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A natural fungicide for the control of *Erysiphe betae* and *Erysiphe cichoracearum*

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Abstract This study examines the effects of a vegetable fungicide on sugar beet powdery mildew (*Erysiphe betae*) and cucumber powdery mildew (*Erysiphe cichoracearum*). The formulations consisting of a dispersion of *Brassicaceae* meal in vegetable or mineral oils on infected leaves of sugar beet, reared in the greenhouse, and of musk melons cultivated under plastic tunnels, were tested in comparison to each oil taken separately. Both formulations containing *Brassicaceae* meals, caused 94% of conidia to be distorted while for the untreated group only 2% were distorted. Furthermore, the leaf area infected by *E. betae* was 56% for untreated plants and 2.7 and 9.9% respectively, for plants treated with meal containing mineral and vegetable oil. Vegetable oil considered separately or with *Brassicaceae* meals showed no phytotoxicity, while the formulations based on mineral oil showed a significantly lower fresh and dry weight on tomato plants. The low level or absence of phytotoxicity of plants treated with vegetable oil formulations suggests that to improve the efficacy of powdery mildew control, they could be used mixed with sulphur. The efficiency of the vegetable formulations in the powdery mildew control observed during these trials encourages further investigation on other parasitic fungi and foliar pathogens.

Keywords Biological control · *Brassica carinata* · Environmentally-friendly products · Glucosinolates · Vegetable oil

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Introduction

In the last century, pesticides were largely adopted to counteract the action of pests and disease and to increase plant health and yield. However, the continuous use of chemical fungicides for plant defence caused great environmental impact, the onset of resistance phenomena within some populations of fungal pathogens as well as acute and general toxicity on humans and non-target organisms. This situation has prompted an increased demand for more environmentally-friendly products in order to reduce the side effects of chemical fungicides in crop protection (Christopherson and Glass 1969; Coats et al. 2003; Homma et al. 1981).

Horst et al. (1992) observed that rose powdery mildew (*Sphaerotheca pannosa* var. *rosae*) and black spot (*Diplocarpon rosae*) were significantly reduced by an aqueous solution of sodium bicarbonate plus 1% oil. Rue extract from *Ruta graveolens* plants significantly reduced tobacco powdery mildew (*E. orontii*) at a concentration of 10^2 g hl^{-1}, while sodium bicarbonate produced the same effect at 10^3 g hl^{-1}

(Lahoz et al. 2001). Emulsified oils appeared to be effective against powdery mildew and other epiphytic fungi (Hagiladi and Ziv 1986), while Martin et al. (2005) showed that mineral, vegetable and fish oil significantly reduced the percentage of leaf area covered with powdery mildew. Northover and Schneider (1993) argued that oil treatments turned out to be more effective as a curative rather than preventative means for grapevine powdery mildew.

Natural oil-based fungicides could represent a good alternative to chemical fungicides (Hagiladi and Ziv 1986; Henriques et al. 1998; Horst et al. 1992 Northover and Schneider 1993; 1996; Osnaya and Schloser 1998; Wicks et al. 1999). In fact, they are effective in controlling some plant pathogens at low doses and induce little or no resistance in target fungi (Martin et al. 2005). Furthermore, they have excellent spreading and leaf surface adhesion characteristics, and due to their rapid biodegradation have a low toxicity for human beings and cause little environmental impact.

Phytotoxicity can, however, occur on some crops, especially if treatments are carried out on stressed plants. Mineral oil applied at concentrations >1% caused some damage on grapevine, whereas no phytotoxic effect of vegetable oils has been observed at concentrations <2% (Wicks et al. 1999).

The purpose of this study is to investigate the efficacy of vegetable oils taken alone and/or after adding *Brassicaceae* meal containing high levels of glucosinolates as an alternative to synthetic fungicides. Glucosinolates are glycosidic compounds characterised in their intact form by the absence of biocidal activity, while their enzymatic hydrolysis degradation products (essentially isothiocyanates and nitriles) are well known for their high cytotoxic activity (Lazzeri et al. 1993; 2004; Marciano et al. 2004; Mayton et al. 1996; Rosa et al. 1997). Studies concerning the fungicidal effects of these purified molecules, confirmed their potential in plant protection (Manici et al. 1997; 1999; 2000; Rongai et al. 2006; Sarwar et al. 1998).

The experiments were carried out in 2005 and 2006 under laboratory and greenhouse conditions. A new formulation consisting of a dispersion of *Brassicaceae* meal in oil and subsequent emulsion in water (Rongai et al. 2006) was evaluated on sugar beet powdery mildew *Erysiphe betae* and musk melon powdery mildew *Erysiphe cichoracearum*. For comparison purposes oil without meal was used. The phytotoxicity of tested products was evaluated on tomato and sugar beet plants treated with oils and formulations.

Materials and methods

Formulation and oil used

The formulation was a suspension of oil; a by-product of Brassica seeds and water were added to the mix before use. 'Formulation A' (mineral oil+2 g l^{-1} of meal) and 'formulation B' (vegetable oil+arabic gum at 10% of oil+2 g l^{-1} of meal) were tested. Both formulations were applied as 1.5% emulsions in water. The mineral oil was a high-purity paraffin oil ('Ultra Fine Oil', Intrachem Bio Italia), characterised by a low phytotoxicity, while vegetable oil was extracted by solvent (hexane) from *Crambe abyssinica* cv. Mario seeds. The meal was obtained from *Brassica carinata* sel. ISCI7 seeds after partial defatting (<2% of oil), grinding and sieving at 0.5 mm; its glucosinolate content was determined essentially by following the procedure proposed for seed analysis in the Official Journal of European Community Regulation (1990), with some minor modifications (Wathelet et al. 2004) and was found to contain 115 μmol g^{-1} of the glucosinolate sinigrin (propenyl-glucosinolate).

Erysiphe betae conidial germination and colony growth on sugar beet leaves

Conidia of *E. betae* were brushed from infected sugar beet leaves obtained from greenhouse plants and collected in 60 ml of distilled water containing 3.6 ml of Tween 20. The spore suspension (at a concentration of 5×10^4 conidia ml^{-1}) was sprayed (1 ml per leaf) on the upper leaf surface of 12 detached sugar beet leaves for the treatments (four replicates of three leaves each). The 60 fully-expanded leaves came from 30 different 3 month-old plants. After 2 h for each treatment (mineral oil, 'formulation A', vegetable oil, 'formulation B' and water) 50 ml of product were sprayed onto leaves, using a hand pump dispenser. The leaves were transferred to plastic beakers with the petioles dipping in water and maintained at 20–25°C, a relative humidity (RH) of 90–100% and weak light (about 80 μE m^{-2} s^{-1}) to favour germination of conidia.

Thirty-six hours after the treatment, three leaf disks (ø=10 mm) were cut from each leaf, flooded in 10 ml^{-1} distilled water and wiped with a brush. Conidia were concentrated by centrifugation at 4,500 *g* for 8 min, mounted on a glass slide and examined under a microscope at 160× magnification to establish the number of shapeless and germinated conidia. On the sixth day, the number of colonies was counted on the upper side of three additional leaf disks from each leaf using a stereomicroscope. The experiment was repeated 30 days later.

Greenhouse and field experiments

Sugar beet (*Beta vulgaris*) plants with a high infection of powdery mildew (*E. betae*) were maintained in 80×80×100 mm pots, containing type B neutral sphagnum peat (Floragard Vertriebs Gmbh für Gartenbau, Germany) in a greenhouse at about 24°C, RH 60–80% and photoperiod of 10/14 h of light/darkness. The formulations were applied (300 ml per group) on five groups of infected sugar beet plants by an aerosol sprayer (operating at 7 kg force cm^{-2}) carrying out two treatments (at 1-week interval). Each group contained 12 plants (three plants per replicate). Plants in the first group were treated with mineral oil, the second with 'formulation A', the third group with vegetable (+arabic gum at 10% of oil) oil and the fourth with 'formulation B'. The control group was sprayed with water. A randomised block experimental design was used.

The treatments with 'formulation A, B' and water were applied on musk melon (*Cucumis melo*) plants naturally infected by powdery mildew (*Erysiphe cichoracearum*) and grown in plastic tunnels at Sermide (Mantova) Italy, from August–October 2005. The trials were repeated (substituting 'formulation A' with wettable sulphur) in August–October 2006. A randomised block experimental design was used. Each group consisted of 48 plants (12 plants per replicate). The plants were sprayed (two l per group) twice (about 30 and 20 days before harvest). Powdered sulphur residues of previous treatments (50 kg ha^{-1} every 10 days) were present on the plants and came into contact with the oil.

Seven days after the last treatment, powdery mildew infection was estimated visually on a scale from 0 to 5. A value of 0 was assigned to plants almost devoid of any infection, 1=1–20%; 2=21–40%; 3=41–60%; 4=61–80% and 5>80%. Leaf samples were detached from the mid-lower section of the plant. Upper and lower leaf surfaces were evaluated separately.

Greenhouse phytotoxicity experiments

Tomato (*Solanum lycopersicum*) and sugar beet (*B. vulgaris*) plants were cultivated in 80×80×100 mm pots using type B neutral sphagnum peat (Floragard Vertriebs Gmbh fur Gartenbau, Germany) and maintained at 24°C day/15°C night with a 16-h photoperiod. Four treatments were tested on tomato and sugar beet plants (vegetable oil, mineral oil, 'formulation A' and 'formulation B') in addition to the untreated control. The plants were 30 days old at the time of treatment. Oils and formulations were applied as 1.5% emulsions in water on 12 plants for each treatment (three plants per replicate). In addition, 12 control plants were sprayed with water. A randomised block experimental design was used. The plants were treated with an aerosol sprayer (operating at 7 kg force cm^{-2}) twice with 7-day intervals, using a 250 ml spray mixture per treatment. Fresh and dry weights were determined 10 days after the last application of products. The samples were dried in a ventilated oven at 60–65°C for 72 h, before collection of dry weight data. Thirty days later, the phytotoxicity trials were repeated.

Statistical analysis

Statistical analysis of the data collected was by ANOVA. The results of both trials are presented with an ANOVA testing for significance of trial and trial× treatment interactions. The data were arcsine-transformed before the analysis. Mean values were compared by Fisher's protected LSD test at $P=0.05$. SigmaPlot version SPW10 was used to create graphics.

Results

Erysiphe betae conidial germination and colony growth

The percentage of normal, shapeless and germinated conidia collected from sugar beet leaves 36 h after the

Table 1 Effects of treatments on normal conidia, distorted conidia, germinated conidia and mean number of colonies of sugar beet powdery mildew (*Erysiphe betae)*

Treatments	Oil ml l^{-1}	Meal g l^{-1}	Normal conidia %	Distorted conidia %	Germinated conidia %	Mean number of colonies n cm^{-2}
Control			87.1a	2d	5.2a	33.6a
Mineral oil	15		45.4c	49.1b	3.5a	9.2b
Vegetable oil	15		63.0b	29.8c	4.8a	8.3b
'Formulation A'	15	2	2.6d	94.4a	1.6a	1.9c
'Formulation B'	15	2	2.8d	94.0a	1.6a	1.5c

Values (means of two trials) with different letters are statistically different (LSD test, $P<0.05$)

treatments are shown in Table 1. 'Formulations A and B' caused 94.4 and 94% of conidia to be distorted while for mineral and vegetable oil the values were 49.1 and 29.8% respectively. Only 2% of distorted conidia were present in the water-treated group. Conidia from plants treated with both formulations were collapsed and with irregular edges, while those collected from water-treated plants were swollen and with straight edges. The percentage of germinated conidia was not significantly different between the untreated and treated plants. Moreover, 6 days after the treatments, the number of colonies was significantly greater on the water-treated plants (33.6 colonies) compared to those treated with mineral and vegetable oil (<10 colonies) and the two formulations (<2 colonies) (Table 1). The ANOVA testing for trial and trial×treatment interactions showed no significant effects.

Efficacy of the formulations against sugar beet and musk melon powdery mildew

Both formulations ('A and B') significantly decreased the percentage of infected sugar beet leaf tissue in the greenhouse. In general, oil treatments alone reduced powdery mildew infection. In fact, the leaf area infected by powdery mildew was 59.6% for untreated plants, and 23.4 and 25.6%, respectively, for plants treated with mineral and vegetable oil. However, when *B. carinata* meal was added to mineral or vegetable oil, the leaf area infected with powdery mildew dropped to 2.7 and 9.9%, respectively, for 'formulations A' and 'B' (Fig. 1) There were no differences between trial and trial×treatment interactions.

Similar results were obtained with the musk melons cultivated in plastic tunnels. The percentage of infected tissue on both leaf surfaces was statistically lower than the unsprayed control in 'formulation B' while no difference was observed on the upper leaf surface of plants sprayed with 'formulation A'. The trial repeated in 2006 confirmed the efficacy of 'formulation B'. There were no significant differences between 'formulation B' and wettable sulphur (Fig. 2).

Phytotoxicity

Vegetable oil and 'formulation B' did not show any phytotoxicity on tomato and sugar beet plants. Fresh and dry weight values did not differ significantly from corresponding control values (Table 2). In contrast, tomato plants treated with mineral oil and 'formulation A', showed a chlorotic appearance and were smaller than control plants. In addition, fresh and dry

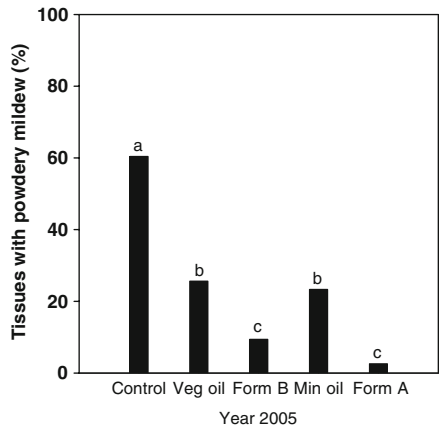

Fig. 1 Percentage of sugar beet upper leaf surfaces with powdery mildew (*Erysiphe betae*). *Values with different letters* are statistically different ($P<0.05$) based on Fisher's protected least significant difference (LSD)

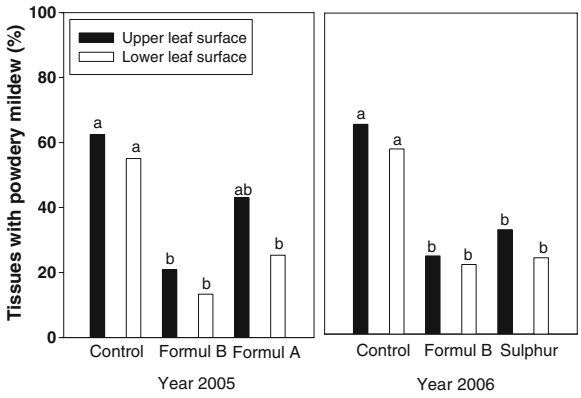

Fig. 2 Percentage of musk melon tissues with *Erysiphe cichoracearum* infection in the years 2005–2006. *Values with different letters* are statistically different ($P \leq 0.05$)

weights were significantly lower, about half of the corresponding control values. No phytotoxic effects were observed on sugar beet plants treated with either 'formulation A' or 'formulation B' (Table 2). The phytotoxicity trials repeated 30 days later produced similar results. There were no differences between trials or trial×treatment interactions.

Discussion

Erysiphe cichoracearum and *E. betae* cause common diseases of cucumber and sugar beet crops and damage the plants in the open field, but especially in the greenhouse. Sugar beet powdery mildew is particularly damaging in areas with arid climates, causing sugar yield losses of up to 30% (Francis 2002), probably because direct damage caused by the fungus combines with indirect damage due to the weakening of the plants, that become more vulnerable after climate stress. In the last few years there has been recurrence of powdery mildew infections, raising many problems for farmers. It therefore appears necessary to find new ecological products that are able to control the spread of this disease.

In our trials, both formulations were effective against powdery mildew of sugar beet in laboratory and greenhouse tests. For musk melons, only 'formulation B' with vegetable oil seemed to be effective under plastic tunnels. Plants treated with the formulations had a significantly lower infected leaf area than controls and plants treated with mineral and vegetable oil.

The results of laboratory tests confirmed that the effects of treatments on the percentage of germinated conidia do not actually depend on the treatments. Thus, it appears that whether conidia were normal or distorted had no influence on their ability to germinate. At this point the question is thus why the number of colonies on treated leaves (Table 1) was significantly lower than on the control group.

Cohen et al. (1996) demonstrated that a soap (Zohar LQ-215) did not reduce spore germination or germ-tube elongation of cucumber powdery mildew but inhibited the later stages of the disease-cycle (i.e. mycelial growth and sporulation). One of the possible explanations could be that the presence of grain meal and oil film on treated tissue tends to inhibit powdery mildew development (Hagiladi and Ziv 1986; Ziv 1983). Another explanation might be that the distorted conidia observed on the 'formulation' treatments (94%) had lost their energy to infect, and after 6 days only a few colonies were counted.

Regarding the plastic tunnel experiments, 'formulation B' was effective in controlling *E. cichoracearum* in the years 2005–2006. In both these years the infection level was up to 60%, in contrast to plants treated by 'formulation B' where the infection level remained <25% (Fig. 2). It should be emphasised that treatments were carried out at the end of the musk

Table 2 Fresh and dry weight of tomato and sugar beet leaves in the greenhouse assay, 10 days after two treatments

Values (mean of two trials) with different letters are statistically different ($P<0.05$) based on Fisher's protected least significant difference (LSD)

Treatments	Oil	Meal	Tomato plants		Sugar beet plants	
	ml l^{-1}	g l^{-1}	Fresh weight g	Dry weight g	Fresh weight g	Dry weight g
Control			10.13a	1.56a	14.2a	0.80a
Mineral oil	15		5.32b	0.73b	14.3a	0.81a
Vegetable oil	15		10.41a	1.47a	14.5a	0.80a
'Formulation A'	15	2	5.10b	0.69b	14.4a	0.79a
'Formulation B'	15	2	9.83a	1.22ab	14.7a	0.80a

melon cycle with sulphur residues of previous sulphur applications present on the crop. Such residues on musk melon leaves could be the cause of the low efficacy of 'formulation A'. This formulation, in fact, contains mineral oil which, combined with sulphur, becomes highly phytotoxic. In fact, all plant leaves treated with 'formulation A' showed many necrotic spots, a typical phytotoxicity sign. These symptoms were mainly observed on leaves exposed to direct sunlight and were probably provoked by the sunshine and the high temperatures (maxima >30°C) during the trials. Severe adverse effects of oil spraying are associated with high volumes applied at low pressure using hand-held sprayers. According to Martin et al. (2005), no unfavourable effects on grapevines should be expected for applications spaced at 14-day intervals and at treatment volumes <600 l ha^{-1} at a rate of 1.5% of oil.

No phytotoxicity effects were noted on tomato, sugar beet or musk melon plants treated with 'formulation B'. Again, since the treatments were carried out on musk melon leaves with sulphur residues, we hypothesise that 'formulation B' mixed with sulphur could be used to control musk melon powdery mildew. It would be interesting to determine if the use of sulphur enhances the efficacy of 'formulation B'. The non-phytotoxicity of vegetable oil, contained in 'formulation B', can be explained by the different composition and greater viscosity (Martin et al. 2005); due to its longer persistence and greater viscosity than refined mineral oil, vegetable oil is certainly less phytotoxic since it does not penetrate the leaf.

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