

A Modified Technique for Inducing Systemic Resistance to Blue Mold and Increasing Growth in Tobacco

S. Tuzun and J. Kuć

Graduate student and professor, respectively, Department of Plant Pathology, University of Kentucky, Lexington 40546. Journal Paper 84-11-159 of the Kentucky Agricultural Experiment Station, Lexington 40546.

This research was supported in part by grants from the R. J. Reynolds Corporation and the Scientific and Technical Council of Turkey, and by Cooperative Agreement 58-7B30-3-540 of the USDA.

Accepted for publication 15 May 1985 (submitted for electronic processing).

ABSTRACT

Tuzun, S., and Kuć, J. 1985. A modified technique for inducing systemic resistance to blue mold and increasing growth in tobacco. *Phytopathology* 75:1127-1129.

Sporangial suspensions of *Peronospora tabacina*, aqueous washings from frozen or fresh leaves of uninoculated tobacco plants, washings from sporangia, ethanol-killed sporangia, autoclaved sporangia, or distilled water were injected into stems of 8- to 10-wk-old tobacco plants grown in the greenhouse. Injections were made either external or internal to the xylem. Plants injected external to the xylem with *P. tabacina* were systemically protected 90-99% (based on the area of necrosis and amount of sporulation) against disease caused by subsequent foliar challenge with the

pathogen. Protection was about 50% 15 days after stem injection and reached a maximum after 21 days. Unchallenged plants injected with isolate 82 of the fungus were about 40% taller, had about 30% increase in fresh weight, about 40% increase in dry weight, four to six more leaves, and about 15% greater leaf area than control plants at the time the former reached 50% flowering. Plants injected with isolate 82 reached 50% flowering about 2 wk before the controls. Plants injected internal to the xylem were systemically protected against blue mold but were severely stunted.

Blue mold, one of the most important diseases of tobacco, has caused severe economic losses in Australia, Central America, North America, and Europe. The blue mold epidemic of 1960 in Central Europe caused a loss estimated at \$25 million (US) (4). Prior to 1979, the disease in the United States was largely responsible for losses in seed beds; in 1979, however, the disease severely damaged tobacco in the field and caused a loss of more than \$250 million (US) in the United States and Canada (7). In subsequent years, the disease has occurred in individual fields, but it has been controlled by applying the systemic fungicide metalaxyl. Strains of *P. tabacina* resistant to metalaxyl have appeared on shade-grown cigar tobacco in several Central American countries (T. Young, Ciba Geigy Corp., *personal communication*).

Induced systemic resistance of tobacco foliage to blue mold was observed in the field by Pont (6) and Cruickshank and Mandryk (3). Plants naturally infected in the field or plants stem-injected with sporangia of *P. tabacina* in greenhouse tests were highly

resistant to disease following subsequent challenge with the fungus. Sporangia of *P. tabacina* applied to the soil surface around the base of tobacco plants also induced systemic protection (2). Protection, based on the area of necrosis and sporulation relative to plants injected with water, was about 50 and 95% at 2 and 3 wk after induction, respectively (2). Plants naturally infected in fields and plants infected by stem injection or application of sporangia to the soil were, however, often severely stunted unless grown with high nitrogen fertilization (2,5). In this paper, we describe a technique for the induction of systemic resistance against blue mold which also increases the growth of tobacco. Preliminary data describing the technique and the response of tobacco have been reported (8).

MATERIALS AND METHODS

Plants. Burley (Ky 14) tobacco plants were grown in the greenhouse (20-26 C in the fall and winter, 20-33 C in the spring and summer) in 2-L pots containing Pro-Mix Bx (Premier Peat Corp., New York, NY 10036). Daylight was supplemented with 16 hr of fluorescent and incandescent light, and the pots were watered to saturation twice a week with Peters fertilizer (W. R. Grace & Co., Fogelsville, PA) solution (14.8 cc of 20-20-20 [N-P-K] soluble fertilizer per 3.8 L). Sucker shoots at the base of the plant were removed by hand.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

Fungus. Two isolates of *P. tabacina* were used for stem inoculations. Isolate 79 was collected from a field near Georgetown, KY, in 1979 and isolate 82 was collected at Spindletop Farm, Lexington, KY, in the fall of 1982. Isolates of the pathogen were maintained on Ky 14 plants grown in growth chambers at 20 C. Frozen, viable sporangia (1) and fresh sporangia were used for inducing and challenge inoculations, respectively. Both frozen and fresh sporangia of both isolates elicited systemic resistance and both were effective for challenge inoculations. The procedure followed was determined by convenience for the investigators. Sporangia were brushed from lesions into distilled water and concentrations were adjusted with a hemacytometer.

Inducing inoculations. Approximately 1 ml of inoculum (5×10^5 sporangia per milliliter of isolate 79 or 82) of *P. tabacina* was injected into stems approximately 5–10 cm above the soil line of 8 to 10-wk-old tobacco plants. Injections were made with a 1-cc tuberculin 26G 3/8 intradermal bevel syringe (Becton, Dickinson and Co., Rutherford, NJ) into the stem external or internal to the xylem. For injecting external to the xylem, the needle was introduced tangentially to the stem and the suspension was introduced over a period of at least 1 min. The needle was introduced at a right angle to the surface of the stem and into the center of the stem when injecting internal to the xylem. Plants were kept in the greenhouse until challenged. Control plants were injected with water or sporangial washings prepared from suspensions filtered to remove sporangia, ethanol-killed sporangia, autoclaved sporangia, or washings from frozen or fresh leaves of uninoculated plants that were brushed as in the preparation of sporangial suspensions.

Challenge inoculations. Freshly produced sporangia of isolate 79 (5×10^4 sporangia per milliliter) were used in all experiments. The upper and lower surfaces of leaves were uniformly sprayed with inoculum (about 30 ml per plant) and plants were covered with brown plastic bags, the inner surfaces of which were sprayed with water. Plants were kept covered in the dark at 18 C for 18 hr. Plastic bags were then removed, and the plants were kept in growth rooms (18–21 C, illuminated for 15 days with cool-white fluorescent lights supplemented with incandescent lights ($75 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)).

Symptoms. Lesion development was recorded 7 days after challenge. The six most heavily infected leaves from each plant were rated on a scale of 0 to 4 for disease (0 = no evidence of disease; 1 = $\leq 10\%$ of leaf area with lesions; 2 = $>10\text{--}<40\%$, 3 = $40\text{--}<75\%$, 4 = $\geq 75\%$). A leaf with even a single lesion was rated 1, and with induced plants this often represented less than 1% of the leaf area.

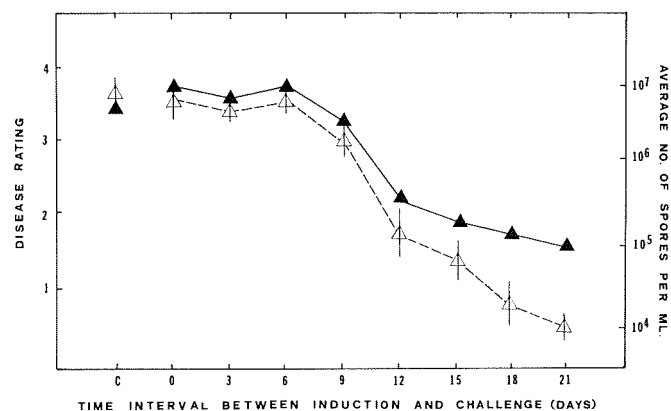


Fig. 1. Effect of the time interval between injecting sporangia of *Peronospora tabacina* (isolate 82) external to the xylem in tobacco stems and challenge inoculation with the fungus (isolate 79) on the level of protection against blue mold. All plants were challenged 21 days after time 0, and injection occurred at different time intervals relative to challenge. Data for disease ratings (Δ --- Δ) are averages from three experiments with three plants per time interval per experiment. Bars represent standard deviations of the means. Sporulation data (Δ --- Δ) are from a single experiment. Ratings: 0 = no evidence of disease, 1 = $\leq 10\%$ of leaf area with lesions, 2 = $>10\text{--}<40\%$, 3 = $40\text{--}<75\%$, 4 = $\geq 75\%$. C = Control plants, challenged 21 days after stems were injected with water.

To measure sporulation, the two most heavily infected leaves from each plant were collected and kept in moistened plastic boxes (21 C for 8 hr). Spores were carefully brushed from leaves into 5 ml of distilled H₂O and sporangia were counted with a hemacytometer and light microscopy.

To determine differences in growth and development, the heights of the plants from the growing point to the soil level were measured and the numbers of leaves, plants flowering, and plants with suckers were determined. Leaf area was determined by using an optical leaf area analyzer (Li-Cor Instrument Corp., Lincoln, NE). Fresh weight was determined by weighing the leaves and stems of each plant. The dry weight was determined after drying leaves and stems to constant weight in a drying oven at 65 C.

RESULTS

Injection of sporangia internal to the xylem in tobacco stems resulted in necrosis internal and external to the xylem, whereas injection into tissue external to the xylem resulted in necrosis only in the tissue external to the xylem. This included outer phloem and cortex and rarely the cambium. Necrosis was apparent 5–7 days after both types of stem injection, and it extended to 30 to 40 cm and 60 to 70 cm in length 21 days and 36 days after injection, respectively. Protection against blue mold, based on the area of lesions and amount of sporulation relative to plants injected with water, was barely apparent 9 days after inoculations with *P. tabacina* external to the xylem of tobacco stems. Protection reached 50% after 12 days and 90 to 99% 18 to 21 days after stem inoculation (Fig. 1). Sporulation was about 100-fold greater on plants injected with water than on those that were fully protected (Fig. 1). Plants injected with aqueous washings from frozen or fresh leaves of uninoculated plants, washings from sporangia, ethanol-killed sporangia, or autoclaved sporangia were not protected against blue mold and did not show increased growth. Plants injected with *P. tabacina* internal to the xylem were equally as protected as those injected external to the xylem. The number of infected leaves decreased and the total number of leaves per plant increased 9 to 21 days after the induction of resistance (Fig. 2). At the time 50% of the unchallenged plants injected with isolate 82 of *P. tabacina* were flowering, they were about 40% taller, had about 30% greater fresh weight, 40% greater dry weight, 15% greater leaf area, and four to six more leaves than controls of the same age that had started flowering (Table 1). Growth differences were still evident when control plants were fully flowering. Protected plants reached 50% flowering about 2 wk before control plants. Stem injection with either isolate produced similar results, but isolate 82 consistently gave somewhat better protection and greater increases in growth. Plants injected with fungus generally suckered earlier and more abundantly than control plants. Injection of sporangia

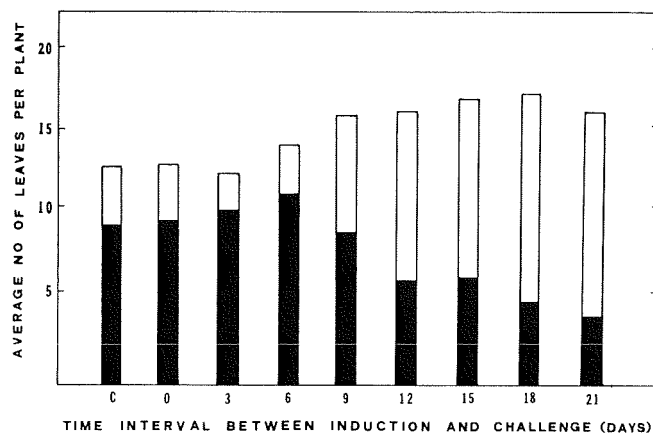


Fig. 2. Average number of leaves and average number of leaves with symptoms of blue mold (shaded bars) on tobacco plants described in Fig. 1. A leaf with even a single lesion is considered infected. C = Control plants, challenged 21 days after their stems were injected with water.

TABLE 1. The effect of injecting sporangia of *Peronospora tabacina* into stems external to the xylem on the growth of tobacco plants

Growth ^a	Treatment ^b	Time after stem injection (days)			
		0	21 ^c	36 ^d	52
Height	I-79	26	53	86	124
Ave per plant (cm)	I-82	28	69	96	127
	C	28	40	65	94
	I-79		253	516	664
Fresh wt	I-82		272	591	729
Ave per plant (g)	C		180	440	543
	I-79		24	44	60
	I-82		29	51	66
Dry wt	C		18	34	45
Leaf area	I-79		5,175	7,926	8,844
Ave per plant (cm ²)	I-82		5,467	8,643	8,949
	C		3,965	7,392	7,582

^aHeight is based on six experiments with at least five plants per treatment per experiment. All other parameters of growth were based on three experiments each with at least six plants per time interval per treatment.

^bI-79, I-82 = stems injected with sporangia of *P. tabacina* isolate 79 and 82. C = stems injected with water.

^cPlants with stems injected with sporangia of *P. tabacina* were systemically protected >90% against blue mold (based on area of necrosis and sporulation) 21, 36, and 52 days after stem injection.

^dThe days required after stem injection for about 50% of the plants to flower for I-82, I-79, C were 36, 43, and 52, respectively.

into stems internal to the xylem caused severe stunting (Fig. 3), but the plants were systemically protected against foliar infection by *P. tabacina*.

DISCUSSION

Our results show that induced systemic resistance to blue mold by stem infection with the fungus can be separated from decreased growth. In earlier experiments (2,3,6) severe stunting was consistently associated with induced resistance. In addition, stem-inoculated plants flowered sooner and had earlier and more numerous development of suckers. Mandryk (5) reported that high levels of nitrogen reduced the stunting of plants stem infected by *P. tabacina*. Plants with resistance induced by using the modified technique, however, grew better than controls, and they also flowered sooner and developed more suckers without high levels of nitrogen fertilization. Besides providing high protection against blue mold, injections of sporangial suspensions external to the xylem increased plant height, leaf area, and fresh and dry weights. Microscopic and macroscopic examination revealed that the modified stem injection technique resulted in necrosis of the outer phloem and cortical tissue. Necrosis was seldom observed in the cambium and, generally, the internal phloem, xylem, and pith were without visible damage. Sporulation was never observed in or on stems injected with the fungus. Injection into stems internal to the xylem, however, resulted in necrosis extending to tissue external to the xylem. This damage may be the reason for stunting. It's likely that the hormonal balance of plants is changed by both techniques of stem injection. Different quantities and interaction of different hormones may result in stunting as well as increased growth in plants injected by the two different techniques. Bakanae disease

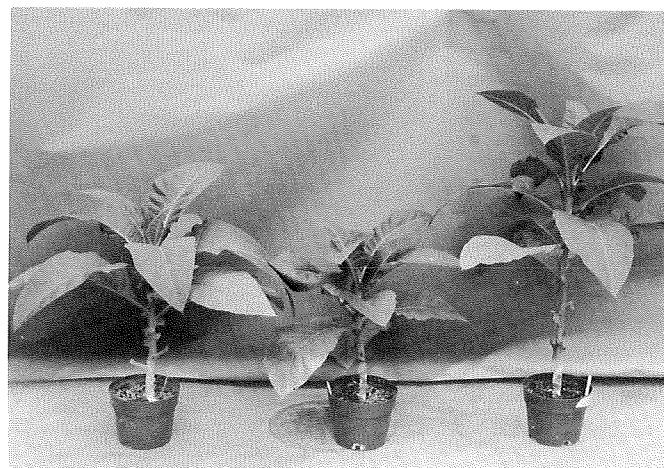


Fig. 3. The effect of injecting sporangia of *Peronospora tabacina* internal or external to the xylem in tobacco stems. Left to right: water injected into stem external to the xylem, sporangia injected into stem internal to the xylem, and sporangia injected into stem external to the xylem. The photograph was taken 21 days after stem injection.

caused by *Gibberella fujikuroi* increased the height of rice plants because gibberellins were released by the fungus (9). However, the phenomenon was associated with increased extension of the internodes and with abnormal growth. To the best of our knowledge, this is the first report of an increase in several parameters of normal growth in plants with induced systemic resistance.

Field tests are in progress to test the effectiveness and stability of systemic resistance to blue mold.

LITERATURE CITED

- Cohen, Y., and Kuć, J. 1980. Infectivity of conidia of *Peronospora tabacina* after freezing and thawing. *Plant Dis.* 64:549-550.
- Cohen, Y., and Kuć, J. 1981. Evaluation of systemic resistance to blue mold induced in tobacco leaves by prior stem inoculations with *Peronospora tabacina*. *Phytopathology* 71:781-789.
- Cruickshank, I. A. M., and Mandryk, M. 1960. The effect of stem injection of tobacco with *Peronospora tabacina* Adam on foliage reaction to blue mold. *J. Austr. Inst. Agric. Res.* 26:369-372.
- Lucas, G. B. 1975. *Diseases of tobacco*. 3rd ed. Biological Consulting Associates, Raleigh, NC. 621 pp.
- Mandryk, M. 1961. The relationship between acquired resistance to *Peronospora tabacina* in *Nicotiana tabacum* and soil nitrogen levels. *Austr. J. Agric. Res.* 13:10-16.
- Pont, W. 1959. Blue Mold (*Peronospora tabacina* Adam) of tobacco in North Queensland. Some aspects of chemical control. *Queensl. J. Agric. Sci.* 16:299-327.
- Schiltz, P. 1981. Downy Mildew of tobacco. Pages 577-599 in: *The Downy Mildews*. D. M. Spencer, ed. Academic Press, London, New York, San Francisco.
- Tuzun, S., and Kuć, J. 1983. A new technique which immunizes tobacco against blue mold (*Peronospora hyoscyami* f. sp. *tabacina*) and increases growth of tobacco. *Phytopathology* 75:823.
- Yabuta, T. J., and Mayashi, T. 1979. Biochemical studies on "bakanae" fungus of rice. II. Isolation of gibberellin, the active principle which produces slender rice seedlings. *J. Agric. Chem. Soc. Jpn.* 15:257-266.