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Efficacy of essential oils and biopesticides on *Phytophthora infestans* suppression in laboratory and growth chamber studies

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Abstract
Control of late blight by alternative products is important for reduction of fungicide inputs and potato production costs. The efficacy of essential oils and biopesticides for inhibition of growth of *Phytophthora infestans* on media and suppression of late blight on potato plants in growth chambers was examined. Growth of pathogen isolates of diverse genotypes was evaluated on Rye B media amended with essential oils (lavender, thyme, thyme borneal, and oregano) and the biopesticide Serenade (*Bacillus subtilis* strain QST 713). Over 90% inhibition of pathogen growth was achieved with oregano and Serenade amendments. The protective foliar application of Serenade, an aerated compost tea (ACT), Effective Microorganism mix (EM), and oregano, resulted in disease suppression of 5–40% relative to the untreated control. ACT had no significant suppressive effects (0–15% reduction), EM resulted in mild suppression (15–30% reduction), and oregano and Serenade consistently resulted in moderate disease reduction (20–40%). No oil or biological treatment produced disease control comparable to the chemical control chlorothalonil, which resulted in disease reductions of 80–98%. Both oregano and Serenade resulted in some phytotoxicity at high doses. These results suggest that the natural products and biological amendments tested are not sufficient for effective late blight control by themselves; however, when used in combination with other established disease control practices, these approaches may contribute to improved, integrated, and more sustainable management options for late blight.

Keywords: *Natural products, biopesticides,* Phytophthora infestans, *disease suppression, essential oils, potato*

Introduction

Potato production in Maine has varied considerably during the last decade. This may be attributed to market fluctuations and the presence of disease constraints (Lambert & Currier 1997; Groves 2002; Larkin & Honeycutt 2006). Among the diseases or pests affecting potato production in Maine, late blight, caused by *Phytophthora infestans* has been the most significant factor (Groves 2002; Olanya et al. 2002). The

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constraints imposed by potato late blight include reduced yield, storage rot and fungicide costs. Although potato late blight can be controlled by using a combination of fungicide applications and host resistance, selection of more resistant varieties is not always a viable option due to the variety requirements of the processing industry and other specific markets. In addition, when conditions are favourable for disease development, fungicide applications are recommended every 5–7 days throughout the growing season (Kirk et al. 2001, 2005). Viable options for reducing the cost, quantity, and number of applications of fungicides currently used for late blight control would improve the sustainability of potato production.

Natural compounds or environmentally benign products have been documented for managing fungal and bacterial diseases and pests in numerous pathosystems. Natural compounds consisting of essential oils and organic acids have been evaluated for their efficacy as seed treatments for seed-borne pathogens (Groot et al. 2005). In this research, thyme (essential oil) exhibited the highest *in vitro* inhibiting activity against two bacterial and fungal seed-borne pathogens (Groot et al. 2005). Natural products or biological amendments (biopesticides) may have value for late blight management provided their effectiveness can be documented and pesticide applications reduced. Natural products, such as plant essential oils, have shown some bioactivity on *P. infestans* (Quintanilla et al. 2002) and on plant pests and diseases (Isman 2000). Plant essential oils have been reported to reduce the growth of fungal and bacterial pathogens such as *Botrytis cinerea, Fusarium* spp., and *Clavibacter michiganensis* (Daferera et al. 2003). A commercial formulation of the bacterium *Bacillus subtilis* (Ehrenberg) Cohn, Serenade™ (strain QST713; AgraQuest, Davis, CA), has been reported to effectively control blueberry flower infection caused by *Monilinia vaccinii-corymbosi* (Scherm et al. 2004). Aerated compost tea (ACT), produced by mixing compost with water in the presence or absence of additives and aerobically incubating for a defined period, has been used to suppress a range of foliar diseases (Weltzien 1991; Scheuerell & Mahaffee 2002). ACT has also been shown to be effective in suppressing seedling damping-off caused by *Pythium ultimum* in container-based studies (Scheuerell & Mahaffee 2004), bacterial spot of tomato (Al-Dahmani et al. 2004), and *Phytophthora nicotianae* and Phytophthora blight in soil and greenhouse trials (Bowers & Locke 2004). In Asia, the concept of Kyusei nature farming has been successful, in which a defined mixture of fermenting yeast, lactic acid bacteria, phototrophic bacteria, and other microorganisms, called Effective Microorganisms (EM), are applied to compost, soil, and foliage to improve productivity and disease control (Higa 1991, 1994). Soil rotation practices and amendments that augment soil microbial communities have been documented to reduce soil-borne potato diseases (Larkin & Griffin 2003; Larkin & Brewer 2005; Larkin & Honeycutt 2006).

Despite the progress in biological control of soilborne and some foliar fungal and bacterial diseases of crop plants, limited information is available on control of potato late blight using natural or biologically based products. Control of tuber blight in storage by using biological agents has been reported. A 20–60% reduction in late blight was observed when *Pseudomonas fluorescens* and *Enterobacter cloacae* were mixed with zoospores and used to inoculate potato tubers under storage conditions (Slininger et al. 2004). Significant inhibition of *P. infestans* in media by a bio-control agent, *Streptomyces violaceusniger* strain YCED-9 was reported as a result of antibiotic production by the actinomycete (Trejo-Estrada et al. 1998). Leaf extracts of a perennial weed *Inula vicosa* (sticky fleabane) were documented to be highly effective

for control of potato or tomato late blight and various foliar diseases such as downy mildew of cucumber, powdery mildew of wheat and rust of sunflower in growth chamber experiments at concentrations ranging from 0.68 to 1% (Wang et al. 2004). Significant control of potato late blight (38–96%) on detached leaves and seedlings was reported when plant extracts from *Galla chinese, G. rhabarbarum,* and *Sophira flavescens* were applied as a protective foliar spray in China (Wang et al. 2004b). Similarly, Chinese medicinal plants were also found to be effective for late blight inhibition on detached leaves (Cao et al. 2004). The use of compost tea for late blight control in organic potato production in Maine and elsewhere has been attempted (J. Gerritsen, organic potato grower, personal communication). Except for these few reports, little has been published on the effectiveness of natural products for late blight control. However, increasing concerns with environmental/health effects, pesticide restrictions, and development of fungicide resistance, along with the desire for more sustainable production practices, have enhanced the attractiveness of alternative products for late blight control. Therefore, the objectives of this study were to: (1) evaluate the effects of natural products and biopesticides on *in vitro* inhibition of *Phytophthora infestans* in lab assays; and (2) determine the effectiveness of these natural products and amendments on the development and suppression of late blight disease on potato plants in growth chamber experiments.

Materials and methods

Essential oils, biological amendments, and isolates of Phytophthora infestans

The essential oils used were commercial products derived from lavender, oregano, thyme, thyme borneal and majoram (Aromaland, Inc., Santa Fe, NM). A commercial biopesticide containing *Bacillus subtilis* strain QST 713 (Serenade; AgraQuest, Davis, CA) was used as recommended by the manufacturer. Effective Microorganisms (EM-1™; EMRO USA, Tucson, AZ, USA), a mixture of fermenting yeast, lactic acid bacteria, phototrophic bacteria, and other microorganisms was obtained as a concentrate, and activated before use. EM was activated by adding EM-1, molasses, and water in a 1:1:20 (volume) ratio, followed by mixing and fermenting in a tightly sealed (anaerobic) container for 3–7 days. The mixture was used after the pH dropped below 3.9. An aerated compost tea (ACT), was aerobically brewed for 24 h using a commercial 83.3-L Earth Tea Brewer™ (Sustainable Agriculture Technologies, Inc., Cottage Grove, OR) from vermi-compost and nutrient additives obtained from and according to manufacturer recommendations. Mefenoxam (Ridomil Gold™, 46% a.i.) and chlorothalonil (Bravo 500™, 40.4% a.i., Syngenta Inc., Greensboro, NC) were used as chemical controls. Isolates of *Phytophthora infestans* representing diverse genotypes were obtained from the culture collection of the USDA-ARS, NEPSWL, Orono, ME (Groves 2002). Genotype designations were based on glucose phosphate isomerase (gpi) allozyme analysis (Goodwin et al. 1995). Most isolates were collected from Maine potato fields. Additional tester isolates for specific genotypes were obtained from Dr W. Fry, Cornell University (isolates 99-1, 99-3, and 99-5). All cultures were maintained on Rye A medium (Canten & Jinks 1968) and subsequently grown on Rye B medium (Canten & Jinks 1968) for use in the *in vitro* tests and growth chambers.

Inhibition of in vitro *growth of* P. infestans

Inhibition assays were conducted on Rye B agar medium in plastic petri plates (9 cm diameter) with and without additions of essential oils, biopesticides, and chemical controls. All essential oils were filter-sterilized prior to use, and were added to molten media just prior to pouring agar. Two experiments were conducted for *in vitro* growth assays. The essential oils (oregano, lavender, thyme, thyme borneal, majoram) and Serenade were added to agar media at concentrations of both 100 and 1000 ppm. Mefenoxam was added to media at 5 and 100 ppm as a chemical control. A control treatment consisted of Rye B media with no additives. After the media was solidified and cooled, isolates of *P. infestans* representing diverse pathogen genotypes were transferred to the center of each plate. In experiment 1, isolates of *P. infestans* used represented the gpi genotype designations 100/111/122 (US-8; isolates 00-109, 00-37, and 02-5), 100/100 (isolate 00-77), 100/122 (US-14; isolate 02-1), and 86/100 (US-1; isolate 99-1). In experiment 2, genotype 100/111/122 was represented by isolates 99-3, 99-73, and 99-46, genotype 100/122 was represented by isolates 99-13A and 99-77, and genotype 86/100 by isolates 99-33 and 99-5. Each experiment was established with a factorial treatment structure consisting of $8 \times 3 \times 6$ (natural product treatments, concentrations, *Phytophthora* isolates, respectively) for experiment 1, and $7 \times 3 \times 6$ for experiment 2, arranged in a completely randomized design (CRD) with five replications. Each petri dish constituted an experimental unit. Inoculated plates were incubated at 18°C, and colony diameter was measured in two directions after 6 days of growth. Measurements of subsequent colony diameter were conducted every 3–4 days thereafter. Pathogen growth inhibition was determined as the percent reduction of growth relative to the nonamended controls.

Efficacy of biopesticides and an essential oil in suppression of late blight in growth chamber experiments

Potato plants (variety Shepody) were grown in the greenhouse for 4 weeks. Each compound or natural product was applied to potato foliage in growth chambers 24 h prior to inoculation with *P. infestans.* These products were applied at different concentrations based on commercial recommendations. In Experiment 1, Serenade was applied at the concentration of 0.8%, EM was applied at 2%, oregano was applied at 0.1%, and compost tea was applied until run-off at 100% concentration. A negative control treatment, in which plants were sprayed with sterile distilled water was also included. In Experiments 2 and 3, chlorothalonil was applied as a protective foliar spray to compare natural products with a commercial fungicide. In both experiments (2 and 3), oregano was applied at the concentration of 1.5%, EM at 10%, Serenade at 8%, chlorothalonil at 0.25%, and sterile distilled water (control) was applied until run-off. Plants were also treated with natural products with no pathogen added to determine any phytotoxic effects.

Plants (three plants/treatment per growth chamber) were inoculated in Experiment 1 with a sporangial suspension of *P. infestans* (US-8; genotype 100/111/122, isolate 00-109) at 300 sporangia/mL at 24 h after treatment application, and placed at 90% RH and 18C in each of three replicate growth chambers. In Experiments 2 and 3, sporangial concentrations were increased to 13,750 spores/mL because late blight levels were low in Experiment 1. The final volume to run-off was 5.5 mL. After 3 days of incubation, potato plants were assessed for whole plant late blight severity, single

leaflet blight severity, late blight incidence, and lesion numbers as follows. Whole plant late blight severity was estimated as the percentage of total diseased leaf area. Single leaflet disease severity represented the percent diseased area on each of three designated leaflets/plant. Disease incidence on leaves (or infection frequency) was measured as the number of diseased leaves divided by total number of leaves (diseased and non-diseased). Defoliation was also quantified as the number of leaves abscised relative to total number of leaves. Single leaflet disease severity and lesion numbers/leaflet assessments were made on three randomly selected leaflets per plant previously selected and tagged prior to late blight assessments. Plant height and phytotoxic effects or foliar damage attributed to natural products were also recorded on plants treated with oregano, Serenade or other biopesticides alone. All experiments were conducted as a randomized complete block design (RCBD) with three replicate blocks (growth chambers)

Effects of product concentration on late blight suppression and plant damage

The relationship of application dose or concentration of each natural product to efficacy of suppression of late blight was evaluated in growth chamber assays. The experiment consisted of 16 treatments arranged in a RCB with three replications. Oregano, Serenade and EM were each applied as protective foliar sprays onto potted plants at low (0.1–2%), moderate (2–10%) and high (20%) application rates at 24 h prior to pathogen inoculation. Chlorothalonil was applied at the recommended rate of 0.75%. Oregano was applied at 0.1, 2, and 20%, representing low, moderate and high concentrations, respectively. EM was applied at: 2%, 10% and 20%. Serenade was applied at 2.5%, 10% and 20% concentrations. Potted plants were incubated under the same conditions as previously described. Based on visual symptoms, the number of diseased leaflets and total number of leaves were quantified for each treatment. Plant damage was assessed as the average percent of leaf area showing symptoms.

Data analysis

Effects of natural product treatment, amendment concentration, pathogen isolate, and assessment date on *in vitro* pathogen growth (colony diameter) and percent reduction of pathogen growth were determined by analysis of variance (ANOVA) with factorial treatment structure. All plant and disease measurements in growth chamber studies were analyzed by ANOVA as randomized complete block designs, with each growth chamber representing a block. The experiment was repeated two times. Mean separation was accomplished using Fisher's protected LSD test. All statistical analyses were conducted using the general linear models procedures of the Statistical Analysis System ver. 7.0 (SAS Institute, Cary, NC).

Results

Inhibition of in vitro growth of P. infestans

No significant experiment by treatment interactions were observed for *in vitro* inhibition of *P. infestans* growth. Therefore, data from two experiments were combined for final analysis. Significant factor effects in the inhibition of pathogen growth were observed for natural product treatments, concentrations, isolates of *P. infestans*, as well

as for treatment × concentrations; treatment × isolate; isolate × concentration; and isolate × concentration × treatment interactions ($P < 0.001$).

All treatments significantly reduced *in vitro* growth of *P. infestans* when media was amended with natural products and biopesticides (Table I). Within each pathogen genotype, similar responses were observed among multiple isolates tested. The reduction of mycelial growth differed among isolates representing diverse genotypes, and ranged from 24.2 to 100% (Table I). When averaged across all pathogen genotypes, the greatest reduction in growth was obtained with oregano and Serenade at 100 ppm (Table I). The essential oils lavender, thyme, and thyme borneal variably reduced growth. When a pathogen isolate of 100/111/122 genotype designation (US 8) was used, reduction of growth was equally high for Serenade, thyme borneal, thyme and oregano treatments and less when mefenoxam was used. Serenade (*B. subtilis* strain QST 713) and oregano (essential oil) consistently inhibited growth of *P. infestans* on Rye B over time (Figure 1). *In vitro* growth of isolates of *P. infestans* on Rye B media amended with oregano and Serenade was inhibited by over 90% relative to the nonamended control. Generally, over 50% reduction in growth was achieved when thyme or thyme borneal was applied. Lavender was less effective in reducing mycelial growth. After 3 weeks of incubation, inhibition of pathogen growth by mefenoxam and lavender was 58 and 40%, respectively, whereas inhibition by Serenade and oregano remained near 100% (Figure 1).

Product concentration significantly affected pathogen growth on amended media. Inhibition of mycelial growth was greater at the higher concentration of 1000 ppm compared to 100 ppm for all treatments, with the exception of oregano and Serenade, which completely suppressed growth at both concentrations (Figure 2). Similarly,

Table I. Effects of amendment of Rye-B media with different natural products (essential oils and a biopesticide) on inhibition (%) of *in vitro* growth of isolates of *P. infestans* from different genotypes.

	Genotypes and isolates of *P. infestans*[x]			
	100/111/122 (00-109)	100/100 (00-77)	100/122 (99-13A)	86/100 (99-1)
Treatment[w]	Inhibition (%)			
Lavender	69.7 b	28.7 c	51.0 b	41.9 b
Thyme Borneal	88.7 a	87.1 a	98.5 a	99.4 a
Thyme	94.4 a	50.3 b	89.4 a	94.1 a
Oregano	99.6 a	82.7 a	99.7 a	99.6 a
Serenade	100 a	100 a	100 a	100 a
Mefenoxam	44.9 c	91.0 a	24.2 c	89.6 a
Means[y]	82.89	73.31	77.13	87.44
$LSD_{(.05)}$	17.6	21.5	26.59	31.78

[w]The essential oils, the biopesticide Serenade (*Bacillus subtilis*), and the chemical control mefenoxam, were all added to Rye B medium at the rate of 100 ppm. [x]The isolates 00-109, 00-77, 99-13A and 99-1 represent the genotypes 100/111/122 (US 8), 100/100, 100/122 (US-14) and 86/100 (US-1) respectively, based on allozyme analysis with glucose 6-phosphate isomerase (gpi). After media was poured and cooled, isolates were added to amended and unamended agar plates and incubated for 14 days at 18 C. [y]Data represent the reduction in radial growth relative to the unamended control on day 16 after inhibition. Means within columns followed by the same letter are not significantly different according to Fisher's protected LSD test ($P < 0.05$). Means represent five replicate plates per treatment and isolate combinations.

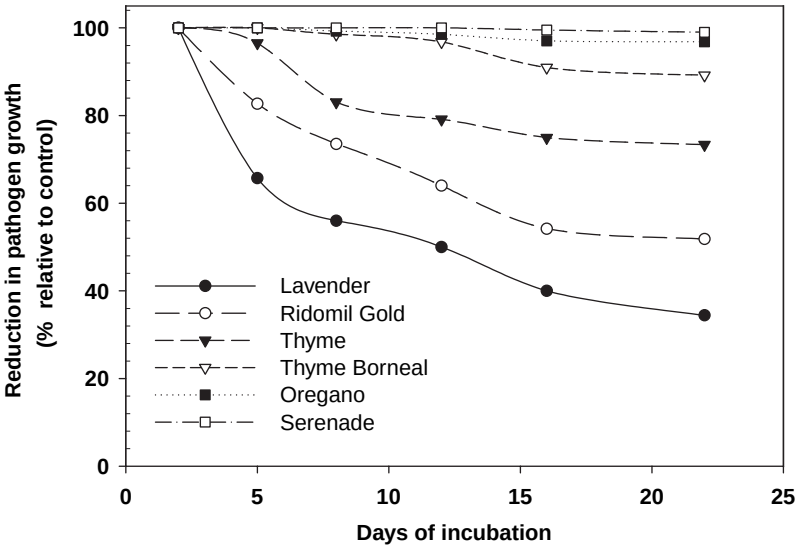

Figure 1. Inhibition of *in vitro* growth of *P. infestans* on Rye B media by essential oils (lavender, thyme, thyme borneal and oregano), a biopesticide (Serenade), and mefenoxam after incubation at 18°C for 21 days. Media was amended with products at 100 ppm and data represents mean of five replicates. The reduction in growth is calculated relative to the unamended control.

inhibition of growth was greater at 100 ppm compared to 5 ppm when mefenoxam was incorporated into medium (data not shown).

Efficacy of biopesticides and an essential oil in suppression of late blight in growth chamber experiments

Late blight development was limited in Experiment 1, with average late blight severity ranging from 27 to 45% diseased leaf area across all treatments after 11 days incubation. However, EM, oregano, and Serenade treatments resulted in significant reduction of late blight (25–40% reduction) (data not shown).

In Experiments 2 and 3, no significant experiment by treatment interaction was detected for late blight suppression, so data from both experiments were combined for analysis. Average late blight severity based on whole plant symptoms increased from 21% to nearly 60% in untreated control plants from 3 to 11 days after inoculation (Figure 3). Late blight progress was slowest and disease severity the lowest when chlorothalonil treatments were applied. Natural product treatments applied to foliage prior to pathogen inoculation suppressed late blight development to varying degrees relative to the untreated control. Over all assessment dates, EM microbial inoculants resulted in reductions in late blight severity (15 to 30%), whereas compost tea had little effect on late blight development (0–15% reduction) (Figure 4). Oregano reduced average late blight severity by 20–38%, and the bio-pesticide Serenade reduced late blight severity by 30–40%. However, none of the natural products or biopesticides (compost tea, EM, oregano, or serenade) resulted in disease suppression comparable to the chemical control, chlorothalonil, which reduced late blight by 90% compared to the untreated control.

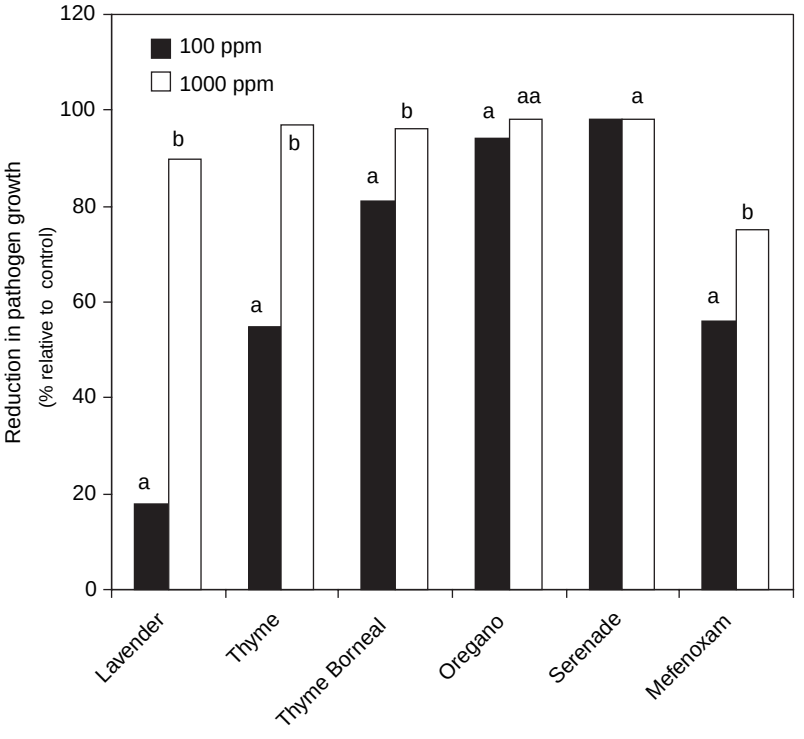

Figure 2. Effects of amendment of Rye B media with lavender, thyme, thyme borneal, oregano (essential oils), Serenade, and mefenoxam on *in vitro* growth. Inhibition of growth of *P. infestans* (US 8 isolate, 100/111/122 genotype) is expressed relative to the unamended control. Plates were amended at 100 and 1000 ppm and incubated at 18°C for 21 days. Data consist of means of five replicates.

Late blight incidence was nearly 100% on pathogen-inoculated control plants, and incidence was significantly reduced on plants treated with EM, oregano, and Serenade, but not with compost tea (Table II). Only Serenade significantly reduced late blight severity based on single leaflet assays. None of the natural product treatments provided comparable control to that of chlorothalonil, which resulted in the lowest incidence and severity of late blight of any treatment (Table II).

Treatments also affected the number of lesions per leaf (Figure 5). All treatments except compost tea significantly reduced the number of lesions relative to the untreated control (Figure 5). EM, oregano, and Serenade treatments reduced lesion numbers by 38, 45, and 52%, respectively; whereas chlorothalonil reduced lesion numbers by 80%. The level of disease suppression decreased over time (3–11 days after pathogen inoculation) for all treatments, except chlorothalonil, which maintained high levels of suppression throughout (Figure 6). Compost tea treatments were not effective at any sampling time.

Effects of product concentration on late blight suppression and plant damage

The application of biopesticides or natural products at different concentrations resulted in variable suppression of late blight. At 2 and 10% concentrations, EM did

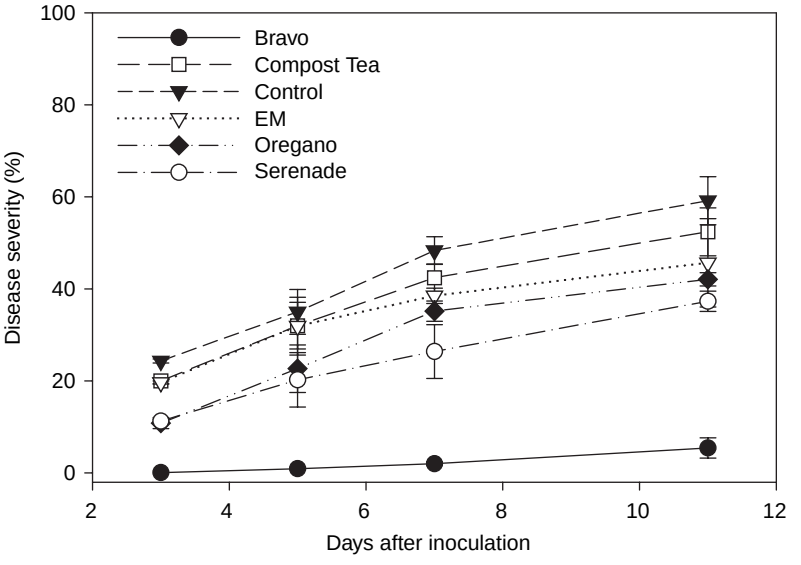

Figure 3. Development of late blight disease severity on potato as affected by foliar application of various natural products and biopesticides in growth chamber assays. Treatments included Compost tea, Effective Microorganisms (EM – *Lactobacillus* sp., phototrophic bacteria and yeast); Serenade (*B. subtilis*); Oregano (essential oil), and chlorothalonil. The chlorothalonil and biopesticides were applied 48 and 24 h prior to inoculation with *P. infestans* (100/111/122 genotype). Data represent average disease severity (%) and standard errors assessed on three plants per treatment in each of three replicate growth chambers at four assessment dates over time.

not significantly affect infection frequency. Application of EM at 20%, oregano at 0.2%, and Serenade at 2.5% resulted in significant reductions in infection frequency and moderate levels of control (50–70% reduction in infection frequency, Figure 7). Application of Serenade at 10 and 20% resulted in greater reductions in infection frequency (80–85%). Oregano exhibited a strong phytotoxic activity when applied at 2 and 20%. At the 20% concentration, 100% plant mortality was recorded. Some phytotoxicity was also noted on plants treated with Serenade at 10 and 20% rates, which appeared as small necrotic or chlorotic specks on leaves (8–16% of leaf area). However, no plant mortality was observed with Serenade at these rates. No phytotoxicity was noted when plants were treated with EM, chlorothalonil, or compost tea.

Relationship of foliar application of selected biopesticides and essential oil on plant height and defoliation

The application of natural products did not significantly affect plant height, whether subsequently inoculated with *P. infestans* or not (Table III). Height of 4-week-old potato plants after 10 days of incubation following biopesticide applications ranged from 23 to 26.5 cm (Table III). No defoliation of potato plants was detected when chlorothalonil, EM, or oregano was applied onto plants without subsequent inoculation with late blight, whereas Serenade applied at a moderate rate (8%) resulted in a small amount of defoliation (2%). Following inoculation with the late blight pathogen, all treatments resulted in some degree of defoliation. Defoliation was

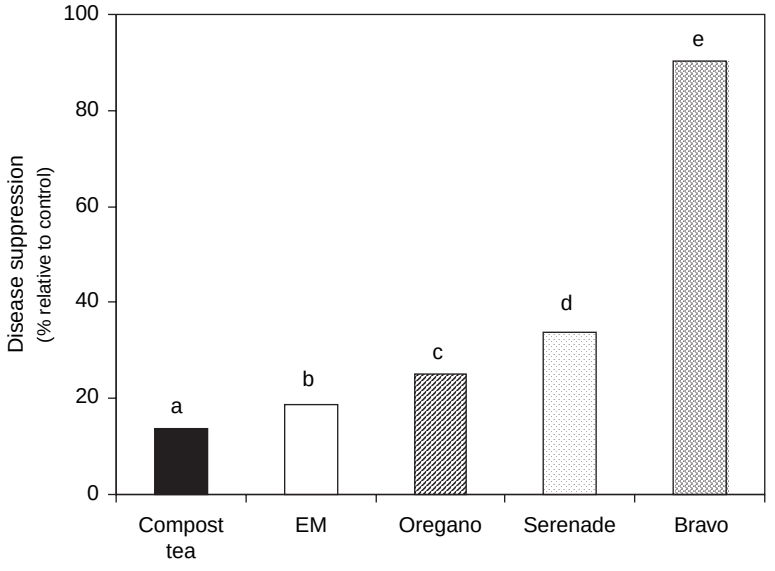

Figure 4. Efficacy of foliar application of natural products in disease suppression of late blight on potato plants. Treatments include Compost tea, Effective microorganisms (EM – *Lactobacillus* sp., phototrophic bacteria and yeast); Serenade (*B. subtilis*); Oregano (essential oil), and chlorothalonil. The chlorothalonil and biopesticides were applied 48 and 24 h prior to inoculation with *P. infestans* (100/111/122 genotype). Data represent reduction of disease severity relative to the untreated control after 11 days of incubation at 18°C, 95% RH in growth chambers. Each experiment consisted of three replicates (growth chambers) and three potato plants (var. Shepody) per replicate. Data represent average of two experiments.

observed in the pathogen-treated control and oregano treatments, with all other treatments substantially reducing the amount of defoliation caused by late blight (Table III).

Discussion

The *in vitro* inhibition of *P. infestans* by Serenade and essential oils indicate their anti-microbial activity and potential for late blight control. The inhibition of growth of the pathogen on media by Serenade suggests strong activity of the active agent *B. subtilis* in the inhibition process. Oregano, thyme and thyme borneal also exhibited moderate to strong inhibition activity against *P. infestans*, whereas lavender was less effective. Essential oils demonstrated significant inhibition at 100 and 1000 ppm. These results are similar to those of other researchers in which variation in the anti-fungal activity of essential oils in response to concentration has been reported (Wilson et al. 1996; Daferera et al. 2003). Regarding Serenade, inhibition of pathogen growth by toxins produced by the bacterium may be a factor. The role of *Bacillus* spp. in biocontrol of plant diseases has been previously identified (Jacobson et al. 2004). The reduction in pathogen growth due to natural products may be attributed to various fungitoxic or fungistatic active constituents of the essential oils. Active anti-microbial compounds, such as thymol, carvacrol, and linalol, have been previously identified and reported in oregano, thyme and lavender respectively (Daferera et al. 2003). The use of essential oils for pest and disease management has been attributed to their fungicidal and insecticidal properties (Isman 2000). Some effectiveness of essential oils on *P. infestans*

Table II. Effects of foliar application of biopesticides and essential oil on disease incidence of *P. infestans* in growth chamber studies.

Treatment[w]	Late blight incidence[x] (%)	Leaflet severity[x] (%)
Control	99.3 a	98.7 a
Compost tea	91.8 ab	95.3 ab
EM	83.8 bc	93.0 ab
Oregano	76.2 c	89.2 ab
Serenade	74.6 c	85.8 b
Chlorothalonil	5.7 d	22.5 c

[w]All treatments were applied to foliage until run-off. Compost tea was aerobically brewed from vermicompost for 24 h, and applied full-strength. Effective Microorganism (EM-*Lactobacillus* sp., yeast and phototrophic bacteria), oregano, and Serenade (*B. subtilis*) were applied at 10, 1.5, and 8%, respectively. Chlorothalonil, a chemical control was applied at 0.25%, and water was applied for the untreated control. [x]Late blight incidence and percent leaf area diseased (three leaflets) on Shepody. The pathogen genotype 100/111/122 (US 8, isolate 00-109) was used. Data represent means of three plants per growth chamber or replication assessed after 10 days of incubation. Data represent means from two experiments, and means followed by the same letter are not significantly different.

has been previously reported (Quintanilla et al. 2002). In that research, as in the present study, essential oils showed greater efficacy for controlling *P. infestans* when tested *in vitro* than *in vivo* assays.

Differences in inhibition of growth of diverse genotypes of *P. infestans* were noted in this study. Observed variation in pathogen growth may be a result of differences in sensitivity to the essential oils among the different genotypes. Although variation also exists among individual isolates *P. infestans*, multiple isolates within each genotype showed similar responses, indicating that some differences in sensitivity may be related to genotype. Interestingly, genotype 100/100 was least sensitive to inhibition by lavender, thyme, thyme borneal, and oregano of all the genotypes tested, but was most sensitive to mefenoxam. On the other hand, genotypes 100/111/122 and 100/122 were least sensitive to mefenoxam, but were highly sensitive to inhibition by oregano, thyme, and thyme borneal. Although this does not suggest that sensitivity to essential oils is in any way related to mefenoxam resistance, it does show how different assemblages of traits may be associated with different genotypes. However, all isolates were completely suppressed by Serenade. Differential inhibition of specific fungal and bacterial pathogens such as *Botrytis cinerea*, *Fusarium* sp., and *Clavibacter michiganensis* subsp. *michiganensis* to some essential oils has also been documented (Daferera et al. 2003). High levels of resistance of genotypes 100/111/122 and 100/122 (also known as US-8 and US-14) to mefenoxam has been previously reported (Groves 2002). Differences in genotypic responses of *P. infestans* to fungicidal compounds also have been previously reported (Deahl et al. 1992; Kato et al. 1997; Fontem et al. 2005). On the other hand, no differences among isolates from different genetic backgrounds to dimethomorph were obtained (Kirk et al. 2005).

Of the biopesticides and essential oils tested for suppression of late blight on potato plants, Serenade, oregano, and EM treatments all reduced late blight to some degree, whereas the aerated compost tea generally had no effect. However, none of the natural products reduced late blight development comparable to that of an effective chemical control, and none produced a level of late blight control necessary for practical effectiveness in the field. No natural product treatment reduced late blight by 50% or more. Given the rapid and potentially devastating nature of late blight epidemics,

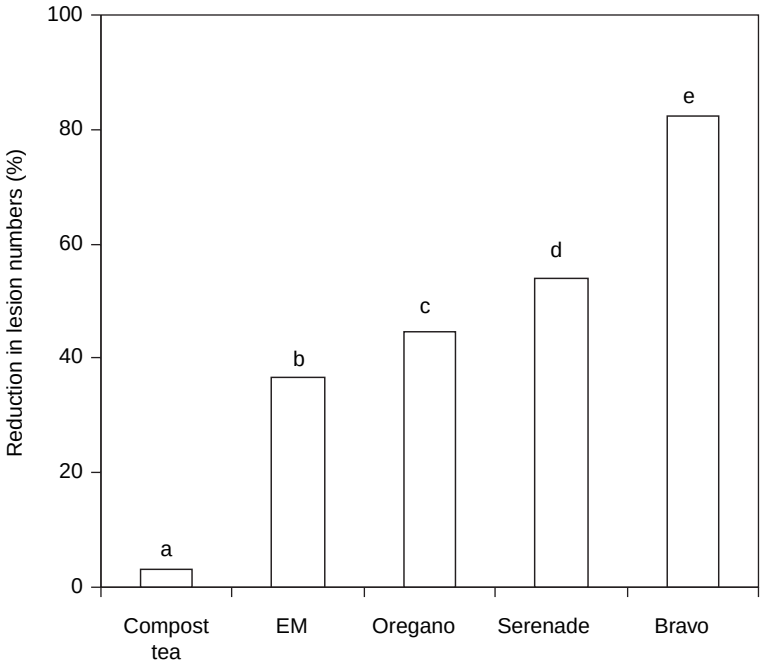

Figure 5. Effect of foliar application of biopesticides and chlorothalonil on reduction of lesion numbers of *P. infestans* on diseased plants. Lesion numbers were calculated relative to the untreated control plants in growth chambers and were assessed on three leaflets per plant randomly tagged for disease assessment.

pathogen or disease suppression must be greater than 80–100% for effective control. Thus, until improvements in efficacy are made, natural products may best be used in conjunction with other proven late blight management practices as an integrated approach to potentially reduce the number and frequency of fungicide applications.

Serenade (*B. subtilis*) was more effective in suppressing late blight than the other natural products tested. The bacterium *B. subtilis* is known to produce lipopeptides or toxins which reduce pathogen growth or is toxic to them. The low suppression of late blight on potato plants by oregano despite the inhibitory *in vitro* activity suggests that this essential oil may have more limited potential for late blight suppression. The difference in efficacy between *in vitro* and *in vivo* (plant) assays may be attributed to the culturing conditions in the Petri dish assays which enable the full toxicity of essential oils to be retained. Similar findings have been observed by other authors (Bang 1995). The high volatility of oregano and other essential oils may result in a low retention of toxic compounds on the leaf surface, and inhibitory activity may not last long enough to provide effective pathogen control on plant surfaces under natural conditions.

The low level of disease suppression by compost tea and EM in our studies may be attributed to their lower inhibitory activity against *P. infestans*, lack of persistence or retention on potato foliage surface long enough to permit disease suppression, low establishment of potential bio-control agents, or that constituent microbes in EM and compost tea were not effective in suppressing infection and disease spread by *P. infestans*. Previous research has documented a rapid loss of microorganisms applied to foliage by compost tea for control of *P. infestans* (Sturz et al. 2004). The lack of

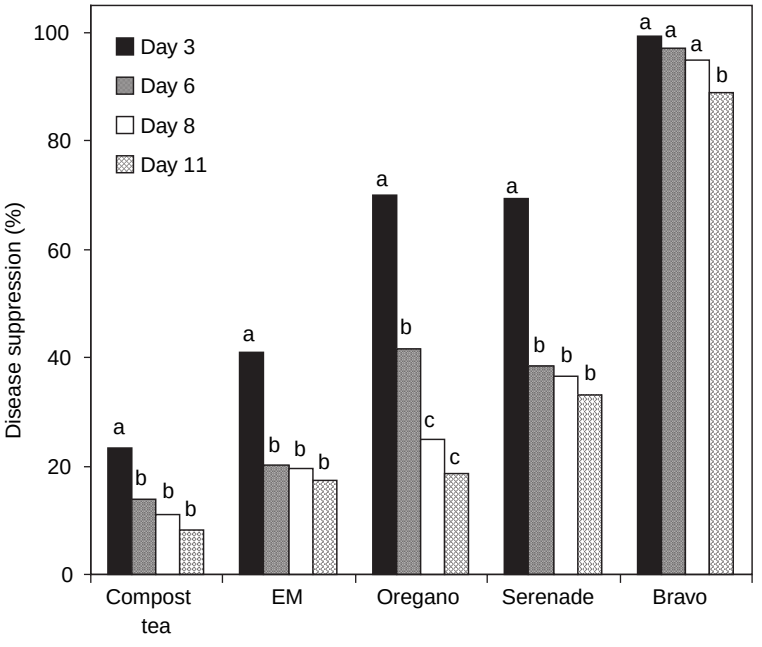

Figure 6. Relative late blight suppression at several disease assessment periods in growth chamber experiments. The biopesticides (Compost tea, Serenade – *B. subtilis*, Effective microorganism – EM); essential oil (oregano) and the fungicide chlorothalonil were applied as protective foliar sprays on potato variety Shepody prior to pathogen inoculation with *P. infestans* (100/111/122 genotype). Disease reduction levels were calculated in relation to the untreated control for each assessment period. Data consist of average of two experiments.

disease control by compost tea was attributed to failure in establishment of potential biocontrol microbes on the leaf surface (Sturz et al. 2004). In our study, we did not quantify initial and final microbial populations in compost tea or EM in the extract or on the phylloplane of potato foliage. However, the constant misting of foliage by the humidifier may have contributed to removal of biocontrol microbes from the leaf surface. The lack of disease control may also be at least partially attributable to the rapid spread known to occur with late blight. The low disease suppression exhibited by compost tea in our study could also be due to lack of the necessary quantity and diversity of microorganisms required for effective disease control. Different composts and compost teas can have very different microbial characteristics which may result in differential activity on pests and diseases (Scheuerell & Mahaffee 2002). Although the same source of compost and brewing recommendations that previously provided high quality compost teas were used, the microbial quality of the tea produced was not tested. However, this same aerated compost tea was recently found to reduce soilborne potato diseases when applied to the soil in the field, but not when applied only to foliage, throughout the potato growing season (Larkin and Brewer 2005). In related tests, EM did not control soilborne potato disease in the field (Larkin and Brewer 2005).

A decrease in disease suppression at subsequent assessments showed that natural products were not effective for extended periods of time. The low bioactivity of compost tea, EM or serenade at subsequent assessments suggests that the active

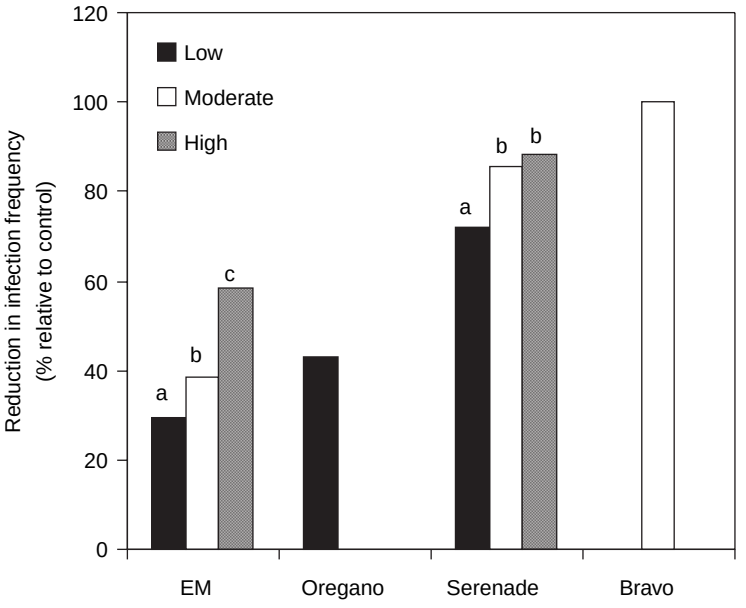

Figure 7. Relationship of natural product concentration of applied treatments to the infection frequency of potato leaves. Low, moderate and high concentration treatments refer to concentrations of 2, 10 and 20% for EM, 0.1, 2, and 20% for oregano and 2.5, 10 and 20% for Serenade, respectively. Oregano treatment concentrations of 10 and 20% resulted in 100% plant mortality, and thus there were no plants to evaluate for infection frequency.

microbial constituents in the products decreased over time. A decrease in bioactivity of plant extracts was detected by Cao et al. (2001). In the case of oregano, the low bioactivity may be attributed to the volatility of the chemical constituents. Perhaps future work should look at multiple applications of essential oils and biopesticides in relation to their efficacy. The efficacy of oregano oil and other biopesticides should be tested particularly under field conditions where relative humidity is normally high.

The suppression of late blight by some of the natural products, particularly Serenade and oregano, is encouraging (Olanya et al. 2004). The natural products or biological amendments present several positive attributes, including reduced risk of resistance development, lack of pesticide residues, applicability to organic agriculture, and potentially greater sustainability. Although the levels of late blight control observed may not be comparable to current chemical control options, nor sufficient for satisfactory disease management at this time, these approaches may potentially be used in conjunction with existing disease management practices to provide improved control. Use of natural products in combination with compatible fungicides may enable reductions in the rate or number of chemical applications required for effective disease control. Continued research and development with natural products and biopesticides may provide substantially improved efficacy through identification of more effective active agents, development of better formulations, enhanced retention on leaf surfaces, and combinations of complementary compounds and agents. Continued work with these types of materials and approaches, alone, and in combination with established control methods under field conditions, is warranted

Table III. Effects of foliar application of biopesticides, with and without subsequent inoculation with *P. infestans* on plant height and defoliation in growth chamber experiments.

Treatment[w]	Plant height[x] (cm)	Defoliation[y] (%)
Control (untreated) + LB[z]	23.0 ± 0.8	43.1 ± 4.5
Chlorothalonil	26.0 ± 1.6	0
Chlorothalonil + LB	25.6 ± 1.4	7.4 ± 5.5
Effective Microorganism (EM)	26.5 ± 0.9	0
EM + LB	24.2 ± 0.9	25.2 ± 9.1
Oregano	25.3 ± 2.6	0
Oregano + LB	24.4 ± 0.9	46.5 ± 7.9
Serenade	24.8 ± 1.8	2.2 ± 1.3
Serenade + LB	25.8 ± 0.5	9.1 ± 3.4

[w] All treatments were applied to foliage until run-off. Effective Microorganism (EM) mixture consists of an anaerobic fermentation of *Lactobacillus* sp., yeast and phototrophic bacteria was applied at a concentration of 10%; oregano (essential oil) was applied at 1.5%. Serenade (*B. subtilis*) was applied at 8%. Chlorothalonil (chemical control) was applied at 0.25%, and water was applied for the untreated control All treatments were applied to plants alone (without inoculation) and also with subsequent inoculation with the late blight pathogen (+LB), *P. infestans* isolate 00-109, genotype 100/111/122. [x]Average plant height on a sample of six plants per treatment. Measurement of plant height was taken on 6-week-old potato plants. [y]Average percentage of leaves defoliated in final assessment, 11 days after inoculation. Defoliation was quantified by assessing the number of leaves abscised relative to the total number of leaves and was measured over multiple sampling dates.

and necessary as we strive for more and better options for sustainable late blight management for the future.

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