# Involvement of pH in the Competition between *Cytospora cincta* and *Pseudomonas syringae* pv. *syringae*

Elke Endert-Kirkpatrick and David F. Ritchie

Former graduate research assistant and associate professor, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616.

Paper 11134 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh 27695-7601.

The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the products mentioned nor criticism of similar ones not mentioned.

Accepted for publication 1 December 1987 (submitted for electronic processing).

## ABSTRACT

Endert-Kirpatrick, E., and Ritchie, D. F. 1988. Involvement of pH in the competition between *Cytospora cincta* and *Pseudomonas syringae* pv. *syringae*. Phytopathology 78:619-624.

Isolations from cankered and freeze-injured peach trees from 1982 to 1985 indicated that *Cytospora cincta* and various yeasts predominated, whereas *Pseudomonas syringae* pv. *syringae* was isolated infrequently. Less than 50% of fluorescent bacteria isolated were pathogenic. Growth and survival of *C. cincta* and *P. s.* pv. *syringae* were monitored during separate and mixed incubation in a liquid medium and after inoculation into bark of dormant 8- and 9-yr-old peach trees. In a liquid medium based on aqueous peach twig extracts, populations of *P. s.* pv. *syringae* declined rapidly in mixed cultures with *C. cincta*. In some replications, viable bacteria were no longer detected after 3–5 days of incubation. Growth of *C. cincta* was not significantly affected by the presence of *P. s.* pv. *syringae*. The pH of the medium declined rapidly during the first 2 days of incubation of *C. cincta* by itself or with *P. s.* pv. *syringae*. Mixed inoculation of *C. cincta* and *P. s.* pv. *syringae* into scaffold limbs of peach trees resulted in slightly larger cankers than did inoculation of *C. cincta* alone; however, *P. s.* pv. *syringae* could not be reisolated from these cankers. *P. s.* pv. *syringae* also failed to survive in sites inoculated with bacteria alone, but naturally occurring *C. cincta* was isolated from most of these sites. Colonization of bark by *C. cincta* reduced the pH of inner bark from pH 5.2 to as low as pH 3.8. We conclude that *P. s.* pv. *syringae* is incapable of long-term survival in a shared environment with *C. cincta*.

Cytospora canker caused by *Cytospora cincta* Sacc. (teleomorph *Leucostoma cincta* (Fr.) Hohn.) and *C. leucostoma* (Pers.) Sacc. (teleomorph *L. persoonii* (Nits.) Hohn) and bacterial canker caused by *Pseudomonas syringae* pv. *syringae* reduce the vigor and longevity of numerous *Prunus* spp. including peach (6,11,15,20,21). Initial symptoms induced by *P. s.* pv. *syringae*, *Cytospora* spp., and freeze injury are variable and often indistinguishable (13), making etiological studies difficult. Bacterial canker is assumed to be an annual disease because *P. s.* pv. *syringae* has been difficult to isolate from cankered bark in summer (15,19). In contrast, Cytospora cankers appear to remain active from one to several years, hence the synonymous name perennial canker (10,18,22). Although mixed infections by both pathogens have not been observed in nature, inoculation of both *P. s.* pv. *syringae* and *C. cincta* resulted in more extensive cankers than did inoculations of either pathogen alone (15). The severity of bacterial or Cytospora canker may also be increased by freeze injury (13,16,22) or nematode infestation (6). Many pathogenic *P. s.* pv. *syringae* strains also are capable of serving as ice nuclei, thereby increasing the sensitivity of colonized tissues to freeze injury (7). It has been hypothesized that freeze-injured tissues are invaded first by *P. s.* pv. *syringae*, and subsequently by *Cytospora* spp. (13,15).

Where the disease complex peach tree short life (13) occurs, *P. s.* pv. *syringae* has been implicated as a cause of tree death (2,3,12). Where peach trees in North Carolina are affected by peach tree short life, they are usually extensively colonized by *Cytospora* spp. and the frequency of isolation of *P. s.* pv. *syringae* has been low (D. F. Ritchie, *unpublished*). Furthermore, strains of *P. s.* pv. *syringae* isolated in North Carolina were less virulent than two strains from California (4).

*Cytospora* spp. from fruit trees characteristically acidify their substrates during growth (10). We reported that the reduction in substrate pH is partially attributed to the excretion of oxalic acid by the mycelium (5). Such acidification in infected peach tissues may inhibit most non-spore-forming bacteria including *P. s.* pv. *syringae*. We conducted the following experiments to determine the interactions between *C. cincta* and *P. s.* pv. *syringae* and possible effects on canker development. Our specific objectives were to 1) compare the relative isolation frequencies of *P. s.* pv. *syringae* and *Cytospora* spp. in apparently healthy and freeze-damaged peach tissues in North Carolina orchards; 2) determine the lowest pH at which *P. s.* pv. *syringae* can remain viable; 3) monitor the growth of *P. s.* pv. *syringae* and *C. cincta* in mixed and separate incubation in vitro; and 4) monitor the viability of *P. s.* pv. *syringae* and *C. cincta* in relation to bark pH and canker development following mixed and separate inoculations into peach trees.

## MATERIALS AND METHODS

**Field isolations.** During late winters and springs of 1982 to 1985, bark and twig samples were taken from apparently healthy, freeze-injured, and cankered peach trees in the Sandhills region of the North Carolina piedmont. The samples were transported to the laboratory on ice and were refrigerated until further processing. Each sample was individually surface-disinfested for 3 min in 0.52% NaOCl, rinsed twice in sterile distilled water, and macerated in 2–3 ml of sterile sodium phosphate buffer (0.02 M, pH 7.0). For isolation of fluorescent Pseudomonads, a 0.2-ml aliquot of the maceration solution was plated onto King's medium B (9) and incubated at room temperature for 2–4 days. Fluorescent bacteria were identified as *P. s.* pv. *syringae* by oxidase reaction, arginine metabolism, gelatin liquefaction, aesculin hydrolysis, and hypersensitive reaction in tobacco leaves (4). Pathogenicity of *P. s.* pv. *syringae* strains was determined either by canker formation in peach shoots in the greenhouse or by lesion formation in cotyledons of peach seedlings (4). *Cytospora* spp. were assayed by taking seven subsamples, approximately 3 × 5 mm, from each bark and twig sample and plating them onto 1% malt extract agar (10 g of malt extract, 2.5 g of yeast extract, 20 g of agar, and 1 L of distilled water) and incubating at room temperature (20–22 C) for 4 or 5 days. Colonies of *Cytospora* spp. were identified by mycelial and pycnidial morphology. Based on the criteria of Willison (21) and Kastirr and Ehrig (8), the vast majority of *Cytospora* isolates

were concluded to be *C. cincta*.

**In vitro interactions between *C. cincta* and *P. s.* pv. *syringae*.** A liquid medium based on aqueous extracts of peach twigs was used to monitor microbial competition in vitro. One-year-old dormant twigs of cultivar Lovell were harvested during winter, weighed, washed, and frozen in 50-g samples. For each 1 L of medium, 25 g of frozen twigs was cut into 2-cm segments and steamed for 1 hr. The extract was clarified by filtration through four thicknesses of cheesecloth, adjusted to 1 L, and amended with 20 g of dextrose and 5 g of yeast extract. The medium was adjusted to pH 7.2 with 1.0 N NaOH and dispensed in 50-ml aliquots into 125-ml flasks before autoclaving. The final pH of the sterilized medium was 6.5.

*P. s.* pv. *syringae* strain B-15$^+$ rif 2 (resistant to rifampin) and *C. cincta* strain 4A were incubated in the liquid medium as separate and mixed cultures. The fungal inoculum consisted of five mycelial disks cut with a 5-mm-diameter cork borer from the edge of an actively growing malt agar culture. Bacteria were grown 48 hr on nutrient agar, harvested, and suspended to $10^7$ cfu/ml in sterile distilled water. Bacterial inoculum consisted of 0.1 ml of the cell suspension. Inoculated flasks were incubated on a shaker at 125 oscillations per minute at room temperature (22–24 C). At daily intervals, one flask from each treatment was harvested to measure the substrate pH, mycelial dry weight of *C. cincta*, and the viable population of *P. s.* pv. *syringae*. All treatments were replicated over time for a total of eight replications. Because the uninoculated liquid medium did not undergo measurable pH changes during incubation, sterile controls were not included among the treatments.

To determine whether saprophytic fungi commonly found in dead peach tissue would behave similarly to *C. cincta*, two of the most commonly isolated fungi were chosen. These fungi, *Schizophyllum commune* Fr. and *Calosphaeria pulchella* (Pers.:Fr.) Schroet. were originally isolated from dead peach trees in North Carolina. Inoculation, incubation, and harvesting procedures were similar to those used for *C. cincta*. Each experiment was replicated over time for a total of eight replications.

**The effect of low pH on survival of *P. s.* pv. *syringae* in vitro.** *P. s.* pv. *syringae* strains B-15$^+$ rif 2, B-3A, and PS-14, originally isolated from almond, peach, and apricot, respectively, were individually exposed to a series of sterile citrate buffers (0.1 M, citric acid/sodium citrate) from pH 3.0 to 6.2 in 0.4-unit increments. The strains were first grown in nutrient broth to a density of $10^8$ cfu/ml and chilled in an ice bath for 30 min. A 0.2-ml aliquot from each culture was pipetted into 5.0 ml of citrate buffer, mixed, and allowed to incubate for 10 min, 48 hr, or 2 wk. After each incubation period, a 0.1-ml sample was transferred to 1.0 ml of sterile sodium phosphate buffer (pH 7.0, 0.2 M) and mixed. A 10-fold dilution series was plated onto King's medium B for colony counts to determine viable bacteria. All treatments were replicated over time for a total of five replications. Bacterial population counts were transformed to a logarithmic scale and analyzed separately for each strain.

Because we had shown that *C. cincta* acidifies its environment by excretion of oxalic acid (5), we formulated a corresponding series of oxalate buffers to match the pH values of the citrate buffers. Although we initially attempted to buffer oxalic acid with calcium oxalate, its low solubility in water resulted in large pH fluctuations after autoclaving. Consequently, we adjusted the pH of 90 ml of oxalic acid (0.11 M) with saturated sodium hydroxide and added sufficient distilled water to yield 100 ml of 0.1 M oxalate buffer. The pH of these solutions remained stable after autoclaving. The procedures for bacterial exposures and data analyses were similar to those used for the citrate buffers.

**In vivo interactions between *C. cincta* and *P. s.* pv. *syringae*.** Competition between *C. cincta* and *P. s.* pv. *syringae* was monitored in bark inoculations on 24 pairs of cultivar Loring peach trees budded to seedling Lovell and Halford rootstocks. Because fall inoculations were reported to be most successful (15), one tree in each pair was inoculated in October 1983 as these trees were completing their eighth growing season. The remaining tree in each pair was inoculated in March 1984. Three scaffold limbs in each tree were wounded by single hammer impacts approximately 20–25 cm distal to the crotch. All three wounds on each tree were treated with one of the following: 1) a 5-mm mycelial disk from the margin of an actively growing culture of *C. cincta* strain 4A, 2) a control consisting of a 5-mm disk of sterile 1% malt agar, 3) 0.1 ml of a $10^7$ cfu/ml suspension of *P. s.* pv. *syringae* strain B- 15$^+$ rif 2, 4) 0.1 ml of sterile distilled water, or 5) a combination of strains 4A and B-15$^+$ rif 2. Inoculated wounds were immediately wrapped with two thicknesses of masking tape to delay desiccation. Each treatment was replicated six times, with the three scaffold limbs per tree serving as three observations per replication, in a randomized complete block design.

Canker development, bark pH, and fungal and bacterial viability were evaluated in April 1984 by destructive sampling. The outer bark at each inoculated site was removed, and the length and width of inner bark necrosis recorded. To measure bark pH, the inner bark was moistened with 0.1 ml of distilled water, and surface readings taken with a flat-surface electrode attached to a portable pH meter. Two pH readings, one 2–3 cm distal and one 2–3 cm proximal to the inoculation site, were averaged for each scaffold limb. The inner bark surrounding the inoculation site was then removed and transported to the laboratory on ice.

Bark samples were individually surface-disinfested for 3 min in 0.52% NaOCl, rinsed twice in sterile distilled water, and cut into 3 × 4-mm segments in 3–4 ml of sterile sodium phosphate buffer (pH 7.0, 0.02 M). Seven bark segments per canker were plated on 1% malt extract agar and incubated at room temperature for 4 or 5 days to detect *C. cincta*. *P. s.* pv. *syringae* strain B-15$^+$ rif 2 was isolated by plating 0.2 ml of the buffer extract onto King's medium B amended with 50 $\mu$g/ml each of rifampin and cycloheximide and incubated as described for field isolations. Fluorescent colonies were randomly selected and subjected to biochemical tests to verify their identity.

The experiment was repeated in November 1984 with two modifications: first, *C. cincta* strain 9.2 was substituted for strain 4A as the fungal inoculum because strain 9.2 reduced the pH of liquid media to a lower endpoint in comparative experiments. Second, a spring inoculation was omitted because cankers did not develop sufficiently in spring 1984 to allow detection of treatment differences. Results were evaluated in May 1985.

## RESULTS

**Field isolations.** Isolations from bark of cankered and freeze-injured trees consistently yielded a variety of microorganisms, predominantly *Cytospora* spp. and yeasts representing several genera, during all 4 yr in which isolations were attempted. *P. s.* pv. *syringae* and other fluorescent bacteria were less frequently isolated than were *Cytospora* spp. Isolations from apparently healthy trees in 1982 and 1983 yielded either saprophytic or no microorganisms. For this reason, sampling of healthy trees was not done in 1984 and 1985.

Because of severe freeze injury, sampling of injured trees was most extensive in 1984 (Table 1). Isolation frequencies comparable to those of 1984 were obtained for *C. cincta* during the other 3 yr. Frequencies during these 3 yr ranged from 58 to 76% of total samples. Of 198 isolates of *Cytospora* examined, only two were identified as not being *C. cincta*. Isolation frequencies for *P. s.* pv. *syringae* in each of the 4 yr were consistently less than those for *C. cincta*. Most of the fluorescent bacteria isolated were not identified as *P. s.* pv. *syringae*, and less than 20% of those identified as *P. s.* pv. *syringae* were pathogenic (Table 1). No organism was consistently associated with any specific type of symptom. Samples from bark with sour sap, which has traditionally been attributed to *P. s.* pv. *syringae* (2,12,13,15), yielded cultures of *Cytospora* spp. and yeasts more frequently than *P. s.* pv. *syringae* or other fluorescent bacteria. The presence of pycnidia near the bark surface could not be used as an indicator of infection by *Cytospora* spp. because fruiting structures were not always formed by the time of sampling (Table 1).

**In vitro interactions between *C. cincta* and *P. s.* pv. *syringae*.** Populations of *P. s.* pv. *syringae* declined rapidly in mixed cultures with *C. cincta* (Fig. 1). In some replications, viable bacteria were no

longer detected after 3–5 days of incubation. Growth of *C. cincta*, as measured by mycelial dry weight, was not significantly affected by the presence of *P. s.* pv. *syringae*. The pH of the medium declined rapidly during the first two days of incubation of *C. cincta* by itself or with *P. s.* pv. *syringae* (Fig. 1).

Results for the two saprophytic fungi, *Calosphaeria pulchella* and *Schizophyllum commune*, were similar; thus, only data for *C. pulchella* are shown (Fig. 2). Mixed incubation of *P. s.* pv. *syringae* with *C. pulchella* resulted in significantly reduced mycelial growth after 4 days. However, bacterial growth was not inhibited by either fungus. Although both *C. pulchella* and *S. commune* by themselves reduced the pH of the substrate, the rate of acidification was markedly slower than that observed for *C. cincta*. Neither *C. pulchella* nor *S. commune* was capable of acidifying the medium in the presence of *P. s.* pv. *syringae* (Fig. 2).

**Effect of low pH on survival of *P. s.* pv. *syringae*.** All three *P. s.* pv. *syringae* strains tested were sensitive to low pH. Two of three strains did not survive a 2-wk exposure to citrate buffers of pH 5.0 or less (Fig. 3). Ten-minute exposure to pH 4.2 or less was lethal to two of these strains, whereas 48-hr exposure to pH 4.2 or less was lethal to all three strains (Fig. 3). Strain PS-10, which was isolated in North Carolina, was less sensitive to low pH than either B-3A or B-15$^{+}$ rif 2, which were originally isolated in California.

Bacterial sensitivity to low pH was less pronounced in the oxalate solutions (Fig. 4). All three strains of *P. s.* pv. *syringae* survived 2-wk exposure to pH 4.6 and greater. Survival was variable in 10-min and 48-hr exposures to pH 3.0–4.2. Again, strain PS-10 was slightly less sensitive to pH's below 4.6 than the other two strains.

**In vivo interactions between *C. cincta* and *P. s.* pv. *syringae*.** Inoculation with *P. s.* pv. *syringae* in fall 1983, either alone or with *C. cincta*, resulted in larger cankers than did inoculation with *C. cincta* alone (Fig. 5A). However, attempts to reisolate *P. s.* pv. *syringae* from these inoculation sites were not successful (Fig. 5A). Of 18 scaffold limbs inoculated with *P. s.* pv. *syringae*, 11 (61%) were colonized by naturally occurring strains of *C. cincta* by the following spring. *P. s.* pv. *syringae* inoculated in spring 1984 did not survive in bark tissue if *C. cincta* was mixedly inoculated into the same site (Fig. 5A). Where *P. s.* pv. *syringae* was inoculated alone, 15 of 18 sites (83%) still contained viable bacteria 1 mo after inoculation. Five of these sites (28%), however, were also colonized by *C. cincta*. In contrast, colonization of wounded, uninoculated sites by *C. cincta* was relatively infrequent in both fall and spring tests (Fig. 5A).

The pH of bark colonized with *C. cincta* strain 4A, as well as by the naturally occurring strains of *C. cincta* that colonized *P. s.* pv. *syringae*-inoculated sites, was reduced significantly below that of control sites ($P = 0.01$) in both fall and spring inoculations (Fig. 5B). In bark with viable *P. s.* pv. *syringae*, the pH was not

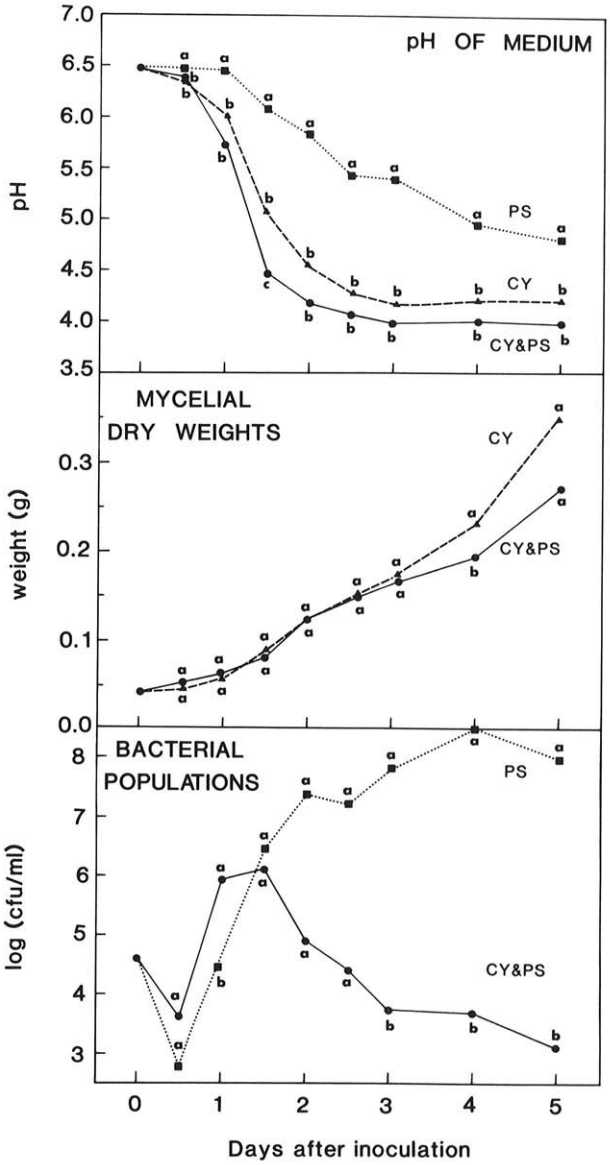


**Fig. 1.** Results of mixed and separate incubations of *Pseudomonas syringae* pv. *syringae* strain B-15$^{+}$ rif 2 (PS) with *Cytospora cincta* strain 4A (CY) in liquid culture. Means of eight replications. Treatment means at a given sampling time labeled with the same letter were not significantly different ($P = 0.05$) using Fisher's protected LSD procedure.

TABLE 1. Results of isolations from 198 freeze-injured and cankered peach trees in North Carolina, February and May 1984

| Sample and symptom | Total samples (no.) | Cytospora fungi: Fruiting bodies Samples (no.) | % | *Cytospora cincta* Samples (no.) | % | Yeasts Samples (no.) | % | Fluorescent Pseudomonads: Fluorescent Samples (no.) | % | Similar to *Pss*[a] Samples (no.) | % | Pathogenic *Pss*[b] Samples (no.) | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Trunk and scaffold limbs | | | | | | | | | | | | | |
| Cambium injured | 173 | 1 | 0.6 | 52 | 30.1 | 73 | 42.2 | 44 | 25.4 | 26 | 15.0 | 15 | 8.7 |
| Bark necrosis | 134 | 3 | 2.2 | 44 | 32.8 | 57 | 42.5 | 30 | 22.4 | 20 | 14.9 | 13 | 9.7 |
| Sour sap | 112 | 1 | 0.9 | 39 | 34.8 | 47 | 42.0 | 25 | 22.3 | 16 | 14.3 | 10 | 8.9 |
| Delimited canker | 32 | 0 | 0.0 | 9 | 28.1 | 6 | 18.8 | 9 | 28.1 | 8 | 25.0 | 4 | 12.5 |
| Callus adjacent to injured cambium | 7 | 0 | 0.0 | 4 | 57.1 | 2 | 28.6 | 2 | 28.6 | 0 | 0.0 | 0 | 0.0 |
| Twigs and buds | | | | | | | | | | | | | |
| Discolored xylem | 37 | 2 | 5.4 | 20 | 54.1 | 32 | 86.5 | 10 | 27.0 | 5 | 13.5 | 3 | 8.1 |
| Bud necrosis | 32 | 2 | 6.2 | 22 | 68.8 | 28 | 87.5 | 7 | 21.9 | 4 | 12.5 | 3 | 9.4 |
| Water-soaking | 10 | 1 | 10.0 | 6 | 60.0 | 9 | 90.0 | 5 | 50.0 | 3 | 30.0 | 2 | 20.0 |

[a]Similar in biochemical characteristics to *Pseudomonas syringae* pv. *syringae*.
[b]See text and (4) for method used to test pathogenicity.

significantly different ($P = 0.05$) from uninoculated control sites, indicating that *P. s.* pv. *syringae* did not alter the bark pH.

To determine whether wounding contributed to changes in bark pH, 17 scaffold limbs on six apparently healthy trees of equal age and scion/rootstock varieties as those inoculated, and within the same orchard, were cut and the pH immediately measured in April 1984. The average bark pH was 5.2 (standard error ± 0.14 units). When *C. cincta*-contaminated control sites were excluded from the data set, the pH of wounded, uninoculated control sites was not significantly different ($P = 0.05$) from the pH of unwounded scaffold limbs (data not shown).

Repetition of the field experiment in fall 1984 produced similar results (Fig. 6A and B). Mixed inoculation of *C. cincta* with *P. s.* pv. *syringae* resulted in larger cankers than did inoculation of *C. cincta* alone, although *P. s.* pv. *syringae* could not be reisolated from the mixed inoculation sites (Fig. 6A). Most of the sites (93%) originally inoculated with *P. s.* pv. *syringae* were colonized by naturally occurring strains of *C. cincta*, whereas 53% of uninoculated control sites were colonized (Fig. 6A). *C. cincta* significantly reduced the pH of the inner bark ($P < 0.01$), both alone and in mixed inoculations with *P. s.* pv. *syringae* (Fig. 6A). Naturally occurring strains of *C. cincta* also reduced the bark pH, as indicated by the reduced pH of *P. s.* pv. *syringae*-inoculated sites of which 93% were colonized by naturally occurring *C. cincta*, but to a lesser extent than did strain 9.2 (Fig. 6B).

## DISCUSSION

Our data show that the growth and viability of *P. s.* pv. *syringae* is inhibited by low pH, specifically by the conditions of low pH created by the vegetative growth of *C. cincta* on organic substrates (Figs. 1–4). The sensitivity of *P. s.* pv. *syringae* to pH 4.0–5.0 made it a poor competitor against *C. cincta* (Fig. 1). The same relationship was observed in trees under field conditions (Figs. 5 and 6). We conclude, therefore, that *P. s.* pv. *syringae* is incapable of long-term survival in a shared environment with *C. cincta*. Additionally, naturally occurring *C. cincta* seemed to prefer colonization of wound sites inoculated with *P. s.* pv. *syringae* versus wounded but uninoculated sites (Figs. 5A and 6A). We have no good explanation for this unless infection by *P. s.* pv. *syringae* creates an environment that *C. cincta* can more readily colonize.

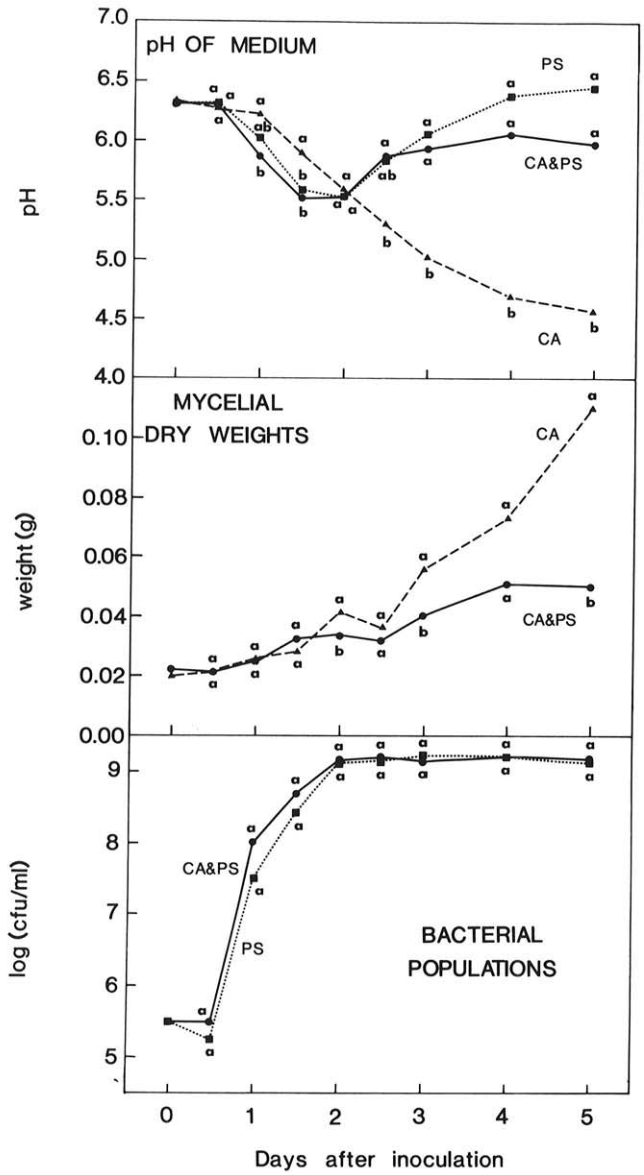


**Fig. 2.** Results of mixed and separate incubations of *Pseudomonas syringae* pv. *syringae* strain B-15$^{+}$ rif 2 (PS) with *Calosphaeria pulchella* (CA) in liquid culture. Means of eight replications. Treatment means at a given sampling time labeled with the same letter were not significantly different ($P = 0.05$) using Fisher's protected LSD procedure.

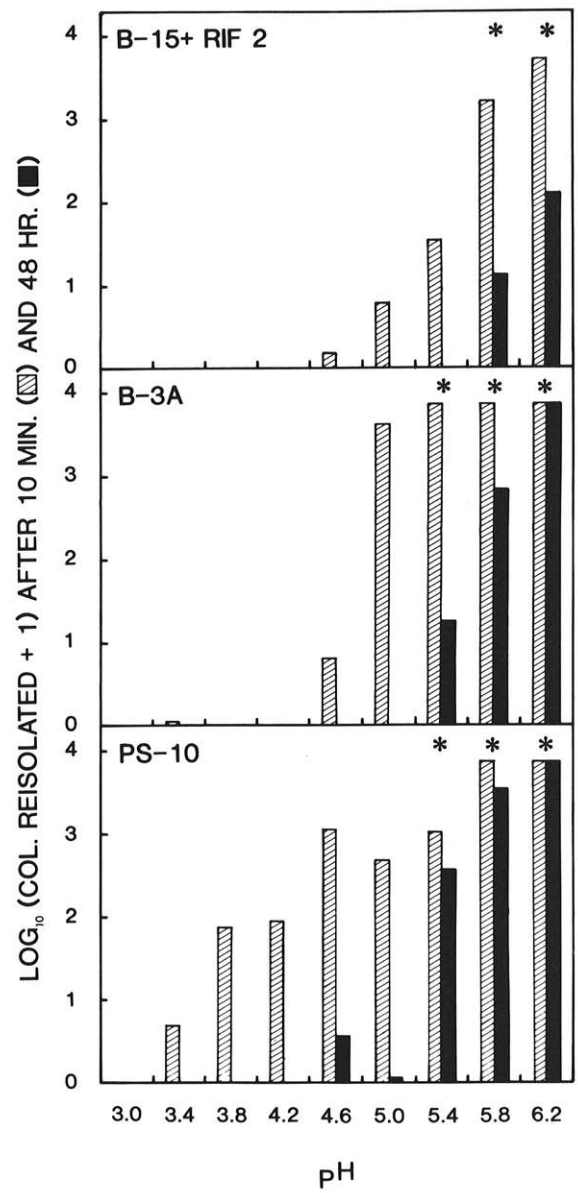


**Fig. 3.** Effect of pH of citrate buffers (0.1 M) on the survival of three strains of *Pseudomonas syringae* pv. *syringae*. Strain B-15$^{+}$ rif 2 was originally isolated from almond, whereas strains B-3A and PS-10 were isolated from peach. Means of five replications. Asterisks indicate bacteria were reisolated after 2 wk.

Wensley (20) and Royse and Ries (14) showed that antagonistic fungi, bacteria, and actinomycetes commonly occur on peach bark and may restrict *C. cincta*. Thus, initial infection by *P. s.* pv. *syringae* may negate the effects of these antagonistic microorganisms. Unlike *C. cincta*, in the in vitro experiments, the two saprophytic fungi, *Schizophyllum commune* and *Calosphaeria pulchella*, did not inhibit *P. s.* pv. *syringae*; in contrast, they were inhibited by *P. s.* pv. *syringae*. We interpret this to suggest that *C. cincta* is more than a casual invader of dead or dying peach tissue.

The possibility that fungal toxins inhibitory to *P. s.* pv. *syringae* were produced was tested by using sterile 5-mm disks of Whatman No. 1 filter paper soaked in cell-free culture filtrates of *C. cincta* and placed on the surface of nutrient agar plates (pH = 7.0) that had been uniformly streaked with a *P. s.* pv. *syringae* strain. Disks soaked in sterile liquid medium adjusted to pH 4.2 were used as controls. After 2 days, no bacterial inhibition was detected around either filtrate or control disks, suggesting that the bacterial inhibition observed in vitro was not caused by fungal toxins. However, this does not preclude the possibility that if toxin assays were performed using other media that a toxin might be detected.

We consistently observed a progressive dehydration of inner bark in cankers caused by *C. cincta*. This observation is consistent with Stanova's report (17) that *C. cincta* causes dehydration and wilting of infected limbs. Both dehydration and acidification may thus be responsible for creating an environment unsuitable for bacterial growth and survival. It has been suggested that *C. leucostoma* is adapted to infect dry bark because infections are most active following moisture stress (1). *C. cincta* may infect under similar conditions.

Rozsnyay and Klement reported that *P. s.* pv. *syringae* differed from *C. cincta* in that *P. s.* pv. *syringae* could only be reisolated from "freshly necrotized tissue," whereas *C. cincta* could be reisolated at any time throughout the year (15). Although we have sampled in late winter and early spring, within hours after freeze injury has occurred, we have found bacterial canker and *P. s.* pv. *syringae* to be rare in North Carolina peach orchards relative to infections by *C. cincta*. It is possible that the canker complex of peach trees involves tree stress or injury followed by colonization by *P. s.* pv. *syringae* and then by *C. cincta*. If this is the sequence of events, and because colonization by *C. cincta* creates an environment unsuitable for *P. s.* pv. *syringae*, this may be an explanation why this bacterium has been difficult to detect in North Carolina peach orchards.

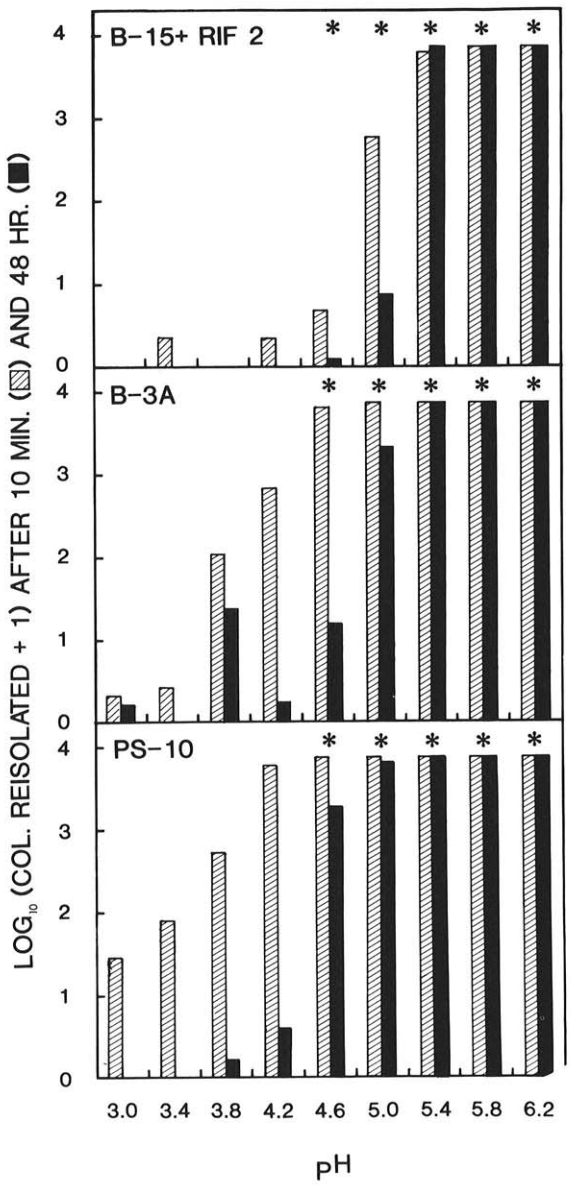


**Fig. 4.** Effect of pH of oxalic acid solutions (0.1 M) on the survival of three strains of *Pseudomonas syringae* pv. *syringae*. Strain B-15$^{+}$ rif 2 was originally isolated from almond, whereas strains B-3A and PS-10 were isolated from peach. Means of five replications. Asterisks indicate bacteria were reisolated after 2 wk.

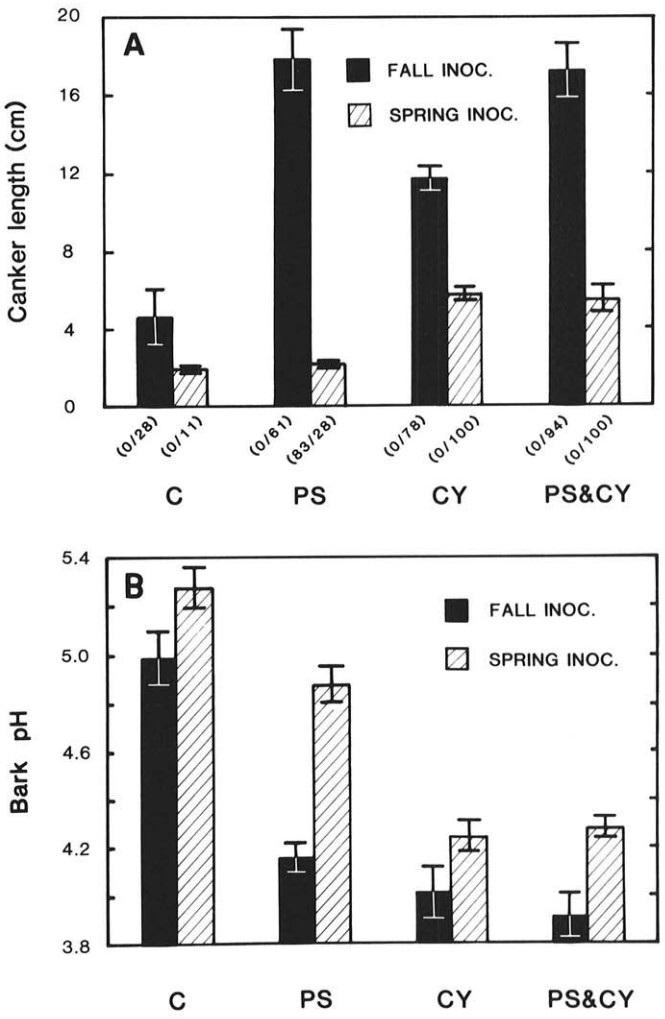


**Fig. 5.** Results of October 1983 (fall inoculation) and March 1984 (spring inoculation) inoculations of *Pseudomonas syringae* pv. *syringae* strain B-15$^{+}$ rif 2 (PS) and *Cytospora cincta* strain 4A (CY) into scaffold limbs of 8-yr-old peach trees. Uninoculated control indicated by C. Each bar represents the mean of 18 scaffold limbs; brackets indicate standard errors about each mean. Numbers in parentheses indicate, respectively, percent reisolations of (PS/CY) in April 1984. **A,** Length of necrosis of inner bark; **B,** pH of inner bark within 2–3 cm of the inoculation site in April 1984.

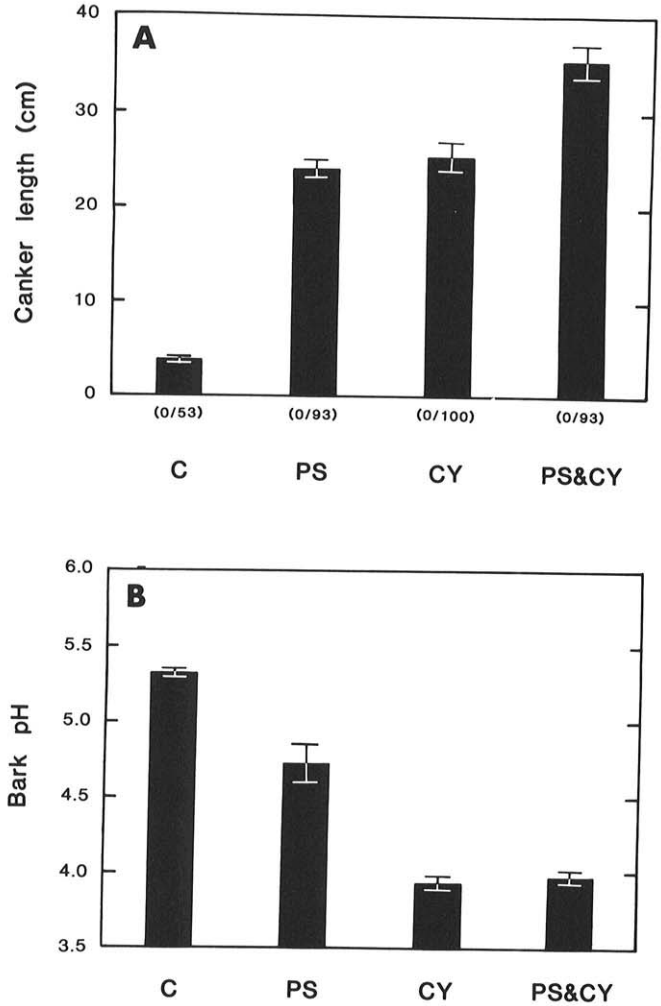


**Fig. 6.** Results of November 1984 inoculations of *Pseudomonas syringae* pv. *syringae* strain B-15$^{+}$ rif 2 (PS) and *Cytospora cincta* strain 9.2 (CY) into scaffold limbs of 9-yr-old peach trees. Uninoculated control indicated by C. Each bar represents the mean of 30 scaffold limbs; brackets indicate standard errors about each mean. Numbers in parentheses indicate, respectively, percent reisolations of (PS/CY) in May 1985. **A,** Length of necrosis of inner bark; **B,** pH of inner bark within 2–3 cm of the inoculation site in May 1985.

## LITERATURE CITED


1. Bertrand, P. F., English, H., Uriu, K., and Schick, F. J. 1976. Late season water deficits and development of Cytospora canker in French prune. Phytopathology 66:1318-1320.
2. Dowler, W. M., and Petersen, D. H. 1966. Induction of bacterial canker of peach in the field. Phytopathology 56:989-990.
3. Dowler, W. M., and Weaver, D. J. 1975. Isolation and characterization of fluorescent pseudomonads from apparently healthy peach trees. Phytopathology 65:233-236.
4. Endert, E., and Ritchie, D. F. 1984. Detection of pathogenicity, measurement of virulence, and determination of strain variation in *Pseudomonas syringae* pv. *syringae*. Plant Dis. 68:677-680.
5. Endert-Kirkpatrick, E., and Ritchie, D. F. 1985. Utilization of pectic substances by *Cytospora cincta* from peach. (Abstr.) Phytopathology 75:1375.
6. English, H., Lownsbery, B. F., Schick, F. J., and Burlando, T. 1982. Effect of ring and pin nematodes on the development of bacterial canker and Cytospora canker in young French prune trees. Plant Dis. 66:114-116.
7. Gross, D. C., Cody, Y. S., Proebsting, E. L., Jr., Radamaker, G. K., and Spotts, R. A. 1984. Ecotypes and pathogenicity of ice-nucleation-active *Pseudomonas syringae* isolated from deciduous fruit tree orchards. Phytopathology 74:241-248.
8. Kastirr, U., and Ehrig, F. 1984. Morphologische differenzierung von Cytospora-arten on apfelgeholzen. Arch. Phytopathol. Pflanzenschutz 20:453-467.
9. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of procyanin and fluorescin. J. Lab. Clin. Med. 44:301-307.
10. Konicek, D. E., and Helton, A. W. 1962. An optimum environment for the culturing of *Cytospora* isolates from stone fruits. IV. Hydrogen-ion concentration. Mycopathol. Mycol. Appl. 16:243-248.
11. Luepschen, N. S. 1981. Criteria for determining peach variety susceptibility to Cytospora canker. Fruit Var. J. 35:137-140.
12. Petersen, D. H., and Dowler, W. M. 1965. Bacterial canker of stone fruits in the southeastern states. Plant Dis. Rep. 49:701-702.
13. Ritchie, D. F., and Clayton, C. N. 1981. Peach tree short life: A complex of interacting factors. Plant Dis. 65:462-469.
14. Royse, D. J., and Ries, S. M. 1978. The influence of fungi isolated from peach twigs on the pathogenicity of *Cytospora cincta*. Phytopathology 68:603-607.
15. Rozsnyay, D. S., and Klement, Z. 1973. Apoplexy of apricots. II. Cytosporal die-back and the simultaneous infection of *Pseudomonas syringae* and *Cytospora cincta* on apricots. Acta Phytopathol. Acad. Sci. Hung. 8:57-69.
16. Scorza, R., and Pusey, P. L. 1984. A wound-freezing inoculation technique for evaluating resistance to *Cytospora leucostoma* in young peach trees. Phytopathology 74:569-572.
17. Stanova, M. 1970. Parasitism and pathogenic activity of *Cytospora cincta* Sacc. on *Prunus aremniaca* L. and on *Prunus persica* L. Batsch. Biologia 25:445-451.
18. Tekauz, A., and Patrick, Z. A. 1974. The role of twig infections on the incidence of perennial canker of peach. Phtyopathology 64:683-688.
19. Weaver, D. J., and Wehunt, E. J. 1975. Effect of soil pH on susceptibility of peach to *Pseudomonas syringae*. Phytopathology 65:984-989.
20. Wensley, R. N. 1971. The microflora of peach bark and its possible relation to perennial canker (*Leucostoma cincta* (Fr.) v. Hohnel (*Valsa cincta*)). Can. J. Microbiol. 17:333-337.
21. Willison, R. S. 1936. Peach canker investigations. II. Infection studies. Can. J. Res. 14:27-44.
22. Willison, R. S. 1937. Peach canker investigations. III. Further notes on incidence, contributing factors, and related phenomena. Can. J. Res. Sect. C-D 15:324-339.