Chapter

Fungicidal Activity of Soybean Oil against Powdery Mildew on Wheat

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Abstract

Products derived from soybean crops are not only global food staples, but are also used in pharmaceuticals, industry, and agriculture. Soybean oil (SBO) and other oils are often used as adjuvants in agricultural sprays to facilitate spread of the active ingredient (a.i.) across the plant surface. This chapter describes original research in which a natural fungicide (biofungicide) was formulated using SBO as the a.i. Antimicrobial activity of SBO against a powdery mildew (PM) pathogen, *Blumeria graminis* f. sp. *tritici*, was measured, as well as effects on plant health and yield of wheat plants. Results were compared with a conventional fungicide and another lipid biofungicide. The mode of action was investigated using scanning electron microscopy. Results showed that SBO provided PM control equal to the conventional fungicide when plants were adequately spaced and caused collapse of fungal structures and extrusion of cell contents. Commercialisation potential of SBO biofungicide is discussed.

Keywords: biofungicide, horticultural oils, powdery mildew, scanning electron microscopy, soybean oil, wheat

1. Introduction

Soybean (*Glycine max*), a species of legume native to East Asia, is economically the most important bean in the world, providing vegetable protein for millions of people and animals and ingredients for hundreds of chemical products including pharmaceuticals, cosmetics and biofuels [1]. Soybeans have a myriad of health benefits for humans including their ability to stimulate metabolism, promote heart health and osteotropic activity, protect against cancer, prevent birth defects, aid digestion, increase circulation and decrease the risk of diabetes [2], but in this chapter we focus on the use of soybean oil (SBO) in agriculture to improve plant health.

Soybean oil is a vegetable oil that is solvent-extracted from pressed seeds of soybean, followed by refinement, blending and optional hydrogenation, and is one of the world's most widely consumed edible plant oils [3, 4]. While plant-derived oils, such as SBO, are predominantly used in agriculture as an adjuvant to aid the spread of pesticides over plant surfaces and also to help the pesticide to stick to the plant surface [5, 6], SBO is also directly antimicrobial against a range of powdery mildew fungi [7–10], *Botrytis cinerea* [11] and bacteria such as *Staphylococcus aureus* and *Escherichia coli* [12]. In addition, SBO has insecticidal activity against mites [13], whitefly and aphids [14, 15] and insects associated with stored grain products [16] . However, there are few commercial products in horticulture that

use plant oils as the active ingredient (the most notable exception being neem oil), with most spray oils comprising mineral oils that are refined from petroleum. Commercial development of SBO as a pesticide offers the advantages of reduced reliance on petroleum products, and the use of an edible oil is considered to be less toxic to human health and the environment. However, fats and oils are often associated with chlorosis and necrosis of plant tissue [13, 17–19], and other problems include inconsistent activity, handling and application difficulties, spoilage and development of unpleasant odours, and these issues need to be considered when developing a SBO fungicide.

Powdery mildew (PM) disease is characterised by fluffy white lesions on the surfaces of aerial plant tissues. It is caused by pathogens from the Erysiphales order and is responsible for significant yield losses globally in crops such as cucurbits, apples, roses, tomatoes, grapes and various cereals such as wheat and barley [20, 21]. PM is one of the most economically damaging plant diseases around the world. For instance, losses to barley PM in the State of Western Australia have been estimated at $30 million (AUD) annually [22]; losses account for approximately 15% of total crop revenue for American North Western hop growers, which equated to over $30 million USD in the year 2000 [23]; and the introduction of PM-resistant grape varieties into the State of California alone has been estimated to yield $48 million (USD) in annual cost savings [24]. There are serious limitations with existing PM control methods, such as pathogen resistance to demethylation inhibitor fungicides [25, 26]. Moreover, synthetic pesticide use has been clearly linked to human health concerns such as increased incidence of respiratory disease and cancer [27]. There are also limitations on the use of sulphur and copper-based fungicides, considered to be more natural fungicides, in organic systems because sulphur can act as a nose and eye irritant [28] and because heavy metals like copper accumulate in soils with intensive copper fungicide use over time, resulting in phytotoxicity [29]. These issues are driving the development of biofungicides (fungicides comprising biological control agents and/or natural products) that are suitable for both organic and conventional growers, considered safer in terms of human health and which provide an environmentally benign option for durable disease control. Furthermore, PM strains mutate and develop resistance rapidly to synthetic pesticides, but there are few documented instances of resistance development to oils [30, 31]. Biofungicides can be used as standalone products, or in integrated disease control programmes that combine treatments with multiple modes of action, to reduce the application number of traditional synthetic pesticides and to delay the onset of resistance.

The principal aim of this study was to investigate the potential of SBO as a biofungicide to control powdery mildew. To achieve this aim, SBO effects on disease control efficacy and plant health and yield were compared under regulated conditions found in a controlled environment room and a glasshouse versus the more variable conditions in a field situation. SBO performance was also compared versus conventional fungicides and another lipid biofungicide-emulsified anhydrous milk fat (AMF) from cows' milk, since SBO and AMF were the two top candidates from a preliminary study investigating lipid biofungicide action against PM [32]. Product mode of action (MoA) was also investigated using scanning electron microscopy (SEM), because knowledge of the MoA permits a product to be used more effectively in relation to timing and mode of application, and helps to manage the risk of target organisms developing resistance to the control product [33, 34]. MoA is also often necessary for product registration. Wheat (*Triticum aestivum*) was chosen as the ideal crop for this study because it is a global food staple for which PM is a common disease problem [35, 36] and

because wheat plants can be easily and quickly grown. Given that the leaf surface of wheat is non-hairy and robust, it is also more likely to produce clear images in SEM following sample preparation by cold stage freezing and sputter coating with gold.

Findings obtained from the data are discussed with respect to the commercialisation potential of SBO biofungicide.

2. Controlled environment (CE) room and glasshouse trials

2.1 CE trial methods

PM-susceptible 'Endeavour' wheat plants, were sown at a density of four plants per 12 cm diam. pot. Plants were maintained in two blocks (1 pot/treatment/block) in a CE room at 20°C with a 16-hour photoperiod. After 1 week, the experimental plants were artificially inoculated by taking potted wheat plants infected with *Blumeria graminis* f. sp. *tritici* (formerly classified as *Erysiphe graminis* f. sp. *tritici*) (wheat PM), and trailing the infected leaves from these plants across the leaf surfaces of the healthy plants, such that spores from lesions on the infected leaves would brush off onto the healthy wheat plants. Treatment application (**Table 1**) commenced when the plants were 2 weeks old and at plant growth stage (PGS) 1, as defined by [37]. Leaves were sprayed to run-off (i.e., the point where the leaf is completely saturated and liquid starts to drip off the leaf) using a hand-held spray bottle (500 mL Garden Trigger Sprayer, Hills, Australia), with a total of 9 spray applications applied over a course of 7 weeks (2 sprays/week for the first fortnight, and 1 spray/week thereafter).

The first disease assessment (designated time 0) was made immediately before the first treatment application, followed by an assessment after 7 weeks (PGS = 8–10). Disease severity on the three most basal leaves of each plant was assessed using percent leaf area infection diagrams (**Figure 1**), and the rating scale shown in **Table 2**. Disease ratings for the three leaves were averaged to give one value per plant. Disease assessments had to be made on different leaves on each assessment date, because as the plants mature, the most basal leaves wither and die,

Treatment	Treatment code
Unsprayed control—no fungicides	Unsprayed
Water control	Water
Amistar® WG fungicide[1] (0.4 g/L)	Amistar
AMF[2] (7 g/L) + DATEM[3] (5 g/L) + Grindox[4] 122™ (1 g/L)	AMF
Soybean oil[5] (20 g/L) + DATEM (5 g/L) + Grindox 122™ (1 g/L)	SBO

[1]*Amistar® WG fungicide, containing 250 g/L azoxystrobin active ingredient, was supplied by Syngenta, Basel, Switzerland, and is effective against both powdery mildew and rust pathogens.*

[2]*AMF = anhydrous milk fat—a highly saturated solid milk fat, obtained from New Zealand Milk Products Ltd. (now trading as Fonterra).*

[3]*DATEM = an emulsifier containing diacetyl tartaric acid esters of mono- and di-glycerides. Sold by Danisco Ltd., Brabrand, Denmark as: Panodan® AL 10.*

[4]*Grindox 122™ = an antioxidant produced by Danisco Ltd., Brabrand, Denmark.*
[5]*Soybean oil (Amco brand) was obtained from the supermarket.*

Table 1.
CE room wheat trial treatments.

so data from each assessment date were analysed separately by SAS, version 8.02 (SAS Institute, Cary, NC), using a nested design, with treatments nested within pots and plants within treatments and pots.

Figure 1.
Standard disease area diagrams to show four severities of wheat powdery mildew infection, from [37].

Rating	Percent leaf area infected
0	No infection
1	1% infection
1.5	1–5% infection
2	5% infection
2.5	5–25% infection
3	25% infection
3.5	25–50% infection
4	50% infection
4.5	>50% infection

Table 2.
Wheat powdery mildew (PM) leaf disease rating scale, from James [37].

2.2 CE trial results and discussion

At time 0, there were no consistent treatment differences evident (**Figure 2**), but after 7 weeks, "Amistar", "AMF" and "SBO" all provided significantly greater control of PM than "Unsprayed" and "Water" treated controls (**Figure 3**), and the amount of disease on plants treated with "AMF", "SBO" and "Amistar" was lower than that recorded at time 0, i.e. before any treatment application (**Figure 2** vs. **Figure 3**).

There were no visual signs of leaf damage associated with the treatments. (**Figure 4**).

Thus, under the controlled conditions of the CE room, SBO could perform as effectively as both the commercial fungicide Amistar and the AMF biofungicide,

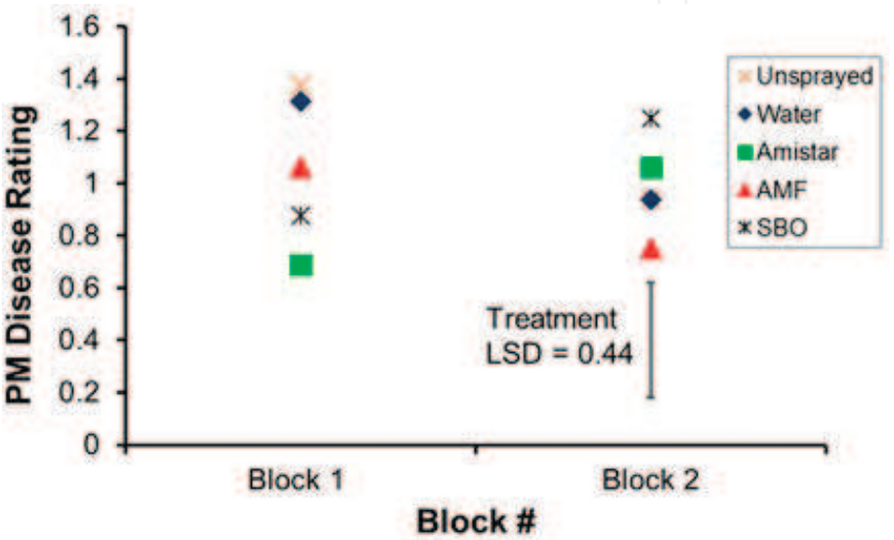

Figure 2.
PM disease severity on the basal leaves of 'Endeavour' wheat plants from the controlled environment (CE) wheat trial at time = 0, i.e. prior to any treatment application. Treatment codes are given in **Table 1.** *The least significant difference (LSD) bar applies to within-column comparisons only, owing to the hierarchical nature of the nested design, where the number of replicate plants (n) for each data point on the graph = 4.*

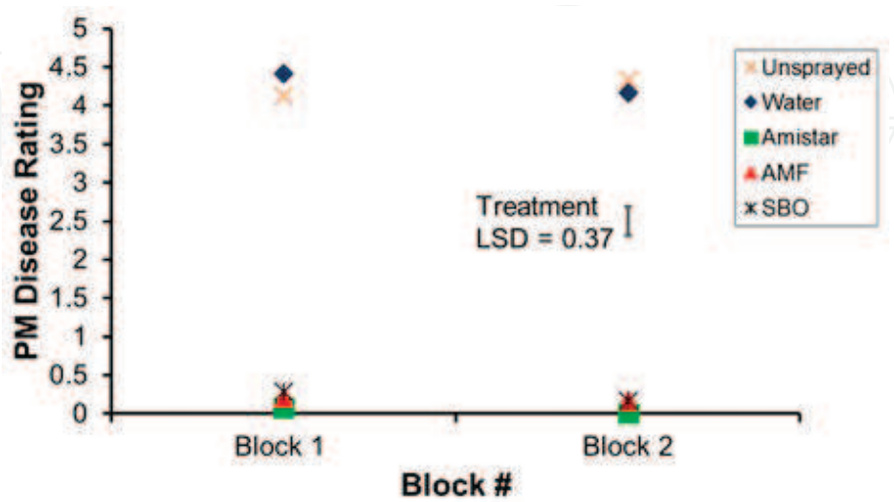

Figure 3.
PM disease severity on the basal leaves of 'Endeavour' wheat plants from the CE trial at time = 7 weeks, i.e. after 7 weeks of treatment application. Treatments are described fully in **Table 1** *and comprised leaving the plants unsprayed, or spraying with water, Amistar® fungicide or emulsified anhydrous milk fat (AMF) or soybean oil (SBO). The LSD bar applies to within-column comparisons only, owing to the hierarchical nature of the nested design, where n for each data point on the graph = 4.*

Figure 4.
'Endeavour' wheat plants in the CE room that have (A) not received any protection against PM (treatments "water" and "unsprayed"); or (B) were sprayed with "AMF", "SBO" or "Amistar" fungicides. The fungicides provided effective control of PM, without any visual adverse effects on plant health.

in terms of disease control and lack of adverse effects on plant health. Given that disease severity measured at the end of the experiment (after 7 weeks of treatment application) was lower than the disease severity measured at time 0 (before treatments commenced), this might suggest that all three fungicides have eradicant as well preventative activity against PM on wheat. However, this conclusion cannot be made for sure until assessments are made on whole plants throughout the course of the experiment, since different leaves were assessed at the start and the end of the CE experiment, owing to natural attrition of the oldest leaves.

2.3 Glasshouse trial methods

For the glasshouse trial (performed during February–March in Hamilton, New Zealand), the setup was similar to the CE trial, except that wheat seeds were sown into 6.75 L black polythene planter bags (PB12, Easy Grow Ltd., New Zealand). There were four replicate bags of four plants/treatment, and one replicate bag from each treatment was randomly positioned on a separate table (block). Treatments were the same as in the CE trial, except for omission of the unsprayed control. A total of seven spray applications were made throughout the course of the experiment (1 spray for the first fortnight, and 1 spray/week thereafter). Treatment application commenced when the plants were 11 days old and at plant growth stage (PGS) 1, as defined by [37].

Disease severity on the three most basal leaves/plant was assessed as described in the CE trial, with the initial disease assessment (designated Time 0) made immediately before the first treatment application, followed by an assessment after 7 weeks (PGS = 9–10.3). At the end of the trial, plants were considerably larger than those in the CE trial, so an additional disease assessment was also made at week 7 on the

whole plant rather than the three most basal leaves, using the scale defined in [38], as shown in **Figure 5** and **Table 3**. Experimental design and statistical analysis was the same as for the CE trial.

2.4 Glasshouse trial results and discussion

Only data from the first (time = 0) and last (time = 7 weeks) disease assessments are presented. At time 0, disease levels in block 1 were significantly higher in the "Water" control than all other treatments, but this trend was not repeated in other blocks, and overall there were no consistent treatment differences evident at the start of the experiment (**Figure 6**). After 7 weeks, "Amistar", "AMF" and "SBO"

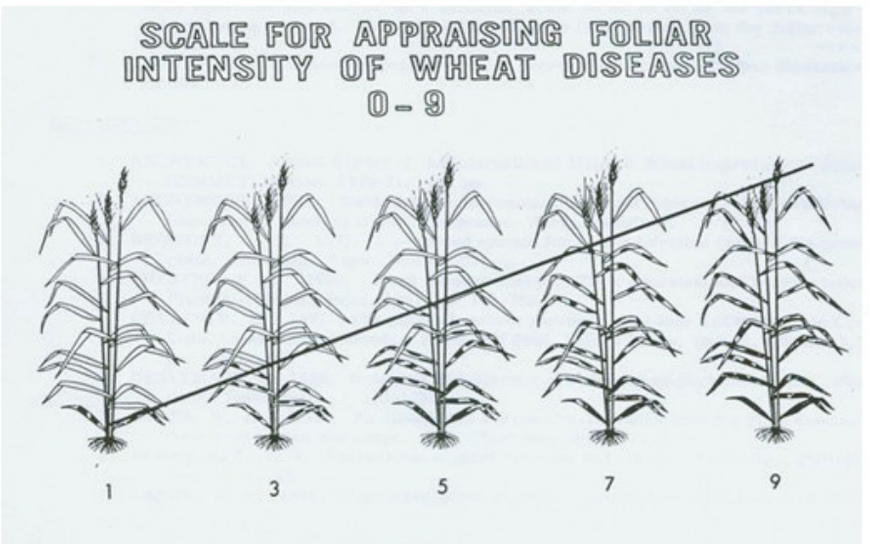

Figure 5.
PM disease severity on whole wheat plants, from [38] .

Numeric scale	Characteristics
0	Free from infection.
1	Very resistant. Few isolated lesions on lowest leaves only.
2	Resistant. Scattered lesion on 2nd set of leaves, with first leaves infected at light intensity.
3	Moderately resistant. Light infection of lower third of plant.
4	Low intermediate. Moderate to severe infection of lower leaves, with scattered to light infection extending to the leaf immediately below the mid-point of the plant.
5	Intermediate. Severe infection of lower leaves, with moderate to light infection extending to the mid-point of the plant, but not beyond.
6	High intermediate. Severe infection of the lower third of the plant, moderate degree on middle leaves, and scattered lesions beyond the mid-point of the plant.
7	Moderately susceptible. Lesions severe on the lower and middle leaves, with infections extending to the leaf below the flag leaf, or with trace infections on the flag leaf.
8	Susceptible. Lesions severe on lower and middle leaves. Moderate to severe infection of upper third of plant. Flag leaf infected in amounts more than a trace.
9	Very susceptible. Severe infection on all leaves, and the spike infected to some degree.

Table 3.
Scale for appraising foliar intensity of wheat diseases on whole plants, from Saari & Prescott [38].

all provided significantly greater control of PM than "Water" treated controls, regardless of whether disease severity was measured on the three most basal leaves (**Figure 7**), or the whole plant (**Figure 8**). All three fungicides performed as well as each other. In all blocks, the amount of disease on whole plants treated with "AMF", "SBO" and "Amistar" was lower than that recorded at the start of the experiment, i.e. before any treatment application (**Figure 7** c.f. **Figure 8**). This suggests that eradicant activity may be possible, under low initial inoculum loads (at the start of this experiment, there was <1% leaf infection) and corroborates the results found in the CE trial. However, the glasshouse environment is still relatively controlled and the plants are more widely spaced than in a field experiment, so field testing was the next step in the research.

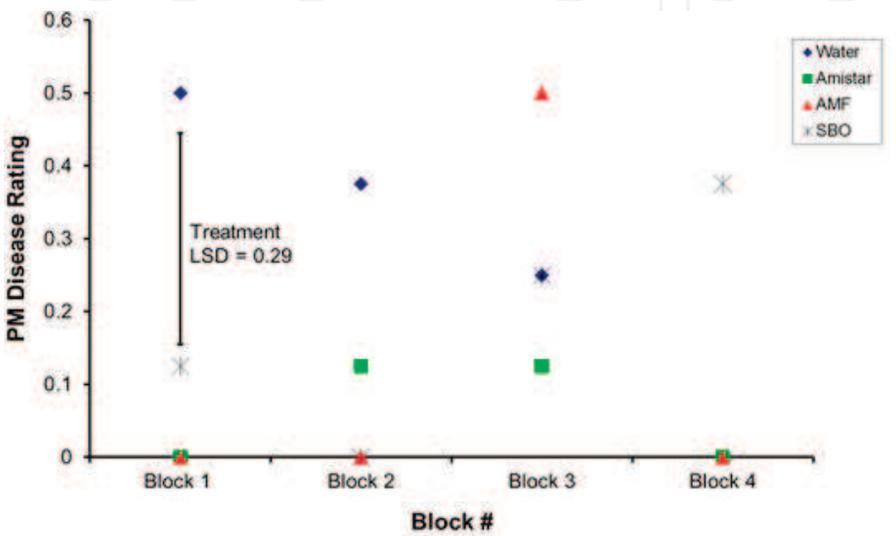

Figure 6.
Average PM disease severity on the basal leaves of 'Endeavour' wheat plants in the glasshouse trial at time = 0, i.e. prior to any treatment application. Treatment codes are given in **Table 1**. *The LSD bar applies to within-column comparisons only, owing to the hierarchical nature of the nested design, n for each data point on the graph = 4.*

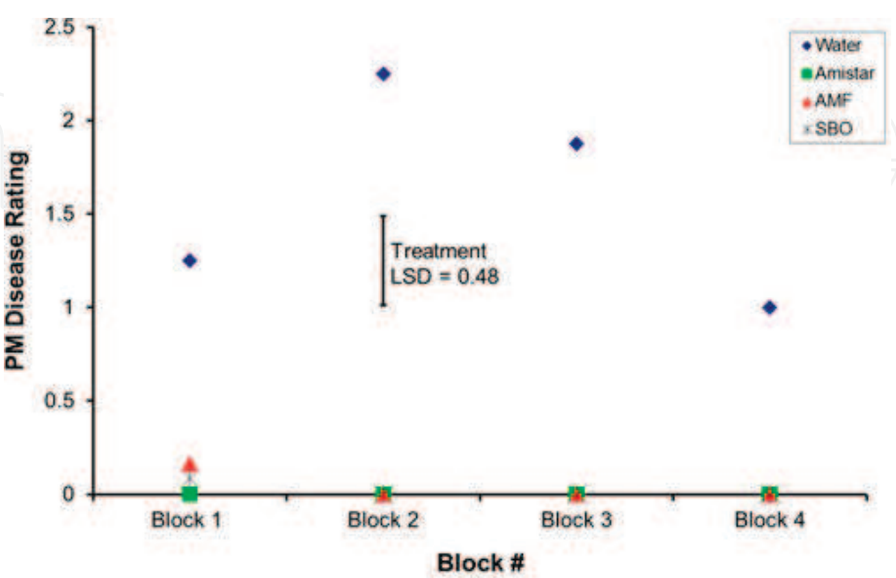

Figure 7.
Average PM disease severity on the basal leaves of 'Endeavour' wheat plants in the glasshouse trial at time = 7 weeks, i.e. after 7 weeks of treatment application. Treatment codes are given in **Table 1**. *The LSD bar applies to within-column comparisons only, owing to the hierarchical nature of the nested design, n for each data point on the graph = 4.*

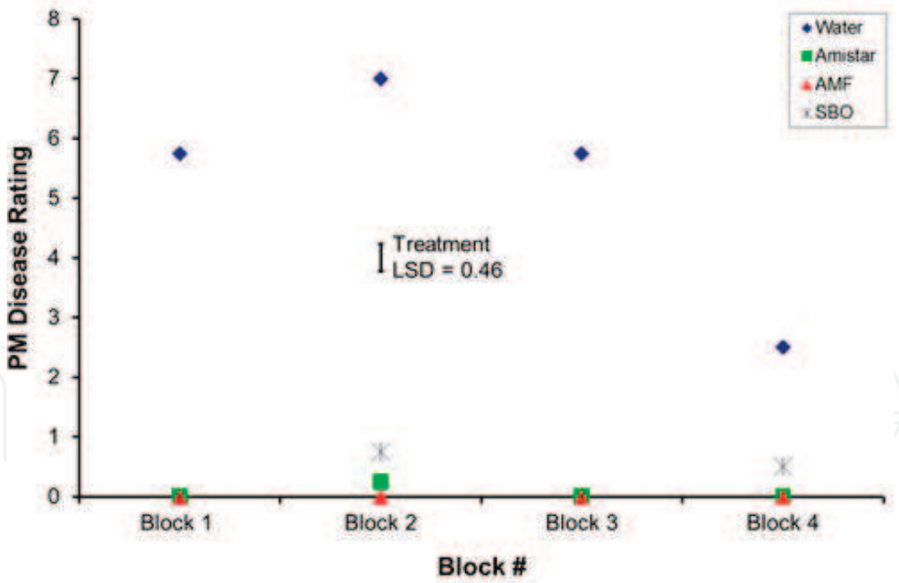

Figure 8.
Whole plant assessments of PM disease severity on 'Endeavour' wheat plants in the glasshouse trial at time = 7 weeks, i.e. after 7 weeks of treatment application. Treatment codes are given in **Table 1***. The LSD bar applies to within-column comparisons only, owing to the hierarchical nested design, n for each data point on the graph = 4.*

3. Field trial

3.1 Field trial methods

Three spring wheat cultivars were used in this trial: 'Janz', an Australian cultivar highly susceptible to PM and resistant to brown leaf rust but susceptible to stripe rust; 'Karamu', a New Zealand cultivar that is susceptible to PM and leaf rust but resistant to stripe rust; and 'Gundaroi', a durum wheat cultivar that is susceptible to PM, but resistant to both rusts.

The three wheat cultivars were sown in separate adjacent areas in early September (spring) in Christchurch, New Zealand. Each cultivar was grown in two strips 24 m long and 1 m wide. Each strip was divided into 20 plots (1.2 m long × 1 m wide) and each plot contained approximately 150 plants. Adjacent, or nearly adjacent plots were randomly assigned to each of the five treatments to make a block and this procedure was repeated to give five blocks (25 plots), spread along the two strips, with block 3 split across both strips. The remaining 15 plots were untreated (buffers). Within each treatment plot five plants were labelled with block and treatment number. These labelled plants were used for repeat disease assessments over the trial period, with data analysed separately for each cultivar as a repeated measures design using SAS version 8.02 (SAS Institute, Cary, NC).

The five treatments were identical to those used in the CE trial, except that Amistar® fungicide was applied at the recommended field rate of 750 mL/ha and 1.4 mL of fungicide liquid concentrate /12 L water. During the growing season, there were five applications applied to designated treatment plots, sprayed to run-off using 20 L backpack pressure spray units (Backpack 435, Solo, New Zealand), of the water and biofungicide treatments (on average 17 days apart), and two applications of Amistar fungicide (7 weeks apart, according to manufacturer recommendations). After treatment application, plants were left to dry for several hours before disease assessments were carried out. At each assessment, the growth stage of each wheat cultivar was noted, as defined by James [37] . The five labelled plants in each plot were assessed according to a PM rating system from 0 to 9

(**Figure 5** and **Table 3**). The same rating system was used for a rust assessment on 'Janz', 123 days after sowing.

Plants were left in the field for 6½ weeks after the last spray until harvest in late February (summer). The trial was harvested with a rice binder (Model 210B, Mitsubishi, Japan) and each treatment/block rep was processed through a thresher (Nursery Master Stationary Thresher, Wintersteiger, Austria) to separate the wheat

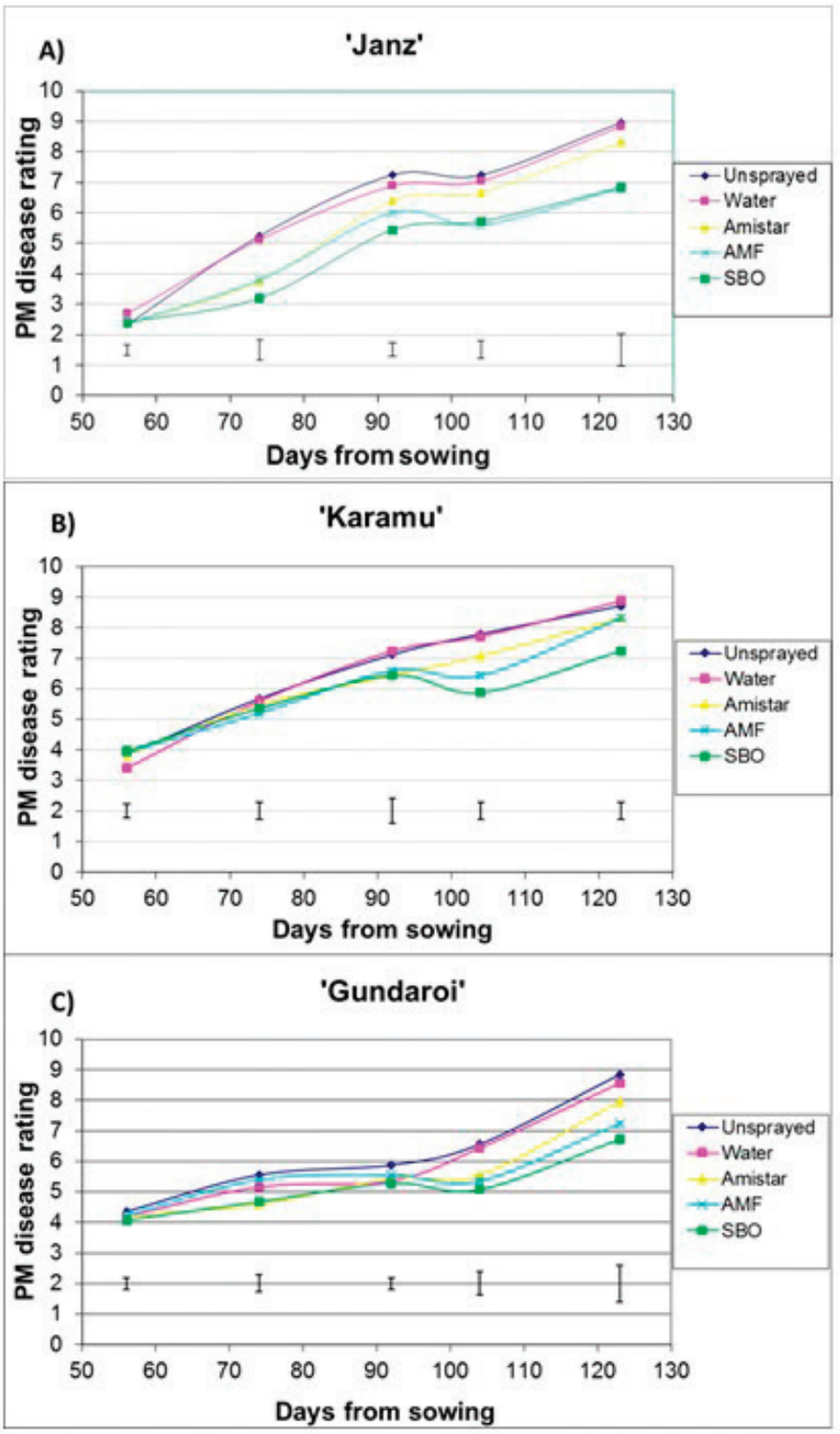

Figure 9.
Powdery mildew disease ratings in three field-grown wheat cultivars following application with Amistar® WG fungicide (750 mL product concentrate/ha at and 1.4 mL of fungicide liquid concentrate/12 L water), emulsified anhydrous milk fat (AMF) at 7 g/L and emulsified soybean oil (SBO) at 20 g/L. control treatments involved spraying the plants with water or leaving them unsprayed. Data were assessed as a repeated measures design with the bars indicating the least significance difference at each assessment date.

grain from the chaff and straw. The grain was placed into paper bags, which were taken back to the field lab and weighed. The following day, each bag was sorted through a 2 mm sieve screen (Endecott, United Kingdom) that separated grain into two lots: seconds (<2 mm) and first grade (>2 mm). The 1000 seed weights were measured on a machine (Numigral II, Tripette & Renaud, France) that automatically counts 250 seeds, which were weighed and then the weight was multiplied by four. Harvest data was analysed as a nested design (with blocks and treatments nested within cultivar) using SAS version 8.02.

3.2 Field trial results and discussion

For all three wheat cultivars, SBO was the most effective fungicide against PM, and provided significantly greater protection than the commercial synthetic fungicide, Amistar, during the middle part of the season, i.e. 90–120 days from sowing (**Figure 9**). The total degree of PM control was not as great as that observed in the CE and glasshouse trial, most likely because the close proximity of plants in the field trial resulted in overlapping growth leading to ineffective spray penetration and possible build-up of inoculum in protected parts of the canopy. The increase in disease was most marked in the final two disease assessments and under these heavy inoculum loads, Amistar was completely ineffective in the most PM-susceptible cultivar 'Janz' (**Figure 9**). No evidence of eradicant activity was observed for any of the products under the heavy inoculum loads and more variable conditions of the field trial.

Rust was only present in the 'Janz' cultivar, and SBO (and AMF) do not appear to provide control of this pathogen (**Figure 10**). Amistar claims to control rust, but there were no significant differences among the treatments (**Figure 10**).

Irrespective of wheat cultivar, SBO was associated with significantly lower harvest yields than all other treatments (**Table 4**). Yields in the AMF treatment were lower but not significantly different from the controls and plants sprayed with Amistar had significantly higher yields than both the other treatments (**Table 4**). This suggests that there is a yield cost associated with SBO and AMF use in the field trial. There are two possible explanations for this. The first is that oil use can be associated with damage to plant tissue, which affects the ability to produce and store photosynthates [13, 17–19]. However, we did not observe any chlorosis or necrosis associated with SBO or AMF use in any of our trials on wheat. More likely

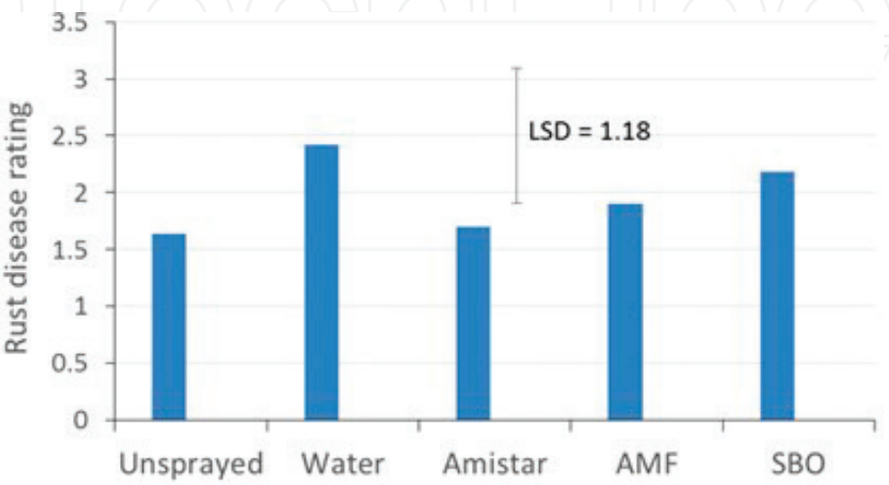

Figure 10.
Stripe rust levels in field-grown 'Janz' wheat, 123 days after sowing. Treatments are the same as described in **Figure 9**. *Data were assessed as a randomised block design with means separation by least significant difference (LSD, P < 0.05).*

Cultivar	Treatment[1]	Total grain weight (g)	First grade weight[2] (g)	Weight 1000 grains (g)
'Janz'	Unsprayed	616	599	44.5
	Water	658	641	45.1
	Amistar	672	656	46.6
	AMF	592	575	42.6
	SBO	481	463	38.4
	LSD[3]	**69.4**	**67.2**	**2.15**
'Karamu'	Unsprayed	619	578	37.5
	Water	609	569	36.7
	Amistar	840	797	42.9
	AMF	605	570	37.2
	SBO	526	479	33.0
	LSD	**49.3**	**50**	**1.62**
'Gundaroi'	Unsprayed	619	578	37.5
	Water	609	569	36.7
	Amistar	840	797	42.9
	AMF	605	570	37.2
	SBO	526	479	33.0
	LSD	**49.3**	**50**	**1.62**

[1]Control plants were left untreated or sprayed with water. Fungicide treated plants were sprayed with Amistar® fungicide (750 mL product concentrate/ha and 1.4 mL of fungicide liquid concentrate/12 L water), emulsified anhydrous milk fat (AMF) at 7 g/L and emulsified soybean oil (SBO) at 20 g/L.
[2]First grade grain has a size >2 mm.
[3]Least significant difference ($P < 0.05$).

Table 4.
Yield data of field-grown wheat: Total grain weight (g), first grade grain weight (g) and weight of 1000 grains (g), harvested approximately 170 days after sowing.

is the second explanation that disease severity has escalated to a greater degree in the plants treated with lipid fungicide than in the Amistar treatment in the 6 1/2 week interval between the final spray application and harvest. Work in other crops (cucurbits and grapes) has shown that SBO and AMF need to be applied at fortnightly intervals to maintain effective disease control (Wurms, Plant & Food Research, unpublished data), whereas Amistar is a systemic fungicide (i.e., it is absorbed into the plant) and provides disease control over a more sustained period, and therefore only needed to be applied twice during the same trial period to provide effective control of PM [39] .

4. MoA studies

4.1 Scanning electron microscopy (SEM) methods

Experimental set-up was the same as for the CE trial, except that there was no water treatment, and there were two spray applications, 3 days apart. Four days after the second spray, lesions from all the treatments were sampled for electron microscopy.

Leaf pieces (5 × 10 mm) were cut from plants and mounted on a copper specimen stub, then processed for observation using a sputter cryo system (Emscope SP2000, Hemel Hempstead, United Kingdom). Mounted samples were first frozen using liquid nitrogen slush and then transferred under vacuum to a preparation chamber. There they were thermally etched for 5 min at −80°C, radiantly etched for 30–60 s, and then sputter coated with gold. The coated material was transferred under vacuum to a cold stage in the specimen chamber of a Philips SEM 505 scanning electron microscope (Philips, Eindhoven, Netherlands) and examined at an accelerating voltage of 15 kV and a specimen temperature of between −150 and −180°C [40].

4.2 SEM trial results and discussion

Amistar®, SBO and AMF fungicides all exhibited eradicant activity via a non-toxic (physical) MoA, as illustrated by direct effects on the fungus, since these treatments caused conidiophores (spore bearing structures) to collapse (**Figure 11**), conidia (asexual spores) to wither (**Figure 12**) and extrude cellular contents (**Figures 12** and **13**) and hyphae to wither/desiccate (**Figure 13**). This is supported by the CE and glasshouse trial data which showed that, under low initial inoculum loads, disease severity decreased on the same wheat plants monitored over time (**Figures 2**, **3, 6–8**). The MoA of SBO is most likely created by disruption of membrane transport of the pathogen since the SEM images indicate that SBO causes plasmolysis of mycelia and cell rupture and leakage of cell contents, especially in conidia

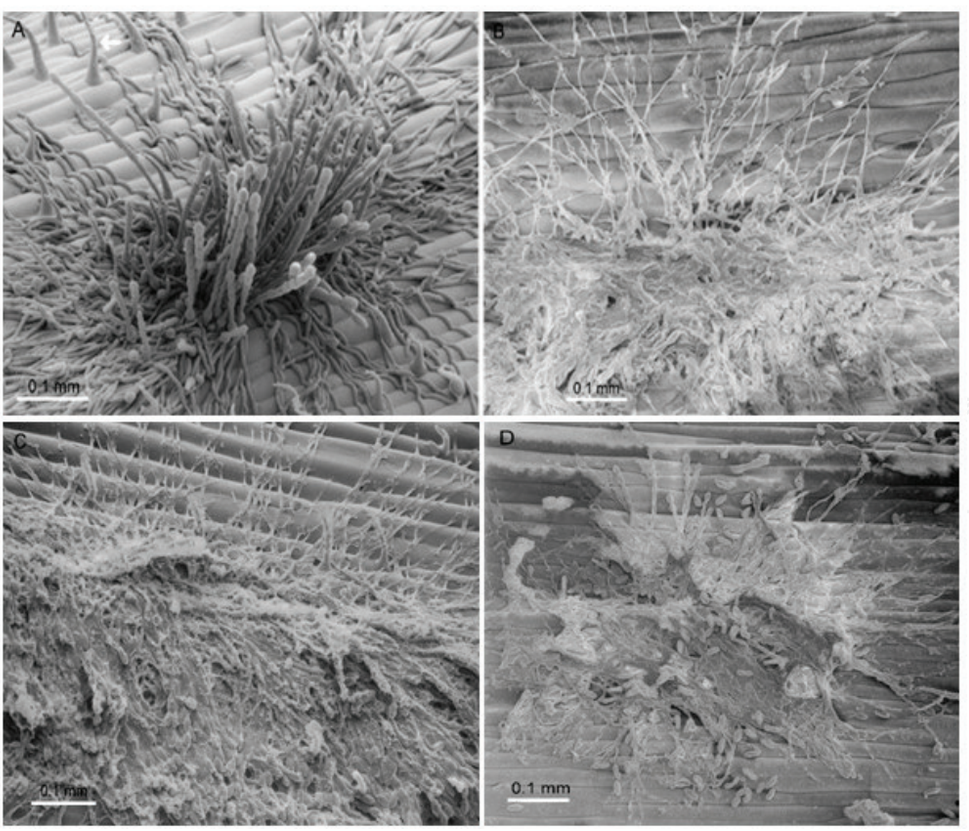

Figure 11.
SEM images of powdery mildew colonies on 'Endeavour' wheat leaves that were either A, untreated; or sprayed with: B, Amistar® fungicide; C, anhydrous milk fat (AMF); or D, soybean oil (SBO). In healthy, unsprayed colonies (A), turgid hyphal threads can be seen growing along the leaf surface in among pointy/tapered trichomes (leaf hairs), an example of which is arrowed in A, and upright conidiophores bearing chains of spherical conidia (asexual spores) are visible extending upwards and outwards from the leaf surface. Conversely, hyphae appear shrivelled and conidiophores have completely collapsed in sprayed colonies (B-D).

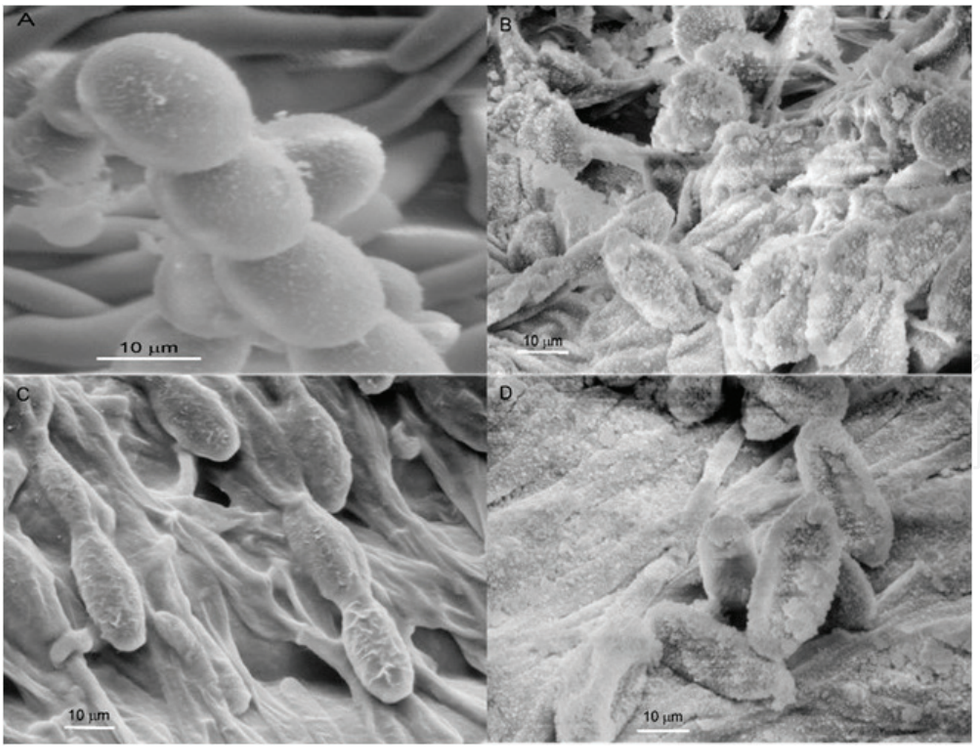

Figure 12.
Higher magnification SEM images of powdery mildew conidia on 'Endeavour' wheat leaves that were either A, untreated; or sprayed with: B, Amistar® fungicide; C, AMF; or D, SBO. In unsprayed plants (A), the conidia are present in chains attached to conidiophores that protrude outwards from the leaf surface. Unsprayed conidia have a plump/turgid appearance and the spore surface appears to be quite smooth. In contrast, conidia on sprayed plants are lying collapsed on the leaf surface and have a withered/dehydrated appearance (B-D). Ridging of the conidial surface is apparent in AMF-treated plants (C), and grainy exudates, most probably cell contents, surround conidia sprayed with fungicide (B) and soybean oil (D).

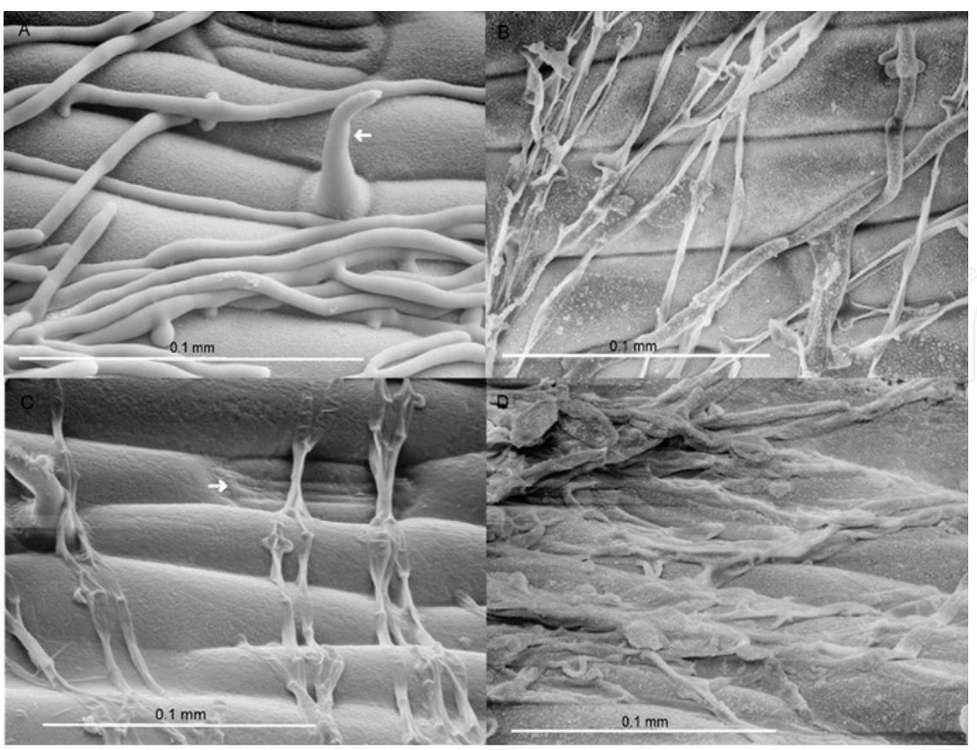

Figure 13.
SEM close-ups of powdery mildew hyphae on 'Endeavour' wheat leaves that were either A, untreated; or sprayed with: B, Amistar® fungicide; C, AMF; or D, SBO. In unsprayed plants (A), the hyphae growing on the leaf surface are plump and turgid. A trichome is arrowed in A. By contrast, hyphae on sprayed plants are completely withered/dehydrated (B-D). A plant stomate (pore for gaseous exchange) on the leaf surface is arrowed in C.

(**Figures 11–13**). This is in agreement with a review on the antimicrobial mode of action of essential plant oils, where antimicrobial action was most commonly due to membrane permeabilization/disruption leading to loss of water, leakage of cell contents and sometimes total lysis [41] . In contrast, AMF may have a different MoA to SBO, as SEM demonstrated that AMF caused deep ridging and distortion of conidia, rather than extrusion of cell contents (**Figures 11–13**). Combination of products with different complimentary MoA has the potential to increase product durability and efficacy, since it is more difficult for the pathogen to develop resistance. In addition, pathogens do not tend to develop resistance to agricultural sprays containing oils [30, 31, 42], because membrane transport is such a fundamental life process. The combination of reduced amounts of SBO and AMF has been shown to be as effective as greater concentrations of each biofungicide on its own [10]. Non-toxic (physical) rather than toxic (chemical/antibiotic) MoAs are also advantageous when it comes to product registration, since the latter higher-risk product group requires expensive and time consuming toxicology testing.

5. Commercialisation potential

SBO biofungicide shows great commercial promise, given that it provided PM control on wheat in both controlled internal conditions and more variable field environments (at least for part of the growing period) that equalled or exceeded that provided by conventional fungicides. Moreover, SBO is cost effective and simple to produce (data not presented), has a physical mode of action (thus making registration easier) and contains Generally Recognised As Safe (GRAS) [43], edible ingredients. However, there are critical factors that must be taken into consideration to ensure optimal performance and success of this product.

The most important consideration for use of this type of fungicide is that it has a direct, non-systemic mode of action [44] and therefore requires direct contact with the plant surface that it is to protect. This explains why disease control was much higher in the CE and glasshouse trials, where plants were more widely spaced, and why PM pustules were observed on the undersides of leaf blades close to the stems of plants in the field trial (data not presented), because heavily overlapping foliage prevented spray access. Our research has also shown that the percentage of grape bunch surfaces directly exposed to spray (i.e., not covered by leaves), as determined by leaf plucking, was a significant factor in the efficacy of SBO fungicide against *Botrytis cinerea* [11]. Consequently spray penetration and the density of plant architecture/growing systems are key considerations to the success of this fungicide.

We believe that other disadvantages that may be associated with SBO can be managed with careful use. Although phytotoxicity is sometimes associated with oils, optimisation of formulation (as well as rate and frequency of application) has been shown to minimise toxic effects [11, 45]. Our SBO formulation [46] has managed to achieve the balance of efficacy without adverse effects on plant health. Phytotoxicity can also be avoided by taking care not to tank mix products such as elicitors [32] or sulphur [44, 47] as these may react together to form plant damaging compounds causing foliar injury and leaf drop. However, these products can still be successfully used together in an integrated spray programme provided that their use is alternated [11]. SBO has also been demonstrated to have a much less adverse effect on plant health than AMF [32]. Another effective option is that SBO can be tank mixed with AMF at much lower concentrations than either product on its own [10]. This offers the dual advantages of reduced cost of goods and greater durability, due to differing modes of action as described in the preceding SEM section. Other recommendations include not spraying below 4°C (40°F), because the emulsion

breaks down, and avoiding sprays on newly emerged foliage or floral tissue, although we have treated rose blooms without any toxic effects [10].

Although SBO exhibited both preventative and eradicant activity in this study, eradicant activity was not effective against heavy, established inoculum loads in the field trial, and consequently SBO is best used as a preventative. Given that horticultural oils degrade readily and are not very persistent [31], they also need to be applied at regular (e.g. fortnightly) intervals. A lack of PM control over the last 6 weeks between spraying and harvest could be the reason for loss of yield in the wheat field trial, although further work would need to be carried out to confirm this by carrying out a PM disease assessment at harvest. SBO is particularly well-suited for use in an integrated pest management (IPM) programme. In New Zealand, our SBO formulation has been registered as MIDI-Zen® by BotryZen 2010 Limited, and is intended to be used a part of a residue-free IPM programme for control of *Botrytis cinerea* on grapes. Although use of MIDI-Zen right up to vintage in grapes has been shown to delay the increase in soluble sugars (°Brix), which would necessitate delaying harvest for 1–2 weeks to allow Brix to rise, this problem is normally avoided by using MIDI-Zen in the middle part of the grape growing season (from pre-bunch closure to version) and another biofungicide in the final 3 weeks leading up to harvest [11].

In summary, the potential for SBO is very exciting as it offers the potential for effective, environmentally benign and durable control. Armed with a good formulation and an understanding of how best to optimise its use and minimise any adverse effects, we should see increased use of SBO in agriculture to improve plant health.

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References

[1] Encyclopaedia Britannica. Soybean plant. [Internet]. 2018. Available from: https://www.britannica.com/plant/soybean [Accessed: 2018-06-01]

[2] Organic Facts. Thirteen amazing benefits of soybeans. [Internet]. 2018. Available from: https://www.organicfacts.net/health-benefits/cereal/soybeans.html [Accessed: 2018-06-01]

[3] Johnson LA. 11-Oil recovery from soybeans. In: Johnson LA, White PJ, Galloway R, editors. Soybeans. Chemistry, Production, Processing and Utilisation. Urbana: AOCS Press; 2008. pp. 331-375. DOI: 10.1016/B978-1-893997-64-6.50014-7

[4] Wikipedia. Soybean oil. [Internet]. 2018. Available from: https://en.wikipedia.org/wiki/Soybean_oil [Accessed: 2018-06-01]

[5] Soil Service Inc. Landoil. [Internet]. 2018. Available from: http://soilserviceinc.com/spray-surfactants/landoil/ [Accessed: 2018-06-06]

[6] Somervaille A, Betts G, Gordon B, Green V, Burgis M, Henderson R. Adjuvants—Oils, surfactants and other additives for farm chemicals. [Internet]. 2012. Available from: https://grdc.com.au/__data/assets/pdf_file/0017/222155/adjuvants-oils-surfactants-and-other-additives-for-farm-chemicals-revised-2012-edition.pdf.pdf [Accessed: 2018-06-06]

[7] Ko WH, Wang SY, Hsieh TF, Ann PJ. Effects of sunflower oil on tomato powdery mildew caused by *Oidium neolycopersici*. Journal of Phytopathology-Phytopathologische Zeitschrift. 2003;**151**:144-148. DOI: 10.1046/j.1439-0434.2003.00698.x

[8] Northover J, Schneider KE. Activity of plant oils on diseases caused by *podosphaera-leucotricha*, *venturia-inaequalis*, and *albugo-occidentalis*. Plant Disease. 1993;**77**:152-157. DOI: 10.1094/pd-77-0152

[9] Wurms KV, Ah Chee A. Control of powdery mildew (*Podosphaera leucotricha*) on apple seedlings using anhydrous milk fat and soybean oil emulsions. New Zealand Plant Protection. 2011;**64**:201-208

[10] Wurms KV, Hofland-Zijlstra JD. Control of powdery mildew on glasshouse-grown roses and tomatoes in the Netherlands using anhydrous milk fat and soybean oil emulsions. New Zealand Plant Protection. 2015;**68**:380-388

[11] Wurms K, Ah Chee A, Elmer P, Agnew R, Wood P. Developing new biologically-based products for control of botrytis bunch rot. Part 1: Developing a new natural product for mid-season botrytis control—NP2 moves closer to the market. Wine and Viticulture Journal. 2011;**26**:64-72

[12] Xuan TD, Gu GQ, Minh TN, Quy TN, Khanh TD. An overview of chemical profiles, antioxidant and antimicrobial activities of commercial vegetable edible oils marketed in Japan. Food. 2018;**7**(2):21. DOI: 10.3390/foods7020021

[13] Moran RE, Deyton DE, Sams CE, Pless CD, Cummins JC. Soybean oil as a summer spray for apple: European red mite control, net CO_2 assimilation and phytotoxicity. Hortscience. 2003;**38**:234-238

[14] Butler GD, Coudriet DL, Henneberry TJ. Toxicity and repellency of soybean and cottonseed oils to the sweetpotato whitefly (homoptera, aleyrodidae) and the cotton aphid (homoptera, aphididae) on cotton in greenhouse

studies. Southwestern Entomologist. 1988;**13**:81-86

[15] Fenigstein A, Eliyahu M, Gan-Mor S, Veierov D. Effects of five vegetable oils on the sweetpotato whitefly *Bemisia tabaci*. Phytoparasitica. 2001;**29**:197-206. DOI: 10.1007/bf02983451

[16] Shaaya E, Kostjukovski M, Eilberg J, Sukprakarn C. Plant oils as fumigants and contact insecticides for the control of stored-product insects. Journal of Stored Products Research. 1997;**33**:7-15. DOI: 10.1016/s0022-474x(96)00032-x

[17] Baysal-Gurel F, Miller SA. Management of powdery mildew in greenhouse tomato production with biorational products and fungicides. In: Paret ML, Vallad GE, Zhang S, Jones JB, editors. IV International Symposium on Tomato Diseases; Acta Horticulturae. Vol. 1069. 2015. pp. 179-183

[18] Finger SA, Wolf TK, Baudoin AB. Effects of horticultural oils on the photosynthesis, fruit maturity, and crop yield of winegrapes. American Journal of Enology and Viticulture. 2002;**53**:116-124

[19] Zhuang QG, Wang LH, Li MZ, Hou TP, Xie Y. Enspray 99′ mineral oils for white peach scale, *Pseudaulacaspis pentagona* and phytotoxicity to 'Hongyang' kiwifruit. In: Huang H, Zhang Q, editors. VIII International Symposium on Kiwifruit; Acta Horticulturae. Vol. 1096. 2015. pp. 363-369

[20] Glawe DA. The powdery mildews: A review of the world's most familiar (yet poorly known) plant pathogens. Annual Review of Phytopathology: Annual Reviews. 2008;**46**:27-51. DOI: 10.1146/annurev.phyto.46.081407.104740

[21] Pasini C, Daquila F, Curir P, Gullino ML. Effectiveness of antifungal compounds against rose powdery mildew (*Sphaerotheca pannosa* var. *rosae*) in glasshouses. Crop Protection. 1997;**16**:251-256. DOI: 10.1016/s0261-2194(96)00095-6

[22] Grains Research & Development Corporation. Barley powdery mildew. Fact sheet [Internet]. 2012. Available from: https://grdc.com.au/__data/assets/pdf_file/0028/24958/grdc-fs-barley-powdery-mildew.pdf [Accessed: 2018-06-06]

[23] Turechek WW, Mahaffee WF, Ocamb CM. Development of management strategies for hop powdery mildew in the Pacific Northwest. Plant Health Progress. 2001;**2**:1. DOI: 10.1094/PHP-2001-0313-01-RS

[24] Fuller KB, Alston JM, Sambucci OS. The value of powdery mildew resistance in grapes: Evidence from California. Wine Economics and Policy. 2014;**3**:90-107. DOI: 10.1016/j.wep.2014.09.001

[25] Colcol JF, Rallos LE, Baudoin AB. Sensitivity of *Erysiphe necator* to demethylation inhibitor fungicides in Virginia. Plant Disease. 2012;**96**:111-116. DOI: 10.1094/pdis-12-10-0883

[26] Lopez-Ruiz FJ, Perez-Garcia A, Fernandez-Ortuno D, Romero D, Garcia E, de Vicente A, et al. Sensitivities to DMI fungicides in populations of *Podosphaera fusca* in south Central Spain. Pest Management Science. 2010;**66**:801-808. DOI: 10.1002/ps.1948

[27] Ye M, Beach J, Martin JW, Senthilselvan A. Occupational pesticide exposures and respiratory health. International Journal of Environmental Research and Public Health. 2013;**10**:6442-6471. DOI: 10.3390/ijerph10126442

[28] Homestead Organics. Sulphur. [Internet]. 2018. Available from: http://www.homesteadorganics.ca/sulphur_pest.htm [Accessed: 2018-06-07]

[29] Brunetto G, Ferreira PAA, Melo GW, Ceretta CA, Toselli M. Heavy metals in vineyards and orchard soils. Revista Brasileira De Fruticultura. 2017;**39**:2(e-263). DOI: 10.1590/0100-29452017263

[30] Horticentre Group. Eco-oil. [Internet]. 2018. Available from: http://www.horticentre.co.nz/page/23-products-services+pest-disease-control+crop-chemistry-biologicals-growth-aids-and-cleaners+insecticides+insecticides-d-f+eco-oil [Accessed: 2018-06-07]

[31] Skinner A. Spraying with horticultural oils. [Internet]. 2017. Available from: http://ucanr.edu/datastoreFiles/268-754.pdf [Accessed: 2018-06-01]

[32] Ah Chee A, George M, Alavi M, Wurms K. Lipid based fungicides for control of powdery mildew in cucurbits. New Zealand Plant Protection. 2018;**71**:262-271. DOI: 10.30843/nzpp.2018.71.123

[33] Australian Wine Research Institute. Understanding chemical 'modes of action' [Internet]. 2010. Available from: https://www.awri.com.au/wp-content/uploads/spray_modes_of_action.pdf [Accessed: 2018-09-10]

[34] McGrath M. Fungicides and mode of action [Internet]. 2005. Available from: https://gpnmag.com/article/fungicides-and-mode-action/ [Accessed: 2018-09-10]

[35] AHDB Cereals & Oilseeds. Powdery mildew [Internet]. 2018. Available from: https://cereals.ahdb.org.uk/cereal-disease-encyclopedia/diseases/powdery-mildew.aspx [Accessed: 2018-06-20]

[36] Salgado JD, Paul PA. Powdery Mildew of Wheat [Internet]. 2016. Available from: https://ohioline.osu.edu/factsheet/plpath-cer-11 [Accessed: 2018-06-21]

[37] James WC. An illustrated series of assessment keys for plant diseases, their preparation and usage. Canadian Plant Disease Survey. 1971;**51**:39-65

[38] Saari EE, Prescott JM. Scale for appraising foliar intensity of wheat diseases. Plant Disease Reporter. 1975;**59**:377-380

[39] Syngenta. Amistar [Internet]. 2018. Available from: https://www.syngenta.co.nz/product/crop-protection/fungicide/amistar [Accessed: 2018-09-13]

[40] Falloon RE, Sutherland PW, Hallett IC. Morphology of *Erysiphe pisi* on leaves of *Pisum sativum*. Canadian Journal of Botany. 1989;**67**:3410-3416

[41] Hyldgaard M, Mygind T, Meyer RL. Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. Frontiers in Microbiology. 2012;**3**:24. DOI: 10.3389/fmicb.2012.00012

[42] Halcomb M, Hale F. General Comments about the Use of Horticultural Oil [Internet]. 2012. Available from: https://extension.tennessee.edu/mtnpi/Documents/handouts/Insect%20and%20Disease%20Control/Horticultural_Oil_Handout.pdf [Accessed: 2018-06-21]

[43] U.S. Food & Drug Administration. Generally recognised as safe (GRAS) [Internet]. 2018. Available from: https://www.fda.gov/food/ingredientspackaginglabeling/gras/ [Accessed: 2018-09-13]

[44] Pundt L. Integrated pest management program. Horticultural oils. [Internet]. 2015. Available from: http://ipm.uconn.edu/documents/raw2/html/831.php?aid=831 [Accessed: 2018-06-21]

[45] Wurms K, Ah Chee A. Product formulation is crucial to the success

of lipid-based bio-fungicides. New Zealand Plant Protection. 2018;**71**:272-284. DOI: 10.30843/nzpp.2018.71.124

[46] Wurms KV, Ah Chee A. Fungicidal composition comprising anhydrous milk fat (AMF) and soybean oil for the treatment of powdery mildew. Horticulture & Food Res Inst assignee. NZ patent NZ534007A; 2007

[47] Extension Toxicology Network. Sulfur. [Internet]. 1995. Available from: http://pmep.cce.cornell.edu/profiles/extoxnet/pyrethrins-ziram/sulfur-ext.html [Accessed: 2018-06-21]