Select *Malus* Clones for Rapid Detection of Apple Stem Grooving Virus

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ABSTRACT

Howell, W. E., Mink, G. I., Hurtt, S. S., Foster, J. A., and Postman, J. D. 1996. Select *Malus* clones for rapid detection of apple stem grooving virus. Plant Dis. 80:1200-1202.

Highly sensitive, rapidly reacting woody plant indicator clones were found when 201 *Malus* clones were screened for response to apple stem grooving virus (ASGV). A clone of *M. micromalus* (GMAL 273.a) displayed diagnostic foliage symptoms within 2 to 4 weeks of bud-inoculation in the greenhouse. Clones of *M. yunnanensis* (GMAL 2342) and *M. tschonoskii* (GMAL 1834) expressed diagnostic symptoms after 6 to 8 weeks. This is in contrast to the 6 to 8 months frequently required for reliable ASGV reactions to appear on inoculated plants of Virginia Crab (*Malus domestica*), the currently recommended ASGV indicator, grown under the same conditions. When Virginia Crab and the three *Malus* selections were screened in two separate years with 19 ASGV isolates from diverse geographic origins, foliar symptoms were produced most consistently on *M. micromalus* GMAL 273.a and least consistently on Virginia Crab. *M. micromalus* GMAL 273.a is superior to Virginia Crab as a rapid woody plant indicator for ASGV.

Apple stem grooving virus (ASGV) occurs commonly in apple (*Malus domestica*) and pear (*Pyrus communis*) cultivars and can be difficult to detect using greenhouse-grown woody indicators Virginia Crab or *Pyronia veitchii*. Inoculated plants must be observed up to 18 months, numerous replications are needed to obtain reliable foliage symptoms (1,6), and occasionally a month delay between budding and inoculation is needed (5). Consequently, tests for ASGV require a much longer time period than do tests used to detect other common apple and pear viruses (3,4).

In this report, three *Malus* clones are identified that are highly sensitive to ASGV; they exhibit diagnostic symptoms within 1 to 2 months.

MATERIAL AND METHODS

***Malus* selections.** One hundred ninety-six *Malus* clones, including 37 species and species hybrids from the "core" subset of the National Clonal Germplasm Repository, Geneva, NY, and five *Malus* clones from the National Research Support Project No. 5 (NRSP5/IR2) located in Prosser, WA, were evaluated for ASGV sensitivity under greenhouse conditions.

Virus isolates. All 201 clones were initially screened for sensitivity to three isolates of ASGV designated 121-13 (originally from Granny Smith apple), B15 (2), and 111-13 (originally from Mutsu apple). These isolates represent a range of symptomatic biotypes on Virginia Crab. Isolates 121-13 and B15 incited severe and mild brown line symptoms, respectively, at the graft union between Virginia Crab indicators and inoculated apple seedling rootstocks. The 111-13 isolate often induced a necrotic "flame" pattern at and just above the graft union.

After the original test, three *Malus* clones were selected for 2 years of additional testing against a panel of 19 ASGV isolates from around the world. This panel included the three ASGV isolates utilized in the first test, 13 from pome fruit selections imported from Japan, Italy, Israel, South Africa, China, Nepal, and Pakistan to the USDA-ARS Plant Introduction Facility in Beltsville, MD, and three additional isolates from the NRSP5/IR2 Project (111-1 in Comice pear, 111-7 in Jonee apple, and 111-9 in Smothee apple). The three clones were also inoculated with various isolates of two other viruses commonly found in apples: apple chlorotic leaf spot virus (ACLSV) isolates B47, B59, and B70, and apple stem pitting virus (ASPV) isolates B30, B39, and B49 (2) and an ASPV isolate from Japan.

Propagation, inoculation, and culture. Dormant buds of each *Malus* selection were chip-budded onto four 1-year-old apple seedling rootstocks, which were simultaneously bud-inoculated with tissue containing one of the virus isolates. A set of four noninoculated control trees was propagated for comparison. One week later, the distal seedling growth was cut off and buds of each test clone were allowed to grow. The test trees were grown between January and September in a glass greenhouse held at 26°C (summer daytime temperatures would occasionally reach 32°C). Each tree was observed during the next 6 months for development of symptoms on foliage and at the graft union.

RESULTS

Reaction of *Malus* selections. Of the 201 *Malus* selections tested during the original screening process, 37 displayed ASGV-associated symptoms at the graft union, on the foliage, or both (Table 1). The symptomatic clones belonged to 18 of the 37 tested species and hybrids. All but four of the sensitive clones reacted to all three ASGV isolates used in the original test (Table 1). A list of clones that remained symptomless during these tests is available from the authors upon request or from the USDA-GRIN computer network.

Symptoms. Several clones exhibited distinct leaf symptoms 4 weeks after inoculation; leaf symptoms consisted primarily of chlorotic spots and often included leaf deformation. Pronounced symptoms developed on the foliage of *M. micromalus* GMAL 273.a, *M. yunnanensis* GMAL 2342, and *M. tschonoskii* GMAL 1834.

Six months after inoculation, bark was stripped from the trees and the graft unions were examined. All but two clones that displayed prominent foliage symptoms on the test indicators also developed symptoms at or near the union of Virginia Crab. Eighteen clones lacking foliar symptoms exhibited prominent symptoms at the union involving a necrotic brown line encircling the Virginia Crab variety just above the graft. A few clones displayed stem grooving. Bark and wood necrosis was not limited to graft union areas on ASGV-infected *M. micromalus* GMAL 273.a trees.

Symptom development on *M. micromalus* GMAL 273.a was very rapid (within 14 to 21 days) and included chlorotic and

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Projects 1262 and 1719.

PPNS Paper No. 0226, College of Agriculture and Economics Research Center, Washington State University, Pullman 99164.

Accepted for publication 16 July 1996.

Publication no. D-1996-0814-05R

necrotic spots, strong leaf epinasty, stem necrosis, and occasionally death of the plant. In comparison, symptom development on *M. yunnanensis* GMAL 2342, *M. tschonoskii* GMAL 1834, and Virginia Crab required 6 to 10 weeks. At that time, severe leaf distortion and necrosis occurred on ASGV-inoculated *M. yunnanensis* GMAL 2342, characteristic chlorotic spots were noted on leaves of *M. tschonoskii* GMAL 1834, and sporadically a chlorotic spot was associated with unilateral leaf deformation on an occasional leaf of Virginia Crab.

Virus sensitivity of selected clones. All 19 ASGV isolates induced diagnostic symptoms on *M. micromalus* (Table 2). In contrast, six of these isolates induced no foliage reaction on Virginia Crab, the standard ASGV indicator. Furthermore, Virginia Crab failed to show any graft union reaction to two isolates from pear until a year later. In general, isolates from pear induced less reaction in all the indicator plants (Table 2); perhaps ASGV isolates often occur at lower titer in pear.

With some clones, only a few plants inoculated with a given ASGV isolate displayed foliar symptoms. The failure of all plants to develop foliar symptoms might be attributed to erratic distribution of the virus within the inoculum source. However, such occurrences happened most often with Virginia Crab and *M. tschonoskii* GMAL 1834, and least often with *M. micromalus* GMAL 273.a and *M. yunnanensis* GMAL 2342, suggesting that these two clones were perhaps more sensitive to ASGV (Table 2).

Sensitivity to other viruses. No symptoms were noted on *M. tschonoskii* GMAL 1834 and *M. yunnanensis* GMAL 2342 inoculated with any of the ACLSV and ASPV isolates. Leaves of *M. micromalus* developed a faint mosaic pattern with ACLSV, which was different from ASGV symptoms. On the other hand, one isolate of ASPV induced chlorotic spots on Virginia Crab similar to but smaller than those induced by ASGV. The other three isolates of ASPV produced symptoms on one or none of four test plants.

DISCUSSION

Reliable detection of ASGV using Virginia Crab in the greenhouse or field requires examination of graft unions after 6 months or more; whereas with *M. micromalus* GMAL 273.a, less than 3 weeks is required. Unfortunately, there is difficulty in propagating *M. micromalus* GMAL 273.a, which apparently is related to very small caliper budwood produced by this selection. This difficulty was circumvented by bud propagation from large budwood only or by cleft graft propagation. In contrast, *M. yunnanensis* GMAL 2342 propagated easily by budding. Other than the propagation problems with small-caliper budwood of *M. micromalus,* no particular culture or pest problems were noted with either of these clones under greenhouse conditions. The partial inconsis-

Table 1. Reactions induced on 37 *Malus* clones after inoculation with three isolates of apple stem grooving virus (ASGV)[a]

		Symptoms associated with ASGV isolate[b]		
Clone	**Species / selection**	**121-13**	**B15**	**111-13**
GMAL 1879	*asiatica*	BL	BL	BL
GMAL 2711	*asiatica*	CS	–	CS,LD
GMAL 2477	*baccata* / Hansen's #2	BL	BL	BL
GMAL 859	*domestica* / Kim McIntosh	CS,LD,BL	–	CS,LD
GMAL 1992	*domestica* / Novosibirski Sweet	BL	BL	BL
GMAL 3479	*domestica* / Novole	BL	BL	BL
IRA 28	*domestica* / Virginia Crab	CS,BL	CS,LD,BL	BL
GMAL 185	*florentina*	CS,BL	CS,BL	CS,BL
GMAL 2887	*halliana*	·	BL	·
GMAL 1315	*halliana* / Parkman	BLF,G,N	·	·
GMAL 55	*hupehensis*	BL	BL	BL
GMAL 1878	*hupehensis*	CS,LD,BL	BL	BL
GMAL 2037	*hupehensis*	NS,LD,BL	·	BL
GMAL 135	hybrid / Almey	BL	LD,BL	BL
IRA 107	hybrid / Ottawa 3	BL	BL	BL
IRA 351	hybrid / Sugar Crab	BL	·	BL
GMAL 46	*mandshurica*	CS,BL	BL	CS,BL
GMAL 273	*micromalus*	E,R,S,N	E,R,S,D	E,R,S,D
GMAL 275	*micromalus*	S,BL	S,BL	CS,BL
GMAL 1497	*micromalus*	BL	BL	BL
GMAL 1847	*sargentii*	NS,LD,BL	NS,LD,BL	BL
GMAL 2333	*sieboldii*	BL	BL	BL
GMAL 365	*sieboldii* / #387	CS,BL	CS,BL	CS,BL
GMAL 202	sp. / Demir	CS,LD,BL,G	CS,BL	CS,LD,G
GMAL 250	sp. / Yellow Autumn Crab	BL	BL	BL
GMAL 215	*spectabilis* / Plena	BL	·	–
GMAL 1831	*toringoides*	BL	BL	BL
GMAL 248	*toringoides* / Cut-Leafed Crab	CS,BL	·	·
GMAL 1869	*transitoria*	·	BL	CS,LD,BL
GMAL 1836	*trilobata*	BL	BL	BL
GMAL 1834	*tschonoskii*	CS,LD,BL	BL?	CS,BL
GMAL 422	× *magdeburgensis*	·	·	BL
GMAL 518	× *robusta* / Korea	BL	BL	CS,LD,BL
GMAL 1867	× *rockii*	CS,LD,BL	CS,LD,BL	CS,LD,BL
GMAL 2480	× *zumi* / Calocarpa	BL	BL	BL
GMAL 1838	*yunnanensis*	LD,E,N	–	–
GMAL 2342	*yunnanensis* / Veitchii	NS,LD	NS,LD	NS,LD

[a] Noninoculated control trees of each were propagated for comparison. None of the ASGV-associated symptoms developed on the control plants.

[b] Foliage symptoms: CS = chlorotic spots, NS = necrotic spots, S = chlorotic and necrotic spots, LD = leaf deformation, E = leaf epinasty, R = leaf reddening. Graft union symptoms: BL = brown line, BLF = brown line and necrotic flame pattern, N = general necrosis, G = stem grooving. · = test clone did not grow or rootstock died. – = no symptoms.

Table 2. Reaction of *Malus micromalus* GMAL 273.a (MM), *M. yunnanensis* GMAL 2342 (YUNN), *M. tschonoskii* GMAL 1834 (TSCH) and Virginia Crab to a range of apple stem grooving virus (ASGV) isolates from around the world and to apple chlorotic leaf spot (ACLSV) and apple stem pitting viruses (ASPV)

			Symptoms on foliage[a]			Virginia Crab	
Virus	Isolate	Source	MM	TSCH	YUNN	Foliage[a]	Union[b]
ASGV	B15	IR2	++++	++++	++++	?	3
ASGV	121-13	IR2	++++	+++	++++	–	4
ASGV	111-13	IR2	++++	++	++++	–	2
ASGV	111-1	IR2	++	+	+	–	0
ASGV	111-9	IR2	++++	++	+	+	3
ASGV	111-7	IR2	++++	++++	++++	++++	3
ASGV	Q26005	Japan	++++	++++	++++	++++	3
ASGV	T21550H	Israel	++++	++++	++++	++	3
ASGV	T21551I	Israel	++++	++++	++++	++	3
ASGV	T22067A	S. Africa	++++	++++	++++	++	3
ASGV	Q24379	China	+++	++++	–	+	1
ASGV	Q24387	China	++++	++++	++++	++++	3
ASGV	Q26702	Nepal	++++	++	++	+	2
ASGV	Q26703	Nepal	+++	–	?	–	0
ASGV	Q26704	Nepal	++	?	+	–	1
ASGV	Q27432	Pakistan	+++	+++	+	–	2
ASGV	Q27647	China	++++	++	++++	+	3
ASGV	Q28034	Japan	+++?	·	+++	++	3
ASGV	Q26753	Japan	+++	·	++++	+	3
ACLSV	123-6	IR2	CM	–	–	–	0
ACLSV	123-1	IR2	CM	–	–	–	0
ACLSV	123-9	IR2	CM	–	–	–	0
ASPV	123-8	IR2	–	·	–	++++	0
ASPV	123-4	IR2	–	–	–	–	0
ASPV	123-13	IR2	–	–	–	+	0
ASPV	Q26003	Japan	–	–	–	+	0
Check	Noninoc		–	–	–	–	0
Check	Noninoc		–	–	–	–	0
Check	Noninoc		–	–	–	–	0

[a] + = one of four test plants with symptoms, ? = one of four test plants with symptoms that may or may not be attributable to the virus, · = test clone did not grow or rootstock died, CM = chlorotic mottle, – = no symptoms.

[b] Symptom intensity at graft union ranged from no symptoms (0) to very severe brown line symptoms (4) on all four test plants.

tency of ASGV expression on *M. tschonoskii* GMAL 1834 renders it on par with Virginia Crab.

Less extensive but similar tests were conducted at the National Plant Quarantine Center in Beltsville, MD, and at the National Clonal Germplasm Repository in Corvallis, OR, and their results (not shown here) complemented those reported above.

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