Differentiation of Syndromes Caused in Apple by Graft-Transmissible, Xylem-Affecting Agents

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ABSTRACT

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Transmission tests in woody and herbaceous hosts demonstrated infection of apple clones by at least three graft-transmissible agents that can induce yxlem aberrations in Virginia Crab apple indicator plants. The syndromes that they induce are stem pitting (SP), brown line decline (BLD), and junction necrotic pitting (JNP). They differ in ability to persist when plants of infected apple clones are heat-treated. SP is expressed on the Virginia Crab stem as xylem pits matched by protrusion of symptomless phloem tissue. Consistent differences in severity of pitting among isolates suggest occurrence of strains, or that different agents can cause pitting. BLD is characterized by a groove in the xylem encircling the union between Virginia Crab and its rootstock, matched by a protrusion of necrotic phloem and sometimes accompanied by scattered longitudinal grooves on the Virginia Crab stem. When severe, the trees decline and die. All BLD-affected trees are infected with apple stem grooving virus. JNP is a syndrome that includes a line of pits encircling the junction matched by necrotic phloem pegs, not accompanied by stem grooving, and not leading to tree decline. The causal agent is not sap-transmissible. Flute fruit symptoms are associated with SP and JNP, but not BLD.

Additional key words: Apple stem grooving virus.

It has been customary to designate all graft-transmissible, xylem-affecting syndromes on apple as either stem pitting or stem grooving (2,5), the former caused by an uncharacterized agent, the latter by apple stem grooving virus (ASGV). For both diseases the standard woody indicator is *Malus domestica* Borkh. 'Virginia Crab,' and diagnostic symptoms are apparent when the bark is lifted so that xylem and phloem interfaces can be examined.

Some confusion has persisted in the diagnosis of these diseases. Apple indexing programs in British Columbia, New York, and elsewhere have yielded variants of stem pitting, stem grooving, junction brown line, and decline syndromes on Virginia Crab, some of which cannot be deemed typical of either stem pitting or the disease induced by ASGV. Moreover, the association of ASGV infection with development of necrosis at graft unions (ie, junctions) has been inconsistent.

The pitting induced by most stem pitting isolates on the woody cylinders of Virginia Crab and *M. domestica* 'Hyslop Crab' is moderately severe within 2 yr, and very severe in the 3rd yr. The pits in the xylem are matched by protrusions of healthy (non-necrotic) phloem. Severely-affected trees are less vigorous than healthy ones, but do not decline or die. Fruits are shortened and have depressions that characteristically extend from the calyx to the stem and (flute fruit) (5,13). Variants were observed in three British Columbia orchards; Hyslop Crab trees displayed mild stem pitting (SP) and flute fruit (FF) symptoms which did not become more severe after three growing seasons. In one of these orchards, 40 such mildly-pitted trees were topworked by the owner with Delicious or Golden Delicious apple, using scionwood from uncertified sources. Within 2 yr the Hyslop portions of all grafted trees developed severe SP symptoms and severe FF symptoms. This suggested that two agents can cause xylem pitting.

Two types of junction browning syndrome, brown line decline and junction necrotic pitting, have been observed at the union between Virginia Crab and rootstock. Both are characterized by xylem depressions filled with brown necrotic phloem.

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Brown line decline (BLD) first was reported as Virginia Crab decline (13). Typically, in the season after inoculation, the leaves turn pale green and drop prematurely. In subsequent seasons, terminal growth ceases and the leaves are small and pale. The Virginia Crab portions of many of the trees die in the second or subsequent seasons; the seedling rootstocks remain symptomless and produce vigorous shoots. At the union between the Virginia Crab and rootstock, affected trees exhibit a groove encircling the xylem cylinder, matched by a ridge of necrotic phloem tissue. Many such trees have scattered grooves elsewhere on the Virginia Crab woody stem, matched by pegs of necrotic phloem. All of our infected clones inducing BLD symptoms also induced SP and FF on Virginia Crab, except those that were heat-treated. In early indexing trials, symptom expression was erratic. Bud inoculation of 1-yr-old, maiden trees of Virginia Crab induced no symptoms, or mild brown line only, whereas simultaneous application of inoculum buds and Virginia Crab buds to apple seedlings yielded brown line symptoms and decline in greater numbers of the test trees.

Junction necrotic pitting (JNP) occurred at the stock-scion junction of Virginia Crab trees that were used to index a number of apple clones. The pitting was much more severe than that on the Virginia Crab trunk. The deep xylem pits at the junction were matched by a continuous ring of brown, necrotic phloem pegs, providing a syndrome that often was difficult to distinguish from BLD. This paper describes tests we have conducted, which have helped to distinguish these syndromes, and to elucidate their etiology.

MATERIALS AND METHODS

Stem pitting—differentiation of syndrome types. Hyslop trees with mild pitting were indexed on Virginia Crab by simultaneous double-budding of inoculum and indicator on apple seedlings. In the 2nd and 3rd yr after inoculation, bark flaps were lifted to allow preliminary rating of SP severity. After 7–9 yr the test trees were removed, and all bark was stripped from the trunks for final readings. A scale of 0 to 10 was used in rating disease severity.

Junction browning syndromes. *Effect of inoculation sequence on symptom expression.* In an effort to ensure consistent expression of symptoms at the junction of indicator and rootstock for JNP and BLD, and decline for BLD, trials were initiated in which Virginia Crab buds were applied to apple seedlings a month or more after bud inoculation. They were compared with trees to which Virginia Crab buds were applied a month or more before inoculation.

Indexing of heated clones. Two of the infected clones, Rome Beauty 1 that induced BLD, and the Stayman 1 that induced JNP, were subjected to heat therapy (14) and bud perpetuations from the heated trees were indexed on Virginia Crab.

Greenhouse assay. Indexing by sap inoculation of *Chenopodium quinoa* Willd. and *Cucurbita maxima* L. 'Zucchini' was designed primarily to determine whether apple clones were infected with ASGV. Inocula were prepared from leaves of apple shoot tips, or from cambial tissues taken from the bases of current season shoots (15). Each type of tissue was triturated in buffer that comprised 3% nicotine, 0.01 M K_2HPO_4 and 0.01 M cysteine HCl, pH 9.1 (8). The resulting macerate was rubbed on mature leaves of the *C. quinoa* and Zucchini squash test plants dusted with 38-μm (400-mesh) Carborundum. After inoculation, leaves immediately were rinsed with tap water. Clones that did not yield ASGV were reindexed five or more times, each time on four or more receptor plants of each indicator species. They also were indexed on *Cucumis sativus* L. 'Marketer.' In other tests, the inoculations of *C. quinoa* and Zucchini squash were made after trituration of tissues in potassium phosphate buffer, 0.01 M, pH 7.2 plus 5% PVP, MW 10,000, or in 2.5% PEG, MW 6,000–7,500.

Two of the isolates that were identified as ASGV by symptom expression on the herbaceous species were concentrated and partially purified from squash leaf tissue and characterized by the techniques of Uyemoto and Gilmer (9).

RESULTS

Stem pitting—differentiation of syndrome types. Three Hyslop crab trees from one orchard and two from another were indexed on four Virginia Crab test trees for each tree that was indexed. SP severities were compared with those induced in Virginia Crab trees on which 16 other apple clones were indexed by the same procedure.

In the 2nd yr, the test trees on which the five Hyslop trees were indexed displayed no pitting or barely-detectable pitting (0–1.0). In the 3rd yr there was detectable but mild pitting on all test trees; in the four to six subsequent years that the trees were retained there was no further increase in severity (Table 1). Pits were small and shallow, and tended to be clustered in scattered patches (Fig. 1). Flute fruit symptoms were mild and occurred on only a small proportion of the fruits. In contrast, the test trees on which 16 other apple clones were indexed developed moderate pitting in the 2nd yr, and severe pitting in the 3rd yr (Fig. 2). Flute fruit symptoms were moderate on most fruits in the 2nd yr, and severe on all fruits thereafter. All uninoculated trees remained free from SP and FF symptoms.

TABLE 1. Stem pitting severities[a] on Virginia Crab, resulting from indexing of Hyslop Crab trees in two orchards, compared with stem pitting severity in indexing of other apple clones

	Period after inoculation		
	2 yr	3 yr	7–9 yr
Orchard 1, Tree 1	0.5[b]	2.5	2.5
Orchard 1, Tree 2	0	1.5	1.0
Orchard 2, Tree 1	0.5	2.0	2.0
Orchard 2, Tree 2	0	1.5	2.0
Orchard 2, Tree 3	1.0	1.5	2.0
Average of 16 other clones	4.6	7.6	

[a]Severity rating scale 0–10 (mild = 1–3; moderate = 4–6; severe = 7–10).
[b]Average readings of groups of four test trees.

Junction browning syndromes. *Effect of inoculation sequence on symptom expression.* Occurrence of severe brown line symptoms and subsequent tree decline was invariable if BLD inoculum buds were applied in August, and those of the Virginia Crab indicator in the following spring. Results were almost as reliable if inoculum buds were applied in early spring, and Virginia Crab buds applied a month or more later.

The same inoculation sequences ensured reliable expression of JNP symptoms on Virginia Crab indicator trees.

The test trees to which indicator buds were applied before inoculum buds, failed to exhibit symptoms.

Indexing of heated clones. Forty-five trees derived from buds of heat-treated Rome Beauty 1, when indexed on Virginia Crab, induced BLD symptoms; none induced SP, although the unheated clone was SP-infected. The constant syndrome induced at the junction of Virginia Crab and rootstock by the heated clones was a sunken xylem groove which was filled with brown, necrotic phloem tissue (Fig. 3) and the trees declined rapidly. Some of the Virginia Crab test trees had scattered xylem grooves which were matched by pegs of necrotic phloem. Fruits were normal on all test trees.

Indexing on Virginia Crab of the trees derived from heated buds of clone Stayman 1 yielded a variety of results (Table 2). Several of these induced SP and JNP (Fig. 4); others induced JNP only (Fig. 5); several induced neither SP nor JNP. The expression of JNP alone was a ring of xylem pits matched by pegs of necrotic phloem in Virginia Crab tissue adjoining the stock-scion junction. Fruits displayed mild FF, usually with wisps of fine russet in the depressions.

Greenhouse assay. The clones that were indexed on herbaceous hosts included a selection of heat-treated and untreated apples, all of which had been indexed on Virginia Crab and most of which had been indexed also on Spy 227 apple and Russian apple R12740-7A (Table 2). The 30 clones were selected to include inocula that had induced the following syndromes on Virginia Crab: Group 1–BLD, SP, and FF; Group 2–BLD only; Group 3–JNP, SP, and FF; Group 4–JNP and mild FF. Infection with ASGV was demonstrated in all clones that induced BLD on Virginia Crab (Groups 1 and 2). No ASGV infection was demonstrated in clones of Groups 3 and 4.

Apple chlorotic leaf spot virus (ACLSV) symptoms were induced on C. *quinoa* by a proportion of inocula from all syndrome groups. The two ACLSV isolates from M.9 (b) and the isolate from St (c) (Table 2) are in the category, "super latent" (4); that is, detectable on *C. quinoa,* but not on the R 12740-7A apple indicator. There was no correlation between the incidence of ACLSV infection and the occurrence of symptoms on Virginia Crab.

ASGV and ACLSV were the only viruses transmitted from the apple clones to herbaceous hosts in our tests.

DISCUSSION

Our observations and experimental results demonstrate that at least three graft-transmissible agents induce wood-pitting of grooving symptoms on Virginia Crab.

Severe stem pitting and mild stem pitting. These two syndromes are both characterized by pitting of the woody stem of Virginia Crab and Hyslop Crab, with no associated phloem necrosis. They differ consistently in the interval between inoculation and the appearance of symptoms, and in the ultimate severity of symptoms on the woody stem and on the fruit.

Causal agents of these syndromes have not been isolated or characterized, and there are no means of determining whether they are caused by different agents, or by "strains" of a single agent.

Brown line decline and junction necrotic pitting. BLD and JNP may be distinguished by several criteria. BLD readily yields ASGV by sap transmission from leaf or cambial tissues. It is characterized on Virginia Crab and several other cultivars by development at the junction of a groove that is filled with necrotic phloem tissue (Fig. 2), frequently (but not invariably) by occurrence on the crab stem of scattered grooves with corresponding protrusions of necrotic phloem, and by absence of fruit symptoms. No symptoms are

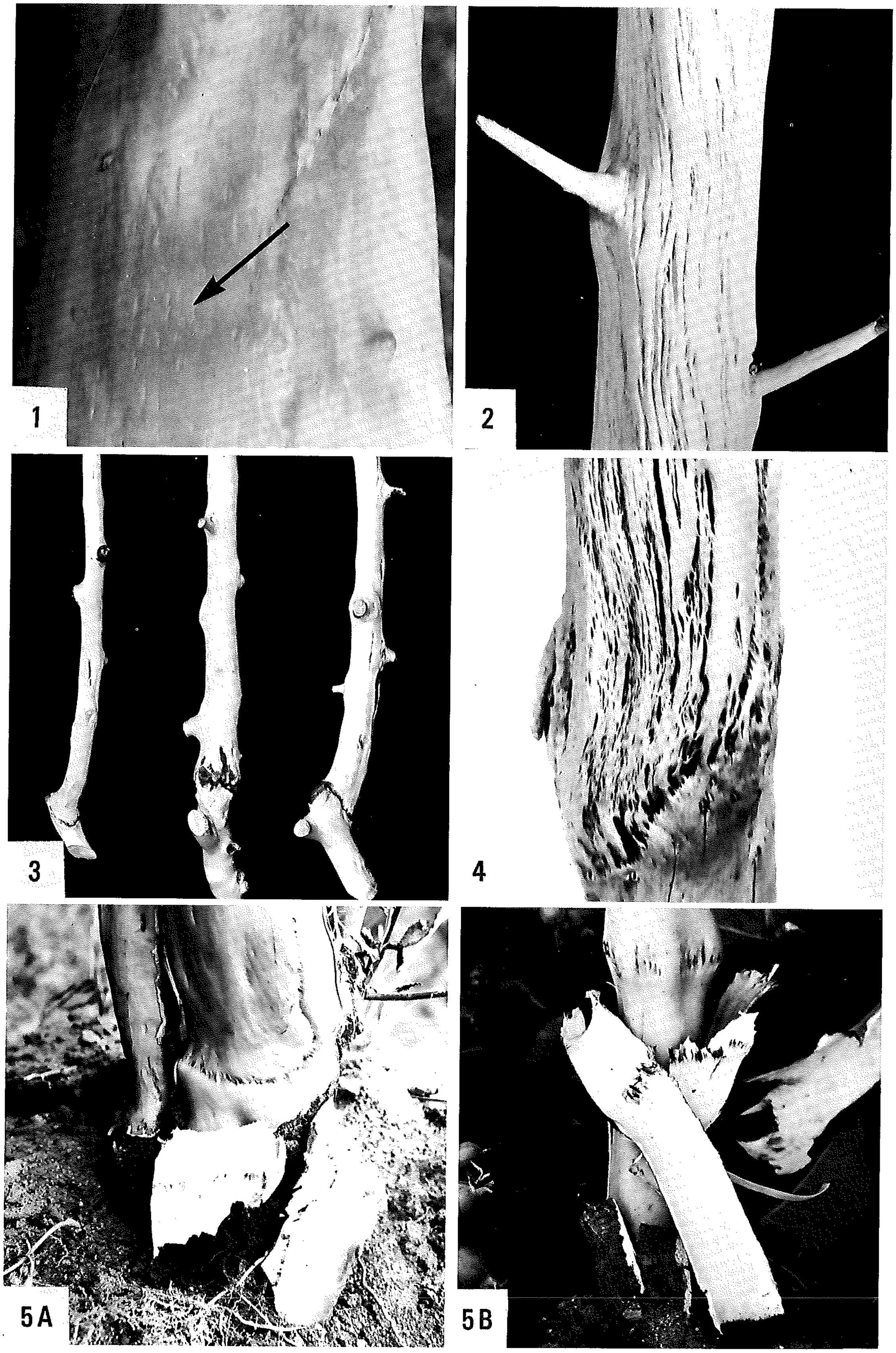

Fig. 1-5. Syndromes caused in apple by graft-transmissible, xylem-affecting agents. **1,** Mild pitting in xylem of trunk of cultivar (Virginia Crab) 6 yr after inoculation by application of buds from cultivar Hyslop Crab. **2,** Moderate xylem pitting on Virginia Crab, induced within 2 yr of bud inoculation from 16 other apple clones. **3,** Brown line decline: showing sunken xylem groove, filled with necrotic phloem, at union of Virginia Crab and seedling rootstock, and scattered grooves on Virginia Crab stem. **4,** Junction necrotic pitting and stem pitting occurring together on Virginia Crab, the former as deep pits filled with necrotic phloem encircling the stock-scion union, the latter as a network of pits not containing necrotic phloem, distributed over the Virginia Crab trunk. **5,** Junction necrotic pitting on Virginia Crab: **A,** densely arranged pits that simulate brown line decline symptoms; and **B,** a ring of distinct pits.

TABLE 2. Correlation of woody host indexing and herbaceous host indexing of apple clones

Clone	Heat-treated	Sources tested (no.)	Reactions of woody hosts: Virginia Crab	Spy 227	Russian R12740-7A	Isolations on herbaceous hosts (*Chenopodium quinoa* and Zucchini squash): ASGV[a]	CLSV[a]
Rome Beauty 1	no	1	SP, BLD, FF[b]	LP, SL	CLS	+	+
RB 1 (a)[c]	yes	2	BLD	neg.	neg.	+	−
M.9 (a)	yes	3	SP, JNP, FF	SL, LP	CLS	−	+
M.9 (b)	yes	2	SP, FF	SL	neg.	−	+
Stayman 1	no	1	SP, JNP, FF	SL, LP	CLS	−	+
St (a)[c]	yes	6	JNP	SL	neg.	−	−
St (b)	yes	2	SP, JNP, FF	SL	neg.	−	−
St (c)	yes	1	JNP, FF	SL	neg.	−	+
St (d)	yes	1	JNP, FF	SL	CLS	−	+
St (e)	yes	3	neg.	neg.	neg.	−	−
Hyslop	no	4	SP, JNP, FF	(no test)	...[e]	−	+
Tydeman Early	no	1	JNP, FF	SL, LP	CLS	−	+
Granny Smith	no	1	SP, BLD	SL, LP	CLS	+	+
Delicious	no	1	SP, BLD	(no test)	...	+	+
Mutsu[d]	no	1	BLD	neg.	neg.	+	−

[a]ASGV, apple stem grooving virus; CLSV, chlorotic leaf spot virus.
[b]BLD, brown line and decline; CLS, chlorotic leaf spot; FF, flute fruit, JNP, junction necrotic pitting; LP, leaf pattern; SL, Spy lethal; SP, stem pitting.
[c]Propagations from heat-treated buds of Rome Beauty 1 and Stayman 1, respectively.
[d]Provided by P. R. Fridlund, Irrigated Agriculture Research & Extension Center, Prosser, WA. 99350.
[e]No test.

induced on Spy 227. ASGV has proved to be heat stable in our tests (14) and in those of Paul Fridlund, Irrigated Agriculture Research and Extension Center, Prosser, Washington (*personal communication*). In our tests, it has persisted in apple clones when the agents causing SP and JNP, and other graft-transmissible agents were eliminated. JNP does not yield ASGV, or any other virus in sap transmission tests. It is characterized by formation of a ring of pits encircling the stock-scion junction (Fig. 5) and by lack of pitting or grooving on the crabapple stem. Fruits of Virginia Crab bear small depressions, or sometimes ridges and flutings. All JNP isolates induced epinasty, bark necrosis, and decline (Spy lethal) symptoms on Spy 227.

The junction grooving that characterizes severe BLD is usually distinguished readily from the ring of pits induced by JNP. However, when BLD symptoms are mild, the junction aberrations may be merely a discontinuous groove or a ring of deep pits, resembling the symptoms of JNP, and rendering diagnosis difficult. Use of *C. quinoa* would detect the presence of ASGV hence BLD.

Our test results suggest that severe BLD symptoms resulting in tree decline can be assured only by establishing a substantial titer of ASGV in test trees before the sensitive component is topworked to it. This appears to account for our own erratic results in early indexing, and for the conflicting published reports of symptoms induced by ASGV on Virginia Crab (1–3, 10–12).

JNP isolates induce FF symptoms on Virginia Crabs, and Spy lethal symptoms on Spy 227, as do SP isolates. However, the differential heat elimination of SP and JNP in Malling 9 and the Stayman clone (Table 1) demonstrates that SP and JNP differ, although there are no means of determining whether they are caused by unrelated agents or by strains of the same agent.

The recently-described union necrosis and decline disease (6) is characterized by imbedding of brown necrotic tissue at the graft union interface, generally resembling the symptoms of BLD and JNP. However, the consistent association of tomato ringspot virus infection (7) with the occurrence of union necrosis and decline disease indicates that its etiology is not the same.

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