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COLLECTION AND EVALUATION OF BACTERIA FOR THE BIOLOGICAL CONTROL OF LATE BLIGHT OF CELERY

(*Septoria apiicola* Speg.)

by

Nancy Lovering ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Master of Science.

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EVALUATION OF BACTERIA FOR THE BIOLOGICAL CONTROL OF CELERY LATE BLIGHT

Nancy Lovering

ABSTRACT

M.Sc. Nancy Lovering Plant Science

COLLECTION AND EVALUATION OF BACTERIA FOR THE BIOLOGICAL CONTROL OF LATE BLIGHT OF CELERY (*Septoria apiicola* Speg.)

Late blight, caused by *Septoria apiicola* Speg., is the most important disease affecting celery in Québec. Biological control was investigated as an alternative to conventional chemical control of late blight. Two hundred and four bacterial isolates were collected from celery leaves, and muck and mineral soils of celery fields in south-western Québec. Two experiments were conducted to screen the bacteria for antagonism toward *Septoria apiicola*: one on agar to test for inhibition of pycnidial formation, and the other on leaf disks to test for inhibition of germination of conidia. From these two experiments, 18 isolates were selected that prevented pycnidial formation in an inhibition zone ≥1.0 cm wide and reduced germination to below 30% of the control. These isolates were re-evaluated for inhibition of germination on leaf disks. A bacterial suspension (10^7 cells/ml) was incubated on leaf disks for 24 hours before a suspension of *S. apiicola* conidia (150,000 spores/ml) was applied, and the disks were incubated for 25 hours. Four isolates reduced germination to ≤19% of the control. These isolates were tested on plants in a greenhouse. None of the isolates was able to reduce the number of late blight lesions compared to the control.

RÉSUMÉ

M.Sc. **Nancy Lovering** **Phytotechnie**

ACQUISITION ET EVALUATION DE BACTERIES POUR LE CONTROLE BIOLOGIQUE DE LA TACHE SEPTORIENNE DU CELERI

(*Septoria apiicola* Speg.)

La tache septorienne est la maladie la plus importante qui affecte le céleri au Québec. L'agent de cette maladie est le champignon *Septoria apiicola* Speg. L'utilisation d'un contrôle biologique a fait l'object d'une étude comme méthode alternative au contrôle chimique conventionnel. Des échantillons de feuilles de céleri, ainsi que des échantillons de sol organique et minéral, ont été recueillis dans des champs de céleri dans le sud-ouest du Québec. De ces échantillons, 204 isolats de bactérie ont été isolés. Deux essais ont été effectués pour sélectionner les bactéries ayant des effets antagonistes au *S. apiicola*. Un premier test fut exécuté pour déterminer quelles étaient les bactéries qui inhibaient la formation de pycnides sur gélose, et un deuxième test, sur l'inhibition de la germination des conidies sur des disques foliaires de céleri en présence de ces bactéries. D'après ces deux essais, 18 isolats se sont avérés antagonistes. Ces isolats, qui ont empêché la formation de pycnides dans une zone inhibitoire d'une largeur d'au moins 1.0 cm et une diminution de germination à moins de 30%, ont été selectionnés et soumis à un deuxième test sur l'inhibition de la germination sur disques foliaires. Une suspension de cellules de bactérie (10^7 cellules/ml) a été soumise à une incubation de 24 heures sur des disques foliaires, puis

une suspension de conidies de *S. apiicola* (150,000 spores/ml) a été ajoutée à ces disques et incubés pour 25 heures. Une diminution de germination à ≤19% a été observée avec quatre isolats. Des essais effectués sur des plantes en serre avec ces isolats n'ont pas reduit le nombre de lesions lorsqu'elles étaient comparées avec témoins.

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I. INTRODUCTION

There is an increasing need for alternatives to chemical control of plant pathogens. The general public and scientists alike have concerns over levels of pesticide residues in the environment, especially in plants used for human or animal consumption; concerns about buildup of fungicide resistance in pathogens; and, after oil prices increased in the early 1970s, concerns about the increasing costs of pesticides on the market (Burge, 1988; Waage and Greathead, 1988; Boland, 1990; Whipps, 1992). Pesticide registrations are being cancelled, and in some cases even approved pesticides cannot realistically be used because of pressure from the public (Harman, 1991; Andrews, 1992). These trends and concerns mean that the current dependence on chemical pesticides must be reduced.

Some of the alternatives to chemical control are crop rotation, deep plowing of debris, flooding, use of resistant cultivars and biological control. Resistant cultivars are generally very effective against pathogens, but breeding for resistance may take many years. Biological control can be developed in a shorter time than it takes to breed for resistance (Burge, 1988). It works well with resistance and other non-chemical controls as part of an integrated approach to pest management. While the use of fungicides will probably never be completely replaced by alternative methods, most are compatible

with fungicide use, and they can help to lessen the amount of fungicide needed to reduce the impact of disease below economic thresholds.

A broad definition of biological control includes any non-chemical means of reducing disease, and in this sense it has been used almost since man began cultivating crops (Campbell, 1989). A narrower definition, such as that from Cook and Baker (1983), is "the reduction of the amount of inoculum or disease-producing activity of a pathogen accomplished by or through one or more organisms other than man". In this sense, biological control has been investigated or used only since the early part of this century, and biological control research has been commonly supported by government and industry research funds only since the mid-1960s (Campbell, 1989).

The two main approaches to biological control are to introduce antagonists to the infection court, or to encourage naturally-occurring populations of antagonists by manipulating the ecosystem at the level of the host plant, or on a microbial level (Blakeman and Fokkema, 1982; Cook and Baker, 1983). The majority of biocontrol research and applications involves introduced antagonists. In this case, a one-time application may be sufficient for a growing season, or, more commonly, many applications may be needed during the season. The ecosystem can be manipulated by altering irrigation, fertilizer, or pH levels.

Septoria apiicola (Speg.) is the causal agent of late blight, a serious, yield-reducing disease of celery. It causes small coppery lesions surrounded by chlorotic halos. The chlorotic area can expand rapidly, reducing the area available for photosynthesis, and eventually blighting the whole leaf, and thus reducing yield. No celery cultivars exist with high levels of resistance, although some cultivars have low levels of resistance or are tolerant to late blight (Mathieu, 1992). The disease is currently controlled with frequent fungicide applications from the time of emergence until harvest. Although it is not registered for such use, some growers may apply benomyl during transplant production in the greenhouse to protect the plants before they are set out in the field. The development of fungicide resistance in *S. apiicola* may become a problem. As early as 1979, benomyl-resistant strains of *S. apiicola* were reported in Australia (Dullahide, 1979).

Biological control could be a good complement to fungicides for control of late blight, especially if applied as a protectant in the greenhouse before transplants are set out in the field. In the celery - *S. apiicola* pathosystem yield loss is directly related to initial inoculum level (Mudita and Kushalappa, 1993). Therefore any reduction in initial inoculum due to a biological control agent would not only diminish yield losses, but would also likely reduce the number of fungicide applications necessary.

The objectives of this work were first to isolate a range of bacteria from both soil and leaf surfaces, and then to screen these bacterial isolates for antagonism toward *Septoria apiicola*.

II. LITERATURE REVIEW

2.1 *Septoria apiicola* and Late Blight of Celery

2.1.1 Celery production

Québec has an important place in celery production in Canada. For the last ten years its annual production has consistently been about half of all the celery harvested in Canada. In 1994, Canada and Québec respectively produced 727 and 432 hectares, with a value of 11.6 and 7 million dollars (Statistics Canada, 1995).

Celery is a very high value crop. In 1993 the Quebec crop was worth $15,191/ha and in 1994 the value was $16,204/ha. In comparison, the farm value of beans and cauliflower grown in Québec was approximately the same as celery, but beans and cauliflower were worth $2336/ha and $8813/ha in 1994, respectively (Statistics Canada, 1995). The high value of the celery crop makes it worthwhile for growers to make frequent fungicide applications to control disease.

In Québec celery is generally grown on muck soil in the southwest area of the province, although some is also grown on mineral soil.

2.1.2 *Septoria apiicola*

The late blight fungus is classified in the subdivision Deuteromycotina; class Coelomycetes, characterized by reproduction by means of conidia borne in dark pycnidia, generally with a circular ostiole (Alexopoulos and Mims, 1979; Hawksworth et al., 1983). Spegazzini classified it for the first time in 1887 in the genus *Septoria* and the species *apiicola*. However, because he had isolated the fungus from wild celery (*Apium australe* Thouars), and not cultivated celery (*Apium graveolens*), this name was not immediately accepted. Instead, two main species (as well as many synonyms) were recognized in the genus *Septoria* as causing late blight. These were *Septoria apii* (Br. and Cav.) Chester, causing small lesions, and *Septoria apii-graveolentis* Dorogin, causing large lesions. In 1964, using a world-wide collection of isolates, Gabrielson and Grogan (1964) showed that there was no difference between these two recognized species, and proposed that the name *Septoria apiicola* should replace them. This work was confirmed by Sheridan (1968).

Septoria apiicola has a world-wide distribution. It was first reported in North America in Delaware in 1891, and within several years it had been reported in all celery-growing areas of the United States (Horst, 1990).

S. apiicola is pathogenic on cultivated celery (*Apium graveolens*), celeriac (*A. graveolens* var. *rapaceum*), and wild celery (*A. australe* Thours). It has not been found

to infect other members of the Umbelliferae, such as parsley (Cochran, 1932).

2.1.3 Disease development

Plants infected with Septoria blight usually produce infected seed. Infected seed often carries both spores on the seed coat and mycelia in the interior (Mukhath and Wood, 1962), and in wheat seed infected with *Septoria nodorum* mycelia have been known to penetrate to the endosperm of badly infected seeds (Agrawal et al., 1985). However, spores on the seed coat, not the mycelia. seem to be the primary source of inoculum (Maude, 1964 and Sheridan, 1966).

Young celery plants may become infected if seed coats carrying *Septoria apiicola* spores or mycelium remain attached to the developing plant (Donovan et al., 1990). Spores may then be washed from the seed coat onto the plant by rain or irrigation water. Germination of spores takes place under conditions of relative humidity above 90% for 48 hours or free water for 24 hours, and germ tubes may penetrate the leaf tissue at any point. Symptoms appear after 7 - 14 days after spore germination. Rounded lesions up to 0.5 inches in diameter develop first on older, outer leaves and then on newer, inner growth. The lesions are dark, may be encircled by chlorotic tissue (Sherf and MacNab, 1986), and are formed from mycelia that penetrate first intercellularly into mesophyll tissue, but eventually intracellularly when leaf tissue is necrotic (Donovan et al., 1990). Black pycnidia which contain filiform, usually three-septate, spores about 30 - 50 μm long are found in the lesions and also on seeds

(MacMillan, 1942). Under wet conditions the pycnidia exude creamy-coloured cirri containing the spores. The cirrus protects the conidia from desiccation. These spores may then be disseminated by water-splash (rain or irrigation), or by workers and machinery coming into contact with the infected plants. In Québec, Septoria blight generally first appears from mid-July to mid-August, about two months after transplanting (Patrick Auclair, pers. comm.).

2.1.4 Overwintering of propagules

The pathogen overwinters in infected debris. Infected debris remaining in the field may be the main source of primary inoculum in Québec as the blight is very rarely seen in the production greenhouses (Patrick Auclair, pers. comm.). Scharen (1964, 1966) showed that dry pycnidia of *Septoria nodorum* on wheat straw exuded viable spores when they were wetted. If the pycnidia were dried and then wetted again more viable spores were exuded, and if the drying and wetting cycle was repeated several times new pycnidia were formed which also exuded viable spores. Thus, under favourable environmental conditions, debris left in the field may act as a substrate for the increase of inoculum potential.

The amount of initial inoculum depends on the degree of decomposition of the debris. Decomposition is faster in warmer climates, and slower in cold climates like that of Québec. Mudita and Kushalappa (1993) showed that the loss in yield is directly related to the level of initial inoculum.

Late blight can cause serious reductions in yield and even crop loss (Marshall, 1960). Early in this century epidemics in California (1908) and in Michigan (1915) resulted in losses of $500,000 and $1 million, respectively (Horst, 1990).

2.1.5 Control

S. apiicola on celery seeds is currently controlled by using seed at least three years old, or by treating seeds with hot water or fungicide (CPVQ, 1987). Several workers (Maude, 1964; Sheridan, 1966; Maude and Shuring, 1970) have found that the incidence of viable spores on seed decreases with the age of the seed. The hot water treatment involves soaking seeds for 30 minutes at 48°C (CPVQ, 1987). However, the procedure must be followed exactly or seed germinability may be reduced or inoculum may not be completely eliminated.

Disease control begins in the greenhouse. Some seedling producers apply benomyl one to three times in the greenhouse, about two weeks before transplanting into the field, although it is not registered for this use. The provincial government recommends using chlorothalonil weekly in the greenhouse once the seeds have germinated. In the field, the government recommends the application of fungicide every 7 - 12 days, depending on weather conditions, starting one week after transplanting. This results in 8 - 14 applications over the cropping season, depending on whether the crop is destined for early or late harvest. They recommend using chlorothalonil, anilazine, maneb, mancozeb, zineb or methiram (CPVQ, 1987). The reseau de

dépistage de sud-ouest de Montréal (PRISME), a private integrated pest management company, recommends no fungicide at all for plants to be harvested before August 10 (early harvest). For later harvest, PRISME recommends spraying every 7 - 10 days beginning at the first sighting of lesions (Patrick Auclair, pers. comm.) This results in four to six applications during the growing season.

It is also recommended to practice at least a two year rotation with other vegetable crops such as carrot, onion or lettuce (CPVQ, 1992). Debris should be deep ploughed after harvest to bury any diseased material which may provide initial inoculum for the next growing season.

2.2 Biological Control

2.2.1 Definition and approaches

Cook and Baker (1983) define biological control as "the reduction of the amount of inoculum or disease producing activity of a pathogen accomplished by or through one or more organisms other than man". These other organisms are almost always bacteria, yeasts or fungi, although there is some evidence that viruses may also provide control of fungal pathogens (Burge, 1988). Many consider biocontrol to have a much wider definition, encompassing breeding for resistance as well as most cultural methods of control (Cook, 1988).

There are two basic approaches to biocontrol. The first is to enhance naturally-occurring populations of microorganisms by manipulating the ecosystem, or simply to use cultural practices to control disease, without specific reference to the microflora. The second is to introduce large numbers of antagonists to the infection court where the pathogen is or may become established (Blakeman and Fokkema, 1982; Cook and Baker, 1983).

Cultural practices were successfully used to reduce the incidence of Fusarium foot rot in the Pacific north-west. After high-yielding semi-dwarf winter wheat cultivars were introduced to this area, severe outbreaks of Fusarium foot rot caused by *Fusarium culmorum* were observed in low to intermediate rainfall areas where the plants were often subjected to high water stress. The disease was most severe where growers were fertilizing for yields greater than those obtainable given the available water. Wheat is generally resistant to foot rot as long as mid-day plant water potentials do not fall below -30 to -33 bars at plant maturity. It was found that by reducing fertilization to levels consistent with growth expected given the available water that the incidence and severity of Fusarium foot rot were dramatically reduced (Papendick and Cook, 1974; Cook, 1988).

Some evidence exists to suggest that naturally-occurring populations may provide a background level of biological control. It has been observed that fungicide treatment may actually worsen diseases caused by fungal pathogens (Leben, 1965;

Rishbeth, 1988). Treating rye with benomyl immediately after flowering reduced saprophyte populations on the phylloplane, and increased infection with *Cochliobolus sativa* resistant to benomyl. *C. sativa* was stimulated to germinate by the presence of pollen on the leaf. The fungicide suppressed mainly *Cladosporium* spp., pink yeasts and *Aureobasidium pullulans*. At least 3000 saprophytic propagules per cm^2 of leaf surface were necessary to suppress disease (Fokkema et al., 1975). In Australia, when copper fungicides were applied to control *Clasterosporium carpophilum*, apricot die-back disease increased, likely due to a decrease in surface antagonists (Hislop, 1976). In other cases, as with suppressive soils, microbial populations may give high levels of disease control even though the pathogen may be present in large numbers. This may be due to the entirety of the microbial population, or to a certain species or group of species. Weller and Cook (1983) found that a group of antibiotic-producing fluorescent pseudomonads were responsible for conferring suppressiveness to take-all of wheat (*Gaeumannomyces graminis* var. *tritici*) in soils in Washington state. Often physical properties of the soil are involved as well. In Hawaii, a conducive soil was rendered suppressive to *Pythium splendens* by amendment with calcium and alfalfa meal. The authors speculated that the resulting high calcium levels and large microbial population contributed to "a strong nutrient deprivation environment in suppressive soil" which prevented the sporangia from germinating (Kao and Ko, 1986).

The inundative approach to biological control has had varying degrees of success. Cullen et al. (1984) found that ascospores of *Chaetomium globosum* applied to

apple trees reduced lesions caused by the scab pathogen *Venturia inequalis* by 22-25% under field conditions, but that inoculations had to be repeated every 1-2 weeks because the antagonist populations declined very quickly after application. *Aureobasidium pullulans* was also used as an antagonist, but had such a low survival rate that it provided no control at all in the field. Burge (1988) lists several reasons why this approach may not work in the field: competition, lack of virulence to overcome fungi not raised under experimental conditions, lack of sufficient inoculum generated or maintained at the site of attack, and difficulty surviving the rapidly fluctuating environmental conditions.

2.2.2 Mechanisms of biological control

There are three basic mechanisms of biocontrol: antibiosis, competition and mycoparasitism. There is a fourth mechanism called induced resistance, which consists of inoculating the plant with a non-pathogenic strain or weak strain of a pathogen before it is exposed to a virulent strain. The weak or non-pathogenic strain stimulates the host defences, one of which may be phytoalexin production (Rishbeth, 1988). These mechanisms are not mutually exclusive, and one antagonist may use a combination of them. For example, if a plant is inoculated with a non-pathogenic strain which subsequently establishes itself on the leaf, it will compete with the pathogen for nutrients or infection sites. Thus both induced resistance and competition are at work (Whipps, 1992).

Antibiosis. Antibiosis is the production, by microorganisms, of low molecular weight substances which are harmful to the growth or activity of other microorganisms, generally bacteria and fungi (Fravel, 1988). Both aerial and soil inhabiting fungi are known to produce antibiotics *in vitro*. *Bacillus subtilis* consistently gave greater than 90% control of bean rust (*Uromyces phaseoli*) when applied five or fewer days before inoculation with the pathogen. It inhibited germination and prevented formation of normal germ tubes. A heat-stable inhibitor present in culture filtrates was composed of 95% protein and 5% carbohydrates (Baker et al., 1983).

Competition. Microorganisms also compete for nutrients, space and in the case of autotrophs, light, on the phylloplane (Campbell, 1989; Andrews, 1990). Many pathogens, such as *Botrytis cinerea* and species of *Alternaria, Cladosporium, Cochliobolus* and *Septoria*, which are sensitive to antagonism, grow saprophytically over the leaf surface before penetrating and infecting the plant. During this growth phase the pathogen relies somewhat on nutrients from the spore, but mainly on those leaked from the leaf or deposited on its surface. At this stage the pathogen is vulnerable to nutrient competition from antagonists, and in the face of strong enough competition, the saprophytic phase may be prevented, leading to a reduction in infection (Blakeman and Fokkema, 1982; Blakeman, 1985; Rishbeth, 1988). It was shown that when beet leaves were wetted for 24 hours before inoculating with *Botrytis cinerea*, populations of the natural microflora increased, germination rate of *B. cinerea* spores decreased from 80% on unwetted control leaves to 17%, and 76% of amino acids present on the leaf

were taken up by the natural microflora. When 10^5 to 10^6 cells/ml of various *Pseudomonas* spp. isolated from beet leaves were applied to beet leaves 24 hours previous to inoculation with *B. cinerea,* there was a 0.94 negative correlation between amino acid uptake and percentage pathogen spore germination. Similar results were obtained with *Phoma betae* and *Cladosporium herbarum* (Blakeman and Brodie, 1977). This suggests that nutrient competition is the control mechanism in this case. Nelson and Powelson (1988) obtained comparable results using a similar strategy to control *B. cinerea* on snap beans. They found that when *Trichoderma hamatum* was applied to bean blossoms 24 hours before the pathogen, the incidence of pod rot was reduced by up to 94%, which was not significantly different from control obtained with the fungicide vinclozolin.

Mycoparasitism. Mycoparasitism occurs when one fungus directly attacks another, and uses it as a food source. Both obligate and facultative mycoparasites exist, but only the facultative ones have potential as biocontrol agents as the obligate ones do not destroy their hosts (Burge, 1988). Mycoparasites must be applied when there is a large quantity of biomass (pathogen) on which they may live (Blakeman, 1985) and high temperature and relative humidity for them to parasitize a large proportion of the pathogen biomass (Campbell, 1989). They are especially useful for the powdery mildews and rusts (Rishbeth, 1988). For instance, *Stephanoascus flocculosus* and *S. rugulosus* have been shown to parasitize and kill powdery mildew of cucumber in less than 48 hours. However temperatures must be above 20°C and relative humidity must

be at least 80%. No antagonism took place at 60% relative humidity or less (Jarvis et al., 1989). *Verticillium lecanii* is a commercial biocontrol agent for aphids and whiteflies, and is also a mycoparasite of various rust species. It does not normally penetrate teliospores of macrocyclic rusts, which limits its effectiveness as a biocontrol agent. However, it may be useful against the mainly microcyclic rusts of many greenhouse crops. *V. lecanii* was found to completely colonize 90% of telia of chrysanthemum rust (*Puccinia horiana*) in five days by penetrating through the germ pore (Srivastava et al., 1985). It was also effective against leek rust (*P. allii*) (Uma and Taylor, 1987), carnation rust (*Uromyces dianthi*) and brown rust of wheat (*P. recondita*) (Spencer and Atkey, 1981). It penetrated the urediniospores directly in all of the last three examples, and was able to control an established infection of brown wheat rust (Spencer and Atkey, 1981).

A variant of parasitism other than by fungi is parasitism by viruses, or virus-like particles. Fungal viruses are generally cytoplasmically transmissible. They reduce pathogenicity, spore-forming ability, spore viability and growth rate. In areas of Italy where *Endothia parasitica* (causal agent of chestnut blight) is no longer a problem many strains of the blight pathogen have been found to contain double-stranded RNA segments (Buck, 1988). *Helminthosporium victoriae*, causal agent of Victoria blight of oats, and *Gaeumannomyces graminis*, causal agent of take-all of wheat, are also known to be susceptible to viral diseases (Burge, 1988).

2.2.3 Biological control in the phyllosphere

In general, there has been more success with biocontrol of soil pathogens than with pathogens of aerial plant parts. In 1992 there were seven commercially available biocontrol agents, all for control of rhizosphere pathogens (Andrews, 1992). This is partly due to the fact that chemical methods generally give excellent, relatively inexpensive control of aerial plant pathogens, so until recently there have been fewer reasons to study biological control in the phyllosphere. It has been more difficult and expensive to use chemical methods on soil pathogens, so researchers had more of a stimulus to study and develop alternative methods to control soil-borne diseases. The other factor involved is the lack of knowledge of phyllosphere ecology. In order to find and develop successful biocontrol organisms, it is necessary to have good, detailed knowledge about the ecosystem in which they exist and will function, and phylloplane ecology has become a popular research area relatively recently (Rishbeth, 1988). Moreover, the current ecological knowledge is primarily autecology (ecology of one species and its environment), but what is important for biocontrol is synecology (ecology of interacting populations or communities in a single locale) (Davenport, 1976; Andrews, 1990).

The phyllosphere may be loosely considered as the surfaces of aerial plant parts including stems, leaves, buds and flowers, as well as the thin boundary layer of air surrounding them (Spurr, 1990). In the phyllosphere environmental conditions such as temperature, radiation, wind, leaf wetness, relative humidity and CO_2 concentration

determine the microclimate. These factors can change quickly, and may cover a wide range. They are controlled by the overall weather; but are modified by the plant structure; density; and the shape, size and surface roughness of various plant parts. For example, the leaf is often cooler at its edge than in the center (Burrage, 1976). These conditions are greatly buffered in the rhizosphere (Blakeman, 1985; Rishbeth, 1988; Spurr, 1990; Andrews, 1992).

Nutrients on the leaf surface are often limiting to the growth of micro-organisms, especially early in the growing season. In the spring they consist mainly of leachates from inside the leaf, such as amino acids, carbohydrates, phenolic compounds and growth regulating substances. As the growing season progresses, the amount of leachates increases as the leaf becomes more "leaky", and is supplemented by air-borne pollen grains (Godfrey, 1976). In contrast, the root tip is nutrient-rich (Campbell, 1989). This means that introduced agents may have more difficulty establishing themselves on the phylloplane than in the rhizosphere, where it is easier to manipulate the surrounding environment to favour their survival and/or growth (Andrews, 1992). This situation is exacerbated by the fact that on a microscopic level the phylloplane may be rough depending on the number of trichomes and the type of wax present, while the root surface is smooth (Campbell, 1989). However, when chitinolytic *Bacillus cereus* with colloidal chitin was applied to peanut leaves, the bacteria reduced early leafspot (*Cercospora arachidicola*) by 63% compared with the untreated control in field trials. The chitin amendment protected the bacteria from desiccation and UV radiation, and

increased the area suitable for their survival and reproduction (Kokalis-Burelle et al., 1992). Thus it was possible to successfully manipulate the phylloplane to favour biological control.

2.2.4 Isolating and screening antagonists

There are two approaches to isolating microbes to begin a biocontrol study. One is to isolate several species representative of an entire community, which may be done by using dilute general media and picking off colonies from dilution plates. The other approach is to isolate micro-organisms from certain genera or with certain characteristics (Andrews, 1985; Blakeman, 1988). In this case it is necessary to have detailed knowledge about the pathogen and host ecology. These isolations may be made by using selective media; by incorporating substances which can be degraded by lytic enzymes, such as chitin or cell wall suspensions, into the media; or by seeding the media with the pathogen and plating dilute washings from leaves, roots, or soil (Andrews, 1985; Campbell, 1989).

Another major consideration is whether to isolate potential antagonists from the target habitat, i.e. the host plant, or from another habitat such as a non-host, or from the soil to control an air-borne pathogen (Boland, 1990).

The primary difficulty in screening micro-organisms for biocontrol ability is to find assays which are cheap, quick and convenient, and yet which are also biologically

relevant. For instance, it is well-known that tests on agar media are poor predictors of *in vivo* activity. It is advisable to carry out several tests, both *in vitro* and *in vivo* (Cullen and Andrews, 1984; Andrews, 1985; Campbell 1989). Yet agar plate techniques may provide clues as to the mode of action, and may also show useful microbes which could be rejected by certain *in vivo* tests. They are also useful for comparing potential antagonists (Andrews, 1985). Agar slide methods are informative on the morphological effect of the potential antagonist on the pathogen, such as inhibition of germination, germ tube length or abnormal germ tube growth (Blakeman and Fokkema, 1982).

Andrews (1992) has devised a protocol for screening potential antagonists. It involves screening organisms concurrently using both *in vitro* assays and *in vivo* assays under controlled conditions, such as in a growth chamber or greenhouse. Antagonists which show activity in both types of assay are carried forward to field trials, as are those which show activity in only the *in vivo* screen. Those which show activity only in the *in vitro* screen are stored. From field testing, promising organisms can be formulated, put into scaled-up production and registered. Andrews considers that testing potential antagonists on the phylloplane under controlled conditions is the key screening assay. It permits isolates which did not inhibit the pathogen in agar plate tests to be detected. Also, it is reasonably sure that if an isolate does not show any inhibition in a controlled environment conducive to its growth, that it will not do so in the field, and so can be eliminated from further testing (Blakeman, 1988).

Once one or more antagonists have successfully passed through the screening process, they are tested under field conditions, and then a scale-up production procedure is developed and the biocontrol agent is formulated and registered.

III. MATERIALS AND METHODS

3.1 General Materials and Methods

Production and maintenance of *Septoria apiicola*

Septoria apiicola (ATCC #96013) was originally isolated from celery in a commercial field, and was maintained on celery plants in the greenhouse in order to ensure its pathogenicity during the course of the work. It was isolated from the plants once a week. Two lesions containing pycnidia were cut from each of ten leaves. The lesions were surface disinfested in 1% NaOCl for 2 minutes, and rinsed twice for 2 minutes in sterile distilled water. Then they were placed in 2 ml sterile distilled water in a Petri dish, and incubated at 22°C for 3 hours to induce the pycnidia to exude conidia. After incubation, a 5-ml syringe (Becton-Dickinson) was used to place 0.5 ml of the conidial suspension on a plate of celery decoction agar (CDA). The plate was shaken back and forth on a flat surface to evenly spread the suspension, and was incubated for 14 days at 22°C. The first 6 days were in the dark, and the remaining 8 days were under fluorescent and near UV light with a 12 hour photoperiod. At the end of this time mature conidia were produced. Cultures were used only if they were not more than 4 weeks old, and there was no sub-culturing (Mathieu, 1992).

Celery decoction agar consisted of 320 ml celery decoction, 4 g potato dextrose broth (Difco) and 15 g Bacto-agar (Difco) in 680 ml distilled water. If necessary, the pH was adjusted to 5.6 with 20% H_2SO_4. Celery decoction was made by placing 50 g of coarsely chopped celery leaves and petioles in 500 ml distilled water and mixing in a blender (Waring) at high speed for 2 minutes. The mixture was strained, boiled for 2-3 minutes, and strained again (Mathieu, 1992).

Production of celery plants

Celery plants of the cultivar Florida 683, which is susceptible to *S. apiicola*, were used for all experiments. Plants used for maintenance of *S. apiicola* were grown in a greenhouse. Seeds were sown on the surface of an artificial growing medium (Promix) in cell packs, and were covered with a thin layer of sand. A clear plastic cover was placed over the cell packs to increase the relative humidity. When the seedlings were 4 weeks old, they were transplanted to 11-cm-diameter pots filled with a medium of 4:1:1 (v:v:v) pasteurized organic soil : peat moss : perlite. The transplants were fertilized with a dilute solution of 10-52-10 (N-P-K) (PlantProd, Plant Products Co. Ltd., Bramalea) to promote root growth, and afterwards every 2 weeks with 15-15-17 (N-P-K) fertilizer (PlantProd, Plant Products Co. Ltd., Bramalea). A calcium chloride solution (8 g/l) was applied twice a week as a foliar spray beginning at the four leaf stage to prevent blackheart.

Inoculation of plants with *S. apiicola*

Plants were inoculated with *S. apiicola* at the five leaf stage. A 5-ml syringe was used to gently wash the surface of a culture plate containing mature *S. apiicola* pycnidia with a 0.01% solution of Tween 20, and the spore concentration was adjusted to 20,000 spores/ml by using a haemocytometer. The conidial suspension was sprayed onto the leaves of the plants with an artist's airbrush (Badger-350, Badger Air-Brush Co., Franklin Park, IL). Then the plants were enclosed in plastic bags which had previously been misted with water on the inside to provide the high relative humidity necessary for infection. The bags were removed after 72 hours, and the plants were returned to the greenhouse.

The plants were watered from above so that water splash would naturally spread conidia onto newly-emerged non-diseased leaves, and thus ensure that the plants remained infected with *S. apiicola* as the older leaves died off. Young plants were inoculated approximately every 5 months.

3.2 Isolation of Bacterial Antagonists

Bacteria were isolated from both soil and leaves, by direct and indirect methods, over two growing seasons.

They were isolated by direct plating in September 1991 from soil collected from

two commercial celery fields in Sherrington and Hemmingford, Québec, and from one experimental plot at an Agriculture Canada substation in Ste-Clothilde, Québec. The commercial fields were located on mineral soils, and the experimental plot was located on muck soil. Soil samples for direct plating were collected in May 1992 from experimental plots at Ste-Clothilde. The plots had been cropped to celery in 1991 and were lying fallow in 1992.

Potential antagonists were isolated by using celery as bait in September 1991 and in June, July and August 1992. The soil in which the bait was placed in 1991 came from the commercial fields in Sherrington and Hemmingford. In 1992 the bait was placed in a fallow Agriculture Canada experimental plot in Ste-Clothilde.

Bacteria were isolated directly from leaf surfaces and by dilution plating in September 1991, using leaf material from the two commercial fields and the experimental plot at Ste-Clothilde. Dilution plating was also used in August 1992 with material from Ste-Clothilde.

Isolation from soil by direct plating

For direct plating, soil samples of 50-80 g were collected from around the base of celery plants to a depth of about 5 cm. The soil in each individual sample was thoroughly mixed, and then small amounts were placed on half-strength nutrient agar with cycloheximide (½ NA+) and King's medium B (KMB) (Tuite, 1969) media. The

plates were kept on the lab bench at room temperature. As bacterial colonies formed, monocultures were made from those colonies with different morphologies, based on such characters as colour and texture, by making transfers to fresh NA and KMB media.

Isolation from soil by baiting with celery

In 1991 the inert coatings were removed from celery seeds of variety Florida 683 by rubbing them on a sieve under running water, and they were placed on the moistened surface of the various soil samples contained in Petri dishes. When the seeds germinated, 20 seeds from each sample were placed in flasks with 99 ml of sterile distilled water, and the flasks were kept on a rotary shaker (Labline Variable Junior, model 3520) at 150 rpm for 2 hours. Serial dilutions of 10^{-3} to 10^{-9} were made, and 50 ml aliquots were placed on ½ NA+ and KMB media. The plates were kept on the lab bench at room temperature. As bacterial colonies formed, monocultures were made from those colonies with different morphologies, based on such characters as colour and texture, by making transfers to fresh NA and KMB media.

In 1992 the bait consisted of three celery petioles and their leaf laminae, both healthy and diseased, which were enclosed in a pouch made from nylon window screening. Both young and senescing plant parts were used. Six pouches were buried at depths of 5, 10 or 15 cm. After 2 weeks they were removed from the soil, and dilution platings were made by placing 1 g of leaf material plus any adhering soil which could

not be lightly shaken off in 99 ml of sterile distilled water in a flask. The flasks were placed in an ultrasonic cleaner (Branson 1200, Branson Ultrasonics Corp.) for 30 seconds to dislodge bacteria from the leaf surfaces. Fifty μl aliquots from serial dilutions of 10^{-3} to 10^{-9} were plated on ½ NA+ and KMB media, and incubated at room temperature. As bacterial colonies formed, monocultures were made from those colonies with different morphologies, as described above.

Isolation from the leaf surface

For direct isolation five small leaf pieces containing both *S. apiicola* lesions and healthy tissue from each sample were placed on the surface of ½ NA+, ½ PDB+, KMB and RB plates. The plates were incubated at room temperature. As bacterial colonies formed, monocultures were made from those colonies with different morphologies, as described above.

For serial dilutions, leaves from each sample were cut into small pieces, and 1 g of leaf material was placed in 99 ml sterile distilled water in a flask. The flasks were held in an ultrasonic cleaner for 30 seconds to dislodge bacteria from the leaf surface. As described previously, the suspensions were serially diluted, plated, and transfers into monoculture were made from those bacterial colonies with different morphologies.

Storage of bacteria

All bacterial isolates were placed in a NB-glycerol (NBGly) medium for long-

term storage at -70°C. The medium was prepared by combining 8 g NB and 100 ml glycerol in 1 litre of distilled water. A sterile cotton swab was used to transfer bacteria from 24-48 hour old culture plates to 1.25 ml NBGly medium in a 1.5 ml microcentrifuge tube. The tubes were incubated at 26°C for 3 hours before being placed in a freezer at -70°C.

3.3 Evaluation of Bacterial Isolates Based on Pycnidial Growth *in vitro*

Bacterial antagonists were grown in 5 ml of NB in 16-mm-diameter test tubes. A loopful of bacteria was placed in each tube and the tube was shaken using a vortex mixer (Thermolyne Maxi Mix II, model 37600) briefly, and then incubated for 24 hours at room temperature on a rotary shaker at 200 rpm. The control consisted of half strength NB, not inoculated with bacteria, and was treated in a similar manner.

A 15,000 spore/ml suspension of *S. apiicola* was prepared with the aid of a haemocytometer. One hundred μl of this suspension was spread evenly over the surface of a celery potato dextrose agar plate (Mudita and Kushalappa, 1991), adjusted to pH 6 (CPDA6), and the plates were sealed and incubated at 22°C for 12 hours in the dark. Then a 50 μl drop of 24-hour-old antagonist suspension was placed in the center of each CPDA6 plate. The plates were returned to the incubator for 10 days. The light regime was continual darkness for 3.5 days, and then a 12 hour photoperiod of both fluorescent and near UV light for 6.5 days.

Pycnidial diameter was measured by using a micrometer in a dissecting microscope (Wild). Eight pycnidia per plate were measured: four at 0.5 cm from the edge of the bacterial colony, and four at 1.0 cm away. A template was used to divide the plate into 90° quadrants, and two pycnidia were measured per quadrant, one at each of the two distances from the edge of the colony. If zones of inhibition of the formation of pycnidia were present, they were also noted as being at least 0.5 cm wide if the width of the zone was 0.5 - 0.99 cm, or as being at least 1.0 cm wide if the width of the zone was 1.0 cm or greater.

The experimental design was completely randomized, with three replicates existing in time. The experiment was performed one replicate at a time using the same incubator. The plates were distributed evenly on the four shelves of the incubator to ensure that they all received the same light conditions. There were four control plates per replicate, one for each shelf, because it was felt that a temperature gradient might exist in the incubator. The experiment was performed once.

When the experiment was planned, the intention was to carry out a statistical analysis of the results. However, when the results were obtained it was clear that there were very few isolates that in any way reduced the formation of pycnidia, and there were clear and striking differences between those which did and those which did not. As well, this was a screening experiment, and the results would be subject to an arbitrary decision for setting a cut-off point beyond which a given treatment was

deemed ineffective at reducing pycnidial formation and growth. For these reasons it was not statistically analysed.

3.4 Evaluation of Bacterial Isolates Based on Conidial Germination on Leaf Disks

The methodology for this experiment was taken from Fernando (1993). Bacterial isolates were grown in 5 ml NB in 16-mm-diameter test tubes for 24 hours on a rotary shaker at 200 rpm in an incubator (Forma Scientific, model 2740) at 22°C. Thirty μl of the bacterial suspension was placed on the upper surface of each of three 1.0 cm diameter leaf disks on moistened filter paper (Whatman #1) in a 4.5 cm diameter glass Petri dish. The bacterial suspension was diluted ten-fold, which resulted in an OD_{600} = 0.1 to 0.2. Half-strength NB was used as a control. The leaf disks used came from celery plants grown in a growth cabinet (Conviron) at 22°C with a 14-hour photoperiod. The fourth and fifth leaves were used to provide leaf disks. The disks were incubated for 24 hours at 22°C, and then 20 ml of a 150,000 spore/ml suspension of *S. apiicola* was applied to them and they were returned to the incubator for 25 hours. Germination was stopped and the disks cleared by placing them on a cotton pad saturated with a solution of 1:1 v:v glacial acetic acid : absolute ethanol for 48 hours. A fresh pad saturated with distilled water was placed beneath the disks for 24 hours. The disks were stained with lactoglycerol-cotton blue to count the number of germinated conidia.

The experimental design was completely randomized, with one Petri dish per treatment. The isolates were randomly divided into six batches of 35 isolates each, and the experiment was performed on one batch at a time. Each of the three disks per dish was a sample. Sixty conidia, twenty from each disk, were evaluated for germination per treatment. The experiment was performed twice. It was not statistically analysed because a given isolate would be carried forward for further screening based on its ability to reduce germination to ≤30% of the control, not based on its ability to reduce conidial germination compared to other isolates.

From the above experiment eighteen isolates which reduced conidial germination to ≤30% of the control were selected to be re-tested, with a few modifications to the method already described. The concentration of the bacterial suspensions was measured with a spectrophotometer, and adjusted to $OD_{(XX)} = 0.1$. (approximately 10^7 cells/ml). Five leaf disks were placed in each Petri dish, and percent germination was counted on 25 conidia on each of four disks. The design consisted of three replicates of 18 isolates with five sample disks per isolate. The experiment was performed twice.

3.5 Evaluation of Bacterial Isolates Based on Disease Severity

Four isolates which reduced germination to ≤15% (123, 131, 143 and 197) were selected from the previous experiment for further testing on plants in the

greenhouse. These isolates were Gram-stained, and identification was attempted with Biolog bacterial identification system (Hayward, California), based on patterns of carbon source metabolization.

Seedlings were produced in a growth chamber, and then transplanted into pots in the greenhouse. Supplemental lighting was used to provide a 14-hour photoperiod. Pest insects were controlled by an integrated pest management program. Control of mealybugs was obtained with ethanol and by spraying the plants with Safer's soap. The following predators were used against thrips: *Hypoaspis miles, Amblyseius cucumeris*, and *Orius insidiosus*. *Hippodamia convergens*, *Aphidoletes aphidimyza*, and *Aphidius* spp. were used against aphids.

The bacterial isolates were grown in 50 ml NB on a rotary shaker (250 rpm) at room temperature, approximately 25°C, for 24 hours. They were then suspended in 0.1 M $MgSO_4$, and the concentration adjusted to $OD_{600} = 0.1$. (approximately 10^7 cells/ ml). At the 6-8 leaf stage three fully expanded leaves per plant were tagged, and were held in a horizontal position by wire loops placed in the soil. The bacterial suspensions were sprayed using an automatic spray chamber (Incom International) at a pressure of 170 kPa with a cone-type nozzle moving horizontally at 0.6 kph (Mudita and Kushalappa, 1993). Controls consisted of plants sprayed with 0.1 M $MgSO_4$. The plants were enclosed in plastic bags previously misted with water, and placed in a growth chamber at 22°C with a 14-hour photoperiod. A wire frame prevented the wet bags

from touching the leaves. After 24 hours the plants were inoculated with a 20,000 spore/ml suspension of *S. apiicola* conidia, using the spray chamber. They were replaced in the plastic bags and returned to the growth chamber. Three days later the bags were removed and the plants were placed in the greenhouse. The number of lesions on the tagged leaves was counted 14 and 21 days after inoculation with the pathogen.

The experimental design was completely randomized with four treatments and a control consisting of 0.1 M $MgSO_4$, with six replicate plants per treatment. The experiment was conducted twice.

IV. RESULTS

4.1 Effect of Bacterial Isolates on Pycnidial Growth *in vitro*

In total, 204 bacterial isolates were collected from organic and mineral soils and celery leaves. All these isolates were screened for their ability to inhibit the production of *Septoria apiicola* pycnidia *in vitro*.

When *S. apiicola* conidia in aqueous suspension were spread on CPDA, they gave rise to clusters or aggregates of pycnidia, rather than individual pycnidia. The diameters of these pycnidial clusters ranged from 23 to 2545 μm (Table 1.)

A total of 61 isolates produced inhibition zones ≥1.0 cm wide, although not consistently in all replicates. Thirty-three isolates produced inhibition zones ≥1.0 cm wide in only one of three replicates. Five isolates had inhibition zones in only two replicates and 23 isolates had inhibition zones in all three replicates (Table 2). However, the majority of the treatments produced pycnidial clusters that did not appear to differ from those of the control.

Table 1. Mean diameter of pycnidial clusters (μm) at 0.5 and 1.0 cm from the edge of the bacterial colony after 10 days incubation on CPDA[a].

Isolate	Replicate 1		Replicate 2		Replicate 3	
	0.5 cm	1.0 cm	0.5 cm	1.0 cm	0.5 cm	1.0 cm
A1	56	568	591	477	636	591
A2	523	704	1000	864	886	795
A3	1886	1409	1136	864	886	727
B1	864	727	932	864	1091	1023
B2	1250	864	568	500	682	614
B3	864	773	1045	1045	1204	545
C1	1250	841	954	109	841	614
C2	205	0	568	727	0	0
C3	977	932	409	591	1250	1045
1	886	636	841	954	795	909
2	477	1136	750	954	364	750
3	1159	1204	954	932	1636	1795
4A	0	318	932	1023	364	523
4B	795	886	773	1000	0	0
4C	0	386	0	0	0	136
5	682	841	1000	864	727	886
7	773	977	795	818	1636	1932
8	1000	1432	864	841	795	568
17	1409	1250	977	1318	500	682
18	750	864	0	45	45	0
20	409	909	750	1114	1136	1000
21	909	977	104	1295	795	591
22	1523	1136	682	750	1659	1795
23	0	0	0	0	0	0
24	0	0	0	0	0	0
28	704	823	886	1068	636	773
30	614	773	886	1091	250	273
31	545	591	0	0	386	773
33	0	0	0	0	0	0
37	250	523	0	0	1091	1341
38	704	886	818	932	500	273
39	614	818	818	750	1000	1091
41	0	0	0	0	0	182
42	932	909	318	159	432	364
44	500	818	500	864	909	818
45	432	682	1023	977	159	227
46	1863	1773	545	455	704	614
47	568	704	795	682	91	455
48	1023	682	273	704	591	795

continued...

Table 1., continued

Isolate	Replicate 1		Replicate 2		Replicate 3	
	0.5 cm	1.0 cm	0.5 cm	1.0 cm	0.5 cm	1.0 cm
49	1204	1409	727	545	455	409
50	1954	1568	455	591	1500	614
52	1295	1409	682	545	1114	1659
53	1045	977	250	227	954	909
54	0	0	0	0	0	0
55	1204	1068	818	795	1500	1500
56	1341	1295	659	704	0	182
58	364	523	727	841	954	795
59	977	1023	818	341	0	0
60	0	0	0	0	0	0
61	0	0	0	0	0	0
64	864	1091	932	1000	0	0
65	591	886	227	477	0	273
66	909	954	864	909	864	909
68	455	1454	0	0	0	0
69	1000	954	977	932	864	932
70	545	773	205	68	1023	1182
71	614	727	727	750	773	1045
74	932	1091	477	386	1341	1545
75	841	795	386	841	0	0
76	682	954	932	909	1159	1318
78	0	0	0	0	0	0
80	0	0	0	0	0	0
83	909	977	704	727	523	364
84	1545	886	1159	1159	318	295
90	1023	1091	1023	1204	954	727
96	0	591	0	841	45	409
99	0	0	0	0	0	0
100	1091	795	1227	1432	0	0
101	0	23	0	0	0	0
102	682	727	0	45	0	0
103	1341	1204	0	0	1276	1523
104	1454	1568	795	864	1364	2432
105	0	0	0	0	0	0
106	1068	1023	0	0	682	727
107	1000	932	0	0	1545	182
108	977	795	523	409	1136	1023
109	1613	1341	841	864	1659	1023

continued...

Table 1., continued

Isolate	Replicate 1		Replicate 2		Replicate 3	
	0.5 cm	1.0 cm	0.5 cm	1.0 cm	0.5 cm	1.0 cm
110	0	0	205	182	749	1023
111	977	954	1159	1136	705	954
112	1091	1023	727	773	1227	1750
113	0	432	409	773	0	114
114	0	0	0	0	0	0
115	1136	1273	727	818	841	591
116	727	909	1273	1273	500	409
117	1250	1068	545	455	682	455
118	91	16	0	0	0	0
119	273	432	909	659	1114	1409
120	0	0	0	0	0	0
121	682	591	0	0	704	91
122	1000	954	682	886	1250	1613
123	0	0	0	0	0	0
124	0	0	0	0	0	0
125	1091	1114	795	1136	1250	1454
126	1114	1182	954	1136	1477	1273
127	704	591	954	932	954	1023
128	0	0	0	91	0	0
129	0	0	818	750	1091	1295
130	636	636	636	545	1523	1318
131	1045	1523	841	795	864	568
132	0	0	0	0	0	0
133	0	0	0	0	0	0
134	0	0	0	0	0	0
135	750	727	364	523	1045	1068
136	1136	1136	909	682	0	0
137	1159	1045	704	704	1068	1091
138	773	864	909	818	841	1227
139	704	1250	1159	954	1045	841
140	500	977	727	500	114	386
141	682	614	636	659	1136	1227
142	750	932	205	954	1000	886
143	0	0	0	0	0	0
144	954	1000	704	636	1318	1386
145	136	523	0	23	91	341
146	614	773	773	864	591	682
147	1045	954	818	954	977	1023
148	1250	1273	864	1114	500	523

continued...

Table 1., continued

Isolate	Replicate 1		Replicate 2		Replicate 3	
	0.5 cm	1.0 cm	0.5 cm	1.0 cm	0.5 cm	1.0 cm
149	0	0	864	1068	409	318
150	795	864	909	1000	227	1000
151	0	2545	1909	1818	0	1568
152	455	545	432	455	0	0
153	0	0	909	886	1227	1182
154	1182	977	773	1114	864	932
155	250	273	273	159	0	0
156	795	1227	932	909	954	2704
157	704	954	773	977	1365	1364
158	909	773	909	818	1136	954
159	1023	1182	704	1000	500	682
160	1227	1114	909	841	818	909
161	0	0	0	0	0	0
162	568	727	591	773	1000	750
163	909	818	1091	1023	1091	1364
164	795	864	909	864	795	364
165	1409	1364	864	1136	795	1000
166	704	795	0	0	1636	1545
167	2000	2068	954	1000	704	1136
168	1682	1818	341	432	1454	1091
169	818	932	909	1068	636	818
170	1159	1273	0	0	1500	1682
171	909	1000	932	1000	0	0
172	1295	1204	727	818	0	659
173	954	1659	0	0	1182	1954
174	1114	886	954	932	1136	591
175	1114	954	0	0	1364	1364
176	909	1000	795	682	727	727
177	932	1182	341	500	1591	2522
178	545	704	500	659	1000	818
179	841	818	795	773	1091	1114
180	568	773	909	864	1273	1409
181	1295	1182	0	0	1182	318
182	750	932	1045	1114	1659	1250
183	2000	1636	977	1409	1341	1432
184	0	409	205	295	954	727
185	1136	1114	636	477	1636	1295
186	1159	1068	704	1114	1545	1318
187	1932	1114	364	386	1432	1204

continued...

Table 1., continued

Isolate	Replicate 1		Replicate 2		Replicate 3	
	0.5 cm	1.0 cm	0.5 cm	1.0 cm	0.5 cm	1.0 cm
188	886	682	954	841	1636	2045
189	841	727	477	523	954	1136
190	773	614	750	795	773	864
191	818	1000	727	659	3681	3500
192	1250	1273	909	909	1159	1091
193	523	682	704	500	0	91
194	932	886	886	659	977	1364
195	1091	1136	1295	1091	2273	1091
196	432	545	682	886	1091	1045
197	0	0	0	0	0	0
199	0	0	0	0	0	0
200	1091	977	182	182	818	568
201	1795	1364	864	795	182	477
202	1409	1227	977	841	2045	2136
203	932	1204	182	182	227	432
204	886	909	841	1068	864	682
205	1204	1023	659	659	2772	2341
206	1365	1114	773	659	1204	295
207	1409	1204	954	1091	1523	1841
208	727	659	773	636	841	818
209	1227	1182	0	0	1454	1727
210	682	954	1068	773	2159	2772
211	841	1091	500	568	2704	2636
212	795	818	909	1023	1454	1409
213	841	977	704	1091	1091	1318
214	773	1091	773	818	1909	3431
215	750	818	682	1091	1114	1114
216	818	1341	636	545	1545	3295
217	1250	1159	1023	1182	1523	1523
219	1182	1068	1091	864	2273	2909
220	1227	1523	909	1273	1954	1159
221	682	682	659	1136	1000	455
222	0	0	0	0	0	0
223	68	250	68	295	0	182
224	954	864	659	932	2318	2636
225	1045	1136	659	704	1909	1409
226	1159	954	455	727	1091	2545
227	0	0	0	0	0	0
228	0	0	704	682	1318	1250

continued...

Table 1., continued

Isolate	Replicate 1		Replicate 2		Replicate 3	
	0.5 cm	1.0 cm	0.5 cm	1.0 cm	0.5 cm	1.0 cm
229	1091	1568	773	841	0	0
230	2136	1341	568	568	1273	1273
232	1045	841	727	727	568	1118
233	545	545	614	682	682	477
235	795	841	0	0	1136	954
236	727	886	0	0	1545	1341
237	818	864	818	864	864	1091
238	136	614	0	0	136	477
Control 1[b]	1204	1295	954	864	295	773
Control 2	818	1045	1227	795	2545	2704
Control 3	704	1045	659	773	1886	1954
Control 4	841	864	886	1023	1636	2477

[a] *S. apiicola* conidia were spread on a plate of CPDA, and 12 hours later a suspension of a bacterial isolate was spotted in the center of the plate.

[b] Four controls, one placed on each shelf of the incubator, were used to observe any variation due to a temperature gradient in the incubator.

Table 2. List of isolates that produced inhibition zones ≥1.0 cm wide in the growth of *Septoria apiicola* on CPDA in one or more replicates[a].

One replicate	Two replicates	Three replicates
C2	41	23
4B	68	24
4C	101	33
31	118	54
37	128	60
59		61
64		78
75		80
100		99
102		105
103		114
106		120
107		123
110		124
121		132
129		133
136		134
149		143
152		161
153		197
155		199
166		222
170		227
171		
173		
175		
181		
209		
228		
229		
235		
236		
238		

[a] A total of 61 bacterial isolates produced inhibition zones.

4.2 Effect of Bacterial Isolates on Spore Germination on Leaf Disks

The germination of the *S. apiicola* conidia varied from 0 to 100 percent on leaf disks sprayed with 204 different isolates of bacteria (Tables 3.1 - 3.6). The germination was expressed as the proportion of maximum germination, where the maximum germination was the number of conidia germinated in the control from each batch and trial. For this reason proportions were sometimes greater than 1, i.e. if a treatment had a higher percent germination than the control. Eight-five isolates reduced germination to ≤30% of the control in at least one trial, while only 15 isolates reduced germination to ≤30% of the control in both trials. These were isolates 7, 21, 38, 60, 65, 71, 90, 96, 100, 101, 102, 123, 128, 131, 143, 149, 162, 196, and 197.

Batch one had extremely low germination for most isolates, including the control, in trial one. Germination in the control was only 1.67%. Therefore only trial two was considered when evaluating the performance of the isolates in batch one.

4.3 Effect of Selected Bacterial Isolates on Spore Germination on Leaf Disks

Eighteen isolates were selected for further testing on leaf disks based on the results from the two mass screening experiments. More weight was given to the results from the *in vivo* assay than those from the agar plate assay because the leaf disk assay more closely resembled a field situation. Fifteen isolates reduced conidial germination

Table 3.1. Proportion of maximum germination[a] of *Septoria apiicola* conidia after 25 hours incubation on leaf disks previously sprayed with a bacterial isolate (Batch 1).

Isolate	Trial 1	Trial 2
Control	1.00	1.00
A1	3.99	0.83
4B	0.00	0.72
7	0.00	0.26
8	2.07	0.74
23	3.21	0.70
39	4.99	0.63
55	1.00	0.59
66	1.46	0.85
69	7.98	0.38
76	2.22	0.57
100	1.00	0.11
109	11.0	0.80
113	5.99	0.91
120	2.35	0.70
121	17.0	0.91
124	8.98	0.59
138	1.00	0.50
139	4.99	0.51
143	1.00	0.13
157	0.00	0.94
174	0.00	0.59
180	2.99	0.94
184	1.00	1.03
190	0.00	0.82
191	3.74	0.65
195	3.99	0.13
199	0.00	0.74
201	4.99	0.35
204	0.00	0.51
208	2.99	0.94
210	5.21	0.81
222	0.00	0.57
225	1.00	0.89
236	0.00	1.02

[a] Maximum germination is the number of conidia germinated in the control, and was 1.67% and 90.00% for trials 1 and 2, respectively.

Table 3.2. Proportion of maximum germination[a] of *Septoria apiicola* conidia in after 25 hours incubation on leaf disks previously sprayed with a bacterial isolate (Batch 2).

Isolate	Trial 1	Trial 2
Control	1.00	1.00
8	0.90	0.92
37	0.24	1.03
42	0.10	1.28
59	1.19	1.23
64	1.43	1.26
71	0.24	0.21
104	0.00	1.31
111	0.19	1.06
117	1.52	1.39
133	0.52	1.37
134	0.52	1.00
136	0.38	1.23
137	0.14	0.69
142	0.05	1.31
145	0.48	0.44
146	1.48	1.15
148	0.64	1.01
149	0.24	0.10
150	0.81	1.33
152	0.05	1.05
154	0.24	1.33
156	0.81	0.33
161	1.59	1.15
164	1.05	1.08
165	1.14	1.31
167	0.90	1.36
169	0.33	1.05
172	0.33	0.28
178	0.81	1.11
188	1.10	1.15
196	0.00	0.18
228	0.86	0.94
230	0.48	0.92
233	1.10	1.46

[a] Maximum germination is the number of conidia germinated in the control, and was 35.00% and 65.00% for trials 1 and 2, respectively.

Table 3.3. Proportion of maximum germination[a] of *Septoria apiicola* conidia after 25 hours incubation on leaf disks previously sprayed with a bacterial isolate (Batch 3).

Isolate	Trial 1	Trial 2
Control	1.00	1.00
A2	0.60	0.47
C1	0.20	0.71
1	0.65	0.89
17	0.68	0.73
44	0.64	0.62
52	0.64	0.81
58	0.00	1.04
74	0.33	0.74
78	0.79	0.87
96	0.23	0.02
99	1.35	0.98
107	0.64	0.84
112	1.08	0.80
115	1.52	0.84
116	1.03	0.84
126	0.69	0.44
127	0.25	0.82
130	0.88	0.69
141	0.93	0.53
163	0.65	0.82
175	1.57	0.87
181	0.54	0.87
182	0.98	0.51
186	1.42	0.93
189	1.03	1.08
193	0.30	0.45
194	1.28	0.74
197	0.30	0.20
200	1.28	0.60
206	1.33	0.81
207	0.59	0.79
216	1.67	0.40
224	0.45	0.89
238	1.82	0.60

[a] Maximum germination is the number of conidia germinated in the control, and was 33.96% and 75.00% for trials 1 and 2, respectively.

Table 3.4. Proportion of maximum germination[a] of *Septoria apiicola* conidia after 25 hours incubation on leaf disks previously sprayed with a bacterial isolate (Batch 4).

Isolate	Trial 1	Trial 2
Control	1.00	1.00
B3	0.32	0.64
C2	0.73	0.80
3	0.59	0.61
4C	0.98	0.07
20	0.27	0.70
21	0.27	0.00
38	0.38	0.16
48	0.14	0.54
65	0.14	0.11
90	0.55	0.23
98	0.09	0.45
102	0.11	0.18
108	0.45	0.45
114	0.27	0.50
118	0.50	0.48
122	0.23	0.36
123	0.14	0.05
128	0.18	0.07
147	0.68	0.82
162	0.18	0.01
166	0.30	0.41
173	0.91	0.82
179	1.18	0.61
185	0.05	0.76
187	0.09	0.73
202	0.61	0.39
203	0.91	0.32
212	0.82	0.36
214	0.12	0.64
217	0.03	0.71
226	0.14	0.47
229	0.05	0.48
NIR	0.18	0.75

[a] Maximum germination is the number of conidia germinated in the control, and was 36.67% and 73.33% for trials 1 and 2, respectively.

Table 3.5. Proportion of maximum germination[a] of *Septoria apiicola* conidia after 25 hours incubation on leaf disks previously sprayed with a bacterial isolate (Batch 5).

Isolate	Trial 1	Trial 2
Control	1.00	1.00
C3	0.15	0.98
18	0.05	0.87
22	0.05	0.99
24	0.05	1.15
41	0.11	0.91
45	0.07	0.37
46	0.10	1.13
47	0.10	0.72
49	0.05	0.82
60	0.00	0.11
80	0.10	1.15
83	0.05	0.98
84	0.07	1.08
97	0.05	0.91
101	0.05	0.28
105	0.15	0.70
119	0.05	1.26
129	0.00	0.98
131	0.12	0.07
135	0.00	1.02
140	0.00	0.93
144	0.15	0.50
151	0.10	1.26
153	0.10	1.02
155	0.05	0.83
159	0.10	0.90
170	0.15	0.74
176	0.00	0.93
209	0.10	1.07
211	0.06	1.27
219	0.05	1.06
221	0.06	1.13
227	0.36	1.04
235	0.00	0.85

[a] Maximum germination is the number of conidia germinated in the control, and was 32.69% and 76.60% for trials 1 and 2, respectively.

Table 3.6. Proportion of maximum germination[a] of *Septoria apiicola* conidia after 25 hours incubation on leaf disks previously sprayed with a bacterial isolate (Batch 6).

Isolate	Trial 1	Trial 2
Control	1.00	1.00
A3	1.05	1.27
B1	1.02	1.24
B2	0.79	1.24
2	0.96	1.24
4A	0.73	0.84
5	1.04	1.13
30	0.88	0.82
31	0.78	0.31
33	0.95	1.11
53	0.86	1.02
54	0.75	1.11
56	0.84	1.13
61	0.93	1.29
68	0.77	0.76
75	0.95	0.00
103	1.07	0.89
106	0.93	0.98
110	0.77	0.98
125	0.63	1.22
132	0.93	1.11
158	0.79	0.93
160	1.02	0.91
168	0.70	0.91
171	1.02	0.98
177	0.59	0.98
183	0.86	0.84
192	0.82	1.22
205	0.91	0.76
213	0.45	0.89
215	0.77	1.00
220	0.71	1.22
223	0.16	0.40
232	0.96	0.00
237	1.03	1.29

[a] Maximum germination is the number of conidia germinated in the control, and was 93.33% and 75.00% for trials 1 and 2, respectively.

to ≤30% or less of the control in both trials (Tables 3.1-3.6), and 13 of these were chosen to be carried to the next level of testing. All 15 should have been carried on, but two isolates were overlooked.

Of these 13 isolates, six also showed inhibition zones of varying widths and in different numbers of blocks in the agar plate assay (Table 2). Isolates 100 and 143 were selected because they reduced germination to ≤30% of the control in trial 2; the results of trial one were not considered because they were anomalous. Although isolates 38 and 90 did not perform well in either screening assay, they were included as controls.

The data from this experiment were arcsin-transformed before performing analysis of variance. The error variances were not homogenous for the two trials, so they were analysed separately. Duncan's multiple range test was also performed on the data.

Isolates 123, 131, 143, and 197 were most effective at reducing germination in both trials (Table 4). In the first trial they reduced germination to 11.0%, 9.8%, 19.0% and 4.2% of the control, whereas in the second trial, they reduced germination to 7.3%, 5.9%, 10.1%, and 7.2% of the control, respectively. In neither trial were these four isolates significantly different from one another. However, these four isolates were significantly different from all the other isolates in trial two only.

Table 4. Mean proportion of maximum germination[a] of *Septoria apiicola* conidia after 25 hours incubation on celery leaf disks previously sprayed with selected bacterial isolates[b].

Isolate	Trial 1[c]	Trial 2[c]
Control	1.00a	1.00a
4C	0.63efg	0.49ef
21	0.76de	0.74bcde
38	0.87bcde	0.90abc
60	0.77cde	0.42f
65	0.88bcde	0.92ab
71	0.83bcde	0.77abcde
90	0.95abc	0.85abc
96	0.87bcde	0.81abcd
100	0.85bcde	0.77abcde
102	0.49fgh	0.81abcd
123	0.11ij	0.07g
128	0.43ghi	0.54def
131	0.04j	0.06g
143	0.19hij	0.10g
149	0.41ghi	0.43f
162	0.93abcd	0.68cdef
196	0.99ab	1.06a
197	0.04ij	0.07g

[a] Maximum germination is the number of conidia germinated in the control, and was 94.00% and 85.33% for trials 1 and 2, respectively. The proportion of maximum germination and statistical analysis were conducted using four significant digits.
[b] The selection criteria were the formation of inhibition zones ≥1.0 cm wide in the growth of *S. apiicola* in the agar plate assay, and the reduction of conidial germination on leaf disks to ≤30% of the check; details in text.
[c] Means with the same letter are not significantly different at $P = 0.05$.

In general, the isolates which were able to reduce germination to ≤30% of the control in the leaf disk assay were not able to do so when the assay was repeated with a more rigorous experimental design. Apart from the four top-performing isolates, the remaining ones did not vary greatly in their ability to reduce conidial germination. Isolates 90, 162 and 196 did not differ significantly from the control in trial 1, and in trial 2 isolates 38, 65, 71, 90, 96, 100, 102, and 196 did not differ significantly from the control.

4.4 Effect of Selected Bacterial Isolates on Disease Severity

Isolates 123, 131, 143, and 197, selected based on their ability to reduce germination on leaf disks (Table 4), were evaluated for their ability to reduce disease severity on plants in the greenhouse. Since the error variances were not homogenous for the two trials, the analysis of variance was conducted separately for each trial. The average number of lesions was derived from the three tagged leaves per plant.

Disease severity was higher in the second trial than in the first as shown by the increase in the number of lesions present. Significant differences between treatments were observed only at 3 weeks after inoculation in trial one (Table 5). Isolate 143 had the fewest mean number of lesions, although not significantly different from the control or isolate 131. There were no significant differences among the treatments at 2 weeks after inoculation in either trial, and at 3 weeks in trial two.

Table 5. Mean number of late blight lesions per leaf of greenhouse-grown celery assessed at 2 and 3 weeks after application of selected bacterial antagonists[a] suspended in 0.1 M $MgSO_4$ and inoculation with *Septoria apiicola* conidia 24 hours later.

	Trial 1		Trial 2[b]	
Isolate	Two weeks[b]	Three weeks[c]	Two weeks	Three weeks
Control	0.22	0.56ab	5.00	7.89
123	0.61	1.61a	4.83	7.00
131	0.33	0.83ab	4.22	7.56
143	0.28	0.50b	3.67	7.39
197	0.61	1.56a	5.11	9.33

[a] The four isolates were selected based on the reduction of conidial germination to ≤20% of the control in both trials of the second experiment on leaf disks (see Table 3); details in text.

[b] There are no significant differences among these means at $P = 0.05$ with trial and time.

[c] Means with the same letter are not significantly different at $P = 0.05$.

The mean minimum temperature was 18°C, and the mean maximum temperature was 28°C during the first trial. During the second trial the mean minimum and maximum temperatures were 21°C and 26°C, respectively.

Isolates 123, 131, and 197 were gram-positive, and isolate 143 was gram-negative. Isolate 131 was tentatively identified as *Pseudomonas fluorescens*, and isolates 123 and 197 were *Pseudomonas* spp.

V. DISCUSSION

The bacterial antagonists applied to the surface of celery leaves 24 hours prior to inoculation with *S. apiicola* were not able to reduce the number of late blight lesions formed as compared with the control. The lack of disease control may have been due to the bacteria not surviving long enough on the leaves to antagonize the *S. apiicola* conidia, or not multiplying, possibly because the pathogen was inoculated too soon after the application of the bacteria. However, bacterial populations were not determined following their application, so this is only conjecture.

There are several factors which affect the survival of bacteria on the leaf surface. High relative humidity is one of the most important influences on the growth and survival of bacteria on the phylloplane (Blakeman, 1985; Andrews, 1992). Because high relative humidity was provided for 96 hours after the application of the bacteria to the leaves, this was probably not a limiting factor in their survival. It is more likely that nutrients were limiting. In the greenhouse the plants would be exposed to fewer exogenous nutrients than in the field, although some nutrients would be leaked from the plants themselves and others would come from aphids (Godfrey, 1976; Blakeman, 1985; Andrews, 1992), which were a pest problem. Supplying simple sugars or amino acids with the solution in which the bacteria were suspended for application could help increase their survival and growth on the leaf (Blakeman, 1985). When mixtures of the

yeasts *Sporobolomyces roseus* and *Cryptococcus laurentii* var. *flavescens* were sprayed with and without nutrients on spring wheat leaves, the populations of yeasts which had been sprayed with nutrients were higher than those which had been sprayed without at two and three weeks after application. The yeasts with nutrients were also able to significantly lower the reduction in chlorophyll content of the leaves by *Septoria nodorum* as compared to control leaves sprayed with water (Fokkema et al., 1979).

A third reason for decreased bacterial survival on the phylloplane is the loss of characteristics which confer fitness for survival in nature. This often occurs with wild-type bacteria that have been cultured on agar media for several generations, and includes traits such as adhesive structures or those that help prevent desiccation (Andrews, 1990).

The lack of disease control by the bacterial antagonists may also have been due to the *S. apiicola* conidia being applied at too high a concentration (2×10^4 spores/ml) for the bacteria to overcome. Or, on the other hand, the concentration of the bacterial suspension may not have been high enough. When *Bacillus cereus* was applied to leek to control leek rust (*Puccinia allii*) at a concentration of 2.5×10^7 bacteria/ml, it gave no control, but reduced frequency of pustules by 93-99% when applied at a concentration of 6×10^9 bacteria/ml (Doherty and Preece (1978) in Rishbeth, 1988).

The timing of antagonist application is also very important. In general, the antagonist should have time to establish itself on the leaf before the arrival of the pathogen. However, this may not always be the case. When *Pseudomonas fluorescens* was applied to pine seedlings in the field to control sceleroderris canker (*Gremmeniella abietina*), a significant reduction in disease incidence was observed only when the antagonist was applied 1 hour before inoculation with the pathogen. If the bacteria were applied even one day before inoculation, there was no significant reduction in disease incidence (Spurr and Knudsen, 1985). The lack of control of late blight observed in the greenhouse may have been due to a similar problem in the timing of the application of antagonist and pathogen.

It is possible that the bacteria and the pathogen may have been at different locations on the leaf (Lukezic et al., 1990). A *S. apiicola* conidium on the top of an epidermal cell can penetrate the leaf directly (Donovan et al., 1990), without the germ tube entering a stomate, while the bacterial antagonists are most likely to be along the junction between cells (Blakeman, 1985; Lukezic et al., 1990; Andrews, 1992).

One explanation for the reduction in germination of *S. apiicola* conidia on leaf disks is that the bacteria may have been taking up nutrients leached from the conidia. Isolates of *Pseudomonas* sp. and *Sporobolomyces* sp. were able to inhibit germination of *Botrytis cinerea* and *Phoma betae* on leaf surfaces, and studies with ^{14}C-labelled amino acids showed a direct relationship between the labelled amino acid uptake by the

bacteria, and the reduction in pathogen spore germination (Blakeman and Brodie, 1977). Bacteria are more able to take up nutrients from water films than fungi; this coupled with the bacteria absorbing nutrients leached from the spore, creates a situation in which amino acids are limiting to the pathogen (Blakeman and Fokkema, 1982).

Microorganisms isolated from the crop plant to be protected and from the plant part to be protected are more likely to be effective biocontrol agents than those which come from other regions or other parts of the plant (Blakeman, 1985; Cook, 1993). This is partly because they do not thrive where they do not occur naturally. However, soil micro-organisms have been able to protect plants from foliar diseases (Andrews, 1992), and with genetic engineering it may be possible to improve a microbe's fitness for survival outside of its native habitat. When the parasite-host associations of biological control of insect pests and weeds were studied it was found that the chances of success were up to 75% greater if the association was a new one (Boland, 1990). Casida and Lukezic (1992) found that *Pseudomonas* strain 679-2, isolated from the soil, reduced severity of leaf-spot diseases caused by *Alternaria solani, Pseudopeziza medicaginis, Phoma medicaginis* and *Stemphylium botryosum* on tomato and alfalfa. In order not to unnecessarily eliminate any possible antagonists, microbes were isolated from both the phylloplane, where they would be well-adapted, and from the soil, where other successful agents on the phylloplane have been found. Bacteria were isolated on a general-purpose medium, nutrient agar, for the same reason.

It is well-known that the effectiveness of an antagonist in agar plate assays is not necessarily correlated with effectiveness at reducing disease under field conditions (Andrews, 1985 and 1992). However, *in vitro* tests can be very useful for identifying the antagonist's mode of action and are an easy way to make comparisons between isolates. Inhibition zones usually result from an antibiotic produced by the biocontrol agent which has diffused through the growth medium, but they may also result from nutrient deprivation (Andrews, 1985). Either one of these modes of action would work well in a biocontrol system with *S. apiicola*, an unspecialized necrotroph (Blakeman, 1988; Andrews, 1992), which grows over the leaf surface for some time before finally penetrating. In the pre-penetration phase it could be antagonized by an antibiotic-producer, or prevented from obtaining the energy necessary for penetration by being out-competed for nutrients by an organism previously established on the phylloplane.

VI. CONCLUSION

The biological control of plant diseases with micro-organisms has great potential to reduce our current dependance on chemical controls. However, there remains much to be learned about microbial ecology before many biocontrol agents can be developed and will be in widespread use (Andrews, 1990). Many micro-organisms are tested for antagonistic activity against plant pathogens, but very few are ever marketed.

To investigate biological control for the control of late blight of celery, 204 bacterial isolates were screened for antagonism toward *S. apiicola*. Four isolates showed promising activity on both *in vitro* and *in vivo* screens, but did not show any antagonistic activity when tested on plants. This does not mean that these isolates should be discarded as possible antagonists, but rather that they should be re-examined under different environmental conditions.

Suggestions for further research include re-evaluating isolates 123, 131, 143, and 197 for their ability to reduce disease severity on plants. If the bacteria were suspended in a buffer containing amino acids or simple sugars as a nutrient source, they may become effective. These four isolates were able to reduce germination on leaf disks, and it is possible that a source of nutrients would enable them to overcome unfavourable environmental conditions on the phylloplane. The creation of mutant or

labelled strains of bacteria would enable their populations to be monitored over time, providing more information about their fate once applied on the plant. These same strains could also be used in mode of action studies. Finally, it is possible that some of these mutants could be more competent on the phylloplane than the wild-types (Boland, 1990).

VII. LITERATURE CITED

Agrawal, K., T. Singh, D. Singh and S.B. Mathur. 1985. Studies on glume blotch disease of wheat I. Location of *Septoria* in seed. Phytomorphology 35:87-94.

Alexopoulos, C.J. and C.W. Mims. 1979. Introductory Mycology, 3rd ed. John Wiley and Sons, New York. 632 pp.

Andrews, J.H. 1985. Strategies for selecting antagonistic micro-organisms from the phylloplane. In Biological Control on the Phylloplane. C.E. Windels and S.E. Lindow, eds. American Phytopathological Society Press, St. Paul, Minnesota. pp 31-44.

Andrews, J.H. 1990. Biological control in the phylloplane: realistic goal or false hope? Canadian Journal of Plant Pathology 12:300-307.

Andrews, J.H. 1992. Biological control in the phyllosphere. Annual Review of Phytopathology 30:603-635.

Baker, C.J., J.R. Stavely, C.A. Thomas, M. Sasser and J.S. MacFall. 1983. Inhibitory effect of *Bacillus subtilis* on *Uromyces phaseoli* and on development of rust pustules on bean leaves. Phytopathology 73:1148-1152.

Blakeman, J.P. 1985. Ecological succession of leaf surface micro-organisms in relation to biological control. In Biological Control on the Phylloplane. C.E. Windels and S.E. Lindow, eds. The American Phytopathological Society Press, St. Paul. pp 6-30.

Blakeman, J.P. 1988. Competitive antagonism of air-borne fungal pathogens. In Fungi in Biological Control Systems. M.N. Burge, ed. Manchester University Press, Manchester. pp 141-157.

Blakeman, J.P. and I.D.S. Brodie. 1977. Competition for nutrients between epiphytic microorganisms and germination of spores of plant pathogens on beetroot leaves. Physiological Plant Pathology 10:29-42.

Blakeman, J.P. and N.J. Fokkema. 1982. Potential for biological control of plant diseases on the phylloplane. Annual Review of Phytopathology 20:167-192.

Boland, G.J. 1990. Biological control of plant diseases with fungal antagonists: challenges and opportunities. Canadian Journal of Plant Pathology 12:295-299.

Buck, K.W. 1988. Control of plant pathogens with viruses and related agents. In Biological Control of Pests, Pathogens, and Weeds: Developments and Prospects. R.K.S. Wood and M.J. Way, eds. The Royal Society, London. pp 185-207.

Burge, M.N. 1988. The scope of fungi in biological control. In Fungi in Biological Control Systems. Burge. M.N., ed. Manchester University Press, Manchester. pp 1-18.

Burrage, S.W. 1976. Aerial microclimate around plant surfaces. In Microbiology of Aerial Plant Surfaces. C.H. Dickinson and T.F. Preece, eds. Academic Press, London. pp 173-184.

Campbell, R. 1989. Biological Control of Microbial Plant Pathogens. Cambridge University Press, Cambridge. 218 pp.

Casida, L.E., Jr. and F.L. Lukezic. 1992. Control of leaf spot diseases of alfalfa and tomato with applications of the bacterial predator *Pseudomonas* strain 679-2. Plant Disease 76:1217-1220.

Cochran, L.C. 1932. A study of two Septoria leaf spots of celery. Phytopathology 22:791-812.

Conseil des productions végétales du Québec (CPVQ). 1987. Légumes protection. Agdex 250/605. Ministère de l'Agriculture, des Pêcheries, et de l'Alimentation du Québec. 112 pp.

Conseil des productions végétales du Québec (CPVQ). 1992. Feuillet technique - légume - céleri. Agdex 251. Ministère de l'Agriculture, des Pêcheries, et de l'Alimentation du Québec. 6 pp.

Cook, R.J. 1988. Management of the environment for the control of pathogens. In Biological Control of Pests, Pathogens, and Weeds: Developments and Prospects. R.K.S. Wood and M.J. Way, eds. The Royal Society, London. pp 61-71.

Cook, R.J. 1993. Making greater use of introduced micro-organisms for biological control of plant pathogens. Annual Review of Plant Pathology 31:53-80.

Cook, R.J. and K.F. Baker. 1983. Nature and Practice of Biological Control of Plant Pathogens. The American Phytopathological Society, St. Paul. 539 pp.

Cullen, D. and J.H. Andrews. 1984. Epiphytic microbes as biological control agents. In Plant-Microbe Interactions: Molecular and Genetic Perspectives. Vol. 1. T. Kosuge and E.W. Nester, eds. Macmillan Publishing Co., New York. pp 381-399.

Cullen, D., F.M. Berbee and J.H. Andrews. 1984. *Chaetomium globosum* antagonizes the apple scab pathogen, *Venturia inequalis*, under field conditions. Canadian Journal of Botany 62:1814-1818.

Davenport, R.R. 1976. Ecological concepts in studies of micro-organisms on aerial plant surfaces. In Microbiology of Aerial Plant Surfaces. C.H. Dickinson and T.F. Preece, eds. Academic Press, London. pp 199-215.

Donovan, A., S. Isaac, H.A. Collin and C.J. Veltkamp. 1990. Ultrastructure of infection of excised leaves of celery caused by *Septoria apiicola*, causal agent of leaf spot disease. Mycological Research 94:548-552.

Dullahide, S.R. 1979. Septoria leaf spot control in celery. Queensland Agricultural Journal 105:317-320.

Fernando, D. 1993. A simple technique to observe conidial germination on leaf surfaces. Mycologist 7:188-189

Fokkema, N.J., J.G. den Houter, Y.J.C. Kosterman and A.L. Nelis. 1979. Manipulation of yeasts on field-grown wheat leaves and their antagonistic effect on *Cochliobolus sativus* and *Septoria nodorum*. Transactions of the British Mycological Society 72:19-29.

Fokkema, N.J., J.A.J. van de Laar, A.L. Nelis-Blomberg and B. Schippers. 1975. The buffering capacity of the natural microflora of rye leaves to infection by *Cochliobolus sativus*, and its susceptibility to benomyl. Netherlands Journal of Plant Pathology 81:176-186.

Fravel, D.R. 1988. Role of antibiotics in the biocontrol of plant disease. Annual Review of Phytopathology 26:75-91.

Gabrielson, R.L. and R.G. Grogan. 1964. The celery late blight organism *Septoria apiicola*. Phytopathology 54:1251-1257.

Godfrey, B.E.S. 1976. Leachates from aerial parts of plants and their relation to plant surface microbial populations. In Microbiology of Aerial Plant Surfaces. C.H. Dickinson and T.F. Preece, eds. Academic Press, London. pp 433-439.

Harman, G.E. 1991. Seed treatments for biological control of plant disease. Crop Protection 10:166-171.

Hawksworth, D.C., B.C. Sutton and G.C. Ainsworth. 1983. Ainsworth and Bisby's Dictionary of the Fungi. 7th ed. Commonwealth Mycological Institute, Kew, Surrey, England. 445 pp.

Hislop, E.C. 1976. Some effects of fungicides and other agrochemicals on the microbiology of the aerial surfaces of plants. In Microbiology of Aerial Plant Surfaces. C.H. Dickinson and T.F. Preece, eds. Academic Press, London. pp 41-47.

Horst, R.K., ed. 1990. Westcott's Plant Disease Handbook. 5th ed. Van Nostrand Reinhold, New York. 803 pp.

Jarvis, W.R., L.A. Shaw and J.A. Traquair. 1989. Factors affecting antagonism of cucumber powdery mildew by *Stephanoascus flocculosus* and *S. rugulosus*. Mycological Research 92:162-165.

Kao, C.W. and W.H. Ko. 1986. The role of calcium and microorganisms in suppression of cucumber damping-off caused by *Pythium splendens* in a Hawaiian soil. Phytopathology 76:221-225.

Kokalis-Burelle, N., P.A. Backman, R. Rodriguez-Kabana and L.O. Ploper. 1992. Potential for biological control of early leafspot of peanut using *Bacillus cereus* and chitin as foliar amendments. Biological Control 2:321-328.

Leben, C. 1965. Epiphytic microorganisms in relation to plant disease. Annual Review of Phytopathology 3:209-230.

Lukezic, F.L., K.T. Leath, M. Jones and R.G. Levine. 1990. Efficiency and potential use in crop protection of the naturally occurring resident antagonists on the phylloplane. In New Directions in Biological Control: Alternatives for Suppressing Agricultural Pests and Diseases. R.R. Baker and P.E. Dunn, eds. Alan R. Liss, New York. pp 793-812.

MacMillan, H.G. 1942. Structure and germination of *Septoria* spores. Journal of Agricultural Research 64:547-559.

Marshall, G.M. 1960. The incidence of certain seed-borne diseases in commercial seed samples. III. *Septoria* leaf spot, or blight, of celery. Annals of Applied Biology 48:27-33.

Mathieu, D. 1992. Effects of temperature and duration of leaf wetness on infection of celery by *Septoria apiicola*, and cultivar screening for partial resistance. M.Sc. Thesis, McGill University, Montreal. 177 pp.

Maude, R.B. 1964. Studies on *Septoria* on celery seed. Annals of Applied Biology 54:313-326.

Maude, R.B. and C.G. Shuring. 1970. The persistence of *Septoria apiicola* on diseased celery debris in the soil. Plant Pathology 19:177-179.

Mudita, I.W. and A.C. Kushalappa. 1991. Effect of media and temperature on sporulation of *Septoria apiicola*, and of inoculum density on septoria blight severity in celery. Phytoprotection 72:97-103.

Mudita, I.W. and A.C. Kushalappa. 1993. Ineffectiveness of the first fungicide application at different initial disease incidence levels to manage Septoria blight in celery. Plant Disease 77:1081-1084.

Mukhath, A. and R.K.S. Wood. 1962. Infection of celery seedlings by *Septoria* spp. Nature 196:493.

Nelson, M.E. and Powelson, M.L. 1988. Biological control of grey mold of snap beans by *Trichoderma hamatum*. Plant Disease 72:727-729.

Papendick, R.I. and R.J. Cook. 1974. Plant water stress and development of Fusarium foot rot in wheat subjected to different cultural practices. Phytopathology 64:358-363.

Rishbeth, J. 1988. Biological control of air-borne pathogens. In Biological Control of Pests, Pathogens, and Weeds: Developments and Prospects. The Royal Society, London. pp 155-171.

Scharen, A.L. 1964. Environmental influences on development of glume blotch in wheat. Phytopathology 54:300-303.

Scharen, A.L. 1966. Cyclic production of pycnidia and spores in dead wheat tissue by *Septoria nodorum*. Phytopathology 56:580-581.

Sherf, A.F. and A.A. MacNab. 1986. Vegetable Diseases and Their Control. 2nd ed. John Wiley and Sons, New York. 728 pp.

Sheridan, J.E. 1966. Celery leaf spot: sources of inoculum. Annals of Applied Biology 57:75-81.

Sheridan, J.E. 1968. The causal organism of celery leaf spot, *Septoria apiicola*. Transactions of the British Mycological Society 51:207-213.

Spencer, D.M. and P.T. Atkey. 1981. Parasitic effects of *Verticillium lecanii* on two rust fungi. Transactions of the British Mycological Society 77:535-542.

Spurr, H.W., Jr. 1990. The phylloplane. In New Directions in Biological Control: Alternatives for Suppressing Agricultural Pests and Diseases. R.R. Baker and P.E. Dunn, eds. Alan R. Liss, New York. pp 271-278.

Spurr, H.W., Jr. and G.R. Knudsen. 1985. Biological control of leaf diseases with bacteria. In Biological Control on the Phylloplane. C.E. Windels and S.E. Lindow, eds. The American Phytopathological Society Pess, St. Paul. pp 45-62.

Srivastava, A.K., G. Défago and H. Kern. 1985. Hyperparasitism of *Puccinia horiana* and other microcyclic rusts. Phytopathologische Zeitschrift 114:73-78.

Statistics Canada. 1995. Fruit and vegetable production. Catalogue 22-033 Seasonal. Ministry of Supply and Services Canada, Ottawa. 65 pp.

Tuite, J. 1969. Plant Pathological Methods: Fungi and Bacteria. Burgess Publishing Company, Minneapolis. 239 pp.

Uma, N.U. and G.S. Taylor. 1987. Parasitism of leek rust urediniospores by four fungi. Transactions of the British Mycological Society 88:335-340.

Waage, J.K. and D.J. Greathead. 1988. Biological control: challenges and opportunities. In Biological Control of Pests, Pathogens and Weeds: Developments and Prospects. R.K.S. Wood and M.J. Way, eds. The Royal Society, London. pp 1-18.

Weller, D.M. and R.J. Cook. 1983. Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. Phytopathology 73:463-469.

Whipps, J.M. 1992. Status of biological disease control in horticulture. Biocontrol Science and Technology 2:3-24.