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**Epidemiology and Control of Bacterial Soft
Rot Diseases in Potatoes: *Erwinia Carotovora*
Pv. Carotovora (ECC), *Erwinia Carotovora*
Pv. Atroseptica (ECA), *Erwinia Chrysanthemi*
(ECH) and *Erwinia* Spp. (ESPP.)**

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Final Report BARD Project No. I-581-82.

Epidemiology and Control of Bacterial Soft Rot Diseases in

Potatoes: Erwinia carotovora subsp. carotovora (Ecc),

Erwinia carotovora subsp. astroseptica (Eca),

Erwinia chrysanthemi (Ech) and Erwinia spp. (Esp).

by

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הספריה המרכזית
למדעי החקלאות
בית-דגן

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Abstract

Bacterial soft of potato caused by strains of Erwinia carotovora, subsp. carotovora (Ecc) E. carotovora subsp. atroseptica (Eca) Erwinia chrysanthemi (Ech) and Erwinia spp. (Esp) were studied to help reduce losses of this important food crop during pre- and post-harvest periods.

Improved methods for detection and identification of soft rot bacteria were developed. These methods include the development of an improved pectate selective medium exhibiting higher recovery and increased selectivity for soft rot Erwinia spp. Other advances developed include the use of three different incubation temperatures as a means of differentiation of species and sub-species of soft rot Erwinia and the development of highly specific monoclonal antibodies to assist in rapid identification and detection of these soft rot bacteria.

By the use of improved techniques, an epidemiological study was conducted in which population dynamics under controlled conditions were analyzed. The disease potential was estimated based on population levels of the different Erwinia spp. on potato seed tubers or in irrigation water required for disease expression in the field. Under Israeli conditions, populations of Eca at levels of 10^3 CFU/g potato peel have the potential to cause economic losses in the field. Bacterial populations of Ecc were found to be unimportant, whereas the Ech population had high disease potential under Israeli conditions. Our study demonstrated the importance of each Erwinia group and the population dynamics of these Esp strains during the two main growing seasons in Israel (Spring and Autumn).

Factors influencing resistance in the potato tuber to bacterial soft rot were also studied. By increasing the calcium content in the tuber or plant tissues, it was possible to increase resistance to bacterial soft rot especially under USA conditions.

In a cooperative project with Prof. H. Frankel, Dr. A. Grinstein, and M. Austerwil of the Institute of Agricultural Engineering in the Volcani Center, Bet Dagan, a new bactericidal application technique was also developed. This technique increased the ability to reduce soft rot potential on potato tubers without immersion, and was better than commercial organic mercury treatments. As a result of this project, semi-commercial Erwinia-free potato seed is now being produced in the Golan Heights, Israel.

Objectives of the Original Research Proposal.

The primary objectives of the proposed research were as follows:

- I. Develop improved methods for detection and quantification of soft rot bacteria associated with water, soil, seed, and plant material (Israel, USA)
 - II. Evaluate new bactericidal and/or bacteriostatic preparations with potential value for application to both seed tubers and potatoes in commercial storage (Israel, USA: Eca, Ecc, Ech and Esp).
 - III. Study population dynamics under controlled conditions to determine the relationship between disease potential and expression, particularly under climatic conditions in Israel (Israel: Eca, Ecc, Ech).
 - IV. Determine factors affecting resistance of potato tubers to bacterial soft rot pathogens. This would permit the development of improved assays for detecting differences in susceptibility between breeding lines (USA: Eca, Ecc, Ech).
- I. Develop improved methods for detection and quantification of soft rot bacteria associated with water, soil, seed and plant material.

Improved methods for detection and quantification of soft rot bacteria associated with soil, water, seed and plant material have been developed. The improved methods are based on: a) Enhancing the selectivity of the crystal violet pectate selective medium (CVP) that was developed by Cuppels and Kelman (1974). b) Improving enrichment methods for the recovery of low populations of Erwinia spp. by using pectate as a sole carbon source and incubating under anaerobic conditions. c) Improving recovery and identification of Erwinia populations by incubating CVP plates at three different temperatures (15°C, 28°C, and 39°C.). d) Improving and modifying the MPN (most probable number) technique for estimating bacterial populations. e) Developing monoclonal antibody techniques for rapid identification of strains of Erwinia carotovora and Erwinia chrysanthemi.

a) A number of inorganic compounds were tested to evaluate their effectiveness in enhancing selectivity of CVP. A range in concentrations of lithium chloride and manganese sulfate were evaluated. At concentrations effective in significantly reducing populations of Pseudomonas fluorescens, strains of Erwinia carotovora were inhibited although to a lesser degree with manganese sulfate than P. fluorescens. The addition of egg albumin and conalbumin enhanced the formation of fluorescence by P. fluorescens strains in the crystal violet pectate medium, as well as in potato dextrose agar, casein hydrolysate, peptone, glucose agar and other standard media. Neither egg albumin nor conalbumin had an adverse effect on growth of soft rot Erwinia strains. Addition of these materials to the various media tested greatly improved the ability to distinguish P. fluorescens and related pseudomonads from the soft rot Erwinia. Increasing the concentration of calcium and the addition of the trisodium salt of citric acid to the CVP medium enhanced the

ability to differentiate strains of P. fluorescens from strains of the soft rot Erwinia. On this medium, pectolytic strains of Erwinia formed typical, distinctive pits characteristic of these bacteria. The size of the pits on this modified medium were smaller than on the standard CVP medium. In sharp contrast, the pectolytic pseudomonads either formed very shallow pits or no pits within 48-72 hr. Furthermore, the medium remained firm and pits did not enlarge as rapidly as they did on standard CVP. Recovery efficiency on modified CVP was equivalent to that on standard CVP. Strains of P. fluorescens are frequently present in soil and decaying plant material and interfere with detection and enumeration of E. carotovora populations. Table 1 summarizes the key diagnostic tests that aid in the identification of Erwinia carotovora subsp. carotovora, Erwinia carotovora subsp. atroseptica and Erwinia chrysanthemi strains.

The crystal violet pectate medium (CVP), has been used effectively to isolate and detect pectolytic Erwinia spp. The advantages and disadvantages of the medium as well as the efforts to improve its selectivity were presented in the 1985 Annual Research Report. Additional attempts to improve this medium were completed in 1986.

In previous work, the addition of sodium thiosulfate slightly enhanced Erwinia carotovora carotovora (Ecc) recovery when added at concentrations of 2.5 mg/ml and 5.0 mg/ml to Di's Modified CVP. Although plating efficiency of one strain of Pseudomonas marginalis was not affected, the addition of the reducing agent decreased the recovery of one strain of P. fluorescens by 30%. In a continuation of this phase of the research, recovery of a strain of Ecc, four pectolytic strains of P. marginalis, and two different strains of P. fluorescens was tested on the modified CVP medium containing the concentrations of sodium thiosulfate used in previous experiments. In all cases the percent recovery of the bacteria tested was not affected, indicating that the results previously obtained with the one strain of Pseudomonas fluorescens were not indicative of the effects on other plant pathogenic pseudomonads.

Tinopal, a brightening agent that was reported to inhibit most plant pathogenic bacteria, was tested by addition to the casamino peptone glucose medium (CPG) at concentrations of 0.47, 2.35, 4.7, 9.4, 18.4, and 46 µg/ml. Ecc, P. phaseolicola, and P. tabaci strains were grown on the CPG medium at these concentrations. Recovery of pectolytic strains of P. marginalis and of P. fluorescens was also evaluated at Tinopal concentrations of 0.47, 2.35, 4.7, and 9.5 µg/ml. Although the addition of Tinopal to the CPG medium did not affect recovery in all cases, concentrations of 4.7 µg/ml and above reduced the colony size of both P. phaseolicola, and P. tabaci; colony diameter of P. tabaci was reduced to about 1/8th of normal size at the 46 µg/ml concentration. The size of colonies of pectolytic pseudomonads was not affected.

In addition to the screening of selective agents, changes in the concentrations of components of the modified CVP medium were also evaluated. One problem in the use of the modified CVP is that addition of increased amounts of CaCl₂ and trisodium citrate reduces the recovery of Erwinia about 10% below that of the standard CVP medium. However, the isolation of colonies

of the pectolytic Erwinia strains from CVP is difficult because this medium rapidly liquifies as colonies develop. Furthermore, because the surface is not firm, it is difficult to streak suspensions on this medium without cutting into the agar.

One modification involved the addition of various levels of CaCl_2 , (0.06, 0.08, 0.10, and 0.12%) without the addition of 2.5 gm of sodium citrate. Unfortunately with levels of CaCl_2 above 0.06%, the medium prepared in flasks solidified so rapidly as it cooled that plates could not be poured.

A second series of experiments involved changes in concentrations of both CaCl_2 and agar. Three basal media tested were A) the standard CVP; B) a modified version of CVP including an increased amount of the CaCl_2 solution (6 ml.) plus a 2.5 gm increase of sodium citrate; and C) CVP plus 4.0 gm of agar/500 ml. rather than 2.0 gm. All three media poured easily. Ease of streaking on medium C was better than on the other two media. Plating efficiency on all three media was tested using two strains each of Ecc, Erwinia carotovora subsp. atroseptica (Eca), and Erwinia chrysanthemi (Ech). When the number of bacteria recovered on medium B was compared to that on the standard CVP, % recovery ranged from 86-100%. Also the plating efficiency of medium C was 85-109% of that on the standard CVP. The modified CVP (medium B) showed the deepest, most distinct pits, followed by the medium C, and then the original CVP.

The media were further evaluated by the dilution plating of a field soil suspension on plates of media A, B, and C. The modified medium decreased nonpectolytic background bacteria to 57% of those isolated on the original CVP; on medium C (the CVP plus increased agar levels) approximately the same number of such bacteria were recovered as on the standard CVP.

In conclusion, CVP modified by increasing the agar concentration is most effective when streaking suspensions to obtain Erwinia colonies. However, the advantages of medium B with its high reduction in background bacteria, and its deep, very distinct pits outweighs the disadvantages of a slight reduction in plating efficiency of Erwinia. Thus, it is the best of the media tested for the isolation of soft rot Erwinia from infected soil and plant material.

b) Enrichment methods for the detection and quantification of the soft rot Erwinia (Eca, Ecc, and Ech) were based on the inoculation of plant or soil material into a minimal salts medium containing pectate as sole carbon source. Incubation was completed under anaerobic condition (jars or incubators) for 72 hr. at 15°C, 28°C, or 39°C. Under these conditions, only two genera of bacteria could be enriched: Erwinia or Clostridium. After 72 hr the enrichment medium was diluted, plated on CVP using the most probable number (MPN) technique, and incubated under aerobic conditions. After this step, only Erwinia spp. were enriched and grown. Estimation of Erwinia populations of 1 to 10 CFU per sample could be detected, whereas by direct inoculation on CVP plates, only Erwinia populations of greater than 50 CFU per ml or gram of sample could be detected.

c) A procedure for the detection and quantification of soft rot Erwinia spp. (Eca, Ecc, and Echr) from potato tubers was developed. Improved methods of detection and enumeration of soft rot pathogens enable us to conduct epidemiological studies and to generate data used for formulating recommendations concerning the improvement of seed certification practices. Forty tubers per lot of seed tubers were removed, peeled (gram fresh wt. determined) and homogenized together in 0.1% ascorbic acid (w/v). This suspension was diluted and plated on CVP or enrichment media and incubated for 3-4 days under N₂ atmosphere at 15°C, 28°C and 39°C. Results were calculated as Erwinia CFU/g peel. Rapid identification of the bacterial populations was dependent on growth at different temperatures and length of incubation. After 72 hr. of incubation at 15°C on improved CVP medium, colonies were identified as Eca. After 48 hr. of incubation at 28°C on CVP media, most colonies were identified as Ecc, whereas only Echr grew at 39°C after 72 hr. of incubation. Colonies sampled from each incubation regime were verified using classical biochemical identification techniques. The data presented in Table 2 illustrate the problem of poor recovery of Erwinia spp. on CVP media, improved recovery on the modified CVP medium, and the need to develop alternate methods for the estimation of low populations rather than direct isolations on CVP plates. This table also demonstrates the rapid differentiation of the three groups of Erwinia spp. by using three different incubation temperatures for given periods of time. Similar recovery levels are achieved using mixtures of the tested pathogen at different ratios in a given population (Table 3).

d) The MPN technique was modified using 10 replicates in petri plates containing 15 ml of the improved CVP medium. On the surface of the plate, 10 circles were drawn and 20 µl of each dilution of a ten-fold dilution series was placed in these circles. The final calculations were completed using the tables of Meynel and Meynel (1970).

For estimation and recovery of low populations, a two step technique has been developed. A population of less than one cell per ml of sample can be isolated, identified and estimated with approx. 10% error (Table 4). Estimations of low populations (i.e. after enrichment) and estimations by the MPN method on improved CVP media were conducted at 28 C. If a final result was obtained in which approximately 1.4×10^3 CFU's were present, this would indicate that there was a population of 0.1-1 CFU per ml or gr. in the source material and the bacteria were probably Ecc.

e) The objective of this phase of the project was to develop monoclonal antibodies (MCAs) to a strain of Ecc pectate lyase from Ecc and alkaline phosphatase from Echr that would be helpful in the detection of these pathogens.

Antibodies were produced by injecting a seven week old Balb/c mouse with 5×10^6 cells of a rifampicin resistant strain of Ecc suspended in either Freund's incomplete, Freund's complete or a saline buffer at various times over a four month period. The mouse was killed and its spleen cells fused with mouse myeloma cells. The fusion product was suspended in Dulbecco's Modified Eagle's medium plus 20% fetal calf serum.

After 7 days, supernates from colony-containing wells were screened for antibody production against the injected bacteria. Hybridomas that screened positive were expanded, cloned, and subcloned by limiting dilution until a single antibody-producing hybridoma colony was obtained.

After the process of cloning and subcloning, seven stable clones were obtained and named El-1, El-2, El-3, El-4, El-5, El-6, and El-7.

Hybridoma supernates were screened against a wide variety of plant pathogenic bacteria isolated from different hosts in different geographical locations. Testing included strains of *Ecc*, *Eca*, and *Echr* from our culture collection and from the collection of G. Kritzman, Israel. Also included were *E. amylovora*, *E. aroideae*, *E. herbicola*, and *P. marginalis*, and a purified pectic lyase preparation.

Ammonium sulfate-precipitated supernates were also screened against 40 serotypically different *Erwinia* including *Ecc* and *Eca* obtained from Solke de Boer, Agriculture Canada, Vancouver, British Columbia.

Both ascitic fluid and supernates were tested for their possible use in the detection of soft rot *Erwinia* in tissues extracted from tubers infected with *Ecc* or *Eca*. Problems with high background, and inherent enzyme activity of the tuber delayed progress in this phase of the study. Titering of the supernates and ascitic fluid was completed.

All of the MCAs appear fairly specific for the *Erwinia carotovora* subsp. *carotovora* group, some more than others. All MCAs tested failed to detect 7 *Echr* strains, and one strain each of *E. aroideae*, *E. amylovora*, *E. herbicola*, and *P. marginalis*. All ten *Eca* strains tested in the original screen were negative. None of the MCAs were able to recognize strains from serogroup 1, the group in which most *Eca* strains are placed. However, some MCAs were able to detect strains in serogroup 18, a group that includes other *Eca* strains.

A screen of the 40 *Ec* serogroups separated the MCAs into 3 to 4 distinct groups. One group El-1, El-3, and El-4 recognized strains from serogroup 29 only. Initial tests showed recognition of serogroups 3 and 19; however, the definitive serogroup tests were completed with purified antibody and are probably more accurate. El-4 weakly recognizes serogroup 28. El-5 and El-6 appear similar, both recognizing serogroups 3, 4, 6, 8, 10, 18, 19, 28, 29, and 31. El-5 also detects serogroup 38. A slightly more general antibody, El-2 recognizes strains from serogroups 3, 4, 6, 18, 10, 15, 18, 19, 28, 29, 31, 37, and 40.

Although all test have not been completed using El-7, this MCA appears to be the least specific of the antibodies tested. Preliminary studies indicate that El-7 detects serogroups 2, 3, 6, 19, 25, 26, 29, 31, 35, 37, and 40. Furthermore, it seems possible that this MAb could detect serogroups detected by some of the other MCAs.

The initial research has been completed on this project. Additional characterization of the MCAs will include serogrouping tests, isotyping, and a more extensive screen of other plant pathogenic and enteric bacteria.

There are three possible practical applications for the MCAs. A gold marker could be linked to the antibodies specific for serogroup 29 and this could be used to locate the rif-resistant strain of Ecc in tissue during spread of the bacterium in the tuber. Of particular interest will be the localization of Ecc in relationship to tissue involved in the wound healing process. Secondly, epidemiological experiments could be designed to follow the spread of serogroup 29 strains in the field. In addition, studies of specific antigenic sites could provide information on correlations between hosts, geographic location of strains, and serogroups.

Finally, additional preparations of MCAs to serotypes not recognized by E-2 or E1-7 could result in a collection of MCAs that could recognize all Erwinia carotovora strains.

In order to improve the identification procedures for Echr, the enzyme alkaline phosphatase, which is unique to Echr, was partially purified. This enzyme differed in optimal activity, pH and temperature from a commercial alkaline phosphatase. Partially purified alkaline phosphatase preparations were injected into rabbits to produce antibodies against specific for alkaline phosphatase from Echr. Serological tests on nitro-cellulose membranes showed that the rabbit antiserum (at 1:1000 dilution) reacted with partially purified alkaline phosphatase from Echr but not with commercial alkaline phosphatase. Possible interactions with contaminating components were not ruled out. Positive reactions were also obtained with four isolates of Ech; however there was no reaction with some strains of Ecc and Eca. This identification method is still in development.

Soft rot bacteria in the genus Erwinia are characterized by their ability to produce large amounts of extracellular pectic enzymes. These enzymes are capable of cleaving pectic substances, or the "cement" that holds plant cell walls together. One of the major extracellular pectic enzymes produced is pectate lyase (PL). Our objectives were to determine whether we could identify and differentiate these soft rot bacteria by comparing their pectic lyases immunologically.

We had previously purified an extracellular endopectate lyase from Erwinia carotovora subsp. carotovora (AK241), a strain isolated from potato field soil in Wisconsin. Approximately thirty monoclonal antibody-producing hybridomas were generated through standard techniques. These were specific for either an endopectate lyase I (MW=41K) (PLI) or an endopectate lyase II (MW=40K) or both PLI and PLII. In addition, polyclonal rabbit antiserum was prepared against a partially purified PLI preparation. These antibodies were used in ELISA and dot blot immunoassays to analyze extracellular pectic enzymes from various pectolytic Erwinia spp.

Pectolytic Erwinia carotovora subsp. carotovora (Ecc) and E. chrysanthemi (Echr) strains isolated from potato seed and stems in Israel were obtained from G. Kritzman. These strains are capable of causing soft rot of potato, but do not cause typical black leg symptoms in potato cultivars in Israel based on observations by G. Kritzman. Forty homologous Erwinia carotovora

cultures, representing forty whole cell serogroups including Ecc and E. carotovora subsp. atroseptica (Eca) strains, were obtained from S. H. DeBoer of Agriculture Canada, Vancouver, British Columbia. Twelve strains of E. chrysanthemi isolated from potato and other hosts were obtained from R. S. Dickey, Cornell University. Additional pectolytic Erwinia spp. were obtained from the collection of A. Kelman at the University of Wisconsin-Madison.

The Erwinia strains were grown in Mount's pectate broth or Luria broth plus sodium polypectate for 48hr at 28 C and frozen at -20 C. The pectolytic enzymes produced in this growth medium were screened by placing 10 μ l of this frozen and thawed cell suspension onto a nitrocellulose membrane (NCM). The NCM was blocked with skim milk to remove all other protein binding sites, and probed with either the rabbit anti-PLI antiserum or the monoclonal antibodies (MCAs). The NCM was washed, and blocked again before probing with either goat anti-rabbit or goat anti-mouse alkaline phosphatase conjugate. The NCM was again washed thoroughly and placed on an agarose gel containing the alkaline phosphatase substrate, 5-bromo-4-chloro-3-indolyl phosphate (BCIP), and incubated overnight at room temperature. Positive detection by the antibodies for the presence of pectate lyase in the cell suspensions was indicated by a dark blue spot. Gradation in the color response from dark blue to no color was classified as strong positive detection, weak positive detection or no detection.

Pectate lyase activity in the cell suspensions was verified by placing the NCM with the cell suspensions spotted on it, face down on an agarose gel containing polygalacturonic acid suspended in a buffer optimal for pectate lyase activity. After 1 hr incubation at room temperature, the gel was stained in a 0.1% (w/v) ruthenium red solution for 20 min and pectate lyase activity determined by observing a cleared area against a ruthenium red-stained pectate agarose background.

All cell suspensions of the pectolytic Erwinia strains grown in the polygalacturonic acid-containing medium displayed pectate lyase activity as determined by the pectate agarose gel technique. A positive reaction of the polyclonal or monoclonal antibodies for detection of pectate lyase in the cell suspensions in a dot blot assay was indicated as a dark blue spot.

The rabbit polyclonal antiserum against PLI detected PL from all pectolytic Erwinia strains tested (Table 5). These strains included the 40 Erwinia serogroups (Table 6) as well as other Ecc and Eca strains and all Ehr strains (Tables 7 and 8). However, this antiserum did not react with non-pectolytic Erwinia spp. including E. amylovora and E. herbicola. Four pectolytic pseudomonads (including P. marginalis) were weakly detected by the polyclonal antiserum.

Of the thirty monoclonal antibodies produced, four (4-7, 4-10, 5-9, 5-12) were used in the studies on the pectate lyases produced by the pectolytic Erwinia spp. (Table 5). MCA 5-9 detected pectate lyases in more strains than the other MCAs. These MCAs could detect PL from the majority of Ecc strains (from both potato and non-potato hosts) including all forty Ec serogroups (Table 6). Six randomly selected Ecc strains (Isr 26, 46, 47, 48, 87, 132)

and one Eca strain (Isr67) from Israel also produced pectate lyases that could be detected by the MCAs.

Detection of pectate lyases produced by Echr strains from potato and non-potato hosts was varied using the MCAs (Table 1). Pectate lyase from all Echr potato strains isolated in Israel and a Taiwan strain, could be strongly detected by the MCAs (Table 3). However, the majority of Echr strains not isolated in Israel produced pectate lyases that were weakly detected or not detected by the MCAs.

Non-potato Ech strains including those that infect corn, E. chrysanthemi pv. zeae (Echrz), were also studied (Tables 1 and 4). The pectate lyases from these strains exhibited less uniformity in detection by the MCAs compared to the Ecc, Eca and Ech potato strains. The only Ech strain from a non-potato host that could be strongly detected by all four MCAs was SR142, a corn strain from Italy. The other Echrz strains produce pectate lyases that vary in their ability to be detected by the MCAs.

The pectate lyases from monocot host strains other than those from corn strains (SR231, SR233, SR234, SR235) could not be detected by the MCAs. The pectate lyases from most Echr dicot strains also could not be detected with the exception of one carnation strain (SR229), one chrysanthemum strain (0862), and a Parthenium strain (1015) all of which could be weakly detected. These MCAs could not detect the presence of pectate lyases from E. amylovora and E. herbicola as well as the pectate lyases from the pectolytic pseudomonads studied.

Immunological relatedness of extracellular pectate lyases in the soft rot Erwinia spp. may be a useful tool in the identification and differentiation of these bacteria. We have produced both polyclonal and monoclonal antibodies against the major extracellular pectate lyase from Ecc and used these antibodies as diagnostic probes to survey the pectate lyases produced by representative strains of Ecc, Eca, Echr and Echrz strains.

The polyclonal rabbit anti-PL serum could detect pectate lyases in all pectolytic Erwinia spp. tested including Ecc, Eca and Ech. In addition, pectolytic pseudomonads produced a PL that could be weakly detected by the antiserum. Although, our antiserum was prepared against a PL from Ecc, PL was still uniformly detected in all Echr strains tested. Ecc PL may contain antigenic sites that are universal for PL from all soft rot Erwinia spp. On the contrary, Ech PL preparations may have dominant antigenic sites not present on Ecc PL that produce high concentrations of antibodies unique for Ech PL.

In our survey of the forty serogroups using the MCAs against an Ecc PL, the MCAs recognized all Ec strains tested. Differences between Ecc and Eca could not be detected by our technique.

Immunological differences in PL from different Ech strains were observed using the MCAs. Detection of PL from potato strains of Ech appeared to be dependent upon geographical location. The Ech strains from Israel and Taiwan produced PL that were strongly detected by the MCAs. However, strains from

Peru and the Netherlands produced PL that were weakly detected or not detected at all. It is not known whether the Israeli strains produce a PL enzyme or isozyme not present in the other potato Echr strains.

Pectate lyases of Ech strains from non-potato hosts were serologically quite diverse in relation to detection by the MCAs against an Ecc PL. The MCAs weakly detected PL from most corn strains (Echrz) tested regardless of geographical or climatic origin. The only exception was a corn strain from Italy that produced a PL that is strongly detected by all MCAs.

Since positive detection of PL in the Ech strains by the MCAs is quite variable, certain questions arise. MCAs recognize a specific region or epitope of a protein molecule. There may be one epitope or many epitopes per molecule. PL preparations from the *Erwinia* spp. that are strongly detected by the MCAs may either contain a high concentration of a particular PL with one epitope or a low or high concentration of a PL containing numerous epitopes per molecule. If a particular strain produces a PL that is weakly detected, can this be interpreted as a high or low concentration of a single epitope PL or a low concentration of a multiple epitope PL molecule? Recognition of PL by these MCAs may also be dependent on 1) strain used 2) cultural conditions for PL production 3) length of artificial storage of culture 4) limitations in sensitivity of technique and 5) different epitopes recognized by the different MCAs.

II. Evaluation of new bactericidal and/or bacteriostatic preparations with potential value for application to both seed tubers and potatoes in commercial storage.

The Departments of Plant Pathology (G. Kritzman) and Pesticide Application (H. Frankel, A. Grinstein and M. Austerwil) have evaluated an alternative method of treating potato seeds to avoid the conventional dipping techniques. Thus far very promising results have been obtained using an alternative chemical to mercury compounds. The new chemical is C-103, a bromine compound, a product of Bromide Compounds Ltd, Israel. The chemical concentration death points for the following pathogens of potato: Eca, Ecc, Echr, *Streptomyces scabies* and *S. spp.*, *Rhizoctonia solani* and *Verticillium dahliae* were determined in relation to the duration of the incubation in the tested chemical concentration. No bacterial isolates survived incubation in 50 ppm of C-103 (2,2-dibromo-3-nitrilopropionamide) (DBNPA) for two minutes at 25°C. The addition of 100 ppm TOG (a mixture of 150g thiabendazole and 150g 8-hydroxyquinoline liter of glycolic acid), resulted in death of all bacteria and fungi after a two minute treatment. Equipment and methods for applying the chemicals in low volumes were constructed, tested and evaluated using biological and analytical procedures. Seed potato tubers naturally contaminated with the above pathogens, were treated with both C-103 and TOG. Estimation of disease potential as well as pathogen populations at various times in the field after treatment, showed that the surfaces of the potato tuber seeds were effectively disinfested. *R. solani*, *V. dahliae*, *S. scabies* and *S. spp.* were controlled as well as surface-borne *Erwinia* spp. Control of tuber-born *Erwinia* spp. by spraying with C-103 and TOG was as effective as dipping the tubers in an organic mercury compound (7-methoxyethyl-mercurichloride). No phytotoxicity resulting from the spray mixtures was detected in subsequent field trials.

An application technique, using very low volume rates in conjunction with an aerosol spray chamber, has been developed. One ton of seed tubers, considered to have a surface area equivalent to 150 m², was treated with approximately 150 ml of concentrated pesticide; this is equivalent to a spray rate of 10 liters per hectare of field crop. Potato tubers were carried through the spray chamber on a roller belt, rotating six to seven times around their own axes. Kinetic energy nozzles (rotary atomizers) at feed rates of approx. 30 ml/min, produced a spray mist with a volume medium diameter of 80 microns. The spray droplets were deposited on the rotating tubers by gravitation, the amount being a function of exposed surface area. An exposure period of approx. 8 seconds resulted in a droplet density of 350-500/cm². Treatment capacity was 1 ton in 12 minutes. No subsequent drying was required after such a low application rate. It has been demonstrated that uniformity of chemical deposition was higher than that obtained with conventional dip or drench treatment.

Results obtained for the control of seed-borne fungal and/or bacterial pathogens with very low volume application of C-103 were equal to or better than those obtained by dipping in mercury-based materials. Significant control, as well as saving in pesticide and labor, was obtained. This method is still in development and other chemicals are being tested.

III. Population Dynamics of Pectolytic Bacteria in the *Erwinia carotovora* Group.

- a) Relation of populations of soft rot bacteria on seed to severity of seed decay:

Most commercial seed potatoes in the world carry populations of either *Erwinia carotovora* subsp. *carotovora* (Ecc) or subsp. *atroseptica* (Eca) or both. In several cases also *Erwinia chrysanthemi* (Ech) is present. Potato seeds are usually produced in cool temperate regions and some are exported to countries with warmer climates unsuitable for seed production. However, in areas with warm climates, *Erwinia* spp. soft-rots may cause severe damage. In Israel, the increase in the population of *Erwinia* spp. is prevalent when tubers from the spring crop are used as seed for the following autumn crop. This pattern of disease incidence and its association with the source of inoculum from the mother tuber were observed in a 3 year study. In 1985-86 a confirmation of earlier results was observed (Tables 10,11). Since 1983, 10 potato varieties were monitored during the spring and autumn growth season. The varieties listed from most resistant to most susceptible, are: Desiree, Cara, Alpha, Draga, Cardinal, Mirka Spunta, Pentland Crown and Maris Bard. Certain potato varieties enhance the development of bacterial populations. As the spring season advances, increasing temperature is associated with a shift of bacterial populations from Eca to Ecc to Ech. Decreasing temperatures at the end of the autumn season were associated with a shift in the populations of the *Erwinia* spp. present; thus, when the Eca population increased the Ecc and Ech populations decreased.

The levels of the pathogen population during the growing season was contingent upon the initial population level, temperature and the host variety. Seed contamination level and the Erwinia spp. involved are major factors in population dynamics of soft rot disease. Rotting seed tubers, and the multiplication of all Erwinia spp. were affected by the initial level of inoculum. A pathogen population level of less than 10^3 Eca per gr peel was found to have very poor potential for creating economic problems in the fields of growers (Table 5). This result is based on randomized sampling of 45 tubers from a given field ($10 \times 1000\text{m}^2$) that was sown with one potato seed lot. The estimation methods are the same as those described previously with the addition of preincubation of the tubers in a mist chamber at 22°C for 48 hr.

b) Decay of cut seed and seed pieces:

A common way of growing potatoes in Israel is by using as seed small whole tubers (35-55 mm dia.) that weigh up to 60 gr. Cutting under Israeli conditions is very risky because of severe problems associated with seed piece decay due to soft rot bacteria. However, cutting seed does not create problems of soft rot, blanking or blackleg if low Erwinia populations are present on the seed and the transferred inoculum from piece to seed piece by cutting knife is eliminated (Table 9).

c) Seed-borne Erwinia spp. and symptom expression in potato plants growing in the field.

Three different disease symptoms have been described related to Erwinia spp. on potato: soft rot, blackleg and wilting. In many potato growing areas of the world, E. carotovora subsp. atroseptica (Eca) and subsp. carotovora (Ecc) were responsible for potato blackleg symptoms, whereas in the tropics Erwinia chrysanthemi (Ech) was also involved. Our findings reveal that only Erwinia carotovora subsp. atroseptica under Israeli conditions, was the casual agent for blackleg symptoms alone or in combination with other Erwinia spp.

Three years of collection yielded more than 50 Ecc isolates that were tested for symptom development alone and with mixtures of Eca, Ech or both. In all cases, when Ecc isolates were tested alone, only soft rot occurred. Ech isolates produced typical wilting symptoms, whereas Eca isolates produced typical blackleg symptoms. All three Erwinia spp. were involved in soft rot of seed tubers or stolon rot below ground level.

d) Dispersal of pectolytic bacteria of the genus Erwinia in irrigation water of Israel.

We have shown for the first time in Israel the following: occurrence of Erwinia spp. in irrigation water, how contaminated irrigation water may influence disease incidence in the field, and the identification and presence of Erwinia chrysanthemi in Israel. Erwinia spp. was detected in 7 different water sources located in Israel. Population levels fluctuated throughout the year (Table 12, 1985 report).

Under laboratory conditions these bacteria survived more than three months in water maintained at 25°C . All strains were found to be poor soil survivors under Israeli conditions in irrigated soil. No Erwinia strains could be

detected beyond 100 days incubation. Our experiments, as well as under commercial conditions, indicated that irrigation water could serve as a sole source of inoculum. Seed contamination problems resulting from soft rot bacteria in irrigation water was solved in Marom Golan by treating the water with UV light prior to passage through the irrigation system.

Details of the research results on this topic have been submitted in the previous annual reports to BARD (1984, 1985). Additional details are summarized in two theses that were submitted for the "Master of Science" degree to the faculty of Agriculture of the Hebrew University of Jerusalem, by 1) Tova Katz, 1985. Dispersal of pectinolytic bacteria causing plant disease in irrigation water 72pp and 2) Y. Gromen, 1985. Population dynamics of pectolytic bacteria of the Erwinia carotovora group, on aerial parts and tubers of potato plants.

IV. Factors affecting resistance of potato tubers to bacterial soft rot pathogens.

a) Tuber skin appearance and periderm thickness

Since calcium improves the structural integrity of cell walls and membranes, the reduction of Erwinia soft rot in high calcium tubers can be attributed in part to these structural enhancements (McGuire and Kelman, 1986). A preliminary study showed calcium fertilization of potato plants may also affect tuber surface structure; such change might also influence susceptibility of potato tubers to bacterial soft rot. The preliminary data indicated that surface netting of tubers was better developed on tubers from calcium treated plots, than on tubers from control plots. Furthermore, tubers with well developed netting had more layers of periderm cells than those tubers with smooth skin.

Soft rotting bacteria do not have the mechanisms to penetrate directly through the thick-walled cells of the periderm of a mature tuber; rather they invade mainly through bruises and to a lesser degree via cuts and lenticels. At the time of harvest, when samples were taken after the tubers have passed from the digger onto a truck and then were washed and graded, over 80% of the tubers decayed under mist chamber assay conditions. In contrast, less than 10% of hand-dug potatoes decayed (Kelman and Maher, 1983). Increased periderm thickness may reduce severity of damage to tubers when they are wounded by bruising and thus, indirectly affect tuber soft rot susceptibility.

To determine whether skin appearance and periderm thickness of Russet Burbank potato tubers was influenced by calcium fertilization, 800 Russet Burbank tubers (200 tubers/treatment) were examined after harvest from plots with four different soil levels of calcium at the Hancock Experiment Station. The data confirmed the initial findings that calcium affects development of surface netting (Table 1). A higher percentage of tubers with well developed netting was obtained from calcium fertilized plots (47.0% to 56.0%) than from the control plot that was not treated with calcium (20.5%). Furthermore, a lower percentage of tubers from calcium treated plots were smooth skinned (11.5% to 17.5%) than tubers from the control plots (35.5%). No difference in degree of surface netting was observed among tubers from plots treated at the rates of 112 to 336 kg/ha Ca, however.

A distinctive pattern of surface netting is a varietal characteristic of Russet Burbank potato tubers. Development of netting is reported to be influenced by various cultural practices and environmental factors. For instance, high levels of nitrogen or potassium fertilization have been associated with production of tubers with a smooth skin; soil temperature also has been reported to affect tuber surface morphology (Blodgett and Rich, 1949; Yamaguchi et al., 1964). However, no previous reports that calcium fertilization affects development of tuber surface netting were found in a comprehensive literature survey.

Periderm thickness of twenty tubers with well developed netting and 20 tubers with smooth skin was examined by scanning electron microscopy. Tubers with well developed netting had more layers of periderm cells than tubers with smooth skin. At mid-point of tubers, in tubers with well developed netting the number of cells in the periderm ranged from 6 to 20 with an average of 13.6; in contrast in tubers with a smooth skin periderm depth ranged from 3 to 14 cells with an average of 9.1. The difference in number of periderm cells between tubers with well developed netting and those with a smooth skin was significant ($P < 0.001$).

In a separate study, high calcium tubers harvested from calcium fertilized plots showed lower weight losses than low calcium tubers from control plots after storage for 7 months (J. Fuller, personal communication). This decrease in weight loss might be related to the thickness of periderm in high calcium potato tubers.

b) Periderm rupture force

In view of the evidence that calcium fertilization affects tuber netting and periderm thickness, the question was raised as to whether there would be a difference in susceptibility to wounding between tubers with well developed netting and those with smooth skins. One approach to measure the possible differences was to determine periderm rupture force by an Instron Universal Testing Instrument (Model 1132).

The averages of periderm rupture forces for tubers with well developed netting vs. those with smooth skin were 47.2 and 42.1 Newtons, respectively. ($P < 0.001$). Periderm rupture force for tubers with well developed netting was approximately 10 % higher than that required for smooth skin tubers. Since the measurements were made after tubers were stored for 6 months, it is possible that turgidity or water status of tubers may affect the results. The difference in periderm rupture force between tubers with well developed netting and those with smooth skins might have been greater if measurements have been made early in the storage period when turgidity of tubers was higher. Presumably high calcium tubers would be more turgid or have a higher water potential because of less water loss than low calcium tubers after an extended storage period. There were no obvious differences in flaccidity because of water loss between the smooth vs. well-netted tubers, however.

c) Degradation of isolated potato cell walls by purified pectic lyase

Tubers from potato plants supplied with nutrient solutions with high levels of calcium had higher calcium concentrations in medullar tissues than tubers from low calcium solutions. Tubers from potato plants watered with Hoagland's solution without calcium (i.e. 0 ppm) for 80 days contained a relatively low concentration of calcium in medullar tissues (Table 2). Cell walls of medullar tissues from high calcium tubers were higher in calcium concentration than those isolated from low calcium tubers. These observations were similar to those made previously by McGuire and Kelman (1986).

Two cell wall preparations with calcium concentrations of 0.012 and 0.076%, respectively, were obtained from tubers of potato plants that received 0 ppm and 250 ppm calcium in nutrient solutions. These cell wall preparations were used to assay their resistance to degradation by a purified pectate lyase (PLI) produced by a strain of Erwinia carotovora subsp. carotovora. The rate of release of degradation products (unsaturated uronides) was significantly lower from high than from low calcium cell walls.

McGuire and Kelman (1986) also reported previously that maceration was more extensive in low calcium tubers than in high calcium tubers, when partially purified culture filtrates of Erwinia carotovora subsp. carotovora containing pectate lyase and polygalacturonase activities were injected into whole Russet Burbank tubers. The activity of pectate lyase in that preparation was 3100 μ mole of product formed per minute per milligram of protein; polygalacturonase activity was 3.9 μ mole of product formed per hour per mgm of protein. Since pectate lyase was the main pectic enzyme in the partially purified culture filtrate preparation that was tested, the decrease in maceration of high vs. low calcium tubers can be explained on the basis that cell walls in high calcium tubers were more resistant to degradation by pectate lyase than tissues in low calcium tubers. Our recent study of degradation of isolated potato cell walls by purified pectate lyase further supported the concept that high calcium cell walls were more resistant to degradation by pectate lyase than low calcium cell walls.

DESCRIPTION OF COOPERATION

In Israel as well as the USA, problems to be resolved were very similar. Questions, cultures and results were exchanged on a regular basis between the two groups during the project period. During preliminary research on soft rot bacteria, the Israeli group observed very poor recovery of Erwinia spp. on CVP media. These problems were solved by the USA group through improvement of this medium. The USA group also improved the technique for preparing and determination of oxidized substances from sucrose. The Israeli group were also able to improve the assays for population estimation by rapid differentiation of the three main Erwinia groups based on incubation at three different incubation temperatures, and the use of enrichment procedures to estimate very small populations. Because of the significant differences in the climatic conditions between these two potato growing zones, each group was able to evaluate the importance of these different bacterial groups under conditions found in each country. The USA group has concentrated on the possibility of the development of a rapid identification of Erwinia spp.

through the use of monoclonal antibodies against Erwinia pectate lyases. These monoclonal antibodies were tested on wide collection of pectolytic bacterial strains including a large number of Israeli isolates. Efforts were made for better identification of the "so called" Erwinia spp. group. These are Erwinia strains that could not be identified to species or subspecies by the usual biochemical and physiological methods. In studies to estimate the Erwinia population on potato tubers, the Israeli group have found that more than 20% of the total Erwinia population cannot be identified as Eca, Ecc or Ech.

A cooperative study was also established to determine the role of Ca^{++} ion in the tuber resistance or susceptibility to the soft rot bacteria. Samples of potato peels from different varieties and growing areas and grown on different soils, were sent to the USA for analysis. Through this cooperation a better understanding of this problem was gained especially the comparison of calcium deficiency in low pH soil (USA) versus alkaline soils (Israel).

MAIN ACHIEVEMENTS OF THE RESEARCH

a) Evaluation of the research achievement with respect to the original research proposal:

This research project covered the primary objectives that were submitted in the research proposal. Improved methods for detection and quantification of soft rot bacteria from soil, seed, plant material and water has been developed. Monoclonal antibodies to pectate lyase were developed as a new approach for identification of Erwinia strains. A new bactericidal preparation and application technique for potato seed tubers has also been developed. This new ultra low volume spray technique was found to be equivalent in control to mercury treatments with a numerous advantages compared to the dipping technique. The new treatment was successfully tested in Israel under commercial conditions.

The population dynamics study under Israeli climatic conditions provided a better estimation of the disease potential of potato seed tubers imported from Europe to Israel. In addition a new inoculum source for soft rot disease, irrigation water was discovered. We showed in several cases, that irrigation water is a primary inoculum source for initiating bacterial soft rot. The study of the population dynamics initiated research in two other areas, production of disease-free seed in the Golan Heights and studying soft rot problems involving the export of fresh potato tubers to Europe and South America.

The relationship of calcium to enhanced resistance of potato tubers to bacterial soft rot was evaluated.

b) Benefit to Agriculture.

This project has been completed with a strong emphasis on the application of the research to the solution of agricultural problems. Specific applications can be cited as follows:

1. The development of improved procedures for detection of very small numbers of Erwinia spp. and estimation of population size in given seed samples provided information that aids in making sound decisions in the areas of potato seed production, import and export as well as guiding decisions on storage and shipment. One of the important findings of the research was the evidence that conventional field inspection of seed potato fields does not provide a meaningful basis for determination of the soft rot potential of seed tubers. It has become clear that it is essential to use laboratory procedures to estimate the numbers of each Erwinia group as CFU/gr. of potato peel.
2. The investigation of the population dynamics of the four Erwinia groups in the field experiments provided important data on the potential crop losses on potato seed with a known level of CFU of each of the pathogens. This finding provided important information on the susceptibility of the main varieties and the level of contamination that may be related to severity of problems such as blanking, blackleg, soft rot or wilting. The decision as to which lot of seed potatoes a farmer will grow in the spring for the production of seed in the autumn could be guided by means of the methods developed on this project. Furthermore, it also provided a basis for determination of risks involved in long or short term storage of specific seed lots or which stocks will be likely to suffer losses when shipped to Europe.
3. The development of new techniques for seed disinfection without dipping may also aid in solving several problems such as : 1) eliminating the continuous use of mercuric compounds that are very hazardous, and can create pollution problems. 2) the ability to make treatments before storage also helps in reducing storage loss and provides more flexible time for the farmer before the planting. 3) elimination of dipping of seed potatoes reduces problems of soft rot of seeds as a result of the anaerobic condition that encourage the soft rot Erwinia and clostridia populations that may be present in lenticels of tubers.
4. The importance of irrigation water in Israel as a primary source of inoculum for Erwinia soft rot diseases was demonstrated for the first time. Furthermore, it was shown that U.V. equipment can be used effectively to eliminate soft rot bacteria from irrigation water in the region where pathogen-free potato seed are produced. This procedure may have application in other situations where sewage water in urban areas may be used for irrigation of other crops susceptible to soft rot bacteria.
5. Rapid identification of Erwinia by using monoclonal antibody and related sensitive serological methods has aided in the detection and identification of new pathogens such as Ech in Israel and U.S.A. These new serological techniques will aid in the operation of programs that require estimation of bacterial populations on seed and cuttings.

6. The methodology that was used in this research can be easily adapted by potato breeders for evaluation of potato varieties for resistance under difference climatic conditions.
7. The study of the mechanisms of soft rot resistance in potato tubers has provided additional information on the role of calcium in potato tissue in resistance maceration by soft rot bacteria. A significant reduction in soft rot susceptibility was obtained by enhancing Ca^{++} absorption by tubers.

Applications of the research have been made as follows:

1. Estimation of Erwinia on potato seed according to our procedures is being followed on a commercial scale by the laboratory in Gilat for the benefit of all the potato growers in the Negev region of Israel.
2. Water treatment for elimination of the problem of irrigated water contamination by Erwinia spp. is being used with success in the Merom Golan.
3. Erwinia-free seed is now being produced in Merom Golan according to the methodology that was developed in this project.
4. Potato seed treatment according to the new technology will replace the organic mercury dipping technique and it is already used on a commercial scale in the Hevel Habsor.
5. Application of non-line forms of calcium is now accepted as an important cultural practice to reduce hazard of bacterial soft rot in storage of tubers harvested from low-cation exchange capacity soils.

List of Publications:

1. Kats Tova. 1985. Dispersal of pectinolytic bacteria causing plant disease in irrigation water. Master of Science Thesis. ARD.
2. Groman, Y. 1985. Population dynamics of pectolytic bacteria of the Erwinia carotovora group on aerial parts and tubers of potato plants. Master of Science Thesis.
3. Klopmeier, M. J., Livingston, R.S. and Kelman, A. 1986. Differentiation of soft rot bacteria using monoclonal antibodies. *Phytopathology* 76:1077-1078. (Abstr.)
4. Livingston, R. S., Galuska, D. A., Klopmeier, M. J., and Kelman, A. 1986. Production and characterization of monoclonal antibodies for pectic lyases. *Phytopathology* 76:401-402. (Abstr.)
5. Tzeng, K. C. 1986. Calcium nutrition of potato plants in relation to bacterial soft rot susceptibility and internal brown spot of potato tubers. Ph.D. Thesis. University of Wisconsin-Madison. 150 pp.

Table 1. Identification of *Erwinia* isolates according to biochemical and physiological characteristics.^a

<u>Assay or Characteristics</u>	<u>Eca</u>	<u>Ecc</u>	<u>Ech</u>
Pectin degradation at:			
15 C	+	-	-
28 C	-	+	+
39 C	-	<u>+</u> ^b	+
Pectate lyase activity	+	+	+
Pathogenicity	+	+	+
Reducing products from sucrose	+	-	-
Phosphatase formed	-	-	+
Tetrazolium chloride reduction	+	-	-
Indole formation	-	-	<u>+</u>
Sensitivity to erythromycin (50 ug/ml)	-	-	<u>+</u>
Growth in 5% NaCl	+	+	<u>+</u>
Production of acid from:			
Trehalose	+	+	-
Lactose	+	+	+
Maltose	-	-	+
Mannitol	+	+	+

^aEach test was replicated 5 times

^b+ = strains were variable

Table 2. The percentage of *Erwinia* spp. recovered on three different media: nutrient agar (NA), and two types of the crystal violet pectate (CVP) medium after incubation at three temperatures.

	Temperature Incubation		NA		CVP (1974)		CVP (1985)	
		Time Hr.	CFU/ml	%	CFU/ml	%	CFU/ml	%
Eca	15 C	48	62 ^a	100	22	35	38	61
	28 C	48	63	100	0	--	0	--
	39 C	48	0	--	0	--	0	--
Ecc	15 C	48	33	100	0	--	0	--
	28 C	48	86	100	34	40	67	78
	39 C	48	0	--	0	--	0	--
Ech	15 C	72	0	--	0	--	0	--
	28 C	72	80	100	0	--	0	--
	39 C	72	78	100	24	31	41	52

^aEach number represents the mean of 3 independent experiment with five replicates each; SD = $\pm$ 10%

Table 3. The percentage of Erwinia spp. recovered and identified from mixtures on nutrient agar (NA) and the approved CVP medium^a when incubated at three different temperatures.

<u>Erwinia</u> spp. alone or in mixtures in suspensions	CFU/ml				Total %
	NA	CVP			
	15 C	28 C	39 C		
Ecc 1	1051	---	824	---	78.4
Ecc 2	67	---	35	---	77.6
Eca 1	1232	747	---	---	60.6
Eca 2	59	36	-	---	61.3
Ech 1	1098	---	---	575	52.4
Ech 2	60	---	---	31	51.8
Ecc 1 + Eca 1	3432	755	831	---	---
Ecc 1 + Eca 2	1093	31	827	---	---
Ecc 1 + Ech 1	2036	---	825	580	---
Ecc 1 + Ech 2	1122	---	820	33	---
Ecc 2 + Eca 1	1311	744	37	---	---
Ecc 2 + Eca 2	120	35	35	---	---
Ecc 2 + Ech 1	1179	32	--	574	---
Ecc 2 + Ech 2	117	36	--	30	---

Table 3 (Cont.)

Ecc 1 + Eca 1 + Ech 1	2266	751	830	569	--
Ecc 2 + Eca 2 + Ech 1	199	34	35	575	--
Ecc 1 + Eca 2 + Ech 1	1068	36	834	580	--
Ecc 1 + Eca 1 + Ech 2	2401	747	829	31	--
Ecc 2 + Eca 2 + Ech 2	192	36	36	30	--
Ecc 2 + Eca 1 + Ech 2	1274	737	35	32	--

^aEcc and Eca were incubated for 48 hr. and Ech for 72 hr. at 15 C, 28 C and 39 C respectively.

Table 4. The effect of the inoculum levels (CFU/ml) on the population recovery levels of Eca, Ecc and Ech after 72 hr. incubation at 28 C for estimation of Ecc, at 15 C for Eca and at 39 C for Ech. An enrichment procedure was followed by the improved MPN technique.

Tested	Erwinia CFU/ml in the tested sample				
Erwinia	0.1-1	1-10	10-20	20-50	50-100
Ecc	1.4x10 ³ ₊₁₁₁ ^a	2.3x10 ³ ₊₁₉₇	8.6x10 ⁴ ₊₇₁₅	3.1x10 ⁵ ₊₂₈₅₀	5.8x10 ⁵ ₊₄₃₅₀
Eca	0.97x10 ³ ₊₇₇	1.8x10 ³ ₊₁₆₈	4.5x10 ⁴ ₊₃₈₀	9.7x10 ⁴ ₊₈₃₂	1.2x10 ⁵ ₊₁₀₂₀
Ech	3.1x10 ² ₊₉	8.4x10 ² ₊₇₀	1.3x10 ³ ₊₉₁	8.70x10 ³ ₊₅₉₁	1.6x10 ⁴ ₊₁₁₃₀

^a Each number represent the mean of 5 independent experiments.

Table 5. Detection of pectate lyase (PL) from various pectolytic and non-pectolytic bacterial strains by rabbit polyclonal anti-PL serum and four monoclonal antibodies (4-7, 4-10, 5-9, 5-12) specific for a PL from *Erwinia carotovora* subsp. *carotovora* (SR319) using a dot blot immunoassay^a.

Strain/Host ^b	Rabbit Anti-PL	# Strains Positive/# Strains Tested			
		Monoclonal Antibodies			
		4-7	4-10	5-9	5-12
Ecc (potato)	55/55	55/55	55/55	55/55	55/55
Ecc (non-potato)	4/4	4/4	4/4	4/4	4/4
Eca (potato)	11/11	10/11	11/11	11/11	11/11
Echr (potato)	20/20	13/20	16/20	16/20	14/20
Echr (non-potato)	24/24	7/24	8/24	10/24	6/24
<i>E. amylovora</i>	0/1	0/1	0/1	0/1	0/1
<i>E. herbicola</i>	0/1	0/1	0/1	0/1	0/1
<i>Pseudomonas</i>	4/4	0/4	0/4	0/4	0/4

^a Cells grown in Mount's pectate broth used as antigen and a 1:100 dilution of the monoclonal antibody or a 1:1000 dilution of rabbit anti-PL with a 1:1000 dilution of goat anti-mouse or goat anti-rabbit alkaline phosphatase conjugate and BCIP substrate. Dark blue spot indicates positive detection of pectate lyase.

^b Ecc = *Erwinia carotovora* subsp. *carotovora*; Eca = *E. carotovora* subsp. *atroseptica*; Echr = *E. chrysanthemi*.

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Table 6. Detection of pectate lyase (PL) in cell suspensions of forty Erwinia carotovora serogroups ^aby rabbit polyclonal anti-PL and four monoclonal antibodies (4-7, 4-10, 5-9, 5-12) specific for PL from E. carotovora subsp. carotovora (SR319) using a dot blot immunoassay^b.

<u>Serogrp</u>	<u>Strain</u>	<u>Subsp^c</u>	<u>Rabbit</u>	<u>Monoclonal Antibody</u>			
			<u>Anti-PL</u>	<u>4-7</u>	<u>4-10</u>	<u>5-9</u>	<u>5-12</u>
I	31	Eca	s	s	s	s	s
II	21	Ecc	s	s	s	w	s
III	71	Ecc	s	+	+	w	s
IV	190	Ecc	s	+	+	w	+
V	26	Ecc	s	+	+	+	s
VI	189	Ecc	s	+	+	w	+
VII	68	Ecc	s	+	+	w	s
VIII	62	Ecc	s	+	+	w	s
IX	63	Ecc	s	+	+	+	s
X	61	Ecc	s	+	+	+	s
XI	193	Ecc	+	+	+	+	s
XII	67	Ecc	+	+	+	+	s
XIII	59	Ecc	+	+	+	w	+
XIV	65	Ecc	s	+	+	w	+
XV	23	Ecc	s	+	+	+	+
XVI	94	Ecc	s	+	+	+	+
XVII	92	Ecc	s	+	+	w	+
XVIII	6	Eca	s	w	w	w	w
XIX	194	Ecc	s	+	+	+	s
XX	196	Eca	s	w	w	+	w
XXI	197	Ecc	s	+	s	w	s

Table 6. continued

XXII	198	Eca	s	w	+	+	+
XXIII	207	Ecc	s	+	+	+	s
XXIV	208	Ecc	s	+	+	+	s
XXV?	260	Ecc?	s	+	+	w	+
XXVI	211	Ecc	s	+	+	w	+
XXVII	360	Ecc	s	+	+	+	+
XXVIII	365	Ecc	s	+	+	+	+
XXIX	380	Ecc	s	+	+	+	+
XXX	209	Ecc	s	+	s	s	s
XXXI	477	Ecc	s	+	+	w	+
XXXII	476	Ecc	s	+	+	+	+
XXXIII	474	Ecc	s	+	+	+	s
XXXIV	475	Ecc	s	w?	+	w	+
XXXV	499	Ecc	s	w	+	w	s
XXXVI	497	Ecc	s	+	+	w	s
XXXVII	498	Ecc	s	w	+	w	w
XXXVIII	500	Ecc	s	+	+	w	+
XXXIX	518	Ecc	s	s	s	s	s
XL	517	Ecc	s	+	+	w	+

^a Strains courtesy of S.H. DeBoer.

^b Cells grown in Mount's pectate broth used as antigen with 1:100 dilution of monoclonal antibody or 1:1000 dilution of rabbit anti-PL and 1:1000 dilution of goat anti-mouse or goat anti-rabbit alkaline phosphatase conjugate and BCIP substrate. Dark blue spot indicates positive detection of pectate lyase in cell suspension. s = strong positive detection; + = good positive detection; w = weak positive detection; - = no detection.

^c Ecc = E. carotovora subsp. carotovora; Eca = E. carotovora subsp. atroseptica.

Table 7. Detection of pectate lyase (PL) from Erwinia chrysanthemi strains isolated from potato by rabbit polyclonal anti-PL serum and four monoclonal antibodies (4-7, 4-10, 5-9, 5-12) specific for PL from Erwinia carotovora subsp. carotovora (SR319) using a dot blot immunoassay.^a

<u>Strain No.</u> ^b	<u>Location</u>	<u>Rabbit Anti-PL</u>	<u>Monoclonal Antibody</u>			
			<u>4-7</u>	<u>4-10</u>	<u>5-9</u>	<u>5-12</u>
SR297	Peru	+	-	w	w	-
SR298	Peru	w	-	-	w	-
1088	Peru	+	-	-	-	-
1686	Peru	+	-	w	w	-
1687	Peru	+	-	w	w?	-
1591	Taiwan	+	+	+	+	+
1669	Netherlands	+	w?	-	-	w
1682	Netherlands	?	-	-	-	-
Isr302	Israel	+	+	+	+	+
Isr305	Israel	+	+	+	+	+
Isr307	Israel	+	w	+	w	+
Isr308	Israel	+	w	w	w	w
Isr309	Israel	+	+	+	w	+
Isr312	Israel	+	+	+	w	+
Isr317	Israel	+	+	+	w	+
Isr321	Israel	+	+	+	w	w
Isr325	Israel	+	+	+	+	w
Isr327	Israel	+	+	+	w	w
Isr329	Israel	+	+	+	w	w
Isr331	Israel	+	w	+	+	w

^a Cells grown in Mount's pectate broth used as antigen with a 1:100 dilution of the monoclonal antibody or 1:1000 dilution of rabbit anti-pectate lyase (PL) and 1:1000 dilution of either goat anti-mouse or goat anti-rabbit alkaline phosphatase conjugate using BCIP as the substrate. Dark blue spot indicates a positive detection of PL in the cell suspensions; + = strong detection, w = weak detection, - = no detection.

^b Strain nos. beginning with SR from A. Kelman; Isr from G. Kritzman; no letters from R. Dickey.

Table 8. Detection of pectate lyase (PL) from *Erwinia chrysanthemi* strains isolated from various hosts by rabbit polyclonal anti-PL serum and four monoclonal antibodies (4-7, 4-10, 5-9, 5-12) specific for a PL from *Erwinia carotovora* subsp. *carotovora* (SR319) using a dot blot immunoassay.^a

Strain ^b	Strain No.	Host	Location	Rabbit Anti-PLI	Monoclonal Antibody			
					4-7	4-10	5-9	5-12
Echrz	SR58	corn	N.Carolina	w	-	-	-	-
Echrz	SR72	corn	Lincoln Co., WI		w	+	+	-
Echrz	SR74	corn	Lincoln Co., WI	+	-	w	+	-
Echrz	SR80	corn	Spooner, WI	+	-	w	w	w
Echrz	SR83A	corn	Spooner, WI		-	-	-	-
Echrz	SR84	corn	India	+	-	-	w	-
Echrz	SR120A	corn	Hawaii	+	-	-	w	-
Echrz	SR142	corn	Italy	+	+	+	+	+
Echrz	SR171	corn	Colombia, S.A.	w	w	w	w	-
Echrz	1030	corn	France	+	w	-	w?	-
Echrz	SR147	grass	Australia	+	-	-	-	-
Echr	SR231	<u>Philodendron</u>	Florida		-	-	-	-
Echr	SR233	<u>Syngonium</u>	Florida	+	-	-	-	-
Echr	SR234	banana	Honduras	+	-	-	-	-
Echr	SR235	<u>Dieffenbachia</u>	Honduras	+	-	-	-	w
Echr	SR32	chrysanthemum	New York	w	-	-	w	-
Echr	0862	chrysanthemum	USA	+	w	w	w?	-
Echr	SR228	poinsettia	Ohio	+	-	-	-	-
Echr	SR229	carnation	Pennsylvania	+	w	w	w	w
Echr	1237	carnation		+	w?	-	-	w
Echr	SR243	tomato	France	w	-	-	-	-

Table 8 continued.

Echr	SR244	<u>Pelargonium</u>	Comores Islands	w	-	-	w	-
Echr	0706	plantain		?	-	-	-	-
Echr	1015	<u>Parthenium</u>	USA	+	w	+	w?	w

^a Cells grown in Mount's pectate broth used as antigen with a 1:100 dilution of monoclonal antibody or 1:1000 dilution of rabbit anti-PL and 1:1000 dilution of goat anti-mouse or goat anti-rabbit alkaline phosphatase conjugate using BCIP as substrate. Dark blue spot indicates a positive detection of pectate lyase; + = strong positive detection; w = weak positive detection; - = no detection.

^b Echrz- E. chrysanthemi pv. zeae; strain nos. beginning with SR from A. Kelman, all others from R. Dickey.

Table 9. Effect of cutting Desiree seed: Erwinia populations in relation to seed piece decay.

Treatments	Planting day				Harvest day	
	log CFU/gr peel				log CFU/gr peel	
	Eca	Ecc	B (%) ^a	Bl (%) ^b	Eca	Ecc
<u>Spring</u>						
whole tubers	2.31	0.72	0	0	0.33	2.67
cut tubers	2.31	0.72	0	0	0.46	2.30
whole tubers	3.58	0.86	3.5	3.0	1.61	2.83
cut tubers	3.58	0.86	4.2	2.8	1.44	2.91
whole tubers	5.61	0.33	13.3	11.0	2.00	3.36
cut tubers	5.61	0.33	14.5	10.2	1.88	2.80
<u>Autumn</u>						
whole tubers	0.96	2.93	0	0	1.68	0.42
cut tubers	0.96	2.93	0	0	2.11	0.31
whole tubers	2.98	2.58	7.6	9.1	4.90	0.20
cut tubers	2.98	2.58	8.1	9.0	4.78	0.14
whole tubers	4.43	1.90	27.5	14.8	8.54	0.33
cut tubers	4.43	1.90	38.4	11.7	8.75	0.41

^a B=blanking

^b Bl-blackleg

Table 10. Populations of Erwinia spp. on commercial potato seed lots on the planting day in the spring and at the harvest time. The potatoes were grown in different commercial fields in Israel.

Variety	Log CFU per g. peel								
	Seed: Harvest day			Tubers: Harvest day			Diseased Percent		
	Ecc	Ecc	Ech	Eca	Ecc	Ech	W ^a	BL ^b	B ^c
Desiree 1	7.63	0.60	0.00	2.22	2.88	0	0	16.5	11.8
Desiree 2	2.54	0.42	0.00	0.09	2.91	0	0	0	0
Desiree 3	3.36	0.39	0.00	1.36	2.70	0	0	2.8	4.5
Desiree 4	2.87	0.21	0.00	0.05	2.66	0	0	0	0
Spunta 1	2.60	0.11	0.00	0.71	3.40	0	0	0	0
Spunta 2	3.56	0.24	0.00	2.48	3.32	0	0	3.9	5.5
Draga 1	3.27	0.09	0.00	0.54	2.00	0	0	2.1	6.0
Draga 2	5.40	0.11	0.00	3.25	2.00	0	0	10.0	14.0
Draga 3	2.81	0.11	0.00	0.06	2.20	0	0	0	0.2
Alpha 1	5.11	0.43	0.00	3.47	3.11	0	0	7.6	3.6
Alpha 2	4.22	0.36	0.00	3.39	3.36	0	0	7.5	4.1
Alpha 3	2.70	0.38	0.00	0.01	3.81	0	0	0	0
Alpha 4	3.44	0.25	0.00	2.34	3.57	0	0	3	1.8
Cara 1	2.58	0.18	0.00	0.09	2.98	0	0	0	0
Cara 2	3.50	0.27	0.00	2.13	3.21	0	0	1.5	0.8
Mirka 1	5.14	0.53	0.13	3.66	4.53	1.80	4.8	27.9	33
Mirka 2	4.01	0.67	0.09	3.71	4.82	2.41	3.3	25.0	34
Mirka 3	3.12	0.44	0	3.37	5.00	0	0	19.9	33
Mirka 4	2.65	0.58	0	1.11	5.00	0	0	0.3	0.1

a: W=wilting; b: B=blackleg; c: B=blanking

Table 11. Populations of *Erwinia* spp. as estimated on commercial potato seed lots at planting date in the autumn and at the harvest time.
The plants were grown in different commercial fields in Israel.

Variety	Log CFU per gr peel						Diseased Percent		
	At the planting day			At the harvest day			W ^a	BL ^b	B ^c
	Eca	Ecc	Ech	Eca	Ecc	Ech			
Desiree 1	0.92	2.71	0	1.83	0.02	0	0	0	0
Desiree 2	3.45	3.33	0	6.00	0.08	0	0	3.4	6.8
Draga 1	1.53	3.00	0	1.70	0.10	0	0	0	0
Draga 2	4.23	2.15	0	6.54	0.07	0	0	4.0	3.6
Alpha 1	2.00	2.14	3.30	2.37	0.11	0.01	2.8	0	5.2
Alpha 2	3.67	2.00	0	7.37	0.25	0	0	8.3	4.5
Cara 1	0.50	3.97	0	0.80	1.13	0	0	0	0
Cara 2	4.51	4.23	0	5.55	1.64	0	0	11.4	8.1
Mirka 1	1.99	4.10	3.58	2.60	2.87	1.08	21	0	4.8
Mirka 2	2.96	3.67	0	8.44	2.80	0	0	17.1	5.0

a: W=Wilting

b: BL=Blackleg

c: B=Blanking

Table 12. Estimation of *Erwinia* spp and Total bacterial in irrigation water of 6 different location during 1986. Total bacteria represents CFU's on plate count agar. *Erwinia* populations were estimated through enrichment and MPN techniques.

Water Source	Total Bacteria CFU/ml	CFU/100 ml			
		<u>Ecc</u>	<u>Eca</u>	<u>Ecc</u>	<u>Esp</u>
Bet Shean	2.5×10^4	108.2	0	11.3	4.1
Near Beer-Sheva	8.3×10^6	280.5	58.8	0.3	18.3
Merom Golan	2.2×10^4	132.0	0	0	10.5
Sea of Galilee	6.9×10^5	18.0	11.4	0.5	8.3
Sea of Galilee At the Aqueduct	9.3×10^5	157.8	23.0	17.1	4.8
Bet Dagan	1.8×10^3	0	0	0	0
Hevel Taanach	3.4×10^5	152.8	0	14.6	3.1