Physical Modes of Action of Petroleum and Plant Oils on Powdery and Downy Mildews of Grapevines

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ABSTRACT

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Three petroleum (mineral) oils and two glyceridic plant oils, applied as emulsions (1% vol/vol) in water, were compared with reference materials for the control of powdery mildew on potted vines of *Vitis vinifera* cv. Chardonnay under greenhouse conditions. The same materials were tested at 15 liters of oil/1,500 liters of water/ha in five-application programs in vineyards of *V. vinifera* × *labrusca* cvs. New York Muscat and Canada Muscat. In a series of greenhouse experiments, the petroleum oils provided moderate protection, excellent pre-lesion and post-lesion curative action, and were antisporulative. The plant oils showed significant action only in pre-lesion treatments and as antisporulants in treatments applied to established lesions. In the vineyard, the petroleum oils, Stylet-Oil, Sunspray UFO, and Safe-T-Side, were as effective as myclobutanil (Nova) in suppressing powdery mildew. Canola and soybean plant oils, emulsified with Agral 90, were no better than Agral 90 alone, and reduced the incidence of disease only marginally in comparison with a water check. Potted Chardonnay vines were inoculated with downy mildew and treated one day pre- or one day postinoculation with Sunspray UFO, safflower plant oil, mancozeb, or water. Only the preinoculation application of mancozeb reduced the incidence of downy mildew. Three petroleum oils and two plant oils were inefficacious against downy mildew in vineyard experiments.

Additional keywords: *Oidium tuckeri, Plasmopara viticola, Uncinula necator*

Powdery mildew caused by *Oidium tuckeri* Berk. (teleomorph *Uncinula necator* (Schwein.) Burrill and downy mildew caused by the Oomycete *Plasmopara viticola* (Berk. & M. A. Curtis) Berl. & De Toni in Sacc. are potentially serious diseases (24) of many grape cultivars in most grape-growing regions, including southern Ontario, Canada. In certain regions of the world, multiple applications of fungicides per growing season to control powdery mildew have resulted in the selection of fungal populations resistant to the benzimidazole and DMI groups of fungicides (11,25,30). The risk of fungicide resistance and the public concern over the use of pesticides on food and beverage crops prompted our interest in the fungicidal efficacy of highly refined petroleum and plant oils (20–23,29). Oils might be used as alternatives to conventional fungicides, and integrated into programs including other necessary materials, thereby reducing the frequency of use of and the risk of resistance to all fungicide groups in the program.

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Calpouzos reviewed the early use of oils as fungicides, principal among which was the use of petroleum oil to control sigatoka disease (*Mycosphaerella musicola)* of banana (2–4). Multiple-application programs of petroleum oils have been effective against powdery mildew diseases of rose (13,18), hop (16), lilac (8), euonymus (34), cucumber (35) and grape (6,19,25, 26). Repeated applications of plant oils have controlled powdery mildew diseases of hydrangea (15), hop (16,17), and apple (22). In our earlier studies, both petroleum and plant oils were efficacious against powdery mildew but not against downy mildew of grapevine (23). Previously, we showed that several plant oils had no protective activity against spinach white rust caused by the Oomycete *Albugo occidentalis* (22). However, our results differ from those of reports on the efficacy of a petroleum oil against grapevine downy mildew (5) and of emulsified plant oils against tobacco blue mold (7) caused by the Oomycete *Peronospora tabacina.*

The knowledge of a fungicide's physical mode of action permits it to be used to greatest advantage in relation to the time of inoculation, infection, lesion appearance, and sporulation, and takes into consideration fungicide movement and residual activity (32). Materials that prevent disease when applied before inoculation and infection are termed protective (prophylactic). Those that suppress an infection before it becomes visible are referred to as having pre-lesion or pre-symptomatic curative (therapeutic) action. A third mode is the deactivation of a visible infection, and is called post-lesion or post-symptomatic curative (therapeutic) action. The reduction of spore production, and hence disease spread, is a fourth mode of action. This antisporulative action may result from curative treatments made either before or after lesion appearance. These four physical modes of action describe the mycological actions of oils and of reference fungicides.

The present studies were initiated in 1992 following our demonstration of the protective and the pre- and post-lesion curative action of plant oils on apple powdery mildew (*Podosphaera leucotricha*) under greenhouse conditions (22). The objective was to define the physical modes of action of petroleum oils against grapevine powdery mildew and downy mildew diseases since these have not been previously described. These data would complement efficacy data of multi-spray, season-long vineyard programs. Furthermore, the modes of action and vineyard efficacy of plant glyceridic oils against both diseases do not appear to have been reported previously, except for our preliminary communication (23). A knowledge of the protective, curative, and antisporulative activity of oils would allow the development of a disease prevention program with application intervals based on these characteristics.

MATERIALS AND METHODS

Spray materials and application methods. Four petroleum oils, three plant oils, one surfactant, and two standard fungicides were examined. The petroleum oils were (i) Stylet-Oil (JMS Flower Farms Inc., Vero Beach, FL), (ii) Sunspray UFO (Sun Oil Co., Marcus Hook, PA), (iii) Safe-T-Side (Grove Trees) (Brandt Chemical, Pleasant Plains, IL), and (iv) Light Mineral Oil (Stanley Pharmaceuticals Ltd., Vancouver, BC, Canada). Selected physical characteristics of these oils are given in Table 1. The plant oils were canola oil, safflower oil, and soybean oil (Daminco Inc., Mississauga, ON, Canada). They were refined, bleached, and decolorized, and were of food grade quality with a bland flavor. They were supplied under nitrogen, without additives, with a maximum peroxide value of 0.5 to 1.0 meq/kg

and maximum free acid of 0.05 to 0.50% (oleic acid equivalent). The oleic and linoleic compositions of canola, safflower, and soybean oils were 58 and 26%, 13 and 78%, and 24 and 54%, respectively (22). The surfactant was Agral 90 (90% nonylphenoxy polyethoxy ethanol, Zeneca, Stoney Creek, ON, Canada), and the fungicides were Nova 40W (myclobutanil) and Dithane 80DF (mancozeb) (Rohm and Haas, Mississauga, ON, Canada). Canola and soybean oils were mixed with Agral 90 in a ratio of 97.5:2.5 (vol/vol). This permitted easy emulsification comparable to that of the three formulated petroleum oils (i–iii). Light Mineral Oil and safflower oil were mechanically emulsified with a homogenizer (Brinkmann Instruments, Rexdale, ON, Canada) and sprayed within 4 min.

For greenhouse experiments, sprays were applied with a compressed air paint sprayer gun (DeVilbiss, Barrie, ON, Canada) as a fine spray deposit of noncoalescing spray droplets on both surfaces of each of the nine youngest, unfolded leaves of each potted vine. In the vineyard experiments, sprays were applied with a custom-built, eight-nozzle, over-row hooded boom, hydraulic sprayer (Rittenhouse and Sons Ltd., St. Catharines, ON, Canada) driven at 5 km/h that delivered 1,500 liters/ha at a line pressure of 2,400 kPa.

Greenhouse studies. Experiments were conducted in greenhouse compartments at mean temperatures of 20 to 25°C, 40 to 60% relative humidity (RH) and a 16-h photoperiod. Vines of *V. vinifera* L. cv. Chardonnay budded on 3309 rootstock from commercial nurseries were grown in potting mix in clay pots 18 cm in diameter. Vines were thinned to a single shoot and staked for support. For each experiment, 35 to 40 vines were used with 4 to 5 vines serving as replicates of each treatment, and arranged in a completely randomized design on greenhouse benches. Vines were used when the shoot had at least nine unfolded leaves. After an experiment, vines were grown to about 1.3 m in height, cut back to six basal buds after leaf fall, overwintered, and reused.

Dry inoculations of groups of potted vines with powdery mildew conidia were conducted in a room maintained at >90% RH, 22 to 24°C, and continuous light (PAR 15 $\mu E\ s^{-1}\ m^{-2}$) from two 40W fluorescent tubes, for 24 h. A group of 35 to 40 vines was placed with non-overlapping leaves in a circle of radius 70 cm. Four 2.5 × 7.5 cm glass microscope slides, lightly covered with white petroleum jelly (Vaseline, Chesebrough-Ponds, Markham, ON, Canada), were supported at the same height as the leaves to be inoculated, to monitor the desired spore deposition of 100 to 200 conidia/cm^2. Pieces of dry flat dialysis tubing were attached to four additional slides with Vaseline, and similarly placed in the vine canopy. These were removed at the end of the 24 h postinoculation incubation period and stained with cotton blue in lactophenol to determine the percentage of conidial germination. To inoculate 40 plants, 5 to 10 leaves heavily mildewed with 2- to 3-week-old lesions were used. Inoculum leaves were individually held over the vines and conidia were dislodged and scattered as showers by means of a fine jet of high velocity air directed from the narrow aperture of a Pasteur pipette attached to a 3-m-long flexible hose. This technique gave good reproducibility of infection of the upper (adaxial) leaf surface.

Chardonnay vines were inoculated with *P. viticola* from leaves with downy mildew collected from a vineyard of *V. vinifera* cv. Chardonnay. Inoculum leaves were incubated overnight at 22°C in darkness in plastic bags containing wet paper toweling. The following day, sporangia were removed using a sterile water spray from a glass atomizer. The suspension was coarse filtered and the density of sporangia was determined with a hemacytometer and adjusted to a concentration of 1×10^5 sporangia ml^{-1}. The inoculum was cooled in a bath of melting ice until required. The lower (abaxial) surfaces of the five youngest unfolded leaves of each vine were inoculated with a fine spray of sporangial suspension using a glass atomizer. The proportion of sporangia that had released zoospores increased from 0% at the time of inoculation (0.5 h after initial inoculum preparation), to 30 and 86% after incubation for 0.5 and 6.0 h at 22°C, respectively, indicating a high degree of sporangial viability. The inoculated plants were incubated in darkness at 22°C for 24 h in a room misted with a domestic ultrasonic humidifier (Bionaire, Inc., Montréal, QC, Canada), and returned to a greenhouse bench.

Each of six greenhouse experiments was conducted at least twice, indicated as A, B, etc., with experiments 1 to 5 involving powdery mildew and experiment 6 being concerned with downy mildew. In the initial protective experiments 1A and 1B, oil treatments were applied 1 day before conidial inoculation. For the pre-lesion curative experiments 2A and 2B, the treatments were applied 3 days after inoculation, corresponding to an interval of 3 days before the initial appearance of powdery mildew lesions. For the post-lesion curative experiments 4A and 4B, vines were inoculated and incubated for 10 days, at which time powdery mildew severity was assessed. The oil and fungicide treatments were applied immediately after this pre-spray assessment. Seven days after treatment, disease severity was assessed again and treatment effects determined by covariance analysis of the pre- and post-spray disease ratings. The effects of post-lesion treatments on the conidial density on the upper surface of leaves were determined by rinsing conidia with 0.05% Tween 20, sprayed from a glass atomizer. The third to seventh youngest unfolded, inoculated leaves on each vine were rinsed and the weight of the resulting conidial suspension determined, and a 20-ml aliquot preserved. The conidial suspensions were concentrated 10-fold by centrifugation of 10 ml (relative centrifugal force [RCF] = 1,800 × *g* for 5 min.) using a Sorvall GLC-1 (Sorvall Inc., Newtown, CT) and resuspension of the conidial pellet in 1 ml of water, before counting with a hemacytometer. The five leaves on each vine from which the conidia were rinsed were detached and their laminal area determined using an electronic area meter (Delta-T Devices Ltd., Cambridge, UK). The conidial recovery data were expressed as conidia/cm^2 of upper leaf surface, and after conversion to natural logarithms were analyzed for treatment differences.

To examine the effects of oil on the mycelia and conidia of *O. tuckeri*, heavily mildewed grapevine leaves were sprayed with 1% (vol/vol) emulsion of Stylet-Oil in water. One day later, several 1-cm-diameter leaf disks were punched from treated leaves and also from heavily infested but nontreated leaves. Fresh leaf disks were mounted on stubs with two-sided tape and, without fixation or coating, were inserted into a Hitachi S-570 scanning electron microscope (SEM) and examined immediately.

Table 1. Selected physical characteristics of four petroleum oils, in their unformulated and formulated conditions.

	Proprietary name of formulated petroleum oil			
	Stylet-Oil	**Sunspray UFO**	**Safe-T-Side**	**Light mineral oil**
Unformulated oil				
Unsulfonated residue (%)	99	92	92	ca. 100
Specific gravity	0.85	0.86	0.86	0.84
Boiling point, mid range at 10 mmHg,°F(°C)	435 (224)	414 (212)	435 (224)	508 (264)
Formulated oil				
Oil content (%)	97.1	98.8	80.0	100[z]
Specific gravity	0.86	0.87	0.89	0.84
Color	Colorless	Light straw	White	Colorless
Odor	Odorless	Odorless	Ammonia	Odorless

[z] Light mineral oil contained no additives.

For experiments 1, 2, and 4, eight treatments were applied consisting of three formulated petroleum oils, Stylet-Oil, Sunspray UFO, Safe-T-Side, two plant oils, canola and soybean, that were mixed with Agral 90, Nova, Agral 90 alone, and a water check. Each oil was used at a concentration of 1% (vol/vol) in water.

A more detailed examination of the protective and pre-lesion curative action of petroleum oils was conducted in experiment 3. The protective action of Sunspray UFO applied 7 days, 4 days, and 1 day before inoculation (experiment 3A) and its pre-lesion curative action when applied 1, 3, and 5 days after inoculation (experiment 3B), were determined in single experiments. These were followed by experiments 3C and 3D, in which Stylet-Oil was applied 3 days, 2 days, and 1 day before inoculation and 1, 3, and 5 days after inoculation. Initial lesion appearance occurred 6 days after inoculation.

The petroleum oil and surfactant system that composed Stylet-Oil were not available separately for determination of their individual curative properties. Accordingly, a nonformulated pharmaceutical grade of light mineral oil was compared with Stylet-Oil, for pre-lesion curative action, when applied 5 days after inoculation, equivalent to 1 day prior to initial lesion appearance. The light mineral oil was mechanically emulsified. Experiments 5A and 5B were conducted using oils prepared at concentrations of 0.25, 0.50, 0.75 and 1.00% (vol/vol) in low conductivity water, emulsified for 1 min. and applied within 2 to 4 min. A water check was included for comparison.

Experiment 6 examined the 1-day protective and 1-day postinoculation pre-lesion curative action against downy mildew of Sunspray UFO, safflower oil (mechanically emulsified), and Dithane, compared with a water check. Chardonnay vines were inoculated with *P. viticola* from a Chardonnay vineyard, as described above. Three vines served as replicates of each treatment and were arranged in a completely randomized design. Disease severity was assessed 12 days after inoculation by means of the rating scale of 0 to 9 described below. Two preliminary experiments are not described here.

Vineyard studies. Two vineyard experiments were conducted to evaluate the efficacy of full-season programs of oils against powdery mildew. Experiments 7A and 7B were conducted on *V. vinifera* × *labrusca* L. cvs. New York Muscat and Canada Muscat, respectively. Row spacing was 3.3 m in underdrained Morley silty clay loam in Vineland, ON, and vines were spaced 2.1 m apart with four vines pruned to a six-cane kniffen system in each treatment replicate plot. The five oils and three standard treatments that were tested in greenhouse experiments 1, 2, and 4 were used in these vineyard evaluations, except that Nova could not be accommodated on Canada Muscat. The treatments were replicated and randomized in three complete blocks, oriented north-south, in both experiments. The application dates and the Eichhorn and Lorenz grapevine growth stages (given in brackets) (10) were 13 July [31] (pea-sized berries), 27 July [32], 10 August [33] (bunch closing), 23 August [35] (color change), and 7 September [36]. Powdery mildew was introduced uniformly on infected potted vines, one pot per plot, on 5 August, and the incidence and severity of infections of the vineyard vines were assessed 20 to 23 September. The harvest dates were 26 and 27 September 1994.

Disease evaluation and data analysis. The severity of foliar infection in the greenhouse experiments was assessed 10 to 12 days after inoculation. A rating scheme modified from Horsfall and Cowling (12) consisted of 10 categories. For each rating, the lower and upper percentage limits are given with the percentage equivalent (in parentheses). The percentage equivalent for each rating was obtained by converting the percentage limits into angles, calculating the mean, and back transforming it to a percentage. The 10 categories with their percentage area limits and the percentage equivalents (in parentheses) were as follows: 0, (0%); 1, trace to 0.2% (0.1%); 2, 0.2 to 0.6% (0.4%); 3, 0.6 to 1.8% (1.2%); 4, 1.9 to 5.5% (3.6%); 5, 5.6 to 16.6% (10.5%); 6, 16.7 to 50% (32.2%); 7, 50 to 83.2% (67.8%); 8, 83.3 to 94.4% (89.5%); and 9, 94.5 to 100% (98.4%).

Disease ratings were made of the six or seven youngest, unfolded leaves (adaxial surface), inoculated with *O. tuckeri*, and of the five youngest unfolded leaves (abaxial surface) inoculated with *P. viticola*. Each potted vine constituted a treatment replicate, and the mean ratings for each of the

Table 2. Protective and pre-lesion curative action of three petroleum and two plant oils on powdery mildew caused by *Uncinula necator* of potted Chardonnay vines

		Leaf area covered with powdery mildew (%)[w]			
		Protective[x]		Pre-lesion curative[x]	
Treatment material	Amount/100 liters	Exp. 1A	Exp. 1B	Exp. 2A	Exp. 2B
Stylet-Oil	1.0 liter	21 b[y]	5 b	0.0 a	0.0 a
Sunspray UFO	1.0 liter	20 b	3 b	0.2 a	0.0 a
Safe-T-Side	1.0 liter	19 b	2 b	2.0 a	0.9 a
Canola-oil[z]	1.0 liter	31 c	13 c	0.0 a	0.1 a
Soybean oil[z]	1.0 liter	48 c	10 c	0.4 a	0.1 a
Agral 90	25 ml	49 c	29 d	6.1 b	9.6 b
Nova 40W	13 g	0 a	0 a	0.0 a	0.0 a
Water control	...	57 c	19 c	11.3 c	13.4 c

[w] Evaluated 10 to 11 days after inoculation.
[x] Protective treatments were applied 1 day before inoculation, and pre-lesion treatments 3 days after inoculation equivalent to 3 days before initial lesion appearance.
[y] Values are the arithmetic means of the percentage equivalents of individual leaf ratings of 0 to 9. Means in the same column followed by different letters differ significantly ($P = 0.05$) by Fisher's protected least significant difference.
[z] Canola and soybean oils were mixed with Agral 90 at a ratio of 97.5:2.5 (vol/vol) prior to emulsification in water.

Table 3. Protective and pre-lesion curative action of Sunspray UFO and Stylet-Oil on powdery mildew caused by *Uncinula necator* at differing degrees of disease severity, on potted Chardonnay vines

		Leaf area covered with powdery mildew (%)[y]			
		Sunspray UFO		Stylet-Oil	
Time of treatment (days)[x]		Exp. 3A	Exp. 3B	Exp. 3C	Exp. 3D
Unsprayed		8.1 b[z]	14.0 c	28.0 c	49.4 d
Protective	−7	5.6 ab	...	...	...
	−4	1.6 a	...	...	...
	−3	...	...	8.1 b	6.5 c
	−2	...	...	8.0 b	5.6 bc
	−1	0.4 a	...	4.7 b	2.2 ab
Curative	+1	...	0.5 b	0.2 a	0.5 a
	+3	...	0.1 a	0.5 a	0.2 a
	+5	...	0.0 a	0.9 a	0.1 a

[x] Before and after inoculation. Both petroleum oils were emulsified in water (1%, vol/vol) and applied as a fine spray to both leaf surfaces at the indicated time intervals before (−) or after (+) dry spore inoculation.
[y] Evaluated 10 to 11 days after inoculation. Initial lesion appearance occurred 6 days after inoculation.
[z] Values are the arithmetic means of the percentage equivalents of individual leaf ratings of 0 to 9. Means in the same column followed by different letters differ significantly ($P = 0.05$) by Fisher's protected least significant difference.

vines were analyzed by analysis of variance (ANOVA) followed by Fisher's protected least significant difference (LSD) means separation test (14,31). In the post-lesion curative experiments 4A and 4B, the treatment effects were determined from covariance analyses of the pre-spray and 7 day post-spray data, and the conidial data were transformed to natural logarithms before the analyses. The responses of two oils at four concentrations, excluding the water check, were compared by regression analyses. In the vineyard experiments, 100 leaves per replicate plot were assessed for disease severity, 50 each from the east and west sides of each plot. For all experiments, the treatment means were tabulated as percentages of leaf area covered by aerial mycelium (including conidiophores or sporangiophores) and were calculated from the individual leaf ratings using the percentage equivalents.

RESULTS

In the initial 1-day protective experiments 1A and 1B, the petroleum oils were only moderately effective compared with the excellent activity of Nova (Table 2). The plant oils provided no significant protection against moderate to high levels of infection. In contrast, when applied as 3-day pre-lesion curative treatments, both types of oils were as effective as Nova (experiments 2A and 2B, Table 2). The Agral 90 surfactant showed moderate pre-lesion curative activity but no protective activity (Table 2). In the subsequent and more detailed examination of the action of petroleum oils, both Sunspray UFO and Stylet-Oil gave moderate control when applied protectively between 4 days and 1 day before inoculation (Table 3). Both oils gave excellent suppression of surface mildew when applied as pre-lesion curative treatments 1, 3, and 5 days after inoculation (Table 3), corresponding to intervals of 5 days, 3 days, and 1 day before the initial appearance of lesions.

In the post-lesion and antisporulative experiments 4A and 4B, the petroleum oil treatments immediately smothered the established lesions and greatly reduced the area covered with typically white mildew colonies (Table 4). Canola and soybean oils also showed an appreciable smothering action immediately after treatment, restoring heavily mildewed leaves to a bright green color. However, within 7 days, when the final evaluation was conducted, much of the mildew had reappeared on leaves treated with plant oils, resulting in an intermediate assessment in experiment 4B, and no activity in experiment 4A (Table 4). The plant oils were comparable to the petroleum oils in reducing by more than 96% the numbers of conidia rinsed from the treated leaves (Table 4). Nova and Agral 90 showed no post-lesion curative nor any antisporulative action in these experiments.

Powdery mildew lesions treated with oils lost their white, iridescent appearance and the leaves became bright green with regions of paler mottling corresponding to the more severely diseased areas. Tissue necrosis was limited to minor flecking, which appeared even on noninfected leaves 1 to 2 weeks after treatment. The SEM examination of heavily infected leaves, either untreated or treated with Stylet-Oil, showed that the oil treatment had not reduced appreciably the amount of surface mycelia or conidia. Compared with untreated tissue, oil-treated mycelia and conidia were less collapsed under the desiccating, high vacuum conditions of SEM examination.

In the comparison of light mineral oil with Stylet-Oil in experiments 5A and 5B, the mechanical homogenization of the light mineral oil produced an unstable emulsion, containing a broad size range of oil droplets and numerous air bubbles, when viewed microscopically. The emulsion obtained with Stylet-Oil was very stable with more numerous droplets that were smaller and more uniform in size and with fewer air bubbles. Despite these major differences, the light mineral oil showed pre-lesion curative activity comparable to that of Stylet-Oil at concentrations of 0.5 to 1.0% (vol/vol), against a moderate infection of 27.6% in the water check (experiment 5A, Table 5). In the repeat experiment (5B) the level of infection was appreciably higher at 56.9%, and the mineral oil emulsion was weaker than Stylet-Oil (Table 5).

In experiment 6, *P. viticola* inoculum collected from a Chardonnay vineyard infected the potted Chardonnay leaves and produced abundant sporangiophores and sporangia when incubated under humid conditions, 12 days after inoculation. Saf-

Table 4. Post-lesion curative and antisporulative action of petroleum and plant oils on grapevine powdery mildew cause by *Uncinula necator* of potted Chardonnay vines[w]

		Upper leaf surface area with mildew (%)[x]		Conidial recovery (/cm²) 7 days post-treatment[y]	
Treatment material	Amount/100 liters	Exp. 4A	Exp. 4B	Exp. 4A	Exp. 4B
Stylet-Oil	1.0 liter	0.4 b	1.0 a	10 ab	8 ab
Sunspray UFO	1.0 liter	0.2 a	0.5 a	5 a	5 a
Safe-T-Side	1.0 liter	1.0 c	0.3 a	10 ab	6 ab
Canola oil[z]	1.0 liter	7.4 d	5.5 b	10 ab	14 b
Soybean oil[z]	1.0 liter	6.1 d	6.1 b	20 b	39 b
Agral 90	25 ml	6.0 d	12.7 c	324 c	712 c
Nova 40W	13 g	5.8 d	21.5 c	217 c	1,152 c
Water control	. . .	6.9 d	14.6 c	478 c	1,005 c

[w] Before treatment, the mean upper leaf surface areas covered with powdery mildew in experiments 4A and 4B were 6.4 and 11.7%, respectively.

[x] Evaluated immediately before treatment and 7 days after treatment using a rating scheme of 0 to 9. Treatment differences were analyzed using covariance. Arithmetic means in the same column followed by different letters differ significantly ($P = 0.05$) by Fisher's protected least significant difference, calculated from the percentage equivalents of individual leaf rating, 7 days after treatment.

[y] Conidia were rinsed from leaves 7 days after treatment. Numbers were transformed to natural logarithms and analyzed. Means in the same column followed by different letters differ significantly ($P = 0.05$) by Fisher's protected least significant difference.

[z] Canola and soybean oils were mixed with Agral 90 in a ratio of 97.5:2.5 (vol/vol) prior to emulsification in water.

Table 5. Comparison of Stylet-Oil with light mineral oil for pre-lesion curative action on powdery mildew caused by *Uncinula necator* of potted Chardonnay vines, at two levels of disease severity[w]

	Leaf area covered with powdery mildew (%)[x]			
	Experimet 5A		Experiment 5B	
Concentration of oil (%)	Stylet-Oil	Mineral oil	Stylet-Oil	Mineral oil
1.00	0.0[y]	0.2	0.0	1.1
0.75	0.2	0.5	0.2	3.0
0.50	0.8	0.8	0.5	4.2
0.25	1.8	11.6	4.7	21.7
Water control	27.6	27.6	56.9	56.9
Regression[z]				
Intercept (*Y* axis)	0.208	0.398	−0.032	1.672
Coefficient (slope)	−3.106	−5.161	−4.117	−3.983
R^2	0.943	0.929	0.983	0.882

[w] Oil and water treatments were applied 5 days after inoculation corresponding to 1 day before initial lesion appearance in greenhouse experiments.

[x] Evaluated 10 to 11 days after inoculation.

[y] Values are the arithmetic means of the percentage equivalents of individual leaf ratings of 0 to 9, of the seven youngest unfolded and inoculated leaves on each of four replicate plants per treatment.

[z] Of mean ratings against $\log_{10}$ oil concentration. The mean disease rating for each treatment was regressed against $\log_{10}$ of the oil concentration, excluding the water check.

flower oil and Sunspray UFO showed neither protective nor pre-lesion curative action against grapevine downy mildew and Dithane provided only protection (Table 6).

In the vineyard experiments 7A and 7B, the five-application programs of petroleum oils provided high levels of powdery mildew control, comparable to that of Nova, judged either by the incidence or by the severity of foliar disease (Table 7). The plant oils mixed with Agral 90, and Agral 90 alone showed only intermediate suppression, and powdery mildew worsened rapidly after the final application. This contrasted with the slow development of powdery mildew in the plots treated with petroleum oils and Nova, despite their close proximity to heavily mildewed plots. A severe and progressive development of downy mildew occurred in August and September during the course of the treatments in both experiments. There was no obvious suppression of the disease by any of the oil treatments compared with the water check, so no detailed assessment was made.

DISCUSSION

Under greenhouse conditions, petroleum oils showed moderate protective action, when applied as early as 4 days prior to conidial inoculation. However, the emulsified plant oils and Agral 90 were ineffective. This contrasted with the high degree of protective action of six plant oils in our earlier greenhouse studies with apple powdery mildew (22). Locke (15) showed a 1-day protective action of formulated neem plant oil. Against sigatoka disease of banana, petroleum oils were considered to have no protective action, and had no effect on spore germination and leaf penetration (2–4). However, Ziv and Zitter (35) showed that Sunspray UFO gave appreciable protective action against powdery mildew of cucurbits.

Initial lesion appearance occurred in the greenhouse 6 days after inoculation with maximum lesion expression occurring after 10 to 11 days. In our studies, both petroleum and plant oils showed excellent pre-lesion curative action when applied 3 days after inoculation, equivalent to 3 days pre-lesion curative action. Stylet-Oil and Sunspray UFO were comparably effective when applied 5 days, 3 days, and even 1 day before lesion appearance, equivalent to 17, 50, and 83% of the elapsed pre-lesion incubation time. Petroleum oil applied to banana suppressed lesion development when applied after 32 days (80%) of a 40-day pre-lesion incubation period (2). Also, in our earlier work with apple powdery mildew, mechanically emulsified canola oil showed a high level of pre-lesion curative action when applied up to 4 days postinoculation, equivalent to 3 days prior to lesion appearance (22), corresponding to 57% of the elapsed pre-lesion incubation time.

As single post-lesion applications, the petroleum oils and plant oils smothered the mildew lesions, obliterating them and restoring the foliage to a bright green color with pale mottled areas corresponding to large mildew lesions. This effect was more persistent with mineral oils than with the plant oils, so that 7 days after treatment, the petroleum oils appeared markedly superior. Martin and Salmon (16,17) showed that a number of emulsified plant oils exhibited post-lesion curative action against hop powdery mildew (*Sphaerotheca fuliginea*), but that paraffinic (mineral) oils were less effective and needed to be used at higher concentrations for an adequate curative action. The post-lesion curative action of canola oil was more evident against apple powdery mildew (22) than against grapevine powdery mildew.

The post-lesion application of both petroleum and plant oils greatly reduced the number of conidia rinsed from leaves 7 days after treatment. This was interpreted as an antisporulative mode of action, which was also described briefly by McWhorter (18) for Volck petroleum oil applied to rose powdery mildew, and by Wilson (33) for petroleum oil applied to *Septoria apii* of celery. However, petroleum oils were not regarded as antisporulants on sigatoka disease of banana (4).

The SEM examination showed that the Stylet-Oil treatment had not removed nor disrupted the fungal mycelia and conidia. The loss of the white reflective appearance of superficial fungal tissue was attributed therefore to the refractive effects of the oil film. Furthermore, the oil film may have stuck conidia to the leaf surface thereby reducing the number that were rinsed free as a measure of antisporulative action. The single rinse procedure did not permit a differentiation between the effect of the treatment on the conidia present at the time of treatment, and the true antisporulative effect of the treatments in reducing the formation of new conidia.

Clayton et al. (7) showed that of several plant oils only those containing the polyunsaturated fatty acids linoleic, linolenic, eleostearic, and licanic acids controlled blue mold of tobacco caused by the Oomycete *Peronospora tabacina*. In our ear-

Table 6. Protective and pre-lesion curative action of safflower oil and Sunspray UFO against downy mildew caused by *Plasmopara viticola* on leaves of potted Chardonnay vines

Treatment material	Concentration (% wt/wt)	Leaf area with sporangiophores (%)[x]	
		Protective[y]	Curative[y]
Safflower oil	1.0	23 b[z]	37 a
Sunspray UFO	1.0	43 b	38 a
Dithane 80 DF	0.2	0 a	31 a
Water control	...	41 b	27 a

[x] After 12 days postinoculation incubation at 20 to 24°C, plants were exposed to >95% relative humidity at 22°C in darkness for 24 h to induce the development of sporangiophores.

[y] The protective and curative treatments were made, 1 day before and 1 day after inoculation, respectively.

[z] The lower surface of each of the five youngest inoculated leaves was assessed for the area covered with sporangiophores, using a rating scheme of 0 to 9. Treatment values are the arithmetic means of percentage equivalents of individual leaf ratings of 15 leaves from three replicate plants per treatment. Means in the same column followed by different letters, differ significantly (P = 0.05) by Fisher's protected least significant difference.

Table 7. Efficacy of five-spray programs of petroleum and plant oils for the control of powdery mildew caused by *Uncinula necator* on leaves of New York Muscat and Canada Muscat in a vineyard

Treatment material[w]	Amount/100 liters	Disease incidence (% leaves infected)[x]		Disease severity (% leaf area infected)[x]	
		New York Muscat	Canada Muscat	New York Muscat	Canada Muscat
Sunspray UFO	1.0 liter	11 a[y]	14 a	3 a	3 a
Stylet-Oil	1.0 liter	8 a	16 a	0 a	2 a
Safe-T-Side	1.0 liter	8 a	14 a	0 a	3 a
Canola oil[z]	1.0 liter	70 b	87 b	17 b	24 c
Soybean oil[z]	1.0 liter	71 b	81 b	15 b	23 b
Agral 90	25 ml	79 b	86 b	16 b	16 b
Nova 40W	13 g	0 a	...	0 a	...
Water control	...	92 c	98 c	29 c	41 d

[w] Applications were made 13 [31], 27 July [32], 10 August [33], 23 August [35], and 7 September 1994 [36]. Eichhorn and Lorenz growth stages (10) are given in brackets. Disease incidence and severity were assessed 20 to 23 September.

[x] One hundred leaves per replicate were examined for the incidence of powdery mildew and its severity using a rating scheme of 0 to 9. Means in the same column followed by different letters, differ significantly (P = 0.05) by Fisher's protected least significant difference. The infected leaf area is the arithmetic mean of percentage equivalents of individual leaf ratings.

[z] Canola and soybean oils were mixed with Agral 90 in a ratio of 97.5:2.5 (vol/vol) prior to emulsification in water.

lier research with white rust of spinach caused by the Oomycete *Albugo occidentalis*, we found that four high-linoleic acid oils and four low-linoleic acid oils were equally ineffective as protective treatments (22). In the present study, safflower plant oil containing a high proportion of linoleic acid and Sunspray petroleum oil showed no activity against downy mildew of grapevines, when used in either a protective or a curative manner. Our negative greenhouse results were corroborated by the failure of both types of oils to control downy mildew in our vineyard experiments. These results contrast with the moderate vineyard activity of Esso Process 95 petroleum oil as reported by Castellani and Matta (5).

The three formulated petroleum oils showed almost identical performance in the four physical modes of action, despite their different compositions, which remained confidential. Safe-T-side was a thick, creamlike invert emulsion, whereas Stylet-Oil and Sunspray UFO were both clear solutions of oil and surfactant. Stylet-Oil emulsified easily in water in contrast to the incomplete mechanical homogenization of the nonformulated light mineral oil. Yet the mineral oil showed a high level of pre-lesion curative action reasonably comparable to that of Stylet-Oil at a concentration of 1% (vol/vol). These close similarities in action are interpreted as showing that petroleum or mineral oils are intrinsically efficacious against grapevine powdery mildew, and that the surfactant components of the three formulated products probably contributed little to their performances except for the ease of emulsification. We showed that Agral 90 had a moderate post-lesion curative action on apple powdery mildew, and slightly reduced apple scab under orchard conditions (22). Against grapevine powdery mildew, Agral 90 showed only a slight pre-lesion curative action, and in a full-season program, it showed a slight to moderate activity. Therefore, the high degree of pre-lesion curative and antisporulative action of the plant oils emulsified with Agral 90 was attributed primarily to the plant oils.

Petroleum (mineral) oils are not intrinsically fungicidal although added surfactants could be toxic. Mineral oils are used for the preservation of fungal cultures (9), and have been used as carriers of rust spores in plant inoculation procedures (27,28). Calpouzos et al. (4) showed that petroleum oils did not prevent spore germination nor infection. Oils decreased photosynthesis and transpiration but increased respiration. This led Calpouzos (2) to speculate that the therapeutic effect of oil on leaves infected by *M. musicola* was not due to a direct effect on the pathogen, but possibly due to an indirect effect by altering the physiology of the host. In our experience, the regrowth and reappearance of powdery mildew lesions after treatment with plant oils, and to a lesser extent with petroleum oils, showed that these oils had a fungistatic rather than a fungicidal effect, possibly indicating a temporary effect on host physiology.

Petroleum oils have moderate activity against *Botrytis cinerea* on cucumber and have greatly increased the fungicidal activity of benomyl and vinclozolin (1). Since *B. cinerea* is also a serious pathogen, causing bunch rot of certain grape cultivars (24), petroleum oils could be used advantageously to simultaneously control bunch rot and powdery mildew. However, the inefficacy of petroleum oils against downy mildew would necessitate the use of specific fungicides within an integrated program. The choice of a suitable fungicide would be determined by its chemical compatibility with oil, and the safety of the fungicide-oil combination on particular grape cultivars.

The petroleum oils were distinguished by their high degree of curative and antisporulative action. These characteristics suit them to monitored programs in which pesticide applications are timed according to disease risk or severity. The first application might be delayed until after the initial ascospore infection has occurred or until a low incidence of powdery mildew is observed on susceptible indicator cultivars. In our vineyard experiments, where conidial inoculum was introduced, petroleum oils applied at 14-day intervals gave excellent prevention of powdery mildew. This was consistent with our greenhouse demonstrations of excellent post-lesion curative and antisporulative actions for at least 7 days after treatment, and by a 5-day pre-lesion curative and moderate protective actions.

In summary, for the control of grapevine powdery mildew, three formulated petroleum oils showed moderate protection for up to 4 days, excellent pre- and post-lesion curative action, and excellent antisporulative activity. In contrast, two plant oils formulated with Agral 90 showed excellence only in pre-lesion and antisporulative modes of action. Neither group of oils showed any activity against grapevine downy mildew. These greenhouse results corroborated those of five-application vineyard programs in which petroleum oils were as effective as myclobutanil and superior to plant oils for preventing grapevine powdery mildew.

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