

INTEGRATED CONTROL OF BACTERIAL WILT OF POTATO

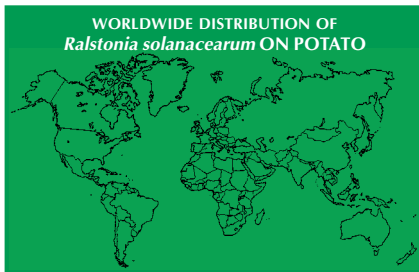
S. Priou, P. Aley, E. Chujoy, B. Lemaga and E. French

INTEGRATED CONTROL OF BACTERIAL WILT OF POTATO

Authors: S. Priou, P. Aley, E. Chujoy, B. Lemaga and E. R. French

Ralstonia solanacearum is the causal agent of the disease known as potato brown rot or bacterial wilt (BW). The bacterium affects more than 30 plant species, the most susceptible crops being potato, tomato, eggplant, pepper, banana and groundnut. Spread of the pathogen worldwide has been associated with its dissemination in latently infected planting material. Bacterial wilt is the second most important constraint to potato production in tropical and sub-tropical regions of the world. Quarantine measures necessary to avoid spread of the disease to BW-free areas often restrict the production of seed potatoes and limit the commercialization of ware potatoes between countries and between infected and non-infected regions within a country. Limited availability of high-quality seed, and lack of farmer knowledge on proper agronomic practices, threaten the sustainability of the potato crop in the developing world. BW incidence can be reduced only if various control components are combined. This involves mainly the planting of

2



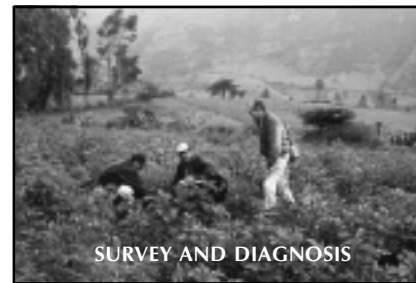
healthy seed in clean soil, planting tolerant varieties, rotation with non-susceptible crops, and, the application of various sanitation and cultivation practices. An integrated disease management approach can lead to significant reduction, or even eradication of BW.

WORLDWIDE DISTRIBUTION OF *R. solanacearum* ON POTATO

Bacterial wilt has spread to most potato producing countries. Its occurrence in Australia and in the southeastern United States has resulted in concentration of scientific research on BW in these areas. In Latin America, the disease has been reported from all potato producing countries except Ecuador. BW occurs throughout central and southern Africa, and is a serious production constraint in Uganda, Ethiopia, Kenya, Madagascar, Rwanda, Burundi, Nigeria and Cameroon. Although not a serious disease in Egypt, latent infection of tubers has resulted in a strong decline of potato exports to Europe. In South Asia, BW occurs in the mid-hills of the Himalayas in India, Pakistan, Nepal and Bhutan; also in the mid-hills and plains of India, where it is effectively controlled. In East and Southeast Asia, bacterial wilt is

important in Indonesia, the Philippines, southern Vietnam, Laos, Japan and southern China. In the early 1990s, BW became a serious threat to potato production in European countries including Belgium, England, France, The Netherlands, Spain, Italy and Portugal. It has also been reported in Russia. Earlier incidence in Sweden was followed by eradication.

3



SURVEY AND DIAGNOSIS

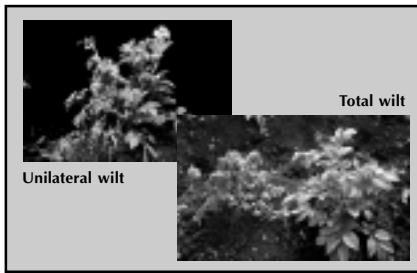
Inspection of potato fields is of prime importance in seed certification schemes. This is the way to identify diseased areas where national crop protection services may apply quarantine and control measures. Likewise, early disease diagnosis allows potato growers to define an appropriate management strategy. Field diagnosis of bacterial wilt disease can be done by identifying the foliage and tuber symptoms during the growing season or at harvest.

4



Foliage symptoms

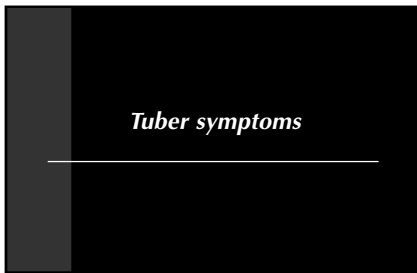
5



Total and unilateral wilting of potato

Above ground symptoms of BW include wilting, stunting and yellowing of the foliage. The browning of vascular bundles may be seen externally, or when the cortex is peeled. Characteristic too, is the initial wilting of only part of the stems of a plant, or even one side of a leaf or stem. If disease development is rapid, the entire plant wilts quickly, without yellowing. Alternatively, the diseased stem can wilt completely and dry up, while the remainder of the plant appears healthy.

6



Tuber symptoms

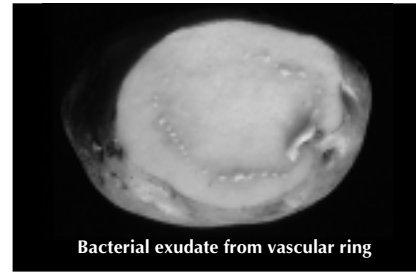
7



Bacterial exudate in tuber eyes

External symptoms on the tuber are visible at harvest when infection is severe. Bacterial ooze collects at tuber eyes or on the end of the stolon, causing soil to adhere to the secretions.

8



Bacterial exudate from vascular ring

Tuber symptom is often described as brown rot. Cut tubers show brownish discoloration of the vascular ring, and slight squeezing forces a pus-like slime out of the ring, or it may exude naturally.

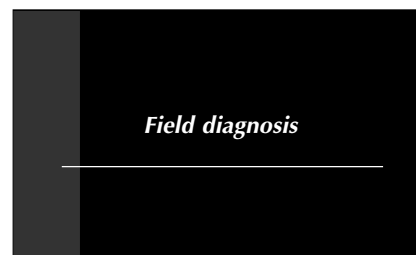
9



Necrosis involving secondary infection

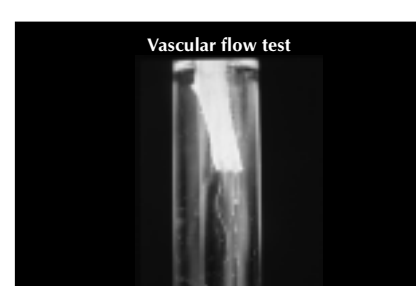
The vascular ring, or the whole tuber, may disintegrate completely at more advanced stages of necrosis development. This often involves secondary infection with saprophytic bacteria (*Erwinia* spp., *Clostridium* sp.) or fungi (*Fusarium* sp., *Pythium* sp.).

10



Field diagnosis

11



The vascular flow test

A diagnostic test is necessary because plant wilting caused by the bacterium *Ralstonia solanacearum* can be confused with symptoms induced by other pathogens such as *Fusarium*

eumartii, *Verticillium* sp., *Erwinia chrysanthemi*, or by insect or mechanical damage at the stem base. Diagnosis in the field can be easily accomplished by cutting a piece of stem 2-3 cm long from the base, and suspending it in clear water in a glass container. The cut stem can be held with an opened paper clip to maintain a vertical position. Within a few minutes, the smoke-like milky threads stream downward from the cut stem. This milky slime exuding from the stem is characteristic of *R. solanacearum* present within the vascular system.

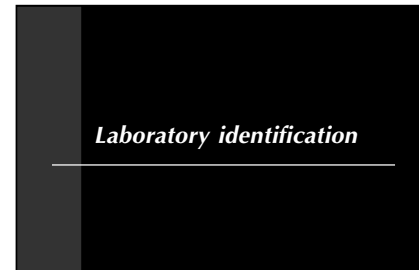
12



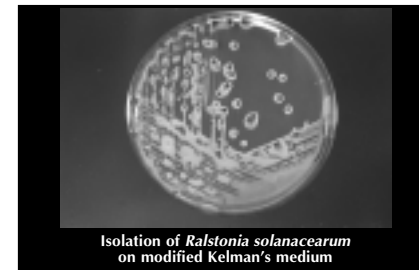
The KOH test

The brown rot symptom on tubers can be confused with ring rot caused by *Clavibacter michiganensis* subsp. *sepedonicus* (previously called *Corynebacterium sepedonicum*). A quick differential test can be performed directly on the bacterial exudate on the tuber to differentiate between the two bacteria. Two drops of 3% potassium hydroxide (KOH) are placed on the ooze and mixed with a laboratory loop or a wooden toothpick for 10 seconds. The formation of a milky thread upon lifting the toothpick indicates the presence of *R. solanacearum* (a Gram-negative bacterium), whereas with *C. michiganensis* subsp. *sepedonicus* (a Gram-

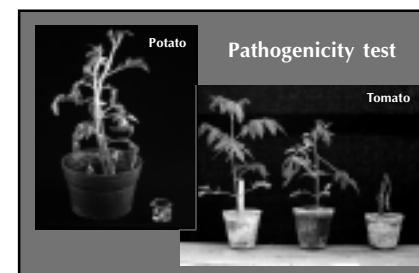
13



14



15



positive bacterium) the thread is not produced.

Laboratory identification

Isolation of *R. solanacearum* on modified Kelman's medium

The BW agent can be isolated from a diseased tuber or stem by plating the bacterial tuber exudate or two drops of the suspension obtained in the vascular flow test on modified Kelman's medium (TZC containing only 2.5 g dextrose). After 48 h incubation at 30°C, the typically fluid, slightly red-tinted colonies of virulent *R. solanacearum* are easily distinguished from other saprophytic bacteria (round shaped, uniformly dark red-colored colonies).

Pathogenicity test

The purified culture of *R. solanacearum* on Kelman's medium can be inoculated to tomato or potato seedlings to confirm its pathogenicity. Young tomato or potato transplants (third to fifth true leaf) are inoculated by injecting

the stems with 20 µl (e.g., a drop) of a bacterial suspension of *R. solanacearum* at 10⁸ cells/ml with a 1 ml-propylene syringe and a hypodermic needle, just above the cotyledons. Another inoculation method is to insert (at the level of the third leaf axil) a toothpick carrying the bacteria from a Kelman's agar plate. Greenhouse conditions that favor disease development are 28 ± 4 °C, 80-90% R.H., and natural daylight. Plants are irrigated normally, except one day before inoculation. If the isolate is a pathogenic strain of *R. solanacearum*, the wilting of tomato or potato seedlings may begin in less than a week, but will certainly appear within 4 weeks. After symptoms appear, the vascular flow test can be performed, and the pathogen can be re-isolated from the vascular flow as described previously.

16

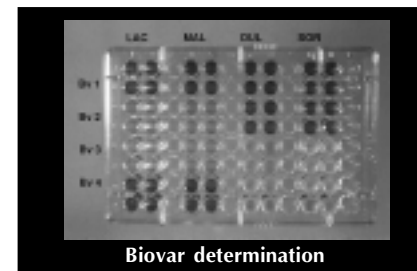
Differentiation of *R. solanacearum* into biovars

BIOCHEMICAL TESTS	BIOVAR				
	1	2	3	4	5
Utilization of: • lactose, maltose and cellobiose	-	+	+	-	+
Oxidation of: • mannitol • sorbitol, dulcitol	-	-	+	+	+
	-	-	+	+	-

Differentiation of *R. solanacearum* into biovars

Two classification systems are used for *R. solanacearum*: the race and the biovar systems. The biovar system consists of biochemical tests based on the ability of the bacterium to utilize three disaccharides and/or oxidize three hexose alcohols. Biovar classification is based on the following:
Bv 1 = utilization of disaccharides

17



negative, oxidation of alcohols negative;
Bv 2 = utilization of disaccharides positive, oxidation of alcohols negative;
Bv 3 = utilization of disaccharides positive, oxidation of alcohols positive;
Bv 4 = utilization of disaccharides negative, oxidation of alcohols positive;
Bv 5 = utilization of disaccharides positive, oxidation of only mannitol.

Biovar determination

These biochemical tests can be performed together in microtitre plates. The utilization of the disaccharide and oxidation of the alcohol result in the acidification of the medium, expressed by the change in the medium color from green (neutral pH) to yellow (acidic pH).

18

Equivalence between biovar and race in *R. solanacearum*

Race	Biovars	Hosts	Location
1	1, 3, 4	Solanaceous + many more	Lowland tropics
2	1, 3	Musaceae = Moko disease	American and Asian tropics
3	2A	Potato, Tomato	Cool climates
4	5	Mulberry	China
Not known	2T	Numerous	Primarily South American lowlands

Equivalence between biovar and race in *R. solanacearum*

The race system is based on host range under field conditions. Four races can be distinguished:

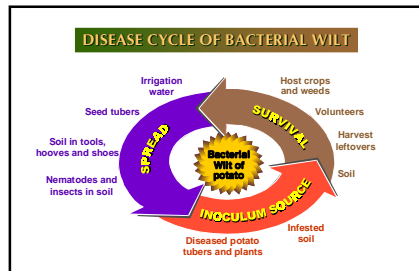
- Race 1 affects a wide range of solanaceous crops including potato and many weeds. Some strains can affect groundnut, ginger and diploid banana. It is prevalent at lower

elevations in the tropics and subtropics.

- Race 2 affects plants of the musaceae family, such as triploid banana, plantain and *Heliconia* spp. in the tropics.
- Race 3 affects mainly potato and occasionally tomato and other solanaceous crops and weeds. It is common in higher elevations or latitudes (cool climates).
- Race 4 affects mulberry and occurs only in China.

There is some equivalence between race and biovar: race 3 coincides with biovar 2A; race 1 strains are of biovar 1, 3 or 4, biovar 1 being the most common on potato. No race has been established for biovar 2T, primarily found in the lowlands of the Amazon basin. It has numerous strains with a wide host range as is the case with race 1; however, biovar 2T strains are less aggressive.

19



DISEASE CYCLE

The source of inoculum can be infected potatoes (seed tubers, harvest leftover and infected plants) or infested soil, or both. The pathogen can survive in soil (mostly on plant debris) and in the rooting system and rhizosphere of many hosts (weeds, other

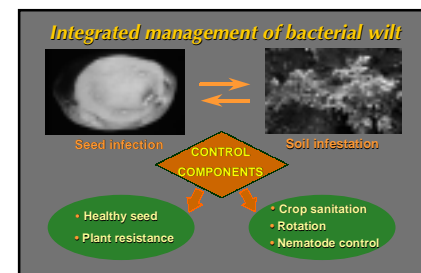
host crops, potato volunteers). *R. solanacearum* is mainly spread by movement of infected seed tubers. Contaminated surface water used for irrigation and infested soil adhered to farmers' shoes and tools also contribute to disease dissemination. Bacterial entrance into potato roots is facilitated by wounds made by tools during post-emergence cultivation, and by nematodes and insects in the soil.

20



CONTROL

21



Integrated management of bacterial wilt

To control and eradicate bacterial wilt, the main components are the use of healthy seed and planting in clean soils. However, many additional factors influence the incidence of the bacterium, such as environmental conditions (temperature and soil moisture), rotation with non host plants, the use of less susceptible varieties and cultural practices (crop sanitation and nematode control).

22

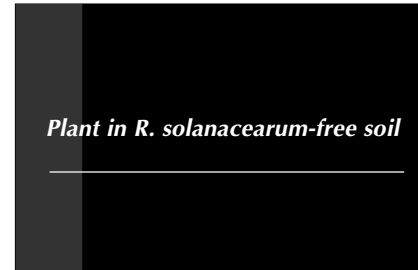


Thus only an integrated control strategy can succeed in reducing BW incidence or eradicating it. This strategy is site-specific. From the existing and available control factors, those that are feasible, suitable and effective should be selected for a given location (see slide #47). Moreover, social and economic factors that influence farmers' decision-making should be factored into the management scheme.

Use of healthy seed

Infected seed tubers are the main means of dissemination of *R. solanacearum* (particularly for race 3 strains). In cool conditions, such as tropical elevations above 2500 m, infected but symptomless plants may harbor the bacterium and transmit it to progeny tubers as latent infection, leading to severe disease outbreaks when grown at warmer locations. In seed certification schemes, no bacterial wilt must be tolerated during the growing season. For seed production, only BW-free seed tubers originating from disease-free areas must be used. The original planting material used in clonal (pre-basic and basic seed tubers) or positive selection multiplication scheme must be tested for latent infection with *R. solanacearum*. In endemic areas

23



where healthy seed tubers are hardly available, an alternative to planting seed tubers is the use of true potato seeds (TPS) or clean cuttings obtained under controlled growing conditions from micropropagation.

Plant in *R. solanacearum*-free soil

After a bacterial wilt infected crop, potato and other hosts must not be planted for at least two years. Rotation with cereals or graminaceous pastures can be implemented to eliminate soil inoculum. The duration of the rotation necessary to eliminate soil inoculum is variable because *R. solanacearum* survivability in soil varies according to environmental conditions (temperature, moisture) and soil characteristics (biotic and abiotic factors). Some soils are suppressive, e.g., the bacterium does not survive over a long period of time, however, the mechanisms responsible for this suppressiveness are unknown.

24

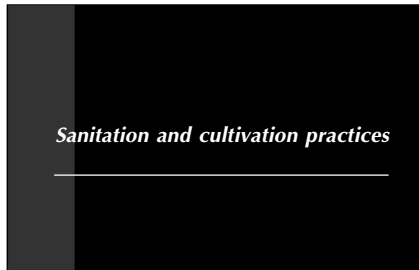


Plant Resistance

Plant resistance is one of the most effective means of controlling BW. However, although many potato varieties have been found to have a degree of resistance to BW, they still transmit latent infection to their progeny tubers. The use of

moderately resistant varieties must be thus coupled with a seed program that provides BW-free seed tubers. Resistance is strain-specific and is overcome when the levels of factors that favor BW are increased: high temperature, excessive soil moisture, wounding of roots and stolons, etc. An essential step in the development of resistant varieties is local screening. Locally grown varieties should be tested for tolerance to BW since some not bred for BW resistance have been found to have lower susceptibility (var. Achat in Brazil, Cruza 148 in East Africa and Peru).

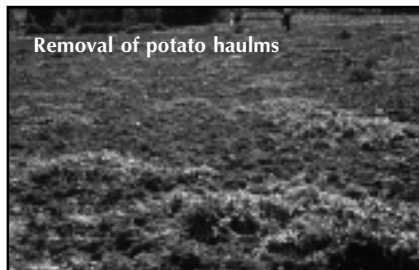
25



Sanitation and cultivation practices

Crop sanitation and cultivation measures aim to avoid or limit pathogen survival and dissemination. These measures are commonly used to manage other potato diseases and pests.

26



Removal of potato haulms

After harvest of a BW-infested crop, potato haulms must be removed from the field and buried deep, down-slope and far from irrigation canals. Alternatively, they can be burned.

27



Removal of rotted tubers

After harvest, sorted-out and leftover diseased tubers must also be removed from the field and destroyed using the same procedure as for haulms.

28



Weeding

R. solanacearum survives in weeds, so weeding must be done before planting potato and any other crop used in the rotation.

29



Roguing volunteer potato plants

Volunteer potato plants are another means of survival of *R. solanacearum* and must be removed in the subsequent crop (potato or any other crop) soon after their emergence.

30



Roguing wilted potato plants

If the incidence of BW is low in a field, wilted potato plants must be removed as soon as they are observed to avoid contamination of healthy neighboring plants. They must be destroyed carefully as

described previously for potato haulms and sorted-out tubers. Tubers from neighboring diseased plants should not be used for seed.

31



Tool decontamination

To prevent movement of soil from an infested to a disease-free field, all tools must be decontaminated by washing with water and calcium hypochlorite (or other available bactericide) or sterilized by flame.

32



Tractor decontamination

Machinery, vehicles, hooves of animals used for traction, and shoes of the personnel coming from an infested field must be washed at least with water before entering another field.

33



Uncontaminated water

The flow of water from an infested field to adjacent fields must be avoided. In infested areas, irrigation by well water is preferred over surface water from rivers or irrigation canals.

34



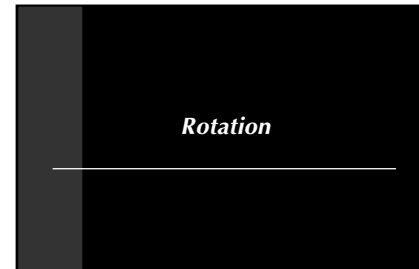
Other cultural practices

- . Minimum post-emergence cultivation
- . Flooding of paddy rice
- . Exposing plowed soil to summer heat

Other cultivation practices

- Minimum post-emergence cultivation: hilling only at planting time to avoid wounding roots with tools. Subsequent hand weeding is preferable for the same reason.
- Flooding of paddy rice: in Asian countries this significantly decreases soil populations of *R. solanacearum*.
- In very warm areas, exposing plowed soil to summer heat also decreases soil infestation.

35

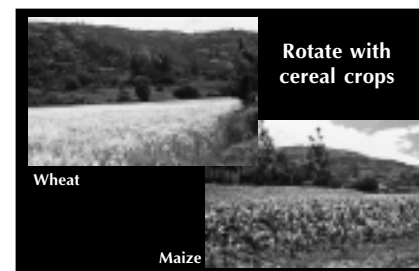


Rotation

Rotation

Rotation with non-host plants is an effective means of decreasing the level of *R. solanacearum* populations in soil, provided volunteer potato plants are continually removed by uprooting the entire plants from the field as they emerge.

36



Rotate with cereal crops

Wheat

Maize

Rotate with cereal crops

Rotation with cereals and gramineous pastures rapidly decreases soil inoculum potential. However, the time necessary for eradication has to be determined in each location.

37



Rotate with liliaceous and brassicaceous

Allium sp. (onion, garlic, leak) and brassicaceous (cabbage, cauliflower) can be planted after a BW-infected potato crop. Furthermore, these crops promote the elimination of weeds and volunteers that maintain the pathogen.

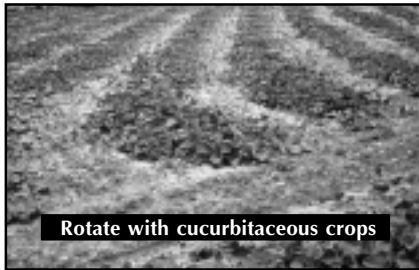
38



Rotate with legumes

Legumes, e. g. fabaceous (pea, bean), can be used as rotation crops for potatoes. Moreover, they have the additional benefit of increasing soil fertility by fixing atmospheric nitrogen. A bean/maize rotation has reportedly reduced soil inoculum potential.

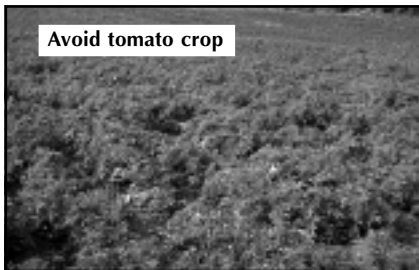
39



Rotate with cucurbitaceous crops

Cucurbitaceous crops (pumpkin, cucumber, zucchini) are not hosts for *R. solanacearum* and can thus be used in the rotation.

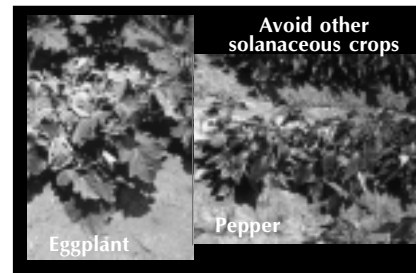
40



Avoid tomato crop

Tomato crop should be avoided since it is highly susceptible to bacterial wilt caused by either race 1 or 3 strains of *R. solanacearum*.

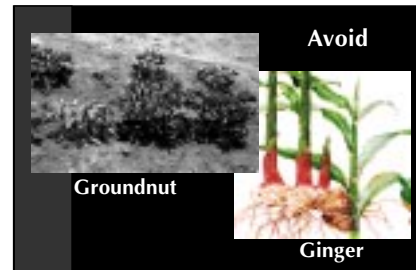
41



Avoid other solanaceous crops

Eggplant, pepper and tobacco must never be planted after a BW infected potato crop

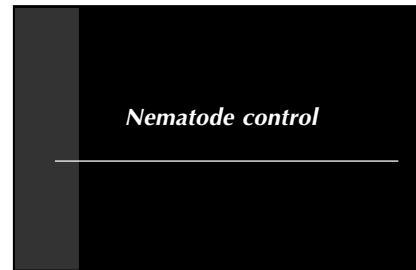
42



Avoid ginger and groundnut

Ginger and groundnut must never be planted after a BW-infected potato crop in infected areas where race 1 strains of *R. solanacearum* are prevalent (low-elevation tropics).

43



Nematode control

Nematodes open a path for the bacterium to enter the plant through root injuries. Therefore, they must be controlled to avoid their interaction with *R. solanacearum*. Major nematode control components are soil fumigation, rotation with cereals, application of high quantities of organic amendments (free of *R. solanacearum*), and planting nematode resistant varieties.

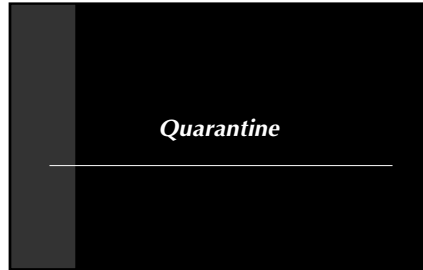
44



Symptoms caused by the root-knot nematode

The major nematode interacting with BW is the root-knot nematode (*Meloidogyne incognita*), occurring mainly in warm climates and sandy soil.

45



Quarantine

Once BW is introduced to an area, quarantine regulations have to be applied to avoid spreading of BW to non-infested areas. Measures restrict the production of seed potatoes and impede the commercialization of ware potatoes to BW-free countries or regions, affecting the economy of the quarantined regions.

46



No transport of infected seed tubers to non-infested areas

Avoid transport of ware or seed potatoes from an infected area to a BW-free to avoid spreading the disease. While it is often difficult to fully control all potato trading, this is one of the best preventative measures available.

47

DE	EGY	
Factors to be rated to formulate a control strategy	Race 3	Race 1
Healthy seed	7	7
Potato resistance or tolerance	4	2
<i>R. solanacearum</i> -free soil	7	7
Suppressive soil	2	2
Rotation with non-hosts	5	3
Roguing wilted potato plants	4	3
Roguing volunteers	4	2
Weed control	2	2
Removal of potato haulms and/or harvest leftovers	3	3
Control of spread in water	2	1
Minimal post-emergence cultivation	4	4
Nematode control	3	3
Tool decontamination, washing of hooves and shoes	2	1
Dry / heat soil	1	3
Flooding of paddy rice	1	3

DEFINING A CONTROL STRATEGY

The most relevant components for the control of bacterial wilt have been presented. Based on that information, the following table

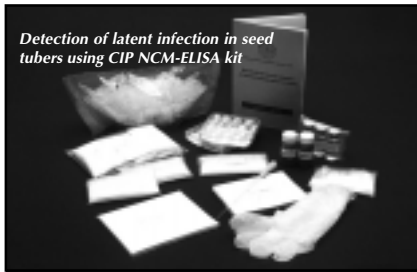
lists the main factors to be considered in developing a strategy for control of bacterial wilt that is caused by either race 3 or 1. Each factor has been rated on a scale of 1 to 7. The higher the number, the more the factor is judged to control the disease. The rates allocated to each factor may change according to the importance of a factor in a given location. A sum of at least 14 would usually be adequate for good control or potential eradication.

Factors to be rated to formulate a control strategy	Race 3	Race 1
Healthy seed	7	7
Potato resistance or tolerance	4	2
<i>R. solanacearum</i> -free soil	7	7
Suppressive soil	2	2
Rotation with non-hosts	5	3
Roguing wilted potato plants	4	3
Roguing volunteers	4	2
Weed control	2	2
Removal of potato haulms and/or harvest leftovers	3	3
Control of spread in water	2	1
Minimal post-emergence cultivation	4	4
Nematode control	3	3
Tool decontamination, washing of hooves and shoes	2	1
Exposure of plowed soil to summer heat	1	3
Flooding of paddy rice	1	3

48



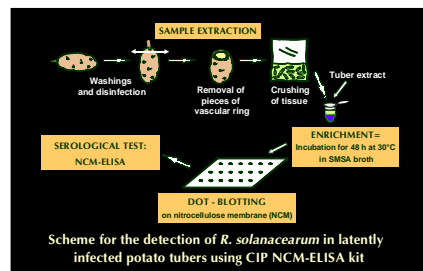
DETECTION METHODS OF *R. solanacearum*



Detection of latent infection in seed tubers using CIP NCM-ELISA kit

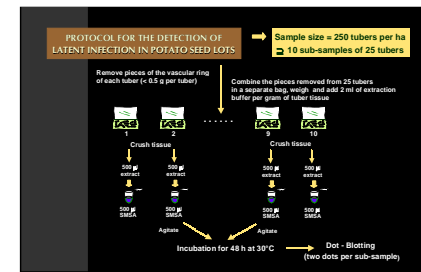
Detection of latent infection in seed tubers

The classical method of detecting tuber infection consists of incubating tubers for 3 to 4 weeks at 30°C and observing oozing from the eyes or stolon ends, or cutting the tubers to observe oozing from the vascular ring. However, this method is time and space consuming and may not reveal low infection rates. Because of this, CIP developed a simple, sensitive, quick and low-cost technique to detect latent infection in tubers that is suitable for seed testing: post-enrichment NCM-ELISA (Enzyme linked immunosorbent assay on nitrocellulose membrane). The kit is being distributed to seed programs worldwide.



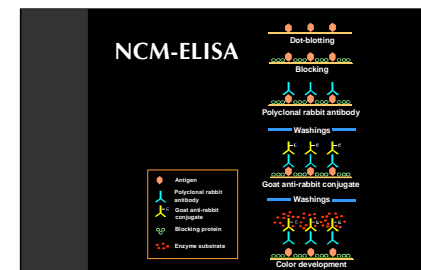
General scheme of seed testing
The main steps of post-enrichment NCM-ELISA to detect *R. solanacearum* in latently infected tubers are:

- 1) Preparation of tuber extracts from the tuber vascular ring;
- 2) Incubation of tuber extracts for 48 h at 30°C in a semi-selective broth (= enrichment procedure); and
- 3) Loading (Dot-blotting) of the enriched samples on nitrocellulose membrane (NCM), and serological test (ELISA).



Evaluation of latent infection in potato seed lots

The minimal sample size required is 250 tubers per hectare (sampled at harvest or in storage) for analysis in ten sub-samples of 25 tubers. The tissue of the vascular ring removed from each of the 25 tubers is crushed in one individual bag after adding the volume of extraction buffer corresponding to double the tissue weight. For each sub-sample, 0.5 ml (500 µl) of the tuber extract is mixed with the same volume of SMSA broth in an Eppendorf tube and incubated for 48 h at 30°C with agitation two times a day. Two dots of each incubated extract are put on the membrane.



The five steps of NCM-ELISA

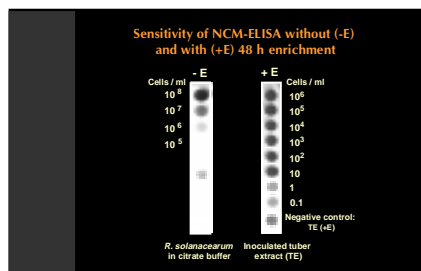
NCM-ELISA is an immuno-enzymatic assay. It uses a nitrocellulose membrane instead of a microtitre plate as support for the samples and reagents. The test consists of the following steps:

- 1) Loading of a very small amount of the tuber extract (20 µl e.g. one drop) on a nitrocellulose membrane (= Dot-blotting);
- 2) Blocking of the area of the membrane that is free of extract with milk casein (1 h incubation);
- 3) Binding of the samples with

R. solanacearum-specific rabbit antibodies (2 h incubation);

- 4) Binding of the *R. solanacearum*-antibodies complex with enzyme-labeled (alkaline phosphatase) goat anti-rabbit antibodies (1 h incubation); and
- 5) Revealing of the bound enzyme by adding the substrate leading to a coloration reaction (5 to 20 min).

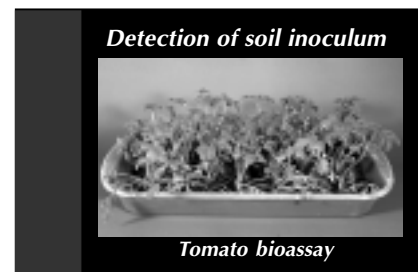
53



Sensitivity of NCM-ELISA with and without enrichment

The presence of *R. solanacearum* in the tuber extract leads to the development of a purple coloration. All races and biovars of *R. solanacearum* can be detected with the polyclonal antibodies. The intensity of the coloration is proportional to the bacterial concentration. After enrichment (e.g., multiplication of the bacterial population in the extract), as few as 10 bacteria per ml of tuber extract (cells/ml) can be detected, whereas 10^7 cells/ml or more are necessary if enrichment is not practiced before conducting the immunoassay. Thus, enrichment increases sensitivity of the serological test by a million-fold, allowing the detection of *R. solanacearum* in potato tubers (or stems) that are latently infected, e.g., with very low infection levels that produce no visible symptoms.

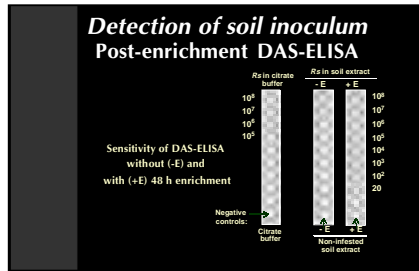
54



Methods to detect soil inoculum

Tomato bioassay

Since tomato is highly susceptible to bacterial wilt at warm temperatures, tomato bioassay is the simplest way to evaluate soil infestation with *R. solanacearum*. However, it requires greenhouse space and is time consuming since it can take up to six weeks to observe symptom development. It can also be done in the field by planting rows of tomato and observing wilt development. For greenhouse tests, 2 kg of soil are placed in a plastic tray and 50 two-week old tomato seedlings (about 10 cm high) are transplanted. For each soil sample, 100 seedlings are transplanted in two trays, and irrigated normally. From 7 days until 45 days, the percentage of wilted tomato plants is recorded two times a week. The test tube assay and the KOH test with the bacteria oozing from the tomato stems can be used to confirm that wilt results from *R. solanacearum* and not from other soil pathogens such as *Pythium* sp., *Verticillium* sp. and *Clavibacter michiganensis* subsp. *sepedonicus*.



Methods to detect soil inoculum Post-enrichment DAS-ELISA

A kit has been set up at CIP to detect low population levels of *R. solanacearum* in soil. The method is based on the simple preparation of soil extracts by mixing 10 g of soil with 90 ml of PBS buffer. After 30 min. constant agitation, the solution is allowed to stand for one minute and 2 ml of the supernatant is removed, mixed in a sterile flask to 38 ml of SMSA broth complemented with potato broth and incubated for 48 h at 30°C with agitation (enrichment procedure). The enriched extracts are analyzed in double-antibody sandwich ELISA (DAS-ELISA) in microtitre plates. As few as 20 bacteria per gram of soil can be detected. For semi-quantification of the populations, the soil extracts can be diluted previous to their enrichment.



The four steps of DAS-ELISA

Double-antibody sandwich ELISA (DAS-ELISA) is an immunoassay conducted in microtitre plates, with the following four steps:

- 1) Coating of the microtitre plate wells with the *R. solanacearum*-specific rabbit immunoglobulins (IgG) (3 h incubation);
- 2) Loading of the samples in the wells (overnight incubation);

- 3) Loading of the *R. solanacearum*-specific rabbit IgG conjugated to alkaline phosphatase (3 h incubation);
- 4) Development of the coloration reaction by adding the enzyme substrate (1 h).



METHODS TO ASSESS POTATO RESISTANCE TO BW

Field evaluation

Screening potato for resistance to BW requires a field with a reasonably uniform and moderate level of infestation (between 30% to 50% wilt incidence in the previous potato crop). The biovar/race of the strains present in the field should be identified (both races 1 and 3 can be present). The plot design should be chosen according to the number of tubers per clone to be tested and the uniformity of the inoculum spread in the field, but at least 20 tubers should be used (4 replications of 5 tubers). During the growing season the number of wilted plants is recorded once a week from 40 days to 80 days after emergence. At harvest, visible infection of tubers (and latent infection if no wilting occurred during the growing season), and yield of marketable sized tubers are also recorded.

Greenhouse inoculation

Apical cuttings of potato plants or cuttings obtained from sprouted tubers are rooted in small pots containing a soil substrate (a mixture of soil, sand and peat 2:1:1). However, the best results have been obtained using the Promix BX® substrate (Premiers Brands, INC, Stamford, Canada). A complete fertilizer (N-P-K 20-20-20, diluted at 0.5% in water) is applied at the time of planting. Plants should be transferred to the greenhouse at least one day before the inoculation. Greenhouse conditions that favor disease development are 28±4 °C, and 85-90% R.H. Plants are irrigated daily, except for one day before inoculation. Each pot containing a one-stem 20-day-old plant (about 15 cm tall) is inoculated with 25 ml of bacterial suspension of *R. solanacearum* at 10⁸ cells/ml. Twenty plants per genotype are inoculated. Plants are incubated for 30 days and the disease indices are recorded weekly on the following scale:

- 1 = no symptoms;
- 2 = wilting of one leaf;
- 3 = wilting of up to 50% of the leaves;
- 4 = wilting of up to 75% of the leaves;
- 5 = complete wilting of the plant.

Cultivars Cruza 148 (resistant to foliage wilt), Molinera (= BR63.65, moderately susceptible), Revolucion (susceptible) and Yungay (susceptible) are used as controls at CIP Lima. At a minimum, Cruza 148 should be used as the less-susceptible control, together with a local susceptible variety.

REFERENCES

- Aley, P., French, E. R. and Nydegger, U. (1994) Methods of greenhouse inoculation with *Pseudomonas solanacearum* to select resistant potato clones. *Fitopatologia* 29:20 (Abstract).
- Anonymous. (1994) Bacterial wilt: the disease and its causative agent *Pseudomonas solanacearum* (eds. Hayward, A. C. and Hartman, G. L.). 259 p. CAB International, Wallingford (UK).
- Anonymous. (1998) Bacterial wilt disease: molecular and ecological aspects (eds. Prior, Ph., Allen, C. and Elphinstone, J.) 447 p. INRA edn, Springer Verlag, Berlin (Germany).
- Commonwealth Mycological Institute. (1977) Distribution maps of plant diseases: *Pseudomonas solanacearum* (E. F. Smith) E. F. Smith on potato (*Solanum tuberosum*), tobacco (*Nicotiana tabacum* and banana (*Musa*) etc. Map No. 138 Edition 5.
- Elphinstone, J. G., Hennessy, J., Wilson, J. K. and Stead, D. E. (1996) Sensitivity of different methods for the detection of *Pseudomonas solanacearum* (Smith) in potato tuber extracts. *EPPO/OEPP Bulletin*, 26 :663-678.
- French, E.R. (1986) Field-screening CIP clones developed for resistance to bacterial wilt. *Technology Evaluation Series No. 1982-6*, 9 p. International Potato Center, Lima (Peru).
- French, E.R. (1994) Strategies for integrated control of bacterial wilt of potatoes. In *Bacterial wilt: the disease and its causative agent Pseudomonas solanacearum* (eds. Hayward, A. C. and Hartman, G. L.) p. 199-207, CAB International, Wallingford (UK).
- French, E. R., Gutarra, L., Aley, P. and Elphinstone, J. (1995) Culture media for *Ralstonia solanacearum* isolation, identification and maintenance. *Fitopatologia* 30 (3): 126-130.
- Hayward, A. C. (1991) Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. *Annual Review of Phytopathology* 29, 65-87.
- Hayward, A. C., El-Nashaar, H. M., de Lindo, L. and Nydegger, U. (1991) The use of microtiter plates in the phenotypic characterization of phytopathogenic *Pseudomonads*. *Proc. 7th Int. Conf. Plant Pathog. Bact.*, Budapest, Hungary, pp 593-598.

Janse, J. D. (1996) Potato brown rot in Western Europe –history, present occurrence and some remarks on possible origin, epidemiology and control strategies. Bulletin OEPP/EPPO Bulletin 26:679-695.

Kelman, A. (1954) The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. Phytopathology 64:693-695.

Martin, C. and French, E.R. (1985) Bacterial wilt of potato: *Pseudomonas solanacearum*. Technical Information Bulletin 13, 8 p. International Potato Center, Lima (Peru).

Priou, S., Gutarra, L. (1998) Video demonstration on the use of CIP NCM-ELISA kit for the detection of *Ralstonia solanacearum* in latently infected tubers. English, Spanish and Chinese versions, 37 min, International Potato Center, Lima (Peru).

Priou, S., Gutarra, L. and Aley, P. (1999) Highly sensitive detection of *Ralstonia solanacearum* in latently infected potato tubers by post-enrichment ELISA on nitrocellulose membrane. EPPO/OEPP Bulletin 29 (1), in press.

Priou, S., Gutarra, L., Fernandez, H. and Aley, P. (1999) Sensitive detection of *Ralstonia solanacearum* in latently infected potato tubers and soil by postenrichment ELISA. CIP Program Report 1997-98, in press, International Potato Center, Lima, Peru.

Priou, S., Gutarra, L., Fernandez, H. and Aley, P. (1999) Sensitive detection of *Ralstonia solanacearum* (race 3) in soil by post-enrichment DAS-ELISA. ACIAR Bacterial Wilt Newsletter 16: 10-13.