eventually disease signs were observed, as noted on the greenhouse cucumber plants treated with Triton X-100-containing treatments 17 days after inoculation (Table 4). Moreover, the fact that the germ tubes of spores on the jojoba wax/ISOL-RB-treated leaves showed no appressorium formation at 48 hours in spite of vigourous germination (Table 5) and germ tube growth (data not shown) indicates that the absence of appressoria on jojoba wax- (and perhaps canola oil-) treated leaves was due to interference with differentiation or the recognition interference, not due to growth interference. The interference of the seed-storage lipids was not diminished in time, as the plants treated with the seed-storage lipid emulsions, and especially those containing jojoba wax, had few disease signs after 17 days (Table 4).

The Triton X-100 surfactant effect of reducing powdery mildew mycelial growth (Table 5) has been observed previously in rice blast fungi (*Pyricularia oryzae* Cav.) *in vivo* [Kim *et al.* 1989] and *Mucor mucedo* (L. ex Fr.) *in vitro* [Reyes 1992]. Cucumber and grape plants treated with the Triton X-100 controls had fewer powdery mildew colonies than water treated plants (Table 4 and 6), and SEM studies showed that *E. cichoracearum* spores on Triton X-100-treated leaves had limited germ tube growth, and that appressorium development was exaggerated (Figure 2C). In addition, the lower powdery mildew disease incidence observed on the plants treated with the 1.0% and 0.2% "pre-veraison"- and the 0.2% "all-season" treatments in the 'Auxerrois' grape field trials at TV may have been due to Triton X-100 alone, as the reduction observed for the Triton X-100-treated plants was not significantly different from that of these jojoba wax emulsion-treated plants (Table 6). Inhibition may have been due to a solubilizing effect of Triton X-100 micelles on the plasma membrane of the growing germ tube. The exaggerated appressorium growth observed seems to corroborate this

idea. Since appressoria are largely anchored to the epicuticle by water tension [Howard *et al.* 1991], the presence of a larger appressorium may have countered the effect of the surfactant in reducing water tension. These actions of the micellar contents on the germ tube plasma membrane would result in the inhibition of germ tube extension, the useless expenditure of cell energy, and the consequent reduction of growth.

The seed-storage lipids must have had an effect either by inhibiting differentiation of the germ tube into appressoria, or by interfering with the recognition processes which triggers differentiation. One possibility for differentation inhibition is that seed-storage lipids interefered with differentiation via a plant-mediated response. Recent research has discussed jasmonic acid as the plant growth regulator [Meyer *et al.* 1984] which mediates plant wound responses [Farmer and Ryan 1992]. Occurring in plants as a response to wounding [Creelman *et al.* 1992], jasmonic acid induces the formation of extracellular proteins which have no antifungal activity on their own [Schweizer *et al.* 1993, Reinbothe *et al.* 1992]. However, jasmonic acid itself may interfere with the appressorial differentiation of the powdery mildew fungal germ tube, as suggested for *E. graminis* [Schweizer *et al.* 1993]. It is a possibility that the seed-storage lipids could have interfered with germ tube differentiation by eliciting a wound response, or by providing a sufficient quantity of the octadecanoid precursors required for the jasmonic acid pathway [Farmer and Ryan 1992]. However, Triton X-100 has been shown to elicit wound responses in plants [Lownds and Bukovac 1989], and canola oil contains large proportions of the 18:2 linoleic and 18:3 linolenic fatty acid precursors used by the jasmonic acid pathway [Ackman 1990, Farmer and Ryan 1992]. If the seed storage lipids were eliciting a wound response, then the leaves treated with Triton X-100- or canola oil-containing emulsions might have shown less powdery mildew disease incidence. This was not the case; leaves treated with the Triton X-100- or canola oil-containing treatments showed significantly more powdery mildew disease than the

jojoba wax emulsion-treated plants (Table 4). If it can be assumed that jasmonic acid would have the same effect on the differentiation processes of *E. cichoracearum* and other powdery mildews, then it seems unlikely that the seed-storage lipids have a significant plant-mediated effect on the differentiation process which produces an appressorium.

While seed-storage lipids could interfere with differentiation of the appressorium by acting on the germ tube directly, this action is morphologically indistinguishable from recognition interference, which also results in no appressorium formation. Thus, the seed-storage lipid emulsions may contain compounds which directly interfere with the production of the appressorium, or they may interfere with the environmental conditions (>99% relative humidity without free moisture [Blaich *et al.* 1989, Heintz 1986, Carver *et al.* 1990]) required for appressorium formation, perhaps by modulating the transpiration gradient as suggested by Waard [1971]. Both types of interference preclude any penetration interference by these lipids (penetration happens after recognition). Therefore, at least for *E. cichoracearum,* and perhaps other powdery mildews, the only thing that can be said for the seed-storage lipids is that they interfere with the development of fungal appressoria.

The efficacy of the lipids in inhibiting appressorium formation may have been a function of the amount of lipid which was released from the spray solution to the surface. Both the canola oil emulsion- and Folicote-treated cucumber leaves showed as little *E. cichoracearum* appressorium formation in the SEM studies as leaves treated with the jojoba wax emulsions (Table 5). However, the canola oil emulsion, Folicote, and even jojoba wax with ISOL-RB did not inhibit *E. cichoracearum* colony formation to the same degree as jojoba wax emulsified with Triton X-100 (Table 4). The varying efficacy of the lipid emulsions may have depended on the way in which each lipid emulsified.

Emulsions are suspensions of particles of one liquid in another liquid in which the former is immiscible; this causes a concomitant increase in the interface area between the two, a state which is inherently thermodynamically unstable [Rosen 1978]. The surfactant forms a micellar film between the two liquids as a consequence of its amphipathic structure, grading from one liquid to the other. This stabilizes the dispersion, as long as hydrogen bonds or Van der Waal forces hold the surfactant micelle together. The fact that the canola oil and Folicote suspensions were stable and semitransparent contributes to the idea that these lipids were not decreasing the stability of the micelles, whereas jojoba wax formed an opaque, unstable suspension with Triton X-100 and ISOL-RB. When the canola oil or Folicote emulsions were dispersed onto the leaf surface, the canola oil and the paraffin wax in the Folicote would be more likely to stay in the micelles of pure surfactant on the epicuticle, and thus be less of an impedance to fungal infection processes. Folicote has previously been observed to cover epicuticles to which it is applied in a discontinuous fashion [Zekaria-Oren *et al.* 1991]; the discontinuities may be due to the lack of separation of Folicote from micelles. The jojoba wax would come out of suspension soon after dispersion, and, when the spray water had evaporated, the jojoba wax would be more likely to coat the epicuticular surface, with micelles of either the Triton X-100 or the ISOL-RB surfactant sitting on top of the coating. (The ISOL-RB-containing suspension was observed to be slightly more stable than the Triton X-100 suspension; thus it likely differed in the actual micellar construction, which may have resulted in its lower efficacy). If this idea is true, then changing the type and length of the chains of the lipid and the surfactant (as long as they are compatible with the plant) may result in increased phytoprotection against powdery mildews and related pathogens.

4.2. Effects of Seed-Storage Lipid Emulsions as Phytoprotectants against Rot-Causing Fungi

In vitro, the seed-storage lipids seemed to have no efficacy in reducing the mycelial growth of either *B. cinerea* and *D. bryoniae* (Table 7). Triton X-100 was the common factor where the greatest reduction (75-92%) of mycelial growth was observed (Table 7). This mycelial growth inhibition by Triton X-100 is consistent with the inhibition observed for powdery mildew germ tubes, as discussed above in section 4.1.2. The fact that the addition of lipids with the Triton X-100 to agar petri dishes seemed to slightly lessen the inhibition of growth observed for the Triton X-100 control-treated petri dishes (Table 7) suggests that the lipids in the surfactant micelles were interfering with the effects of Triton X-100 against the fungal germ tubes, perhaps by interfering with the stability of the micelles, or simply getting in the way.

However, the *in vivo* efficacy of Triton X-100 was not consistent between the *B. cinerea*-grape and the *D. bryoniae*-cucumber systems. One factor which may have contributed to the efficacy of the Triton X-100-containing treatments was the moisture in the system. The cucumber plants treated with the lipid emulsions showed no reduction in black rot disease incidence (Table 8), whereas grape clusters treated with lipids emulsified with Triton X-100 at ACRS showed a ~80% reduction in bunch rot disease incidence, as compared to water and commercial control-treated berry clusters (Table 9). Indeed, plants treated with any of the three lipid-containing treatments at ACRS showed equivalent reductions in the amount of bunch rot disease incidence in spite of the varying concentrations of lipid (Table 9), a fact which suggests that the emulsifying surfactant, Triton X-100, was responsible for the reduction. The contrast between the two *in vivo* systems is in spite of the fact that the amount of either *D. bryoniae* or *B. cinerea*- growth for the lipid emulsions-treated petri dishes was equivalently reduced in

the *in vitro* trials (Table 7). However, the *D. bryoniae*-cucumber petiole system is inherently wet; the petioles were observed to bleed sap for up to 24 hours after the injuries were caused, meaning the added lipid emulsions were probably washed away by the sap. This is opposed to the dry nature of the *B. cinerea*-grape berry system. Future trials could take place on systems of the latter character, not the former, in order to check the effect of moisture on the efficacy of Triton X-100 in reducing mycelial growth.

4.3. Overall Discussion

The seed-storage lipid emulsions tested did provide protection against disease caused by fungal pathogens, protecting the grape and cucumber plants sprayed by at least 60% as compared to water control-treated plants, and often protecting the plants at a degree greater than 90%. However, this control did depend on the plants being free of the fungal pathogen in question previous to spraying, as only plants completely covered with the lipid emulsions remained free from disease. Also, control was wrought by different parts of the emulsion for different pathogens. For the cucumber powdery mildew at least, the lipid component of the emulsion provided the majority of the protection, whereas the surfactant component of the emulsion provided the protection against the rot-causing fungi. This was likely due to differences in the nature of the two plant fungal systems, and the pre-penetrative mechanism by the two different classes of fungi.

For the powdery mildew systems, the evaporation of the spray water from the lipid emulsions probably left a layer of lipid with isolated surfactant micelles sitting on the epicuticular surface. The differentiation of the germinated powdery mildew spores, while encountering occasional surfactant micelles, seemed mostly affected by the lipid layer,

which may have directly interfered with appressorium differentiation, or altered environmental conditions required for appressorium formation. Alternatively, the rot-causing fungal system required free moisture for germination; this moisture (as long as it did not wash the lipid emulsion away) would preclude the formation of a lipid layer, and the surfactant micelles would be able to move. The differentiation of the germinated rot-causing fungal spores seemed mostly affected by the surfactants, which may have solubilized the plasma membrane of the growing germ tubes. In both systems, the lipid emulsions effected control, but different components of the emulsions were the active component in each case. Because of the ability of the lipid emulsion (and particularly the jojoba wax emulsion) to affect both classes of fungi studies, these emulsions may be used agricultural situations on plants which are attacked by fungi of both types. In fact, the jojoba wax/Triton X-100 emulsion used in this study has been patented under the trademark JojobaShield.

These emulsions may be used to suggest the role of various epicuticular-cuticular lipids in plant-fungal interactions. For instance, jojoba wax is composed of an ester similar in chain length to esters found in the epicuticle, whereas Folicote and canola oil are similar to compounds found in the cuticle (paraffin wax, a long chain hydrocarbon, and oleic acid triacyglycerides, respectively). Perhaps the particular efficacy of the jojoba wax emulsion against powdery mildews can be attributed to a similarity between the jojoba wax and the ester components of plant epicuticles. A further extension would be to suggest that esters in the epicuticle contribute particularly to the natural passive defense of plants against powdery mildews. This idea could be tested by spraying plant epicuticles with synthetic versions of components of the epicuticle, and determining which are most protective against powdery mildews.

The lipid emulsions might also be used as tools to discover what triggers are used to initiate germ tube morphogenesis for different fungal pathogens. For instance, surfactants like Triton X-100 might be used to determine the amount of surface tension required between an appressorium and an epicuticular surface before penetration is attempted. Also, fungi similar to the rot-causing fungi studied here might be placed on surfactant-treated host plants to determine the amount of the susceptibility to the proposed solubilization of the germ tube plasma membrane. These experiments (and certainly others) would provide additional knowledge as to how fungi interact with their plant hosts, and which possible interferences of those interactions might be most effective.

However, with respect to the effect of the seed-storage lipids on the powdery mildew fungi, this research indicated the need to discover the cause of the appressorium formation inhibition. An *in vitro* experiment on lipid-emulsion-covered media done under optimum environmental conditions for appressorium formation should clear up the question. If appressorium formation occurs, then a direct inhibition of the seed-storage lipid emulsions on appressorium differentiation can be ruled out. Also, if appressorium formation does not occur, the interference of the microenvironmental recognition factors by the lipid emulsions can be ruled out. This experiment would make more certain the effects of the seed-storage lipid emulsions, and its results would direct the application of the seed-storage lipid emulsions to further research concerning the powdery mildew fungi.

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6. APPENDICES

Appendix 1. Number of *E. cichoracearum* colonies per six-week old "Chicago Pickling" cucumber leaf (mean for three samples per plant) both two weeks after application of exogenous lipid emulsions

	1992 Trials	
Treatment	Trial 1	Trial 2
Water control	46.9 A[1]	39.4 A
0.5% ISOL-RB control	39.7 ABC	31.4 ABC
0.5% Triton X-100 control	42.3 AB	27.1 BCD
4.0% Folicote	-	25.4 CD
1.0% Canola/0.5% Triton	31.3 BC	19.9 DE
10% WiltPruf	-	3.8 FG
1.0% Jojoba/0.5% ISOL-RB	39.3 ABC	11.9 EF
1.0% Jojoba/0.5% Triton	26.9 C	9.4 FG
Commercial control	3.1 D	0.0 G

1. For trial 1, n=6; for trial 2, n=8. Column means sharing the same letter are not significantly different according to Duncan's multiple range test, $p<0.05$.

Appendix 2. Percent of experimental jojoba wax emulsion-treated field-grown 'Auxerrois' grape plants in a particular growth stage as recorded throughout the growing season in 1991

	Treatments applied					
Date	Water control	Commercial control	"All-season"	"Bloom"	"Pre-veraison"	"Post-veraison"
May 9	56%[1] 3-8 cm shoots	88% Bud burst	58% 3-8 cm shoots	55% 3-8 cm shoots	28% 3-8 cm shoots	48% 3-8 cm shoots
May 15	29% tight cluster	97% 3-8 cm shoots	17% tight cluster	27% tight cluster	10% tight cluster	15% tight cluster
May 23	29% tight cluster	26% tight cluster	23% tight cluster	46% tight cluster	10% tight cluster	60% tight cluster
Jun 5	20% bloom	10% bloom	8% bloom	8% bloom	17% bloom	10% bloom
Jul 7	29% berry set	29% berry set	25% berry set	28% berry set	31% berry set	26% berry set
Jul 27	26% colour change	24% colour change	22% colour change	26% colour change	31% colour change	26% colour change
Aug 10	39% colour change	36% colour change	26% colour change	37% colour change	37% colour change	38% colour change
Sep 7	51% berry ripening	49% berry ripening	34% berry ripening	46% berry ripening	51% berry ripening	52% berry ripening
Sep 14	58% berry ripening	58% berry ripening	52% berry ripening	50% berry ripening	53% berry ripening	55% berry ripening
Sep 23	69% berry ripening	71% berry ripening	63% berry ripening	65% berry ripening	68% berry ripening	65% berry ripening

[1]. n=24. Data with significant differences between treatments are listed in Table 13. Growth stage was measured by determining for the most advanced stage the number of vines per treatment that were in that stage.

Appendix 3. Stomatal resistance (s mol^{-1} m^{-2}) measurements from the centre of experimental jojoba wax emulsion-treated field-grown 'Auxerrois' grape leaves on the south and outer side of grape plant canopies as recorded throughout the growing season in 1991

Date	Treatments applied					
	Water control	Commercial control	"All-season"	"Bloom"	"Pre-veraison"	"Post-veraison"
Jun 5	2.82[1]	2.83	2.85	2.85	2.71	2.70
Jun 15	4.11	3.95	4.29	4.42	4.28	4.21
Jun 29	2.08	2.11	2.07	2.14	2.25	1.94
Jul 7	3.98	3.88	4.38	4.13	4.45	4.13
Jul 20	5.77	5.09	4.92	5.52	5.10	5.21
Jul 27	3.25	2.89	3.31	3.71	3.49	3.63
Aug 10	2.40	2.13	2.91	2.40	2.56	2.43

1. n=24. Data showing significant differences between treatments are listed in Table 13.

Appendix 4.1. Number of leaves per shoot of experimental jojoba wax emulsion-treated field-grown 'Auxerrois' grape plants throughout the growing season in 1992

Date	Treatments applied					
	Water control	0.2% "pre-veraison	1.0% "all-season"	0.2% "all-season"	1.0% "pre-veraison"	Triton X-100 control
Jun 12	15.4[1]	14.3	15.5	15.0	13.9	16.1
Jun 18	19.8	20.9	20.2	20.5	18.9	21.6
Jun 26	33.8	33.2	33.0	32.9	31.3	36.1
Jul 3	46.6	42.5	41.9	40.2	40.0	46.7
Jul 10	49.4	46.9	47.9	46.3	43.7	50.4
Jul 18	38.6	40.8	43.0	44.8	42.9	48.4
Jul 24	53.7	54.6	56.4	54.5	52.7	60.5
Jul 31	45.2	48.4	54.1	50.4	50.8	55.9

1. n=16 for the Triton X-100 control, and both "Pre-veraison" treatments, n=24 for both "All-season" treatments, and n=64 for the water control. Means are averages of two shoots per plant.

Appendix 4.2. Number of internodes per shoot of experimental jojoba wax emulsion-treated field-grown 'Auxerrois' grape plants throughout the growing season in 1992

Date	Treatments applied					
	Water control	0.2% "pre-veraison"	1.0% "all-season"	0.2% "all-season"	1.0% "pre-veraison"	Triton X-100 control
Jun 12	12.3[1]	12.5	11.7	12.0	11.5	11.9
Jun 18	13.5	14.0	13.7	13.7	13.8	14.2
Jun 26	18.4	18.0	17.3	17.5	17.3	18.0
Jul 3	22.0	20.8	20.4	20.6	20.4	21.8
Jul 10	23.4	21.9	22.0	21.8	21.8	24.0
Jul 18	21.0	19.7	21.0	21.3	21.7	23.7
Jul 24	22.8	22.5	22.2	22.0	22.7	23.9
Jul 31	22.3	22.0	23.5	21.1	23.1	24.4

1. n=16 for the Triton X-100 control, and both "Pre-veraison" treatments, n=24 for both "All-season" treatments, and n=64 for the water control. Means are averages of two shoots per plant.

Appendix 4.3. Length of the fifth internode (cm) on shoots of experimental jojoba wax emulsion-treated field-grown 'Auxerrois' grape plants throughout the growing season in 1992

Date	Treatments applied					
	Water control	0.2% "pre-veraison"	1.0% "all-season"	0.2% "all-season"	1.0% "pre-veraison"	Triton X-100 control
Jun 12	7.6[1]	7.4	5.5	8.7	7.8	9.3
Jun 18	7.7	7.1	6.1	8.4	8.2	9.3
Jun 26	8.2	7.6	6.1	8.7	8.3	9.6
Jul 3	8.5	7.7	6.2	8.1	8.7	9.6
Jul 10	8.4	7.7	6.6	8.9	8.4	9.8
Jul 18	7.8	7.0	5.9	8.5	8.0	8.5
Jul 24	8.1	7.2	6.1	8.6	8.3	9.7
Jul 31	7.5	6.9	6.0	8.7	8.0	8.4

1. n=16 for the Triton X-100 control, and both "Pre-veraison" treatments, n=24 for both "All-season" treatments, and n=64 for the water control. Means are averages of two shoots per plant.

Appendix 4.4. Length of the 10th internode (cm) on shoots of experimental jojoba wax emulsion-treated field-grown 'Auxerrois' grape plants throughout the growing season in 1992

	Treatments applied					
Date	Water control	0.2% "pre-veraison"	1.0% "all-season"	0.2% "all-season"	1.0% "pre-veraison"	Triton X-100 control
Jun 12	6.8[1]	5.6	6.0	6.1	6.2	6.9
Jun 18	8.0	6.6	6.7	7.2	6.6	8.2
Jun 26	7.9	6.8	6.5	7.4	6.3	7.7
Jul 3	7.8	6.9	6.6	7.6	6.8	7.9
Jul 10	8.5	7.0	6.5	7.6	6.5	8.2
Jul 18	8.2	6.3	6.2	7.9	7.2	8.1
Jul 24	7.8	7.1	6.1	7.4	6.5	7.9
Jul 31	8.3	6.0	6.1	7.7	7.1	8.2

1. n=16 for the Triton X-100 control, and both "Pre-veraison" treatments, n=24 for both "All-season" treatments, and n=64 for the water control. Means are averages of two shoots per plant.

Appendix 4.5. Mean area of the 10th leaf (cm^2) on shoots of experimental jojoba wax emulsion-treated field-grown 'Auxerrois' grape plants throughout the growing season in 1992

	Treatments applied					
Date	Water control	0.2% "pre-veraison"	1.0% "all-season"	0.2% "all-season"	1.0% "pre-veraison"	Triton X-100 control
Jun 12	114.5[1]	108.2	81.7	107.7	105.5	130.4
Jun 18	143.5	140.4	106.3	143.3	143.5	168.0
Jun 26	166.7	168.0	128.8	175.6	177.7	196.6
Jul 3	173.7	176.1	133.2	180.1	185.8	198.8
Jul 10	179.8	165.9	135.0	176.6	175.0	194.9
Jul 18	159.8	169.0	141.9	177.7	176.6	191.3
Jul 24	178.5	182.3	131.6	177.7	180.1	192.7
Jul 31	164.4	169.0	143.3	163.3	169.0	191.3

1. n=16 for the Triton X-100 control, and both "Pre-veraison" treatments, n=24 for both "All-season" treatments, and n=64 for the water control. Means are averages of two shoots per plant.

Appendix 4.6. First cluster rachis length (cm) on shoots of experimental jojoba wax emulsion-treated field-grown 'Auxerrois' grape plants throughout the growing season in 1992

Date	Treatments applied					
	Water control	0.2% "pre-veraison"	1.0% "all-season"	0.2% "all-season"	1.0% "pre-veraison"	Triton X-100 control
Jun 12	9.4[1]	7.9	6.2	9.2	7.9	10.2
Jun 18	10.3	8.6	6.5	9.8	8.6	10.6
Jun 26	10.9	9.6	7.5	11.1	9.2	11.7
Jul 3	12.1	10.3	8.9	11.8	10.8	13.3
Jul 10	12.7	10.2	8.9	12.9	10.9	13.5
Jul 18	12.6	10.4	8.8	12.7	11.9	12.5
Jul 24	11.8	10.6	9.1	13.0	11.5	13.8
Jul 31	13.0	10.8	9.9	12.7	12.6	13.6

1. n=16 for the Triton X-100 control, and both "Pre-veraison" treatments, n=24 for both "All-season" treatments, and n=64 for the water control. Means are averages of two shoots per plant.

Appendix 4.7. Berry volume in mm^3 from berries sampled at the bottom of first clusters on shoots of experimental jojoba wax emulsion-treated field-grown 'Auxerrois' grape plants throughout the growing season in 1992

Date	Treatments applied					
	Water control	0.2% "pre-veraison"	1.0% "all-season"	0.2% "all-season"	1.0% "pre-veraison"	Triton X-100 control
Jun 12	0.0[1]	0.0	0.0	0.0	0.0	0.0
Jun 18	0.0	0.0	0.0	0.0	0.0	0.0
Jun 26	0.0	0.0	0.0	0.0	0.0	0.0
Jul 3	110.6	140.6	68.9	157.5	91.1	157.5
Jul 10	262.1	373.2	185.2	421.9	300.8	389.0
Jul 18	250.0	456.5	262.1	493.0	571.8	493.0
Jul 24	274.6	571.8	-	830.6	493.0	857.4
Jul 31	456.5	551.4	493.0	884.7	804.4	941.2

1. n=16 for the Triton X-100 control, and both "Pre-veraison" treatments, n=24 for both "All-season" treatments, and n=64 for the water control. Means are averages of two shoots per plant.

Appendix 4.8. Amount of chlorophyll in mg/cm^2 for experimental jojoba wax emulsion-treated field-grown 'Auxerrois' grape leaf samples taken from the south and outside of grape canopies at the 1.5 m level throughout growing season in 1992

	Treatments applied					
Date	Water control	0.2% "pre-veraison"	1.0% "all-season"	0.2% "all-season"	1.0% "pre-veraison"	Triton X-100 control
Jun 12	0.39[1]	0.45	0.48	0.50	0.45	0.49
Jun 18	0.53	0.52	0.51	0.54	0.52	0.51
Jun 26	0.52	0.51	0.53	0.53	0.51	0.54
Jul 3	0.53	0.59	0.55	0.60	0.55	0.59
Jul 10	0.50	0.54	0.54	0.56	0.53	0.49
Jul 18	0.47	0.51	0.55	0.57	0.52	0.53
Jul 24	0.42	0.49	0.49	0.49	0.55	0.47
Jul 31	0.49	0.44	0.39	0.44	0.45	0.45

1. n=16 for the Triton X-100 control, and both "Pre-veraison" treatments, n=24 for both "All-season" treatments, and n=64 for the water control. Means are averages of two shoots per plant.

Appendix 5.1. Number of leaves per shoot of experimental seed-storage lipid emulsion-treated field-grown 'Riesling' grape plants throughout the growing season in 1992

	Treatments applied				
Date	1.0% jojoba wax	1.0% canola oil	Commercial control	Water control	0.5% canola oil
Jun 12	13.3[1]	13.3	14.0	14.3	13.3
Jun 18	21.5	21.4	21.6	23.7	30.9
Jun 26	32.0	34.1	33.4	37.1	33.9
Jul 3	35.6	36.1	36.3	40.5	37.1
Jul 10	38.3	40.5	41.9	46.3	42.8
Jul 18	55.3	52.4	50.6	56.9	56.3
Jul 24	47.2	49.6	50.8	54.8	54.4
Jul 31	49.1	53.0	51.3	55.7	59.7
Aug 12	51.9	58.3	53.7	58.7	65.5
Aug 21	59.2	63.9	55.1	58.6	64.3
Sep 7	68.7	68.7	57.8	64.3	79.0

1. n=12

Appendix 5.2. Number of internodes per shoot of experimental seed-storage lipid emulsion-treated field-grown 'Riesling' grape plants throughout the growing season in 1992

Date	Treatments applied				
	1.0% jojoba wax	1.0% canola oil	Commercial control	Water control	0.5% canola oil
Jun 12	12.2[1]	12.6	13.1	13.3	12.3
Jun 18	14.7	14.8	15.7	15.9	14.9
Jun 26	19.0	20.3	20.5	20.7	19.7
Jul 3	22.3	23.1	22.8	22.9	22.4
Jul 10	21.5	23.6	23.0	23.7	22.4
Jul 18	24.8	24.2	25.2	25.5	23.9
Jul 24	22.2	24.6	24.6	24.2	23.7
Jul 31	22.5	24.8	25.0	24.2	24.9
Aug 12	21.7	25.1	25.2	23.8	25.0
Aug 21	23.9	27.8	25.7	24.2	27.3
Sep 7	26.6	26.5	26.0	25.0	25.3

1. n=12

Appendix 5.3. Length of the fifth internode (cm) on shoots of experimental seed-storage lipid emulsion-treated field-grown 'Riesling' grape plants throughout the growing season in 1992

Date	Treatments applied				
	1.0% jojoba wax	1.0% canola oil	Commercial control	Water control	0.5% canola oil
Jun 12	5.1[1]	5.9	5.6	6.6	5.4
Jun 18	-	6.3	6.4	6.4	5.6
Jun 26	5.4	6.4	6.3	6.3	5.5
Jul 3	5.6	6.3	6.5	6.4	5.7
Jul 10	5.6	6.3	6.3	6.3	5.6
Jul 18	6.4	6.6	6.7	7.0	5.9
Jul 24	5.5	6.4	6.5	6.4	5.6
Jul 31	5.5	6.1	6.3	6.3	5.4
Aug 12	5.1	6.1	6.3	5.9	5.4
Aug 21	5.4	6.4	6.4	6.3	5.2
Sep 7	6.2	6.5	6.6	6.6	5.4

1. n=12

Appendix 5.4. Length of the 10th internode (cm) on shoots of experimental seed-storage lipid emulsion-treated field-grown 'Riesling' grape plants throughout the growing season in 1992

Date	Treatments applied				
	1.0% jojoba wax	1.0% canola oil	Commercial control	Water control	0.5% canola oil
Jun 12	3.7[1]	4.2	4.8	5.6	4.5
Jun 18	4.4	4.4	5.1	6.1	5.0
Jun 26	4.5	4.8	4.9	6.1	5.3
Jul 3	4.5	4.8	5.3	3.0	5.3
Jul 10	4.4	5.1	5.2	6.1	5.3
Jul 18	5.1	5.0	5.5	6.8	5.6
Jul 24	4.5	5.0	5.2	6.1	5.3
Jul 31	4.5	4.9	5.0	6.0	5.1
Aug 12	4.8	-	5.1	6.4	5.4
Aug 21	4.3	4.8	5.0	5.9	5.1
Sep 7	5.0	4.7	5.2	6.5	5.5

1. n=12

Appendix 5.5. Area of the 10th leaf (cm^2) on shoots of experimental seed-storage lipid emulsion-treated field-grown 'Riesling' grape plants throughout the growing season in 1992

Date	Treatments applied				
	1.0% jojoba wax	1.0% canola oil	Commercial control	Water control	0.5% canola oil
Jun 12	59.0[1]	63.1	76.9	77.3	76.3
Jun 18	86.0	86.9	94.4	106.1	98.9
Jun 26	93.4	94.4	110.6	119.3	116.6
Jul 3	96.0	97.6	106.1	116.2	114.1
Jul 10	95.5	98.5	109.3	120.6	115.7
Jul 18	111.3	105.3	119.7	133.3	126.6
Jul 24	95.8	104.9	112.1	120.3	117.2
Jul 31	96.8	103.6	111.1	117.8	115.6
Aug 12	92.5	99.0	112.7	120.7	126.0
Aug 21	101.9	99.9	110.5	117.6	112.8
Sep 7	108.4	94.1	120.7	128.7	128.5

1. n=12

Appendix 5.6. First cluster rachis length (cm) on shoots of experimental seed-storage lipid emulsion-treated field-grown 'Riesling' grape plants throughout the growing season in 1992

Date	Treatments applied				
	1.0% jojoba wax	1.0% canola oil	Commercial control	Water control	0.5% canola oil
Jun 12	6.7[1]	7.2	6.5	7.1	7.0
Jun 18	7.4	8.0	7.4	8.1	7.8
Jun 26	8.0	8.7	8.0	8.9	9.0
Jul 3	8.3	8.9	8.7	9.1	9.1
Jul 10	8.9	9.3	8.6	9.4	9.8
Jul 18	9.2	9.7	7.3	8.8	9.8
Jul 24	9.0	-	9.1	9.3	10.1
Jul 31	8.9	9.4	8.5	9.5	9.7
Aug 12	8.9	9.5	9.0	10.2	9.8
Aug 21	9.0	9.6	8.8	9.7	10.0
Sep 7	9.7	10.1	8.6	9.3	10.7

1. n=12

Appendix 5.7. Berry volume (mm^3) from berries sampled at the bottom of first clusters on shoots of experimental seed-storage lipid emulsion-treated field-grown 'Riesling' grape plants throughout the growing season in 1992

Date	Treatments applied				
	1.0% jojoba wax	1.0% canola oil	Commercial control	Water control	0.5% canola oil
Jun 12	0.0[1]	0.0	0.0	0.0	0.0
Jun 18	0.0	0.0	0.0	0.0	0.0
Jun 26	39.4	45.5	45.1	46.5	60.6
Jul 3	184.6	183.5	168.3	191.1	178.3
Jul 10	283.9	264.3	274.8	272.1	292.2
Jul 18	313.6	369.7	300.7	351.4	350.4
Jul 24	428.5	395.0	416.6	441.2	490.0
Jul 31	400.4	355.6	396.3	414.0	365.2
Aug 12	-	564.5	466.7	545.0	-
Aug 21	532.5	514.1	544.2	619.8	549.9
Sep 7	664.2	676.7	607.6	674.3	683.9

1. n=12

Appendix 5.8. Amount of chlorophyll in mg/cm^2 for experimental seed-storage lipid emulsion-treated field-grown 'Riesling' grape leaf samples taken from the south and outside of grape canopies at the 1.5 m level throughout growing season in 1992

	Treatments applied				
Date	1.0% jojoba wax	1.0% canola oil	Commercial control	Water control	0.5% canola oil
Jun 12	0.49[1]	0.50	0.48	0.50	0.46
Jun 18	0.51	0.48	0.49	0.44	0.47
Jun 26	0.50	0.53	0.53	0.54	0.55
Jul 3	0.56	0.53	0.54	0.60	0.55
Jul 10	0.48	0.51	0.51	0.54	0.51
Jul 18	0.54	0.56	0.53	0.53	0.51
Jul 24	0.58	0.57	0.56	0.58	0.58
Jul 31	0.53	0.55	0.53	0.54	0.54
Aug 12	0.57	0.48	0.58	0.54	0.58
Aug 21	0.47	0.50	0.49	0.49	0.48
Sep 7	0.47	0.47	0.46	0.49	0.49

[1]. n=12

Appendix 6. Weight of harvested grape clusters sampled from experimental seed-storage lipid emulsion-treated field-grown 'Auxerrois' grape plants in 1992

Treatment	Cluster weight (g)
0.2% "All-season"	77 A[1]
1.0% "All-season"	60 AB
0.2% Triton X-100 control	60 AB
1.0% "Pre-veraison"	69 AB
0.2% "Pre-veraison"	54 B
Water control	19 C

[1]. n=16 for the Triton X-100 control, and both "Pre-veraison" treatments, n=24 for both "All-season" treatments, and n=64 for the water control. Means are averages of two shoots per plant. Column means sharing the same letter are not significantly different according to Duncan's multiple range test, $p<0.05$.