

Section 1

Important bacterial diseases in potato seed-tuber production

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Introduction

The three most important bacterial diseases in potato seed tuber production are:

1. Bacterial wilt caused by *Pseudomonas solanacearum*. Race 3 (biovar 2) is most common in potato and very often associated with latent infection.
2. Bacterial soft rot and black leg caused by *Erwinia carotovora* (Ecc) and *E. chrysanthemi* (Ech).
3. Common scab caused by *Streptomyces scabies*.

Symptomatology

1. Bacterial wilt

Foliage wilt can develop early, specially when transmission occurs by planting tubers with latent infection in high temperatures at the early stage of the crop-growing season. This often happens with the autumn crop. Initial wilt symptoms are generally unilateral on leaflets on one side of the leaf leaves of only one side of a stem, or just some of the stems.

Young plants will collapse in warmer environments that promote bacterial growth. In colder climates, wilting is slower and plants are stunted. A symptom that sometimes develops along with wilting is a slight yellowing of foliage. We can also observe dark stripes corresponding to an infected vascular ring in the stems and tubers. Brown discoloration in the vascular tissue varies in intensity depending on the bacteria strain and the variety of the potato. Severe wilt will cause dryness and brown color of leaves.

Signs of the pathogen include a yellow-gray exudate from the vascular ring of dormant buds that accumulates in the eyes and causes soil adherence. The exudate can also be seen as mucous drops at the end of the excised vascular tissue when cutting stems or infected tubers. The exudate appears at the end of the excised vascular tissue.

2. Soft rot bacteria and black leg

Symptoms are similar for the three *Erwinia* species that affect potato. Predominance of *Erwinias* depends on their adaptation to temperature low for Eca, intermediate for Ecc and warm for Ech.

Bacterial soft rot in potato tuber is characterized by a maceration of the parenchymatose tissue, resulting in a creamy or brown soft rot. Secondary organisms cause an accompanying fetid smell. The seed-tuber may rot before stem emergence and cause fails in the furrow. Stems develop ring from infected seed-tubers show different symptoms depending on the climate. Under dry weather conditions, leaves become chlorotic and stems break and dry. Under high humidity conditions, wilting and stunting of plants and chlorotic leaves with rolled edges are commonly observed symptoms. In presence of free water, a black and mucilaginous rot usually extends over soil surface and may rapidly advance in apical direction.

A cross section above the lesion normally shows darkening of the vascular tissue due to a substance causing wilt. In aerial stems, black rot may appear (not connected to the base) when infection is initiated by mechanical damage or by insects, and it is spread by rain or insects. This is called aerial rot.

In the field, the disease appears as patches, which usually coincide, and humid areas.

3. Common scab

Common scab affects mainly potato tubers, and occasionally the stems and stolons. In tubers, damage may vary

from circular lesion of 5 to 10 mm diameter to irregular lesion usually formed by an aggregate of two or more of the former. Lesions may be protuberant up to 2 mm on the surface, or appear as depressions down to 7 mm. Their colors go from light to dark brown except for deep hollow lesions which are even darker.

The color of damaged underground stem and stolon tissues varies from light to dark brown. The lesions are oval when they originate in lenticels, and nearly circular when they form as a result of submerging roots or are due to stem cracking.

4. Differences between *Pseudomonas* and *Erwinia* symptoms

Under certain environmental conditions, the symptoms of bacterial wilt and black leg are similar.

In these cases, an initial diagnosis using a selective test is recommended. The test is shown in the following table.

Screening for early infection by *Ralstonia solanacearum* (bacterial wilt), soft rot and black leg in potato.

	<i>Ralstonia</i>	<i>Erwinia</i>
Mother tuber	Will not disintegrate, except by secondary infections. If the vascular ring shows exudate, bacterial wilt has begun.	Mother tuber rot originates wilt and black leg. Soft, dark rot is occasionally fetid.
Foliage	Wilt commonly unilateral. Slight absent yellowing. Late necrosis.	Generalized wilt; upward rolled leaflets; yellowing and early necrosis.
Plant	Slow infection causes stunting. Fast infections results in premature death.	Dwarfism, dark wet rotting of stem, rapid death (if anaerobiosis persists).
Stem, longitudinal cut	Brown vascular ring.	General darkening under anaerobiosis. Vascular tissue is dark to black when infection is arrested.
Stem section	Brown vascular rings mucous exudate (fusiform in water),	Either whole or only vascular ring is dark to black (in water "cloudy" diffusion)'.
Tuber	Brown vascular ring; yellowish grey exudate from buds or stolon end. exudate from vascular ring cutting will form momentary filaments.	Under excess soil humidity (anaerobiosis), soft and dark rot progressing from stolon to the whole tuber.

Epidemiology and its application to Seed Production

1. Bacterial wilt

We deal mainly with race 3, which generally affects only potatoes. Race 1 prevails in warmer regions, has more hosts and survives better in the soil and weeds.

Race 3, through its longer association with the potato crop that is usually grown in cool regions, is better adapted to low temperatures than other strains that normally prevail in warmer areas. However, its optimal temperature is high (27-28°C). This explains why it grows slowly and remains as a latent infection in cold potato areas.

A very counteractive seed production cycle would be to plant (healthy) seed in infested soil, where soil average at 15 cm depth is 14°C or higher, but where air temperatures are not high, or about 18°C as maximum average. Infection will occur in those conditions, but very little or no wilting will be noticed in the foliage, nor symptoms will be observed in the tubers.

When infection occurs, it is latent. If hosted seed tubers are planted in warmer conditions, then the disease appears with severity. A more counteractive procedure is to sort seemingly healthy tubers from a symptomatic crop in foliage, tubers or both. Wilt incidence can be high in warm areas, moderate in mild areas and low in cool regions. However, if they are used as seed in the next cropping season, the latent infection they carry would range from moderate to very high, depending on the temperature.

The second main factor causing bacterial wilt is its survival in the soil. Some soils foster incidence of the disease while others suppress the bacteria or show a tendency to make the bacteria disappear. Survival is also fostered by the presence of volunteer plants from tubers of the previous crops or voluntary carriers. Other factors promote bacterial wilt but they are less important. They will be covered in the "Integrated Control" section.

2. Soft rot and black leg

Normally all potato tubers' lenticels carry latent infection of one or more *Erwinias*. Potato tubers with latent infection planted in good environmental conditions will not develop the disease.

However, when excess humidity saturates the soil and a water film covers the tubers, the anaerobic condition will promote first soft rot of mother tubers. When anaerobiosis persists, black leg will develop.

In tropical climates, the bacteria remains in the field from one crop to another in the rhizosphere of weeds and other crops, infecting volunteer plants. The bacteria spread through water irrigation, rainy splashes, aerosols, and from the mother tuber to offspring through the potato plant rhizosphere or rhizoplane.

To eliminate latent infection, a tuber-less crop must be grown using nodes or material multiplied *in vitro*.

3. Common scab

The *S. scabies* pathogen is present in nearly all soils of potato areas worldwide where it may be native or introduced through seed. However, for unknown reason it is of little or no importance in Peru.

Once this filamentous bacterium is introduced, it will remain in its saprophytic phase on rotting plant material or in any of several plant roots, including the fleshy roots of other crops (beet-root, sugar beet, radish, rape, rutabaga, carrots and parsley), to which it does not cause economic loss.

Adequate soil humidity discourages the development of *S. scabies*. Effective scab control can be achieved through appropriate irrigation.

Integrated Control

1. Bacterial wilt

P. solanacearum race 3 can be controlled and even eradicated by **the** combination of two or more factors. As long as healthy seed is sown in pathogen free soils, prevention is secured. However, numerous factors can effectively contribute to control if the most appropriate measures may not be applied. These are given in Fascicle 1.2 Integrated Control of Bacterial Wilt of Potato.

2. Soft rot and black leg

Erwinias are found protected in tuberoses lenticels or in the vascular system. Therefore, it is difficult to attack them with chemicals. Small tuber quantities kept wet in an environment where lenticels proliferate can be effectively treated with sodium hypochlorite at 1 % or antibiotics.

Pathogen-free tuber production begins with in vitro cultures (cuttings, mini-tubers), followed by aseptic multiplication in greenhouse, shed or field for irrigation, it is important to use water free from *Erwinias*. The water may need filtration, or chemical or ultraviolet-ray treatment.

Plant in well-drained fields and irrigate sparingly.

Plant non-susceptible crops in rotations and weed carefully to eliminate volunteer potato plants from the root.

When planting for seed, pull out symptomatic plants and harvest early after destroying foliage with chemicals. Avoid hitting or cutting tubers at harvest or during transportation. Clear the field of all tubers, especially damaged or rotten ones, which must be incinerated or buried.

Storage in diffused light or in well-ventilated warehouses with regular temperature is recommended.

The rot potential of a seed potato lot can be determined by 4-day incubation at 20-25 C in anaerobic conditions (nebulization chamber or covered with wet towel paper and placed inside plastic bags). The number of rotten tubers and the intensity of rotting indicate rot latent potential. Disinfect warehouses, boxes, bags and equipment with quaternary ammonia or another appropriate bactericide.

Integrated control provides a number of measures to ensure low levels of seed-tuber contamination. Healthy tubers give healthy plants, but plants can be contaminated with bacteria in water, aerosols or carried by insects to lesions, etc.

3. Common scab

- Avoid using seed with scab, especially if potato will be planted in a new field.
- Use long rotations to reduce inoculum potential.
- Use the most tolerant varieties.
- Keep a high level of humidity in the soil (field capacity) during tuber development and growth.
- Avoid applying excess lime that increases soil pH and encourages pathogen growth. Use sulfur and acid-forming fertilizers. Pentachloronitrobenzene reduces inoculum potential when mixed with the soil, but does not have a lasting effect.
- Do not incorporate organic material that serves as nutrient for *S. scabies* in the saprophytic phase.

Soil disinfestation

Serious problems arise from insufficient understanding of the desirable methods for pathogen elimination in soil mixtures for rooting (substrate). The most important considerations are as follows:

If ingredient sources are pathogen free, they can be used without disinfesting. Moss, river sand and deep soil are some examples. They must be monitored throughout potato planting.

Steam sterilization is ruled out because it kills beneficial saprophytic bacteria and may increase mineral content to phytotoxic levels. If steam is needed, use an air mix system for soil pasteurization at approximately 70°C.

Chemical fumigants are effective in general, depending on the pathogens and nematodes to be eliminated. Although they do not have secondary effects, some are toxic to humans and animals. See Fascicle 1 .3.

Methods for Diagnosis and Identification

1. Bacterial wilt

Based on the symptomatology described, the presence of wilt caused by *P. solanacearum* can be suspected. For a complete diagnosis, we must determine presence of a mucous exudate from the vascular ring. This may be evident in tuber eyes, or cut tuber surface on which drops of exudate form around the sectioned vascular ring.

In young plants without tubers, cut a piece of the stem of 1 to 3 cm length near the soil and place it on the top of a glass with water using a paper clip. Leave it to settle a few minutes. Bacterial wilt is revealed by the presence of a slowly disappearing mucous filament exudate.

Preliminary pathogen identification also results from the Gram-negative characterization of the bacterium. The exudate is transferred with a toothpick from the cut tuber surface to a microscope glass slide. Then, it is mixed with 3% KOH in equal amounts (2 drops of each one). After stirring for 20 seconds, the toothpick is lifted slowly. If a relatively long filament is formed without breaking, the bacterium is Gram negative.

Crop serological test and others to determine the presence of *P. solanacearum* are given in Fascicles 1 .4 and 1 .5.

2. Soft rot and Black leg

Diagnosis based on symptoms must take into account similarities and differences already given for bacterial wilt. In mother tubers, rot is usually complete, but sometimes it is difficult to detect.

In anaerobic conditions, black leg unmistakably appears on the stem. However, if humidity is low, a dark color of the vascular ring is externally visible in clear stems or by longitudinal section. The transversal cut does not result in exudate, but flow test can result in rapid bacteria dissemination in water without the filament stage.

Erwinia isolation is easily accomplished beginning from the vascular ring and using Crystal Violet Pectate (CVP) as a selective medium. Isolation from a rotten tuber is difficult because of secondary microbes. However, if latent infection of an apparently healthy tuber is activated, in anaerobic conditions, it will isolate easily from the symptomatic lenticel.

Detection and identification methods for *Erwinias* are given below.

Erwinias Detection

a) Selective Medium

Burnett and Perombelon Bulmer Crystal Violate Pectate (unpublished) are appropriate for *Erwinias* isolation from soil and water plants.

Ingredients

NAOH 1.0 M (8 g/200 ml water)	4.5	ml
CaCl ₂ H ₂ O 10%	6.8	ml

Na NO ₃	1.0	g
Agar (Difco)	2.0	g
Na Polypectate	9.0	g
Crystal violet 0.075%	1.0	ml
Trisodium citrate dehydrate	2.5	g
Tryptone	0.5	g
Novobiocine (sodium salt BDH)	2.0	ml
Distilled water	500	ml

Procedure

- Pour 500 ml of boiling distilled water in a blender vase, previously washed with hot water.
- Mix NaOH, CaC₁₂.2H₂O NaNO₃ crystal violet, tryptone dehydrate of citrate trisodium and agar at low speed.
- Add sodium polypectate slowly (H. L. Kluft & Co. Ltd., Wisconsin, U.S.A.).
- Mix at high speed.
- Sterilize at 121°C for 25 minutes.

In this medium, Erwinia colonies of bacterial soft rot are easily recognized for their morphological characteristics and the deep holes they form in 48 h at 27°C. These caverns must not be mistaken with less deep and concave holes caused by *Pseudomonas fluorescens*. Best results are obtained if the medium is allowed to dry at air temperature, before using the slides. Stewart selective medium (1962) or Cuppels and Kelmans CPG (1974) are useful to purify Erwinia colonies developed in CVP. This pure strain can be maintained in nutritive inclined agar at air temperature.

Enrichment procedure

Small Erwinia populations, in soil, water or in plant, tissues may grow in an enriched pectate medium (McCarter-Zorner et al., 1984) for easier detection.

Ingredients

MgSO ₄ .7H ₂ O	0.32g
(NH ₄) ₂ SO ₄	1.08g
K ₂ HPO ₄	1.08g
Sodium polypectate	2.70g
Distilled water	100 ml

Procedure

- Dissolve each salt in 1/3 distilled water.
- Add KHPO solution and mix well.
- Add sodium polypectate slowly and place it in steam or water bath for one hour.

- d) Adjust pH to 7.2
- e) Sterilize at 121 °C for 15 minutes

Erwina sp. can be cultivated by incubating the sample in anaerobic conditions in a pectate-enriched medium at 27°C for 48 h and then planting some medium in CPVP

Identification of soft rot *Erwinias*

a) Routine identification: soft rot *Erwinias* may be identified routinely without purification through their variable capacity to grow in a CVP at 27°C and 33.5°C. The presumed individual colonies of *Erwinias* that develop in CVR are punctiliously inoculated on the medium on three CVP slicks. Before plaquing, erythromycin at a concentration of 35 ppm is added to one of the plaques. The antibiotic free plaque is incubated at 27 C while the other two are incubated at 33.5°C. The three bacterial soft rot *Erwinias* form characteristic colonies at 27°C but only *Ecc* and *Echr* develop in CVP at 33.50C and from these only *Ecc* develops when erythromycin is present.

b) Standard identification test: To verify the results from the temperature test, perform either the dye (1969), Graham (1972), or Lelliot (1974) tests summarized in the following table a representative sample of purified isolates.

Biochemical and culture tests to screen Erwinias and bacterial soft rot.

Diagnosis test	<i>Ecc</i>	<i>Eca</i>	<i>Echr</i>
Acid of:			
Lactose	+	+	+ or -
Maltose	-	+	-
Alpha methylglucoside	-	+	-
Palatin	-	+	-
Trehalose	-	-	+
Glucose gas	-	+	-
Saccharose reducing substances	-	-	+
Use of:			
Malonate	-	-	+
Tartrate	-	-	+
Indol	-	-	+
Development in NaCl So.	+	+	+ or -
Lecitinase	-	-	+
Phosphatase	-	-	+
Sensitivity to Eritromicine	-	-	+
Blue Pigment	-	-	+ or -
Development temperature (°C)			
Minimum	6	3	3
Optimum	38-30	27	34-37
Maximum	37-42	35	45

c) Serology: All three bacterial soft rot Erwinias are related serologically, *Ecc* and *Eca* closer than *Echr*. Numerous serotypes have been identified for each organism (De Boer et al. 1 979; Yarkrus and Schaad, 1979.)

3. Common scab

In the spite of its filament-types development, *S. scabies* is not a fungus but bacterium of acariotic features. Nearly 1 micron in width, it is much smaller then fungi.

Therefore, microscope observation and isolation are difficult and experience is required. It isolates better from a yellowish better from a yellowish translucent tissue found under lesions. Planting is done in saccharose -potato-agar (at 0.5% saccharose) and incubation at 25-3°C (See Compendium of Potato Disease, 1980).

Symptoms vary according to bacteria tuber susceptibility and environmental condition. Therefore, of *S.scabies* are not always easy to diagnose.