"Final Project Report to the NYS IPM Program, Agricultural IPM 2002–2003."

Title: Identification of crops which regulate soil population levels of *Burkholderia cepacia* causing bacterial canker and sour skin of onions.

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Type of grant: Biological control and pest biology.

Project location(s): The findings of this study could be applied on organic soils cropped to onion in New York and elsewhere in Northeastern North America.

Abstract.

Two approaches (field sampling and miniculture) were used to identify vegetable and field crops and other plant species which regulate population levels of *Burkholderia cepacia* in organic soils cropped to onions, and for rotation with onions to reduce the occurrence of bacterial canker and sour skin of onions caused by the pathogen. It was found that crops such as corn and soybean may aggravate disease incidence if used in rotation to onion, whereas other crops such as radish, lettuce, beets, carrots, millet, and carrot and turnip may offer profitable and disease-ameliorating rotation alternatives. Furthermore, baseline trends are becoming apparent that allow the comparison of cropping strategies from one field to another as relating to the population levels of *B. cepacia* that the fields support.

Background and justification.

B. cepacia is a bacterium which can infect onion plants at different stages in their development resulting at first in bacterial canker of growing and maturing plants and later in sour skin of onion bulbs immediately prior to harvest or after harvest in storage [6]. The bacterium is resident in the organic soils of New York fields cropped to onions. Recent research has indicated that population levels of the bacterium in the soils are regulated by the cropping histories of the soils. In addition to the typical pathogenesis of *B. cepacia* to onion, another aspect of the pathogenesis of the bacterium is the ability to rapidly invade wounds on leaves of onion plants [1] caused by hail storms accompanied by rain which splashes particles of soil containing the bacterium into the wounds resulting in rapid decay of the leaves and the developing bulb tissue. This scenario can result in the partial or complete destruction of an onion crop depending on the severity of the hail and rain storm. In recent years New York onion growers have suffered severe losses due to *B. cepacia*. Control of *B. cepacia* has been considered a high priority during the annual summer and winter meetings of the New York Onion Industry Council in recent years. Field studies since 1999 and a miniculture procedure developed during 2001, with both approaches utilizing a selective medium effective in quantifying population levels of *B. cepacia* in soils previously cropped to onion after growth of other vegetables and field crops, have identified several vegetable and field crops which have the potential to reduce or increase the levels of *B. cepacia* in the soils. Corn greatly increases

levels of the bacterium while wheat appears to decrease levels of the bacterium. Continued studies to identify crop plants which regulate the levels of *B. cepacia* in the soils would provide onion growers with the information they need to utilize those crops which reduce levels of *B. cepacia* in rotation with onion and avoid those crops which increase the levels of *B. cepacia* and thereby reduce the threat of *B. cepacia* as an onion pathogen. This approach to control *B. cepacia* would avoid the contamination of ground water by the use of pesticides [4] which growers have used in the past to attempt control of the bacterium.

Objectives.

The objectives of the proposed research were: (1) to determine the levels of *B. cepacia* in New York soils cropped to onion and potential rotational crops which regulate the level of the bacterium in the soil and (2) to determine by utilizing a miniculture procedure under controlled environmental conditions those vegetable and field crops (other plant species as feasible) which regulate (increase, maintain, or decrease) populations of the bacterium in the soils. Identification of crops which reduce the populations of *B. cepacia* to low levels in the soils would provide control (partial or considerable) of the pathogenesis of *B. cepacia* on subsequent crops of onion following rotation to the identified crops. The basis for evaluation of the success of the proposed project would be the identification of a number of crops which decrease *B. cepacia* in the soils and could be used as cash crops in the rotation sequence. Successful results from the proposed research would impact all commercial onion growers in New York and the 12,000–13,000 acres of onions the growers presently cultivate to onions each year in the state.

Procedures.

The population levels of *B. cepacia* in soils cropped to onion and other vegetable and field crops (other plant species as feasible) were determined to identify those crops and plant species which regulate (increase, maintain, or decrease) the levels of the bacterium in the soils. This was accomplished by sampling the soils of fields of grower cooperators and also by a recently developed and successfully tested miniculture procedure utilized under controlled environmental conditions.

(1) For field studies, 5–8 soil samples from each field surveyed were mixed by hand and then sifted through a number 16 sieve (1.1 mm). Five to 9 gm of the soil were dried at 50°C overnight, then reweighed. The ratio of dry weight (DW) to fresh weight was calculated. In the procedure, fresh soil (0.5–0.7 g) was added to a screw-cap tube containing 25 ml of sterile 0.1% (w/v) aqueous peptone. The soil suspension was shaken for 30 minutes on a wrist-action shaker at 375 cycles per minute. Aliquots (0.02 and 0.10 ml) were removed from the suspension and spread over the surface of PCAT agar (a patented, selective medium for *B. cepacia*) [2]. The assay plates were incubated at 37°C for 2 days and the resulting colonies of *B. cepacia* were counted. The number of colony forming units (CFU) per gram (DW) of soil was calculated using the bacterial counts multiplied by the sampling dilution factor and divided by the dry weight of the input soil.

(2) In the miniculture procedure, seeds were pre-germinated on water-saturated filter papers in the dark. Disposable syringe tubes of 10 ml size were arranged in a test-tube rack. A circle of Whatman 2 filter paper was placed in the bottom of each tube to retain the soil. Two and one-half grams (DW) of soil was added to the tube and packed to the 10 ml level to result in a soil density of 0.25g (DW)/cm^3. Water was added to the tube and the soil was permitted to drain by gravity. The pre-germinated seeds were then pressed into the soil surface (root radical downward) and the tubes were placed in a growth chamber at 20°C (constant) with a 16 hour day length. The resulting plantlets were watered on an as-needed basis. Support of the syringe tubes in the test-tube rack permitted drainage without cross-contamination of the tubes. After 2–3 weeks growth, the soil and plant in each tube were air-ejected from the tube, the plantlet cut off at the soil line, and the entire volume of the soil and root system added to 50 ml 0.1% peptone in a 125 ml Erlenmeyer flask. The soil suspension was shaken on a wrist-action shaker as above for 30 minutes. Aliquots (0.25 ml) of suspension were removed from the flask and

were added to 25ml 0.1% peptone to effect a 1/100 dilution. Samples (0.02 and 0.10 ml) were then taken from the dilution solution and plated on the PCAT medium. The number of resulting colonies of *B. cepacia* was multiplied by the sampling dilution factor and divided by 2.5 to determine the number of CFU per gram (DW) of soil.

Results and discussion.

SURVEYS OF FIELD SOILS AND QUANTIFICATION OF *B. CEPACIA*.

Soil samples were taken from fields cropped to onion and non-onion plants at various times during the growing season and assayed for *B. cepacia*. The results of these determinations are expressed as mean CFU/g (DW) soil. Because only a single viable cell of the bacterium may be required to initiate infection after gaining entry to onion tissues, and because each CFU results from the presence of a single viable cell of the bacterium, these values represent the potential inoculum load in the soil being assayed, per unit mass of infested soil. *B. cepacia* is a naturally occurring environmental organism in agricultural soils, riverbeds [3], and other soil ecosystems. However, previous studies in this laboratory have found it to be undetectible in newly cleared organic soil (never planted to any crop). After a season of onion cultivation, however, low levels of *B. cepacia* can be observed (data not shown). Previous studies have established that *B. cepacia* is capable of overwintering in infested soil, but little has been conclusively established concerning the competitive fitness of *B. cepacia* in infested soils under the influence of non-onion crops; i.e., whether there exist some plant species that exert a diminishing force on the *B. cepacia* population, or whether other plant species may amplify the population levels of the pathogen in the soil.

The results of the field soil assays are shown in Table 1. As might be expected, there is considerable variation in the observed population levels. To some extent this is owing to differences in sampling date, and it must be borne in mind that under ideal conditions a

Table 1. Onion and Rotation Crop Soils Sampled.

Crop Grown (field); notes	Month	CFU/g soil
Leek	August	205,000.
Onion (field 1)	June	108,000.
Pumpkin (field 1)	August	65,200.
Green Onion	October	53,200.
Corn (field 1)	August	39,900.
Onion (field 2)	August	34,300.
Corn (field 2); plants dry/unharvested, (see following spring, below).	October	27,800.
Onion (field 3)	June	23,500.
Onion (field 4)	September	20,400.
Green Bean	August	20,300.
Corn (field 3)	October	13,500.
Pumpkin (field 2)	September	12,200.
Radish; (field 1); 4 year history of radish cultivation.	October	8,230.
Radish (field 2)	July	5,410.
Soybean (field 1); sampled after leaf senescence, before harvest.	October	4,560.
Pumpkin (field 3)	September	2,280.
Corn (field 2); field tilled and yet unplanted, after season above.	April	2,370
Radish & Lettuce; alternating crops over 4 years, sampled after tilling.	October	1,300.
Soybean (field 2) ; sampled after leaf senescence, before harvest.	October	969.
Squash	September	825.
Radish (field 2); newly planted, first of several cycles this year.	April	647.
Turfgrass (field 1); sampled after the sod had been stripped.	August	635.
Turfgrass (field 2); sampled before sod had been stripped.	October	456.
Turfgrass (field 3); sampled before sod had been stripped.	August	0.

bacterial population may increase several-fold within a very short time (hours). However, there are clear trends that can be identified in the data, and these differences can be attributed to the species under cultivation, the cropping history of the field, and the maturity of the plants.

Typically, Allium species (storage onions, green onions, leeks) supported *B. cepacia* populations of 20,000 to 200,000 and higher CFU/g DW soil during the summer months (Table 1). Similarly, green bean, corn, and pumpkin cultivation on soils with a history of cropping to onion resulted in significant pathogen infestation. Other fields, such as those planted to radish repeatedly, radish and lettuce in succession, squash, soybean, and turfgrass showed moderate or very low levels of *B. cepacia* infesting the soil. It is notable that these relationships appear to persist, even after an overwintering; in the case of corn field 2 (April) the early spring *B. cepacia* levels (2,300 CFU/g) are higher than those found in radish field 2 (April; 647 CFU/g), which parallels the differential observed later in the season for the same two fields (27,300 *vs*. 5,410 CFU/g). Thus it is conceivable that cultivation of crops such as radish, lettuce, squash, soybean, and turfgrass may be useful in reducing inoculum levels of *B. cepacia* in fields routinely planted to onion.

INFLUENCE OF NON-ONION CROP SPECIES UPON *B. CEPACIA* POPULATIONS IN MINICULTURE.

In order to examine *B. cepacia* population dynamics in the rhizosphere of a wider variety of plant species than available in fields presently under cultivation, the miniculture system was utilized. This system permits assay of the bacterial profile associated with a single, isolated, plantlet, under controlled environmental conditions, with a known input inoculum load. After cultivation, the resulting *B. cepacia* population load in the soil was determined. Because the initial level of *B. cepacia* in the soil varied from one experiment to another, in order to make a meaningful comparison, the final population in each tube was divided by the input level to calculate a percentage increase (or decrease) under the influence of the plant being tested. The results of these experiments are shown in Figure 1.

As expected, there were great differences found among the crops tested. Some, such as corn and soybean, elicited over 500% increases in the soilborne *B. cepacia* population levels, whereas others such as millet, carrot, and beets actually reduced the levels of the pathogen after 3 weeks' cultivation. Other species found to maintain low levels of *B. cepacia* were turnip, radish, tomato, lettuce, pepper, and wheat. These results generally agree with the field data, although there are some differences, such as with soybean. However, in the soybean fields sampled, the plants and soil were quite dry, and it is likely that biological activity in the soil had

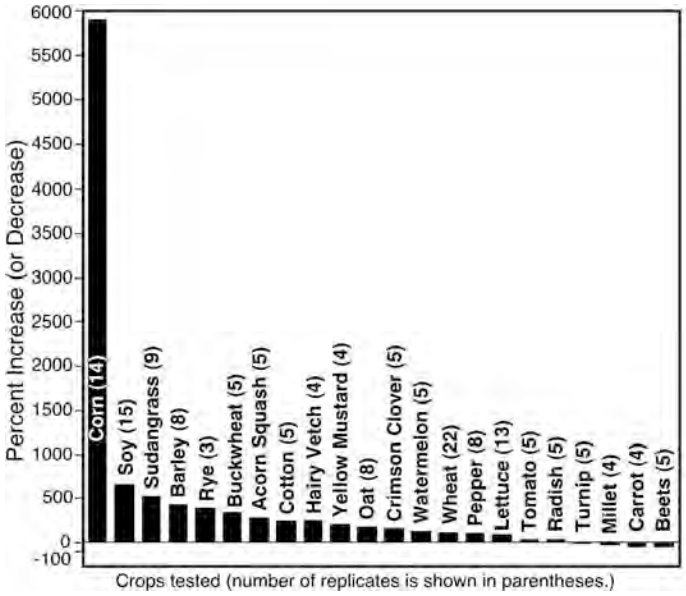

Figure 1. Population levels of *B. cepacia* in miniculture after 2–3 weeks' cultivation. CFU/g (DW) were determined for the soil both before and after cultivation, and the change was expressed as a percent increase or decrease from the input load. In some cases, several cultivars were tested, such as with wheat, but since the results were similar, the averaged data are shown for each species.

slowed in general, whereas the moisture levels in the miniculture soils were maintained higher, to promote plant growth. It is also possible that the large quantities of carbohydrate and protein stored in soybean and corn seeds exerted disproportionate influence on the observed *B. cepacia* levels owing to the relatively small volume of soil employed in these studies.

The results of the miniculture experiments illustrate the usefulness of this system as a preliminary screening technique before field trials are conducted to identify possible rotation crops. We are in the process of testing an array of additional plant species by miniculture, and expect that the data generated will provide a basis for subsequent field studies.

B. CEPACIA POPULATION BASELINE TRENDS DURING THE GROWING SEASON.

Crop field soils are far from homogeneous compounds, and are influenced horizontally and vertically by myriad factors, such as row and bed spacing, plowing, planting, spray patterns, rainfall and shading, water table rise and fall, rainfall percolation, root penetration and decay, solar heating, and changing soil composition. Therefore, it is reasonable to expect a considerable amount of variability in microbe populations both from sample to sample within a field and from field to field. Nevertheless, it was possible to discern an overall *trend* in the *B. cepacia* population levels, both spatially, according to the crop and rotation scheme (above), and temporally during the growing season. Indeed, an ongoing aspect of our work [5] has involved the annual monitoring of onion fields both continually cropped to onion, and managed by crop rotation in order to evaluate any cyclic phenomena that may be apparent (data not shown).

To provide a baseline reference for these multiseason studies, we repeatedly sampled the same sites of two adjacent onion fields (not subject to rotation management) at monthly intervals, and quantified the *B. cepacia* levels present in the soil. Because bacterial growth is an exponential function, the assayed counts were converted to logarithms for graphical presentation. These results are shown in Figure 2. Both fields exhibited the same dynamic

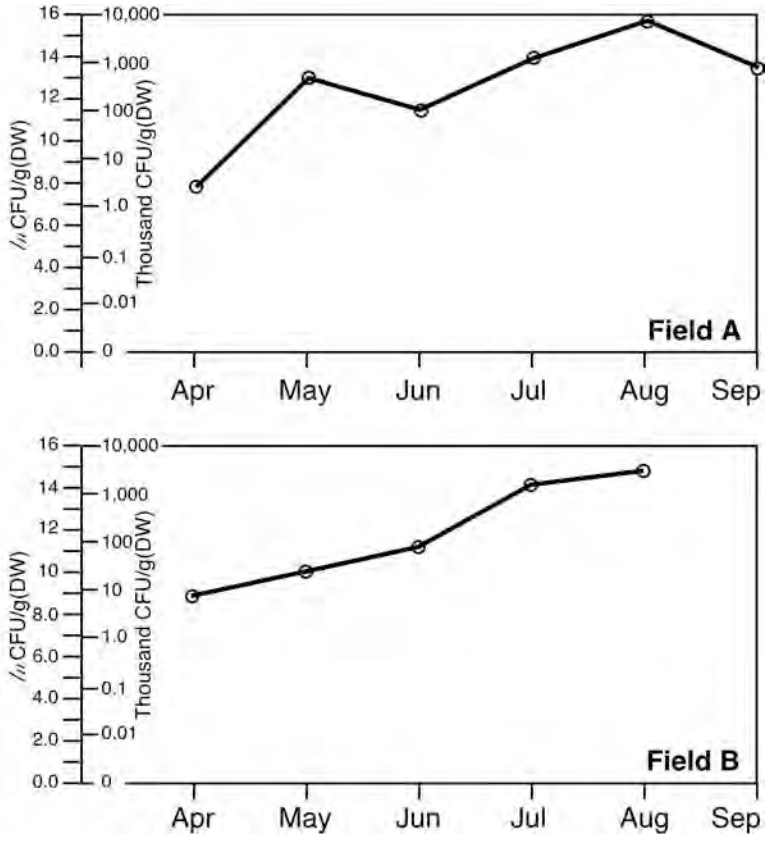

Figure 2. *B. cepacia* population counts during the 2002 growing season as determined in two adjacent onion fields not under rotation management. The data are plotted on a logarithmic scale. Eight sampling sites were revisited in field A, and five were revisited in field B. The averaged value across the field is plotted.

trend and similar population levels, having 2–8 *thousand* CFU/g (DW) soil in the spring at the time of planting, and rising to 2–8 *million* CFU/g (DW) soil by late summer. In field A (Fig. 2), although there appeared to be a transient drop in June, *B. cepacia* population levels continued to rise the rest of the summer, until the final sampling in September, by which time the plants had matured and were essentially dry. The crop in field B was pressured by a mite infestation during 2002, which visibly reduced yields, but did not mask the overall trend in pathogen levels. As expected, there was considerable sample to sample variation in the CFU/g (DW) measured for each field, each month. The standard deviation of the montly samples in field A ranged from 45–237% of the measured *B. cepacia* population level, and was 92% overall. The standard deviation in field B ranged from 0.3–45% of the measured *B. cepacia* population level, and was 31% overall. For comparison, the standard deviations measured in miniculture experiments also ranged from 12–55% of the measured CFU/g (DW) value (50% in unplanted controls). Although seemingly large, this range represents less than a single generation of bacterial growth, and does not mask the overall trends in the population dynamics of *B. cepacia.*

CONCLUSIONS AND PRACTICAL APPLICATION OF FINDINGS.

The identification of several crop species that positively and negatively influence the soilborne levels of *B. cepacia* both in miniculture and in the field context suggests that a management program may be developed for the control of the bacterial canker-sour skin disease of onion. At present, some onion growers have implemented rotation schemes that utilize lettuce, radish, spinach, and squash as commercially viable options to onions. As additional candidate crops are identified by further surveys and ongoing miniculture evaluation, it is hoped that growers will plant partial or entire fields that have been under continuous onion cultivation to such alternative crops, in order to assess the large-scale and field-condition commercial benefit. It is also possible that winter cover crops might find use to aid in reducing *B. cepacia* population levels. Further work needs to be done to identify potential cover crops and green manures that reduce the soilborne levels of *B. cepacia.* Miniculture may provide a route to that goal. The value of reducing soilborne *B. cepacia* populations lies in the reduction of pre- and postharvest losses (because this pathogen is largely resistant to antibiotics and copper formulations [4] that are commonly utilized) and in the avoidance of more severe control measures, such as fumigation, which may incur adverse environmental consequences.

References.

1. S.O. Kawamoto and J.W. Lorbeer, 1974. Infection of onion leaves by *Pseudomonas cepacia*. Phytopathology **64**(11):1440–1445.

2. R.D. Lumsden and M. Sasser, 1986. US patent No. 4,588,584.

3. L.G. Leff, R.M. Kernan, J.V. McArthur, and L.J. Shimkets, 1995. Identification of aquatic *Burkholderia (Pseudomonas) cepacia* by hybridization with species-specific rRNA gene probes. Applied and Environmental Microbiology **61**(4):1634–1636.

4. G.L. Mark and J.W. Lorbeer, 2001. The response of three phenotypically variable *Burkholderia cepacia* isolates to copper-based bactericides. pp315–318. *In:* Plant Pathogenic Bacteria. Edited by S.H. DeBoer. Published by Kluwer Academic Publishers, Dordrecht, The Netherlands. 454pp.

5. G.L. Mark and J.W. Lorbeer, 2001. Population dynamics of the onion phytopathogen *Burkholderia cepacia* in organic soils cropped to onion. *In:* Plant Pathogenic Bacteria. Edited by S.H. DeBoer. Published by Kluwer Academic Publishers, Dordrecht, The Netherlands. 454pp.

6. G.L. Mark, R.D. Gitaitis, and J.W. Lorbeer, 2002. Bacterial diseases of onion. pp. 267–292. *In:* Allium Crop Science: Recent Advances. Edited by H.D. Rabinowitch and L. Currah. CABI Publishing, Wallingford, U.K. 515pp.