Sustained Cytospora-resistance in Italian Prune Cultivars Infected with Prunus Ringspot Virus

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ABSTRACT

Italian prune trees (*Prunus domestica*) infected with *Prunus* ringspot virus (PRSV) and PRSV-free trees were inoculated with *Cytospora cincta* to determine the magnitude of induced resistance to subsequent infections by *Cytospora*. Depressed expansion rates of secondary infections indicated that (i) *Cytospora* invasion was more vigorous in Italian prune trees on peach rootstock (*P. persica*) than on plum rootstock (*P. cerasifera*), (ii) PRSV-induced resistance to *Cytospora* was stronger in trees on peach root than in trees on plum root, and (iii) PRSV-induced resistance to *Cytospora* was additive with new *Cytospora*-induced resistance, particularly in trees growing on peach rootstock.

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Additional key words: rootstock effect on resistance, host-pathogen interaction.

Cytospora-resistance has been elicited in standard Italian prune trees (*Prunus domestica* L.) with *Cytospora cincta* Fr. (4) and PRSV (*Prunus* ringspot virus) infections (2) and in J. H. Hale peach trees (*P. persica* L.) with *Cytospora* infections (1). Mass-action effects seem to apply in this host-pathogen interaction in that the expansion rates of individual cankers has been found to vary inversely with the number of infections initiated simultaneously (3). Therefore, further studies were undertaken to determine (i) whether the resistance response would persist in the presence of a systemic nonnecrosis-producing pathogen such as PRSV and (ii)

whether the persisting resistance, if present, would be additive with that produced by a newly invading necrosis-producing pathogen such as *Cytospora cincta* in the same trees.

MATERIALS AND METHODS.—Test cultivars were Richards Early Italian and standard Italian prune trees growing on Lovell peach (*P. persica* L.) or the California CEA-2 selection of Myrobalan plum (*P. cerasifera* Ehrh.) rootstocks. All trees were 10 yr old and free of known viruses (except PRSV as indicated below) at the time of the study. PRSV-infected trees had been infected since the spring of the second yr with Idaho strain PI-19, rated as a moderately severe strain on the basis of symptoms produced in: (i) Buttercup squash (*Curcurbita maxima* L.) in a greenhouse, (ii) Lovell and Muir peach seedlings (*P. persica* L.) in greenhouse and field, (iii) in plot-grown Shirofugen flowering cherry trees (*P. serrulata* Lindl.), and (iv) in standard Italian prune trees of various age in the field. The *Cytospora* isolate used was Idaho code Cy-59, rated as a vigorous invader of stone fruit cultivars.

Cytospora infections were of three types: the first infection initiated in a given tree was the inciting infection; the second infection, initiated later in that same tree, was the challenge infection (i.e., the systemic-resistance-evaluating infection); and the third infection was the control infection, which was initiated in a previously uninfected tree at the same time as the challenge infection. Depression of expansion rates of challenge infections, in comparison with corresponding control infections, served as a measure of the resistance-effect attributable to the inciting infection.

On 8 June, an inciting *Cytospora* infection was initiated on each of five major scaffold branches on each of four PRSV-free and four PRSV-infected Richards trees on plum root. In like manner, eight PRSV-free and eight PRSV-infected standard Italian trees on plum root also were infected. Like groups of Richards trees and standard Italian trees on Lovell peach root received inciting *Cytospora* infections at the same time.

On 21 June, a Cy-59 challenge infection was initiated 18 cm directly below each of the inciting infections. At the same time, Cy-59 control infections were initiated in comparable locations in like numbers of PRSV-free and PRSV-infected trees of both varieties on both rootstocks. Induced-resistance effects were considered to have occurred when challenge cankers were smaller at any observation date than corresponding control infections.

Size (mm^2) of all cankers was recorded weekly throughout the growing season. Statistical significance was determined via analysis of variance ($P = 0.05$) utilizing details of data not presented in Tables 1 and 2.

During the study, all trees were re-indexed on Shirofugen. Those inoculated 8 yr earlier with PRSV still were positive for PRSV on Shirofugen; those not inoculated with PRSV still yielded Shirofugen-negative responses.

RESULTS AND DISCUSSION.—Figures in Tables 1 and 2 are corrected to represent actual *Cytospora* necrosis; i.e., the area of tissue damaged in the inoculation

TABLE 1. PRSV-induced resistance to *Cytospora* in 10-yr-old Richards Early Italian prune trees on Lovell peach and CEA-2 Myrobalan plum rootstocks[a]

Root-stock	Observation date	Canker area in mm^2					
		Inciting		Challenge[b]		Control	
		PRSV absent	PRSV present	PRSV absent	PRSV present	PRSV absent	PRSV present
Lovell							
	June 15	1,627	1,512				
	June 22	2,530	2,463				
	June 29	3,380	3,492	504	484*	506	560
	July 13	4,351	4,209	1,649	1,462*	2,237	2,275
	July 20	4,744	4,380	1,972	1,761*	2,706	2,714
	July 27	5,040	4,658	2,219	2,009*	3,057	3,106
	August 8	5,260	4,700	2,153	1,969*	3,413	3,113
	August 10	5,304	4,820	2,124	1,841*	3,606	3,153
	August 17	6,219	4,895	2,110	1,745*	3,853	3,181
CEA-2							
	June 15	1,315	1,298				
	June 22	2,105	2,123				
	June 29	2,646	2,845	545	485	608	572
	July 13	3,229	3,355	1,309	1,570	1,930	1,587
	July 20	3,572	3,683	1,759	1,910	2,458	1,913
	July 27	3,725	3,699	2,049	2,111	2,627	2,202
	August 8	3,836	3,799	2,028	2,028	2,733	2,240
	August 10	3,944	3,284	1,967	1,971	2,488	1,982
	August 17	4,107	3,148	1,869	1,842	2,353	1,887

[a]Five *Cytospora* (isolate Cy-59) inciting infections initiated on each of four PRSV-free trees and four PRSV-infected trees on 8 June; on 21 June, Cy-59 challenge infections were initiated 18 cm directly below the 8 June infections; comparable Cy-59 infections were initiated on four additional PRSV-free trees and four additional PRSV trees on 21 June to serve as controls in each case; each figure represents the average of 20 replicate-cankers equally distributed among five trees; significance of difference determined by analysis of variance $P = 0.05$.

[b]Asterisk indicates significant difference from corresponding control infections; see text for significance of difference between inciting infections on PRSV-free and PRSV-infected trees.

procedure has been deducted.

Cytospora inciting infections on Richards trees were significantly smaller by the end of the study (17 August) on PRSV-infected trees than inciting infections on PRSV-free trees when growing on Lovell rootstock; they were numerically smaller, but not significantly smaller, on plum rootstock (Table 1). This indicates a depressing effect on primary *Cytospora* invasion by the PRSV present, particularly in the case of trees growing on Lovell rootstocks in which *Cytospora* invasion was more aggressive. No such reductions in inciting infections on PRSV-infected standard Italian trees occurred (Table 2). For both cultivars, however, average canker size on trees on plum root was significantly smaller by the end of the study (17 August) than for trees on Lovell root, both in PRSV-free and PRSV-infected trees, indicating that the plum root exercises a depressing influence on expansion of *Cytospora* cankers independent of that provided by PRSV or *Cytospora* invasion. Or, conversely stated, *Cytospora* invasion of prune trees growing on Lovell peach root is more vigorous than in comparable trees growing on Myrobalan plum root, whether or not PRSV is present.

Cytospora cankers commonly reach maximum dimensions in mid-to-late summer in Idaho, particularly in young trees, following which the dimensions decrease due to moderate encroachment on the canker-area by healing callus at the margins. This is less common in older trees suffering from other stresses; it also depends on the vigor of the invading *Cytospora* strain. No such peaking-out of canker-size curves was observed for inciting infections in either cultivar except those trees infected with PRSV and growing on plum rootstock. For these, it occurred on 8 August (Tables 1, 2). Challenge infections peaked-out earlier (20-27 July) for Richards trees but later for standard trees (8-10 August), suggesting that the host-physiology processes associated with earliness (Richards) or lateness (standard) also influence the activity of *Cytospora* pathogens. Control infections failed to peak-out for either cultivar growing on Lovell rootstock, whether or not they were PRSV-infected, but peaked-out for both during the period 8-10 August when growing on plum root. Apparently scion-rootstock interactions are biochemically different for the two cultivars.

Challenge cankers were numerically smaller than corresponding control cankers for both cultivars in all cases. For Richards, these differences were significant only in PRSV-infected trees growing on Lovell rootstock (significant at every observation data) where stem invasion by *Cytospora* was more vigorous than in trees on plum rootstock (Table 1). The differences were significant for standard trees, whether or not the trees were infected with PRSV (Table 2). The induced-resistance effects of PRSV and of *Cytospora* therefore were additive for both cultivars.

Under the conditions of the study, the following can be concluded: (i) *Cytospora* invasion of Richards Early Italian and standard Italian prune varieties is not as vigorous when those trees are growing on plum

TABLE 2. PRSV-induced resistance to *Cytospora* in 10-yr-old standard Italian prune trees on Lovell peach and CEA-2 Myrobalan plum rootstocks[a]

Root-	Observation date	Canker area in mm^2					
		Inciting		Challenge[b]		Control	
		PRSV absent	PRSV present	PRSV absent	PRSV present	PRSV absent	PRSV present
Lovell							
	June 15	1,443	1,571				
	June 22	2,644	2,973				
	June 29	3,911	4,453	486	460	489	521
	July 13	5,771	6,540	1,236	643*	1,462	1,370
	July 20	6,008	7,329	2,016	914*	2,220	2,029
	July 27	6,414	8,041	2,187	1,083*	2,411	2,282
	August 8	6,743	8,875	2,288	2,106*	2,531	2,859
	August 10	7,272	9,388	2,476	2,293*	3,593	3,211
	August 17	8,690	9,883	2,303	2,139*	4,121	3,543
CEA-2							
	June 15	1,169	1,240				
	June 22	2,087	2,376				
	June 29	3,005	3,302	432	372	447	480
	July 13	4,259	4,411	881	866*	968	1,244
	July 20	4,463	4,661	1,457	964*	1,778	1,305
	July 27	4,833	4,981	1,578	1,002	1,954	1,341
	August 8	4,977	5,438	1,634	1,013*	2,016	1,477
	August 10	5,003	5,315	1,673	993*	1,862	1,482
	August 17	5,203	5,270	1,580	794*	1,704	1,219

[a]Five *Cytospora* (isolate Cy-59) inciting infections initiated on each of four PRSV-free trees and four PRSV-infected trees on 8 June; on 21 June, Cy-59 challenge infections were initiated 18 cm directly below the 8 June infections; comparable Cy-59 infections were initiated on four additional PRSV-free trees and four additional PRSV trees on 21 June to serve as controls in each case; each figure represents the average of 20 replicate-cankers equally distributed among five trees; significance of difference determined by analysis of variance $P = 0.05$.

[b]Asterisk indicates significant difference from corresponding control trees; see text for significance of difference between inciting infections on PRSV-free and PRSV-infected trees.

rootstock as when they are on Lovell root; (ii) PRSV-induced resistance-to-*Cytospora* is stronger in trees on peach root than in trees on plum root, though the difference is less in standard trees than in Richards trees; (iii) PRSV-induced resistance is additive with *Cytospora*-induced resistance in both cultivars, especially when growing on peach rootstock.

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