Conidial Germination and Appressorial Formation of *Colletotrichum capsici* and *C. gloeosporioides* Isolates from Pepper

J. B. MANANDHAR, Research Fellow, International Rice Research Institute, P.O. Box 933, 1099 Manila, Philippines; G. L. HARTMAN, Research Plant Pathologist, USDA, ARS, University of Illinois, Urbana 61801; and T. C. WANG, Associate Specialist, Asian Vegetable Research and Development Center (AVRDC), Shanhua, Tainan 741, Taiwan, ROC

ABSTRACT

Manandhar, J. B., Hartman, G. L., and Wang, T. C. 1995. Conidial germination and appressorial formation of *Colletotrichum capsici* and *C. gloeosporioides* isolates from pepper. Plant Dis. 79:361-366.

Conidial germination and appressorial formation of *Colletotrichum capsici* and *C. gloeosporioides* were examined on pepper fruits and in association with some inorganic and organic compounds. Conidial germination and appressorial formation were greater on immature green or ripe red fruits of pepper (*Capsicum annuum*) variety Long Fruit than in water droplets on glass slides. Conidial germination was significantly ($P = 0.05$) higher for both fungi as concentrations of either sucrose or KCl increased. Appressorial formation for *C. capsici* was highest when sucrose was 10 mM and for *C. gloeosporioides* at 0.1 mM. Appressorial formation was reduced and mycelia formed for both fungi at higher sucrose concentrations, but not at 1-100 mM of KCl. Among six compounds tested for induced conidial germination and appressorial formation, $CaCl_2$ and sucrose caused a significant ($P = 0.05$) increase, KCl an intermediate one, and $AlCl_3$ was lowest. β-resorcilic acid did not stimulate conidial germination and appressorial formation, and Fe-resorcilic acid stimulated conidial germination only for *C. capsici*. Three test melanin biosynthesis inhibitors at 1 μg per milliliter stimulated conidial germination but varied in causing appressorial formation for both fungi. Appressorial formation of *C. capsici* was completely inhibited by tricyclazole and stimulated by fthalide and isoprothiolane; appressorial formation of *C. gloeosporioides* was completely inhibited and stimulated by isoprothiolane and tricyclazole, respectively, and only slightly inhibited by fthalide. Inorganic and organic compounds that affect conidial germination and appressorial formation may play a role in the preinfection process of *Colletotrichum* spp. on pepper fruits.

Colletotrichum capsici (Syd.) E. J. Butler & Bisby and *C. gloeosporioides* (Penz) Penz. & Sacc. in Penz. are the two main causal agents of pepper (*Capsicum annuum* L.) anthracnose in the hot humid tropics of Asia (15). Conidia of *Colletotrichum* spp. often do not germinate in situ because of the presence of germination-inhibitors in the spore matrix (13), but will germinate after being washed or rain-splash disseminated (14). In order for conidia to infect plants, there are pre- and post-infection phases associated with disease development on the plant surface (19,21). Conidia are dispersed along with water droplets on the plant surface and germinate to form adhesive appressoria at the end of germ tubes (7) by means of hemicellulose mucilage (11) to initiate direct penetration. Water droplets on the plant surface cause an increase in conductivity that leaches compounds such as free sugars, amino acids, minerals, and antifungal substances from the fruit (23,24). Formation of appressoria is a prerequisite for direct penetration of the host surface. Appressoria also may play an important role in survival (4). Conidial germination, appressorial formation, and the formation of infection hyphae are three independent processes that interact with stimulators or inhibitors present in plant exudates. Red pepper fruit exudates promoted higher conidial germination of *C. gloeosporioides* than did green fruit exudates (6). In another study (1), green fruit exudates stimulated the formation of appressoria of *C. capsici* and *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk (1), suggesting that conidial germination and appressorial formation depended upon the quality and quantity of nutrients in the leachates. Ca^{+2} and K^{+} are major cations in plant leachates (24), while aluminum was reported to inhibit calmodulin that promotes Ca-intake in *Colletotrichum* spp. (25). An intermediate iron-binding compound of anthranilic acid, 2,3-dihydroxybenzoic acid, exuded from banana fruits, stimulated conidial germination and appressorial formation of *C. musae* (Berk. & M. A. Curtis) Arx (22). Melanin biosynthesis inhibitor, a curative systemic fungicide, interfered with appressorial melanization and reduced or destabilized infection peg formation (8,10,26). Relative importance of these cations and organic compounds in conidial germination and appressorial formation of *C. capsici* and *C. gloeosporioides* is not known. The precise mechanism and physiological process of conidial germination and appressorial formation of *Colletotrichum* spp. are not well understood. The objective of this study was to compare conidial germination and appressorial formation of *C. capsici* and *C. gloeosporioides* in association with inorganic and organic compounds, and on pepper fruits.

MATERIALS AND METHODS

One isolate each of *C. capsici* and *C. gloeosporioides* was obtained from infected red fruits of a field-grown hot pepper (*Capsicum annuum* L.) breeding line PBC 595 from the Asian Vegetable Research and Development Center (AVRDC), Shanhua, Tainan, Taiwan in August 1992. Single conidial isolates of each species were obtained by picking conidia from sporulating acervuli and placing them in water. A 0.1-ml conidial suspension was placed on acidified (pH 5) 2% water agar and spread throughout the dish. Single germinating conidia were selected with the aid of a dissecting microscope. Isolates were maintained in acidified (pH 5) potato-dextrose agar at 28 C. These isolates were frequently re-isolated from inoculated detached pepper fruits to maintain their pathogenicity.

For all experiments, glassware was dipped in concentrated HCl acid for 3 h and rinsed two or more times in sterile distilled water. Nail polish diluted 1:1 with acetone was used to make each of three 5-mm inner diameter circles on sterile microscope slides. Conidial suspensions of *C. capsici* and *C. gloeosporioides* were harvested from 5-day-old potato-dextrose agar cultures grown under 12-h light per day at 28 C by brushing the colony surface with a sterile artist brush with 10 ml of sterile distilled water. Conidial suspensions were passed through two layers of cheesecloth, resuspended in 50 ml of sterilized distilled water, and centrifuged at 15,000 rpm for 10 min before discarding the supernatant. This was repeated three times, after which suspensions were adjusted to 10^5 conidia per milliliter using a hemacytometer. All test compounds were sterilized at double the test concentration. Ten microliters of a test compound and the conidial suspension of either *C. capsici* or *C. gloeosporioides* were

Accepted for publication 19 December 1994.

poured in a sterile test tube, vortexed for 5 s, and a drop of suspension spotted with a micropipette within either the nail polish circle on microscope slides or inside a pen-marked circle on pepper fruits depending upon the experiment. Slides were placed on inverted 10-cm-diameter glass dishes containing two layers of wet No. 1 Whatman filter paper and 2% water agar on the upper dish. These were incubated near 100% relative humidity in the dark at 28 C. At the end of the incubation period, slides and pepper fruits were dried on a slide drier at 45 C for 5–10 min or in a transfer hood at room temperature for 3–10 min, respectively. Nail polish was applied on the spotted circles of fruits and peeled off after drying under a hood. Peeled tissues were placed in a drop of 0.3% cotton blue in lactophenol on microscope slides. A total of 200 conidia was counted for each isolate using a compound microscope. Conidia were considered germinated when germ tubes were longer than the width of the conidium. Appressoria were counted if swollen structures were delimited by septa at the end of germ tubes. The effect of chemical compounds on conidial germination and appressorial formation was determined by evaluating the percentage of conidial germination and appressorial formation per 200 conidia in each of the three circles per slide. Each experiment had two replications unless otherwise stated and was repeated two to three times.

Conidia germination and appressorial formation. A 10-µl conidial suspension of either *C. capsici* or *C. gloeosporioides* was placed within the nail polish circles on glass slides, transferred to glass dishes and incubated as previously described. Two slides each were removed 0, 6, 12, 18, and 24 h after incubation, dried on a hot plate for 5–10 min, and stained with 0.3% cotton blue in lactophenol.

Immature green or ripe red fruits (15 cm long × 15 mm diameter at the fruit midpoint of var. Long Fruit) were harvested from greenhouse-grown plants, washed in running tap water, wiped dry with paper towels, and placed firmly on moist paper towels set on top of galvanized iron mesh in plastic boxes (27 × 18 × 9 cm deep) with sterilized distilled water on the bottom. Three 5-mm-diameter circles 1 cm apart were marked with a pen on the visual midsection of fruits. A 10-µl conidial suspension of each isolate was spotted within each circle on two lots of pepper fruits and incubated in a germinator near 100% in the dark at 28 C. One fruit lot was removed after 12 h of incubation, while the other was removed after 24 h of incubation. Fruits were dried, and nail polish applied to pen-marked areas, which were peeled off when dry and immersed in stain. Within each circle 200 conidia were counted for germination and appressorial formation. Five immature green or ripe red fruits were used as replications and were not dried or imbibed. Five glass slides were used as a control.

Sucrose and KCl concentrations. Ten mM of sucrose and KCl, made in deionized distilled water and autoclave sterilized, were diluted to 0.01, 0.1, 1, and 10 mM by adding sterilized distilled water. Equal volumes of a conidial suspension (10^5 conidia per milliliter) of each isolate and 0, 0.02, 0.2, 2, and 20 mM of either sucrose or KCl were poured in a sterile test tube and vortexed for 5 s yielding a final conidial suspension of 5×10^4 conidia per milliliter and a final concentration of either sucrose or KCl of 0, 0.01, 0.1, 1, and 10 mM. A 10-µl drop (500 conidia) either with sucrose or KCl concentration was spotted within each nail polish circle on a microscope slide, transferred to dishes, and incubated as previously described. Slides were dried, stained, and conidial germination and appressoria counted.

Efficacy of inorganic and organic compounds. Aluminum, calcium, potassium chloride, sucrose, 2,4-dihydroxybenzoic acid (β-resorcilic acid, Sigma), and β-resorcilic acid with ferrous sulfate (Fe-resorcilic acid) were compared with water for their effects on conidial germination and appressorial formation. Aluminum, calcium, potassium chlorides, and sucrose at 2×10^{-3} M were autoclave-sterilized. Fe-resorcilic acid was prepared by adding equimolar β-resorcilic acid and $Fe_2(SO_4)_3$ according to Swineburne (22). β-resorcilic acid and Fe-resorcilic acid at 2×10^{-3} M were sterilized by passing through 0.2 µm Millipore filter. Equal volumes of a conidial suspension of each isolate (10^5 conidia per milliliter) and the test compound were mixed, vortexed, and a 10-µl drop was spotted within nail polish circles on glass slides.

Efficacy of melanin biosynthesis inhibitors. Fthalide (4,5,6,7-tetrachloroph-

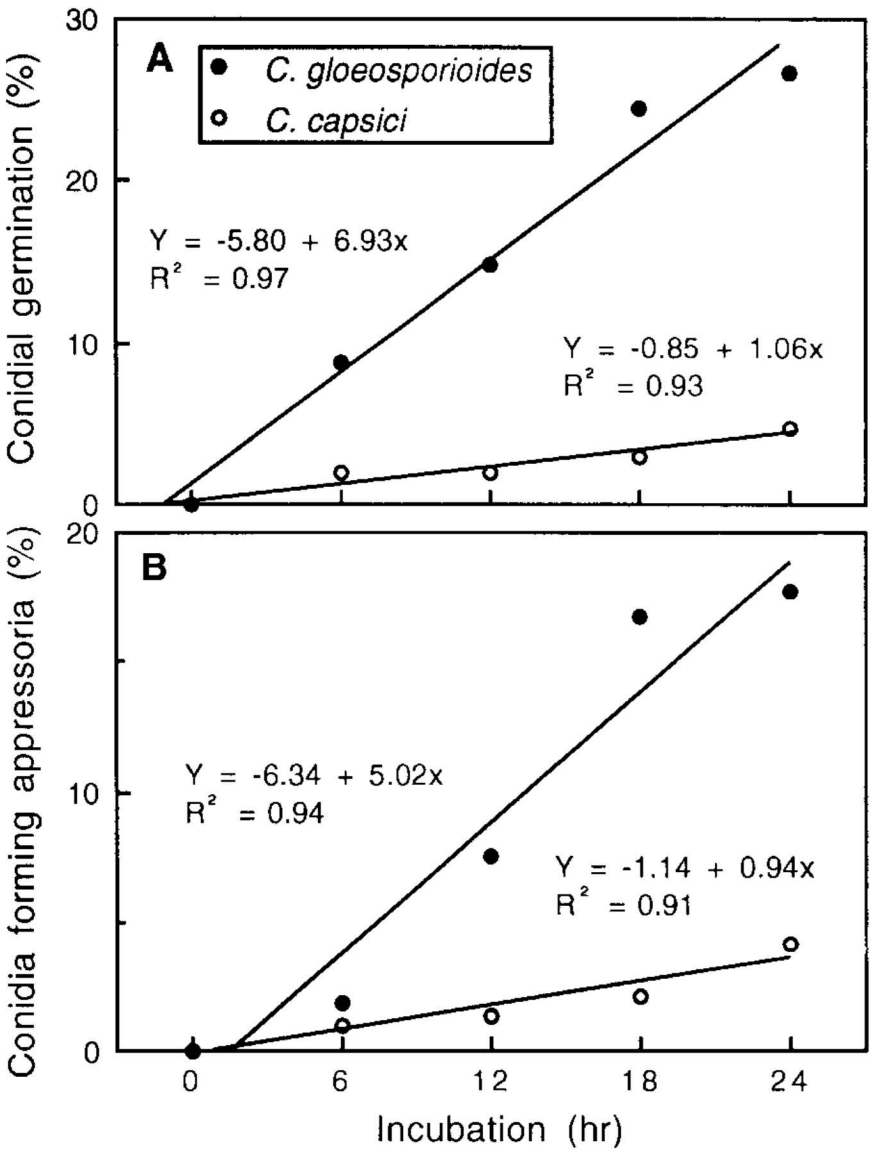

Fig. 1. Conidial germination and appressorial formation of *Colletotrichum capsici* and *C. gloeosporioides* on glass slides at different incubation periods.

thalide 50% wettable powder, Wu-Yue); isoprothiolane (diisopropyl-1,3-dithiol-2-ylidenemalonate 40% liquid, Chenghong); and tricyclazole (5-methyl-1,2,4-triazolo-(3,4,b) benzothiazole, Beam 75 W, DowElanco) at 1 μg a.i per milliliter were prepared by making stock solutions of 2 μg a.i per milliliter. The solutions were filter sterilized through a 0.2-μm Millipore filter and stored at 5 C until used. Equal volumes of a conidial suspension (10^5 conidia per milliliter) of each isolate and either fthalide, isoprothiolane, tricyclazole, or sterilized distilled water were poured into a sterile test tube to make a final conidial count of 5×10^4 conidia per milliliter in 1 μg a.i per milliliter of test compound. A 10-μl drop of conidial suspension was spotted within the nail polish circle, incubated for 24 h and stained.

RESULTS

Incubation period. Conidial germination of *C. gloeosporioides* on glass slides was significantly ($P = 0.05$) higher than *C. capsici* after 6, 12, 18, and 24 h (Fig 1A). Appressorial formation of *C. gloeosporioides* was significantly ($P = 0.05$) higher than *C. capsici* after 12, 18, and 24 h (Fig 1B).

Although conidial germination and appressorial formation on fruit surfaces was significantly ($P = 0.05$) higher than on glass slides (Table 1), there was no difference in conidial germination or appressorial formation between immature green or ripe red fruit surfaces.

Effect of sucrose and KCl. Conidial germination of *C. capsici* and *C. gloeosporioides* generally increased as concentrations of sucrose increased from 0 to 100 mM (Fig. 2A). Appressorial formation was highest at 10 mM for *C. capsici* and 0.1 mM for *C. gloeosporioides* (Fig. 2B). Appressorial formation for *C. gloeosporioides* declined at concentrations above 0.1 mM and was almost nil at 100 mM sucrose. The slight decline of appressoria of *C. capsici* at 100 mM sucrose was not significantly ($P = 0.05$) different from 10 mM sucrose. At all sucrose concentrations, conidial germination of *C. capsici* and *C. gloeosporioides* was significantly ($P = 0.05$) higher than in the water control. Similarly, *C. capsici* appressoria formation at all sucrose concentrations was significantly ($P = 0.05$) higher than in the water control. *Colletotrichum gloeosporioides* had significantly more germinated conidia at 0.1 and 1 mM sucrose, did not differ from the water control at 10 mM, but differed at 100 mM sucrose.

Conidial germination of both *C. capsici* and *C. gloeosporioides* increased significantly ($P = 0.05$) as concentrations of KCl increased (Fig. 3A). Similarly, more appressoria formed at each of the KCl concentrations than with the water control. There was no significant difference in the number of appressoria formed for either *C. capsici* or *C. gloeosporioides* between 1, 10, and 100 mM KCl (Fig. 3B).

Effect of inorganic and organic compounds. Significantly ($P = 0.05$) more conidia germinated for both *C. capsici* and *C. gloeosporioides* in calcium chloride or in sucrose than in the water control (Table 2). In potassium chloride and Fe-resorcilic acid, significantly ($P = 0.05$) more conidia germinated for *C. capsici* but not for *C. gloeosporioides*, compared with the water control. More appressoria formed in calcium chloride, sucrose, and potassium chloride than in water (Table 2). Appressoria of *C. gloeosporioides* did not form in β-resorcilic and Fe-resorcilic acid. Aluminum chloride and Fe-resorcilic acid did not differ from the water control in appressoria counts for *C. capsici*. Appressoria formed in water and calcium chloride were alike except in pigmentation. Those in water were lightly pigmented while those formed in calcium chloride were heavily pigmented (Fig. 4A, B). In aluminum chloride, chlamydospore-like structures formed at the end of short germ tubes for *C. capsici* (Fig. 4C) and thickenings developed at one side of the conidium after septation for *C. gloeosporioides* (Fig. 4D). In sucrose, three or more appressoria formed in one germ tube for *C. capsici* (Fig. 4E) while for *C. gloeosporioides* usually two appressoria formed at each end of two germ tubes produced by a conidium.

Effect of melanin biosynthesis inhibitors. Conidial germination of *C. capsici* in all the fungicide treatments was significantly ($P = 0.05$) greater than in the water control (Table 3). Germination of

Table 1. Mean percent conidial germination and appressorial formation of *Colletotrichum capsici* and *C. gloeosporioides* on immature green and red fruits, and on glass slides[w]

Surface	Conidial germination (%)		Appressorial formation (%)	
	Cc[x]	Cg[y]	Cc	Cg
Immature green fruit	14 a[z]	21 a	35 a	46 a
Ripe red fruit	15 a	22 a	33 a	52 a
Glass slide	10 b	13 b	17 b	24 b

[w] Means based on three samples in each of two replications and two experiments.
[x] *Colletotrichum capsici*.
[y] *C. gloeosporioides*.
[z] Numbers in a column followed by the same letter are not significantly different using Duncan's multiple range test at $P = 0.05$.

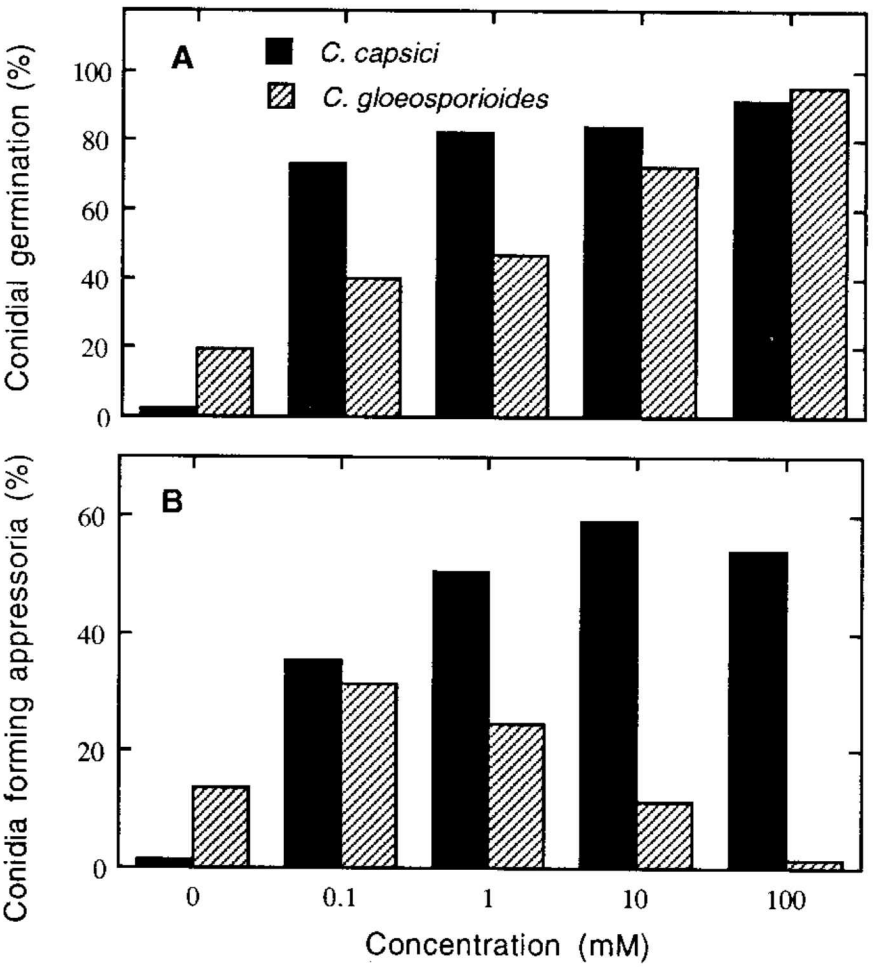

Fig. 2. Conidial germination and appressorial formation of *Colletotrichum capsici* and *C. gloeosporioides* on glass slides at different sucrose concentrations.

C. capsici conidia was significantly higher in tricyclazole while significantly lower in the water control. Germination of *C. gloeosporioides* conidia was significantly higher in isoprothiolane. Significantly more appressoria of *C. capsici* formed in fthalide and isoprothiolane than in the water control, but formed only occasionally in tricyclazole. No appressoria of *C. gloeosporioides* formed in isoprothiolane and tricyclazole, and there was no significant difference in appressorial formation between fthalide and the water control. Appressoria were normal in appearance in fthalide and in isoprothiolane for *C. capsici* (Fig. 4F, H) and *C. gloeosporioides* (Fig. 4I, K). Appressorial-like structures of *C. capsici* and *C. gloeosporioides* (Fig. 4G, J) that were not delimited by septa occasionally formed in tricyclazole.

DISCUSSION

Conidia of *C. capsici* and *C. gloeosporioides* germinated and formed appressoria in water after 6 h on glass slides and germination continued to increase linearly up to 24 h. Resuspended conidia after centrifugation had lower germination percentage and produced fewer appressoria than nonresuspended conidia (3,6; J. B. Manandhar, *unpublished data*). Conidial germination and appressorial formation were greater on pepper fruits than on glass slides, indicating that the fruit surface or nutrients from fruit exudates are important in the preinfection process. It was shown that red fruit exudates stimulated conidial germination and appressorial formation of *C. gloeosporioides* compared with green fruit exudates (6). In our study, conidia of *C. capsici* and *C. gloeosporioides* germinated and formed appressoria competently on the surface of both immature green and ripe red fruits even though the incidence of pepper fruit anthracnose occurs more at purple and ripe red fruit stages (15). Germination of *Colletotrichum* spp. conidia has been reported over a wider range of temperatures (6,17,20) than has appressorial formation. Nutrition (6) and chemical stimulation also were reported to be important in conidial germination (1,22). The process of conidial germination and appressorial formation are independent in that each may have a different set of stimuli. Appressoria that formed on immature fruits may remain quiescent until ontogenic changes occur in the fruits, and symptoms observed on different age fruit may be due to differences in the formation of infection hyphae and not to differences in conidial germination and appressorial formation.

Conidial germination was stimulated at the lowest concentration of sucrose and increased 15- and 25-fold for *C. capsici* and *C. gloeosporioides*, respectively, at 0.1 mM sucrose compared with the water control. Conidial germination reached 90–95% at 100 mM in 12 h. Grover (6) reported that *C. gloeosporioides* reached its highest rate of conidial germination at 100 mM and declined at 250 mM and higher concentrations. *Colletotrichum capsici* was reported to grow best in starch, followed by lactose, glucose, galactose, maltose, and sucrose (3), whereas *C. gloeosporioides* was reported to grow best in sucrose (6). In our study, appressorial formation increased nearly 10-fold for *C. capsici* and twofold for *C. gloeosporioides* in 0.1 mM sucrose compared with water. Appressoria of *C. capsici* formed at a wider range of sugar concentrations than did appressoria of *C. gloeosporioides*. Sucrose concentrations beyond minimal requirements stimulated conidial germination and the growth of mycelia instead of appressoria. Mycelial growth in the natural environment may be detrimental to fungal success and survival (16). Number of appressoria per conidium increased at 0.1 mM and 1 mM sucrose, suggesting that low concentrations of sucrose or possibly other sugars may aid in appressorial development and fungal survival.

Appressorial formation of *C. capsici* increased as concentrations of potassium increased in response to thigmotropism (18). In our study, conidial germination

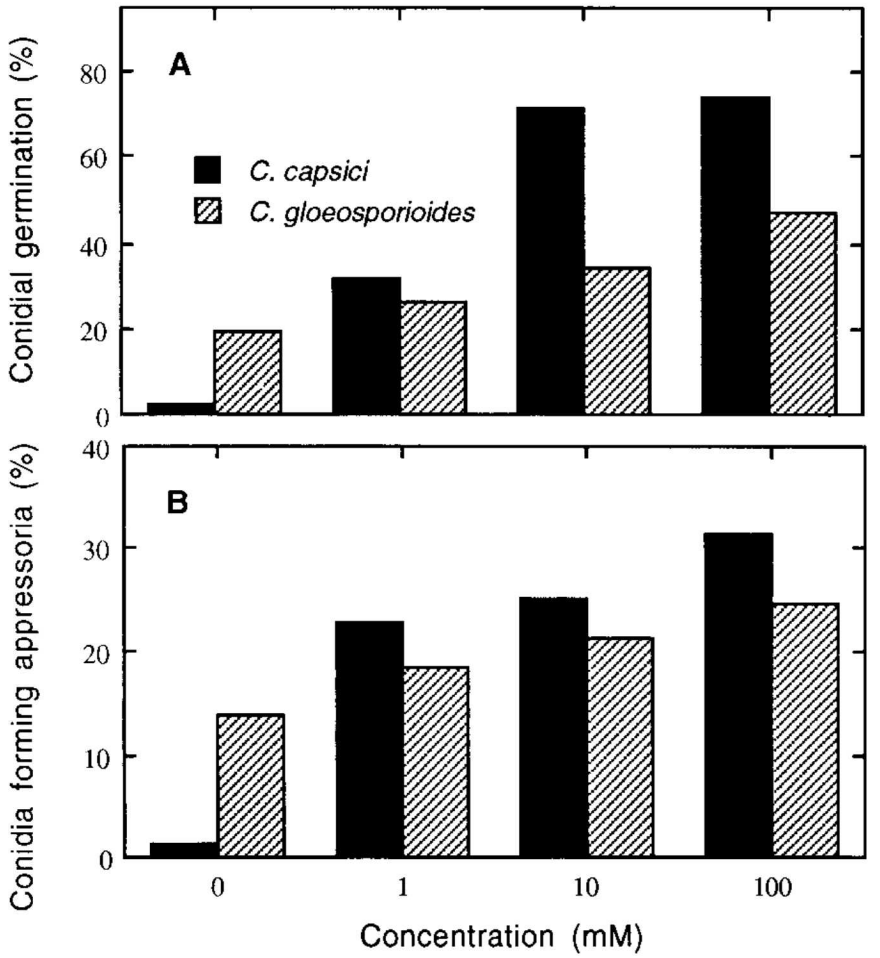

Fig. 3. Conidial germination and appressorial formation of *Colletotrichum capsici* and *C. gloeosporioides* on glass slides at different potassium chloride concentrations.

Table 2. Mean percent conidial germination of *Colletotrichum capsici* and *C. gloeosporioides* after 12 h, and percent conidia and appressorial formation 24 h after incubation near 100% relative humidity at 28 C[w]

	Conidial germination (%)		Appressorial formation (%)	
Compounds	Cc[x]	Cg[y]	Cc	Cg
Aluminum chloride	7 c[z]	30 bc	1 e	7 c
Calcium chloride	86 a	54 a	44 ab	35 a
Potassium chloride	35 b	24 bc	31 bc	24 ab
β-resorcilic acid	12 c	6 c	22 cd	0 c
Fe-resorcilic acid	44 b	10 bc	13 c–e	0 c
Sucrose	83 a	39 ab	54 a	29 ab
Water	2 c	14 bc	3 de	19 b

[w] Means based on three samples in each of two replications and two experiments.
[x] *Colletotrichum capsici*.
[y] *C. gloeosporioides*.
[z] Numbers in a column followed by the same letter are not significantly different using Duncan's multiple range test at $P = 0.05$.

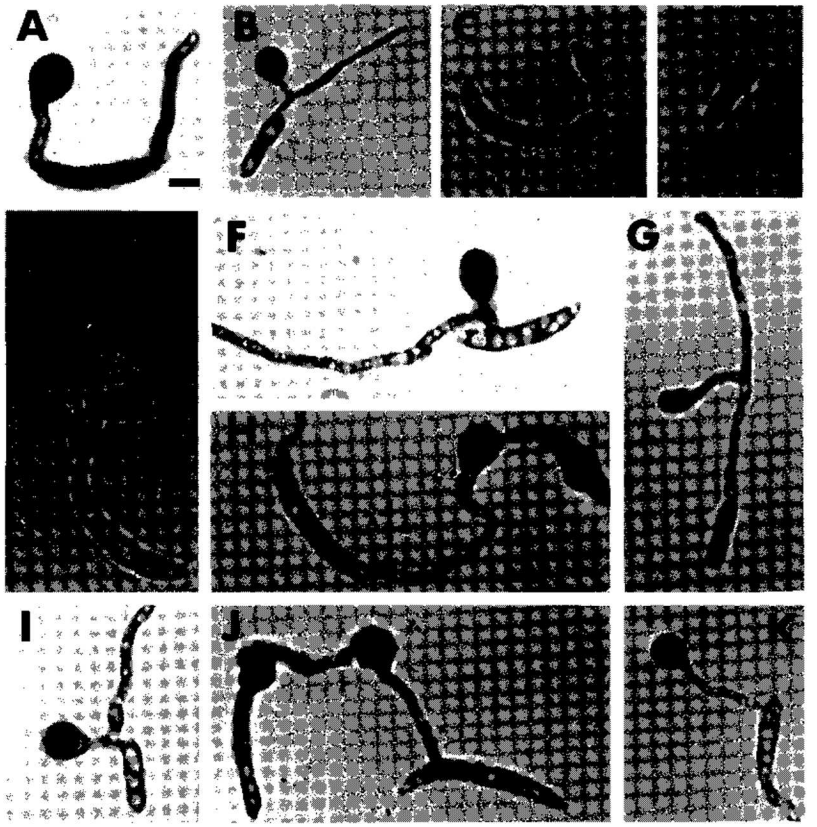

Fig. 4. Appressorial formation of *Colletotrichum capsici* and *C. gloeosporioides* in association with different compounds. **(A)** *C. capsici* in $CaCl_2$ at 10^{-3} M; **(B)** *C. gloeosporioides* in $CaCl_2$ at 10^{-3} M; **(C)** *C. capsici* in $AlCl_2$ at 10^{-3} M; **(D)** *C. gloeosporioides* in $AlCl_2$ at 10^{-3} M; **(E)** *C. capsici* in sucrose at 10^{-3} M; **(F)** *C. capsici* in 1 μg a.i. per ml of isoprothiolane; **(G)** *C. gloeosporioides* in μg a.i. per ml of tricyclazole; **(H)** *C. capsici* in 1 μg a.i. per ml of fthalide; **(I)** *C. gloeosporioides* in 1 μg a.i. per ml of fthalide; **(J)** *C. capsici* in 1 μg a.i. per ml of tricyclazole; **(K)** *C. gloeosporioides* in μg a.i. per ml of isoprothiolane. Bar in **(A)** is ca 7 μm for all figures.

Table 3. Mean conidial germination and appressorial formation of *Colletotrichum capsici* and *C. gloeosporioides* 24 h after incubation near 100% relative humidity and 28 C in several melanin biosynthesis inhibitors[w]

	Conidial germination (%)		Appressorial formation (%)	
Compounds	**Cc[x]**	**Cg[y]**	**Cc**	**Cg**
Fthalide	49 b[z]	24 b	18 a	11 a
Isoprothiolane	37 b	52 a	16 a	0 b
Tricyclazole	78 a	17 b	4 b	0 b
Water	5 c	28 b	0 b	17 a

[w] Means based on three samples in each of two replications and two experiments.
[x] *Colletotrichum capsici.*
[y] *C. gloeosporioides.*
[z] Numbers in a column followed by the same letter are not significantly different using Duncan's multiple range test at $P = 0.05$.

increased as KCl concentrations increased, but the percentage of conidia that formed appressoria did not differ when the concentration was 1 mM KCl or greater.

The effect of cations Fe^{+3} (1,22), Ca^{+2} (25), and K^{+} (18) on the infection process has been described. However, their relative importance to conidial germination and appressorial formation is not known for *Colletotrichum* spp. In our study, sucrose and calcium increased conidial germination and appressorial formation. Fruits of susceptible peppers have more reducing, nonreducing, and total sugars (2), and both Ca^{+2} and K^{+} occur in plant leachates (23,24). In our study KCl increased conidial germination and appressorial formation almost as much as Ca^{+2} and sucrose. Since washed conidia of *C. capsici* had low conidial germination and appressorial formation, significant inhibition due to Al^{+3} was not detected. Al^{+3} was not inhibitory to conidial germination but was inhibitory to appressorial formation of *C. gloeosporioides*. Fe-chelating compounds from banana fruit exudates affected conidial germination and appressorial formation of *C. musae* (22). In our study, β-resorcilic acid and Fe-resorcilic acid did not differ from the water control for *C. gloeosporioides* conidial germination and appressorial formation, but β-resorcilic acid increased appressorial formation and Fe-resorcilic acid increased conidial germination for *C. capsici*. It is difficult to explain why the behavior of these compounds differently from that in other reports (1,22). β-resorcilic acid may not be effective in binding iron present in the conidial walls of *C. capsici* and *C. gloeosporioides* strains of peppers or iron-binding compounds may be different for pepper isolates of *Colletotrichum* spp. The different response of the pepper isolates of *Colletotrichum* may be due to a different form of 2,3-dihydroxybenzoic acid reported earlier (22). Diseased fruits are secondary sources of inocula that cause plant-to-plant spread in pepper fields. Conidia produced from pepper fruits are unlikely to be Fe-depleted. Phylloplane microorganisms compete for the least available Fe^{+3} on the plant surface and stimulate the production of siderochrome that has more affinity with iron. Siderochrome is utilized by germinating *Colletotrichum* spp. conidia to remove iron from conidial walls (12). In addition to siderochrome, phylloplane bacteria produce unknown stimulants that cause conidial germination of *Colletotrichum* spp. (9). However, β-resorcilic acid was not different from the water control in causing conidial germination of *C. capsici* and *C. gloeosporioides*, and Fe-resorcilic acid was superior to β-resorcilic acid in causing conidia germination of *C. capsici* but not of *C. gloeosporioides*. Effects of preformed antifungal compounds present in leachates (24) on conidial germination and appressorium formation of *Colletotrichum* spp. are not known. One possible mechanism that may account for the chemotropic stimulus that initiates the infection process during the prepenetration phase (5) is that appressoria remain quiescent on green fruits, then develop lesions only when fruits become ripe red (1,6,22).

Colletrotrichum capsici conidia germinated at low concentrations of isoprothiolane but not in fthalide. Tricyclazole stimulated germination of *C. gloeosporioides* conidia. Melanin biosynthesis inhibitor is known for reducing appressorial melanization, thereby reducing or destabilizing the formation of infection pegs (8,10,26). Appressorial formation was also stimulated by fthalide and isoprothiolane for *C. capsici*, but not for *C. gloeosporioides*. Tricyclazole also inhibited appressorial formation of *C. capsici*. Tricyclazole inhibited appressorial formation for both *C. capsici* and *C. gloeosporioides*, whereas isoprothiolane inhibited appressorial formation only for *C. gloeosporioides*.

LITERATURE CITED

1. Adikaran, N. K. B., Brown, A. E., and Swinburne, T. R. 1983. Observations on infection of *Capsicum annuum* by *Glomerella cingulata* and *Colletotrichum capsici*. Trans. Br. Mycol. Soc. 80:395-401.
2. Azad, P. 1991. Fate and role of chemical constituents of chili fruits during infection with *Colletotrichum capsici*. Indian Phytopathol. 44:129-131.
3. Chung, B.-K., and Lee, S. B. 1986. Effect of

conidial number and nutrition on the germination of conidia in *Colletotrichum dematium* f. sp. *capsicum* causing red pepper anthracnose. Korean J. Plant Prot. 25:41-46.
4. Emmett, R. W., and Parbery, D. G. 1975. Appressoria. Annu. Rev. Phytopathol. 13:147-167.
5. Flentze, N. T. 1959. The physiology of penetration and infection. Pages 76-87 in: Plant Pathology - Problems and Progress 1908-1958. C. S. Holton et al, eds. The Wisconsin University Press, Madison, WI.
6. Grover, R. K. 1971. Participation of host exudate chemicals in appressorium formation by *Colletotrichum piperatum*. Pages 509-518 in: Ecology of Leaf Surface Microorganisms. T. F. Preece and C. H. Dickinson, eds. Proc. Internl. Symp., Univ. Newcastle upon Tyne. University of Newcastle upon Tyne.
7. Hamer, J. E., Howard, R. J., Chumley, F. G., and Valent, B. 1988. A mechanism for surface attachment in spores of a plant pathogenic fungus. Science 239:288-290.
8. Hirooka, T., Miyagi, Y., Araki, F., Kunoh, H., and Ishizaki, H. 1982. The effect of isoprothiolane on the emergence of infection pegs from appressoria of *Pyricularia oryzae*. Pestic. Sci. 13:379-386.
9. Koomen, I., and Jeffries, P. 1993. Effects of antagonistic microorganisms on the postharvest development of *Colletotrichum gloeosporioides* on mango. Plant Pathol. 42:230-237.
10. Kubo, Y., Suzuki, K., Furusawa, I., and Yamamoto, M. 1982. Effect of tricyclazole on appressorial pigmentation and penetration from appressoria of *Colletotrichum lagenarium*. Phytopathology 72:1198-1200.
11. Lapp, M. S., and Skoropad, W. P. 1978. Nature of adhesive material of *Colletotrichum graminicola* appressoria. Trans. Br. Mycol. Soc. 70:221-223.
12. Lenne, J. M., and Parbery, J. M. 1976. Phyllosphere antagonists and appressorium formation in *Colletotrichum gloeosporioides*. Trans. Br. Mycol. Soc. 66:334-336.
13. Louis, I., Chew, A., and Lim, G. 1988. Influence of spore density and extracellular conidial matrix on spore germination in *Colletotrichum capsici*. Trans. Br. Mycol. Soc. 91:694-699.
14. Madden, L. V., Wilson, L. L., and Ellis, M. A. 1993. Field spread of anthracnose fruit rot of strawberry in relation to ground cover and ambient weather conditions. Plant Dis. 77:861-866.
15. Manandhar, J. B., Wang, T. C., and Hartman, G. L. 1995. Microdrop inoculation of *Colletotrichum gloeosporioides* and disease development on pepper fruits. Plant Dis. (In press.)
16. Mercer, P., Wood, R. K. S., and Greenwood, A. D. 1970. The effect of orange extract and other additives on anthracnose of French bean caused by *Colletotrichum lindemuthianum*. Ann. Bot. (Lond.) 34:593-604.
17. Miehle, B. R., and Lukezik, F. L. 1972. Studies on conidial germination and appressorium formation by *Colletotrichum trifolii* Bain & Essary. Can. J. Microbiol. 18:1263-1269.
18. Muruganandam, V., Venkatachalam, S., and Suryanarayanan, R. S. 1991. Potassium enhances thigmotropically stimulated appressorium formation in *Colletotrichum capsici*. Cryptogam. Mycolog. 12:155-159.
19. Parbery, D. G., and Emmett, R. W. 1977. Hypothesis regarding appressoria, spores, survival and phylogeny in parasitic fungi. Rev. Mycol. (Paris) 41:429-447.
20. Skoropad, W. P. 1967. Effect of temperature on the ability of *Colletotrichum graminicola* to form appressoria and penetrate barley leaves. Can J. Plant Sci. 47:431-434.
21. Stoessl, A. 1983. Secondary plant metabolites in preinfectional and postinfectional resistance. Pages 71-121 in: The Dynamics of Host Defense. J. A. Bailey and B. J. Deverall, eds. Academic Press, NY.
22. Swinburne, T. R. 1976. Stimulants of germination and appressoria formation by *Colletotrichum musae* (Berk. & Curt) Arx. in banana leachates. Phytopathol. Z. 87:74-90.
23. Tukey, H. B., Jr. 1966. Leaching of metabolites from above-ground plant parts and its implications. Torrey Bot. Club Bull. 93:385-401.
24. Tukey, H. G., Jr., Tukey, H. B., and Wittwer, S. H. 1958. Loss of nutrients by foliar leaching as determined by radioisotopes. Am. Soc. Hortic. Sci. 71:496-506.
25. Warwar, V., and Dickman, M. B. 1992. Calcium and calmodulin are involved in appressorium development of *Colletotrichum trifolii*. (Abstr.) Phytopathology 82:1064.
26. Wolkow, P. M., Sisler, H. D., and Vigil, E. L. 1983. Effect of inhibitors of melanin biosynthesis on structure and function of appressoria of *Colletotrichum lindemuthianum*. Physiol. Plant Pathol. 22:55-71.