EVALUATION OF PROCEDURES FOR DETECTION OF PECTOLYTIC *ERWINIA* SPP. ON POTATO TUBERS[1]

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Abstract

The effectiveness of a tuber incubation method for detection of *Erwinia carotovora* var. *atroseptica* and *E. carotovora* var. *carotovora* in potato tubers was compared with a lenticel sampling procedure. In the first method, tubers were injured by puncturing lenticels with sterile toothpicks, then wrapped in moist paper towels and polyvinylidene film, and placed in closed chambers flushed with N_2. In later experiments, wrapping tubers in two layers of polyvinylidene film and incubation in air was found to be as effective as the single layer of polyvinylidene and incubation in chambers flushed with N_2. Isolations were made on a selective crystal violet pectate (CVP) medium from homogenized samples of tissue removed from soft rot lesions developing around injured lenticels. In the second method, 10 lenticels/tuber were aseptically removed with a scalpel and homogenized in distilled water; the suspension was plated on CVP. The first method was less tedious and slightly more effective than the lenticel sampling method. In a preliminary survey, these methods were used to detect *Erwinia* infestations in small samples of certified seed potato tubers from Maine, Minnesota, Montana, New York, North Dakota, and Wisconsin. Pectolytic *Erwinia* spp. were detected in at least one sample from each state except Montana. The percentage of tubers with *Erwinia* infestations varied from 0-100% among samples. Characterization of *Erwinia* isolates showed that both *E. carotovora* var. *carotovora* and *E. carotovora* var. *atroseptica* were present. Pectolytic *Erwinia* spp. on symptomless potato seed tubers may serve as inoculum sources for blackleg and soft rot diseases.

Resúmen

Se comparó la eficacia de un método de incubación de tubérculos con otro de muestreo de lenticelas para detectar *Erwinia carotovora* var. *atroseptica* y *E. carotovora* var. *carotovora*. Para el primer método, los tubérculos fueron heridos punzando las lenticelas con mondadientes esterilizados, luego fueron envueltos en toallas de papel húmedas y en una lámina de polivinilideno (Pv.) y colocados en cámaras cerradas inundadas con N_2. En los experimentos posteriores, se determinó que era tan efectivo

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envolver los tubérculos en dos capas de Pv. con incubación en aire como la capa simple de Pv. con incubación en cámaras inundadas con N_2. Se hicieron aislamientos en un medio selectivo de cristal violeta pectato (CVP) de muestras homogenizadas de tejido extirpado de lesiones de podredumbre blanda en desarrollo alrededor de lenticelas heridas. En el segundo método, se extirparon asepticamente 10 lenticelas por tubérculo con un bisturí, y se homogenizaron en agua destilada; la suspensión fue sembrada en CVP. El primer método fue menos tedioso y ligeramente más efectivo que el método de muestreo de lenticelas. En una encuesta preliminar, estos métodos fueron usados para detectar infestaciones de *Erwinia* en pequeñas muestras de semilla certificada procedente de los Estados de Maine, Minnesota, Montana, Nueva York, Nor-Dakota y Wisconsin. Se determinó *Erwinia* spp. en por lo menos una muestra de cada Estado, exceptuando a Montana. El porcentaje de tubérculos con *Erwinia* varió de 0-100% entre muestras. La caracterización de los aislamientos de *Erwinia* demostraron la presencia de ambos *E. carotovora* var. *carotovora* y *E. carotovora* var. *atroseptica*. Las especies pectolíticas de *Erwinia* en tubérculos de semilla de papa sin síntomas pueden actuar como fuentes de inóculo para las enfermedades piema negra y podredumbre blanda.

Introduction

Seed potato tubers contaminated with *Erwinia carotovora* var. *atroseptica* (Van Hall) Dye and *E. carotovora* var. *carotovora* (Jones) Dye are considered the main source of primary inoculum for the blackleg and soft rot diseases in Scotland and England (6, 10). Initial studies in Wisconsin (1) and Colorado (M. Harrison, personal communication) support the evidence obtained in Scotland that soft rot *Erwinia* do not survive overwinter in the soil. Use of tubers free of blackleg bacteria for planting should significantly decrease the incidence of blackleg and tuber decay. Programs to produce such tubers require a sensitive procedure for detection of contaminated tubers. Recently, Perombelon (11) described a method that involves wrapping tubers in wet tissue paper, placing them in polythene bags and incubating them in a nitrogen atmosphere for 6 to 7 days. The objective of this investigation was to compare the efficiency of a modification of Perombelon's method with a direct lenticel sampling method. In addition, these methods were tested in a preliminary survey for pectolytic *Erwinia* on certified seed potatoes from different regions in the U.S. A preliminary report has been presented (4).

Materials and Methods

Tuber incubation method—Russet Burbank and Sebago potato tubers, commercially grown in Wisconsin, were used for these studies. The tubers

were rinsed under running tap water to remove soil, then wrapped individually in moist paper towels and covered with polyvinylidene film (Saran Wrap, Dow Chemical Company). In most experiments, the tubers were injured prior to wrapping by puncturing 10 lenticels/tuber with a single sterilized toothpick. The wrapped tubers were placed in a plexiglass chamber (9" x 15" x 18") that was flushed with nitrogen gas for 2 hours on the first 2 days of incubation to reduce the oxygen level to about 1%. A 0.25 ml gas sample was removed and used to measure the 0_2 concentration with a Varian Aerograph (Model 90-P) gas chromatograph. Alternatively, the tubers were wrapped with a second layer of polyvinylidene film and incubated without N_2. After 5 days at 20 C (68 F), tubers were examined for soft rot lesions. Isolations were made by removing a small piece of tissue from the edge of several soft rot lesions, homogenizing the sample in 0.2 ml distilled H_20 and plating the suspension on a crystal violet pectate medium (CVP) (1). After 2-3 days incubation, the plates were examined under oblique light for deep cup-like depressions formed by colonies with cross-hatched markings peculiar to pectolytic *Erwinia* spp. (1).

Lenticel sampling method—Russet Burbank and Sebago tubers from several states were used. Ten lenticels/tuber were removed at random from the tuber surface with a sterile scalpel and homogenized together in 0.1 ml of sterile distilled water with a glass rod on a porcelain spot plate. A loopful of homogenate was streaked on each of two CVP plates.

Preliminary survey—Certified seed potato tubers from Maine, Minnesota, Montana, New York, North Dakota and Wisconsin were supplied by P. J. Eastman, J. Jevning, L. Claflin, E. D. Jones, R. Johansen and H. M. Darling, respectively. Samples of 10-30 tubers were tested for *Erwinia* infestations by the tuber incubation method.

Erwinia spp. were identified on the basis of depression type and colony morphology on CVP. *E. carotovora* var. *atroseptica* was differentiated from *E. carotovora* var. *carotovora* by absence of growth at 36 C (96.8 F), production of reducing sugars from sucrose and acid from *a*-methyl glucoside (5), and reaction with a fluorescent antibody stain specific for *E. carotovora* var. *atroseptica* (Allan and Kelman, unpublished data). Furthermore, distinct black zones usually developed at the margin of decayed areas in tuber slices inoculated with *E. carotovora* var. *atroseptica*, whereas this black pigmentation was typically less evident in tuber slices inoculated with *E. carotovora* var. *cartovora*.

Results

The tuber incubation method was effective for detecting *Erwinia* spp. on potato tubers. Placing the tubers in an N_2 atmosphere increased the ability to detect *Erwinia* by almost 10% over the use of a single layer of

polyvinylidene film. Use of a double layer of polyvinylidene film obviated the need for incubation in N_2 without reduction in efficiency of detection. *Erwinia* was recovered from 42% of the tubers for each procedure. Injuring the tubers through the lenticels increased the frequency of soft rot by about 60% and subsequent isolation of *Erwinia* spp. by about 30% when compared to uninjured controls (Table 1). Injuring the lenticels also decreased the time required for soft rot to appear. If incubated for 10 days, all tubers rotted in these tests whether or not they were injured.

The lenticel sampling method was less sensitive for detection of *Erwinia* than the tuber incubation method; however, the difference was small and probably not significant (Table 2).

TABLE 1.—*Effect of injury on the effectiveness of the tuber incubation method.*

Treatment	No. of days incubated	No. of tubers	Tubers decayed (%)	*Erwinia* infestation (%)
Injured	5	66	100	61
Not injured	5	66	37	32

TABLE 2.—*Comparison of tuber incubation method with lenticel sampling method for detection of pectolytic Erwinia on Russet Burbank and Sebago potato tubers.*

Method	No. of tubers	*Erwinia* infestation (%)
Tuber incubation	410	24.0
Lenticel sampling	410	20.5

Certified seed potatoes from all states tested, except Montana, had *Erwinia* infestations (Table 3). *Erwinia* infestations were also present in foundation seed potatoes from two of the six states. Of 71 *Erwinia* isolates selected at random from the five regions, 32 cultures were identified as *E. carotovora* var. *atroseptica* and 39 as *E. carotovora* var. *carotovora*. Both *Erwinia* spp. were present in potatoes from all states except Maine from which only *E. carotovora* var. *carotovora* was obtained.

TABLE 3.—*Detection of pectolytic Erwinia spp. by the tuber incubation method in certified potato seed tubers.*

State	Variety	Date tested	No. of samples	No. of tubers/sample	*Erwinia* infestation (%)*
Maine	Russett Burbank	May 21	2	30	0, 3
Minnesota	Russet Burbank	April 17	2	25	0, 48
Montana	Russet Burbank	March 26	1	25	0
New York	Sebago	May 9	9	10	0, 0, 10, 10, 30, 40, 50, 90, 100
North Dakota	Russett Burbank	May 21	2	30	7, 4
Wisconsin	Russet Burbank	February 2	1	25	12
	Sebago	January 26	1	25	92

*Percentage for each sample.

Discussions

The tuber incubation method provides conditions for rapid multiplication of soft rot bacteria permitting subsequent isolation of *Erwinia*. Induction of bacterial soft rot is enhanced by decreasing the 0_2 concentration around the tuber (7, 9) and this was achieved by wrapping the tubers in polyvinylidene film. Since polyvinylidene (Saran Wrap) has very low permeability to 0_2 ($P0_2 = 0.053$), it is particularly useful for this purpose (14). More nearly anaerobic conditions were achieved with a second layer of polyvinylidene film or a N_2 atmosphere for incubation. There was no difference in efficiency between these two procedures, but using the second layer of polyvinylidene film precluded the requirement for special incubation chambers and a source of N_2.

The optimum incubation time for detection varied from 4-6 days. Shorter incubation time was usually sufficient for immature tubers, whereas longer times were required for stored mature tubers. When a high percentage of the tubers (80% or above) was infested with *Erwinia*, an incubation period of 4 days was usually adequate.

Lenticels are important sites of *Erwinia* infestation (2, 12). Injuring tubers via the lenticels inoculated resident bacteria into susceptible tissue. The soft rot lesions that developed during incubation usually centered around the injured lenticels. Sometimes, however, the lesion originated from an uninjured lenticel.

Although soft rot usually developed in incubated tubers, *Erwinia* or other pectolytic organisms could not always be isolated on CVP medium. Other pectolytic bacteria such as strains of *Clostridium* and *Bacillus* also can cause decay under these conditions (8). Soft rot lesions differing from those induced by pure cultures of *Erwinia* with respect to odor, color, wetness, gas evolution and amount of slime produced were observed and

these differences are probably attributable to modification of the rot symptoms by secondary organisms.

The lenticel sampling method was slightly less efficient in detecting *Erwinia* infestations than the tuber incubation method. Since this method is also more tedious, the tuber incubation method has been adopted for our routine survey studies.

This preliminary survey of certified seed potatoes indicates a possible widespread infestation of certified seed tubers with pectolytic *Erwinia* in the U.S. However, samples were small and taken at various times after storage. For example, the Wisconsin samples were tested about 3 months after harvest, while others were not tested until May of the following spring (Table 3). Although it is known that *Erwinia* can survive in tuber lenticels during storage, the *Erwinia* population or the ability to detect them decreases substantially with time (De Boer and Kelman, unpublished data).

Even a low percentage of infestation is a source of concern because a single infested seed piece can serve as the inoculum source for infestation of a large number of daughter tubers (2, 3, 13). Thus, the presence of *Erwinia* on certified seed potatoes would be a problem even when foundation seed potatoes have only a very low level of infestation.

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