Induction of Reciprocal Resistance in Prunus persica by Cytospora cincta and Agrobacterium tumefaciens

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ABSTRACT

Branches of 4-year-old (cultivar Redhaven) and 3-year-old (cultivar Golden Jubilee) peach (*Prunus persica*) trees were artificially infected with *Agrobacterium tumefaciens* in the spring. At various intervals thereafter, secondary infections by *Cytospora cincta* were initiated on the same branches to determine whether infection by *A. tumefaciens* would influence development of the cankers caused by *C. cincta,* and (conversely) whether the presence of cankers incited by *C. cincta* would influence development of the galls caused by *A. tumefaciens.* Expansion rates of both galls and cankers were significantly depressed in the vicinity of the other infective agent under most test conditions of the 2-year study, demonstrating that the bacterium induces a nonspecific resistance to the fungus in *P. persica* and vice versa.

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Additional key words: Peach resistance, host-pathogen interaction, pathogen-pathogen interaction, phytoalexin.

Previous reports have shown that infection of Italian prune trees (*Prunus domestica* L.) (3, 4) and peach trees (*P. persica* L. Batsch. 'J. H. Hale') (1) with *Cytospora cincta* Fr. results in suppression of canker expansion rates of subsequent Cytospora infections in the same trees. A similar phenomenon resulted from an interaction of *C. cincta* and Prunus ringspot virus (PRSV) (2). Since this evidence suggested a broad nonspecific host-pathogen interaction, additional studies were undertaken to determine whether a bacterium could produce the same systemic resistance against Cytospora infections in peach trees and whether secondary Cytospora infections would influence development of primary bacterium infections. *Agrobacterium tumefaciens* (E. F. Sm. & Towns.) Conn. was selected as the test bacterium because of its widespread occurrence in stone fruit orchards and because Agrobacterium infections produce overgrowths that are reasonably easily measured.

MATERIALS AND METHODS.—For the first year's study, 4-year-old Redhaven peach (*P. persica* L. Batsch.) trees on Halford rootstock were selected for uniformity and for the presence of four scaffold branches of approximately equal size per tree. Four branches on each of three trees were artificially infected with *A. tumefaciens* on 16 June and reserved as REFERENCE trees. Six other trees were infected in like manner at the same time (the INCITING infections, Table 1). The inoculation technique consisted of placing a standard-size piece of potato-dextrose agar medium cut from a bacterial colony under a standard-size T-cut bark flap

TABLE 1. Concurrent development of galls incited by *Agrobacterium tumefaciens* (*Agr.*) and cankers incited by *Cytospora cincta* (*Cyt.*) on 4-year-old Redhaven peach trees[a]

Test date and classification	Gall and canker size at various times following inoculation[b]				
	25 July	1 Aug	8 Aug	15 Aug	22 Aug
25 July test					
Agr. reference galls	47.6 C	67.8 C	97.5 C	101.2 C	109.1 C
Agr. inciting galls	38.8 C	57.0 C	70.1 D	76.0 C	82.1 C
Cyt. challenge cankers	11.7 E	15.0 E	15.4 E	15.8 E	16.1 E
Cyt. control cankers	14.1 F	17.7 F	18.6 F	18.8 F	19.8 F
8 August test					
Agr. reference galls	47.6 C	67.8 C	97.5 C	101.2 C	109.1 C
Agr. inciting galls	44.7 C	65.3 C	92.8 C	100.3 C	103.3 C
Cyt. challenge cankers				10.3 E	13.9 E
Cyt. control cankers				11.6 F	15.0 F

[a]All reference galls and inciting galls were initiated 16 June with *A. tumefaciens;* challenge (*C. cincta*) cankers were initiated 18 cm directly below inciting galls on separate groups of trees on 25 July and 8 August. Control cankers were initiated by inoculating separate groups of healthy trees with *C. cincta,* also on 25 July and 8 August.

[b]Figures represent the average of four galls (cm^3) or four cankers (cm^2) on each of three trees. Significant differences (analysis of variance, $P = 0.05$) within columns for each test are indicated by different letters (C is different from D is different from E is different from F).

and binding with elastic tape. On 25 July (mid-summer test), three of the six trees also were infected with *C. cincta* (Idaho isolate Cy-59; the CHALLENGE infections) so that each of the three trees now sustained eight infections, a Cytospora CHALLENGE infection 18 cm directly below each of the four Agrobacterium INCITING infections. The inoculation technique for *C. cincta* consisted of pressing a standard-size fragment of a Cytospora colony on malt agar into a standard-size impact wound and binding with elastic tape. At the same time the CHALLENGE infections were initiated, three healthy trees (no Agrobacterium infections) were inoculated in four comparable locations with *C. cincta,* providing the CONTROL infections with which depressing effects of the INCITING infections on the CHALLENGE infections could be determined. This process was repeated on 8 August (early-fall test), with the remaining three of the six Agrobacterium-infected trees and three healthy trees, so that two separate tests were conducted under differing conditions of climate and host-physiology.

In the second year's study, 3-year-old Golden Jubilee peach (*P. persica* L. Batsch.) trees on Halford rootstock were used. Three branches of each of three trees were artificially infected with *A. tumefaciens* on 16 May and reserved as REFERENCE trees. Thirty other trees were infected in like manner at the same time (the INCITING infections, Table 2). At weekly intervals beginning 19

TABLE 2. Cankers incited by *Cytospora cincta* on 3-year-old Golden Jubilee peach trees in the presence or absence of galls incited by *Agrobacterium tumefaciens*[a]

26 June	*7.5*									
	9.8									
3 July	*12.6*	*9.5*								
	*	11.1								
	18.3									
10 July	*13.8*	*14.6*	*8.1*							
	*	17.0	8.0							
	21.0									
17 July	*19.0*	*16.2*	*12.9*	*10.8*						
	*	19.8	12.8	9.4						
	22.9									
24 July	*19.5*	*17.7*	*14.2*	*15.3*	*7.0*					
	*	22.3	15.0	14.4	6.3					
	25.8									
31 July	*20.6*	*18.3*	*14.8*	*17.0*	*10.4*	*8.7*				
	*	*	14.4	15.4	8.9	9.1				
	27.0	23.4								
7 August	*23.0*	*19.8*	*16.8*	*21.0*	*10.9*	*13.9*	*10.2*			
	*	*	17.0	19.7	12.5	16.3	12.5			
	28.4	26.2								
14 August	*24.9*	*21.4*	*19.5*	*22.8*	*11.3*	*15.2*	*16.9*	*13.4*		
	*	*	20.0	23.6	13.6	17.4	21.4	15.2		
	32.0	26.7								
21 August	*25.6*	*2.3*	*20.6*	*25.3*	*14.5*	*16.9*	*19.4*	*17.9*	*10.5*	
	*	*	23.8	26.5	15.0	18.8	*	22.3	10.5	
	32.2	28.6					24.5			
28 August	*26.7*	*24.6*	*22.4*	*26.4*	*15.8*	*17.3*	*20.3*	*18.9*	*14.0*	*11.8*
	*	*	24.0	27.6	15.9	19.1	*	*	15.1	11.4
	32.7	30.5					25.3	23.3		
4 September	*27.4*	*25.4*	*23.0*	*27.1*	*16.4*	*17.7*	*20.9*	*19.1*	*15.3*	*14.0*
	*	*	24.3	28.3	16.6	19.4	*	*	16.2	14.8
	33.1	32.1					26.4	24.9		

[a]Challenge cankers were initiated 18 cm directly below Agrobacterium galls. Agrobacterium infections were initiated 16 May, and Cytospora control cankers were initiated in separate groups of healthy trees at intervals corresponding with initiation of challenge cankers.

[b]Each number represents the average of three cankers in each of three trees. The italicized number at each data pair is the challenge-canker average, and the other number the control-canker average. Each challenge canker was located directly below an Agrobacterium gall on the same stem. Significant difference ($P = 0.05$) for each data-pair is indicated by *.

[c]Cankers measured also on 11 September and the differences were significant.

[d]Cankers measured also on 11 and 18 September and the differences were not significant.

[e]Cankers measured also on 11, 18, and 25 September and the differences were not significant.

June and ending 21 August, groups of three of these trees were infected also with *C. cincta* (the CHALLENGE infections) so that each of the three now sustained six infections, a Cytospora CHALLENGE infection 18 cm directly below each of three Agrobacterium infections. Each time CHALLENGE infections were initiated, three previously uninfected trees were inoculated in three comparable locations with *C. cincta*, providing the CONTROL infections with which depressing effects of the INCITING infections on the CHALLENGE infections could be determined.

Other trees were wounded in the standard Agrobacterium inoculation procedure (the nurseryman's T-cut) both years, but not infected, then CHALLENGED with *C. cincta* (impact wounds) as above. Since no effect of the uninfected wounds on development of CHALLENGE cankers was observed, this information was omitted from Tables 1-2.

Size of all Agrobacterium galls was recorded in cm^3 each week; size of all Cytospora cankers was recorded in cm^2. The measurements taken were relative rather than exact in that maximum measurable length × width × height-above-the-stem-surface was recorded as cm^3 for Agrobacterium galls, and length × width of observed Cytospora necroses was recorded as cm^2. Statistical significance was determined via analysis of variance ($P = 0.05$).

RESULTS AND DISCUSSION.—Figures for Cytospora cankers in Tables 1-2 are corrected to represent actual Cytospora necrosis; i.e., areas of tissue damaged in the inoculation procedure have been subtracted. No tissue was destroyed in the Agrobacterium inoculation procedure, and no comparable subtractions were made for resulting galls. Agrobacterium galls developed rapidly in all tests, indicating that climatic differences among tests, and resulting differences in host physiology, did not markedly affect bacterial activity and/or host response to that activity.

In the 25 July test of the first year's study, CHALLENGE cankers were significantly smaller than CONTROL cankers in all cases (Table 1). INCITING galls were significantly smaller than REFERENCE galls only at the 8 August observation, but they were numerically smaller than REFERENCE galls throughout the period of the study. In the 8 August test, CHALLENGE cankers again were significantly smaller than corresponding CONTROL cankers; INCITING galls were numerically smaller than REFERENCE galls throughout the test, but none of these differences was significant.

In the more elaborate study of the second year, expansion rate of CHALLENGE cankers of the first spring test (19 June; Table 2) was significantly depressed within 2 weeks. The effect continued throughout the growing season, demonstrating that a strong resistance to *Cytospora* resulted from the primary (INCITING) Agrobacterium infection. This effect still was evident in the 26 June test but was delayed (as indicated by statistical significance; CONTROL cankers numerically larger than CHALLENGE cankers at all observation dates). Significant differences were not evident in the mid-season tests conducted 3, 10, 17 and 24 July or in the late-season tests conducted 14 and 21 August (though again CONTROL cankers consistently were larger in size than corresponding CHALLENGE cankers). Reasons for the return of significant differences in the intervening 31 July and 7 August tests are not apparent, but here again CONTROL cankers always were numerically larger than corresponding CHALLENGE cankers, and in most cases the differences were significant. Collectively these data verify the results of the first-year study, and demonstrate that the presence of *A. tumefaciens* in a peach tree does diminish the aggressiveness of later infections by *C. cincta* in the same tree.

The second-year study also provided new evidence that secondary Cytospora infections (CHALLENGE infections) can diminish the virulence of preceding Agrobacterium infections (INCITING infections) in the same tree. Inciting galls were smaller than reference galls for every observation date in all tests. These differences were statistically significant ($P = 0.05$) in the:

19 June test (starting with the 17 July observation),
26 June test (starting with the 17 July observation),
3 July test (starting with the 17 July observation),
17 July test (starting with the 24 July observation),
24 July test [starting with the 31 July observation; significant ($P = 0.01$) on and after 21 Aug],
31 July test (starting with the 21 Aug observation), and
7 August test (starting with the 28 Aug observation).

Similar (but not significant) numerical differences were observed for the 10 July, and 14 and 21 August, tests.

Primary Agrobacterium infections depressed expansion rates of secondary Cytospora cankers in peach trees when the infections were separated by a distance of 18 cm, and the Cytospora infections in turn diminished the aggressiveness of the prior Agrobacterium infections. The two kinds of infections apparently influence each other by means of a systemic-chemical product of the host-pathogen interaction which is nonspecific both as to mechanism(s) of production in vivo, and in terms of pathogens affected. This nonspecific inhibition of the development of one kind of disease by another disease present in the same tree could account for many examples of lingering decline in stone fruit orchards.

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