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ORIGINAL CONTRIBUTION

Green peach aphid [*Myzus persicae* (Sulzer) (Hemiptera: Aphididae)] control using Brassicaceae ethyl ester oil sprays

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Abstract

Control of green peach aphid (*Myzus persicae*), a globally important pest, using plant-derived oils is a promising alternative to conventional insecticides. Although various plant-derived oils are potentially useful for insect control, dose–response studies and efficacy comparisons among oils have not been widely reported. Our objective was to compare *M. persicae* control by plant-derived oils, focusing on oils derived from Brassicaceae species that exhibit rotational and environmental quality benefits. We thus applied sprays of emulsified ethyl esters from the seed oils of yellow mustard (*Sinapis alba*), oriental mustard (*Brassica juncea*) and rapeseed (*Brassica napus*) to *M. persicae* in a laboratory bioassay. A dose–response relationship was modelled for the *S. alba* spray yielding LD_{50}/LD_{95} values of $18.2 \pm 0.87/128.1 \pm 5.10$ μg ester per cm^2 ($P < 0.0001$). Ethyl esters of oils from all three species and soybean (*Glycine max*) ethyl ester were compared to determine the efficacy of Brassicaceae oils relative to the dominant plant-oil spray currently available. All ethyl esters were equally efficacious despite measured differences in fatty acid profiles among the oils. Oils derived from mustards *B. juncea* and *S. alba* are potentially useful feedstocks for the production of insecticidal sprays, and testing on additional insects is warranted.

Introduction

Increasing public and scientific concerns about the negative environmental impacts of synthetic, petroleum-based products have increased the need for plant-derived insecticidal oil sprays. In contrast to petroleum, plant oils are a renewable resource that can be utilized in a sustainable way. Studies have shown that emulsions of non-aromatic plant oils applied as sprays are effective in controlling a range of plant pests (Sams and Deyton 2002; Marcic et al. 2009; Nicetic et al. 2011). Historically, insecticidal plant-oil sprays have been more expensive than comparable mineral-oil spray formulations (Ebbon 2002). Prior to the recent increase in consumer demand for bioproducts to reduce synthetic pesticide use, the lack of economic incentives thus limited the development of an infrastructure capable of providing plant-oil feedstocks.

The insecticidal and miticidal efficacy of mineral oil is well documented (Herron et al. 1998b; Agnello 2002; Herron and Barchia 2002; Johnson and Hodgkinson 2002), with the most recent studies showing effects on two-spotted spider mite (*Tetranychus urticae* Koch) (Chueca et al. 2010), citrus red mite [*Panonychus citri* (McG.)], citrus rust mite (*Phyllocoptruta oleivora* Ashmead), leafminer, (*Phyllocnistis citrella* Stainton) (Chen et al. 2009), European corn borer (*Ostrinia nubilalis* Hübner) (Al Dabel et al. 2008), red scale [*Aonidiella aurantii* (Maskell)] (Liang et al. 2010; Garcera et al. 2012), purple or mussel scale [*Lepidosaphes beckii* (Newman)] (Liang et al. 2010), fruit flies [*Bactrocera tryoni* (Froggatt)] (Meats et al. 2012) and cotton aphid (*Aphis gossypii* Glover) (Najar-Rodriguez et al. 2007a,b). It has been demonstrated that unlike synthetic insecticides, pest control by mineral oils is achieved without negatively impacting parasitoids of the insect pest (Liang et al. 2010). As such, natural

enemies of the insect pest are maintained, thereby providing a second mechanism for reducing insect damage. Another benefit of mineral-oil application is the potential control of virus transmission through impacts the oil has on insect vectors (Martin et al. 2004; Ameline et al. 2010; Martoub et al. 2011). Various modes of action including direct effects on the insect and indirect effects relating to feeding-behaviour changes have been suggested (Ameline et al. 2010; Martoub et al. 2011).

One of the major modes of action of insecticidal oil sprays is anoxia-induced mortality in the target organism (Taverner 2002, 1998). Insect-pest control relying on suffocation is especially promising, because it holds the potential to circumvent toxicant-selected resistance at the genomic level. Despite the fact that other modes of action have been suggested (Najar-Rodriguez et al. 2008, 2007a,b; Stadler and Buteler 2009), spray oils are the only class of widely used insecticides and miticides to which no insect or mite species has developed resistance after decades of use (Rock and Crabtree 1987; Agnello et al. 1994).

In the present study, we evaluated the insecticidal efficacy of ethyl esters derived from the seed oils of three high oil-yielding Brassicaceae species: *Sinapis alba* L., *Brassica juncea* (L.) Czern. and *Brassica napus* L. These species are especially useful as rotational crops, because of their capacity to suppress plant pests (Brown and Morra 1997). Of these three economically important oilseed species, *S. alba* requires the least amount of water and lowest insecticidal inputs for production, making it an attractive species for oil production in environments where lower inputs are a grower priority (Brown et al. 1997; Ross et al. 2008). All of the esterified Brassicaceae oils have a short residence time in the environment and thus a potentially low environmental impact (Zhang et al. 1998; Makareviciene and Janulis 2003). Our motivation for using ethylated Brassicaceae oils rather than methylated oils was that methylation typically relies on trans-esterification using petroleum-derived methanol, whereas ethanol can be derived from renewable resources. We found ethylation more compatible with the requirements for sustainability that come with 'organic' agriculture, the major market sector for plant-oil insecticides.

We selected green peach aphid [*Myzus persicae* (Sulzer)] as a target organism because of its commercial importance globally as a pest causing damage in a large number of crops mainly through plant–virus transmission (Cervantes and Alvarez 2011; Tian et al. 2012). It is well known that mineral-oil sprays are effective against aphids and that mineral oils suppress virus transmission (Herron et al. 1998a; Martin et al. 2004). It has also been established that certain plant oils can be used to control aphids and suppress virus transmission. For example, Martín-López et al. (2003, 2006), achieved levels of control of green peach aphid with esterified oils of rapeseed (*B. napus*) and soybean [*Glycine max* (L.) Merr.] that were comparable with control obtained with mineral oil. Although the level of suppression varies, reduced virus transmission through the application of rapeseed oil has also been reported (Martin et al. 2004; Martín-López et al. 2006; Wrobel 2012). These studies, however, did not establish a dose–response relationship for any of the investigated plant oils nor has there been any testing of *S. alba* or *B. juncea* oils.

As soybean-based oil sprays dominate the market (Sams and Deyton 2002), there is a need to compare the insecticidal efficacy of Brassicaceae ethyl esters with ethylated soybean oil. Although it has been shown that both the chemical characteristics of raw and processed oils influence arthropod control and phytotoxicity (Agnello 2002; Taverner 2002; Ouyang et al. 2010; Nicetic et al. 2011), many published reports provide few specific details about the oils tested. Information on oil chemistry is important in establishing a link between oil chemistry and observed results, proposing mechanisms responsible for observed activity and comparing current results with past and future research findings.

Our objectives were to (i) establish a dose–response for *S. alba* ethyl ester in controlling *M. persicae* and (ii) determine whether ethyl esters of *S. alba*, *B. juncea* and *B. napus* oils provide control of *M. persicae* comparable with ethyl ester derived from soybean oil. Primary emphasis was placed on providing a dose–response curve for *S. alba*, because this crop has low water and pesticide input requirements and the seed meal remaining after oil expression has potential utility as a bioherbicide (Borek and Morra 2005). The possibility of using both the oil and seed meal as biopesticides increases the likelihood of achieving economic viability for crop production through the marketing of two distinct products. Chemical characteristics of the various oils are provided to determine whether ester chain length and degree of saturation can be used to explain any observed differences in pesticidal activity of the oils.

Materials and Methods

Aphids, plants and cages

Aphids were obtained from a clonal colony established in the Department of Plant, Soil, and

Entomological Sciences at the University of Idaho, Moscow, ID. *Physalis floridana* (Rydberg) plants were provided as a food source for the aphids.

Aphid cages for conducting the bioassays were constructed from 100 × 15 mm clear plastic Fisherbrand Petri dishes (Fisher Scientific, Pittsburgh, PA). To ensure ventilation, 80-mm-diameter holes were cut in the lid and bottom of the dishes, over which cloth mesh mosquito netting was glued. A slot was made in the edge of the lid to enable a leaf petiole to protrude outside each cage, thereby permitting hydration during the bioassay (fig. 1).

Fresh *Physalis* leaves of consistent size were removed from uninfested plants grown in the greenhouse. One fresh-cut leaf was placed inside each aphid cage, with the petiole protruding through the slot in the lid (fig. 1). Aphid-infested leaves were then separated from *Physalis* plants and placed inside the cages in direct contact with the fresh leaves. The Petri dishes were sealed around the edges with clear Scotch® brand tape to prevent insect escape.

The sealed cages were placed in a vertical position on top of cylindrical 250-ml plastic containers filled with tap water, with the petiole of the fresh leaf protruding downward (fig. 1). The cages were left for 48 h during which time the unhydrated aphid-infested leaves wilted and the aphids relocated to the fresh, hydrated leaves in search of a new food source. This incubation period yielded complete relocation of the aphids and took less time and led to lower mortality rates than occurred with manual transfer. The water level in the containers was adjusted as needed throughout 48 h to ensure uninterrupted leaf hydration. After incubation, the wilted leaves were removed, and the aphids on the incubated leaves were counted immediately before spray treatment. When the number of aphids exceeded our ability to make accurate counts in a timely manner or was radically different compared to aphids on other leaves in the same experiment, individuals were removed using a small artist's paint brush. One hundred aphids per leaf were considered ideal and 35 aphids per leaf a minimum. In a few experiments, sample numbers above 300 aphids per leaf were used (maximum 420 aphids per leaf), but this was found an impractical number to count for large numbers of bioassays. The instar of the counted specimen was not identified under the assumption that a range in instars was represented in each assay. The infested leaves were then returned to the cages and hydrated.

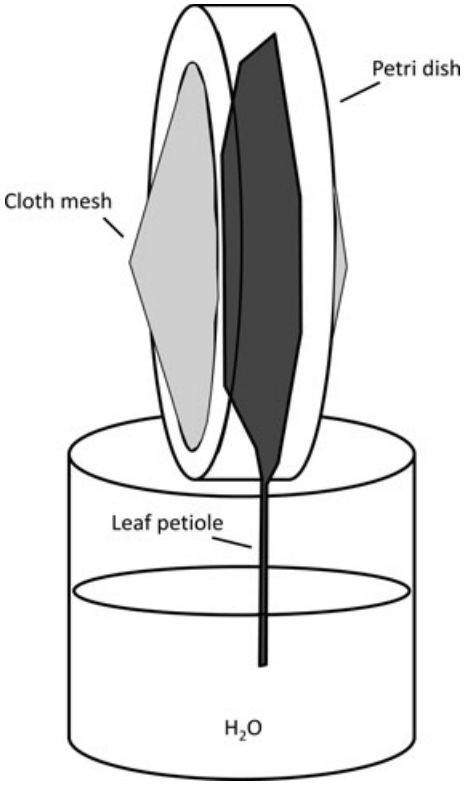

Fig. 1 *Myzus persicae* bioassay showing aphid cages containing a *Physalis* leaf with its petiole immersed in water to maintain hydration.

Spray-delivery system

A commercial 207–345 kPa suction-type Touch-Up Spray Gun (Buffalo Tools, St. Louis, MO) with a 237-ml cup volume and a 1.5-mm nozzle was used as the spray-delivery system. The sprayer was mounted to a ring stand and connected to a regulated air compressor. At a pressure of 70 kPa, the spray system was found to deliver a spray mist that minimized aphid mortality. The mean aphid mortality after 24 h in controls sprayed with pure de-ionized (DI) water at 70 kPa and a distance of 40 cm remained approximately 20% throughout the experiments.

We sprayed N, N-dimethyl formamide on oil-sensitive paper (Syngenta, Basel, Switzerland) to estimate the size and distribution of spray droplets. Stereoscopic examination of the recorded spray patterns yielded a droplet size range of 13 μm–0.31 mm, with the distribution strongly skewed to the lower tail of the range.

Spray was administered in quantifiable amounts by pushing the trigger of the spray gun a number of times in rapid succession to obtain the desired oil loading rate per unit area. For each experiment, the sprayer was calibrated by weighing the amount of

water delivered per unit area to a piece of filter paper (Whatman 41, 11 cm diameter).

Throughout all experiments, the treatment volume to be delivered was selected to ensure wetting of the leaf to the point of dripping. Based on the combined calibration data collected from the 18 separate experiments, we modelled the 95% confidence interval for total spray deposition as 1.69 ± 0.54 mg H_2O/cm^2. From this estimation of the treatment volume, we calculated the amount of emulsified ester deposited and expressed it in units of $\mu g/cm^2$.

Fatty acid profiles of esterified oils

Fatty acid content of the esterified oils used in the study was determined using gas chromatography as described by Hammond (1991). For each ethylated ester, a 100-μl sample was added to 5 ml of hexane contained in a 15-ml test tube. A subsample from this tube was injected into a Hewlett–Packard Model 5890 Series II gas chromatograph (Hewlett–Packard Company, Palo Alto, CA) equipped with a split injection port set to achieve a 100 : 1 split ratio, a J and W 30 m $\times$ 0.25 mm I.D. DB-23 column (0.25 μm film thickness) and a flame ionization detector. Injection-port temperature was maintained at 250°C and detector temperature at 300°C. An initial oven temperature of 215°C was held for 3 min, then increased at a rate of 3°C/min until a final oven temperature of 230°C was reached. Helium at a flow rate of 1 ml/min was used as a carrier gas, with N_2 makeup gas being supplied to the detector at 30 ml/min. The area percentages of the fatty acid ethyl esters were quantified using a Hewlett-Packard 3396 Series II integrator (Hewlett-Packard Company), and the amount of each major fatty acid ethyl ester was expressed as an area percentage of total ethyl esters for triplicate analyses.

Surfactant

In collaboration with Wilbur-Ellis Company (San Francisco, CA), a proprietary surfactant was selected for its appropriate performance as an emulsifier of the ethyl esters in water (Surfactant 8577-76C, Experimental; Huntsman Petrochemical Corporation, The Woodlands, TX). The average CMC of triplicate evaluations was found to be 198 mg/L using a Fisher Scientific Surface Tensiomat 21.

Control efficiency of *S. alba* ethyl ester

Spray emulsions were obtained by mixing *S. alba* ethyl ester with 5% (by weight) of surfactant. The mixture was then pipetted into 50-ml volumetric flasks that were subsequently filled with de-ionized water to obtain treatment doses of 0.13%, 0.25%, 0.5%, 1.0%, 1.5%, 1.75%, 2.0%, 2.5%, 2.75%, 3.0%, 3.25%, 3.5%, 4.0% and 8.0%. A de-ionized water spray was used as a control. Aphid mortality was assessed 24 h after treatment by visual counting under a stereoscope as described later. Seven separate dose–response experiments were conducted to span the range of 14 doses required for model development. Each experiment included from three to eight doses of the total 14 to ensure that sufficient data were obtained for each dose to yield the desired level of confidence. DI water served as the control, and each treatment was performed in duplicate by spraying two leaves with the same dose. Duplicate leaves were paired to obtain similar numbers of aphids. A dose–response relationship for the control efficiency of *S. alba* ethyl ester was modelled by nonlinear regression based on the combined mortality data from the seven experiments using SAS® software and the PROC NLMIXED procedure (SAS Institute 2005). Each dose–response was quantified using five to seven replicates, and a total of 85 points were used for model development.

Spray application

At the time of spray application, each aphid-infested leaf was removed from the cage and held by the petiole 40 cm from the nozzle orifice in front of a flat steel mesh screen to support the leaf during spray application. A Whatman 41 filter paper (11 cm diameter) was placed behind the leaf in front of the screen to provide an even surface and ensure a dry background without contamination from previous dose treatments.

Assessment of aphid mortality

Immediately following spray application, leaves were returned to their respective numbered cages with their petioles protruding from the slot in the edge of the Petri dish. Cotton was inserted between the slot and the petiole to seal the exit, and the Petri dishes were sealed around the edges with Parafilm to prevent aphid escape. The sealed cages were then placed perpendicular with respect to the top of the plastic water containers and taped into position (fig. 1). The cages were left for 24 h and the water level adjusted as needed throughout the incubation to ensure hydration of the leaves.

Twenty-four hours after spray application, the leaves were removed from the cages and placed on a glass Petri dish under a stereoscope. Live and dead aphids were visually counted. Aphids were considered dead if they were in an obvious state of dissolution or showed signs of discoloration (darkening). Aphids that were not moving after prodding with a set of forceps were considered dead or moribund. Mortality was defined as the fraction of dead and moribund aphids observed after 24 h of the total count of aphids at 24 h. We did not observe large population changes between the initial (0 h) number of aphids and the 24-h total count in untreated controls. We concluded that a 24-h post-treatment period was long enough to ensure aphid death but short enough to avoid major reproductive population change. Replications in which the total number of aphids counted (dead and moribund + living) was <85% of the total observed at 0 h were eliminated from the analysis.

Comparing control efficiency of Brassicaceae and soybean ethyl esters

The control efficiencies of Brassicaceae and soybean ethyl esters were compared by applying treatments consisting of *S. alba*, *B. juncea*, *B. napus* or *G. max* ethyl esters in separate emulsions at the same concentration as that determined for the LD_{50} of the ethyl ester of *S. alba* ($0.75 \pm 0.018\%$, 95% confidence interval for ester by volume of final spray emulsion), or 18.2 ± 0.87 $\mu g/cm^2$ wet deposit of ester; $P < 0.0001$). A DI water treatment was used as a control. All emulsions were prepared and applied to duplicate leaves infested with aphids, and mortality was assessed by visual counting under a stereoscope after 24 h as previously described. The results were evaluated by one-way ANOVA ($\alpha = 0.05$) using SAS® software and the PROC GLM procedure (SAS Institute 2005). The experiment was repeated twice, and all four replicates were used in the statistical analyses.

Results

Fatty acids in the ethylated oils

The most prominent difference in fatty acids was the long chain fatty acids containing more than 20 carbon atoms as found in the two ethylated mustard oils from *S. alba* and *B. juncea* (table 1). Approximately 30% of the fatty acids in these two ethylated oils were 22:1, whereas the longest fatty acids in *B. napus* contained 20 carbon atoms and those in *G. max* oils contained only 18.

Control efficiency of *S. alba* ethyl ester on green peach aphid

Figure 2 shows a dose–response relationship for green peach aphid sprayed with emulsified *S. alba* ethyl ester (model $P < 0.0001$). Aphid mortalities ranging from 4% to 100% were observed. It is possible that instar differences contributed to some of the variability observed for replicates treated with the same ethyl ester dose. Mean control mortality was $20 \pm 7\%$ (one standard error about the mean). Significant population changes for untreated aphids on leaves in bioassay cages incubated as previously described were not found after 24 h, indicating that both mortality and reproduction in untreated controls remained near 0% for 24 h. The likely reason for 20% baseline mortality was kinetic energy of the delivered spray droplets. As a result, lower-tail data from the dose–response distribution could not be obtained, a typical sigmoidal relationship was not observed and only the linear and upper-tail portions were modelled (fig. 2).

The PROC NLMIXED maximum-likelihood simulation algorithm was used instead of a transformation-based model such as Probit, because PROC NLMIXED relies on fewer initial parametric assumptions than the traditional transformation models and more accurately simulates the upper and lower tails of the relationship. The LD_{50} for the *S. alba* ethyl ester on

Table 1 Fatty acid content of ethyl esters of oils from the four tested plant species expressed as a peak-area percentage of total fatty acid content

	Fatty acid chain length/double bonds										
Species	14 : 0	16 : 0	18 : 0	18 : 1	18 : 2	18 : 3	20 : 1	22 : 0	22 : 1	24 : 1	NI[1]
Sinapis alba	ND	2.4	4.5	24.4	8.1	7.3	9.7	ND[2]	30.2	ND	ND
Brassica napus	ND	3.7	8.9	55.4	16.8	7.8	1.1	ND	ND	ND	2.3
Brassica juncea	ND	2.5	4.1	24.4	8.4	7.9	9.6	4.4	29.7	2.0	1.1
Glycine max	1.3	9.4	6.5	20.2	46.9	6.2	ND	ND	ND	ND	6.3

[1]Not identified (NI) using gas chromatography.
[2]Fatty acid was not detected (ND).

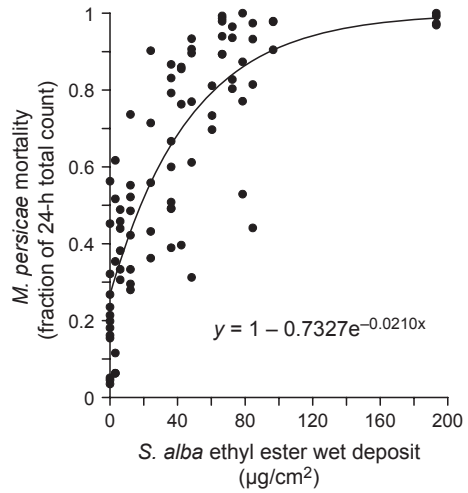

Fig. 2 Dose–response relationship of emulsified *S. alba* ethyl ester applied as a spray to *M. persicae* (model $P < 0.0001$).

M. persicae was found to be 0.75 ± 0.018% (95% confidence interval for ester by volume of final spray emulsion) or 18.2 ± 0.87 µg ester per cm^2 ($P < 0.0001$). The LD_{95} was 5.28 ± 0.104% (95% confidence interval for ester by volume of final spray emulsion) or 128.1 ± 5.10 µg ester per cm^2 ($P < 0.0001$).

Comparing control efficiency of Brassicaceae and soybean ethyl esters

Compared control efficiencies of *S. alba*, *B. juncea*, *B. napus* and *G. max* ethyl esters are shown in fig. 3. Analysis of the data using a one-way ANOVA yielded a $P < 0.0001$ and an $F_{4,4} = 9.63$. Mean mortalities (24-h post-treatment) with one standard error about the mean for the *S. alba*, *B. juncea*, *B. napus* and *G. max* esters were 67.0 ± 10.0%, 69.1 ± 3.2%, 67.9 ± 8.7% and 71.9 ± 6.7%, respectively. A control mortality of 20.3 ± 3.9% was observed. No significant difference was found in the mean aphid-mortality response to Brassicaceae and soybean ethyl ester treatments using Fisher's LSD *t* tests ($\alpha = 0.05$; eight replications).

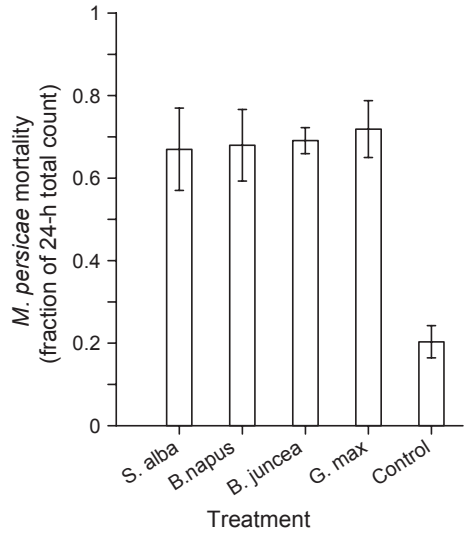

Fig. 3 Mean ± SEM of mortality resulting from spray application of emulsified ethyl esters to *M. persicae*.

Discussion

The LD_{50} for *S. alba* ethyl ester on *M. persicae* of 18.2 ± 0.87 µg/cm^2 and the LD_{95} of 128.1 ± 5.10 µg/cm^2 were obtained using a numerical maximum-likelihood simulation procedure. In contrast to transformation-based models, a numerical maximum-likelihood simulation procedure like PROC NLMIXED allows for flexibility in the response distribution (Rainbolt et al. 2005). Because it relies on maximum-likelihood regression rather than transformation and fitting to a given distribution, this procedure also gives a more accurate approximation of the upper tail of the relationship, which is more difficult to model than the linear range. For these reasons, the PROC NLMIXED procedure was found appropriate for the present study where the lower tail of the relationship is missing in the data (fig. 1), and we are interested in obtaining an LD_{50} as well as information on the effect of doses near or at LD_{95} to compare our results with previously published studies.

Herron et al. (1998a) modelled the dose–response of *M. persicae* treated by spray application with three commercial mineral-oil formulations: C20 Total Citrole, C21 Caltex Lovis and C23 Ampol D-C-Tron NR. Treatments were applied using a Potter spray tower calibrated to give an aqueous deposit of 9.93 ± 0.31 mg/cm^2 at 47 kPa. Based on Probit analysis, the authors reported LC_{50} and LC_{95} values, respectively, of 29 and 107 µg oil per cm^2 for the C20 formulation; 24 and 147 µg oil per cm^2 for the C21 formulation; and 11 and 87 µg oil per cm^2 for the C23 formulation. Thus, the control efficiency of the ethyl esters in our study was found to be of the same order of magnitude as those reported for petroleum sprays applied to the same target organism with a relatively similar application procedure (the water deposit in our present study was lower; however, 1.69 ± 0.54 mg/cm^2 as compared to 9.93 ± 0.31 mg/cm^2). This indicates that insecticidal spray oils derived from *S. alba* should be equally effective as traditional mineral oils for controlling aphids.

It should be noted that cross-comparison between modelled dose–response relationships is problematic for several reasons. Firstly, control efficiency (LD_{50}, LD_{95}) is system specific and not universally applicable in situations that are non-identical to the given experimental conditions of the study. A dose–response relationship obtained by experimentation in a laboratory is also likely to differ from a relationship modelled for the same toxicant and target organism based on a field study.

Secondly, there are a number of procedures commonly used to model dose–response relationships. Transformation-based procedures (Probit, Logit) became popular in medical and agricultural research before the advent of high-capacity computers that easily facilitate numerically intensive simulations (Streibig et al. 1993). With the computational power available today, iteration using a maximum-likelihood algorithm can yield a dose–response relationship within seconds without reliance on distribution assumptions. This can be a great advantage, especially in a situation with incomplete data to cover the response range such as in the present study. However, the majority of published dose–response studies rely on the older procedures that are still routinely used in many academic and industrial fields. These limitations should be considered when making comparisons of dose–response data obtained from different studies.

We found emulsified ethyl esters derived from the seed oils of *S. alba*, *B. juncea* and *B. napus* to be equally efficacious as ethyl ester of soybean that dominates the market of plant-oil sprays. With mean mortality responses ranging from $67.0 \pm 10.0\%$ to $71.9 \pm 6.7\%$ at an application rate of 0.75% (by volume of final emulsion), all four ester emulsions applied were effective control agents of *M. persicae*. We did not observe differential efficacy among the Brassicaceae and *G. max* esters used in our study, despite the presence of longer chain fatty acids (C22) in ethylated oils of *S. alba* and *B. juncea* (table 1). This is significant given that unsaturated fatty acids with chain lengths of greater than C20 do not exist in any of the major botanical oils used for pest control (Sams and Deyton 2002).

Brassica napus oil is the only Brassicaceae seed oil previously investigated for pesticidal performance (Sams and Deyton 2002; Martin et al. 2004; Martín-López et al. 2006; Marcic et al. 2009; Wrobel 2012). Beattie et al. (2002) evaluated two spray adjuvants based on *B. napus* oil (Codacide® and Fasta®) for control of citrus leafminer [*Phyllocnistis citrella* (Stainton)]. Both products were found efficient as control agents, but less effective than the *n*C24 horticultural mineral oil Caltex D-C-Tron Plus®. Both *B. napus* and *G. max* oil have been found effective for control of powdery mildew (*Microsphaera* sp.) (Northover and Schneider 1996; Pasini et al. 1997). Leeson (2002) achieved highly effective control of green peach aphid using Eco-oil®, an emulsion containing *B. napus* oil and essential oils of *Eucalyptus* spp. and *Melaleuca alternifolia*. Our findings support the conclusion that *B. napus* ethyl ester is an effective control agent for *M. persicae* and that oils from *B. juncea* and *S. alba* are equally effective as *B. napus* ethyl ester.

A number of different modes of action may have been responsible for aphid death in our experiments including anoxia leading to asphyxiation, fumigant action from volatile components, nervous system disruption, cellular disruption resulting from contact and desiccation (Taverner 2002). The relative importance of each mode of action remains poorly understood, although research on this topic has concerned researchers for almost a century (Taverner 2002; Najar-Rodriguez et al. 2008, 2007a,b; Stadler and Buteler 2009; Martoub et al. 2011). At least a portion of the confusion is caused by the differing chemical and physical characteristics of the oils, and the relationship of these differences to pesticidal activity (Agnello 2002; Nicetic et al. 2011). For example, whether the oils are dominated by aliphatic, aromatic, saturated or unsaturated hydrocarbons will impact such variables as oil volatility and viscosity, which in turn control the mode of action.

We can use the oils' chemistry to propose the least likely modes of action taking place in our bioassays. Fumigation in the case of mineral oils occurs only with oils containing relatively volatile chemical constituents having carbon chain lengths <10 (Taverner 2002). The shortest chain esters in our formulations were C14, and the dominant chain lengths were C18 to C22 (table 1). It is therefore unlikely that a fumigant effect was responsible for observed aphid toxicity. In addition, anoxia is promoted by oils having viscosities of >50 Saybolt universal seconds (SUS) (Taverner 2002). Ethyl and methyl esters of rapeseed oil and mustard oils are reported to have viscosities of approximately 42–46 SUS (University of Idaho 2011; Hoekman et al. 2012), thus making them lower in viscosity and less effective in blocking spiracles. Especially in treatments with high ester concentrations in the spray, discoloration and/or dissolution of aphid tissue was visibly present. In some severe cases (all in the higher ester–dose range), the bodies of individual aphids were hard to distinguish visually because of their progressed stage of dissolution. This might be explained by the solubilization of membrane lipids

(Taverner 2002; Stadler and Buteler 2009), but whether this was the cause of toxicity or a secondary effect is unknown. Although fumigation and anoxia are unlikely, further discussion on the modes of action is beyond the scope of this study. We recommend that future research should include detailed chemical data that will allow any observed differences in toxicity to be linked or correlated with oil chemistry. This is especially important with respect to plant-derived oils given that most of what we know about modes of action is based on studies with mineral oils that have a much more complicated mixture of hydrocarbons (Agnello 2002).

The equivalent efficacies among ethyl esters of oils from all three Brassicaceae species and *G. max* ethyl ester demonstrate that oils derived from mustard and rapeseed are potentially useful feedstocks for the production of insecticidal sprays. This is the first evidence indicating the comparable utility of ethylated oils from *S. alba* and *B. juncea* with those from *B. napus* and *G. max*. A major advantage of using ethylated mustard oils is the lower requirement for the use of pesticides in growing the respective crops and the reduced need for plant-available water as compared to that required for growing both *B. napus* and *G. max* (Brown et al. 1997; Ross et al. 2008). Decreased production inputs offer ecological advantages, especially attractive for an oil-based insecticide targeting lower input or organic agriculture. Additional testing of ethylated mustard oils on other insect pests is warranted.

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