OZONE DAMAGE TO TOBACCO IN CANADA[1]

F.D.H. Macdowall[2], L.S. Vickery[3], V.C. Runeckles[4], and Z.A. Patrick[5]

Abstract

Tobacco weather fleck has caused significant losses of flue-cured tobacco in southern Ontario since 1955. Fleck damage was greatest near the coast of Lake Erie and decreased progressively inland. Ozone has been shown to be one of the most important incitants of the fleck response in tobacco whereas parasitic fungi, bacteria and viruses were proven not to be implicated as causes of the disorder, The inherently susceptible variety White Gold, used in all tests, was rendered more susceptible by irrigation and nitrogen deficiency. It was also more susceptible during flowering and when producing lateral shoots. The degree and duration of stomatal opening were important factors in determining the amount of injury. Concentration of ozone in the air was shown experimentally to affect the speed of fleck response as well as the severity of symptoms. Statistically significant correlations between ozone concentrations and fleck damage were obtained from field data when the response of highly susceptible tissues only was considered, The merits of several visual rating methods are compared and discussed.

Introduction

Tobacco weather fleck has been a disorder of economic importance in Canada since 1955 (10, 14, 21). Its sudden appearance and increasingly wide distribution on this continent is associated with the general increase of air pollution one ingredient of which, ozone, has now been shown to be the causal agent (4, 5, 6, 8, 9, 13, 17, 20). Ozone was suggested to be the cause of weather fleck in Canada in 1957, and the Ontario Research Foundation measured air pollution in southern Ontario through the 1958 and 1959

---

[1] Contribution No. 316 from the Plant Research Institute, Canada Department of Agriculture, Ottawa, Canada.

[2] Plant Physiologist, Plant Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ont,

[3] Superintendent, Experimental Farm, Research Branch, Canada Department of Agriculture, Delhi, Ont.

[4] Plant Biochemist, Research Laboratory, Imperial Tobacco Company of Canada Ltd., Montreal, Que.

[5] Plant Pathologist, Research Station, Research Branch, Canada Department of Agriculture, Harrow, Ont.

growing seasons (12). Fleck responses usually followed high ozone concentrations, but in general there was no significant correlation between ozone concentrations and fleck in the field (10). However, correlative information was obtained in ozone fumigation tests (8), and better field evidence was obtained with improved methods of measurement of both ozone and fleck. This causal aspect was studied in collaboration with the Meteorological Branch, Department of Transport and the Department of Health and Welfare (9, 11). Preventive measures were also intensively investigated (3, 20). This paper is presented as a background for further reports on the problem. The amount and distribution of damage in Canada, the nature of fleck, the involvement of ozone, the importance of stomata, cultural conditions and methods of rating will be discussed.

Materials and Methods

Seasonal Damage and Geographical Distribution

The severity of weather fleck damage in terms of the whole tobacco crop in southern Ontario was estimated by extension officers of the Ontario Department of Agriculture, graders of the Imperial Leaf Tobacco Co. of Canada Ltd., 2nd research officers of the Canada Department of Agriculture. The individual estimates of leaf damage obtained by the graders for each year were totalled to provide an assessment of the extent of the overall damage. Accuracy in distinguishing this disorder from other leaf spots was increased by providing the graders with photographs of typical leaves affected with each type of spot for comparative use in the field. As a result, fairly reliable information was obtained as to the numbers of farms on which weather fleck occurred. This information was used to determine the percentage of the total acreage in each grader's territory occupied by crops showing weather fleck. The geographical distribution was obtained and compared from year to year. In addition to making a general survey of the extent of destruction of or partial damage to leaves the extension and research officers rated fleck damage at five specific locations. These were in a line from Port Burwell on Lake Erie (Fig. 1), inland. Five consecutive plants in each corner and in the center of a field at each location were rated according to the number of leaves affected and percent area of each leaf injured, In 1961, plants of the variety White Gold were grown to near maturity in pots at the Experimental Farm, Delhi, and ten were transplanted, Still potted, to each of five meteorological stations ranging inland from the lake. Individual fleck occurrences were rated at each station when possible.

Pathological Tests

For pathological tests tobacco plants, of the varieties White Gold, Hicks, and Delcrest, were collected from the Delhi and Harrow areas of southern Ontario as soon as flecking was observed. On each sampling date leaves were collected from (a) apparently healthy plants, (b) plants on which flecks had just begun to appear and (c) plants which had older lesions, The samples also included stalk and roots from both weather flecked and healthy plants. Each sample was examined microscopically. Fresh sections were mounted in distilled water and examined for active fungus mycelium and living bacterial cells within the tissues. Sections were also stained in boiling lactophenol-cotton blue, cleared in lactophenol and examined for evidence of mycelium or bacterial cells within the flecked areas. Epidermal strips were also taken from fresh leaf samples and examined for crystalline inclusions indicative of virus infection.

Isolations were made from sections of leaves washed and plated an potato dextrose agar (P.D.A.) adjusted to pH 4.0 and P.D.A. containing 1/30,000 parts rose bengal and 5% oxgall. In other tests the tissue were surface-sterilized by treating for 1/2 to 1 minute in a 30% hypochlorite-alcohol solution, and washed 3 times in sterile water before plating. The organisms which grew on the agar plates were isolated and identified. The virus group was identified by symptoms on the leaves and by mechanical inoculation of several differential hosts: cowpea, , glutinosa, N. samsum, and N. tabacum vars. Harrow Velvet, Delcrest, and White Gold.

Fleck Rating

A special site was selected each year within a mile of Lake Erie, and a small crop of the very susceptible White Gold variety of flue-cured tobacco was grown for the experimental study of weather fleck, The same site was used in both 1959 and 1960. The crop usually consisted of 5 ranges each having 52 rows of 24 plants, with north-south orientation of the rows. In addition, a row each of the Greek varieties Basma Serez and Ravici Drama was grown each year to indicate impending damage, since these two were the most susceptible to weather fleck, of a number of species of Nicotiana and 32 varieties of N. tabacum g-own at the site in 1958,

In each experimental crop, a representative number of @arts in randomly-distributed 10-plant sections of rows, was used for rating fleck damage. In the years 1959, 1960 and 1961, 50, 200 and 100 index plants, respectively, were used. All plants were topped in 1959, half of them in 1960 and none in 1961.

Several fleck rating methods were devised to appraise the severity of individual fleck occurrences for correlation with quantities of suspected casual agents. For reference purposes these are listed by number as follows:
1) Percent leaf-kill per plant. The percentage of dead tissue in the upper epidermis was estimated, Standard cards with known fractions blackened by flecks were prepared photographically. Each area, of a given fleck density on a leaf was rated by matching with a card and an index for the leaf was assigned after totalling these ratings. The percentages of damage on all the leaves were summed and averaged for each plant, daily, to give the cumulative fleck rating, Fresh fleck was estimated by subtracting the previous day's rating.
2) Fleck index per plant. Fresh fleck damage, distinguishable in the early part of the day as blue-black spots in the mesophyll, was rated daily for each leaf according to an index ranging from 0 to 5. The indices were broadly

interpreted to include a composite evaluation of density of flecking and area of leaf involved. The maximum value of 5 was applied to leaves completely covered with dense flecking. This could recur more than once on a leaf. The totalled leaf indices for each day were divided by the number of plants.

3) Percent flecked area per plant. The percentage area encompassed by fresh weather flecks within each leaf was recorded. The data for each day were totalled and divided by the number of plants examined.

4) Number of leaves flecked per plant.

5) Percent plants flecked.

6) Maximum fleck index per leaf. The ten highest leaf fleck indices of the day were averaged.

7) Maximum number of leaves flecked per plant. The ten plants having the greatest number of freshly-flecked leaves on a given day were assessed for that day.

8) Percent leaf area severely flecked per plant. To assess the effects of cultural treatments on damage, accumulated fleck was rated for each leaf longer than 5 inches on each index plant at several times during growth and at the time of harvest. This rating procedure involved the assessment, on each leaf, of the percentage area that was lightly flecked and the percentage area that was severely flecked. The average density of the former was about one-fifth of the latter, so the total damage was expressed as severe fleck after appropriate computation.

Attempts were made to record the time course of development of fleck by time-lapse photography. Three camera units (19) were set up, each with a single susceptible leaf in view, before forecast fleck occurrences. The sequence of appearance of flecks were later examined in the developed film by means of an analysis projector.

Ozone Tests

Greenhouse-grown tobacco plants of the variety White Gold were experimentally fumigated with ozone. Ozone was generated by passing tank oxygen through a high voltage gap, after which it was admixed with carbon-filtered air being blown at 40 cu. ft. per min. into a 72 cu. ft.-glass-walled chamber in the greenhouse. About 25 pphm ozone was obtained in the chamber with an oxygen flow of 100 cc/min. and with a 15,000-volt transformer fed by a variable transformer set at 100 V.

Measurements of ozone polluting the air in the tobacco crop were made continuously in 1960 and 1961 in a field laboratory operated by the Canada Department of National Health and Welfare. A Kruger "72" Double Analyzer, which measured photometrically the iodine liberated by the oxidation of neutral. potassium iodide, was used. The infiltration method used to measure stomatal opening has been described elsewhere (7).

Results

Seasonal Damage and Geographical Distribution

Estimates of the amount of tobacco damaged by weather fleck since 1955 are presented in Table 1. Damage was highest in 1955 and 1957, considerable damage occurred in 1961, and there was almost no loss in 1962.

Table 1. - Seasonal damage by tobacco weather fleck in Canada

Year	Damage to Tobacco (lb. x/1000)	
	Destroyed	Destroyed + Partial Damage (± 500)
1955	-	5,000
1956	1,012	3,500
1957	2,562	6,000
1958	-	2,500
1959	1,203	4,000
1960	432	4,000
1961	2,186	4,000
1962	2	500

The geographical distribution of the disorder varied considerably from year to year although some areas were regularly affected. Figure 1 comprises a series of maps of the tobacco-growing area of southern Ontario indicating the variation in the incidence of weather fleck from year to year. The circles, each centred approximately in the middle of a grader's territory, indicate the percentage of the territory's tobacco acreage in which weather fleck was observed, There were 100 to 150 farms in each territory. Figure 1 shows that the area to the southwest of Port Dover was the most severely and consistently affected. Crops close to Lake Erie were affected to a greater extent than those further inland.

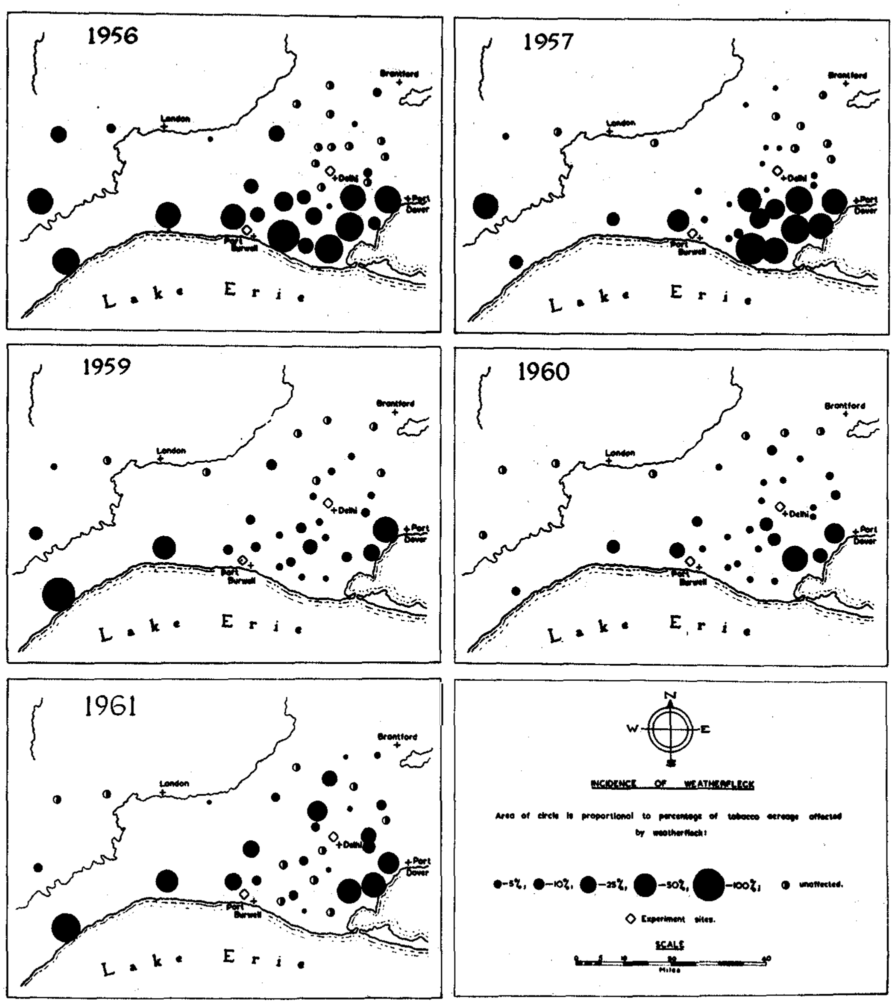

Figure I. Geographical distribution of tobacco weather fleck in southern Ontario. Incidence of fleck represented as percentage of tobacco acreage in which fleck appeared.

The latter conclusion is substantiated by the data in Table 2. The data given for 1959 were obtained from partially-primed plants of the varieties Hicks and White Gold growing beside satellite meteorological stations (11). The data for 1960 were obtained by examining a large number of crops. Half of those closest to the lake consisted of the more tolerant variety Delcrest, but further inland a quarter to a tenth of the farms grew Delcrest. For this reason the damage was not as great close to the lake (Table 2, 1960 data). The damage from a single fleck occurrence in 1961 is also shown in Table 2. This involved the rating of 50 White Gold plants set out at the meterorological stations after growth to maturity in a greenhouse.

Damage to plants was usually heaviest on the south and west sides of fields and in outside rows. For example, 32 out of 38 growers describing north, south, east and west damage in 1958 reported most damage on the south or west sides of fields. This was statistically confirmed in 1960 (unpublished). The consideration of ozone as the damaging agent led to its identification in air meteorologically advected from the southwest (9, 10).

Table 2. - Decrease of fleck damage with increasing distance from Lake Erie.

1959		1960		1961	
Dist. (miles)	No. Flecked Leaves/Plant	Dist. (miles)	Av. Loss ($/acre)	Dist. (miles)	Attack of Aug. 12 (Fleck Index/Plant)
2	3.6	0 to 5	49	0.3	21
4	2.1	6 to 10	53	2	13
6	0	11 to 15	46	4	11
10	1.5	16 to 20	34	6	0
13	0.4	31 to 35	27	8 and 12	0

Non-Parasitic Nature

Intensive microscopic examinations, isolation experiments and tests for mechanically-transmissable viruses during several seasons proved that tobacco weather fleck is non-parasitic in origin. Flecks examined within 48 hours of their formation were mostly free of bacteria, fungi and viral crystalline inclusions. Some typical data are shown in Table 3. As the flecks become older bacteria and fungi probably increase the severity of leaf damage.

These organisms are however, assumed to be secondary. Isolation tests were made to determine whether some known plant pathogens were constantly associated with weather fleck. The results of one series of tests are given in Table 4, Many organisms known to be pathogenic to tobacco leaves were

Table 3 - Results of microscopic examinations of sections from flecked and non-flgcked leaves showing relative proportions of fungi, bacteria and virus inclusions in the tissues.

Material	Number of Sections			
	Examined	+Fungi	t Bacteria	t Inclusions
Flecked Leaves				
a) Fresh Lesions	35	9	3	5
b) Old Lesions	35	21	12	6
Non-Flecked Leaves	25	5	1	3

Table 4. - Relative numbers of microorganisms isolated from tobacco leaves with and without weather fleck.

Material	Number			Parasites (Times Isolated)	Virus (Times Isolated)
	Isolations	Fungi	Bacteria		
Flecked Leaves					
A) Fresh Lesions	50	12	2	I *Alternaria longipes* (7) II *Cercospora nicotianae* (2) III *Pseudomonas tabaci* (1)	I Mosaic gp. (5) II Etch gp. (1) III Ringspot gp. (2) IV Potato Y (2) V Unk. mixt, (5)
B) Old Lesions	50	32	6	I (15) II (5) III (2)	I (3) II (2) V (10)
Non-Flecked Leaves	25	4	1	I (2)	I (3) II (1) IV (2) V (5)

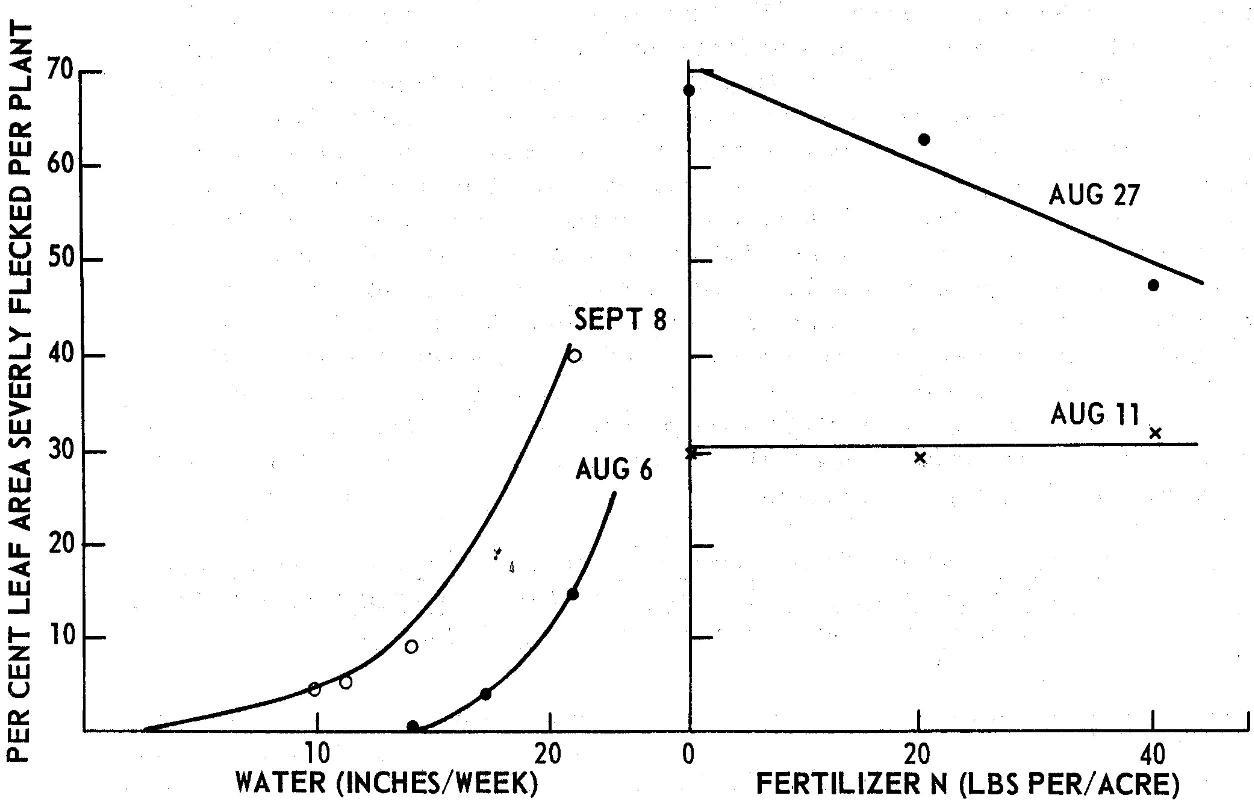

Figure 2. The effects of the amount of applied water (rainfall *t* irrigation), left side, and amount of fertilizer N applied with 120 lbs. P and 120 lbs. K, right side, on the amount of weather fleck accumulated on plants (by rating method number 8 in text).

associated with fleck-like areas, but many of the flecked areas also appeared to be free of microorganisms. None of the isolated organisms produced the typical weather-fleck symptoms when re-inoculated into tobacco leaves. Similarly, inoculation experiments with mechanically-transmitted viruses failed to produce these symptoms.

Influences of Cultural Practices

Information on flecking as a physiological disorder was obtained from experiments employing different cultural practices as well as from differences in varietal response (3). Experiments over 3 years revealed a direct relation between amounts of fleck and applied water (21). The relation was exponential (Figure 2). Nutrition experiments, involving different rates and proportions of commercial fertilizer ingredients, and also foliar sprays and drenches of a wide variety of elements, have established an inverse relation between applied nitrogen and amount of weather fleck (Figure 2). This effect became more pronounced later in the season. The prevention of floral development by the commercial practice of topping and suckering, or by topping and applying maleic hydrazide (MH_{30}), an inhibitor of lateral shoot growth, reduced the amount of weather fleck (Table 5). The treatments apparently acted by delaying the onset of leaf maturity (8). However, the maleic hydrazide treatment is known to adversely affect the quality of tobacco leaf, hence its use is undesirable as a control measure. The suppression of weather fleck by the foliar application of certain antioxidants (20) may also have some detrimental side effects.

Table 5. - Effects of manual and chemical sucker suppression, after topping plants of the White Gold variety, on the amount of weather fleck, as percent leaf area severely flecked (1958) and as the same per plant (1959).

Treatment	1958	1959	
	Fort Burwell	Port Burwell	Delhi
None	217	26	6
Hand suckering	67	15	2
1.125 lbs. MH_{30}/acre 1 week before and at topping	2	11	0

Symptomatology

The symptom known as tobacco weather fleck (Figure 3) (1, 14, 21) can be produced by experimentally generated ozone (Figure 4) (4, 5, 6, 16, 20). However, the fleck symptom is not specific to ozone damage. A fleck response was elicited by sulfur dioxide (15, 22). We have unpublished data

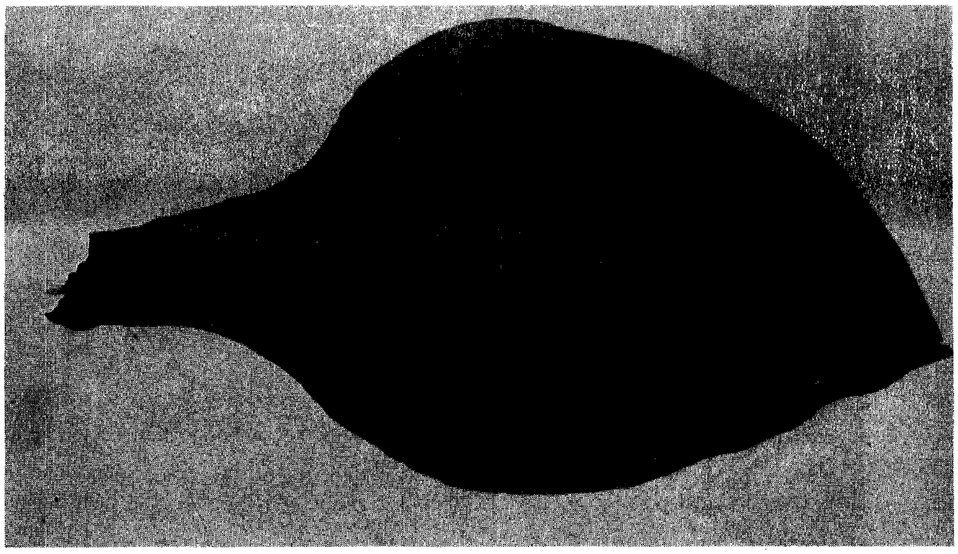

Figure 3. Tobacco weather fleck

confirming this (Figure 4). Tobacco leaves may also fleck as a result of the application of 0.01 M solutions of certain amino acids, particularly serine and alanine, or 0.1 M of alkaline ammonium acetate, either as a foliar spray or as a soil drench.

The histology of weather flecks in Canadian flue-cured tobacco has been described (14). Macroscopically the colour, size and pattern of flecks are variable. The primary lesion, consisting of a darkened (blue-green) group of palisade cells, extends to include the contiguous cells of the upper epidemis. The fleck changes through black to brown as autolysis and dessication occur, and may be bleached in leaves that are sufficiently green. All colour conditions may be seen on one leaf.

Susceptibility changes occur as the leaf develops and are especially evident between the completion of expansion and the yellowing of the leaf (8). The largest individual flecks, up to 20 square mm, occur on the oldest susceptible leaves. An excessive dose of ozone produces large necrotic patches on the leaves as a result of fusion of numerous flecks (Figure 4, B).

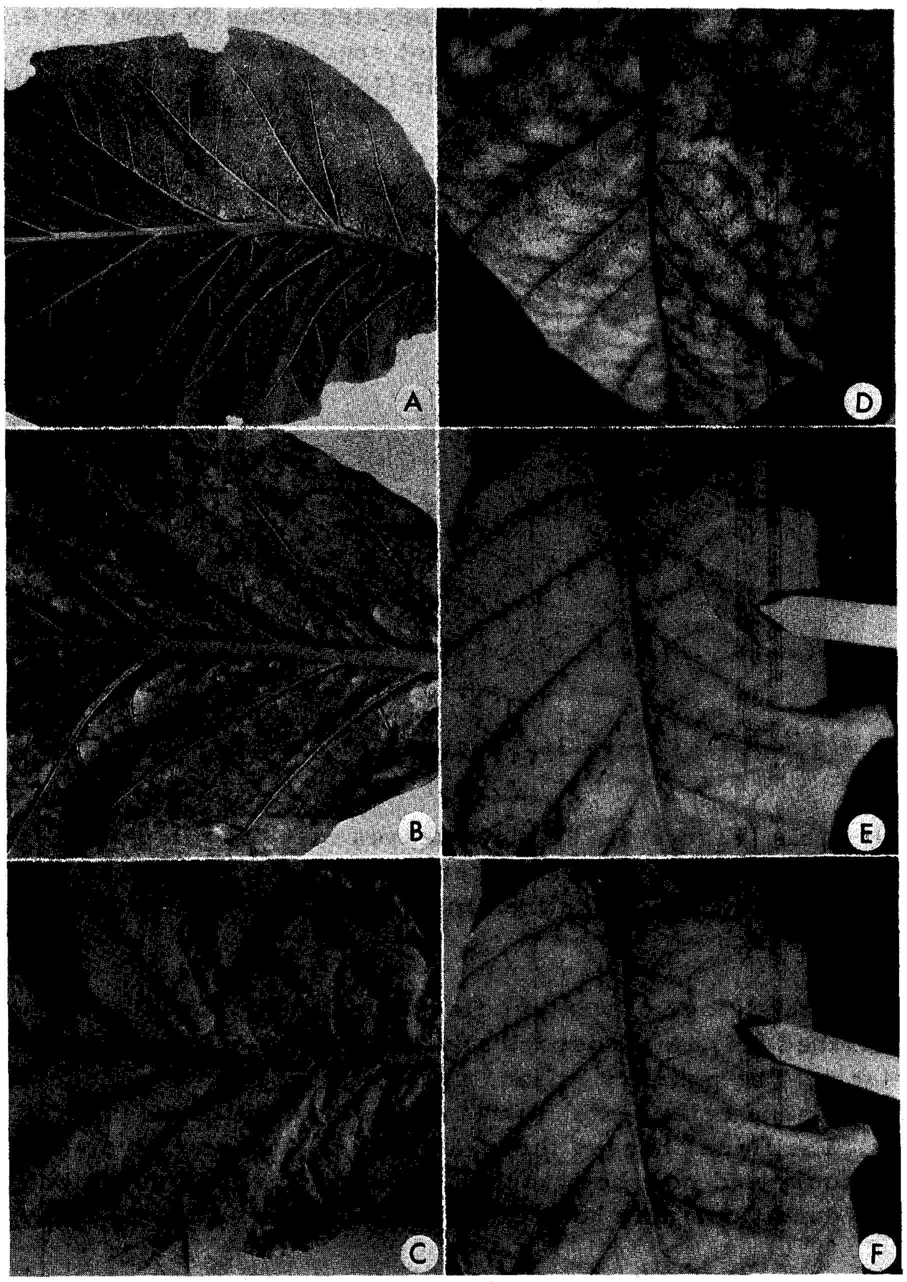

Figure 4. Damaged tobacco leaves from fumigation of greenhouse material with ozone or sulfur diozide. A) Bleached ozone flecks. B) Effect of ozone overdose. C) Ozone response of leaf having water deficit. D) E) and F) Leaves flecked by action of sulfur dioxide, showing (E and F) protective effect of overlying leaflet.

No leaf damage followed exposures to ozone under conditions inducing stomatal closure or blockage. A 6-hour fumigation with 45 pphm ozone in daylight damaged plants severely, but the same treatment in darkness was without effect. Similarly, two turgid planta that were treated with 45 pphm ozone for 6 hours developed 85% and 100% damage, respectively, whereas two wilted planta escaped injury. In the same test a wilted plant, watered before fumigation, recovered sufficient turgidity to suffer 30% damage. The flecks lined the midribs and veins of leaves under moisture-stress (Figure 4, C), and damage increased with leaf position from base to top of such a plant (Table 6).

Table 6. - Distribution of damage (Fleck Index) among the leaves of a turgid plant and a partially wilted plant fumigated with 45 pphm ozone for 6 hours.

Plant Condition	Leaf Number from Base												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Turgid	5	5	5	5	5	4	5	3	4	2	4	3	-
Partially Wilted	0	0	1	0	1	1	1	2	2	2	4	3	4

These symptoms differed from the turgid plant's more heavily-damaged mature leaves and intercostal leaf-damage. The protective effect of a leaf overlapping another, attributed to stornatal closure (4), was a common observation in the field and in experiments with ozone or sulfur dioxide [Figure 4, E and F). A plastic bag over a leaf or a tent over a plant (9) also'afforded protection from air-polluting ozone. Using impervious coatings on leaf surfaces we observed that, as in natural weather flecking (4), experimentally-generated ozone entered the leaf primarily through the. lower epidermis. Since lower guard cells were considerably more responsive to light and other stimuli than those in the upper epidermis (7) the accumulation of a damaging dose of ozone depended primarily on the lower epidermal stomata. Stomatal response to the microenvironment, including sunlight (Table 7), may be responsible for the occasionally-observed concentrations of weather fleck on one side of a row of tobacco plants.

Table 7. - Relation between damage (Fleck Index) by ozone and amount of leaf directly illuminated during fumigation with 35 pphm O_3 for 8 hours.

Leaf number from base	1	2	3	4	5	6	7	8	9	10
% Leaf in direct sun-light	100	0	100	0	100	100	0	100	50	50
Damage Index	2	1	4	0	1	4	0	4	0	2

There was a time lag between fumigation of the plants with ozone and the appearance of the fleck symptom (Figure 5). Flecking was characteristically first observed in the morning following the day of fumigation. Moreover, flecks appeared most quickly at the higher dosages. Figure 6 shows counts of fresh flecks in parts of a single leaf during three successive fleck occurrences of increasing severity. The data were obtained from a time-lapse photographic film. The fleck index per plant of 20 contiguous plants was 17, 18 and 20 on July 18, 19 and 23 respectively. The data show that the sooner the flecks appear the more damage to the plant. On days of heavy air pollution, very susceptible leaves developed visible flecks before dark, Statistical support for the time lag between dose and response was found'in the correlation coefficients for weather fleck on ozone dose, for various time lags (Table 8). The effect of a day's pollution was measured routinely'on the next day. In chamber experiments with greenhouse-grown tobacco plants, the amount of ozone damage increased with the dosage until stomata1 closure interfered. Typical results are giv'en in Table 9. In agreement with observations on weather fleck, the time between removal from fumigation and the appearance of symptoms decreased with increasing dose.

Comparison of Methods for Rating Fleck

The correlations between methods of rating fresh weather 'fleck and ozone incidence are given in Table 10. The lack of any significant correlation in 1959 is attributable to inaccurate ozone and flecking data. The complexity of the rating of accumulated fleck, as percent leaf tissue killed, caused a large error, such that some of the values for fresh damage, obtained by subtracting the day to day cumulative values, were negative. Also, the changing susceptibility of the growing crop was not taken into account in 1959.

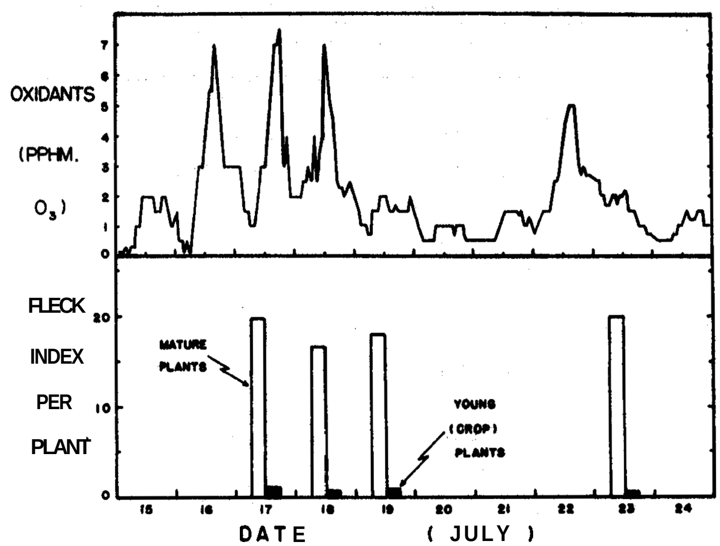

Figure 5. The weather fleck response of tobacco plants (histograms) in relation to high concentrations of ozone (upper curve). Data of 1960 at Port Burwell site.

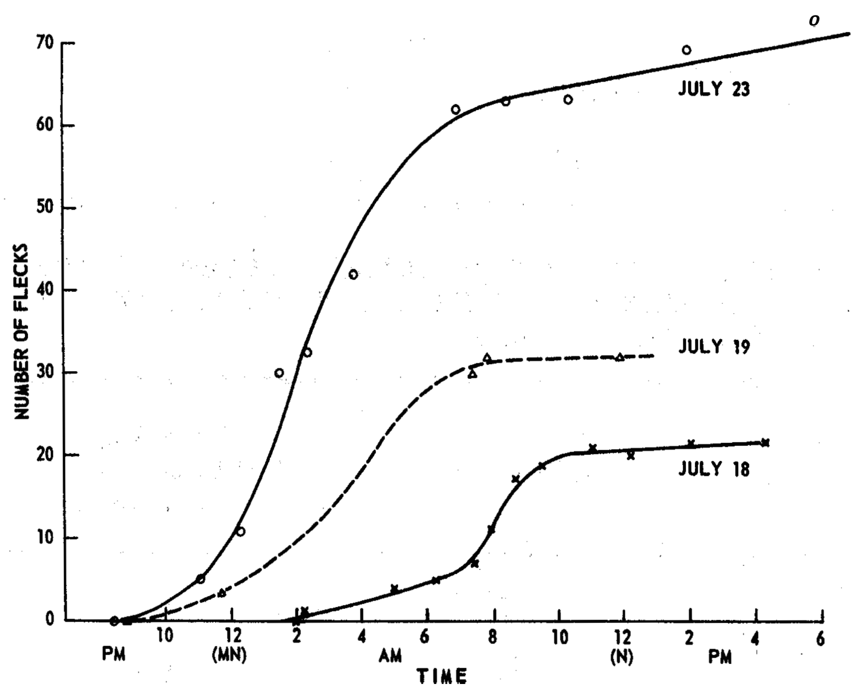

Figure 6. The time course of'the appearance of weather flecks on parts of a single leaf subjected to three successive attacks of increasing severity. Data from time-lapse photography.

Table 8. - Correlation coefficients of daily weather fleck (Max, Fleck Index/-Leaf) on daily ozone dose (pphm-hours), for successive time lags from the day of fleck appearance. 1960.

Time Lage Ozone Dose to Fleck (days)	Correlation Coefficient	Degrees Freedom	Significance
5 before	+ 0.167	66	
4 "	- 0.273	67	* $0.01 < P < 0.05$
3 "	- 0.254	68	* "
2 "	- 0.076	69	
1 "	+ 0.648	70	*** $P < 0.001$
0	+ 0.262	71	* $0.01 < P < 0.05$
1 after	- 0.087	70	
2 "	- 0.247	69	* "
3 "	+ 0.008	68	
4 "	t 0.009	67	
5 "	t 0.145	66	

Table 9. - Effects of increasing time of exposure to 45 pphm ozone, on the damage (% destruction of plant) and on the time required for the first appearance of damage,

Time of Exposure (minutes)	% Damage	Time-lag End of Exposure to Visible Symptoms
30	3	1 day
75	70	X hour
135	75	10 mins.
240	90	0 mins.

Table 10. - Correlations between data on weather fleck, rated daily by different methods, and daily ozone dose for three different seasons at experimental sites near Port Burwell.

Rating Method (see text)	Correlation Coefficients and Significance 1959	1960	1961
1) % Leaf Kill/Plant	- 0.106	--	--
2) Fleck Index/Plant	--	0.257*	0.245
3) % Leaf Area Fld./Plant	--	0.217	--
4) No. Leaves Flecked/Plant	-0.103	0.293*	0.266
5) % Plants Flecked	0.007	0.410***	0.220
6) Max. Fleck Index/Leaf	--	0.468***	0.315*
7) Max. No. Leaves Fld. /Plant	0.132	0.381***	0.262
Degrees Freedom	48	70	42

* $0.01 < P < 0.05$. ** $0.001 < P < 0.01$. *** $P < 0.001$.

Table 11 - Correlation coefficients and significance of the relationship between daily ozone doses and maximal fleck indices for two seasons with and without divisions into two time classes.

Date	Correlation Coefficient	d.f.	Significance
1960 Data			
July 12-Aug. 15	t .814	33	*** P < .001
Aug. 16-Sept, 22(two points omitted)	t .599	34	*** P < .001
Total (July 12-Sept, 22)	t .648	70	*** P < .001
1961 Data			
Aug, 1-Aug. 18(two points omitted)	t .548	14	* .01 P < .05
Aug. 20-Sept. 13	t .567	20	** .001 P < .01
Total (Sept. 1-Sept. 13)	+ .315	42	* .01 P < .05

Judging from the significance of correlation in 1960, the simplest methods for evaluating fleck (Table 10, methods 4 and 5) were as useful as the more detailed ones (Table 10, methods 2 and 3). There was no significant correlation of ozone with any of these sets of rating data in 1961. However, when the change in susceptibility of the crop was ignored and only the most susceptible leaves considered (Table 10, method 6), the correlation coefficients were improved to more significant levels. Further improvement of correlations resulted when on the basis of statistical and graphical examinations of the data, the seasons were divided into 2 time classes (Table 11).

The use of mature plants for rating individual fleck occurrences early in the season, as illustrated in Figure 5, is a practical way to evade the ontogenetic change in susceptibility of the crop. Very significant seasonal correlations were obtained in 1960 by rating July damage on a aet of mature, greenhouse-grown plants transferred to the field in June.

Discussion

The first occurrence of weather fleck in epidemic proportions in 1955 resulted from increasing pollution of air, widespread use of susceptible tobacco varieties, changed cultural practices, and appropriate combinations of meteorological factors. A reversion to the more tolerant variety Delcrest may partly account for the subsequent decrease in severity, but the continued increase of air pollution has caused an increasingly wide distribution of damage. The latter effect has been especially evident in areas beyond those covered in Figure 1. The inland dilution of damage reflects the lifting and breaking of pollutant-retaining inversions, as a result of convection currents from the land (11). The geographical and local distributions of fleck damage thus appeared to result from the effects of meso-and micro-meteorological factors on the dissemination of the catalysts of ozone formation.

Daily ratings of weather fleck were required for the study of its relation to environmental factors. The rating methods used'initially were cumulative; the day to day differences representing fresh flecking. However, a full index describing a severely-flecked leaf excluded a record of future attacks to that leaf, thus accentuating the exponential relationship between damage and visual rating (18). Therefore, an attempt was made to obtain daily estimates of the actual amount of dead tissue (method 1). This method was tedious, time-consuming and subject to errors that were on the same scale as the daily damage. These methods were abandoned in favour of rating fresh fleck only. A simple index, including severity and area-coverage (method 2), was applied and further simplifications proved to be still better measures of response to ozone doses. However, the most detailed method of rating fresh weather fleck, the leaf fleck index per plant, provided the best data on crop response from which to select maximal daily damage in order to evade the problem of changing susceptibility. The maximal fleck index, being the average index for the 10 most severely-damaged leaves on each day, represented the most susceptible tissue only, the nature of which was assumed to be the same in that respect from beginning to end of the season. The best correlations between fleck and ozone dose were obtained with this expression of flecking when the seasons were divided into 2 time classes. A physiological basis is implied by the coincidence of the time classes with the vegetative and flowering phases of development of the crop.

The correlations obtained between rated damage and ozone dose, particularly in 1960, were surprisingly good considering the day to day anomalous effects wrought by internal factors of susceptibility (8) and by external micrometeorological factora (9), and alao considering the non-linear relations between visual damage ratings and damage _per se_ (18).

Substitution of the crop by successive seta of identically grown mature plants, in successive intervals of time through the season, would provide the ideal material for the rating of fleck occurrencea, Some success was met with this method in 1960, but repetition in 1961 waa not successful as the greenhouse-grown plants of that year were much more tolerant. The conditions of preliminary growth required for maximum susceptibility deserve to be worked out.

As a result of many experiments in the early stages of this study a number of factors were tentatively excluded from a causal role in weather flecking. These included pathogenic microorganisms, certain classes of virus, soil type, natural gas and root exudation. However, it was early recognized that many factors are involved in the final expression of weather fleck, many of which are still not completely understood. These factors influence one or both of the two requirements, namely the susceptibility of the plants (8) and the toxicity of the air (9). Field and laboratory experiments strongly supported the thesis that ozone and its reaction products are the initiating agents of the weather fleck response. The concentration of ozone required to bring about the fleck response appears to be dependent on the physiological condition of the host and particularly of the leaf tissue. On one day a relatively low concentration of pollutant brought about injury while on another a much higher concentration was required. This cast some doubt on the causative role of ozone in the field. Nevertheless, with the use of especially defined parameters of dose and of damage, statistically good correlations have been obtained between ozone and weather fleck. Daily variations are attributable to the influences of micrometeorological factors on ozone flux (9) and stomatal opening. The importance of the latter has been unduly criticized (2). The variety of the tobacco and the nutrition and water economy of the plant are primary factors in the development of the physiological state of susceptibility (8).

Acknowledgements

The authors wish to thank Mr. A.F.W. Cole and Dr. Morris Katz, Director, Environmental Assessment, Department of National Health and Welfare for the use of data from continuous ozone measurements for the seasons of 1960 and 1961, and Mr. E.I. Mukammal and Dr. C. C. Boughner, Director, Climatology Division, Meteorology Branch, Department of Transport for associated meteorological studies. We gratefully acknowledge the work of Mesrrs. E. K. Walker, J. M. Elliot, and E. C. Birch, Department of Agriculture, on irrigation, nutrition and suckering experiments, respectively. Our thanks are also due to many others involved in thia project for their collaboration and asaiatance.

Literature Cited

1. BURK, L.G., and H.E. HEGGESTAD. 1956. Weather fleck in Nicotiana tabacum. Plant Dis. Reptr, 40: 424-427.
2. DUGGER, W.M., Jr., O.C. TAYLOR, EUGENE CARDIFF, and C. RAY THOMPSON. 1962. Stomatal action in plants as related to damage from photochemical oxidants. Plant Phyaiol. 37: 487-491.
3. HASLAM, R.J., and B. POVILAITIS. 1962. Note on the flue-cured tobacco variety - Delhi 61. Can. J. Plant Sci. 42: 212-213.
4. HEGGESTAD, HOWARD E. and JOHN T. MIDDLETON. 1959. Ozone in high concentrations as cause of tobacco leaf injury. Science 129: 208-210.
5. HILL, A.C., M.R. PACK, M. TRESHOW, R.J. DOWNS, and L.G. TRANSTRUM. 1961. Plant injury induced by ozone. Phytopathology 51: 356-363.
6. LEDBETTER, M.E., P. W. ZIMMERMAN, and A.E. HITCHCOCK. 1959. The histopathological effects of ozone on plant foliage. Contribs. Boyce Thompson Inst. 20: 275-282.
7. MACDOWALL, F.D.H. 1963. Midday closure of stomata in aging tobacco leaves. Can. J. Bot. 41: 1289-1300.
8. MACDOWALL, F.D.H. Predisposition of tobacco to ozone damage. Can. J. Plant Sci. In press.
9. MACDOWALL, F.D.H., E.I. Mukammal and A.F. W. Cole. Direct correlation of ozone and tobacco weather fleck. Plant Physiol. In press.
10. MACDOWALL, F.D.H., L.S. VICKERY, Z.A. PATRICK, E.I. MUKAMMAL, V. C. RUNECKLES, and H.B. MARSHALL. 1960. Tobacco weather fleck in Canada. Abstr. Meeting of Can. Soc. Plant Physiol. Univ. Toronto.
11. MACDOWALL, F.D.H., A.F.W. COLE, E.I. MUKAMMAL and MORRIS KATZ. 1962. Response of tobacco near Lake Erie to photochemical pollution as influenced by meteorological factors. Abstr. Ann. Meeting Amer. Chem. Soc., Atlantic City.

12. MARSHALL, H.B., B.C. NEWBURY and W.A. PETRIE. 1960. Pollution levels in rural southern Ontario. Abstr. 53rd Ann. Meeting of Air Pollution Control Association, Cincinnati.

13. MIDDLETON, JOHN T. 1961. Photochemical air pollution damage to plants Ann. Rev. Plant Physiol. 12: 431-448.

14. POVILAITIS, BRONIUS. 1962. A histological study of the affects of weather fleck on leaf tissues of flue-cured tobacco. Can. J. Bot. 40: 327-330.

15. TAYLOR, G.S. 1959. A possible cause of tobacco fleck (Abstr.) Phytopathology. 49: 229.

16. TAYLOR, G.S., W.G. De ROO and PAUL E. WAGGONER. 1960. Moisture and fleck of tobacco. Tobacco Sci. 150: 22-28.

17. TAYLOR, GORDON S., and SAUL RICH. 1962. 'Antiasonant-treated cloth protects tobacco from fleck. Science 135: 928.

18. TODD, GLENN W. and WILFRED N. ARNOLD. 1961. Evaluation of methods used to determine injury to plant leaves by air pollutants. Bot. Gaa. 123: 151-154.

19. VOISEY, PETER W. 1962. Movies record tobacco fleck. Research for Farmers. 7 (4): 11.

20. WALKER, E.K. 1961, Chemical control of weather fleck in flue-cured tobacco. Plant Dis. Reptr. 45: 583-586.

21. WALKER, E.K. and L.S. VICKERY. 1961. Influence of sprinkler irrigation on the incidence of weather fleck on flue-cured tobacco in Ontario, Can. J. Plant Sci. 71: 281-287.

22. WYGA, P. 1956. Weather fleck and air pollution. Abstr. Cigar Mfrs. Ann. Res. Seminar.