Management of Late Blight with Alternative Products

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ABSTRACT

Controlling highly destructive plant diseases such as potato and tomato late blight, caused by the oomycete Phytophthora infestans, with non-fungicidal alternative products is a difficult but necessary task that needs to be accomplished. Given the rapid development of potato and tomato late blight epidemics, for many years control strategies relied solely upon the application of fungicides. There are many reports of alternative strategies for managing several plant diseases, however reports on late blight control with non-fungicidal products are recent and appeared in the last 20 years. The most commonly used strategy to control the disease has been the prevention of establishment of P. infestans in the host plant, mainly by using organisms capable of producing chemical compounds that inhibit spore germination. Nevertheless, the epidemiological characteristics of late blight makes the adoption of a “silver bullet approach” risky. To enhance the chances of success of alternative products, a combination of compounds and microorganisms with different modes of action should be employed beginning at the early stages of the host-pathogen interaction. Specifically, when effective options are available, one should consider the use of phylloplane and endophytic organisms combined with resistance induction mediated by plant growth promoting rhizobacteria. Late blight control with alternative products is likely to positively impact both conventional and organic production systems.

Keywords: biological control, disease, organic, Phytophthora infestans, potato, tomato
Abbreviations: PGPR, plant growth-promoting rhizobacteria

INTRODUCTION

Late blight is a challenging plant disease to be tackled with alternative products. The major economic host crops – potato (Solanum tuberosum L.) and tomato (S. lycopersicum L.) – are considered the most important horticultural crops in the several countries and both are severely affected by the disease. The causal agent of late blight is an oomycete, Phytophthora infestans (Mont.) De Bary, which is well-known for its explosive development when environmental conditions are suitable and host plants susceptible to infection (Mizubuti and Fry 2006). Reports of complete field destruction due to late blight epidemics are relatively common in the literature and estimated worldwide economic losses due to the disease vary from 3 to 5 billion dollars annually (Raman et al. 2000; Guenthner et al. 2001; Judelson and Blanco 2005; Haldar et al. 2006).

What makes P. infestans such an aggressive pathogen and late blight such a difficult disease to manage? The genus Phytophthora literally translated from Greek means Phyton = plant and Phthora = destroyer; and in the case of P. infestans this name makes sense. The pathogen has a sophisticated weaponry, including effector molecules coded by avirulence genes that allow rapid infection and host tissue colonization (Kamoun and Smart 2005; Haldar et al. 2006). Once inside host tissue, a complex set of compounds such as metallopeptidase, cutinase, and other proteins with no identifiable function required for cell killing and nutrient uptake are promptly activated (Lee et al. 2006). To date, 10 avirulence (Avr) proteins are known to be involved in
pathogenesis and act as effectors that are delivered inside the plant cells (Haldar et al. 2006). These effector proteins can either trigger resistance response or induce disease in the plants. In addition, different types of proteases inhibitors can be produced by the pathogen to avoid defense proteins formed by the plant. Recently, a new class of such protease inhibitors were described in P. infestans (Tian et al. 2007). Despite milestones achievements related to the understanding of basic biology of P. infestans, numerous mechanisms involved in the pathogenesis are not completely resolved yet (Lee et al. 2006). Nevertheless, it is well-known that the time required for P. infestans to complete its life cycle, i.e. from infection to sporulation (production of new propagules), can be as short as three days. After this short incubation period, a single lesion may give rise to thousands of spores.

Late blight management has been heavily based on fungicide application and in many areas fungicide applications have increased over the last decade due to the introduction of new, more aggressive genotypes of the pathogen (Kato et al. 1997). At the same time, two-counter-balancing factors have also grown: societal pressure for reducing pesticide use on crops and acreage of organically-grown food crops, potato and tomato included (Bettiol et al. 2004; Ghorbani et al. 2004; Obach 2007). Innovative and effective control measures are needed if fungicide use is to be reduced or, in the case of organic production, eliminated.

Organic production of potato and tomato has for many decades depended on the application of copper-based fungicides (e.g. Bordeaux mixture, fixed-copper hydroxide, copper oxide, and copper oxychloride) for control of late blight. In Brazil (Bettiol et al. 2004) as well as in the USA (Koenig and Baker 2002) and Japan (Notification No. 59 of the Ministry of Agriculture, Forestry and Fisheries 2000 - www.maff.go.jp/soshiki/syokuhin/hinshitu/organic/yukishiki/how.pdf) these compounds can be used in certified organic fields. However, there has been increasing pressure to find substitutes for these products because of environmental contamination caused by copper residues. Currently in the European Union only 6 kg of elemental copper per ha per year is allowed in organic production (Ghorbani et al. 2004). As soon as reliable alternatives to manage late blight are available, a complete ban of copper compounds should take place (Duncan 2003). Thus, there is enormous interest in finding effective non-chemical alternatives to protect potato and tomato fields against their most threatening foliar disease.

The term “alternative” can have multiple meanings and its interpretation varies accordingly. In this review we will use it in a relatively “loose” sense that includes natural compounds produced by plants or by microorganisms and also synthetic substances that do not directly affect pathogen development per se. Other non-fungicidal alternatives to late blight management such as cultural (fertilization, planting density, irrigation, etc.) and physical (irradiation, heat, etc.) control methods will not be addressed. Rather, only practices that involve applications of non-fungicidal compounds or their potential producers will be discussed.

**BIOLoGY AND POPULATION GENETICS OF P. INFESTANS – IMPLICATIONS FOR EPIDEMIOLOGY OF LATE BLIGHT**

Important aspects of the biology and population genetics of P. infestans should be considered for thorough analysis and proper development of alternative procedures aiming at controlling late blight. From a historical perspective, late blight has devastated potato and tomato crops for more than 150 years (Turner 2005). The pathogen, P. infestans, belongs to the kingdom Stramenopila, phylum Oomycota, family Pythiaceae. These organisms are morphologically similar to fungi but phylogenetically related to brown algae (Sogin and Silberman 1998). P. infestans can infect foliage, stems, potato tubers and tomato fruits at all stages of plant development. Several wild solanaceous plants can also be infected by P. infestans (Forbes and Landeo 2006). Eggplant has also been identified as a host, but our efforts to find infection on foliage in South America have proven unsuccessful. Eggplant fruits appear to be easily infested (unpublished data). Initially lesions on potato or tomato foliage appear as irregular, water-soaked spots, which may enlarge rapidly into pale green to brown; covering extensive surface of large leaves and stems (Stevenson 1991; Fry et al. 2001). In potato tubers, lesions are reddish brown, dry, and granular, and can develop in inner tissue (Fry et al. 2001). When humidity is high, lesions can be covered with a gray to white moldy growth, which consists of specialized hyphae called sporangiophores that produce the asexual propagules known as sporangia (Stevenson 1991; Fry et al. 2001). Flagellate zoospores, formed inside sporangia, can swim, encyst (lose its flagella) and form a germ tube, which can penetrate host tissue. The pathogen can also produce oospores, which are thick-walled sexual spores capable of surviving in the absence of host plants. Both the A1 and A2 mating types are needed for production of oospores, although presence of both types does not always lead to sexual reproduction, indicating that there are levels of incompatibility.

P. infestans is extremely aggressive on potato and tomato plants under favorable weather conditions, which include average temperature between 18-22°C and high relative humidity (80-100%) (Stevenson 1991; Fry et al. 2001). Determining what is optimal in the complex variable temperatures of nature is more difficult. A study using a late blight forecast model within a geographic information system (GIS) presented a global map of late blight severity based on the number of fungicide sprays that would be needed to control the disease (Hijmans et al. 2000). Using global weather data bases, a number of areas with apparently different climates had high disease severity, including Northern Europe, parts of the Himalayas and many areas within the highland tropics. Nonetheless, disease simulation indicates that temperature regimes from the temperate summer (northern United States for example) cause disease to develop faster than do the lower temperature regimes of the highland tropics (unpublished data).

The pathogenesis process begins with the germination of oospores, sporangia or zoospores. Sporangia are the most abundantly dispersed propagules and germination can be either (i) direct by development of a germ tube or (ii) indirect, by the release of zoospores. Temperature regulates the mode of germination of sporangia. Usually, direct germination takes place at temperatures from 18 to 24°C, whereas indirect germination takes place at temperature lower than 18°C (Croizier 1934). The pathogen can penetrate plant tissue through the cuticle and epidermal cells and develops specialized structures that can extract nutrients from host cells. Necrosis follows host tissue colonization by P. infestans. In green tissue surrounding necrotic areas, sporangioles are formed through the stomata and produce sporangia. These propagules are generally dispersed by the wind, but also by rain over a short distance.

In areas where sexual reproduction does not occur or is rare, the pathogen survives from season to season in infected tubers and to a lesser extent on crop debris. In areas where viable oospores are formed, this propagule can survive in soil for years (Zwankhuizen et al. 2000; Stromberg et al. 2001; Fernández-Pavía et al. 2004; Lehtinen and Hancock 2004). In the highland tropics, host tissue is available all year.

The occurrence of sexual reproduction also affects the population dynamics of the pathogen. Two contrasting scenarios for the genetic structure of populations of P. infestans can be envisioned: (1) a panmictic population is comprised of a large number of distinct genotypes of P. infestans; and (2) a clonal population structure with a limited number of clonal lineages is established across large areas. The impact of control measures can vary according to the
predominant genetic structure of the population (Grünwald et al. 2006). For panmictic populations, high genotype diversity can provide greater potential for local adaptation to a changing environment, which makes disease management more difficult (Sukjowski et al. 1994). In areas where the population is clonal, the risk of new diversity being introduced grows as seed trade becomes more globalized. Ideally, strategies for using alternative products should also consider the population biology of the pathogen; however, we are unaware of clear indications that this has happened. In most situations, research programs are oriented towards the search for products effective against all possible genotypes of *P. infestans*.

**NATURAL PRODUCTS DERIVED FROM PLANTS**

In recent years there has been an increased interest in using natural substances derived from plants for different industrial purposes, for example as food seasoning and natural medicine (Isman 2000; Cao et al. 2004), and also for pest management in agriculture (Mansingh 2004). In most cases, it would appear that natural products derived from plants are safer than fungicides because they have low acute toxicity and because they are readily biodegradable into non-toxic products (Tripathi and Dubey 2004). Consequently, these compounds can be considered as substances of reduced risk to environment and human health (Duke et al. 2003). For example, in the study of mammalian toxicity, some pure essential oils of plant origin that were fed in large quantities to rats did not cause mortality (Isman 2000).

The plant-derived products most commonly used to control plant diseases are essential oils and extracts. The two types of plant-based products have many similarities but also differ for some characteristics. Essential oils are oily liquids obtained from plants through fermentation, enfleurage, extraction, and steam distillation (Burt 2004). Plant extracts, in contrast, are dried plant products obtained by filtration, distillation, and evaporation (Wang et al. 2004). The main compounds that have been investigated to date include phenols, flavonoids, quinones, tannins, alkaoids, saponins, and sterols (Halama and van Haluwin 2004). These products can have fungicidal or fungistatic effects on plant pathogens, or they can provide conditions favorable to the establishment and increase of antagonistic microorganisms on host plants (Scheuerell and Mahaffee 2002).

Essential oils, aromatic compounds, and products of hydrolytic reactions with antagonism activity against several plant pathogens have been investigated for their antimicrobial properties, including anti-oomycete activity (Mari et al. 2003). The information about the use of natural products derived from plants to control late blight is limited (Soylu et al. 2006). The majority of reports are restricted to bioassays conducted under laboratory conditions and often recording a single, but not necessarily reliable, variable: inhibition of *P. infestans* mycelial growth on culture media.

**Plant extracts**

The search for plant extracts with anti-oomycete activity has increased over the last years, and efficacy of plant extracts against *P. infestans* has also been demonstrated. Several preliminary studies were conducted mainly in China and India but, unfortunately, these are brief reports of mostly in vitro tests (Jiang et al. 2001; Cohen et al. 2002; Cao et al. 2003; Deepa et al. 2004). Some of them have great potential to be used as alternative compounds to synthetic fungicide. Another interesting aspect is that in many studies conducted in controlled environment these compounds were as efficient as synthetic fungicides in retarding growth of *P. infestans* in vitro or in reducing late blight severity on host plants. The results of these more detailed studies are discussed below.

Extracts of 88 plant species, distributed among 44 botanical families, were tested for their capacity to inhibit zoospore formation or the *in vitro* growth of *P. infestans* (Wang et al. 2001). In that study, extracts of 19 species were effective in reducing both variables. One of the most effective was extract of garlic cloves, which at 1 or 2% completely inhibited zoospore formation (Cao and van Bruggen 2001; Wang et al. 2001) and colony growth of *P. infestans* (Cao and van Bruggen 2001). In another study, extract of long pepper (*Piper longum*) was assessed against *P. infestans* on tomato plants under greenhouse conditions (Lee et al. 2001). Long pepper extract was compared with chlorothalonil, which is a synthetic fungicide. The extract was partitioned into hexan, chloroform, ethyl acetate, butanol, and water soluble portions, which were dissolved and diluted in dimethyl sulfoxide (DMSO) and water, respectively. The portion obtained from the hexan fraction at 1 mg/ml reduced mortality of inoculated plants by 60% (Lee et al. 2001). A similar experiment was conducted to assess the effect of curcumin, a polyphenol compound present in the rhizome of turmeric (*Curcuma longa*). Tomato plantlets were treated with a curcumin solution of 500 or 1000 mg/l (which was dissolved and diluted in DMSO and water, respectively) and later inoculated with *P. infestans*. All treated plants survived and the level of late blight control achieved with curcumin was similar to that in plants treated with the fungicide chlorothalonil (Kim et al. 2003).

The key to successful trials should be carefully evaluated since most tests were conducted under conditions not similar to those experienced by farmers in the field. For instance, *in vitro* tests suggested that extracts made of clove and garlic controlled late blight (Cao and van Bruggen 2001; Wang et al. 2001). Thus, based on previously reported results, field trials were set up to quantify the efficacy of three mixtures of plant extracts in the control of tomato late blight: chili pepper, black pepper, clove, turmeric, and garlic; black pepper, clove, and garlic; and clove, turmeric, and garlic. Variables related to late blight progress such as severity at half of epidemic duration (Y50); final severity (Ymax); area under the disease progress curve (AUDPC); and disease progress rate (r) were quantified (Diniz et al. 2006).

Unfortunately, under field conditions in Brazil, none of the plant extract combinations reduced any of the variables related to late blight intensity (Diniz et al. 2006). Low concentrations of the putative active ingredients allysulfites and disulfite (allyldisulfites and allylmethyl disulfites) (Cao and van Bruggen 2001), pipernonaline (Lee et al. 2001) and curcumin (Kim et al. 2003) in the extracts and/or their chemical and physical degradation products could have interfered with the tested compounds, reducing their efficacy in the field.

There is variation in the way compounds are extracted and also regarding the solvents used to obtain them. Plant extracts have been formulated as botanical fungicide dissolved in DMSO (Kim et al. 2003), ethanol benzene, diethylether, toluene (Deepa et al. 2004), acetone (Wang et al. 2004), water (Wang et al. 2004; Stephan et al. 2005), sodium citrate and sodium chloride (Burt 2004). However, there is limited information about the persistence and putative toxicity on plants sprayed with plant extracts obtained with these solvents. Temperature, ultraviolet light, pH on treated plant parts, rainfall, and other environmental factors may exert a more or less negative influence on the active principles (Schmutterer 1990). For instance, the residual effect of neem-based products is, in general, restricted to a few days, mostly around five to seven days (Schmutterer 1990). Nevertheless, the residual effect of some protective fungicides on plants is around one week, which does not compromise the use of neem compounds as natural fungicide.

*Inula viscosa* is a common medicinal plant native to the Mediterranean Basin. In addition to its therapeutic properties, this plant has antimicrobial activity against several plant pathogens. In a growth chamber study, extracts diluted in acetone or water at 1% (w/v) reduced late blight severity more than 90% on potato and tomato plants (Wang et al. 2004). Several potentially active compounds were identified in paste samples: tomentosin, inuvicoside, costic acid, and...
isocastic acid. Furthermore, four thin-layer chromatography regions were highly inhibitory to *P. infestans*. However, compounds from these regions were not identified (Wang et al. 2004).

Medicinal plants native to China were evaluated against *P. infestans* in a detached potato leaf bioassay and in the field. Plant species included in this study were: *Terminalia chebula*, *Anemarrhena asphodeloides*, *Allium sativum*, *Gal- la chinensis* and *Hyoscyamus niger* in a detached-leaf trials, only *T. chebula* and *G. chinensis* were selected for field experiments (Cao et al. 2004). The efficiency of these products to control late blight on potato plants in the field was low compared with a copper-based fungicide. The percentage of inhibition of late blight with the fungicide was around 60% and 80% in potato cvs. ‘Agria’ and ‘Nicola’, respectively. In potato cultivar ‘Nicola’ treated with *T. chebula* and *G. chinensis*, the percentages of inhibition were around 30% and 10%, respectively. There was no control of late blight in potato cv. ‘Agria’ treated with *G. chinensis*. However, *T. chebula* did control late blight (40%) in potato cultivar ‘Agria’. Poor rainfastness of plant extracts was proposed as the main factor for the limited late blight control in the field (Cao et al. 2004). Protection of field-grown potato and tomato plants may depend on the association of effective plant extracts with adhesive adjuvants. On the other hand, use of extracts without adhesives might be suitable for greenhouse-grown tomatoes.

The commercial plant preparation Elot-Vis® (Prophyta GmbH, Germany) and extracts from *Rheum rhabarbarum*, *Solidago canadensis*, *Artemisia vulgaris*, *Impatiens parviflora*, and *Urtica dioica* reduced late blight severity on detached potato leaves (Stephan et al. 2005). These treatments were repeated in a second experiment, but only *S. canaden- sis* and Elot-Vis®, applied 24 h before inoculation with *P. infestans* reduced disease development on potato leaves. Nevertheless, *R. rhabarbarum*, applied 1 h after inoculation with the pathogen, was efficient in controlling late blight. When the plant extracts were tested at different concentrations on detached potato leaves *S. canadensis* reduced the infection of *P. infestans* at 0.1, 1, and 5% w/v, while *R. rhabarbarum* inhibited *P. infestans* at 1 and 5% w/v. Although extracts of *S. canadensis* and *R. rhabarbarum* are more efficient in controlling late blight at 5%, these products were phytotoxic at this higher concentration (Stephan et al. 2005).

In the same study, the authors demonstrated that *R. rhabarbarum* and *S. canadensis* also reduced late blight severity on potato plants in a growth chamber. Extracts were more effective when applied up to 3 days before inoculation with *P. infestans*. Late blight severity in plants treated with *R. rhabarbarum* and *S. canadensis* was around 40% and 50%, respectively, while severity in control plants, inoculated only with the pathogen, was above 60%. According to the authors, these products will be tested in combination with non-chemical agents and forecasting models in field experiments (Stephan et al. 2005).

Water-based extracts of fermented soil and plant compost derived from plants referred to as compost teas, have been tested as control products for many plant diseases including late blight. Compost teas purportedly improve soil fertility and at the same time control pests and plant pathogens. Microbial populations in compost tea are likely to be the “active ingredients” responsible for the efficacy of the compound (Scheuerr and Mahaffee 2002). The effects of a commercially available compost tea (Jolly Farmer®) and a form feed extract (Hydrex Seaplants Inc.) derived from powdered kelp (*Ascophyllum nodosum*) on the bacterial community of potato foliage was assessed in field trials (Sturz et al. 2006). These products were applied on potato plants at 5- to 10-day intervals throughout the 2003 and 2004 growing seasons. Epiphytic bacteria were sampled and identified by fatty acid analysis and 16S ribosomal RNA genes. Population densities of bacterial community were greater in plants treated with the compost tea than in powdered-kelp treated plants. Bacterial density was lowest on potato foliage treated with the fungicide mancozeb. In another bioassay, bacterial isolates recovered from plants treated with both compost tea and a foliar feed extract inhibited growth of *P. infestans* on culture media (Sturz et al. 2006). These alternative products are less toxic to the microbial community than fungicides. Nevertheless, more studies on the biology and ecology of microorganisms associated with these compounds are required to evaluate their potential role in the control of late blight.

Galls caused by an aphid, *Schlechtendalia chinesis*, are commonly found in nutgall sumac tree (*Rhus javanica*). Interestingly, these galls can be a source of several antimicrobial compounds, which have attracted interest from the chemical and pharmaceutical industries. Several of these compounds have high fungicidal activity. Methanolic extracts obtained from these galls reduced late blight severity on tomato plants by more than 90% (Ahn et al. 2005).

Other compounds also reported to have inhibited growth of *P. infestans* under laboratory conditions are extracts derived from *Pseudarthria viscida* (Deepa et al. 2004), *Cassia tora* (Kim et al. 2004) and *Catalpa ovata* (Cho et al. 2006). Bryophyte extracts from *Bazzania trilobata* and *Diplophyllum albicans* also reduced late blight severity by more than 70% on tomato plants in growth chamber (Dekuria et al. 2005). Extracts obtained from lichens, such as *Evernia prunastri*, *Hypogymnia physodes*, and *Cladonia portentosa* were also reported as capable of inhibiting *P. infestans* in vitro (Halama and van Haluwin 2004).

**Essential oils**

Neem (Azadirachta indica L.) is a widely used and well-known plant from which seed extracts and oils are commonly used to control insects and pathogens. A high content of azadirachtin, its active ingredient, can be found both in the oil and in the extract (Mordue and Nisbet 2000). In tomato crops, neem oil and extract have been used to control whiteflies (*Bemisia tabaci*) (Kumar and Poehling 2006), nematodes, fungi (Abbas et al. 2005), and also *P. infestans* (Diniz et al. 2006; Rani et al. 2006). Late blight severity at 1% was similar on plants treated with neem oil (50%) and Bordeaux mixture (1%), but Y_max on neem-treated plants (44%) was higher than on plants treated with Bordeaux mixture (14%). The disease progress rate “r” (0.16) and AUDPC (533) values were lower in plots treated with neem oil than in the controls (r = 0.21 and AUDPC = 1186) and similar to the Bordeaux mixture plots (r = 0.16 and AUDPC = 130) (Diniz et al. 2006). Neem oil is potentially useful and its efficacy may be improved at higher concentrations or in combination with other compounds or microorganisms.

Essential oils from 19 plants were evaluated for their efficacy in controlling *P. infestans* on potato in both *in vitro* bioassays and greenhouse experiments. The most inhibitory compounds in vitro were essential oils derived from thyme (*Thymus vulgaris*), peppermint (*Mentha piperita*), dill (*Anethum graveolens*), caraway (*Carum carvi*), and hyssop (*Hys- sopus officinalis*) (Quintanilla et al. 2002). In the greenhouse experiments, the essential oils reduced late blight severity on potato plants, although the essential oil from hyssop was the most effective. Maximum late blight severity values on treated plants were 30% and 15% for cultivars ‘Mandel’ and ‘Kerr’s pink’, respectively. In the control treatment, where plants were inoculated with *P. infestans*, severity was above 65% in both potato varieties. Phytoxicity was observed on potato plants treated with compounds from *caraway* and *thyme* (Quintanilla et al. 2002). We are not aware of any trials designed to assess the efficacy of any of these particular essential oils under field conditions.

The anti-oomycete activity of essential oils derived from *Origanum syriacum* var. beevami, *Thymbra spicata* subsp. *spicata*, *Lavandula stoechas* subsp. *stoechas*, *Rosmarinus officinalis*, *Foeminculum vulgar* and *Laurus nobilis* was assessed against *P. infestans* in assays designed to test both contact and volatile effects. The contact effect was
Antagonism by microorganisms

Preparations made of saprophytic, epiphytic, and endophytic organisms have been assessed as potential biocontrol agents to late blight management and a compilation of the studies will be presented. We define direct antagonism as the suppressive effects of biological control agents against _P. infestans _due to antibiotics, hyperparasitism, and competition. Although 65% reduction for _P. acaule _is achieved, the mechanisms of action of biocontrol agents were not elucidated, there are several evidence that suggests the involvement of direct antagonism.

Microorganism preparations applied to protect plant tissues

One of the first published reports of biocontrol of late blight was on the use of conidial suspensions (10^4 spores/ml) of _Penicillium aurantiogriseum _and _Stachybotrys atro (= _S. chartarum _) applied to leaflets of greenhouse-grown potato plants 12 h prior to the inoculation with _P. infestans _(Jindal _et al. _1988). Late blight intensity was reduced by 93% and 84%, respectively. Simultaneous application of the biocontrol agents with pathogen inoculum also resulted in late blight 48% reduction for _P. aurantiogriseum _and _S. atra _, respectively. When culture filtrates of both organisms were applied 12 h before or at the time of inoculation, late blight severity was also reduced. Possible explanations for the observed results were antibiosis and competition for space and nutrients (Jindal _et al. _1988), however, no detailed studies were conducted to investigate the mechanisms of control. _S. atra _is a cellulose-decaying fungus (Chapman _et al. _2003), thus its cellulolytic activity could affect the integrity of _P. infestans _cell wall. One negative aspect of using _S. atra _as a biocontrol agent is the well-known capability of this species to produce trichotheccene, a mycotoxin that can affect human health (Chapman _et al. _2003). Durable and consistent control of late blight was reported from a series of experiments carried out under field conditions in Germany (Weltzien _et al. _1991). Compost tea made from horse or cow manure, amended or not with seven microorganisms, was applied to potato foliage to control late blight. In plants treated with compost tea amended with microorganisms, final late blight severity was 11% and did not differ significantly from that recorded in plants treated with a mixture of the fungicides metalaxyl and mancozeb. Using microorganisms to the compost tea significantly enhanced disease control (Weltzien _et al. _1991). Apparently variation in the composition of compost microflora can contribute to consistency in late blight control. In the Netherlands, the two most effective isolates among more than 200 microorganisms isolated from compost extract (a mixture of compost + water 1:9 w/w) or from the phyllosphere of potato plants were used in field trials designed to assess their biological control capabilities. The two bacterial isolates, one fluorescent _Pseudomonas _sp. and one _Bacillus _sp., did not control late blight under field conditions either when applied alone or in combination with compost extracts (Jongeblod _et al. _1993). Thus, addition of effective antagonists to the compost extract did not increase efficacy of the extract. The interactions among organisms “native” to the compost with those amended to it seem to play a role in disease control. However, for practical purposes, this is likely to be a difficult factor to be taken into account and control applied. Biocological control of tuber blight (infection of tubers by _P. infestans _) has also been investigated (Clulow _et al. _1995). Tubers grown in wet substrates (soil or compost) were less susceptible than those formed in dry conditions (Stewart _et al. _1993). Inhibition of tuber blight was not due to _Streptomyces _sp., _Penicillium _sp., _Trichoderma _sp., _Gliocladium _sp., or _Rhizoctonia _sp., however, bacteria isolated from the surface of tubers and then cultivated in compost were capable of inhibiting growth of _P. infestans _in vitro. Higher numbers of bacterial isolates were recovered from tubers
kept in wet conditions, mainly for the more resistant cultivar (Clulow et al. 1995). Unfortunately, no characterization of the bacterial isolates was done and we are not aware of any further developments towards using these agents under field conditions.

Fungal and bacterial isolates from the phylloplane and rhizoplane of cultivated and wild tomatoes were able to reduce late blight lesion size on detached leaflets and in whole tomato plants (Garita et al. 1998). One bacterial isolate of Serratia sp. and isolates of Trichoderma sp., Fusarium sp., and Penicillium sp. were selected as potential biocontrol agents, but none were effective in reducing late blight severity in the field (Garita et al. 1999).

Among the many trials conducted so far, the most consistent results of biological control of late blight have been achieved with the application of Xenorhabdus spp. (Li et al. 1995; Ng and Webster 1997; Yang et al. 2001). The antagonist is a Gram-negative member of the Enterobacteriaceae, commonly found in mutual relationship with entomopathogenic nematodes (Kaya et al. 2001). Formulations based on the complex of nematode and bacterial symbiont are available commercially and used against soil pests. Metabolites produced by species of Xenorhabdus have been evaluated against Phytophthora sp., including P. infestans (Kaya et al. 2006). Different types of antibiotics can be produced by Xenorhabdus spp.: indole derivatives; xenorhabdins, which are organically soluble dithiolopyrrolones; and the xenocoumacins, which are water soluble benzopyran-1-one derivatives (Li et al. 1995). The organic fraction of the supernatant of a tryptic soy broth culture of X. bovienii coming from the nematode Steinernema feltiae was tested against potato late blight. Both in vitro and in vivo trials were conducted and phytotoxicity of the metabolites to potato plants was evaluated. The organic fraction at 0.1 and 1.0 mg/ml completely prevented mycelial growth of P. infestans in vitro and the size of late blight lesions was reduced when detached leaflets were treated with 10 or 50 mg/ml (Ng and Webster 1997). Leaflets treated with the compounds had alterations and slight phytotoxic effects were detected in those treated with 10 mg/ml and, more intensively, with 50 mg/ml. In whole plants, application of the metabolites of X. bovienii at 10 mg/ml resulted in control level similar to that achieved with the protectant fungicide chlorothalonil (Ng and Webster 1997). A summary of the results is presented in Fig. 1.

Aminoglycoside antibiotic compounds have been reported to reduce the intensity of diseases caused by oomycetes (Jones and Samac 1996; Xiao et al. 2002), including P. infestans (Lee et al. 2005). Four purified commercial aminoglycoside antibiotics: neomycin, paromomycin, ribostamycin, and streptomycin were tested against P. infestans. Paromomycin was the most active compound against the pathogen in vitro and the estimated effective dose to reduce mycelial growth by 50% was approximately 10 μg/ml (Lee et al. 2005). Subsequent tests were done on tomato plants inoculated with P. infestans and then treated with either commercially available paromomycin or with the culture filtrate of a paromomycin-producing actinomycete Streptomyces sp. (strain AMG-P1). Outstanding tomato late blight control was reported when the concentration of paromomycin was adjusted to 100 μg/ml. Strain AMG-P1 produced this antibiotic at a rate of 25 mg/l. A freeze-dried culture extract used at the rate of 125 μg/ml gave effective disease control against tomato late blight. Nevertheless, even though no phytotoxicity was mentioned, tomato plants treated with 125, 250 and 500 μg/ml apparently had prominent chlorosis (see Fig. 3 in Lee et al. 2005).

In another study, metabolites from the culture broth of X. nematophilus isolated from the nematode S. carpopusae were also tested for the control of late blight in potted potato plants. Metabolites in this study, the whole supernatant of culture broth) at 25 and 50 mg/l were effective in reducing late blight intensity (Yang et al. 2001). However, plants were inoculated with sporangial suspension of P. infestans as shortly as 2h after metabolite application. It would be interesting to assess longer time intervals between metabolite application and pathogen inoculation; also an assessment of the efficacy of treatment with bacteria, instead of the metabolites, could provide useful insights.

An interesting study demonstrated that a yeast-like fungus Pseudomyza floculosa inhibited growth of P. infestans in vitro by means of cis-9 heptadecenoic acid (CHDA), a fatty acid molecule. Detailed biochemical analyses indicated that CHDA was incorporated in the membrane of P. infestans and affected its permeability. The authors postulated that altered membrane permeability would lead to an increase in electrolyte and protein loss and even cytoplasmic disintegration of cells (Avis and Bélanger 2001).

Fourteen of 83 bacterial isolates, mostly Pseudomonas spp. and Bacillus spp., were capable of preventing growth of P. infestans in vitro (El-Sheikh et al. 2002). Overall, Pseudomonas spp. isolates were more effective than Bacillus spp. isolates and both were more effective when applied preventively. Three isolates (2 Bacillus sp. and 1 Pseudomonas sp.) had good antagonistic properties, but caused tuber soft rot and were discarded. High levels of late blight control were reported in this study for application of antagonists, but no fungicide treatment was included for comparison.

Isolates of the bacterial genera Bacillus, Pseudomonas, Rahmelia, and Serratia contributed to a reduction in late blight severity in potato plants in controlled conditions. This study is noteworthy because a highly aggressive isolate of the US-8 clonal lineage of P. infestans was used for inoculations. Several mechanisms of inhibition were thought to jointly act to reduce late blight intensity (Daayf et al. 2003). An isolate of Pseudomonas putida did not inhibit in vitro growth of P. infestans, but induced systemic resistance in potato plants, whereas an isolate of Serratia plymuthica inhibited in vitro growth of P. infestans by antibiosis, but did not induce systemic resistance to the pathogen. However, both bacteria were effective in controlling late blight (Daayf et al. 2003).

**Use of endophytic organisms**

Another line of investigation in the general area of biocontrol is the use of endophytic organisms to control pathogen development. Control of late blight was attempted with arbuscular mycorrhizal fungi (AMF) (O’Herlihy et al. 2003). Potato plantlets originating from tissue culture were transplanted to the field and commercial inoculum of AMF was applied in-furrow at planting. The authors claimed that the late blight epidemic on AMF treated potato plants was delayed, but careful analysis of the disease progress curves revealed that the major epidemiological effect of AMF ap-
plication was a reduction of the disease progress rate. For polycyclic diseases such as late blight this is the most effective strategy to reduce crop losses. In this experiment, even though final late blight severity was high (around 80%), tuber yield in AMF-treated plots did not differ from the most effective treatment: application of fungicide plus chitosan (see O’Herlihy et al. 2003).

Rhizobacteria

Crop growth, yield, and disease resistance can be enhanced by plant growth promoting rhizobacteria (PGPR) (Pieterse et al. 2003). Two PGPR, Bacillus pumilus and Pseudomonas fluorescens, induced resistance to P. infestans and there was reduced zoospore formation and germination (Yan et al. 2002). The in vitro and in vivo tests have shown that species of Bacillus, Pseudomonas, Rahmella, and Serretia can lessen late blight symptoms by a combination of antibiosis and induced resistance against P. infestans (Daayf et al. 2003). When phylloplane isolated organisms were tested in combination with B. cereus, a PGPR, late blight severity was significantly reduced compared with application of epiphytes alone. Curiously, application of the PGPR alone was not effective in reducing tomato late blight intensity, indicating an apparent synergistic effect (Lourenço Jr. et al. 2006).

Combination of potential antagonists

As noted previously, combining antagonists with different modes of action can lead to better control (Punja 1997; van Lenteren 2000). With the objective of developing several strategies to manage late blight in both conventional and organic production, potentially useful biocontrol agents for late blight management were isolated in Brazil. Many phylloplane microorganisms and rhizobacteria isolated from conventional or organically grown tomato plants were tested for antagonistic activity against P. infestans. Based on in vitro inhibition of sporangia germination and detached leaflet bioassays, four phylloplane microorganisms, Aspergillus sp., Cellulo monas flavigena, Candida sp., and Cryptococcus sp. were selected (Lourenço Jr. et al. 2006).

A strategy of selecting antagonists that could hamper distinct stages of P. infestans pathogenesis was implemented at the screening stage of candidate microorganisms. C. flavigena and Cryptococcus sp. inhibited sporangia germination, but did not reduce late blight severity in detached leaflets (Lourenço Jr. et al. 2006). Aspergillus sp. and Candida sp. reduced both sporangia germination and disease severity, probably, through reduced infection frequency due to low sporangial germination and/or inhibited zoospore germ tube formation.

The observation of limited infection of P. infestans on potato tubers from some susceptible varieties in the Tolpuca Valley incited researchers to search for microorganisms as possible antagonists to this pathogen. Isolates of Pseudomonas spp., Burkholderia spp., Streptomyces spp., and Trichoderma spp. were obtained from stems, leaves, tubers, and rhizosphere of potato plants. The suppressive activity of these microorganisms to A1 and A2 mating type isolates of P. infestans was assessed on potato leaves kept in a moist chamber, and also plants grown in a greenhouse and in the field (Lozoya-Saldaña et al. 2006). In the first experiment, the microorganisms were evaluated individually or in combinations on detached potato leaves inoculated with zoospores and sporangia of P. infestans. Reduction of late blight severity occurred with Burkholderia spp., Streptomyces spp., and Pseudomonas spp., applied individually and in combination and among species (Lozoya-Saldaña et al. 2006).

Strains of Pseudomonas spp. selected in the detached-leaf assay were tested, along with several isolates of Trichoderma spp. for late blight control on potato plants in a greenhouse. The value of the area under disease progress curve (AUDPC) in the control plants, which were inoculated only with the pathogen, was 770, while that of the treatments with mixed bacterial strains, combined isolates of Trichoderma spp., and a commercial formulation of Trichoderma spp. (Biopack-F), were 313.3, 373.3, and 366.3, respectively (Lozoya-Saldaña et al. 2006).

Results obtained under greenhouse conditions were not repeatable in the field. In two field experiments done 2001 and 2002, only the treatments with mixed strains of Pseudomonas were efficient in reducing disease. In 2001, a mix of strains of Pseudomonas spp., Burkholderia spp., and Trichoderma spp. tested in 2002 the most effective treatment was a mix of Pseudomonas spp. and Burkholderia. The AUDPC values of the control plants were 1698.2 and 716.1, while those of the treatment with bacterial strains were 1172.5 and 520.8, during 2001 and 2002, respectively. Preparations based on the antagonists used in these experiments could be implemented as a control measure in greenhouses or in areas where the late blight epidemics are less severe, mainly when combined with other available methods of controlling P. infestans (Lozoya-Saldaña et al. 2006).

Under controlled conditions, tomato late blight severity was reduced when a combination of antagonists was used. The best results were achieved when roots of tomato plants were treated with the rhizobacterium B. cereus concomitantly with the treatment of foliage with epiphytic microorganisms (Silva et al. 2004; Lourenço Jr. et al. 2006). B. cereus is postulated to have induced systemic resistance in tomato to P. infestans, since it has been shown to induce non specific resistance to other pathogens in tomatoes (Silva et al. 2004).

The impacts of a potentially effective biocontrol agent against late blight would enhance disease management in organic cropping systems and its contribution would be of great relevance. Bordeaux mixture and other copper-based fungicides are still used to control late blight in organic crops in most countries. However, it is expected that copper-based fungicides will be reduced or banned in the near future. A combination of biocontrol agents with products such as neem oil, which was demonstrated to be effective in reducing tomato late blight severity (Diniz et al. 2006), could be another option to reduce crop losses caused by the disease.

Use of commercial products based on biological control agents

Several commercial formulations of biocontrol agents have been tested for efficacy against late blight. Of many trials involving different microorganisms, including Trichoderma harzianum, Bacillus subtilis, Streptomyces sp., Coniothyrium minitans and a pool of undetermined effective microorganisms (EM 5), the most effective was the B. subtilis based-product Serenade®. Curiously, bacterial cells were not directly responsible for the inhibition of P. infestans. A cell-free culture extract contained metabolites that were active against P. infestans (Stephan et al. 2005). Caution must be exerted when using biocontrol agents capable of producing metabolites with antibiotic activity, B. subtilis, an ubiquitous plant, can produce antibiotic compounds (Romero-Tabarez et al. 2006) and little is known about the persistence of these molecules on plant products or in the environment.

In a similar study, commercial formulations of three well-known antagonist species, Trichoderma harzianum (Plant Shield HC®), Gliocladium virens (G41), and Bacillus subtilis (Rhapody AS®) were tested for the control of late blight on tomato plants, in greenhouse and field rotations. Biocontrol agents were not effective in controlling late blight on either host, but on petunias, which is less susceptible to late blight than tomato, the results were more promising (Becktell et al. 2005). These results highlight the potential of integrated management by adjusting biocontrol agent applications as a function of host resistance and environmental conditions.
NON-FUNGICIDAL CHEMICAL INDUCERS

When extract of dried mycelium of the penicillin-producing fungus Penicillium chrysogenum was sprayed on tomato plants in a greenhouse and on potato plants in the field, late blight severity was reduced by 71% on tomatoes but there was no reduction on potatoes (Thuerig et al. 2006). No penicillin residue was present in the extract (mycelium was dried for 3 h at 140°C and the antibiotic is thermo-labile) and the extract had no direct effect on in vitro growth of P. infestans. The authors proposed that differences in response were plant-mediated, in that dried mycelium induced resistance in tomato, but not on potato. In a complementary study, an extract of P. chrysogenum was used to induce resistance against P. infestans in tomato plants (Unger et al. 2006). A high level of late blight control (>90%) was achieved with two foliar sprays of the extract. The induction of resistance was positively correlated with activity of peroxidase, an enzyme known to participate in resistance response processes in several plant-pathogen interactions (Unger et al. 2006).

Chitosan, a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine, is reported to act as an antimicrobial compound and also boost the ability of plants to defend against infections (Rabea et al. 2003). Late blight epidemics were delayed on plots that received 8 sprays of 0.1% (w/v) chitosan (N, O-carboxymethyl chitosan) compared to the control and the treatment was at least as effective as the fungicide metalaxyl. When chitosan application was alternated with metalaxyl, there was an apparent synergistic effect and the number of metalaxyl sprays was reduced by 50% (from 8 to 4) (Rabea et al. 2003). This promising result should be further evaluated under different environmental conditions to assure consistency.

Cao and Forrer (2001) classified different antagonists/compounds for their efficacy in controlling late blight. Two organisms were classified as the most effective: A Pseudomonas strain used in a product identified as Immunofit M. biomonas (Filippov and Kuznetsova 1994) and Fusarium equisetii (Jindal et al. 1988). Among the chemical inducers, arachidonic acid, eicosapentaenoic acid, linoleic acid, and salicic acid were ranked as the most effective compounds. The activity of synthetic amino-n-butoxyc acids (BABA) were assessed against P. infestans in tomato plants and in vitro tests (Cohen 1994). Generally, BABA did not reduce sporangia germination and mycelial growth of P. infestans. Nevertheless, these compounds, applied at 2000 ppm, reduced the late blight severity in tomato plants. The level of control provided with the application of 3-ABA, 2-ABA, and 4-ABA, were 84%, 36%, and 15%, respectively. Nevertheless, apparently no additional studies were carried with these synthetic amino acids regarding late blight control.

OTHER PRODUCTS

Diluted cow milk (10 or 20%) was reported as an effective alternative product to control powdery mildew on zucchini (Bettiol 1999) and raw milk was effective in reducing the severity of sorghum downy mildew (Peronosclerospora sorgbi) (Arun et al. 2004). It was postulated that diluted cow milk (20%) could also control P. infestans, another oomycete, however at this concentration it did not reduce the severity of tomato late blight. Values of r and AUDPC on plots treated with milk were similar to those in the control plots (Diniz et al. 2006).

Homeopathic preparations have been tested for the control of various diseases and pests on different crops. In tomatoes, post-harvest fruit rot caused by Fusarium roseum was reduced with preparations made from Arsenicum album (C1), Kali iodatum (C149), Phosphorus (C35), and Thuja occidentalis (C87). The latter inhibited spore germination, while fungal colony growth was inhibited by Kali iodatum (C149) and Thuja occidentalis (C87) (Khanna and Chandra 1976). The incidence of tomato powdery mildew (Oidiosis cinctae) was 46.4% in plants treated with Kali iodatum (C100) whereas it reached 58% in untreated control plants (Rolim et al. 2001). To date there is no report of successful late blight control with homeopathic preparations. In the early 1990’s a homeopathic product was tested for the control of potato late blight with no success (van Bol et al. 1993). Recently, a nosode preparation made of P. infestans-infected tomato leaves was tested for control of tomato late blight. There was no reduction of late blight severity at either the midpoint or the end of the epidemic. Also, no reduction in AUDPC and r values were observed when plants were treated with the homeopathic product (Diniz et al. 2006). Given the peculiar characteristics of pathosystems involving P. infestans and potato or tomato, the potential for using homeopathic preparations to reduce epidemics under field conditions would appear unlikely.

CONCLUSIONS

As stated in the beginning of this review, the way P. infestans infects and kills host tissue and its very high multiplication rate present major barriers for late blight management with alternative compounds. Most strategies used so far are focused on preventing pathogen establishment in the host plant by reducing germination/infection processes; most of them combining organisms with similar modes of action; e.g. antibiosis (Ng and Webster 1997; Garita et al. 1998). For many pathosystems this might be appropriate; but the epidemiological characteristics of late blight makes the adoption of a “silver bullet approach” risky. P. infestans propagules are produced in high numbers and promptly dispersed by wind. Additionally, inoculum deposition on plant surface is a stochastic process (Aylor 1986). For these reasons, active antagonists should be present in sufficient number and well distributed on host tissue. Under field conditions, however, the combination of these conditions is unlikely to be always met. Thus, a safer approach to late blight biocontrol would be to use a combination of compounds and microorganisms with different modes of action beginning at the early stages of the host-pathogen interaction. For example, a potentially useful scheme would be protection conferred by antagonists applied on the phylloplane with effective endophytic treatments that could help prevent pathogen establishment combined with resistance induction mediated by PGPR before pathogen infection takes place.

Other important aspects related to the biological control of late blight are:

- Biocontrol can be enhanced by implementation of cultural practices, which create environmental conditions favorable to antagonists, and by activation of plant resistance with chemical or biological inducers.

- Under highly disease-conducive conditions, i.e. favorable environmental conditions and high susceptibility, more effective treatments should be applied. On the other hand, under less conducive conditions, complementation with biological control preparations may contribute to reduce the number of fungicides applications.

- There is a knowledge gap regarding biocontrol agents capable of reducing viability of survival structures of the pathogen. For regions where sexual reproduction occurs and spores contribute to the epidemics, acceleration of the decomposition of survival structures would reduce the potential of primary inoculum.

Preventing the establishment of infection is perhaps the most interesting strategy for biocontrol of late blight. Delaying pathogen development after infection has occurred would be difficult, at least based on results presented so far. Application of control measures that could reduce the survival period, the effectiveness of the source of initial inoculum; prevent sporangia germination (gern tube formation)
and/or zoospore germination should be evaluated in more detail. As a complement to biocontrol, activation of plant resistance should be effective.

Biological control can become an integral part of management programs aimed at controlling late blight in both conventional and organic cropping systems. In conventional cropping systems, the association of biocontrol agents with fungicides may be an option for late blight control and also contribute to reduce selection pressure exerted by fungicides to increase populations of fungicide-resistant individuals. Another possibility is to combine biocontrol, fungicide, and forecast system to schedule applications of the biocontrol agent, fungicide or a mixture of the two. This approach was shown to be valid for management of B. cinerea in cucumber and tomato cultivated in greenhouses in Israel (Shitienberg and Elad 1997). Late blight forecast systems have been validated in many regions of the world and adapting their usage to a biocontrol agent should not be complicated. For organic production it seems risky to rely solely upon biological control and application of alternative products. To date, apparently no alternatives can assure effective late blight control under favorable conditions and a combination with copper-based fungicides is still required to manage the disease in many regions.

Regarding the cropping system, alternative control of potato and tomato late blight should be implemented under a holistic approach. However, a continued search and screening of potential biocontrol agents is needed. We hope that the recent advancements in the field of metagenomics will contribute to the search for antagonists, especially those that are not easily cultivated under laboratory conditions but that could be capable of producing compounds effective against the pathogen. Finally, there should be more support for research oriented towards alternative methods to control tomato and potato late blight.

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