Alternative treatments for late blight control in organic potato: Antagonistic micro-organisms and compost extracts for activity against *Phytophthora infestans*

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Summary

The effects of various fungi, bacteria and different compost extracts on foliar infection of potato (*Solanum tuberosum*) with *Phytophthora infestans* were tested in detached leaf bioassays in 2001–2003. Application of microorganism inocula and compost extracts as well as copper oxychloride to excised leaves resulted in different degrees of blight control. In general, suppression of blight lesion growth was not improved by applying the antagonists before the leaves were inoculated with *Phytophthora* spores. There was some evidence that extracts made from different compost feed stocks of different ages suppressed leaflet infection with blight. However, results were very limited and inconsistent. Moreover, the effects were much smaller than where copper oxychloride was used. Improved efficacy of acceptable alternatives to copper fungicides especially in organic farming is required. No promising effective alternative to the use of copper fungicides to reduce late blight infection in organic potato production systems was identified in the experiments.

Introduction

Late blight (*Phytophthora infestans*) is one of the most serious diseases of potato worldwide that can result in complete destruction of potato crop. It infects the plant under humid conditions, causing the foliage to die and the tubers to rot very rapidly. Throughout the EU, late blight causes substantial economic losses in organic potato production systems (Elad et al., 2002). Experience in both organic and conventional agriculture has shown that under suitable environmental conditions, late blight will spread very rapidly even when fungicides are used. The proposed ban on the use of copper fungicides in organic farming in the EU will substantially increase economic losses of farmers, unless suitable alternative blight management strategies are developed. Several novel approaches to disease control that are likely to be acceptable for inclusion in the EU Organic standards are currently under investigation.

The problem associated with the use of hazardous chemicals for plant disease control has received increasing attention worldwide, because pathogens become resistant to chemical pesticides, environmental pollution and ecological imbalances may occur. In sustainable agricultural systems, non-renewable petrochemical resources should be replaced by biologically-based renewable inputs (Quimby et al., 2002). Biological control of late blight is one alternative treatment to chemical control that deserves more research. Jindal et al. (1988) studied the antagonistic effect of six phylloplane fungi against *Phytophthora infestans* in the laboratory and greenhouse. They found that maximum inhibition in vitro was caused by *Penicillium aurantiogriseum* followed by *Fusarium equiseti*, *Mucor hiemalis*, *Trichoderma koningii*, *Epicoccum purpurascens* and *Stachybotrys atra*. Application of spores of all the test fungi individually on potato plants significantly reduced the intensity of late blight and a maximum disease control of 93 percent was achieved when spores of *P. aurantiogriseum* were applied 12 hours prior to pathogen inoculation. Zhinong et al. (2002) found that *Bacillus puimilus* and *Pseudomonas fluorescens* elicited systemic protection against late blight on tomato and reduced disease severity. Results of studies carried out by Daayf et al. (2003) showed that bacteria with biocontrol activity were from the genera *Bacillus*, *Pseudomonas*, *Rahnella*, and *Serratia*. Mechanisms of inhibition characterized included those occurring directly, through antibiosis, and (or) indirectly, through the induction of plant defence systems. There are now several biological control products with activity against fungal plant pathogens, but so far none of these products has shown promising activity against potato late blight.

In many studies, application of compost extracts (compost teas), which are filtrated solutions of mixtures of compost materials and water, showed promising results on crop protection after a soaking period referred to as extraction time. Also, organic fertilizers such as liquid pig manure, matured cattle manure and sugarcane husks applied directly to the soil showed promising results for control of bean damping-off (*Sclerotinia sclerotiorum*) (Viana et al., 2000). DeCeuster and Hoitink (1999) reported that *Pythium* and *Phytophthora* root rots can be controlled most effectively in container media amended with composted bark. However, some reports showed that using organic fertilizers increased development of some diseases. For example, Chauhan et al. (2000) found that increasing application of farm yard manure from 25 to 75 t/ha, increased disease severity of stem rot (*Rhizoctonia solani*) in cauliflower.

The effects of compost application either as extracts to the foliage or as soil amendments on plant disease control may be due to direct antifungal or resistance inducing/plant strengthening effects. However, the mechanisms by which compost extracts work are not well known but seem to vary depending on the host/pathogen relationship and the mode of application. Goldstein (1998) reported that composts and compost extracts activate disease resistance genes in plants. These genes are activated in response to the presence of a pathogen. They mobilize chemical defences against the pathogen invasion, although often it is too late to avoid the disease. Plants growing in compost may have these disease-prevention systems already running (Sullivan, 2001). Brinton et al. (1996) examined compost teas in relation to their development and use for controlling plant pathogenic fungi such as late blight in potatoes. They found that the key factors influencing effectiveness were the age of the compost (extracts from older composts were more effective than those from younger extracts), and the nature of its source ingredients.

In this study, the objectives were to isolate and screen antagonistic micro-organisms against *Phytophthora infestans* activities in laboratory bioassays and to study the efficacy of compost extracts, made at different stages of the composting process and from different types of compost feed-stocks, on potato late blight development.

Material and methods

Plant materials. Potato tubers (cv. Nicola) were planted in 15 cm diameter pots filled with John Innes No. 3 compost and grown for 5–6 weeks in a glasshouse. Potato leaves of the 5^{th} to 7^{th} compound leaf from 42–45 day old plants (flower bud appearance or flowering) were excised and used in the bioassay tests.

Media for Phytophthora culture (Rye extract agar). Rye grains (60 g) were soaked in distilled water for 36 hours and then the supernatant was poured off and retained. The grain was macerated and extracted in distilled water at 50 °C for 3 hours. The mixture was filtered through two layers of muslin and the sediment was discarded. The original supernatant was added to the filtrate, together with 15.5 g of Agar Number 1 (LAB M^{tm}, UK) and 20.0 g of Sucrose (Sigma, USA), and made up to one litre with distilled water. The mixture was then autoclaved for 15 minutes at 121 °C and poured into Petri dishes. The pathogenic fungus *P. infestans* isolated from potato leaves, by Arlene Cameron in the Scottish Agricultural Science Agency (SASA), was maintained on the Rye agar. Due to reducing the pathogenicity of *P. infestans* after 3^{rd} generation, it was cultured in potato leaves and re-isolated and maintained in Rye agar.

Isolation, processing and application of antagonist microorganisms. Diseased potato leaves infected with *P. infestans* were collected from plants grown in greenhouses and fields in Newcastle upon Tyne, UK. Infected leaf tissues were placed in three different agar media (potato dextrose agar, nutrient agar, agar bacteriological). All fungi and bacteria present on the leaves were isolated and purified by sub-culturing in the fresh agar media. Culture collections of 102 fungal and 24 bacteria strains in 2001 and 91 fungal and 6 bacterial isolates in 2002 were collected individually. Cultures were placed in cabinets for 15–20 days kept at 20 °C in order to produce sufficient spores. Activity of each isolate for biological control of late blight was assessed in 2002 and 2003. Twelve antagonistic isolates that showed greatest activity in leaf bioassays together with three recommended biocontrol agents were tested in the field in 2003. Spores from cultures of *P. infestans* and the antagonists were harvested by Tween 40 (Sigma, Germany) solution (200 µl of Tween 40 and 180 ml of distilled water). Spore concentrations were adjusted to 10^5 ml^{-1} spores (for fungi) and 10^6 cfu ml^{-1} (for bacteria). For control treatments, acidified nitrite (Chidburi, 1999) and copper fungicide (435 g l^{-1} copper oxychloride) were used.

Detached leaf bioassays. In the laboratory bioassay tests, potato leaves were placed onto steel wire mesh covered with soaked filter paper in 17×11×4 cm clear plastic

Fig. 1. Bioassay boxes containing inoculated potato leaflets with biocontrol agents and *Phytophthora.*

boxes and kept at 15–18 °C (Fig. 1). The undersides of potato leaves were inoculated with 40 µl of the prepared antagonist microorganism suspensions either 24 hours before or 30 minutes after applying 20 µl *P. infestans* suspensions (10^5 sporangia/ml). Lesion diameters and percentage of the leaf surface affected were assessed at regular intervals starting 2 days after inoculation and continued up to 10 days.

Compost extracts. Three kind of composts including green-waste (UFAMS, Newcastle City Council), house-hold waste (UFAMS, Newcastle City Council) and cattle-manure (Nafferton Farm, University of Newcastle) were extracted with water (1:10 w/v) using a commercial compost extractor (Growing Solution Inc., USA). The three composts extracted at three different compost developmental stages, 2-weeks, 3-months and one-year old for green-waste and household waste composts and 2-weeks, 4-weeks and 4 months old for cattle manure compost. Undersides of potato leaves were inoculated with 40 µl of compost extracts either 24 hours before or 30 minutes after applying 20 µl *P. infestans* suspensions (10^5 sporangia/ml).

Statistical analysis. All experiments presented here were repeated at least twice and the data from repeated experiments were pooled if statistical analysis showed no significant difference between repeat experiments. In all experiments, five leaves per treatments were scored and disease development data were analysed by analyses of variance (ANOVA). Significant differences between treatments were considered at the $P<0.05$ level.

Results

Biocontrol agents. The potential of 193 fungal and 30 bacteria strains against *P. infestans* development were evaluated during 24 bioassay studies in 2002 and 2003 (Fig. 1). The antagonists numbered 1, 2, 12, 19, 36, 48, 49, 50, 53, 62, 69, 76 and 77 showed between 10 to 50 percent reduction in blight development compared to control plants (Fig. 2). In general, results suggested that efficacy of antagonist was not related to application time. Some were more effective when they were applied before

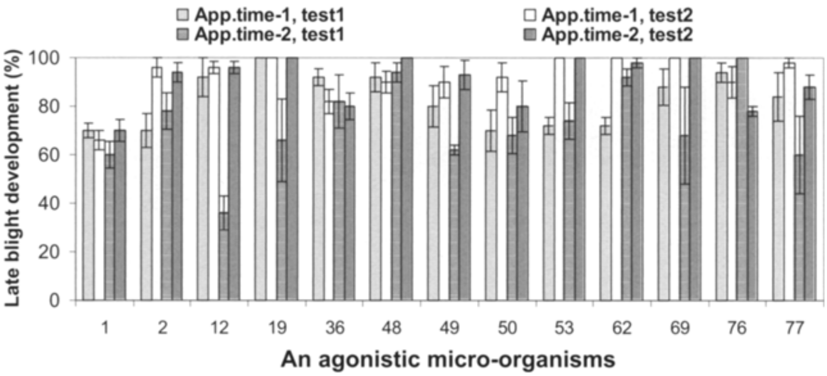

Fig. 2. Results of selected micro-organisms in two repeated tests. (effect of antagonistic micro-organisms on percentage of blight development in the leaf area (%) at two application times (30 minutes after inoculation with *Phytophthora* (application time 1) and 24 hours before inoculation with *Phytophthora* (application time 2)). Each point represents the mean of 5 measurements, and error bars are ±SE of the mean.

Phytophthora; others were more effective when applied after inoculation with *Phytophthora*. Moreover, the efficacy of some micro-organisms was inconsistent in the repeated bioassay.

Compost extracts. In the first experiment, aqueous extract from one month old cattle manure reduced disease development by about 30–40% (Fig. 3). However, this effect

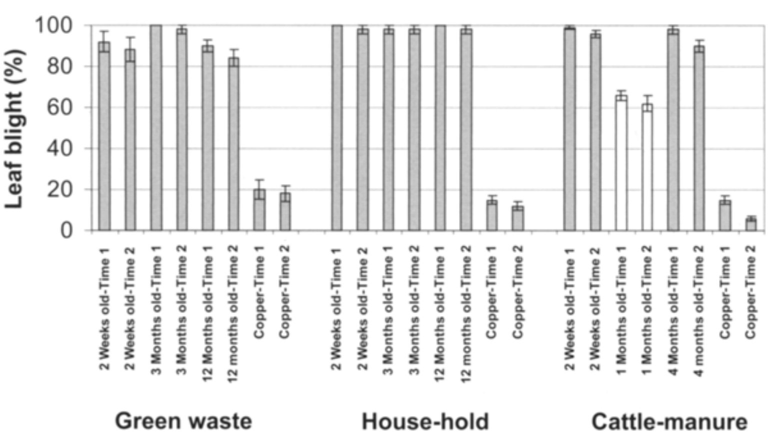

Fig. 3. Effect of compost type, compost developmental stage and application time (30 minutes after inoculation with *Phytophthora* (time 1) and 24 hours before inoculation with *Phytophthora* (time 2)) on blight suppression in detached leaf bioassays (Experiment 1). Each point represents the mean of 5 measurements, and error bars are ±SE of the mean.

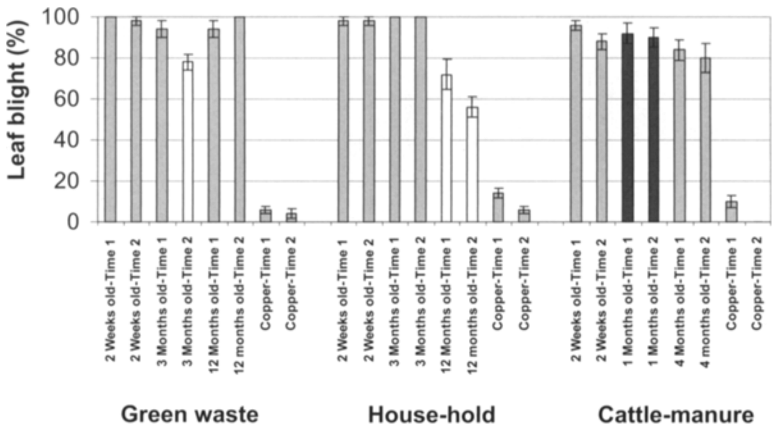

Fig. 4. Effect of compost type, compost developmental stage and application time (30 minutes after inoculation with *Phytophthora* (time 1) and 24 hours before inoculation with *Phytophthora* (time 2)) on blight suppression in detached leaf bioassays (Experiment 2). Each point represents the mean of 5 measurements, and error bars are ±SE of the mean.

was not achieved in the second repeated experiment. In the second assay, mature (12 months old) house-hold waste compost showed 30–45% reduction in late blight although no effect had been evident in the first experiment (Fig. 4). Compost extracts decreased infection of leaves with blight to a much lesser extent than copper oxychloride, which decreased blight infection by over 80% in every bioassay.

Discussion

There was some evidence based on results from laboratory leaf-bioassays that aqueous extracts containing different antagonists, or extracts from various compost feedstocks and of different ages suppressed leaflet infection with blight. However, the effects were very limited, inconsistent and much smaller than where copper oxychloride was used. Copper oxychloride was invariably superior in all bioassays. In 2003, a field experiment was conducted at Nafferton farm (University of Newcastle) in order to study the effect of the 12 most effective antagonists against blight in potato grown in the field. Due to dry conditions, however, blight failed to develop even in the control plants although they were inoculated by *Phytophthora infestans*. Field experiments, and further bioassay tests could identify more superior antagonists. Neither antagonistic micro-organisms nor compost extracts appeared to give effective protection against late blight. Successful use of biological control or compost extracts as blight control agents depends upon identifying those with greatest activity, applying them at the correct time and frequency and producing these extracts consistently to ensure reproducibility of effects. The achievement of consistent effects is dependent upon producing extracts that are exactly same from batch to batch from an individual compost feed-stock and this is a key challenge. Our results also confirm the need for strongly enhanced activities to develop other acceptable alternatives for copper in organic farming.

The incorporation of organic matter such as compost in the soil may be a viable alternative to chemicals for plant disease control, and be more effective than compost extracts applied to the foliage. This is because effective compost teas give immediate but short-term control of surface-spreading pathogens whereas solid composts applied to soil act more slowly over a longer period of time. For example, application 5 t ha^{-1} of farm yard manure, once every three years, reduced dry root rot (*Macrophomina phaseolina*) to 32% in groundnut (*Arachis hypogaea*) compared with untreated plants (Harinath & Subbarami, 1996). The recommended approaches to increase suppressiveness of compost are; curing the compost for four months or more before using it; incorporating the compost in the field soil several months before planting and inoculating it with specific biocontrol agents (Hoitink et al., 1997). Composts' contribution to nitrogen fertility must also be taken into account because nutrient effects may influence the severity of pathogens. Potato plants treated with increased concentrations of nitrogen and potassium showed increased susceptibility to infection with *P. infestans* (Phukan, 1993).

There are other alternatives for late blight management that are recommended in the literature. To improve the consistency of disease control using composts, biological control agents (BCAs) can be added to compost amendments (DeCeuster & Hoitink 1999; Hoitink et al., 2001; Nakasaki et al., 1998; Ryckeboer, 2001). Two of the more common beneficial organisms used to inoculate compost are strains of *Trichoderma* and *Flavobacterium*, added to suppress *Rhizoctonia solani* in potatoes. *Trichoderma harzianum* acts against a broad range of soil-borne fungal crop pathogens, including *R. solani*, by production of anti-fungal exudates (Sullivan, 2001). Mixtures of bacterial and fungal biocontrol agents are more effective than single BCAs (Ryckeboer, 2001). The possibility of inoculating composts with BCAs for controlling soil-borne pathogens in vegetable crops and container plants is currently being examined in different EU funded projects.

Another important consideration related to alternative strategies for controlling late blight is related to weed presence in the field. Lesions of *Phytophthora infestans* were found on woody nightshade (*Solanum dulcamara*), black nightshade (*S. nigrum*) and *S. sisymbriifolium* during a nationwide late blight survey in the Netherlands in 1999 and 2000 (Flier et al., 2003). Therefore these plant species should be regarded as alternative hosts for the late blight pathogen. Oospore production in ageing *S. nigrum* and *S. dulcamara* plants in autumn, however, may generate a considerable source of (auto) infections in following years.

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