Disease Control and Pest Management

Effects of Stem Injections with *Peronospora tabacina* on Growth of Tobacco and Protection Against Blue Mold in the Field

S. Tuzun, W. Nesmith, R. S. Ferriss, and J. Kuć

Department of Plant Pathology, University of Kentucky, Lexington 40546.

Journal Series Paper 85-11-226 of the Kentucky Agricultural Experiment Station, Lexington 40546.

This work was supported in part by grants from the Ciba-Geigy Corporation, R. J. Reynolds Corporation, U.S.D.A. Cooperative agreement 43YK-5-0030 and the Scientific and Technical Research Council of Turkey.

We thank Etta Nuckles, Nancy Doubrava, George Braman, Marty Wiglesworth, Serapion Jenofonte, Louis Real, and Mark Henry for technical assistance. We appreciate the help of those farmers who allowed us to use their facilities for these experiments; we also wish to thank the supporting county extension agents. The assistance of the technical staff of Eden Shale, Spindletop, and South Farm research farms in Kentucky and Gurabo Experiment Station in Puerto Rico are appreciated.

Accepted for publication 10 April 1986 (submitted for electronic processing).

ABSTRACT

Tuzun, S., Nesmith, W., Ferriss, R. S., and Kuć, J. 1986. Effects of stem injections with *Peronospora tabacina* on growth of tobacco and protection against blue mold in the field. Phytopathology 76: 938-941.

The effects of stem injections with *Peronospora tabacina* on growth of tobacco and susceptibility to blue mold were investigated in eight field experiments in Kentucky and three in Puerto Rico during 1983, 1984, and 1985. Burley tobacco was used in Kentucky; cigar tobacco and burley tobacco were used in Puerto Rico. Metalaxyl-treated plants were used as a positive control in some experiments, and untreated plants served as negative controls in all experiments. Plants receiving stem injections had significantly greater height and fresh weight than control plants unless black shank was present or injections were made at very early stages of plant growth. Marketable yield was increased by up to 25% over the controls at Kentucky locations. Stem injections significantly decreased the severity of blue mold in the six experiments where the disease was observed.

Blue mold, incited by *Peronospora tabacina* Adam, has caused severe economic losses in tobacco (*Nicotiana tabacum* L.) during the last two decades (5). Estimated losses were over $250 million in the United States and Canada during the 1979 epidemic (5,9). Since 1979, a decline has occurred in disease incidence in the United States because of less favorable weather conditions for disease developement and extensive use of the sytemic fungicide metalaxyl. Strains of *P. tabacina* resistant to metalaxyl, however, have been found on shade-grown cigar tobacco in several Central American countries (T. R. Young, Ciba-Geigy Corp., *personal communication*), posing a new blue mold threat. Alternative means for control, therefore, will be needed.

Development of induced resistance of tobacco foliage to blue mold was first observed by Pont (8) on plants with naturally infected stems in the field. Cruickshank and Mandryk (3) and Cohen and Kuć (2) observed the same phenomenon on plants artificially stem infected with *P. tabacina*. Protection was greater than 95% compared with controls 3 wk after stem injection; however, in all cases the plants were stunted and showed growth abnormalities. High N fertilization was reported to partially overcome this stunting (6). A modified technique was developed in our laboratory for stem injection of tobacco with *P. tabacina* that gave a high level of protection against blue mold and also increased growth of tobacco in the greenhouse (4,11). In this study, we investigated the effects of this modified stem injection technique on growth of tobacco and protection against blue mold under field conditions. Preliminary results have been reported (12).

MATERIALS AND METHODS

Plants, preparation of fields, and chemical treatments. The investigation consisted of experiments conducted at three field sites in Puerto Rico and eight in Kentucky during 1983, 1984, and 1985. A burley cultivar (Ky 14), a burley hybrid (MS 14× L8), and

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.

© 1986 The American Phytopathological Society

a cigar tobacco cultivar (PR5-65) were used (Table 1). Seedlings were grown in seedbeds at the locations of the experiments according to standard procedures used in the burley industry (10), except in the 1984 Puerto Rico experiments (experiments 2 and 3). In these experiments, seedlings were grown in a greenhouse at the Gurabo Experiment Station. Seedlings were grown at 28–33 C in peat pots containing a Pro-Mix Bx (Premier Peat Moss Corp., New York, NY):soil:sand mixture (1:1:1, v/v/v), drenched with thiophanate-methyl/ethazole (Banrot 40% WP; Mallinckrodt Inc., St. Louis, MO)(0.24 g/L) to control damping-off (*Pythium* spp.) and black shank (*Phytophthora parasitica* f. sp. *nicotianae*). Plants were hardened under shade outside the greenhouse for 1 wk before transplanting. At all Kentucky test sites the soils were fertilized according to University of Kentucky Cooperative Extension Service recommendations (10). Preplant soil tests were conducted by the University of Kentucky Soil Testing Service, and lime and/or fertilizer were applied before planting to bring each soil to approximately pH 6.5 and N, P, and K greater than 335, 90, and 420 kg/ha extractible nutrient, respectively. At the Puerto Rico locations, 2,240 kg of 8-6-10 and 157 kg of N as NH_4NO_3 per hectare were applied in two applications at 20 and 45 days after transplanting. Weed control was maintained through cultivation and the application of preplant-incorporated herbicides [pendimethalin (Prowl, American Cyanamid Co., Wayne, NJ) or pebulate (Tillam, Stauffer Chemical Co., Westport, CT)]. Seedlings were transplanted 45 cm apart in rows spaced 100 cm apart. Treatments were arranged in randomized complete block designs. The number of blocks varied according to space availability and condition of the field (Table 1). Soil was drenched with thiophanate-methyl/ethazole (0.58 g/L) in the Puerto Rico experiments immediately after transplanting and at weekly intervals for 3 wk to control damping-off. Where high blue mold pressure occurred, all plants were sprayed once or twice weekly with mancozeb (Manzate 200, E. I. du Pont de Nemours & Co., Wilmington, DE) (0.36 g/L) until 3 wk after stem injection. At the time of stem injection, metalaxyl (Ridomil 2E, Ciba-Geigy Co., Greensboro, NC) was applied to the soil (15 ml/L) and to leaf surfaces (7.5 ml/L) in some experiments as a positive control. Suckers were controlled by hand, except in experiments 5 and 6, where maleic hydrazide was used. Plants were not irrigated at the Kentucky locations, except in experiments 6 and 9, where irrigation was done twice weekly. Overhead irrigation was used in the Puerto Rico experiments for 10 min every morning, starting 3 wk after stem injection, in order to prolong the period of leaf wetness. Plants were topped by removing the flower panicle and all the leaves shorter than 15 cm in length. Topping was done when approximately 50% of the plants in the field reached flowering, except in experiment 9, where plants were topped individually when each reached flowering.

Fungus and inducing inoculations. Isolate 82 of *P. tabacina* (collected at Spindletop research farm, Lexington, KY, in 1982) was used for stem injections. The fungus was maintained on greenhouse grown Ky 14 tobacco plants as previously described (11). Inoculum was produced on plants grown in growth chambers (11). Infected leaves with abundant sporulation were collected and frozen immediately after collection (1). Within 1 hr before stem injections, inoculum was prepared by brushing sporangia from frozen leaves into water cooled to 5 C. The inoculum suspension was kept in an ice bath during injections. For the experiments in Puerto Rico, frozen leaves containing sporangia were shipped in dry ice from Lexington, KY, to San Juan, Puerto Rico, and kept in a freezer until inoculum preparation.

For stem-injected plants, 0.3–1.0 ml of inoculum (5×10^5 sporangia per milliliter), depending on the size of the plants at the time (11), was injected into the stem of each plant external to the xylem using a 1-cc tuberculin syringe (26 G 3/8 intradermal bevel, Becton, Dickinson and Co., Rutherford, NJ). Stem injections were performed 3–6 wk after transplanting. Average plant size at the

TABLE 1. Sites and procedures used in field experiments to evaluate the effects of stem injection on tobacco growth and blue mold severity

Experiment	Year	Site	Cultivar[a]	Blocks (no.)	Plants/block (no.)	Stem injections[b] (no.)	Mean height at first stem injection (cm)	Time of vegetative height measurement (d.a.s.)[c]	Blue mold measurement
1	1983	Gurabo, Puerto Rico	PR5-65	4	40	2	10	21	Percentage of infected leaves
2	1984	Gurabo, Puerto Rico	PR5-65	10	40	2	16	21	No. of lesions/leaf
3	1984	Gurabo, Puerto Rico	Ky-14	10	40	1	26	21	No. of lesions/plant
4	1984	Owen Co., KY	Ky-14	2	40	1	9	42	Percentage of leaf area with lesions
5	1985	Owen Co., KY	Ky-14	4	20	2	37	20	No. of lesions/plant
6	1985	Fayette Co., KY	Ky-14	3	20	1	20	···	No. of infected leaves/plant
7	1983	Lawrence Co., KY	Ky-14	7	40	1	30	21	Blue mold not present
8	1983	Owen Co., KY	MSL8 × 14	3	40	1	25	···	Blue mold not present
9	1984	Fayette Co., KY	Ky-14	5	10	1	23	20	Blue mold not present
10	1984	Clark Co., KY	Ky-14	6	40	2	8	30	Blue mold not present
11	1984	Jessamine Co., KY	Ky-14	4	40	1	28	23	Blue mold not present

[a] PR5-65 = cigar tobacco cultivar; Ky-14 and MSL8 × 14 = burley cultivars.
[b] Stem injection was performed 3–6 wk after transplanting in all experiments. Additional stem injection performed approximately 2 wk after the first one in some experiments.
[c] d.a.s. = Days after first stem injection.

time of stem injection varied among the experiments (Table 1). A second stem injection was performed approximately 2 wk after the initial injection as a booster inoculation in some experiments where apparent stem necrosis did not develop. Controls were not injected with H_2O or brushings of leaves because of limited availability of time and labor, and because such treatments did not induce resistance in greenhouse experiments (2,11).

Challenge inoculations. Because of concern over spreading inoculum to other tobacco-growing sites, we relied on natural blue mold inoculum. In experiments 2, 3, 5, and 6, however, infected plants were placed uniformly around the experimental plots, once natural disease occurred in the vicinity, to provide additional inoculum. The experimental sites chosen were in fields that had a history of blue mold. Overhead irrigation was applied where available in order to prolong the period of leaf wetness.

Evaluations. Heights of plants were measured from the soil line to the growing point at the time of stem injection, during vegetative growth and/or just before topping. Individual plants were weighed immediately after harvesting to determine fresh weight. Cured weight (marketable tobacco) was determined for bulked plants from each plot following a natural air cure for burley. Measurements of blue mold severity varied depending on the amount of blue mold present and the availability of time (Table 1). The number of blue mold lesions per leaf, number of lesions per plant, area of necrosis, or the number of leaves per plant with one or more lesions were used for disease evaluations. Except in experiments 4 and 5, treatment effects on plant height were evaluated by analysis of covariance using individual plant height at the time of stem injection as the covariate. Differences in adjusted treatment means were evaluated using this analysis of covariance or the Duncan–Waller *K*-ratio *t* test where appropriate. In two experiments where blue mold was only present on control plants, a *t* test was performed to confirm that this amount was significantly different from zero (the value for the other treatments).

RESULTS

Foliar lesions of blue mold developed in six of the 11 experiments. In the Puerto Rico experiments (experiments 1, 2, and 3), flooding and black shank both affected tobacco growth so much that yield data were not taken. Severe drought affected experiment 8. In experiment 9, some lower leaves of stem injected plants had fallen off before harvest.

In all experiments, stem injection resulted in necrosis of the stem tissue external to xylem, but the length of necrosis varied from plant to plant and field to field. In general, necrotic areas were larger on burley tobacco than on cigar tobacco. Sporulation was never observed on or around necrotic areas on stems. The development of classic symptoms of systemic blue mold (5,9) because of stem injections occurred only in experiment 10, in which very small plants were injected.

The vegetative and/or flowering height of stem injected plants was significantly greater than that of control plants in six of the 10 experiments where height data were recorded (Table 2). Flowering occurred 1–3 wk earlier on stem injected plants. The height of metalaxyl-treated plants was significantly greater than that of untreated plants in two experiments, significantly greater than that of stem injected plants in one experiment, and significantly less than that of stem injected plants in two experiments (Table 2).

Stem injected plants had significantly greater fresh weight than controls in the four experiments where fresh weight data were taken and had greater cured weight than controls in all experiments where yield data were taken except experiment 10, where systemic blue mold developed in stem injected plants

TABLE 2. The effect of stem injections with *Peronospora tabacina* and metalaxyl treatment on growth of tobacco and severity of blue mold

Experiment	Treatment	Height (cm)[v]		Fresh wt. (kg/plant)	Cured wt. (kg/ha)	Blue mold[w]
		Vegetative	Flowering			
1	Stem injection	172a[x]	...[y]	...	...	1.4c
	Metalaxyl	165b	...	...	...	3.6b
	Control	160b	...	...	...	43.1 a
2	Stem injection	82ab	...	...	...	0
	Metalaxyl	89a	...	...	...	0
	Control	81b	...	...	...	2.0[z]
3	Stem injection	81b	...	...	...	0
	Metalaxyl	87a	...	...	...	0
	Control	79b	...	...	...	1.5[z]
4	Stem injection	...	108a	2.42a	4,041a	1.2b
	Control	...	91a	1.89b	3,212b	10.7 a
5	Stem injection	130a	...	...	...	8.6b
	Control	107b	...	...	...	78.2a
6	Stem injection	...	...	...	...	0.8b
	Control	...	...	...	...	6.3a
7	Stem injection	108a	133a	1.98a	3,228a	...
	Metalaxyl	100b	116b	1.58b	2,905b	...
	Control	101b	117b	1.71b	3,013b	...
8	Stem injection	...	115a	1.262a	2,385a	...
	Control	...	87b	0.824b	1,865b	...
9	Stem injection	98a	149a	2.68a	3,553a	...
	Control	79b	124b	2.05b	2,998b	...
10	Stem injection	87a	...	...	1,565b	...
	Control	98a	...	...	2,424a	...
11	Stem injection	72a	128a	...	...	...
	Control	53b	104b	...	...	...

[v] Times of vegetative height measurement varied with experiment and are listed in Table 1.
[w] Blue mold measurements varied with experiment and are summarized in Table 1.
[x] For each experiment and type of data, means followed by the same letter are not significantly different ($P \leq 0.05$) according to analysis of variance or the Duncan-Waller *K*-ratio *t* test.
[y] Data not taken.
[z] Mean is significantly different ($P = 0.05$) from zero according to Student's *t* test.

(Table 2).

Stem injected and metalaxyl-treated plants had significantly less blue mold than untreated controls in experiments where the disease was present, and stem injected plants had significantly less blue mold than metalaxyl-treated plants in experiment 1.

DISCUSSION

The increases in tobacco growth and protection against blue mold associated with stem injection in these field experiments are similar to those that were observed in greenhouse experiments (4,11). Protection against foliar blue mold by stem injection with *P. tabacina* was observed in previous field experiments (3,7,8); however, in these cases, plants became systemically infected and were severely stunted. Previous greenhouse experiments indicated that whether a stem-injected plant becomes stunted or not depends on which vascular tissue system is necrotic (4,10). The modified stem injection technique that we used usually results in infection of vascular tissues external to the xylem of tobacco stems, but not of internal tissues. However, if the infection is too deep or if very small plants are inoculated, all vascular tissues become infected, resulting in a spreading necrosis of the vascular tissues and stunting of the plants. We observed such systemic infection and stunting in one experiment where small plants (8 cm high, much smaller than those we used in greenhouse studies) were stem injected (experiment 10), but not in two others (experiments 1 and 4). These results suggest that whether or not small plants become stunted after stem injection may depend on other factors in addition to plant height.

Significant increases in height and/or fresh weight were associated with stem injection in all but three of the nine experiments. A high incidence of black shank, lethal to many plants, was present in the two experiments in Puerto Rico where no growth increase was observed (experiments 2 and 3). The growth increases associated with use of metalaxyl in these experiments were probably due to control of this disease. Although quantitative data on black shank incidence and severity were not taken, these results suggest that black shank is not controlled by stem injection with *P. tabacina*. Overall, these observations indicate that stem injection consistently results in increases in tobacco growth under a wide range of field conditions except in those cases where black shank or systemic development of blue mold occur.

Our results indicate that stem injection could be a valuable technique for producing healthier, better-growing tobacco plants. At present, the technique requires a considerable amount of skill, time, and labor, and consequently, it would not be economically feasible to use in most commercial tobacco production situations. Stem injections can only be made in larger plants because injection of smaller plants can result in systemic infection and 2–3 wk are needed for development of full resistance. These might be the biggest drawbacks at present; however, the technique still may have some practical application value because small plants can be relatively easily protected with fungicide applications. Some growers have shown interest in using the technique in the production of high-value, cigar-type tobacco and organically grown crops. It is possible that machinery could be developed that would facilitate the stem injection procedure; however, because heat- or ethanol-killed sporangia did not protect the plants (2,11), problems associated with working with inoculum of a virulent pathogen would still need to be addressed. Ideally, if chemicals could be found that mimic the effects of stem injection, it might be possible to protect plants against blue mold utilizing their natural mechanisms for resistance. Such attempts would minimize fungicide residue problems and also the problems associated with development of fungicide resistance due to extensive use of systemic fungicides such as metalaxyl.

LITERATURE CITED

1. Cohen, Y., and Kuć, J. 1980. Infectivity of conidia of *Peronospora tabacina* after freezing and thawing. Plant Dis. 64:549-550.
2. Cohen, Y., and Kuć, J. 1981. Evaluation of systemic resistance to blue mold induced in tobacco leaves by prior stem inoculation with *Peronospora hyoscyami* f. sp. *tabacina*. Phytopathology 71:783-789.
3. 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. Aust. Inst. Agric. Sci. 26:369-372.
4. Kuć, J., and Tuzun, S. 1983. Immunization for disease resistance in tobacco. Recent Adv. Tob. Sci. 9:179-213
5. Lucas, G. B. 1975. Disease of tobacco, 3rd Ed. Biological Consulting Associates, Raleigh, N.C. pp. 621.
6. Mandryk, M. 1961. The relationship between acquired resistance to *Peronospora tabacina* in *Nicotiana tabacum* and soil nitrogen levels. Aust. J. Agric. Res. 13:10-16.
7. Nesmith, W., Cohen, Y., Kuć, J., and Spurr, H. 1982. Field evaluations of systemic resistance to blue mold in tobacco induced by inoculations with the blue mold pathogen. (Abstr.) Phytopathology 72:360.
8. 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.
9. 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.
10. Smiley, J. H., Nesmith, W., Townsend, L. H., Duncan, G. A., and Hourigan, W. W. 1983. Tobacco Handbook. University of Kentucky, College of Agriculture, Cooperative Extension Service. 39 pp.
11. 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.
12. Tuzun, S., Nesmith, W., and Kuć, J. 1984. The effect of stem injections with *Peronospora tabacina* and metalaxyl treatment on growth of tobacco and protection against blue mold in the field. (Abstr.) Phytopathology 74:804.