

Effect of Metalaxyl on Capsidiol Production in Stems of Pepper Plants Infected with *Phytophthora capsici*

BYUNG KOOK HWANG, Professor, Department of Agricultural Biology, Korea University, Seoul, Korea, and NAK KYU SUNG, Department of Plant Pathology, Institute of Agricultural Sciences, Suwon, Korea

ABSTRACT

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Capsidiol production, associated with control of *Phytophthora* blight of pepper plants by the systemic fungicide metalaxyl, was determined in the infected stems of pepper cultivars resistant and susceptible to *Phytophthora capsici*. Increasing concentrations of metalaxyl in soil treatments 1 day before stem-inoculation with *P. capsici* gradually retarded the lesion development on the stems of pepper plants, but stimulated capsidiol production in the infected stems. Metalaxyl treatments did not change the rapidly growing stem lesions into brownish necrotic ones of the hypersensitive reaction. In particular, the accumulation of capsidiol by metalaxyl treatment was more pronounced in the resistant cultivar Kingkun than in the susceptible cultivar Hanbyul. At metalaxyl concentrations of 1 and 5 $\mu\text{g}/\text{ml}$, lesions appeared on the stems 2 days after inoculation, with a maximum production of capsidiol. As the stem lesions developed and enlarged, the production of capsidiol in metalaxyl-treated, infected stems declined to a final base level similar to that in the infected control stems 5 days after inoculation. The metalaxyl treatments of 1 and 2 days after inoculation produced more capsidiol in the susceptible seedlings than before inoculation.

Phytoalexins are produced by plants not only in response to interactions with fungi, bacteria, viruses, nematodes, and other living organisms, but also following treatment with many chemicals, irradiation by ultraviolet light, and exposure to the products of microbial metabolism (1). In addition, phytoalexin production can also be induced by fungicides. Reilly and Klarman (7) demonstrated that several fungicides induced the phytoalexin, glyceollin, in soybean (*Glycine max* (L.) Merr.) hypocotyl tissues. Cartwright et al (3) also reported that dichlorocyclopropanes may exert their systemic fungicidal activity against the rice blast disease by causing rice to synthesize the phytoalexins, monilactones A and B.

Recently, it was demonstrated that control of *Phytophthora* rot of soybean hypocotyls by the systemic fungicide metalaxyl caused a hypersensitive response accompanied by glyceollin production (2,12). Subsequently, it was found that in plants only partially protected by metalaxyl, glyceollin concentrations exceeded the EC_{50} in vitro in all lesions. However, spread of the pathogen was not restricted (5), suggesting that glyceollin may not play a significant role in inhibiting spread of the fungus in metalaxyl-treated seedlings.

Stössl et al (9) first reported that pepper fruits produced an antifungal

sesquiterpenoid phytoalexin, capsidiol, in response to infection by several pathogenic and nonpathogenic fungi. The relation between capsidiol concentration and speed of fungal invasion in stems of pepper cultivars susceptible or resistant to *Phytophthora capsici* Leonian has been assessed by Molot et al (6). However, they could not demonstrate a convincing connection between capsidiol induction at the infection front and inhibition of lesion development, nor a clear difference in the reaction of the resistant cultivar.

It has not been demonstrated that applications of the systemic fungicide metalaxyl for control of *Phytophthora* blight of pepper plants affect capsidiol production in infected pepper plants. In the experiments reported here, we examined capsidiol production in stems of pepper plants treated with metalaxyl and inoculated with *P. capsici*. We also compared capsidiol production in the susceptible and resistant cultivars treated with metalaxyl.

MATERIALS AND METHODS

Plant, fungus, and fungicide. The pepper (*Capsicum annuum* L.) cultivars used were Hanbyul and Kingkun, differing quantitatively in susceptibility to *Phytophthora* blight. Ten plants per pot were grown in plastic pots (5 \times 15 \times 10 cm) containing a mixture of steam-sterilized loam soil, sand, and peat (3:5:2, v/v). Fertilizer was applied at the rate of 0.27-0.27-0.13 g of actual N-P-K per pot at 3 wk after planting. Plants were maintained at 25 ± 2 C during a 16-hr light period from 0600 to 2200 hr of

about 5,000 lx and at 16 ± 2 C during darkness until they had eight or 10 expanded leaves.

An isolate of *P. capsici* was cultured on V-8 agar for 7 days at 25 ± 1 C. Zoospores were produced as described previously (4), and a zoospore suspension was used as inoculum. Zoospores in suspensions were counted with a hemacytometer, and the concentration was adjusted to $1 \times 10^5/\text{ml}$ with sterile tap water.

The fungicide used in this study was metalaxyl, formulated as a 25% a.i. wettable powder. All concentrations are given as active ingredient (Ridomil, Seoul Agricultural Chemical Co.). Metalaxyl suspensions were prepared in sterile tap water to give appropriate concentrations. Metalaxyl treatments were performed by pouring the 20 ml of metalaxyl suspensions per pot uniformly over the surface 1 day before stem-inoculation with *P. capsici*, except in the experiment on the effect of time of metalaxyl application.

Inoculation procedures and disease assessment. A longitudinal wound, about 1 cm long and 1 mm deep, was made with a razor blade on each stem of pepper plants, approximately 2 cm apart from the soil surface. A small quantity of sterile cotton soaked in zoospore suspension ($1 \times 10^5/\text{ml}$) for 30 min was placed on the wounded sites of stems. The inoculation sites were then covered with plastic tape to maintain the moist condition. Metalaxyl suspensions at various concentrations were poured into the soil before or after stem-inoculation with *P. capsici*.

Disease development, as measured by lesion length, in pepper plants was rated every day after inoculation. The data are the means of three replicates from the disease ratings of 15 infected plants.

Extraction and estimation of capsidiol. Fifteen stems of pepper plants per treatment replicate were harvested at various intervals after inoculation. Three replicates of each treatment were used. The infected stems were sliced transversely in 3-mm-thick sections for 4 cm upward from the inoculated sites. Comparable areas of healthy, metalaxyl-treated stems also were similarly sliced. The sliced stem sections were placed in 250-ml Erlenmeyer flasks with 15 ml/g fresh weight of 40% aqueous ethanol and were then vacuum-infiltrated. The flasks were stoppered and placed on a recip-

rocal shaker (110 strokes per min) for 7 hr. After agitation by shaking, the stem tissues were removed by filtration and then dried overnight at 95 C. The filtrate was vacuum-concentrated at 40 C to approximately one-half volume. The concentrate was partitioned twice with ethyl acetate. The pooled ethyl acetate layer was dehydrated with $MgSO_4$ and evaporated to dryness at 40 C. Following transfer of the residue to vials with peroxide-free diethyl ether and evaporation of ether under nitrogen, the dry residue was dissolved in 0.5 ml of ethyl acetate and then stored at -20 C until used for gas chromatographic analysis of capsidiol.

All capsidiol analyses were made by gas-liquid chromatography, according to Ward et al (14). The ethyl acetate sample was evaporated under nitrogen. The final residues were dissolved in a solution of methyl myristate (4 mM) in ethanol, the ester serving as an internal standard. Aliquots (2-3 μ l) from each ethanol solution were injected into a Packard model 419 gas liquid chromatograph fitted with a glass column (182 cm long, 2 mm inside diameter) containing Gas Chrom Q (80-120 mesh) coated with 3% SE30. The column was kept at 162 C, the injector at 192 C, and the flame ionization detector at 230 C. The carrier gas was nitrogen at a 40-ml/min flow rate with hydrogen and air at 30 and 300 ml/min, respectively. Retention times were 7.5 and 14.5 min for methyl myristate and capsidiol, respectively. Capsidiol in the samples was identified by cochromatography with authentic capsidiol, which was obtained from Dr. A. Stössl, Agriculture Canada, Research Center, London, Ontario, Canada.

RESULTS

Effect of concentration of metalaxyl on capsidiol production. When metalaxyl was applied to the soil 1 day before stem-inoculation of zoospore suspension of *P. capsici*, lesion development from the inoculation site of the stem decreased in the two cultivars, Hanbyul and Kingkun, with increasing amounts of metalaxyl (Fig. 1A). Four days after stem-inoculation at the eight-leaf stage, susceptible Hanbyul was more severely diseased than resistant Kingkun at 1 and 3 μ g/ml. As the concentration of metalaxyl increased, disease progress gradually decreased, the decline being more pronounced in Hanbyul than in Kingkun. In particular, at the drench treatment of 11 μ g/ml of metalaxyl, lesion development was strikingly inhibited to 1-cm lesion length in the two cultivars.

In contrast to the disease development in both cultivars, the production of capsidiol in pepper stems increased with increasing amounts of metalaxyl (Fig. 1B). In comparable inoculated, drenched plants, resistant Kingkun always

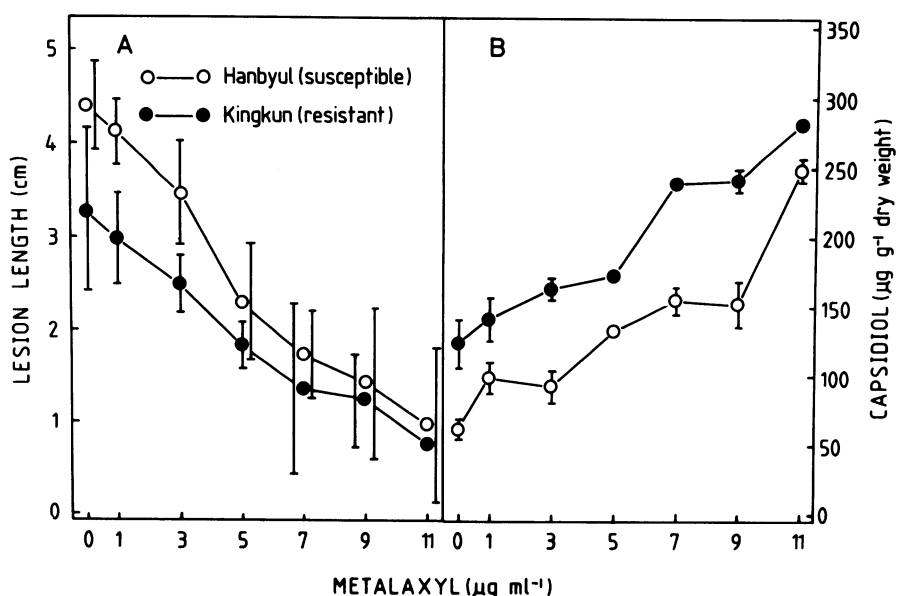


Fig. 1. Influence of concentration of a metalaxyl drench in soil on (A) disease development and (B) capsidiol production in the stems of pepper cultivars Hanbyul (susceptible) and Kingkun (resistant). Plants were inoculated at the eight-leaf stage with zoospore suspensions of *Phytophthora capsici* at the bottom of the stem. Data were obtained on day 4 after stem-inoculation. The vertical bars represent standard deviations.

contained more capsidiol in stems than susceptible Hanbyul, irrespective of metalaxyl concentration. However, capsidiol accumulation by metalaxyl was more noticeable in Hanbyul than in Kingkun. At 11 μ g/ml of metalaxyl, the two cultivars had similar levels of capsidiol. Metalaxyl treatment did not induce capsidiol in healthy, uninoculated plants of both cultivars.

Effect of the time of metalaxyl application on capsidiol production. When metalaxyl was applied to soil at 7 μ g/ml 3 or 7 days before and at the time of stem-inoculation on pepper plants at the 10-leaf stage, *Phytophthora* blight was inhibited in resistant Kingkun and greatly restricted in susceptible Hanbyul (Fig. 2). Small quantities of capsidiol accumulated similarly in the two pepper cultivars. When the soil drench of metalaxyl was delayed after inoculation, lesion development gradually progressed in both pepper cultivars and capsidiol accumulated in large amounts. In particular, the accumulation of capsidiol in metalaxyl-treated plants was much more marked in susceptible Hanbyul than in resistant Kingkun 1 or 2 days after inoculation.

Time course of capsidiol production by metalaxyl. As previously described, the retardation of lesion development was directly proportional to the amount of metalaxyl applied (Fig. 3). The accumulation of capsidiol in stems of the inoculated, undrenched controls increased to a maximum at 3 days after inoculation in the two cultivars and declined afterwards. At 1 or 5 μ g/ml of metalaxyl, accumulation of capsidiol increased more than in the inoculated, undrenched control, with a maximum at 2 days after inoculation in both cultivars.

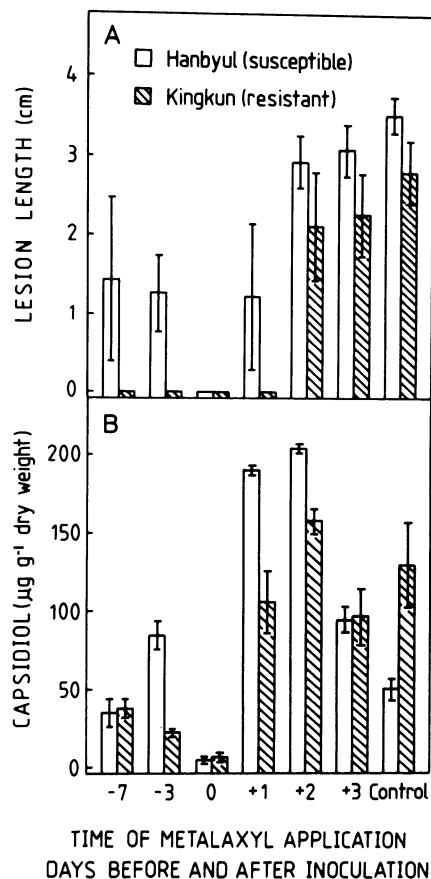


Fig. 2. Influence of the time of soil drench of metalaxyl relative to the time of inoculation on (A) disease development and (B) capsidiol production in the stems of pepper cultivars Hanbyul (susceptible) and Kingkun (resistant). Plants were inoculated at the 10-leaf stage with zoospore suspensions of *Phytophthora capsici* at the bottom of the stem. Data were obtained on day 4 after stem-inoculation. The vertical bars represent standard deviations.

In the cases of these treatments, there were no large differences between the two cultivars in capsidiol accumulation by metalaxyl. Capsidiol production in both cultivars was about three times higher at 5 $\mu\text{g}/\text{ml}$ of metalaxyl than at 1 $\mu\text{g}/\text{ml}$ of metalaxyl at 2 days after inoculation. The concentration of capsidiol in stems decreased after the maximum accumulation 2 days after stem-inoculation, regardless of metalaxyl treatment and pepper cultivar.

DISCUSSION

In addition to the efficacy of metalaxyl for control of *P. capsici* on pepper plants (11), metalaxyl treatment stimulated capsidiol production in pepper stems infected with the fungus. Whereas some fungicides are themselves capable of inducing phytoalexin production in plants (7), it seems likely that metalaxyl itself does not elicit capsidiol production in pepper stems but, rather, increases the capacity of pepper plants to synthesize capsidiol in response to infection by *P. capsici*. This observation is supported by the absence of capsidiol production in uninoculated, metalaxyl-treated plants in this study. Application of metalaxyl in pepper plants infected with *P. capsici* retarded disease development but did not

change the rapidly growing stem lesions into brownish necrotic ones of the hypersensitive reaction. In contrast to the findings of Ward et al (12) that control by metalaxyl of *P. megasperma* f. sp. *glycinea* in soybean hypocotyls resulted in a hypersensitive resistant response accompanied by glyceollin production, our results suggest that metalaxyl can also stimulate phytoalexin production even in a susceptible response to *Phytophthora* infection in plants, even though there is no hypersensitive resistant response. In the control of rice blast by dichlorocyclopropanes, Cartwright et al (3) also suggested that the compound acts by sensitizing the plant such that subsequent inoculation with *P. oryzae* Br. & Cav. results in a resistant reaction.

With increasing metalaxyl concentrations, capsidiol production was stimulated in infected pepper stems. The increase followed similar patterns in the susceptible and resistant cultivars (Fig. 1). Inhibition of disease development, and also stimulation of capsidiol production by metalaxyl appear to be greatly influenced by the degree of resistance of pepper cultivars to *P. capsici*. Therefore, resistant pepper cultivars may possess a genetic basis for producing more

capsidiol than susceptible ones, although the difference between the susceptible and resistant responses of pepper plants to *P. capsici* is quantitative rather than qualitative (4). Whereas large quantities of glyceollin produced in soybean by an incompatible host-pathogen interaction remained constant over a range of metalaxyl concentrations (12), it is of interest that capsidiol amounts in pepper increased significantly in infected stems of resistant cultivars with increasing doses of metalaxyl.

When compared with inoculated, metalaxyl-untreated controls, metalaxyl application before inoculation prevented disease development but did not stimulate capsidiol production. However, larger quantities of capsidiol, especially in susceptible Hanbyul, were produced in infected stems of pepper plants treated with metalaxyl at intervals during disease development (Fig. 2). These results suggest that metalaxyl may cause pepper stems to accelerate capsidiol production by affecting the host-pathogen interaction after the pathogen spreads into stem tissue. Our earlier finding, that metalaxyl is very effective for inhibition of mycelial growth of *P. capsici* (11), raises the possibility, although unproven, that fungal cell wall polymers, which elicit capsidiol accumulation, may also be released in infected stem tissue from mycelium of *P. capsici* by metalaxyl treatment, as observed by Yoshikawa et al (15) in the *P. m. f. sp. glycinea*-soybean system.

The stimulation of capsidiol production by metalaxyl reached its maximum at 2 days after inoculation, when typical symptoms began to appear in wound-inoculated stems (Fig. 3). These results provide evidence that metalaxyl plays a critical role in inducing capsidiol production in the early stages of host-pathogen interaction. In contrast, capsidiol production in the inoculated, metalaxyl-untreated controls increased, with a maximum at 3 days after inoculation when considerable disease progress occurred, thus suggesting that *Phytophthora* infection may be important for stimulation of capsidiol production in the later stages of the host-pathogen interaction.

The amount of capsidiol decreased to base levels after the maximum accumulation. In particular, the effectiveness of metalaxyl treatment was conspicuous in reducing the amount of capsidiol in infected stems. The disappearance of capsidiol with time may not be due to degradation by *P. capsici* (10,13) but, rather, due to the metabolism of it by pepper tissue (8) or possibly to a reaction with metalaxyl. Although unproven, it is also possible that some unknown factors at a late stage of the host-pathogen interaction may degrade or otherwise modify capsidiol in infected, metalaxyl-treated stems. A further

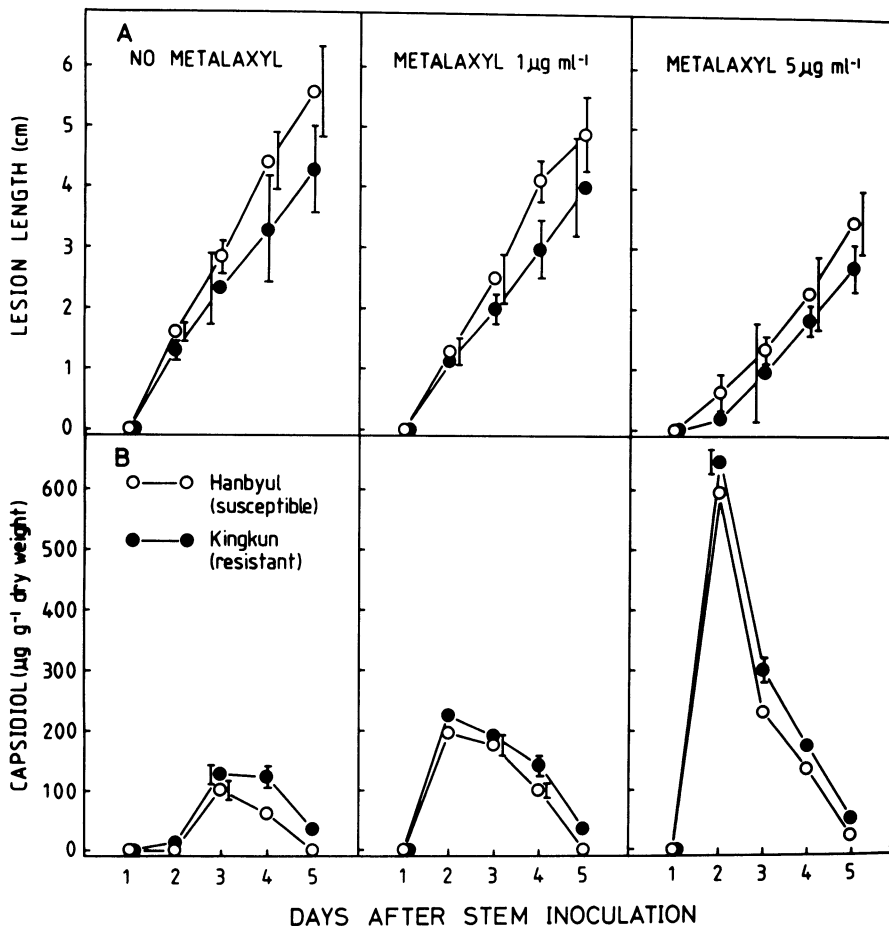


Fig. 3. Time courses of (A) disease progress and (B) capsidiol production in the stems of pepper cultivars Hanbyul (susceptible) and Kingkun (resistant). Plants were inoculated at the eight-leaf stage with zoospore suspensions of *Phytophthora capsici* at the bottom of the stem after soil treatment with metalaxyl. The vertical bars represent standard deviations.

detailed investigation of the relative rates of fungal expansion and capsidiol production and degradation at the metalaxyl-treated infection site is needed.

ACKNOWLEDGMENT

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