The Role of Twig Infections on the Incidence of Perennial Canker of Peach

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ABSTRACT

An investigation of canker infections of 1-yr-old peach twigs revealed that *Leucostoma cincta* is the primary parasite causing that disease in the Niagara peach-growing region of Ontario, Canada. This species was isolated from 98.0% of the nodal twig infections sampled, while only 2.0% yielded cultures of *L. persoonii*. Autumn inoculations of simulated leaf scars with a conidial suspension of *L. cincta* resulted in nodal twig lesions, but inoculation of buds did not result in infection. Excision of leaves, buds, or both from twig nodes was carried out prior to the dormant season and the effect on natural infection was monitored. Twigs with nodes completely removed had seven times fewer infections than control twigs. The effect of leaf or bud removal on infection suggest that either site can act as an infection court during the dormant season. No differences were detected in the relative susceptibility of nodes on different parts of a twig to canker infection. Because the lowermost leaves on each twig tend to abcise first, the time available for leaf-scar healing prior to the onset of the dormant season differs on parts of the same twig. On 1-yr-old twigs the *Leucostoma* pathogens could be isolated up to 2 cm in advance of the macroscopically visible canker lesion. Microscopic examination revealed hyphae of the fungus in the pith, xylem, and cortical tissues of the infected twigs. Xylem vessel walls show browning in advance of the macroscopically visible lesion. Gum plugs in the vessel lumens are common. The progression of tissue discoloration in the twig suggests that the xylem tissues are invaded by the pathogen prior to those of the bark or cortex. Most perennial cankers have the remnants of a twig at their center. This suggests that such cankers are the result of twig infections that have spread to the subtending branch. The twig phase is thus of major importance in the peach canker disease complex.

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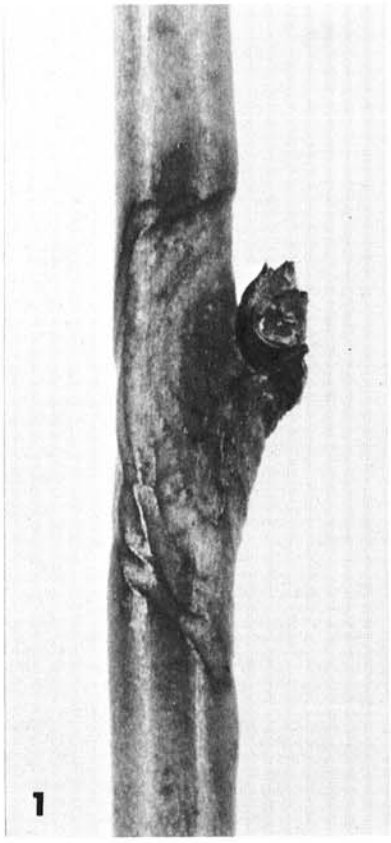
Perennial canker is an important disease of peach trees (*Prunus persica* (L.) Batsch) in the Niagara Peninsula of Ontario, as well as in many other areas of the world. Other stone fruits can also be affected by the disease (2, 4, 5, 7, 8, 11, 12). Two fungi, *Leucostoma cincta* (Pers. ex Fr.) Hohn. [= *Valsa cincta* (Pers. ex Fr.) Fr.] and *L. persoonii* (Nits.) Hohn. [= *Valsa leucostoma* (Pers. ex Fr.) Fr.] are the causal pathogens (1, 5, 9, 10, 18). The imperfect states of the two pathogens are most commonly encountered in the field. These are *Leucocytospora cincta* (Sacc.) Hohn. (= *Cytospora cincta* Sacc.) and *Leucocytospora leucostoma* (Pers.) Hohn. (= *Cytospora leucostoma* Sacc.). Peach canker has been studied by various investigators (9, 14, 17, 18, 19) since it was first discovered in the Niagara Peninsula in 1908 by Caesar (1). Various control measures have been proposed (9, 17) and attempted, but peach canker is more prevalent today in the Niagara Fruit Belt than ever before. At present the disease is of considerable economic importance and is often the limiting factor for this crop.

The disease is characterized by extensive perennial cankers on large limbs. These limbs eventually become girdled and die. Successful invasion by the pathogen requires the presence of wounds or dead tissue on the host (2, 9, 17). Mechanical and winter injuries are thought to play a major role in determining the severity of the disease. In the Niagara peach growing region, most new infections begin on 1-yr-old twigs. Because the results of pathogenesis on these twigs are not immediately apparent, the twig phase of the disease has not been studied in detail. We believe, however, that the twig infection phase may be considerably more important in the disease syndrome than has been suspected. The purpose of this study, therefore, was to investigate aspects of the twig-infection phase of the disease, and to assess what role it plays in the overall canker disease.

MATERIALS AND METHODS.—Most of the experiments were conducted in an orchard of the Horticultural Research Institute of Ontario, Vineland Station. This orchard was planted in 1958, and contained blocks of two peach cultivars, 'Veteran' and 'Elberta', separated by rows of mixed cultivar trees. This orchard was already heavily infected with canker by 1967, and remained so into 1970 when these experiments were conducted. Throughout the experiments, normal horticultural management was maintained in the orchard.

The twigs used for the experiments were those commonly referred to as "suckers", which grow out of much larger and older branches in the central, lower portion of the tree.

Isolation of the pathogens from infected twigs.—Isolations were made from diseased host material to obtain cultures of the pathogenic organisms and to assess the relative frequency of the two species. One-yr-old twigs from Elberta and Veteran trees were brought to the laboratory and were surface-sterilized with 95% ethyl alcohol. The epidermis at the transition zone (TZ) between healthy and diseased tissue was peeled off with a sterile scalpel, and a small wedge-shaped section of tissue,



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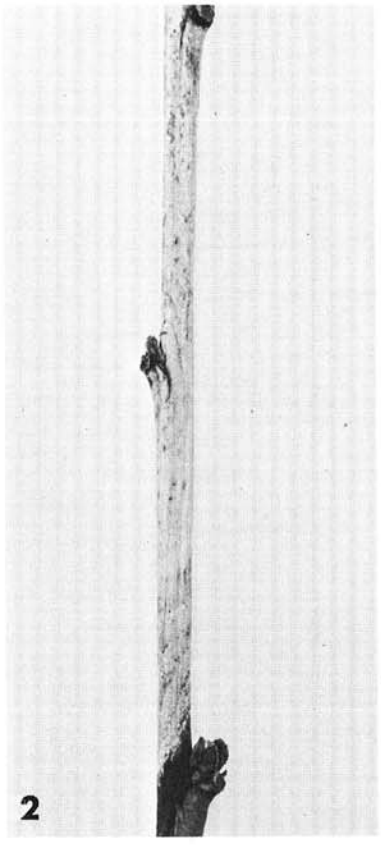

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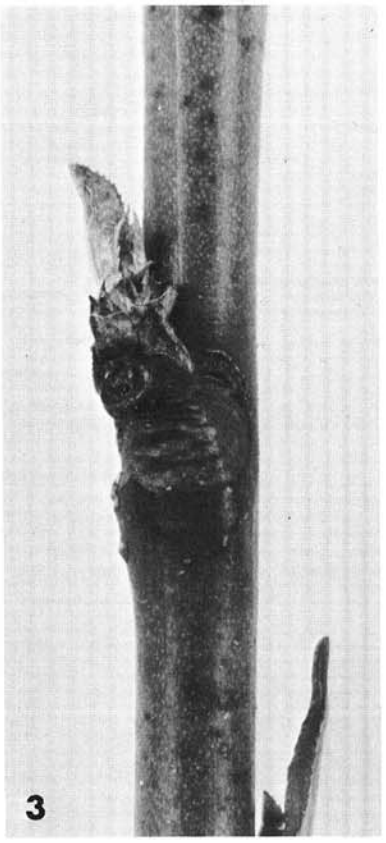

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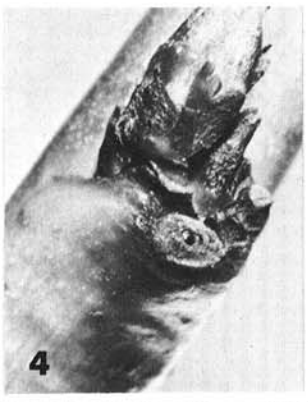

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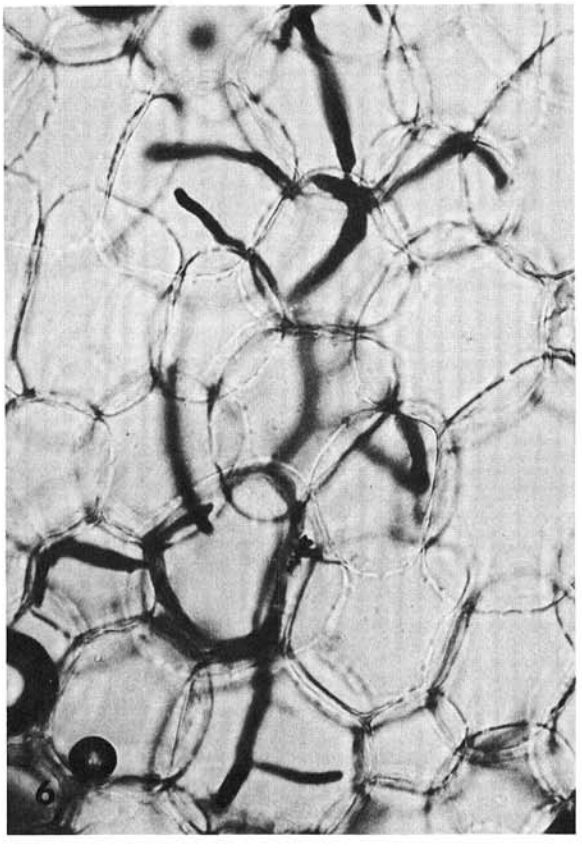

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reaching to the pith, was removed aseptically. The sections were incubated on potato-dextrose agar (PDA) or modified oatmeal agar (2) at room temp. On these media the two *Leucostoma* species could readily be separated and identified (9, 17).

Inoculation of the pathogen into buds and simulated leaf scars.—Buds and simulated leaf scars were inoculated with one of the pathogens to test whether either site can serve as an infection court in the autumn. Leaf scars of the peach cultivar Veteran, and buds of Veteran and Elberta, were inoculated with a spore suspension of isolate P-9 of *Leucostoma cincta* grown on oatmeal agar. A concn of 4.5×10^6 spores/ml was used and a drop of approximately 0.02 ml was applied at each site.

The leaf scar inoculations were made on 40 current year's twigs located on 20 trees. Twigs which had all of their leaves intact were selected. Every fourth leaf was cut off with a scalpel at a position where natural abscission was seen to occur, and was inoculated immediately by applying a drop of the spore suspension on the exposed surface. Similarly, in a staggered position, on the same twig, every fourth leaf scar was inoculated with a drop of sterile distilled water. The remaining leaf scars on each twig were not treated, and were used as controls to assess natural field infections.

Buds on 40 Veteran and 14 Elberta twigs were inoculated in a similar manner by placing a drop of spore suspension on their surfaces. A single bud was inoculated at the specified nodes. Nodes with and without attached leaves were used. Inoculated nodes were surveyed bi-weekly in the spring for canker development.

Excision of the leaves and buds.—An experiment was conducted to determine whether the leaf scars, the buds, or both, are the entry sites for the pathogen in twigs in field infections. At the beginning of September, six weeks prior to the onset of natural leaf fall, all the leaves, the buds, or the complete nodes of the current year's twigs were removed with a scalpel. The compressed nodes at the tip were not treated. One or two twigs were used per treatment on each of 30 trees of cultivars Veteran and Elberta. In a subsequent experiment, 64 trees of each cultivar and 56 mixed cultivar trees were utilized. Treated twigs were surveyed bi-weekly in the spring for canker development.

Histological technique.—The location of the pathogen in field-infected twigs was determined using free-hand sections. Transverse and longitudinal sections were stained in cotton blue and mounted in lactophenol. Tissues from within the cankered area; from the transition zone (TZ); and from apparently healthy tissue beyond the TZ were examined. Only twigs with tip dieback were sampled, because in these the lesions are advancing in a basal direction.

RESULTS.—*Relative frequency and species of the pathogens isolated from diseased twigs.*—In the spring, a nodal twig infection appears as a slightly sunken, discoloured lesion often showing concentric zones of spread (Fig. 1). The bud(s) are usually dry and do not sprout. Small amounts of gum may be present at the infection sites. Girdling results in the dealth of the distal portion of the twig. The advancing edge of the spreading lesion may infect the subtending branch. In twigs with tip dieback, the original point of infection is normally visible and is centered about a node (Fig. 2).

Two culturally separable fungi were isolated from the 650 diseased twigs sampled. They corresponded to Defago's (2) and Willison's (18) descriptions of *Leucostoma cincta* and L. *persoonii*. *L. cincta* was isolated from 637 (98.0%) of the twigs sampled, and *L. persoonii* from 13 (2.0%). The two species were not isolated from the same lesion.

On PDA, all isolates of either species were similar in appearance. On oatmeal agar, isolates of *L. persoonii* were all identical, but *L. cincta* colonies exhibited variation. Colony colour, amount of aerial mycelium, and size and number of pycnidia, differed among isolates. Variations between isolates have been reported for both species (2, 3, 21).

Inoculation of buds and simulated leaf scars with one of the canker pathogens.—The results of the inoculations are shown in Table 1. The number of twig lesions resulting from leaf scar inoculations was significantly different from the water-inoculated controls, $P = 0.05$.

Several of the leaf scar inoculations were made at the bases of side branches on the current year's twigs. Since these sites have no buds(s), the possibility of inoculum redistribution from leaf scar to bud (and vice versa) is minimized. All inoculations at such sites resulted in twig lesions. Other leaf-scar-inoculated nodes, when surveyed in May at the time of flowering, indicated that some buds were healthy and had sprouted, even though a lesion was evident around the node (Fig. 3).

When a drop of spore suspension or water was applied to a freshly exposed leaf scar, it adhered readily to the wound. The inoculum drops appeared to be sucked into the leaf scars and were no longer visible 10 min following application. In contrast, inoculation drops were difficult to apply to buds because they did not stick readily to the bud scale surfaces.

Effect of excision of nodal elements on canker development from natural infections.—An assumption based on previously reported data (2, 19) was incorporated into the experimental design. It was assumed that if infection occurs at the leaf scars, it would take place in the autumn, during or following leaf fall when these sites are thought to be susceptible; whereas if it occurs at the buds, infection would take place in late winter or spring if some of the buds had been injured by low temp.

Earlier observations showed that wounds made on the treated twig nodes were healed over when natural leaf fall

Fig. 1-6. 1-3) Diseased 1-yr-old peach twigs collected in early May. **1)** Young nodal lesion showing a dead bud, a simulated leaf scar, and typical zonation. **2)** Older lesion that has resulted in tip dieback. The zonation indicates that the infection originated at a node. **3)** Young nodal lesion showing a healthy, sprouting leaf bud. **4)** Peach leaf scar in March showing a crack in the surface over the central vascular bundle. **5,6)** Transverse sections of a diseased 1-yr-old peach twig stained with cotton blue in lactophenol. 5) Fungal mycelium present in the xylem vessels and 6) in the pith.

began in mid-October. The twigs entered the dormant season with either one, or both, of the nodal elements (leaves and buds) unavailable as infection sites.

The data summarized in Table 2 indicate that the node is the major site of infection on the twig. Complete removal of the nodal elements significantly reduced the number of lesions. Premature leaf removal had no effect on the number of lesions, thus the buds are implicated as one of the primary infection sites. Since bud excision resulted in significantly more lesions than when all nodal elements were removed, infection through leaf scars apparently also occurred.

A large proportion of leaf scars developed deep cracks on their surfaces during the dormant season (Fig. 4). This suggests that some leaf scars may be susceptible to infection during the winter and early spring when the protective surface layer that forms following leaf fall collapses or cracks. Early healing of leaf scars may not eliminate these sites as potential infection courts, and thus some of the infections attributed to buds may have originated at leaf scars that had cracked during the dormant season.

The cultivar Elberta was more susceptible than Veteran to twig infections.

Effect of position of node on the twig, and its susceptibility to infection in the field.—Weaver (13) stated that the earlier defoliation observed in Elberta peach trees compared to other cultivars could explain in part the resistance of this variety to canker in southwestern Ontario. This cultivar also defoliates early in the Niagara Peninsula, but in this location, it is very susceptible to canker.

The last leaves to fall from a peach twig in the autumn are those at the tip, and in general, the lower leaves fall off first. If early defoliation is responsible for a reduction in the number of infections, then the upper nodes of a twig should be most susceptible to canker. A comparison was made of the number of lesions which occurred on different parts of the twig using the data recorded for control twigs in the excision experiments.

Statistical analysis of the data presented in Table 3 revealed no differences between the mean number of infections in five-node twig segments. This indicates that all the nodes on a twig are equally susceptible to canker infection. The apparent decreased amount of infection in the last twig segment reflects the fact that many of the twigs did not have more than 20 nodes.

This experiment shows that early defoliation by itself does not confer resistance to canker infection; therefore the conclusion reached by Weaver (13) was not substantiated. The disease might, however, operate differently in southwestern Ontario than it does in the Niagara Peninsula.

Pathological anatomy.—Hildebrand (5) stated that in peach tissue the pathogen is present only within the boundaries of the visible cankered zone. Willison (18) suggested, however, that the pathogen may extend beyond these limits. The location of the pathogen in field-infected 1-yr-old peach twigs with tip dieback was determined in the present study by isolation and microscopic examination.

Isolations were made at three locations on each twig, namely: (i), at the transition zone between healthy and diseased tissue; (ii), 1 cm beyond the TZ in apparently healthy tissue; and (iii), 2 cm beyond the TZ. Tissue pieces were plated on potato-dextrose agar and incubated at room temp.

From 150 isolations at each site, *Leucostoma cincta* or *L. persoonii* were obtained 122, 80, and 40 times at the TZ, 1 cm beyond the TZ, and 2 cm beyond the TZ, respectively.

Within the cankered area, microscopic observations revealed that fungal mycelium is present in the cortical tissues, the phloem, the xylem vessels, and the pith (Figs. 5, 6). Hyphae were not seen either in the outer bark tissues or in the medullary rays. The mycelium was intracellular in the xylem vessels and in the pith. The pathogen appeared to spread through the pits in the walls of these tissues. In the phloem and cortex, fungal growth was intercellular. In this region, mats of mycelium separating adjacent rows of cells could often be seen, especially in sections near the original center of infection.

At the transition zone, fungal mycelium was rare in the cortical tissue, phloem, and pith; but could always be found in the xylem vessels. Up to 1 cm beyond the margin of the canker, where bark tissues appear healthy, fungal mycelium could only be seen in the xylem vessels. A brown discoloration was noted in the walls of xylem vessels in sections which were up to 2 cm beyond the margin of the canker. This discoloration is confined primarily to the vessels near the pith, but extends throughout the wood as the canker margin is approached. The lumens of many xylem vessels become plugged with a gum which darkens with age. Within the cankered region, occlusion of xylem vessels with gum was also common.

The ability of fungal hyphae to pass through wound gum plugs has been reported (5, 16), but was not seen in this study.

DISCUSSION.—*Leucostoma cincta* is the primary canker pathogen of 1-yr-old peach twigs in the Niagara Peninsula of Ontario. *L. persoonii* though isolated rarely from such twigs, is commonly present in perennial cankers on older branches and limbs (9, 14, 17). This species is reported to be a weak parasite by Hildebrand (5) and Willison (19), but other workers (14, 21) have found it to be an aggressive invader.

TABLE 1. Number of infected nodes on 'Veteran' and 'Elberta' peach twigs inoculated at simulated leaf scars or the buds with a spore suspension of *Leucostoma cincta*[a]

Treatment	% Infection	
	Leaf scars	Buds
Inoculated with spore suspension	16	3
Control -1 (H_2O)	6	3
Control -2 (natural field infection)	3	2

[a]Leaf scars on the current year's twigs were inoculated 29 October 1969; buds were inoculated 5 November 1969; isolate P-9 was used at 4.5×10^6 spores/ml.

TABLE 2. Number of field infections at nodes of peach twigs following various treatments of the cultivars 'Veteran' and 'Elberta'

Treatment	Infections/twig					
	1968/69		1969/70			
	Veteran	Elberta	Veteran	Elberta	Mixed	Combined mean and standard error
leaves removed	0.44	0.82	0.64	1.25	1.22	0.87 ± 0.11 ac[z]
buds excised	0.18	0.57	0.55	0.91	0.65	0.57 ± 0.11 a
node excised	0	0.22	0.11	0.23	0.23	0.16 ± 0.11 b
control	0.70	1.21	0.82	1.24	0.88	0.97 ± 0.11 c

[z]Means followed by the same letter are not significantly different $P = 0.05$, by analysis of variance.

TABLE 3. Number of field infections on peach twigs as affected by the node position

Twig Segment	Position	Infections per five-node twig segment			Combined mean and standard error
		Veteran[x]	Elberta[x]	Mixed[y]	
1	Tip- 5	29	53	13	19.0 ± 4.7 a[z]
2	6-10	30	39	15	16.8 ± 4.7 a
3	11-15	17	42	14	14.6 ± 4.7 a
4	16-20	19	31	21	14.2 ± 4.7 a
5	20+	20	14	18	10.4 ± 4.7 a

[x]Total of 1968/69 and 1969/70 data.
[y]1969/70 data.
[z]Means followed by the same letter are not significantly different, $P = 0.05$, by analysis of variance.

Most twig infections in nature occur at the nodes. The application of *L. cincta* spores to simulated leaf scars resulted in twig lesions the following spring. The number of successful infections produced was relatively low (16%). The premature excision of leaf petioles likely resulted in leaf scars that are not identical to those produced during natural leaf fall. In addition, Veteran, the cultivar used, is relatively resistant to canker infections of this type.

Buds inoculated in the autumn did not result in the production of twig lesions. But in these experiments, the buds appeared to be healthy when they were inoculated and only damaged buds have been reported to act as infection sites (2, 5, 15). Few of the buds were damaged during the dormant season, and when surveyed the following spring, most produced leaves and/or flowers. Some of the inoculated buds were at nodes at which a leaf was still attached. The possibility existed of inoculum redistribution from the bud to a naturally formed leaf scar. This apparently did not occur, or if it did, the inoculum was not viable at the time.

The removal of the complete node, though producing a substantial wound on the twig, resulted in a 7-fold reduction in number of infections in comparison to the control twigs. This shows the importance of the nodal elements as the primary infection sites on young twigs.

The results indicate that both buds and leaf scars act as natural entry sites for the canker pathogens. The observation that many of the leaf scars crack during the dormant season is especially significant because these may be important infection sites, and may explain some of the contradictory results obtained in this study and by others (13).

Most new field infections were centered around nodes where dead buds were observed in the spring. In some cases, only one or two buds were dead, and the others produced leaves or flowers. On rare occasions, all of the buds at a node sprouted, even though a distinct lesion was present on the twig around the node. In such cases, infection must have occurred through the leaf scar. This was also observed by Willison (19).

In twigs exhibiting tip dieback, the pathogen was isolated from tissues in advance of the macroscopically visible lesion. It is likely, too, that the pathogen extends beyond the visible limits of perennial cankers in larger branches and limbs of peach.

The histological observations imply that once an infection is established in a twig, subsequent advance occurs first in the inner tissues, in the xylem, and then spreads to the bark (cortex and dermal tissues). If the bark tissues were invaded first, the pathogen would be present in the xylem behind the margin of the visible lesion.

If pathogenesis proceeds in a similar manner in larger limbs, *L. cincta* and *L. persoonii* may be classified as sapwood parasites. This would be similar to Hubbes' (6) observation on *Hypoxylon* canker of aspen, which had previously been described as a bark disease.

Leaf scars provide direct access to xylem tissues which are not normally exposed at the surface of the host. During leaf fall, the xylem elements in the vascular cylinder of the petiole are ruptured mechanically (20) and provide the pathogen with access to the woody tissues of the twig.

Lesions on young twigs can spread in a basal direction and infect the subtending branch. This can occur rapidly

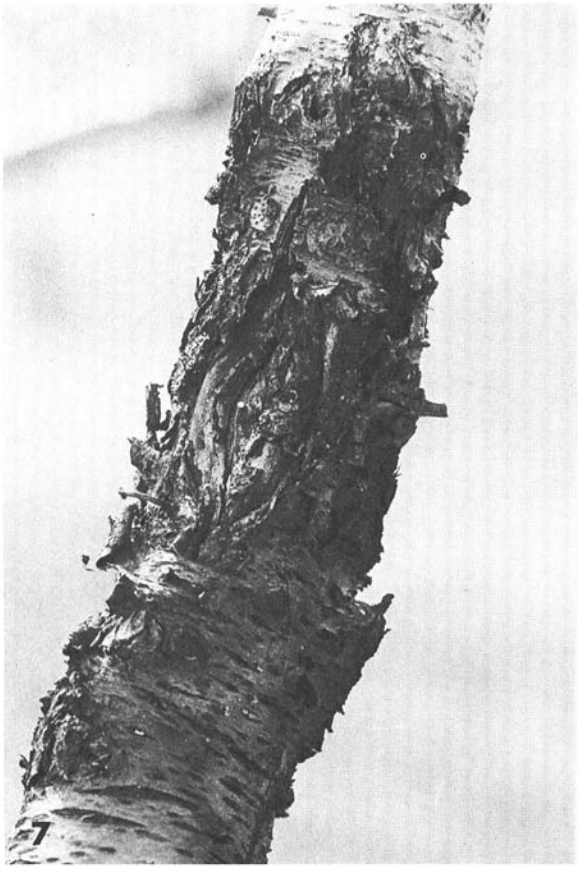

Fig. 7. Perennial canker on a peach limb showing the remnants of a small twig at the center of the lesion.

if the initial infection is at one of the lower twig nodes and could result in a perennial canker on a large limb in the center of the tree. This could subsequently result in the death of a large portion of the tree. The frequently observed presence of remnants of a dead twig at the center of perennial cankers (Fig. 7) supports this interpretation of the disease process.

The present study shows that infection of 1-yr-old twigs plays a major role in the canker disease complex of peach in the Niagara Peninsula of Ontario. If nodal twig infections can be prevented, successful control of this disease might be possible.

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