Inhibition of Photosynthesis Diminishes Antibacterial Action of Pepper Plants

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ABSTRACT

Populations of *Xanthomonas phaseoli* declined rapidly when inoculated pepper plants were maintained in the light. Populations of the bacterium increased when pepper plants were either maintained in the dark, or treated with 3-(*p*-chlorophenyl)-1,1-dimethylurea (CMU) to inhibit photosynthesis. Occurrence of the hypersensitive response (HR) in peppers inoculated with *X. phaseoli* was noted in plants maintained in the light, in the dark, and in those treated with CMU. The HR does not seem to be the controlling factor in the resistance of pepper to *X. phaseoli*, but photosynthetic capacity does appear to influence resistance.

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Inoculation with phytopathogenic bacteria may result in progressive disease (compatible response) of a host plant or in a hypersensitive reaction (incompatible response) in a nonhost plant (5). The plant hypersensitive reaction (HR) is considered to be a defense mechanism which limits bacterial spread and causes a decrease in populations of incompatible bacterial pathogens (5). Incompatible pathogen-host combinations do not always result in HR (7) and there are sometimes initial bacterial population increases in these situations (4).

The HR has been suppressed through use of calcium salts (1), bacterial proteins (12), cycloheximide (11), and cytokinins (9), but the effects on bacterial populations were not reported. Lozano and Sequeira (8) found that tobacco leaves covered with aluminum foil to exclude light did not give a typical HR. They also reported that foil-covered tobacco leaves were not protected from HR by pretreatment with heat-killed *Pseudomonas solanacearum* E. F. Sm. as they were in the light (8). Lovrekovich (6) reported that *P. fluorescens* Migula (a saprophyte) was able to multiply and cause a progressive necrosis of tobacco leaves in the dark and at high relative humidity (RH).

Stall and Cook (15) extracted a bacterial inhibitor from pepper leaves undergoing HR, while Lozano and Sequeira (7) reported a similar extract from tobacco leaves following HR. A causal relationship between the HR and plant production of a bacterial inhibitor was suggested (5, 7), but not specifically proven.

In this paper, the effects of light and of a chemical inhibitor of photosynthesis on a bacterial disease, on the HR, and on plant production of a bacterial inhibitor are examined.

MATERIALS AND METHODS.—Virulent isolates of *Xanthomonas phaseoli* (E. F. Sm.) Dows. (Xp-517) and *X. vesicatoria* (Doidge) Dows. (Xv-728) were grown on lima bean agar. Inocula were prepared by washing 48-h-old cultures from the agar, centrifuging at 4,000 *g* for 5 min and resuspending the pellet in sterile distilled water to a bacterial concn of ca. 10^8 cells/ml. Inoculations were performed by hypodermic syringe injection of the bacterial suspension into the intercellular spaces of the leaves. Bacterial populations in leaves were sampled by cutting 2 disks from each leaf as soon as possible (0 h) and then at 12-h intervals. The 10-mm diam leaf disks were triturated using a mortar and pestle and 10-fold serial dilutions were plated.

Pepper (*Capsicum annuum* L. 'Early Calwonder') plants were grown in steam-treated soil in pots on a greenhouse bench. The fourth leaf from the base of 60-day-old plants was used for each treatment. Light and dark treatments of the plants were accomplished by placing the plants in growth chambers that were maintained at 24 ± 2 C and 65% RH. In the lighted chamber, a light intensity of 23,760 lux (2,200 ft-c) was maintained by use of cool-white fluorescent lamps supplemented with incandescent bulbs. The plants were pretreated for 12 h under the light or dark conditions prior to inoculation with bacteria.

The influence of monuron [3-(*p*-chlorophenyl)-1,1-dimethylurea] (CMU) on the growth rate of the two bacteria was assayed in vitro. Nutrient broth containing 0.125 mM CMU was compared to nonamended nutrient broth as a growth medium for *X. phaseoli* and *X. vesicatoria* in shake cultures at 24 ± 2 C.

A preliminary study of CMU indicated that when 0.125 mM CMU was injected into pepper leaves, it was at least 10-fold below concentrations that caused visible damage within 72 h. This concn is only slightly higher than that used to inhibit photosynthesis in isolated chloroplasts (2). Treatment with CMU was performed 6 h prior to introduction of the bacteria.

Photosynthesis in water-injected leaves and in leaves injected with 0.125 mM CMU was measured with a polarographic oxygen monitor. For each assay, six leaf disks, 6 mm in diam, were vacuum-infiltrated with a solution containing 0.26 M dibasic sodium phosphate and 0.0137 M sodium bicarbonate. Following establishment of a respiration reference line in the dark, illumination at 34,455 lx (3,200 ft-c) was provided by a General Electric 300-W reflector spot incandescent lamp.

All treatments were replicated five times and compared using "Students" *t*-test.

RESULTS.—The in vitro growth rates of *X. phaseoli* or of *X. vesicatoria* in nutrient broth were not significantly different from their growth rates in nutrient broth to which 0.125 mM CMU was added. Respiration of water-injected leaves was not significantly different from that in CMU-injected leaves (Table 1). Photosynthesis was apparently totally inhibited in the CMU-treated leaves within 1 h and there was no evidence of recovery within the 48-h experimental period.

Populations of *X. vesicatoria* in light-treated pepper leaves were not significantly different from those in dark treated leaves (Fig. 1), as they increased from ca. 8×10^5 to ca. 6×10^7 during the 48 h period of the experiment. The leaves remained symptomless during the experiment.

Xanthomonas phaseoli populations in pepper leaves in the light had declined from an initial level of ca. 6×10^5 to ca. 3×10^5 by the 12-h assay. Populations continued to drop, reaching ca. 3×10^2 at 48 h following inoculation. A pronounced HR was observed at 12 h following inoculation, and a gradual drying and bleaching of the inoculated area occurred. *Xanthomonas phaseoli* in dark-treated leaves increased almost 10-fold in population during the 48-hr experiment. At 12 h, the dark treated leaves also exhibited watersoaking and tissue collapse characteristic of the HR, but the area did not gradually bleach as did the light-treated leaves. The CMU-treated leaves in the light showed the HR by 12 h after bacterial inoculation, but the tissue remained similar in appearance to the dark-treated leaves. Populations of *X. phaseoli* in CMU-treated leaves were not significantly different from those of dark-treated leaves which had not received CMU.

DISCUSSION.—Populations of *X. vesicatoria* increased in pepper plants, with the bacteria in light-treated plants having a slightly faster growth rate than those in plants in the dark. These results are similar to the observations of Smith and Kennedy (13) who suggested that their results, with *Pseudomonas glycinea* on soybean might be due to production of additional photosynthetic products that could serve as a nutritive source for bacterial growth.

The population decline of *X. phaseoli* in pepper leaves in the light is in agreement with the generally accepted concepts about incompatible pathogen-host combinations (5, 14), and with the findings of Hsu and Dickey (3) for *X. phaseoli* in tomato leaves. Pepper plants that were pre- and postinoculation dark-treated exhibited a hypersensitive-type tissue collapse within 12 h of inoculation, but atypically, coloration in the HR area remained a dark greenish-brown. Following the HR in leaves injected with *X. phaseoli,* the inoculated areas gradually dried, which could have accounted for the observed leveling off of the growth rate. Smith and Kennedy (13) reported that preinoculation and postinoculation darkness treatments did not alter development of the HR of soybean leaves inoculated with avirulent races of *Pseudomonas glycinea.* Lozano and Sequeira (7) reported that light was necessary for development of the HR. However, they excluded light by covering the leaf with aluminum foil and thus would have reduced gas exchange and raised the RH within the small chamber created by the foil covering.

TABLE 1. The influence of 0.125 mM 3-(*p*-chlorophenyl)-1,1-dimethylurea (CMU) on respiration and on photosynthesis in pepper leaves at 1 h or 48 h after treatment

Treatment	Respiration	Photosynthesis
H_2O (1 h)	-0.14 ± 0.02[a]	$+0.20 \pm 0.04$
H_2O (48 h)	-0.14 ± 0.01	$+0.21 \pm 0.03$
0.125 mM CMU (1 h)	-0.15 ± 0.02	-0.14 ± 0.02[a]
0.125 mM CMU (48 h)	-0.14 ± 0.02	-0.14 ± 0.02

[a]Units are in μl O_2/ml/min evolved at 24 C. Values are the means of five replicates and the standard error of the means. Six leaf disks, each 6 mm in diam constituted a sample. Illumination at 34,454 lux (3,200 ft-c) was provided for photosynthesis measurement.

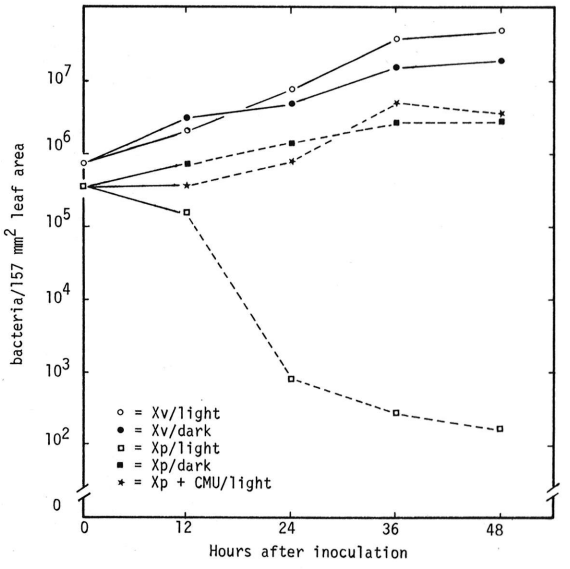

Fig. 1. Populations of *Xanthomonas vesicatoria* (Xv) and *Xanthomonas phaseoli* (Xp) in pepper leaves in the light or in the dark. The plants were in the light or dark for 12 h prior to inoculation. Injection of 3-(*p*-chlorophenyl)-1,1-dimethylurea (CMU) was 6 h prior to inoculation. The hypersensitive reaction was observed at 12 h postinoculation with the *X. phaseoli* treatments. Populations of *X. vesicatoria* in light treated plants were significantly higher than those in the dark at 36 and 48 h postinoculation. Populations of *X. phaseoli* were not significantly different in dark-treated or CMU+light-treated leaves. Populations of *X. phaseoli* in light-treated leaves were significantly different from the other two treatments at 24, 36, and 48 h postinoculation.

The increase in populations of *X. phaseoli* in pepper leaves in the dark appears similar to results reported by Lovrekovich (6) for *Pseudomonas fluorescens* in tobacco. He found that the saprophyte required a very high RH to increase in population, whereas *X. phaseoli* was able to do so in pepper leaves at a RH of 65%. Lozano and Sequeira (7) reported increases of incompatible bacteria in tobacco leaves in the dark, whereas the bacteria were killed within 36 h when the leaf was exposed to light. The

loss of field resistance in guar (10) and sesame (16) under conditions of low light intensity, seems to indicate the light dependence of the resistance mechanism and is in agreement with the results of Lozano and Sequeira (7) and with the results reported here. Light modifies the resistance of plants to some pathogens and the question of identification of the controlling function of light in host plant resistance deserves further study.

Treatment of the pepper leaves with CMU allowed populations of *X. phaseoli* to increase similar to increases found in pepper plants maintained in the dark. Since CMU had no detectable effect on *X. phaseoli* growth rate in vitro, it can be assumed that action of CMU on the pepper plants, probably by blocking photosynthesis (2), is the basis for the plant's failure to prevent population increases of *X. phaseoli*. Lozano and Sequeira (7) considered the presence or absence of the HR to be the controlling factor in their system, but this is not supported by the results of our research. Their suggestion that plant-produced substances are the cause of death for both host and pathogen cells is apparently not borne out by our results unless differential sensitivity of the two organisms is a factor. Occurrence of the HR in the dark without concomitant killing of the incompatible bacteria suggests that the HR is not causally related to production of an antibacterial substance. Similarly, occurrence of the HR in CMU-treated plants in the light, accompanied by increases in populations of an incompatible bacterium, suggests that products of photosynthesis are necessary for antibacterial action by the plant.

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