TOBACCO, A SENSITIVE MONITOR FOR PHOTOCHEMICAL AIR POLLUTION

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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CONTENTS

ABSTRACT

The development of a technique by which the sensitive tobacco variety, Bel W3 is used as a monitor for photochemical air pollution is discussed. The technique uses the plant as an indicator of the oxidant complex in both urban and rural areas. Two pilot studies that were conducted over a 3-year period during the development of the monitoring technique are included in the discussion. Attention is given to an explanation of the proper procedures for planting, transplanting, fertilizing, and caring for mature plants. The methods used in determining and recording injury to plant leaves is included; the studies showed almost daily injury to monitoring plants.

KEY WORDS: tobacco, monitor, photochemical, oxidant, plant, and air pollution

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INTRODUCTION

Indigenous vegetation has been used to identify the photochemical complex, to help map the extent of photochemical pollution, and to provide some evidence in support of control programs. The use of natural vegetation as a pollution monitor, however, is limited by variations in sensitivity because of differences in naturally occurring cultural and environmental conditions, nonspecificity in injury symptoms because of poor care of plants, and lack of uniform distribution of species within a monitoring area.

Various attempts have been made to assess naturally occurring pollution loads and to identify specific "smog" components by special planting of a specific variety of plant under more or less controlled conditions.[1] The most comprehensive program of this type was attempted in Los Angeles with annual bluegrass and petunia as the monitoring species.[2] In a similar program, pinto bean plants were used.[3] The monitoring plants were grown under controlled conditions in both studies and exposed to ambient conditions for a 24-hour period. Attempts to correlate injury with oxidant level were only partially successful. MacDowall et al.[4] correlate sensitivity of tobacco with oxidant level by using an empirical factor (the coefficient of evaporation). This factor was developed from several meteorological factors and has been used with some success in forecasting oxidant injury to tobacco.

At present no technique that combines ease of use and relatively uniform sensitivity of plant material over a long period of time has been developed for use by an inexperienced operator. The development of such a technique employing Bel W3 tobacco is discussed herein.

PILOT STUDY I

Air pollution problems that relate to greenhouse management prompted a study to determine the utility of tobacco as a monitor of ambient oxidant. Tobacco is excellent for this type of study for several reasons: It has been more intensively studied than any other photochemically sensitive species; it has an indeterminate growth habit during vegetative stages; mature leaves are most sensitive and show fairly uniform sensitivity at a given stage of growth; new injury is readily separated from old injury; it can be grown under uniform cultural conditions with a minimum of care; a highly susceptible variety, Bel W3, has been developed;[5] its symptoms of oxidant injury are characteristic, easily identifiable, and quite specific. Figures 1 and 2 show characteristic oxidant (ozone) fleck on Bel W3 tobacco.

Figure 1. Characteristic oxidant (ozone) injury to Bel W3 tobacco variety showing complete destruction of older leaves.

Figure 2. Single tobacco leaf shows 20 to 25 percent injury from oxidant (ozone) fleck.

A pilot study was conducted in the metropolitan areas of Boston, Cincinnati, and St. Louis during the summer months to determine whether Bel W3 tobacco could be used as a simple field monitor and to answer three basic questions:

1. Are oxidant concentrations in metropolitan areas, which presumably are from photochemical sources, sufficient to produce markings on the leaves of sensitive vegetation?

2. Can estimates of the frequency of injury be obtained?

3. Can a quantitative estimate of injury be related to concentration or dose of oxidant?

Fifty-five greenhouse operators in the three metropolitan areas agreed to participate in the study. Each grower was asked to situate two tobacco plants inside his greenhouse. He was to provide at least a bushel of his normal potting soil, water the plants as needed, and follow his standard disease control and fertilizer practices. Each grower was instructed in the identification of the symptoms of oxidant injury to the tobacco and was requested to examine the plants daily and record new injury by both leaf and plant number. Cincinnati locations were inspected every 2 weeks by a National Air Pollution Control Administration staff member, and detailed percentage injury indices were obtained.

Detailed data obtained from the growers in the three metropolitan areas cannot be reported because of the variability in cultural conditions and care of plants and because of the irregularity and doubtful reliability of observations by the greenhouse personnel. Thus, that the sensitivity of exposed material throughout the area was the same or that results noted were uniformly recorded is not known. As a result, no estimates can be made in regard to the regional distribution of any one episode of oxidant injury nor can estimates of differences in severity among episodes be made. From the data obtained, however, some conclusions can be drawn. On some occasions during the season, oxidant concentrations were sufficiently high to cause markings on sensitive vegetation. All locations were subject to episodes of elevated oxidant concentration sufficient to mark sensitive vegetation. There appeared to be some correlation between the occurrence of high oxidant concentrations at the Continuous Air Monitoring Program Stations and reports of tobacco injury within the next 3 days in both Boston and St. Louis. Some of the growers correlated appearance of tobacco injury with appearance of similar injury patterns on other greenhouse crops. However, the use of observers and different cultural conditions in this type of study is of only limited value.

The sensitive variety of tobacco used in the study offers communities a method of detecting the presence of phytotoxic concentrations of oxidant and provides a method for determining the frequency of occurrence and general estimates of regional distribution and level of oxidant. However, greater control over cultural conditions, exposure, and collection of data must be exercised.

Results of the biweekly inspection of the Cincinnati plants are summarized for the period from July 7 through September 9. Injury as a percentage value for each leaf was determined and reported on the basis of total plant injury (average of two plants). Possible environmental factors that affect sensitivity were obtained and are included with the injury indices in Table 1. Results suggest that evaporative cooling has had the greatest effect on sensitizing plants to ambient oxidant. This correlates with lower maximum greenhouse temperatures and is probably a moisture stress effect. No evidence of correlations between sensitivity and fertilizer schedule, and distance and direction from town was found. However, correlation of shade, soil texture, type of house, and care of plants was noted. Thus growth and exposure conditions are important to such a study. Results suggest no significant difference in oxidant concentrations within an 8- to 14-mile radius of the center of Cincinnati.

Table 1. INTERACTION OF FACTORS ON RESPONSE OF TOBACCO TO OXIDANT POLLUTION IN METROPOLITAN CINCINNATI

Environmental factors	Number of locations	Injury index[a]
Average injury index	24	545 (0-1820)
Temperature		
Evaporative cooling 95°F	6	1200
Fan only 105°F	2	565
No cooling 115°F	16	300
Soil texture		
Light	8	645
Medium	9	630
Heavy	7	320
Type of house		
Florist	16	700
Vegetable	8	230
Care of plants		
Good	11	895
Medium	8	425
Poor	9	175
Fertilizer schedule		
None	8	410
1-8 applications	12	625
10 or more applications	4	590
Shade, percent		
0-10	10	365
15-45	10	630
above 50	4	790
Distance from center of town		
7 miles or less	14	520
7 to 13-1/2 miles	10	580
Direction from town		
W-NW	11	595
N-NE	12	475
SE	1	840

[a]Values are reported as percent cumulative leaf injury per plant. Each plant value is obtained by adding the individual injury percentages from each leaf.

Factors affecting tobacco sensitivity were determined from the study and separated into controllable and non-controllable variables (Table 2). These factors were considered when setting up the second study.

Table 2. FACTORS AFFECTING THE SENSITIVITY OF TOBACCO TO PHOTOCHEMICAL AIR POLLUTANTS[a]

Factors	Controllable	Not controllable
Light intensity	Use a 50 percent shade cloth so plants are not exposed to full sunlight	Natural variations
Soil structure and compaction	Use a light, friable mix with high water-holding capabilities	Natural variations
Nutrient level	Use regular fertilizer with high nitrogen content	
Soil moisture	Water daily, the light soil mix will drain well and give good aeration	
Insects and diseases	Minimize and schedule use of controls	
Light duration		Natural variations
Light quality		Natural variations
Temperature	Partial reduction of leaf temperature using shade cloth	Natural variations
Humidity		Natural variations
Wind speed	Partial reduction with shade cloth	Natural variations
Ambient pollutants		Natural variations

[a]These factors were determined from the first pilot study. They affect sensitivity during the growth of the plant as well as during the exposure period.

PILOT STUDY II

Results of the first study enabled researchers to develop a second study. Its procedure, modified following its completion, appears in the Appendix. The second study was designed to answer four questions:

1. What is the best soil mix and nutrient combination to use for maximum plant sensitivity and good growth?

2. How long is the tobacco sensitive and what growth habit is preferable?

3. What shade factor should be used?

4. What is the area of influence of the photochemical complex in the Cincinnati area?

SOIL AND NUTRIENT COMBINATION—1

Three soil types (peat-perlite, peat-perlite-soil, and peat-perlite-soil-manure) and four nutrients (Hoagland, Plant Marvel,* Plant Marvel and Cal-Mag, and tap water) were used in this experimental design. Each nutrient except tap water was used with each soil type. Tap water was used only with the peat-perlite-soil-manure soil to give ten soil-nutrient combinations. Four replicates were used per treatment combination, and the complete set of ten soil-nutrient combinations was tested seven times during the summer period, for a total of 28 replications per treatment.

Tobacco was seeded, transplanted after 4 weeks into the different soil types in 4-inch plastic pots, and grown for 1 week with the appropriate nutrient in a greenhouse in which the air was charcoal filtered. The plants were then placed outside under a 50 percent shade cloth, and exposed to the ambient air for 2 weeks. A new experimental replicate was started each week to allow an overlap. Injury indices were recorded every day for 2 weeks, then the plants were discarded. Indices for average injury per leaf for each plant were obtained and used to determine results shown in Table 3. The plants grown with tap water showed poor growth with obvious nutrient defiency; and although they were the most sensitive, their poor growth made them poor monitors for air pollution. Plants grown in the Hoagland nutrient were more sensitive than those fertilized with Plant Marvel and Plant Marvel plus Cal-Mag but did not grow as well. Considering health of plants, sensitivity, and work involved in care and maintenance of monitoring sites, peat-perlite-soil with a 20-20-20 nutrient addition (this could be the Plant Marvel) is recommended for use with the monitoring plants.

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*Mention of company or product name does not constitute endorsement by the Department of Health, Education, and Welfare.

Table 3. INTERACTION OF SOIL AND NUTRITION ON RESPONSE OF TOBACCO TO AMBIENT OXIDANT LEVELS[a]

Soil	Hoagland	Plant Marvel	Plant Marvel Cal-Mag	Tap Water	Average Soil
Peat-perlite (1/1)	41	31	35		36
Peat-perlite-soil (1.5/1.5/1.0)	43	37	34		38
Peat-perlite-soil-manure (1/1/1/1)	43	37	35	(54)[b]	39
Average nutrients	42	35	35		

[a]Values are reported as average injury per leaf on a percentage basis for those leaves showing injury from ambient pollution. All values are averages of 28 replications. Mean values connected by lines are not statistically different. Summary shows no difference between the soils, although there appears to be an interaction between nutrient and soil in the case of Plant Marvel and peat-perlite. The Plant Marvel and Plant Marvel plus Cal-Mag are not different from each other, but are different from the other nutrients. Hoagland and tap water are different in the peat-perlite-soil-manure mix.

[b]The value listed for tap water was not included in the average for the peat-perlite-soil-manure mixture.

SOIL AND NUTRIENT COMBINATION—2

Two soil types (peat-perlite-soil and peanut hulls-soil) and two nutrients (Kapco and Plant Marvel plus Cal-Mag) were used in this experimental design. Kapco was used only with the peat-perlite-soil; the second nutrient was used with both soils. The tobacco was grown with initial transplant as detailed in the Appendix. The second transplant into bushel baskets was into the experimental soil type with the appropriate nutrient. Each treatment was replicated 12 times in a randomized block design and was an integral part of the study on growth effects. Plants were exposed to the ambient air under a 50 percent shade cloth.

Injury indices were obtained daily (Monday through Friday), and cumulative injury indices for each plant were reported after 4, 8, 12, and 15 weeks. Mean values (12 replications) for each treatment are reported in Table 4. No evidence of a nutrient effect was noted. After the first 4-week period significantly less injury to plants grown in the peanut hulls-soil mixture than to those grown in the peat-perlite-soil mixture was noted. This was probably due to the longer transplant recovery period in the heavier peanut hull-soil mixture. No other 4-week period showed significant differences, although the last 3-week period showed greater injury to plants in the heavy mix. This may have been due to the greater amount of uninjured tissue on the plants in the heavier soil treatment prior to the last 3-week period. Seasonal values for the three treatments are the same. The heavier soil is not recommended for monitoring purposes, because severe wilting at transplant results in a slower start. The peat-perlite-soil mixture with a 20-20-20 nutrient addition is recommended.

Table 4. EFFECT OF FERTILIZER AND SOIL TYPE ON SENSITIVITY OF BEL W3 TOBACCO TO AMBIENT POLLUTION[a]

Soil/fertilizer	Cumulative injury indices			
	7/13 (4 week)	8/10 (8 week)	9/7 (12 week)	9/28 (15 week)
Peat-perlite-soil (1.5/1.5/1.0) Plant Marvel 20-20-20 Alternate Cal-Mag	915	1255 (2170)	830 (3000)	555 (3555)
Peat-perlite-soil (1.5/1.5/1.0) Kapco 20-20-20	870	1285 (2155)	905 (3060)	515 (3575)
Peanut hulls-soil (1/4) Plant Marvel, 20-20-20 Alternate, Cal-Mag	695[b]	1275 (1970)	865 (2835)	670 (3505)

[a]Values are reported as percent cumulative leaf injury per plant. Each plant value is obtained by adding the individual injury percentages from each leaf.

[b]Value is statistically different from the other two values listed on 7/13 at the 0.01 level.

GROWTH INTERACTIONS

Tobacco was chosen as a monitoring plant in part because it produces new leaf tissue throughout most of the growing season. In conjunction with the nutrient study, three growth habits were studied for each soil-nutrient combination for a total of 12 replicates per growth habit. These were grouped in studying the growth effects, because the soil-nutrient had no effect. One group of plants grew normally throughout the season producing, after 8 to 10 weeks, tall, rather unwieldy plants that were difficult to handle. A second group was topped after 8 weeks and the regrowth was studied. A third group made use of new plants every 4 or 8 weeks. With 12 plants per nutrient treatment, each growth treatment contained four plants. In the three growth treatments (one per soil-nutrient combination) in which new plants were used, the four plants per treatment were started together. Two of the four plants in one soil-nutrient combination were replaced with young plants after 2 weeks. Two of the four plants in each of the other two soil-nutrient combinations were replaced by young plants after 4 weeks. Thereafter, the two older plants in the first soil-nutrient combination were replaced every 2 weeks and the two older plants in each of the other two combinations were replaced with young plants every 4 weeks.

Cumulative injury indices were obtained as in the soil-nutrient study and are reported in Table 5. Because it takes a week to 10 days for newly transplanted plants to show maximum sensitivity, the cumulative indices for the replacement plant series were obtained from only two plants for the 10 days after plants were replaced. Values were not different after the first 10 days. Plants were topped after 8 weeks, and results showed that it took about 4 weeks for these plants to regain sensitivity. Plants grown during full season and replacement series plants were no different after 12 weeks. Plants transplanted during September developed slowly and did not produce as much sensitive tissue. Thus, they show less injury during the last 3-week period. The regrowth was well started after 4 weeks and contained considerable sensitive tissue during the last 3 weeks. Plants grown full season were in flower, and all leaves, including the floral bracts, showed injury at the end of the 15-week exposure period.

Table 5. EFFECT OF GROWTH PERIODS ON TOTAL INJURY TO BEL W3 TOBACCO FROM AMBIENT POLLUTION[a]

	Cumulative injury indices[b]			
Growth period	7/13 (4 week)	8/10 (8 week)	9/7 (12 week)	9/28 (15 week)
Full season	815	1285 (2100)	915 (3015)	660 (3675)
4- and 8- week growth alternating replacement every 2 and 4 weeks	840	1235 (2075)	820 (2895)	**425 (3320)
Topping after 8 weeks with regrowth	820	1300 (2120)	**190 (2310)	670 (2980)**

[a]Values are reported as percent cumulative leaf injury per plant. Each plant value is obtained by adding the individual injury percentages from each leaf.

[b]Values with asterisks are statistically different from the other two values listed for the same date at the 0.01 level.

Plants that were allowed to develop for a full season were the most severely injured; however, the injury was not much greater than that seen in the replacement series. Because of difficulties inherent in working with large plants, a replacement sequence of 6 weeks - alternating replacement of two of the four plants every 3 weeks is recommended in the procedural outline in the Appendix.

SHADING INTERACTIONS

Four plants were grown as detailed in the Appendix on an 8 to 4 replacement basis with final transplant into bushel baskets under 0, 35, 50, and 80 percent shade. Injury indices were obtained and reported as in the earlier studies. Results are given as cumulative average plant values for the paired plants in the different shade treatments (Table 6). Results suggest that plant sensitivity is reduced if plants are left in the sun during the mid-summer months, possibly because of higher temperatures, light intensity, and drought conditions, all of which may cause soil moisture stress. A 50 percent shade cloth is recommended for all monitoring work to protect the plants.

FIRST SUMMER MONITORING PROGRAM

Plants were grown as detailed in the Appendix on a 4-week alternating replacement basis at seven locations in the vicinity of Cincinnati, Ohio. Cumulative leaf injury on a percent basis for each plant was obtained on a weekly basis at each location. Continuous oxidant readings were recorded at

Table 6. EFFECT OF SHADE ON SENSITIVITY OF TOBACCO BEL W3 TO AMBIENT OXIDANT LEVELS[a]

Date replaced	Length of exposure, weeks	Plants (paired)	Cumulative injury indices, percent shade			
			0	35	50	80
7/06/66	4	odd	490		615	608
8/03/66	8	even	1500		1902	2070
8/31/66	8	odd	1658	1673	1553	1680
9/28/66	8	even	1313	1280	1295	1293

[a]Values are reported as percent cumulative leaf injury per plant. Each plant value is obtained by adding the individual injury percentages from each leaf. Definite reductions are noted with the 7/6 and 8/3 comparisons between 0 shade and both the 50 and 80 percent shades. These are somewhat tenuous because only two plants were used for each data point.

the laboratory (Mast) and corrected to 2 percent neutral KI values. The weekly oxidant index was recorded as the cummulation of hourly averages that exceeded 3.0 parts per hundred million (pphm) of oxidant during the hours between 6 a.m. and 10 p.m. All hourly averages from the time the oxidant level reached 3.0 pphm were included through the last one above 3.0, even if some of the mid values fell below 3.0. The total was then arbitrarily divided by 2 to obtain the final index. Plants were lost at two locations from high winds. Although the recorded oxidant injury was similar to injury to plants at the other five locations, data from the two locations are not included in the summary table. Table 7 includes the weekly oxidant index, the weekly plant injury index for the major study (Tables 4 and 5), and the weekly plant injury index for five of the monitoring sites. Distance and direction from the center of Cincinnati are included as well as the season's total cumulative indices. The injury index to oxidant index ratio was determined for the two groups of plants located at the oxidant monitoring site.

No consistent relationship between oxidant values and plant injury was found. Meteorological factors were not included in this study. Possibly an inclusion of these would produce a correlation between oxidant and injury. The levels of sulfur dioxide and nitrogen dioxide would be of interest in view of the reported synergism between sulfur dioxide and both ozone and nitrogen dioxide. The data suggest that within the limits of the study there is little difference in the phytotoxic potential of the pollution complex at any location monitored. There is a suggestion that at certain times one station has a higher phytotoxic potential or that meteorological conditions are such that plants show variable sensitivity at the five locations. Injury to the two groups of plants at the laboratory was remarkably uniform.

SECOND SUMMER MONITORING PROGRAM

Plants were grown as detailed in the Appendix on a 3-week alternating replacement basis at five locations east of Cincinnati, Ohio. Stations were located at 5, 7, 25, 50, and 75 miles east of the center of the city. Injury indices and oxidant indices were generated as outlined, and results are shown in Table 8.

Table 7. RESULTS OF FIRST CINCINNATI AREA SUMMER MONITORING PROGRAM[a]

Location	Distance (miles) and direction from Cincinnati	Weekly accumulated plant injury or oxidant indices													
		6/17	6/24	7/1	7/8	7/15	7/22	7/29	8/5	8/12	8/19	8/26	9/2	9/9	Total
Oxidant (laboratory)[b]	5(E)	140	240	280	230	215	195	220	295	255	165	100	195	125	2655
Laboratory - 1 (Tables 4 and 5)	5(E)	10	90	225	310	270	400	290	200	250	255	190	125	190	2805
Laboratory - 2 (Monitor site)	5(E)	10	180	175	350	300	340	255	100	245	330	190	125	130	2730
Dunning	18(E-NE)	65	330	80	200	270	235	260	100	120	305	235	190	55	2445
Matthis	25(E)	20	190	200	260	295	290	235	165	165	270	160	175	200	2625
Clark	30(NE)	50	300	200	255	305	250	225	245	190	325	85	175	110	2715
Ross	30(SE)	15	190	205	130	310	250	200	255	105	225	175	255	195	2510
Ratio:															
Injury/oxidant	Laboratory - 1	0.06	0.38	0.81	1.33	1.26	2.06	1.31	0.69	0.97	1.58	1.92	0.66	1.53	1.06
	Laboratory - 2	0.06	0.74	0.74	1.52	1.38	1.77	1.16	0.34	0.97	2.04	1.89	0.66	1.04	1.03

[a]Values are reported as average percent cumulative new leaf injury per plant (average of four plants). Each plant value is obtained by adding the injury percentages from each leaf. Differences, which might be due to area distribution of oxidant and/or to localized meteorological conditions, do occur among locations.

[b]The oxidant index is the accumulation of hourly averages that exceed 3.0 pphm of oxidant (KI) during the hours 6 a.m. to 10 p.m. When the oxidant level increases to 3.0 pphm, all hourly averages are included through the last one above 3.0 pphm, even if some of the middle values fall below 3.0 pphm. The total is then divided by 2 to obtain the oxidant index.

Table 8. RESULTS OF SECOND CINCINNATI AREA SUMMER MONITORING PROGRAM[a]

Location	Distance (miles) and direction from Cincinnati	Weekly accumulated plant injury or oxidant indices													
		6/13	6/20	6/27	7/5	7/12	7/19	7/26	8/2	8/9	8/16	8/23	8/30	9/5	Total
Oxidant (laboratory)[b]	5(E)	310	200	90	35	110	40	80	210	170	135	150	95	110	1735
Laboratory	5(E)	10	60	50	200	85	230	95	270	290	180	110	200	175	1955
Benken	7(NE)	40	125	200	185	110	120	75	255	260	240	160	80	135	1985
Matthis	25(E)	15	65	130	215	340	80	80	265	225	205	140	115	280	2155
Garrison	50(E)	10	65	240	230	255	115	65	205	255	75	75	120	140	1850
Shoemaker	75(E)	0	25	85	285	125	260	225	140	270	205	55	120	230	2025
Ratio: Injury/Oxidant	Laboratory	0.03	0.30	0.56	5.70	0.77	5.80	1.19	1.29	1.71	1.33	0.74	2.10	1.59	1.13

[a]Values are reported as average percent cumulative new leaf injury per plant (average of four plants). Each plant value is obtained by adding the injury percentages from each leaf. Differences, which might be due to area distribution of oxidant and/or localized meteorological conditions, do occur among locations.

[b]The oxidant index is the accumulation of hourly averages that exceed 3.0 pphm of oxidant (KI) during the hours 6 a.m. to 10 p.m. When the oxidant level increases to 3.0 pphm, all hourly averages are included through the last one above 3.0 pphm, even if some of the middle values fall below 3.0 pphm. The total is then divided by 2 to obtain the oxidant index.

No consistent relationship between oxidant values and plant injury was found during the 1967 monitoring season. The data suggest that within the limits of the study little difference exists in the phytotoxic potential of the pollution complex with distance from Cincinnati. During any given week there are real differences in amount of injury among sites. It is suggested that Cincinnati is not the major source of pollution for the two distant sites and may have relatively little effect on the site farthest from the city. However, no area of southeastern Ohio is free of phytotoxic oxidant pollution. Considerable new injury was recorded during each week of the test period.

DISCUSSION

The technique for a relatively simple plant monitoring system for the photochemical complex has been developed. The system employs the sensitivity of Bel W3 variety tobacco grown under controlled soil conditions and 50 percent shade. Methodology was worked out and duplicated over two seasons of actual exposures. Although correlations with oxidant levels are poor, the injury resembles ozone injury to tobacco under controlled exposures and the field injury is associated with the photochemical complex.

Results presented in this paper suggest that the impact of the photochemical complex is not just an urban problem but is a problem in rural areas as well. Probably all areas east of the Mississippi have sufficient photochemical pollution to produce injury to sensitive plants at certain times during their development.

The use of this monitoring system would give a community estimates of the frequency of the occurrence of phytotoxic levels of oxidants, an estimate of severity of each fumigation, and some estimate of areal distribution of phytotoxic potency in both time and space.

APPENDIX
TOBACCO MONITORING PROGRAM PROCEDURE

I. Plants

A. Tobacco

1. Scientific name - Nicotiana tabacum, L.

2. Variety Bel W3 (from Dr. H. E. Heggestad, USDA, Beltsville, Maryland).

B. Culture

1. Seedlings and transplants must be grown in an enclosure in which the air is charcoal filtered.

2. Seeding

a. Fill a 4-inch pot with vermiculite and wet the vermiculite.

b. Spread 30 to 50 seeds over the top of the vermiculite. This will yield about 30 good seedlings per pot.

c. Place pot in a saucer.

d. A special growth chamber in which temperature can be regulated (70°F night temperature and 80°F day temperature) and day length can be regulated (8-hour day with a light intensity of 2000 ft-c) is recommended for growing seedlings.

e. Water seedlings pot from the bottom (in the saucer) with 1/2-strength nutrient (see III) until after emergence.

f. After emergence, seedlings can be watered from the top with 1/2-strength nutrient.

3. Transplanting

a. When plants are 25 to 30 days old, transplant into individual 4-inch pots in a peat-perlite mix (see II-A).

(1) Separate plants carefully, allowing some vermiculite to remain on the roots.

(2) Hold the plant by the stalk in the center of an empty 4-inch pot and add the peat-perlite mix. The mix should be packed gently to provide good contact with roots. The mix should be added to within 1/4-inch from the top of the pot and the plant held at a height so that the leaves are exposed to the air.

(3) Wash mix from the leaves with distilled or deionized water.

(4) Soak each pot with full-strength nutrient (100 150 cm^3) immediately after transplanting.

(5) Place pots into trays to which full strength nutrient is

added. The trays should be washed thoroughly at least once a week to control algae growth and be refilled with full-strength nutrient. Distilled or deionized water can be added to trays when needed.

(6) These transplants are grown in the greenhouse where the temperature is 80° to 95°F during the day and 70°F at night.

(7) After the seedlings have been transplanted into individual pots and have three good-sized leaves (about 2 weeks after transplanting), remove any lower leaves less than 5 inches in length (measured from the stalk to the tip of the leaf).

b. At this stage of growth (at least 3 good-sized leaves), transplant the tobacco into bushel baskets.

(1) Fill the baskets to approximately 2 inches from the top with the peat-perlite-soil mix (see II-B).

(2) Remove plant with the peat-perlite mix from the 4-inch pot and place it into a hole made in the soil. Pack the soil firmly around the plant and moisten the soil in the basket with 2 gallons of 20-20-20 fertilizer solution (see I-B-5-a (2).

4. Use four plants per location.

a. After the first 4 plants have been in baskets for 3 weeks, replace two of them.

b. Thereafter, every 3 weeks replace the two oldest plants.

5. Daily care

a. Recommended watering schedule

(1) Every other week with Cal-Mag Special.

(a) Mix 1 tablespoon of the fertilizer per 2 gallons of water.

(b) Water each plant with 2 gallons of the solution.

(2) Alternate 20-20-20 with Cal-Mag every other week.

(a) Mix 1 tablespoon of the fertilizer per 2 gallons of water.

(b) Water each plant with 2 gallons of the solution.

(3) Water plants with tap water at least twice a week in addition to the nutrient. Soak soil until water comes out base of the basket (2 gallons).

(a) Soil must be kept moist at all times or tobacco will lose sensitivity.

(b) Peat mulch may be used to help keep the soil moist.

b. Disease and insect control

(1) There is a need for a general spray program for insect control.

(a) Chlordane sprayed in the basket area (not on the plant) will offer some help.

(b) Plants may be sprayed with Isotox and Malathion (each mixed at the rate of 1 tablespoon per gallon of tap water) whenever insects appear, or on a regular schedule.

(c) A systemic insecticide may be used in place of the spray. Add every month.

(2) If a plant becomes diseased, discard plant and basket.

II. Soil

A. Peat-perlite-mixture

1. Materials needed

a. Peat moss

b. Perlite

c. Lime (superfine limestone) and $CaSO_4$ or gypsum

d. Distilled or deionized water

e. 10-quart pail

f. Small cement mixer preferred method of mixing

2. Procedure mix in cement mixer

a. 2 pails (10 qts. each) of peat moss (level full)

b. 2 pails (10 qts. each) of perlite (level full). To each pail of perlite add 2000 cm^3 of distilled or deionized water until all perlite is wet. This equals 4000 cm^3 per mix.

c. When the peat moss and perlite are well mixed (approximately 5 minutes) add: 72 grams lime and 48 grams $CaSO_4$ or gypsum. Add slowly by hand (tends to stick to sides of mixer).

d. Mix for 20 to 30 minutes, stopping mixer two or three times to break up lumps.

e. Let mix age for 2 weeks and check pH. Mix pH should be between 5.5 and 6.0. If pH is below 5.2 remix using another 72 grams of lime. This should bring the pH to above 5.5.

B. Peat-perlite-soil mixture for bushel baskets

1. Use three parts peat-perlite with one part top soil.

2. Rich loam soil is recommended.

3. Mixing is done in cement mixer following the same general

outline as listed for mixing the peat-perlite but with no water or mineral additions. The pH should be between 5.5 and 6.5.

III. Nutrient solution (Hoagland's) for plants in 4-inch pots. All of the chemicals needed for making the nutrient should be mixed with distilled or deionized water to form concentrated solutions. From these concentrated solutions, use the required amounts needed to make the nutrient. Technical grade KNO_3, $Ca(NO_3)_2 \cdot 4H_2O$), $MgSO_4 \cdot 7H_2O$, KH_2PO_4, and K_2HPO_4 is sufficient.

Chemical	Grams per liter of concentrated solution	cm^3 of concentrate per liter of water (for nutrient solution)
KNO_3	101	5
$Ca(NO_3)_2$ $4H_2O$	200	5
$MgSO_4 \cdot 7H_2O$	246	2
KH_2PO_4	102 } Mix these two to form one concentration	1
K_2HPO_4	43 }	
Fe chelate		0.05 gm

Solution B
(Minor elements)

Chemical	Grams per liter of concentrated solution	cm^3 of concentrate per liter of water
H_3BO_3	2.87 } These form one concentrated solution	1
$MnCl_2 \cdot 4H_2O$	1.80 }	
$ZnSO_4 \cdot 7H_2O$	0.22 }	
$CuSO_4 \cdot 5H_2O$	0.08 }	

1/2-strength nutrient equal parts of distilled or deionized water and full-strength nutrient solution.

IV. Enclosure frame for housing 4 plants

A. 8 by 4 by 6 feet high

B. Recommended construction

1. Use 1- by 4-inch lumber
2. If the program is to continue for one year, redwood should be used.
3. Brace corners with metal braces and bolts.
4. Frame should be <u>well</u> anchored.

C. Shading

1. North side open - all other sides covered with shade cloth
2. 50 percent shade needed

3. Shade material

   a. Saran shade material of 52 percent calculated shade recommended.

   b. Comparable material may be substituted for the saran.

4. Location

   a. Open area - no natural shade   south exposure on hills

   b. Have access to water

   c. Protect from possible vandalism

   d. Plants must be arranged in the enclosure so all have same exposure.

V. Injury indices

A. Reading

1. All plants should be read weekly, daily, or on some regular schedule.

2. The same individual should do all the reading.

3. The percentage of injury on each leaf of each plant should be determined. This is a subjective measure.

B. Summary

1. The percentage of area injured on each leaf is totaled for each plant at every reading.

2. New injury on each plant for any given period is determined by the difference in cumulative readings between the beginning and end of the periods.

3. Site averages are obtained by summing the four plant values and dividing by four.

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