Pseudomonas-like early blight on sweet cherries

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Sweet cherry trees in an experimental planting at Summerland, British Columbia, developed late bud opening and other symptoms including chlorotic patches on leaves and blight with gumming and dieback on current year growth. This disease was not caused by any known virus; however, the antibiotic streptomycin reduced disease incidence. The possibility that the causal agent was a bacterium was tested by inoculating tobacco plants with a suspension of bark from an infected tree and with a bacterium isolated from infected bark. Both treatments produced similar lesions on tobacco plants. Furthermore, a tissue suspension made from necrotic lesions on tobacco plants injected, or applied by budding, into 2-year-old cherry trees caused abnormal development. The bacterium has subsequently been identified as an unknown *Pseudomonas* spp. with similarity to *P. cepacia* and *P. gladioli.*

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En Colombie-Britannique a Summerland, dans une plantation experimentale, des cerisiers sucres ont developpes une ouverture tardive des bourgeons, des regions chlorotiques sur les feuilles et des brûlures avec de la gommose et du deperissement sur les nouvelles croissances de l'année. Cette maladie n'a pas ete causee par un virus connu; neammoins, la streptomycine a reduit l'incidence de la maladie qui impliquait une bacterie comme agent causal possible de la maladie. On a évalué la possibilite de la presence d'une bacterie en inoculant des plants de tabac avec une suspension d'ecorce d'un arbre infecte et d'un isolat de bacterie provenant d'une ecorce infectee. Les deux traitements ont produit des lesions similaires sur les plants de tabac. De plus, une solution en suspension composee de tissus necrotiques de tabac a ete injectee, ou appliquees durant une methode de propagation par bourgeonnement, a des cerisiers vieux de deux ans, et cette inoculation a provoque un developpement anormale de ces derniers. Par la suite, la bacterie a été identifiee comme etant une espece iinconnue de *Pseudomonas* ayant une similarite avec *P. cepacia* et *P. gladioli.*

In the Spring of 1990 and 1991, virus-free sweet cherry trees *(Prunus avium* L., var. Bing) and Japanese flowering cherry trees *(Prunus serrulata* Lindl., var. Kwanzen) at the Agriculture Canada, Research Station, Summerland, British Columbia, developed symptoms which are new to this area. There has been no documented report of this disorder from commercial orchards in the Okanagan-Similkameen Valleys of British Columbia. The symptoms appeared to be somewhat similar to those caused by *Pseudomonas syringae* van Hall on stone fruit (Cameron, 1962; Davidson, 1973). Specifically, the infected branches showed late bud opening, a few chlorotic patches on fully expanded leaves, followed by blight early in the spring (May-June). On current year's growth a gumming and dieback extended in both directions. These symptoms occurred on the same trees every year and slowly spread to adjacent trees. Infected trees developed cankers on the trunk and eventually died if the infected branches were not removed in time. Infected trees were tested for virus by sap transmission to herbaceous hosts and by bud inoculation to woody hosts with negative results. It can be concluded that this disease is not caused by any known virus.

Streptomycin has been used in New York State to control blister spot caused by *Pseudomonas syringae* pv. *papulans* (Rose) Dhanvantan, in commercial orchards (Burr, 1990). Proebsting (1988) reported that frequent sprays of bactericides such as streptomycin usually kept *P. syringae* populations near undetectable levels. Infected trees at the Experimental Station that were sprayed with streptomycin (0.6g/L and 3000 L/ha) in late September and early March showed reduced symptoms, and there was no new infection on healthy trees nearby.

Three experiments were conducted to attempt to confirm that this disease was caused by *fseudomonas* spp. Three 6-year-old trees (var. Bing) were chosen in July, 1990 for this study:

Tree 1 had branches with symptoms, some leaves still attached, and had green buds.

Tree 2 had branches with symptoms, without leaves, and had brownish but living buds.

Tree 3 served as a control tree and had leaves and healthy buds.

In the first experiment, infected branches collected from Tree 1 and Tree 2 and control branches from Tree 3 were surface sterilized in 10% bleach for ten min, rinsed with dis-

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tilled water three times and allowed to dry. Their bark was then ground in 0.05M phosphate buffer (pH 7.0) with mortars and pestles. A plastic syringe (Plastipak®) was used to inject 0.5 ml of the buffered bark suspension from each test tree into the underside of the leaves of *Nicotiana tobaccum* L. (var. F2C1) via lateral veins as described by Klement (1963). Four leaves on each of four test plants were inoculated and grown in the greenhouse with a 16 h photoperiod and temperature of 20 ± 2°C.

Brown patches, 5-10 mm in diameter were observed, near the areas that were injected with the bark suspensions prepared from Tree 1 and 2, four days after inoculation. Only small brown necrotic spots appeared where the needle had pierced the leaf on the control. The development of large brown patches indicated that the inoculum from Tree 1 and 2 were pathogenic.

In the second experiment, small pieces of leaf tissue (approx. 1 cm in diam.) were collected from the edge of the brown necrotic lesions on the inoculated tobacco plants and from control leaves. The tissue was ground in 0.05M phosphate buffer.

Treatment 1: The suspension (0.05 ml) from diseased tobacco tissue was injected with a hypodermic syringe during the growing season into each of six leaves of two, 2-year-old cherry trees var. Bing. Treatment 2: The suspension used in Treatment 1 was also applied to the trunk of a 2-year-old Bing tree after a "T-cut" was made and wrapped with budding bands. Treatment 3: Healthy tobacco leaf suspensions were injected into six leaves of a Bing tree as control.

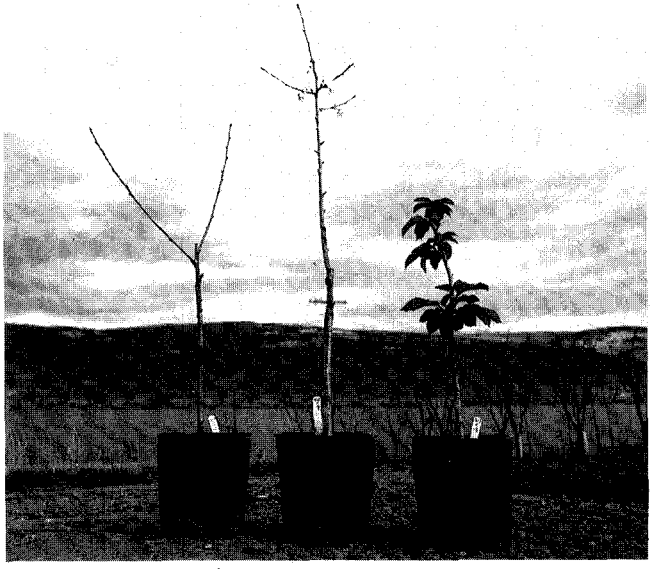

Fig. 1. Effect of tobacco leaf suspension on cherry trees. Right: Control: Leaves inoculated with tobacco leaf suspension from healthy leaves. Middle: T-cut area freshly soaked with tobacco leaf suspension from necrotic lesions and tied with a budding band. Left: Tobacco leaf suspension from necrotic lesions injected into mature leaves.

The inoculated trees were kept in the greenhouse without heat over the winter and by the beginning of March 1991, the leaves on the control tree had grown to 6 cm long (Fig. 1). The tree with the "T-cut" soaked with infected suspension (Treatment 2) had flowers, but leaf buds remained closed. The trees with leaves injected with infected suspension during the previous growing season were still dormant.

In the middle of June 1991, the control trees had grown normally while trees both from Treatments 1 and 2 lacked new annual growth and had small leaves on 2-year-old wood. Light brown gumming occurred around the dead buds below new leaves.

In the third experiment, sterilized bark sections were inoculated on potato dextrose agar (PDA) and bacteria were isolated aseptically. Sub-cultures of the bacteria growing from the bark were isolated and streaked on PDA. Three loops of bacteria, from a culture grown at 22°C for two days, were mixed with 5 ml of 0.05M phosphate buffer and injected into four tobacco leaves with 0.5 ml/leaf. Brown patches, similar to those indicated in Experiment 1, started to show at the injection site four days after inoculation.

A bacterial isolate was sent to Dr. G.S. Saddler of the International Mycological Institute, Ferry Lane, Kew, Surrey, TW9 3AF, U.K. for identification, but it could not be positively identified as any known species within the genus *Pseudomonas.* Dr. Saddler pointed out that it was non-fluorescent and showed some similarity to *Pseudomonas* cepacia and *P. gladioli.* Further studies are needed to characterize this causal agent, explain its mode of spreading in the orchard and to develop an effective control for this disease.

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