Ingress and Spread of *Pseudomonas* in Stems of Peach and Apricot Promoted by Frost-Related Water-Soaking of Tissues

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ABSTRACT

Vigouroux, A. 1989. Ingress and spread of *Pseudomonas* in stems of peach and apricot promoted by frost-related water-soaking of tissues. Plant Disease 73:854-855.

A freezing-thawing cycle created a water-soaked condition in the bark of shoots of peach and apricot that induced a quick (1 min) ingress and a noticeable spread (15-35 mm) of bacteria and dyes in tissues. This phenomenon may explain the decisive effect of low temperature on several diebacks and cankers of fruit trees caused by pseudomonads.

In severe peach dieback in France caused by Pseudomonas syringae pv. persicae (Prunier, Luisetti, and Gardan) Young, Dye, and Wilkie, low temperature has a decisive effect on infection development (9,10). Other diebacks caused by pseudomonads appear to behave similarly (4,7,11). Explanations of sugar content decrease (5) or bacterial ice nucleation (12) have been proposed but are not convincing. Freezing and thawing of the superficial tissues of sour cherry leaves have recently been reported to induce air displacement and the sucking of bacteria through stomata (8). In this paper, I propose that freezing and thawing cause the water-soaking of tissues that promotes the ingress and spread of Pseudomonas in fruit tree stems. This may initiate and favor extensive dieback.

During winter observations on peach and also on apricot (affected by P. s. pv. syringae), we noted that disease progress was especially pronounced after each period of slight frost (-3 C to -5 C),

Accepted for publication 31 May 1989.

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when stems appeared greasy and frozen. We also observed an association of the frozen appearance of peach tissues with disease development after artificial inoculations of excised dormant 1-yr-old shoots (9).

Many plants adapt to low temperatures (-1 C to -10 C) by transferring cellular water to intercellular spaces, where it freezes (2,6). In peach, the accumulation of ice between cells produces a frozen appearance (1). When the temperature rises and the ice thaws, continuous films of water form. The tissues remain water-soaked until the cells absorb the intercellular water. The phenomenon apparently recurs with each freezing period.

A water-soaked condition related to atmospheric high humidity or heavy rain has long been known to allow the ingress and spread of bacteria in tissues of many plants (3). We therefore sought to determine whether water-soaking related to freezing and thawing led to similar phenomena.

Six-month-old potted peach (*Prunus persica* (L.) Batsch 'GF 305') and apricot (*P. armeniaca* L. 'GF 1236') seedlings grown in an unheated (+10 C to +30 C) greenhouse were placed outdoors (+5 C

to +12 C) for 1 wk. Five seedlings of each species were then moved to a refrigerated chamber for 24 hr at -4 C; the others were left outdoors. Two minutes after the refrigerated seedlings had been transferred to the laboratory (+20 C), the bark tissues began to thaw and the stems appeared water-soaked. They were paired with nonrefrigerated stems, and four or five internodes of each were superficially punctured (1 mm) with a needle. A 5-µl drop of erythrosin solution, black ink, or a suspension of P. s. pv. persicae (109 cells/ml) was then micropipetted on each puncture. Thin cortex samples of stems inoculated with P. s. pv. persicae were removed 1 min after drop deposition at 5-mm intervals from the puncture to evaluate spread of the pathogen through the tissues. In January, similar tests were performed with acclimated dormant 1-yr-old shoots of 6-yr-old peach trees (cv. Springtime) grown in an open field. Only ink was used, however, and the shoots had to be peeled to detect absorption and diffusion.

Only a very limited penetration of dyes and bacteria was observed with the control stems, whereas with different types of thawing stems, not only an ingress but a spread in the tissues was observed in a few seconds (Table 1).

Thus, water-soaking associated with thawing and that associated with other climatic conditions led to similar ingress and spread of bacteria or dyes in the tissues. No difference was noted in the behavior of the pathovar on the two tree species, confirming the mainly physical nature of the phenomenon. The thawing-

Table 1. Effect of frost-related water-soaking on diffusion of black ink and bacteria in bark of peach and apricot stems

	Unfrozen stems			Water-soaked stems after frost		
Diffusion indicator*	Mean extent of diffusion ^b (mm)	Range of values (mm)	No. of measure- ments	Mean extent of diffusion ^b (mm)	Range of values (mm)	No. of measure- ments
Black ink	1.2	1-2	15	21.3	7-25	19
Bacterial suspension	1.5	1-5	8			ií
Black ink	1	1	20			20
Bacterial suspension	1	1	9			10
Black ink	3	2–4	38	11.5	4-22	36
	Black ink Bacterial suspension Black ink Bacterial suspension	Diffusion indicator* Black ink Bacterial suspension Black ink Bacterial suspension Black ink Bacterial suspension Black ink Bacterial suspension Bacterial suspension Bacterial suspension	Diffusion indicatoraMean extent of diffusionb (mm)Range of values (mm)Black ink1.21-2Bacterial suspension1.51-5Black ink11Bacterial suspension11	Diffusion indicatoraMean extent of diffusionb of values (mm)Range of values (mm)No. of measurementsBlack ink 1.2 $1-2$ 15 Bacterial suspension 1.5 $1-5$ 8 Black ink 1 1 20 Bacterial suspension 1 1 9	Diffusion indicator* Mean extent of diffusionb (mm) Range of values (mm) No. of measurements Mean extent of diffusionb (mm) Black ink 1.2 1-2 15 21.3 Bacterial suspension 1.5 1-5 8 24.4 Black ink 1 1 20 17.6 Bacterial suspension 1 1 9 22.5	

^aIn 6-mo-old peach and apricot stems, diffusion of black ink was determined by direct observation through the epidermis and diffusion of bacterial suspension was determined by germ isolation every 5 mm from the entry point. In 1-yr-old peach shoots, diffusion was determined by observation after peeling the superficial opaque tissues.

mediated ingress of inoculum promotes a massive inoculation in which potentially larger pathogen populations become deposited in ideal infection courts. This may explain infections initiated in peach and apricot orchards by winter pruning wounds; we found such infections to be especially severe when pruning was done just before a frost period. In addition, the trend of canker spread in orchards and late canker appearance around contaminated leaf scars, both related with low temperature events (10), suggest that the thawingrelated water congestion may be responsible for the resurgence of latent internal populations or for the promotion of expansion of existing cankers. We are currently studying this aspect.

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Longest of the two directions of diffusion, observed 1 or 2 min after deposition of the indicator.