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Bacterial leaf spot, a new disease of lettuce in Quebec caused by *Xanthomonas campestris* pv. *vitians*

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Bacterial leaf spot of lettuce (BLSL) was reported for the first time in 1918 in the United States (Brown 1918). Since this first mention, the disease has been observed in different parts of the world including New York State (Burkholder 1954), Australia (Harrison 1963), California (Schroth *et al.* 1964; Umesh *et al.* 1996), Natal (India) (Wallis and Joubert 1972), New Zealand (Boesewinkel 1977), France (Allex and Rat 1990), Italy (Pennisi and Pane 1990), Florida (Pernezny *et al.* 1995), and Ohio (Sahin and Miller 1997). In Quebec, BLSL was observed for the first time in 1994 and a severe outbreak occurred in 1996 (Toussaint *et al.* 1998b). Losses reached up to 100% in some lettuce fields south-west of Montreal. The disease is now observed sporadically in the different lettuce production areas of Quebec.

In most of the cases in Quebec, BLSL of lettuce was observed on Romaine types. However, in 1998, the disease was also observed on Crisphead cultivars. In the United States, the disease is present on both Romaine and Crisphead cultivars (Pernezny *et al.* 1995; Sahin and Miller 1997). In France, the disease is mainly observed on Butterhead cultivars (Allex and Rat 1990).

DESCRIPTION OF SYMPTOMS

In the development of BLSL, the first apparent symptoms are water-soaked lesions at the margin of the leaves, which are noticeable only when the leaves are wet. Sometimes, these symptoms can also be observed on lettuce transplants in the greenhouse mainly on cotyledons. These water-soaked lesions collapse and appear almost transparent with a dark-brown coloration. When the disease progresses, lesions appear on the midrib of the leaves. The plant tissue around these lesions will turn light green followed by a yellowing of the entire leaves. When more than 50% of the leaf is affected, the leaf rapidly dies. The symptoms of BLSL are limited to leaves and have never been observed on stems or roots. When symptoms are restricted to the older leaves, these leaves can be removed at harvest and yield losses remain low. However, if the environmental conditions are favourable, the disease will progress to the heart of the plant, rendering the plant unmarketable. In cases of severe outbreaks, economic losses are important as an entire field can be

Note du rédacteur : le texte ci-dessus est présenté tel que soumis / Editor's note : the above text is presented as submitted.

destroyed. From our experience, it has been observed that lettuces with BLSL symptoms are more susceptible to *Botrytis cinerea, Sclerotinia sclerotiorum* and *Rhizoctonia solani*. BLSL alone will rarely kill a plant.

THE PATHOGEN

BLSL is caused by *Xanthomonas campestris* pv. *vitians* (Brown) Dye. This bacterium is an aerobic Gram negative non-sporulating rod with one polar flagellum. Its optimal growth temperature *in vitro* is 28°C. Contrasting with several other pathovars of *Xanthomonas campestris*, *X. campestris* pv. *vitians* does not hydrolyse starch.

According to the descriptive chart of the Society of American Bacteriologists, the organism was originally named *Bacterium vitians* (Brown 1918). Later, the name was changed to *X. vitians* (Brown) based on the classification of phytopathogenic bacteria (Dowson 1939). In 1974, Dye reclassified the genus *Xanthomonas* including; the pathogen responsible for bacterial leaf spot was then named *X. campestris*. pv. *vitians* (Dye and Lelliot 1974). In 1995, Vauterin *et al.* reclassified the genus *Xanthomonas* based on DNA homology. Strains of *X. campestris* pv. *vitians* were divided into two species : *X. hortorum* pv. *vitians* and *X. axonopodis* pv. *vitians*. However, only the type strain (ATCC19320) was included in the taxon *X. axonopodis* pv. *vitians* (Vauterin *et al.* 1995). All the other strains tested were classified as *X. hortorum* pv. *vitians*. This recent reclassification is still not accepted by the scientific community and recent literature still identify the pathogen as *X. campestris* pv. *vitians* (Pernezny *et al.* 1995; Sahin and Miller 1997; Sahin *et al.* 1997; Toussaint *et al.* 1998b; Umesh *et al.* 1996). To avoid confusion, and until evidence on the classification of the pathogen is confirmed, the name of *X. campestris* pv. *vitians* will be used throughout this communication.

In the BIOLOG database (version 3.7), the strains of *X. campestris* pv. *vitians* are classified in one of three types, A, B, and C. The BIOLOG system identifies bacteria based on respiratory activity in the presence of different nutrients (95 nutrients). Characterisation of 30 strains of *X. c.* pv. *vitians* from Ohio by fatty acid methyl ester (FAME) analysis, protein electrophoresis, the BIOLOG GN microplate assay, indirect ELISA with a set of eight monoclonal antibodies (MAbs), amylolytic and pectolytic activity, and pathogenicity tests resulted in three groups, A, B, and C. Group A included only the reference strain LMG937 (ATCC19320), group B represented 17% of the 30 strains tested and group C, a new group according to authors, included 83% of the strains tested (Sahin *et al.* 1997). In our identification based on BIOLOG system of strains from Quebec, Florida, Greece, Spain, and France, 57% of the strains were identified as *X. campestris* pv. *vitians* type C, 20% as *X. campestris* pv. *carotae*, and 23% as other identifications (Toussaint *et al.* 1998b). For the latter strains, the identification should be interpreted with care because some strains of *X. campestris* pv. *vitians* grow slowly, and consequently, the purple colour that develops in BIOLOG microplate during respiration is not strong enough in some wells to be detected. This can result in a wrong identification. These limitations of the BIOLOG system must be considered in the characterisation of bacterial strains.

EPIDEMIOLOGY

BLSL is observed under warm, humid and rainy environmental conditions. These weather requirements explain why the disease is rarely observed during the spring and in September in Quebec, but occurs more frequently in July and August, when the climate is propitious to the development of the disease. This disease is thought to be seed borne, but trials done to re-isolate the pathogenic bacteria from commercial lots of seed failed (Ohata *et al.* 1982; Umesh *et al.* 1996). However, it has been proven that it was possible to produce contaminated seed from contaminated plants (Sahin and Miller 1997; Umesh *et al.* 1996).

Dispersion of the bacteria in the greenhouse

Wellman-Desbiens (1998) showed that a large number of seedlings in greenhouse can be contaminated by overhead irrigation. In her experiments, infected plants were produced from seed contaminated with a rifampicin-resistant strain of *X. campestris* pv. *vitians*. An infection focus composed of six contaminated plants was placed at one end of a greenhouse table containing 12 000 non-contaminated lettuce seedlings. The seedlings were watered by overhead irrigation once a day and the dispersion of the bacteria was followed by imprints of lettuce leaves on a medium supplemented with rifampicin. Wellman-Desbiens (1998) demonstrated that after only 7 days, two-third of the seedlings were contaminated. Therefore, only a few contaminated seeds in a greenhouse lettuce transplant production would be enough to contaminate a large number of seedlings before the transplantation in the field. This might explain some of the recent outbreaks of BLSL.

ECOLOGY

X. campestris pv. *vitians* can survive as an epiphyte on leaf surfaces before inducing symptoms. Scanning electron microscopy of asymptomatic lettuce leaves contaminated by the pathogenic bacteria showed the presence of the bacteria at the surface of the leaf. Moreover, the pathogenic bacteria were recovered from asymptomatic lettuce leaves by dilution plating after leaves were macerated in phosphate buffer.

When the first symptoms appear on a leaf, a total of 10^7 CFU cm^{-2} of leaf can be recovered. However, even if the disease progresses on a leaf, no more than 10^8 CFU cm^{-2} could be recovered, which probably corresponds to the leaf carrying capacity. The limit in bacterial population could be due to a lack of nutrients or/and the amount of available space on the leaf (Blakeman and Brodie 1977; Derridj 1996; Fokkema 1984).

The population of *X. campestris* pv. *vitians* is highly dependent of the environmental conditions. During the days following a heavy rain or cool temperatures, a decrease of the bacterial population is observed (Toussaint *et al.* 1999b). However, the bacterial population has the capacity to re-establish itself rapidly when the environment becomes favourable again.

CONTROL OF BLSL

There are no bactericides that are recommended in the "Répertoire des traitements de protection des cultures" from Conseil des productions végétales du Québec for bacterial disease of lettuce. Pesticides allowed on other vegetables for control of bacterial diseases were tested for the control of bacterial leaf spot of lettuce. The most efficient treatments reduce disease severity by only 50% of that observed on non-treated plants (Carisse 1997; Carisse *et al.* 1999; Toussaint *et al.* 1998a, 1999a). Moreover, the use of some copper bactericides caused symptoms of phytotoxicity on lettuce plants.

For controlling bacterial leaf spot of lettuce, different approaches must be considered. An integrated disease management program should include the use of resistant cultivars of lettuce, seed treatments, an early detection of the bacterium on seedlings, and a biological and/or chemical control to reduce the pathogenic bacterial population in greenhouses.

Susceptibility of cultivars

Romaine, butterhead, crisphead, and green leaf types were tested for their susceptibility to BLSL. The Romaine types were the most susceptible types followed by the crisphead. However, within a same lettuce type, differences were observed among. For instance, in Butterheads, the cultivar Bella Green was highly susceptible and the cultivar Optima showed a low disease severity. Nevertheless, symptoms of BLSL were observed in all cultivars tested, but the green leaf types were the least susceptible (Carisse *et al.* 1999).

Seed treatments

Different seed treatments were tested for disinfecting contaminated seeds. Most of these treatments significantly

reduced the incidence of contaminated seed. The most efficient treatment was 1% of sodium hypochlorite for a soaking time of 5 to 20 min. However, treatments using dry heat or hot water for more than 1 h reduced significantly the seed germination as compared to the untreated seeds (Carisse *et al.* 1999).

Detection, and chemical and biological control

Considering the results obtained by Wellman-Desbiens (1998), showing that the pathogenic bacteria could be spread rapidly to a large greenhouse surface when overhead irrigation is used, it becomes important to develop a diagnostic test for early detection of the bacteria. If *X. campestris* pv. *vitians* is detected on seedlings before transplantation, the transplants could be destroyed or chemical and/or biological control could be applied to reduce the pathogenic bacterial population below the threshold for disease development.

CONCLUSION

In conclusion, *X. campestris* pv. *vitians* can survive as an epiphyte on lettuce plant without showing symptoms. Consequently, it is difficult for seed manufacturers to produce uncontaminated seeds without a very sensitive detection test. Hence, like most bacterial diseases, prophylactic measures and integrated pest management must be favoured for the disease control.

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