Ozone Injury to Tobacco in the Field Influenced by Soil Treatments with Benomyl and Carboxin

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ABSTRACT

In field plots under shade tents, soil treatments of carboxin at 5 and 10 μg/g and benomyl at 25 μg/g were tested for their ability to protect tobacco cultivar Conn. 7272 against flecking caused by ozone. Plants growing in soil treated with carboxin had significantly less fleck on their first three leaves than did nontreated plants; but on most of the subsequent leaves the carboxin-treated plants had more fleck than the untreated plants. The plants growing in soil containing carboxin at 10 μg/g were stunted and their leaves developed yellow margins. In the benomyl treated plots, the plants had significantly less flecking on their first eight leaves, but the upper leaves were not protected later in the season. These plants were more vigorous than those in either the carboxin-treated or untreated plots, appeared to have many more fine, white, feeder roots and their roots contained fewer tobacco cyst nematodes.

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Shade-grown, cigar wrapper tobacco (*Nicotiana tabacum* L.) is particularly vulnerable to air pollution damage because its leaves must be free of visible blemishes to be commercially acceptable. Susceptible cultivars become flecked and spotted when levels of ozone reach 5 μliters/100 liters of air or more for a few hours (1). In most years, this concn is present in the air 10 to 20 times during the growing season in Connecticut (8). Development of commercial cultivars resistant to about 10 to 15 μliters ozone/100 liters of air has allowed continued production of wrapper leaves in the Connecticut Valley (2). However, the selection and breeding of commercially desirable plants resistant to present levels of air pollutants may not be able to produce cultivars sufficiently resistant to injury if levels of oxidants in polluted air should increase (2, 9). Another way to increase the ozone-resistance of plants is to grow them in soil treated with certain chemical compounds (4, 5, 6, 10, 11).

The discovery that the systemic fungicide benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] could protect plants against ozone injury has opened a new area of investigation (4, 5, 6, 7, 12). Recently, another systemic fungicide, carboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide), also has been found to protect plants against ozone (S. Rich, et al. (*unpublished*). We report here on field trials that compare the ability of benomyl to protect plants against ozone with that of carboxin.

MATERIALS AND METHODS.–*Seedbeds.*–The best concn of benomyl to be used in the field was known from previous tests, but the proper concn for carboxin was determined by tests in seedbeds. Soil in a tobacco seedbed was rototilled and fertilized with 4.8 kg of 10-6-4 fertilizer per 100 m^2. On 12 April 1972, 3.3 m^2 plots replicated four times were treated with carboxin as follows: emulsion (12%) in 9.5 liters of water per plot applied with sprinkling cans to provide 10 μg of active ingredient per gram of soil; granular (10%) 10 μg carboxin/g of soil raked into the top 3 cm or left on the soil surface; and granular (10%) 2 μg carboxin/g of soil, raked in. The rate calculations were based on an assumed weight of 2.2 kt of soil per ha in a layer 6 cm deep. On the same day, half of each plot was seeded with tobacco cultivar Conn. 7272, very susceptible to ozone, and the other half with Fowler's Special, a commercial cultivar moderately susceptible to ozone. The plots were watered immediately after they were sown, and the seedbeds were then covered with glass sash. Plants were observed periodically for general growth and plant stand.

Oxidant levels exceeding 5 μliters ozone/100 liters of air (as measured with a Mast Model 724-1 ozonemeter equipped with an SO_2 trap) occurred on 1, 4, 9, and 19 June. Leaf injury was recorded on 9 and 21 June. Each leaf on 10 randomly selected plants from each plot was scored on a scale which ranged from zero for no visible injury, to 5 for spotting of the entire leaf. Scores for all leaves on a plant were summed to give the plant score. The total number of leaves was essentially the same on all plants.

Field.–Field plots were established at Windsor, Conn., under a standard shade tent like those used for the commercial production of cigar-wrapper tobacco. The plots were plowed, disked, and fertilized according to standard commercial practices. The field, a Merrimac sandy loam, was known to be infested with the tobacco cyst nematode *Heterodera tabacum* Lownsbery and Lownsbery. Plots 2.4 m long by 2.0 m wide were prepared in six replications on 14 June as follows: carboxin (10% granular) at 5 and 10 μg/g soil and benomyl (50% WP) at 25 μg/g soil were applied evenly to the soil surface and immediately raked in to a depth of approximately 8 cm. Six additional replicated plots, as checks, were not treated with either fungicide, but were raked. The same day, seven plants of cultivar Conn. 7272 were planted along the middle of the long axis of each plot and similar rows were placed between the plots to serve as buffers. Standard cultural practices were followed, except that the leaves were not harvested.

Using the method described previously, fleck was recorded on the five middle plants of the center rows in each plot on 17 July, 12 August, and 7 September. A reference leaf was labeled on each plant so that individual leaf response could be followed. Excessive rain in June delayed growth so that most plants were less than 40 cm tall on 17 July. By 7 September, some lower leaves had died or dropped off and could not be recorded. Plant height (in cm) was measured from the soil surface to the top of the highest leaf on 17 July and 16 August. Wilting was rated on 21 July, a hot, dry day, using a scale which ranged from zero for no wilting, to 4 for severe wilting. Stem diam in mm was measured at 2.5 cm above the soil line on 31 October.

To assess the condition of the root systems, the plant with the largest stem diam in the middle row of each plot was dug on 31 October, the root system washed and then ranked visually on a scale which ranged from 1 for the greatest number of fine roots and least browning, to 4 for the smallest number of fine roots and the most browning. In addition, a single plant from a border row between a benomyl and a check plot was dug on 26 September and the root system washed free of soil. The root system from each plot was dried at 80 C for dry wt determination.

To assess root invasion by the tobacco cyst nematode, four core samples, each 2.5 cm diam, were taken from the root zone of the plants in the middle row of each plot. Two replicates were sampled on 25 August and an additional two replicates on 1 September. The roots were washed out of each sample, stained in hot acid fuchsin, and the nematodes counted in ten 2-cm-long root segments for each plot.

Using a ventilated diffusion porometer (13) stomatal conductance as a measure of relative stomatal opening was measured on both surfaces of leaves 5-8 of plants in the benomyl and untreated plots on 21 July between 0200 and 0210 (EST). The afternoon was partly sunny during the period of stomatal measurement, and the Mast ozonemeter reading was 4.8 pphm.

TABLE 1. Fleck scores for seedling tobacco plants grown on seedbed soil treated with carboxin.

Treatment (μg/g soil)	Fleck score[y,z] 9 June	21 June
Liquid (12%) drench 10	0.5 a	4.6 a
Granular (10%) surface 10	0.7 ab	4.1 a
Granular (10%) incorporated 10	0.8 ab	4.9 bc
Granular (10%) incorporated 2	1.3 b	5.8 c
Untreated check	2.0 b	9.4 d

[y]Total score per plant from summing individual leaf scores of zero for no fleck, to 5 for the entire leaf area spotted.

[z]Means followed by the same letter do not differ significantly, $P = 0.05$, by Duncan's multiple range test.

TABLE 2. Fleck score per plant for groups of leaves on 17 July, 12 August, and 7 September on plants growing in soil amended with benomyl or carboxin[z]

Treatment (μg/g soil)	17 July (leaves 1-6)	12 August (leaves 5-10)
Benomyl 25	8.7 a	2.8 a
Carboxin 5	14.8 b	11.2 b
Carboxin 10	15.2 b	17.8 c
Untreated check	22.4 c	11.7 b

[z]In vertical columns, means followed by the same letter are not significantly different, $P = 0.05$, by Duncan's multiple range test.

TABLE 3. Relative growth of tobacco plants growing in soil treated with benomyl or carboxin[v]

Treatment (μg/g soil)	Wilt rating 21 July[w]	Plant height 16 August[x]	Stem diam 31 October[y]	Root system ranking[z]
Benomyl 25	1.0 a	163 a	3.77 a	1.0 a
Carboxin 5	1.6 a	128 b	3.19 b	2.0 b
Carboxin 10	2.6 b	101 c	2.81 c	3.4 c
Untreated check	1.4 a	142 b	3.34 b	3.6 c

[v]In vertical columns means followed by the same letter do not differ significantly, $P = 0.05$, by Duncan's multiple range test.

[w]Based on scale of zero for none, to 4 for extreme wilt.

[x]Centimeters from soil to tip of highest leaf.

[y]Centimeters diam at a point 2.5 cm above the soil line.

[z]Ratings based upon a range from 1 for greatest fine root retention and whitest roots to 4 for fewest and brownest roots. Data were transformed to logarithmic form before being analyzed.

RESULTS.–*Seedbeds.*–The cultivar Fowler's Special had too little fleck to provide reliable data. Fleck scores for cultivar 7272 are given in Table 1. On 9 June, the emulsion treatment was the only one significantly better than the check. On 21 June, all of the carboxin treatments gave significantly better protection against fleck than did the check. The emulsion treatment and the unraked 10 μg carboxin/g soil granular treatment were significantly better than the 2 μg carboxin/g soil granular treatment, and the unraked 10 μg carboxin/g soil granular treatment was significantly better than the raked granular treatments at 2 and 10 μg carboxin/g soil. However, the unraked 10 μg carboxin/g soil granular treatment reduced plant stand and caused slight yellowing of some leaf margins.

Field.–Mean fleck scores per plant are shown in Table 2 for 17 July and 12 August. One replication damaged by excessive rain in June was excluded from the data analysis.

Based on plant scores for leaves 1-6 on 17 July, the plants growing in the benomyl and carboxin plots had significantly less fleck than did the check plants, and the benomyl-treated plants had significantly less fleck than did the carboxin-treated plants.

By 12 August, benomyl continued to reduce total-plant fleck significantly. Total-plant fleck in the 5 μg carboxin/g soil plots did not differ significantly from that of the checks, and the plants in the 10 μg carboxin/g soil plots had significantly more fleck than did the check plants. When individual leaves were examined, the benomyl-treated plants had significantly less fleck on leaves 5-8 than did comparable leaves on either the carboxin-treated or the check plants. Leaves 9 and 10 were not significantly protected from fleck by either benomyl or carboxin. In fact, 10 μg carboxin/g soil significantly increased the fleck on leaves 8, 9, and 10.

By 7 September, there were no significant fleck differences between treatments on leaves 10-20.

The measurements expressing relative growth are given in Table 3. Carboxin at 10 μg/g soil significantly reduced plant height and stem diam, and significantly increased severity of wilting. A yellowing of leaf margins appeared on all carboxin-treated plants and was especially severe at the higher rate.

Benomyl-treated plants appeared more vigorous than the check plants throughout the season, and both the plant height and stem diam of the benomyl-treated plants were significantly greater than those of the check and carboxin-treated plants. The root systems of the benomyl-treated plants had significantly more fine roots with less browning than did the root systems of the carboxin-treated or check plants. On the plant dug from the buffer row, the portion of the root system growing into the benomyl-treated soil had higher mean dry weights (12.8g) than did the opposite portion of the same root system growing into the check plots (7.0 g).

The mean counts of nematodes per 2 cm of roots from the plants in the plots treated with benomyl, carboxin 5 μg/g soil, carboxin 10 μg/g soil, and in the checks, respectively, were 1.6, 7.6, 6.1, and 4.1. Variability was high. Although the mean number of tobacco cyst nematodes in the roots of benomyl-treated plants was significantly less than in roots of the carboxin-treated plants, it was not significantly different from that of the check plants.

Mean stomatal conductances for lower and upper leaf surfaces, respectively, were 2.00 and 0.36 cm sec^{-1} on plants in the benomyl-treated plots, and 0.67 and 0.08 cm sec^{-1} for plants in the check plots.

DISCUSSION.–Our results show that on certain tobacco strains growing in light, sandy soil, benomyl and carboxin can each reduce the amount of visible injury resulting from air pollution. Although carboxin was active at 5 to 10 μg/g soil in our Merrimac sandy loam soil, it also caused a distortion and yellowing of leaf margins on at least the first six leaves. Plant growth was also suppressed at 10 μg carboxin/g soil. Carboxin below 5 μg/g soil was not very effective on tobacco seedlings in our test, but has been reported to reduce fleck on greenhouse plants (S. Rich, *unpublished*). Perhaps continuous application of small amounts applied to the soil periodically through the season would provide protection without causing phytotoxicity. Spray applications of carboxin did not reduce fleck on wrapper tobacco in preliminary trials conducted in 1970 and 1971, (G. S. Taylor, *unpublished*).

Although benomyl was active at 25 μg/g soil in the present experiment, others have reported that up to 400 μg/g soil were required in soils with high organic matter.

The cultivar of tobacco used may be important to our results. Preliminary trials in 1970 and 1971 (G.S. Taylor, *unpublished*) indicated that the highly susceptible tobacco cultivar, Bel W-3, was not significantly protected by 50 μg benomyl/g soil. A highly fleck-resistant cultivar (Sumatra X G 4) in the same tests had some fleck on untreated plants, but benomyl in the soil did not eliminate fleck completely.

Wilted plants are protected from ozone because stomata are closed. Well-watered, turgid plants are generally more susceptible to ozone injury. Under our conditions, plants grown in benomyl-treated soil were more turgid than those in untreated soil, had better root systems, harboring fewer cyst nematodes, grew taller, and had larger stem diam. In addition, the stomata of plants in the benomyl plots were more wide open during a smoggy afternoon than were the stomata of check plants. Benomyl, then, provides for a greater exchange of gases between the leaves and the atmosphere which would be expected to make plants more susceptible to ozone. Instead, benomyl confers resistance to ozone. Thus, the ozone-resistance provided by benomyl must be produced internally, rather than by closing stomata.

Miller (3) demonstrated that benomyl at 7.5 μg/g soil will suppress the invasion of tobacco roots by the tobacco cyst nematode. He found that the suppression lasted only a few weeks. It is possible that in our field plots the benomyl-treated plants were protected from these nematodes during the active root-producing phase of plant growth, and that the differences in nematode invasion tended to disappear by the time the samples were taken, 10 wk after treatment and planting. This protection from nematodes would also delay the onset of the usual root problems such as Rhizoctonia root rot and black root rot. This, plus the direct fungicidal action of benomyl, as well as its ability to protect against ozone, must all contribute to the increased growth of the tobacco plants in the benomyl-treated plots.

These experiments also demonstrate that, although carboxin can protect plants against ozone, soil treatments with carboxin are too phytotoxic to shade-grown tobacco to be used in Connecticut tobacco fields.

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