Production of Ammonia by Tobacco and Soybean Inoculated with Bacteria

Clyde Hurst, B. W. Kennedy, and L. Olson

Research Specialist and Professor, Department of Plant Pathology, University of Minnesota, St. Paul 55101; and Assistant Professor, Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, respectively. Present address of first author: Ecological Services Laboratory, National Park Service, Washington, D.C. 20242. Present address of third author: Christopher Newport College, College of William and Mary, Newport News, Virginia 23601.

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ABSTRACT

Ammonia was consistently produced by tobacco leaves inoculated with *Pseudomonas tabaci*, but not by tobacco leaves inoculated with *P. pisi*, even though the symptoms caused by the two species of bacteria were indistinguishable. There was no correlation between susceptible, resistant, or hypersensitive reaction and evolution of ammonia by tobacco or soybeans. Soybean leaves and callus tissue inoculated with bacteria did not produce more ammonia than did the controls.

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The wildfire symptom caused by *Pseudomonas tabaci* (Wolf & Foster) Stevens or the hypersensitive reaction caused by *Pseudomonas pisi* Sackett can be simulated by exposure of tobacco leaves to ammonia evolved either from an inorganic source or from the action of *P. tabaci* on nutrient agar (2, 3). Ammonia was also designated as a symptom-causing factor in bacterial blight and in the hypersensitive reaction evoked by *P. tabaci* in cotton (1, 4) and in apple shoots inoculated with *Erwinia amylovora* (5). The purpose of this investigation was to determine whether the hypersensitive symptom produced by inoculation of resistant (hypersensitive) soybean leaves with *Pseudomonas glycinea* Coerper could be simulated by ammonia, and whether the reaction results from ammonia evolution during pathogenesis. Furthermore, we wondered if tissue cultures of soybean inoculated with virulent and avirulent races of *P. glycinea* responded similarly; this would indicate to us whether or not callus tissues retain specificity similar to the host from which they originated. The question arose as to whether production of ammonia was a cause or an effect of chemical events occurring in hypersensitive reactions.

MATERIALS AND METHODS.—A total of five bacterial species were chosen and evaluated for their ability to produce free ammonia after being placed on soybean callus, into tobacco or soybean leaves, and also while growing on nutrient agar. These included *Pseudomonas fluorescens* (a saprophyte), *Pseudomonas tabaci* (a pathogen of tobacco), *Pseudomonas syringae* van Hall (a pathogen of wide host range but not including tobacco or soybean), *Pseudomonas pisi* ATCC No. 11055 (a pathogen of pea), and *Pseudomonas glycinea*, races 1, 2, and 5. Races 1 and 2 are pathogenic to soybean cultivar Acme, race 2 is pathogenic to cultivar Chippewa, and race 5 is pathogenic to neither. Bacteria were grown for 24 to 48 hr on nutrient agar plus 1% dextrose, washed from agar surfaces, and suspended in distilled water to a concentration of 10^9 cells/ml as estimated by use of a MacFarland scale. For evaluation of the reaction of tobacco, leaves were inoculated by infiltration with a bacterial suspension similar to methods by Lovrekovich et al. (3). In the case of soybeans, unifoliolate leaves of *Glycine max* L. 'Acme' and 'Chippewa' were inoculated by a gentle spraying of leaf undersurfaces with bacterial suspensions until water congestion was evident in approximately half the leaf; callus tissue of the two cultivars was inoculated by application of drops of bacterial suspension directly to pieces of the callus tissue removed from agar substrates. All experiments except those evaluating callus and nutrient agar cultures were made in the greenhouse.

In experiments with tissue culture, we trapped ammonia by passing acid (0.1 N H_2SO_4) -scrubbed air over the callus and into an acid trap, or by using a closed Conway system with inoculated callus in the outer ring and acid in the middle ring of sealed plastic dishes. In some experiments, soybean leaves were detached and were placed on a nylon screen which was supported above acid in closed petri dishes. In other experiments, the acid trap was placed inside plastic bags enclosing intact leaves. All experiments with tobacco (*Nicotiana tabacum* L. '402') were performed by the latter method. Production of ammonia on nutrient agar was determined by use of 20-ml serum bottles containing 5 ml of agar, ammonia being trapped in a drop of 5 N H_2SO_4 on a glass rod in the stopper. In all cases, the amount of ammonia collected in acid traps during 3 days of incubation was determined by nesslerization.

RESULTS.—All five bacteria produced ammonia while growing on nutrient agar. The data from six experiments involving tobacco plants and three species of bacteria were averaged and are presented in Table 1. Some of these results supported the work of

Lovrekovich et al. (4), which indicate that ammonia is produced after infiltration of tobacco leaves with *P. tabaci*. Although we consistently obtained ammonia from this combination, we rarely detected production of ammonia when tobacco leaves were infiltrated with *P. pisi*, even though the culture was obtained from Lovrekovich and symptoms produced by the two bacteria were indistinguishable in appearance and rate of development. We did not find appreciable amounts of ammonia being produced by *P. fluorescens* in tobacco. Except for one experiment in which there was poor symptom development and no ammonia above that of the background produced by any treatment, incubation with *P. tabaci* always resulted in ammonia production from each leaf treated. *Pseudomonas pisi, P. fluorescens*, and water treatment (control) occasionally showed comparatively small but detectable amounts of ammonia in the traps.

Inoculation of detached soybean leaves of either of the two cultivars, Acme or Chippewa, with *P. fluorescens, P. syringae, P. tabaci*, or *P. glycinea* races 1, 2, or 5 did not result in evolution of ammonia in amounts above the controls. However, if the leaves were killed by hot water immediately prior to our spraying them with bacteria, both parasitic and saprophytic bacteria caused production of ammonia. An average of results obtained in two experiments using three bacteria were as follows: *P. tabaci*, 596 μg/g fresh wt, *P. syringae*, 216 μg/g; and *P. fluorescens*, 71 μg/g. Inoculated soybean callus failed to release ammonia in quantities above controls.

DISCUSSION.—In these tests, ammonia was not consistently associated with resistant or hypersensitive reactions. This conforms to a recent report by Stall et al. (7), who made similar conclusions regarding hypersensitive reactions in pepper resulting from inoculation with *Xanthomonas vesicatoria*. They also concluded that production of ammonia may be related to susceptibility. The fact that ammonia was produced when bacteria was placed on dead (hot water-killed) soybean leaves and on nutrient agar might suggest that production of ammonia is simply the result of bacterial action on dead tissue, and thus ammonia may ultimately be the result (rather than the cause) of symptoms. This would be a logical deduction from a recent report from Goodman (6). We then cannot account for the difficulty in obtaining a consistent positive test for ammonia from tobacco leaves infiltrated with *P. pisi* where hypersensitivity was clearly and consistently obtained. Furthermore, we were unable to obtain ammonia from any combination using detached leaves of soybean or from tissue cultures of soybean. The relationship of ammonia to development of symptoms in tobacco and soybeans inoculated with certain bacteria is still a question.

TABLE 1. Ammonia production from tobacco leaves infiltrated with three species of *Pseudomonas*

Inoculum (10^8-10^{10} cells/ml)	μg NH_3/g fresh wt[a] (3 days)
Water	0.14
P. fluorescens	0.22
P. pisi	2.34[b]
P. tabaci	32.8

[a] Data averaged from six experiments.

[b] Results inconsistent; two leaves of a total of 18 leaves in six experiments produced ammonia higher than controls.

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