Effect of Chlorine on Peronospora trifoliorum Sporangial Production and Germination

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ABSTRACT

Tap water (500 ml containing 0.5 μg/ml chlorine) placed below, but not in contact with, infected alfalfa plants in a 12.5-liter airtight chamber apparently released chlorine and other unidentified volatile chemicals that reduced *Peronospora trifoliorum* sporangial production 83% and sporangial germination 40%. The tap water was nontoxic after addition of sodium thiosulfate, or 24 hours of exposure to air in a shallow tray. When chlorine gas was added to deionized water at rates of 0, 0.25, 0.5, and 1 μg/ml, 44, 28, 20, and 0 sporangia × 10^3/plant, respectively, were produced that germinated 86, 78, and 62%, respectively.

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Additional key words: air pollution.

Peronospora trifoliorum d By. produces sporangia on infected alfalfa (*Medicago sativa* L.) only during darkness and at a high relative humidity (RH) (2). To insure the high RH requirements for sporangial production, we placed flats of infected seedlings on 3-cm-thick blocks in trays and added tap water to the trays to about 1 cm deep. Then we placed an airtight plastic container over the flats and inside the tray to form a water-sealed humidity chamber. We noted when tap water was omitted from the trays (provided there was adequate moisture in the planting medium), or replaced by deionized water, sporangial production increased and was much less variable. This suggested that one or more volatile substances in tap water affected sporulation and led to the research reported here.

MATERIALS AND METHODS.—Alfalfa (cultivar Kanza) was seeded 1 cm deep in steam-sterilized masonry sand in 5.5 × 5.5 × 5 cm plastic pots (40 seeds per pot, in three rows), and placed in growth chambers maintained at 20 C with about 5,400 lx continuous fluorescent lighting. Four days later, a water suspension containing 100,000 *P. trifoliorum* sporangia per ml was sprayed onto the seedlings to run-off. The inoculated plants were placed immediately into trays composed of the inverted lids of plastic storage boxes. The boxes (25 × 35 × 16 cm) became the covers, and were inverted over the pots and fitted upside down into their lids to give a near airtight seal. These humidity chambers were placed in dark growth chambers. Fifteen hours later the covers were removed and the growth chamber lights were turned on. On the 10th day after seeding, sporulation was induced by replacing covers over the plants and turning off the lights. For sporulation studies, 500 ml of water was poured into each tray and the pots were supported on blocks so that water never contacted the pots.

Inoculum was prepared by cutting off mildewed seedlings (16 hours after inducing sporulation) below cotyledons, placing them in a jar with deionized water, shaking to dislodge the sporangia, and passing the sporangial suspension through a tea strainer to remove the plants.

The average number of sporangia produced per inoculated plant was determined by measuring the absorbance at 625 nm of a sporangial suspension from 30 plants (washed three times in 10 ml of deionized water) with a Bausch and Lomb Spectronic 20 spectrophotometer. Values for the standard curve were determined by actual sporangial counts with a hemacytometer.

Sporangia were germinated at 20 C in drops of deionized water (concentration adjusted to about 35,000 sporangia/ml) on microscope slides kept in petri dishes with a filter paper saturated with deionized water in the bottom. Percentage germination figures given are the means of four replications of 300 observations each.

Chlorine content of water was measured by the orthotolidine method with a Hach Test Kit, Model DR-EL, manufactured by Hach Chemical Company, Ames, Iowa 50010.

RESULTS AND DISCUSSION.—*Peronospora trifoliorum* produced three to four times more sporangia of higher viability in humidity chambers with either deionized or no water than with fresh tap water (Table 1). Mean percentages of alfalfa seedlings with visible sporulation were 87, 85, and 11 when humidity chambers contained deionized, no water, or tap water, respectively. Neither sporangial production nor viability was increased, however, by using deionized water instead of tap water for plant irrigation previous to inducing sporulation.

Because the plants never contacted the water in the humidity chambers, it was hypothesized that one or more volatile substances in tap water apparently reduced sporulation. Also, tap water exposed to air in shallow trays for 24 hours before being used in humidity chambers did not reduce sporulation.

We first investigated chlorine because about 1 μg/ml of

TABLE 1. Effects of water sources used for plant irrigation prior to sporulation and in humidity chambers during sporulation by *Peronospora trifoliorum* on sporangial production and germination

Water source		Sporangia produced per inoculated plant (mean no.)	Sporangial germination (mean %)
Plant irrigation	Humidity chambers		
Deionized	Deionized	46,500	76.9
	None	45,500	78.4
	Tap[a]	15,600	65.2
Tap[a]	Deionized	41,800	79.4
	None	49,200	76.8
	Tap[a]	10,700	60.0
LSD ($P = 0.05$ ANOV)		6,300	6.7

[a]Tap water chlorine content = 0.5 μg/ml.

chlorine gas is added to the Manhattan, Kansas, municipal water supply. Tap water in our laboratory averaged 0.5 μg/ml and ranged from 0.46 to 0.78 μg/ml during periodic sampling for 3 months. Fresh tap water treated with sodium thiosulfate (to bind the chlorine) and placed in humidity chambers did not reduce sporangial production or germination.

To determine the effects of chlorine on sporulation, we added chlorine gas to deionized water used in the humidity chambers during a 15-hour sporulation period. Chlorine concentrations in the trays of water after the sporulation period were about 10% of the amounts added. At rates of 0, 0.25, 0.5, and 1 μg chlorine per ml water 44, 28, 20, and 0 [LSD ($P = 0.05$) = 7] $\times 10^3$ sporangia per plant were produced that germinated 86, 78, and 62% [LSD ($P = 0.05$) = 9], respectively. In chambers with fresh tap water (0.5 μg chlorine per ml) 8,000 sporangia per plant were produced that germinated 53%. Thus, only 40% as many sporangia were produced in chambers with fresh tap water as in those having the same concentration of chlorine in deionized water. This suggests that the tap water contained toxic volatile materials in addition to chlorine, and that these materials (e.g., chloramines) which were bound by sodium thiosulfate reduced *P. trifoliorum* sporulation.

The humidity chamber air volume (excluding the area occupied by water and pots of plants) was about 12.5 liters, or 25 times that of the 500 ml of water used. We did not determine the chlorine concentration in the air inside the humidity chamber. However, if all chlorine from the 1 μg/ml concentration in the water, which prevented sporulation, was dispersed in the chamber space, the average concentration could not have exceeded 0.04 μg/ml.

Among higher plants, alfalfa is one of the most sensitive to chlorine, being visibly injured by 2 hours of exposure in an atmosphere containing 0.1 μg/ml (1). We never observed chlorine damage on the alfalfa seedlings, even when kept 15 hours in chambers having 5 μg chlorine/ml water in the trays. Therefore, *P. trifoliorum* sporulation is inhibited by a somewhat lower atmospheric chlorine concentration than required to cause visible injury to the alfalfa host.

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