

Effect of Inoculation Date on Induction of Resistance to *Cytospora* in Italian Prune Trees by *Cytospora cincta*

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ABSTRACT

Six-year-old Italian prune (*Prunus domestica*) trees were infected with *Cytospora cincta* on eight separate occasions during a single growing season and reinfected two weeks later to evaluate the magnitude of the *Cytospora* induced-resistance reaction when initiated at different times of the year. Strong, season-long resistance was induced by May infections; unusually strong resistance resulted from July

infections. This suggested that studies requiring long observation periods therefore should be initiated in May (under Moscow, Idaho, conditions) and those requiring highest levels of induced resistance should be initiated in July.

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Additional key words: host-pathogen interaction.

Strains of *Cytospora cincta* Fr. ranging from avirulent to highly virulent are present in western stone fruit orchards (2). Numerous studies conducted in Idaho with the more virulent strains have shown that infection of stone fruit species with *C. cincta* results in a host-pathogen interaction of a systemic nature which renders the infected host more resistant to later infections by *C. cincta* (1, 3, 5, 6, 7). This induced-resistance phenomenon has been measured by comparing expansion rates of the later (challenge) infections with those for comparable *Cytospora* infections initiated at the same time on healthy trees (7). The extent of expansion-rate reduction has been observed to vary inversely with number of inciting infections initiated simultaneously (4). The induced resistance, once incited, continues at a significant level throughout the growing season; the strongest response from single inciting (primary) infections occurs approximately one month after the inciting infection is initiated, and this is most evident when the challenge (secondary; evaluating) infection is initiated approximately two weeks after the inciting infection (6, 7). Irregularities and inconsistencies have been observed during such studies, however, and new studies were begun to identify causes of such inconsistencies. The first of these studies was concerned with timing of the inciting infections; i.e., would seasonal or physiological conditions prevailing at the time inciting infections were initiated influence the intensity and duration of the induced-resistance phenomenon.

MATERIALS AND METHODS.—Host trees were 6-year-old Italian prune (*Prunus domestica* L.) trees on Myrobalan plum (*P. cerasifera* Ehrh.) rootstock. The infective agent was Idaho isolate Cy-59 of *Cytospora cincta* Fr. The inoculation procedure consisted of smashing the bark, inverting 0.5 cm² of mycelium-bearing malt agar in the wound (mycelium next to the smashed bark), and binding with elastic electrician's tape as previously described (2). Each tree was inoculated on three comparable branches of approximately the same

diameter, and three trees were inoculated on each of the eight incitation dates (see Table 1). Two weeks following each incitation date, a challenge infection was initiated 18 cm directly below each of the inciting infections for all three trees of that group. Comparable inoculations were made simultaneously on three control trees for each group of challenging infections. Differences in expansion rates between challenge cankers and control cankers would reveal any effects on canker expansion attributable to the preceding inciting infections.

Canker area for all infections was recorded every two weeks during the study as previously described (6). Other trees were given the wound-and-agar treatment (no fungus) and challenged for each test, but since no effects on development of challenge cankers were observed in these trees, no data are presented for them in Table 1. Variation in canker size within test categories was minor; data in Table 1 therefore represent averages for each group of nine cankers (three on each of three trees).

RESULTS AND DISCUSSION.—Striking differences in the degree of induced resistance were noted among the eight different dates of primary (inciting) inoculation (Table 1).

The previous studies conducted in Idaho have shown that a *Cytospora*-resistance effect (induced by infection with *Cytospora*) can be expected to be most prominent approximately four weeks following the initial infection. However, this maximum effect apparently ranges from strong to nonexistent, depending on the time of year that the primary infections are initiated.

In the spring (May) test, the induced-resistance effect appeared within approximately 1 month as expected (significant at $P = 0.05$) and continued at a high level until observations were halted on 5 September (Table 1). Tests conducted in late spring (6 June) and early summer (20 June) failed to result in measurable resistance induction. On the contrary, challenge infections often were larger than comparable control cankers (not significantly so), which suggests newly optimum conditions for *Cytospora*

TABLE 1. Effect of timing on the *Cytospora*-induced-resistance phenomenon in 6-year-old Italian prune trees^a

Observation date ^b	Canker area for inciting infections initiated ^b (% of control)							
	May 23	June 6	June 20	July 4	July 18	August 1	August 15	August 29
June 20	92.6							
June 27	73.5*							
July 4	68.4*	93.7						
July 11	62.6*	81.4						
July 18	68.0*	105.3	84.5					
July 25	68.3*	110.7	101.9					
August 1	67.7*	113.6	104.7	80.2				
August 8	68.6*	114.0	104.4	66.9*				
August 15	67.6*	110.2	103.2	54.7**	89.1			
August 22	67.0*	109.7	100.7	51.8**	66.2**			
August 29	65.7*	115.5	99.7	47.6**	64.6**	100.2		
Sept. 5	66.0*	113.4	99.1	46.3**	71.5**	84.6		
Sept. 12					69.5**	94.3	87.7	
Sept. 19						100.7	76.7*	
Sept. 26						107.0	77.0**	124.0
October 1							72.7**	118.8

^aTests initiated at 2-week intervals beginning on 23 May; Idaho *Cytospora* isolate Cy-59 used for all inoculations; inciting infections initiated on three branches of each of three trees for each test and challenge infections initiated 18 cm directly below the inciting infections two weeks later; for each test, comparable control infections were initiated on healthy trees at the same time that the challenge infections were initiated.

^bEach value represents the average for nine separate challenge infections expressed as percent-of-control; significance of difference for each data-pair (challenge cankers and corresponding control cankers) determined by analysis of variance ($P = 0.05$, *; $P = 0.01$, **). Variation in size of inciting cankers was negligible, therefore those figures were omitted.

invasion (i.e., rapid development of challenge infections) in already-infected trees. This was especially indicated by the fact that these challenge cankers tended to streak rapidly along the stem longitudinally instead of producing the usual elliptical canker. In mid-summer (the 4 July and 18 July tests), the resistance reaction appeared again approximately 1 month following primary infection and continued as long as the trees were observed. In this mid-summer period, the suppression of expansion rates for challenge cankers was highly significant ($P = 0.01$). Differences of this magnitude occurred again in the late-summer test initiated on 15 August (an impractical time for induced-resistance studies due to approaching fall conditions)—but the tests conducted immediately before (1 August) and immediately after (29 August) again produced nonsignificant results.

It appears, therefore, that the induced-resistance phenomenon produced in Italian prune trees fluctuates, and that it depends on as yet unidentified changes in host physiology. For Italian prune trees near Moscow, Idaho, the optimal periods for *Cytospora*-induced resistance apparently fall in the months of May (for studies requiring long observation periods) and July (for studies requiring strongest resistance reactions).

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