Graft Transmission and Host Range of the Pear Decline Causal Agent

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ABSTRACT

The pear decline causal agent was not transmitted by scions taken from *Pyrus* sp. 'Variolosa' held at 4 C for dormancy chilling. The agent was transmitted with scions from nondormant Variolosa and Comice trees having leaf symptoms consisting of brown veins or curl. Own-rooted Angers Quince A trees were severely stunted beginning with the season of inoculation, and leaf veins became brown. Growth of *Pyrus serotina* seedlings was drastically retarded the season following inoculation, and leaf reddening and chlorosis occurred. Phytopathology 60: 204-207.

Pear decline (Moria disease) is caused by a graft transmissible and psylla-vectored causal agent, the nature of which is obscure (2, 8, 10, 15, 17, 18, 19, 22). Varieties of French pear (*Pyrus communis* L.) currently are often grafted with "domestic French" seedling rootstocks such as Bartlett or Winter Nelis; however, before the onset of the pear decline epiphytotic, rootstocks were often other than domestic French seedlings. Associated with pear decline is graft-union phloem failure that affects trees with certain rootstocks previously used such as *P. serotina* Rehd., *P. ussuriensis*, and *Cydonia oblongata*, and some seedling individuals of "imported French."

The initial and basic injury involved in graft-union pathology is necrosis of rootstock sieve tubes where they are joined to the scion sieve tubes (16). This necrosis blocks translocation, and causes the fibrous roots of affected trees to die for want of carbohydrates (1); then, if the weather turns hot and dry, the leaves may wilt and die or abscise, a process called "quick decline". More often, there is a slow decline typified by a suppression of shoot elongation and small, pale green leaves. On slow decline trees, "leaf roll" may occur in which opposing halves of the laminae roll upward. In the fall, leaves may become orange-red rather than the normal yellow (3, 11), a condition regarded as indicative of, but not diagnostic for, pear decline.

During the pear decline epiphytotic which began in 1959 in California, own-rooted trees of a cultivar of unknown species called Variolosa and seedling individuals of *Pyrus serotina* died or were stunted (6). At Riverside, the pear decline causal agent was transmitted to Variolosa by grafting and by the pear psylla. In the greenhouse, a browning of major lateral leaf veins occurred (21), while in the lath house, leaves curled, became thick, brittle, and sometimes prematurely red, but often did not exhibit brown veins.

A disease called "pear leaf curl" was observed in California surveys soon after the onset of the pear decline epiphytotic. It was originally reported as a disease of scion-rootstock combinations not susceptible to pear decline (11, 13). Leaf symptoms were downward curling of tips and margins, deep reddish-purple discoloration very late in the fall, undulations, and abscission. Using grafting procedures or psylla vectors, transmissions from either pear decline trees or pear leaf curl trees produced pear leaf curl in trees with *P. communis* rootstocks and pear decline or pear leaf curl in trees with *P. serotina* rootstocks (9, 12, 14). Data by Kaloostian & Jones (9) indicate that pear decline and pear leaf curl are caused by different agents, because psylla from a single greenhouse culture produced leaf curl in trees with *P. communis* rootstocks, but no disease in trees with *P. ussuriensis* rootstocks. Griggs et al. (7) made tests to determine whether psyila that had not been fed on trees with pear decline might produce toxins that cause pear decline; they found that pear decline was not produced in trees with *P. serotina* rootstocks, although a curling of leaves directly below branches with psylla cages did occur. Apparently the other symptoms of pear leaf curl syndrome were absent. The authors considered this to be a successful transmission of a proposed pear-leaf-curl virus that was different from the pear decline causal agent. It was not stated whether the curling subsequently became systemic; therefore, the possibility of a psylla toxin as opposed to a virus as a cause for this particular localized curling should be considered.

Reports concerning graft transmissibility and perpetuation of the pear decline disease have been conflicting. In an attempt to perpetuate the pear decline disease, Griggs (5) brought *Pyrus serotina* seedlings in containers to the orchard in March 1964 and 1965, and grafted dormant scions onto them immediately upon their removal from pear decline Bartlett trees. The trees were then returned to a psylla-free screenhouse for incubation. None of the resulting trees developed symptoms of pear decline. Shalla (17) performed a similar experiment, except that he used the double bud transmission technique and effected the budding in the fall, apparently before winter chilling had occurred. Three of 10 trees inoculated with Bartlett buds from diseased trees became infected, a percentage of infection comparable to that obtained in summer bud transmission experiments by California and Washington investigators working independently (3, 18).

Pear psylla were much more efficient in the transmission of pear decline than grafting, although they have not given 100% infection (8, 10).

The purpose of this paper is to report new procedures that will assure graft transmissions and perpetuation, to report additional species that can be used for the study of pear decline etiology, and to further define the kinds of symptoms shown by an expanded host range.

MATERIALS AND METHODS.—Several greenhouse experiments were performed in which one control tree was included for each inoculated tree. For inoculations,

either approach grafts or side grafts on the trunk at 10 to 30 cm above the soil level were used immediately after removal from the donor tree. After allowing several weeks for the scions to unite with the host tree, the trees were cut back to within a few buds above the uppermost scion to force new growth. The scions on some trees grew out 15 or 20 cm, after which further growth was nipped back.

In three of the greenhouse experiments, scions were taken from dormant trees that had been in a 4 C cold room for 60 days or longer. In the other experiments, scions were taken from nondormant trees, either in the greenhouse or in the field, that were showing pear decline or leaf curl symptoms.

Healthy propagative materials for the experiments were obtained as follows: cuttings of the cultivar Variolosa, from W. H. Griggs, Department of Pomology, Univ. Calif., Davis; cuttings of Angers Quince A from Robert M. Gilmer, N.Y. State Agri. Exp. Sta., Geneva; seeds for propagating *Pyrus serotina* 'Chojuro', from K. Kishi, Horticultural Research Station, Hiratsuka, Kanagawa, Japan; other seeds for propagating *Pyrus serotina* from the seedsman, F. W. Schumacher, Sandwich, Mass.

RESULTS.—*Effect of scion dormancy on PDV transmission.*—Scions from dormant, chilled, pear-decline Variolosa trees were top-grafted directly onto trunks of 4 own-rooted healthy Variolosa trees for the purpose of perpetuating the disease. Surprisingly, the trees that resulted did not develop brown veins or other symptoms of pear decline. Also, two Variolosa trees and three *P. serotina* seedlings inoculated with side grafts did not develop brown veins and other symptoms of pear decline, even though donor trees on the same greenhouse bench did show symptoms. At the same time, infected Variolosa scions were regularly transmitting the causal agent in many other experiments.

After examining the results of all experiments described in this paper and results reported by others, it was concluded that in successful transmissions the donor trees were in leaf, and in unsuccessful transmissions the trees were in winter dormancy and had had 60 or more days of chilling.

It must not be conveyed that scion wood from dormant trees never transmits pear decline, because twigs taken from dormant, apparently healthy Anjou trees at Davis, California, produced some pear decline-diseased trees when used for propagation and for indexing for vein yellows. Small veinlets of infected Anjou and Comice trees in the greenhouse become brown.

Transmissions from curl diseased Comice trees.—Scions were taken from curl-diseased Comice trees that were in leaf in plots of George Nyland, Univ. Calif., Davis. These transmitted the causal agent of pear decline to Variolosa, and it was perpetuated in scions top-worked on *P. serotina* and on *P. pashia.* The two Variolosa trees that were inoculated by using side grafts showed brown veins and were stunted. The four trees formed by top-working, curl-diseased Comice on serotina died. Three trees formed by top-working diseased Comice on *P. pashia* were extremely stunted.

Quince as a host for pear decline virus.—On 9 November 1967, branches of pear-decline-diseased Variolosa trees were approach-grafted to own-rooted Angers Quince A trees. After the inarches had made a union, the trees were cut back to about 10 inches above the point of attachment, and kept in the greenhouse through the winter of 1967-68 with no interval of chilling (quince does not require chilling). The new growth from two of the cut-back quince trees, with infected approach grafts, was severely retarded, and attained a height of only 1 m as compared with 4 m for check trees during 1968 (Fig. 1-A). A third infected tree was not so severely stunted; it reached 3 m as compared with 4 m for its check tree; however, the trunk diam were 11 mm and 27 mm, respectively. The average trunk diam for the three diseased trees on 9 January 1969 was 9 mm; for the three controls, 26 mm. The leaves on the infected trees were small, and showed symptoms of mineral deficiencies. Major lateral veins on some leaves became swollen and brown, and sections revealed sieve-tube necrosis and excessive secondary phloem formation similar to that in Variolosa (20).

In a second experiment, own-rooted quince trees were inoculated with diseased and healthy Variolosa side grafts on 25 April 1968. By winter they were obviously stunted, and were placed in the cold room from 23 January to 8 March 1969. The following summer on 14 August 1969 the average diam of the four inoculated trees was 12.4 mm as compared to 19.0 mm for the four control trees. Between 22 July and 14 August 1969 the diam of the inoculated trees increased 0.3 mm as compared to 3.8 mm for the controls. Some of the leaf veins were beginning to turn a beige color on 14 August 1969.

In a third experiment on 7 June 1968, own-rooted quince trees were inoculated with combinations of side grafts as follows: two pear-decline Variolosa and two vein yellows Anjou side grafts; two pear-decline Variolosa and two healthy Variolosa grafts; two vein-yellows Anjou and two healthy Variolosa grafts; and four healthy Variolosa grafts (controls). The experiment was replicated twice for a total of eight trees. Control trees and the trees receiving vein yellows scions but not pear decline scions were not noticeably affected. The four trees receiving pear decline scions were uniformly stunted by the winter of 1968-69; and by 14 August 1969, their trunk diam were an average of 18.0 mm as compared to 28.8 mm for the four trees not receiving pear-decline scions. The leaves on all of the pear-decline Variolosa scions and the quince on which they were growing showed symptoms by 2 December 1968. The trees were not chilled during the winter of 1968-69.

Pyrus serotina as a host for pear decline virus.—*Pyrus serotina* seedlings were successfully inoculated in several experiments; the results of two are reported here.

In one experiment, dormant seedlings of the Schumacher source and dormant Variolosa trees were taken from the cold room on 28 February 1968; two branches from either diseased or healthy Variolosa were approach-grafted to the trunks of each of 10 serotina seedlings. By 30 July, leaf veins of the diseased Variolosa grafts

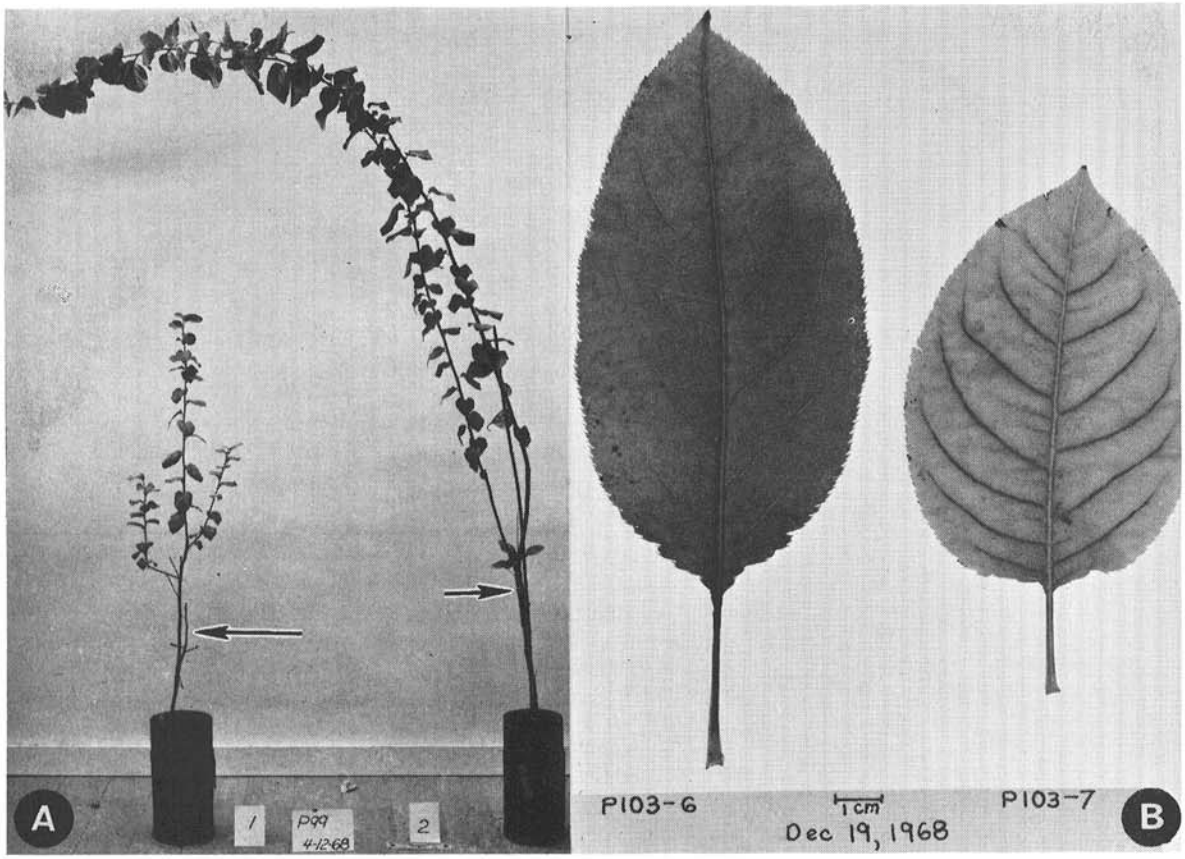

Fig. 1. A) Effect of the pear decline causal agent on Angers Quince tree. Tree 1 (left) received one infected Variolosa scion (arrow) by approach grafting on 9 July 1967. The scion was dead on 2 December 1968. Tree 2 (right) received a scion (arrow) from healthy Variolosa tree. (× 0.05). **B)** Enlarged brown veins on pear-decline-affected leaf of seedling of *Pyrus serotina* 'Chojuro' (right). Leaf on left from healthy Chojuro seedling tree.

had become brown, indicating presence of the causal agent. By this time, the foliage on a number of serotina plants, including checks, was chlorotic, and considerable defoliation had occurred. Because of these untimely signs of dormancy characteristic of seedlings from this seed source, the plants were pruned six inches above the uppermost of the two inoculating scions and placed in the cold room. On 16 October 1968, they were returned to the greenhouse, where day length was supplemented until midnight with incandescent lights. Leaves on the first growth of both check and inoculated plants were variously buckled, deformed, and mottled. These deformities were apparently due to some nutrient and hormonal unbalance attributable to the artificial deviation from the normal annual seasonal rhythm of growth and rest periods. This phenomenon had been observed earlier during manipulations of our stock of healthy *P. serotina* seedlings. The continuing shoot growth became normal after 1 December; but on the diseased plants it soon terminated, and chlorosis and reddening of leaves followed. Between 16 October 1968 and 14 March 1969, the trunks on the five diseased trees did not increase in diam, while the five check trees averaged 3 mm. When the plants were discarded on 29 April, the root systems were examined. At most, there were only a few new white fibrous roots on the diseased plants, and the root systems were much smaller than those of the controls. On the control plants, there were many new fibrous roots and larger pioneer roots. Thus, although shoot growth occurred on infected plants, radial (cambial) growth and root growth were suppressed.

In a second experiment, seeds of *P. serotina* 'Chojuro' were planted on 8 January 1968; each plant was inoculated with two leafy diseased or healthy Variolosa side grafts on 24 April 1968. By the middle of May, all the plants were 4-5 feet tall with large luxuriant leaves; they were cut off at 18 inches. By October, brown veins were present on leaves of all inoculating scions; however, new growth on the inoculated plants was comparable to the checks except for one plant that had spectacular brown veins (Fig. 1-B). The plants had not grown for some time, and were placed in the cold room from 18 December 1968 to 6 May 1969. By 23 July, the four inoculated plants had made a flush of chlorotic shoot growth, then stopped growing while the controls were still growing luxuriantly. Leaves on the tree that showed brown veins in 1968 were again showing them on 8 June 1969.

Discussion.—The results of these experiments, as

well as in the literature cited, indicate that the pear-decline causal agent does not move readily from inoculating scions taken from small dormant branches that have been subjected to winter chilling. The infectious agent might be inactivated during chilling, or it might be confined to degenerated, nonfunctional sieve tubes and companion cells, and thus not be translocated. All sieve tubes, except possibly the youngest, degenerate during winter dormancy with no reactivation when growth is resumed; they are replaced with new phloem in the spring (4). I have observed that, after chilling, diseased Variolosa trees make a normal flush of growth. The new shoots are apparently later invaded by the infectious agent, possibly from larger branches or roots of the plant where it may remain viable and mobile.

The experiments reported here and in the literature indicate that there is a relationship between the presence or absence of symptoms on leafy twigs and the percentage of successful transmissions. Variolosa twigs bearing leaves with brown veins and Comice twigs with curled leaves transmitted in all cases. However, buds that were apparently taken from twigs with symptomless leaves of Bartlett in experiments by Shalla (17) and Shalla et al. (19) and by Blodgett et al. (2), successfully transmitted in only about one-third of the cases.

When either quince or *P. serotina* is used as a rootstock for French pear varieties, the resulting trees are susceptible. Likewise, as own-rooted trees, quince and serotina were extremely susceptible when graft-inoculated in the greenhouse. The inoculating grafts were not removed from the plants after infection occurred, and they might possibly have caused the symptoms. However, the scions were intentionally left attached, because it was feared that the plants might be immune due to extreme hypersensitivity, and might recover if not subjected to continuous inoculation. The inoculating scions died on two of the infected quince trees of the November 1967 experiment; but the quince trees did not recover. Griggs et al. (6) observed in their experimental plantings that *P. serotina* became naturally infected. Therefore, in our experiments, infected Variolosa scions probably served only as a vehicle of infection, and were not the cause of the disease.

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