タバコにおけるオゾン処理と障害との関係

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The Relation between Ozone Treatment and the Injury in Tobacco*

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Since 1965 one of the non-parasitic leaf spots of unknown origin has occurred on flue-cured tobacco in Kinki and Kitashikoku tobacco areas and a severe damage to tobacco leaf production has been recorded. In 1969 such a fleck injury was observed in many tobacco areas all over the country.

Kuroda et al. (1970)[10,17] reported that two popular types of fleck injuries (Type II and III[9]) were caused by air-born oxidants and identical with tobacco weather fleck[6,15,16] observed in U.S.A. and Canada. The greater part of air-born oxidants has been identified as ozone[1,11]. Then an environmentally-controlled ozone fumigation chamber was constructed in order to investigate the effects of environmental factors upon the responses of tobacco plants to ozone.

The present investigations were undertaken to examine the uniformity of micro-environment in the fumigation chamber and also relations between the ozone dose (time × concentration) and responses of tobacco to ozone.

MATERIALS AND METHODS

Design of equipment: The system of equipments for fumigation consists of four units; fumigation chamber, growth cabinet, controller of ozone concentration and cleaning unit for exhausted air.

The growth cabinet (Osaka Reiki, Type TGL-101L) is equippd with provisions for light (23 Klx), temperature (5°～35°C) and relative humidity (55～75%) control. Mixed light of eight Yoko lamps (Toshiba D-400) and two fluorescent mercury lamps (Toshiba H250) provides 23 Klx at the plant level in the fumigation chamber. The fumigation chamber (inside dimention; 80×90×100 cm) settled inside the growth cabinet is made of acrilite plate (7 mm) and at the bottom of it ozone generators (Toyo Rika ultraviolet mercury arclamps) and a variable sirocco fan for air circulation are installed (see fig. 1). Ozone concentration in the fumigation chamber is recorded by Toyo Rika oxidants meter (Type OMR-2C) and controlled with electrically automatic system at the expected level (0～60 pphm*) within 3～5 minutes. The fan equipped with the fumigation chamber provides the ozone-polluted air moving through the plant fumigation area at velocities of 0.5～1.2 m/sec. Most of air polluted with ozone is circulated within the fumigation chamber passing

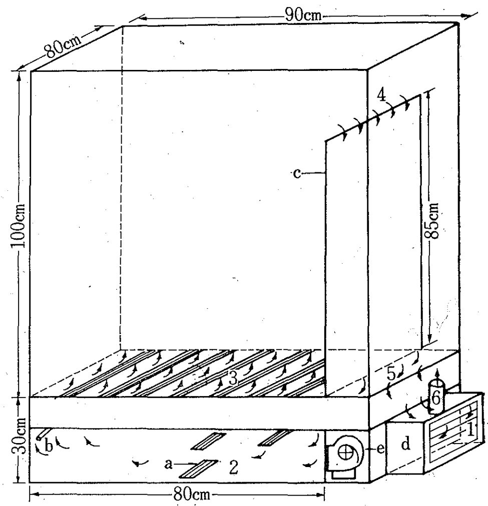

Fig. 1 Schematic design of the fumigation chamber

1. fresh air inlet, 2. air polluted with ozone, 3. polluted air driven to blow slantwise through the crevices, 4 and 5. circulating polluted air, 6. exhausted air.
a. ozone generators, b. air-sampling tube, c. acrilite screen, d. activated charcoal filter, e. sirocco fan.

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* 1 pphm = 0.01 ppm

through the space installed the ozone generators, a part of polluted air (80～150 *l*/min.) being exhausted via a centrifugal blower and, instead, polluted air flowing freshly into the fumigation chamber. The exhausted air is washed and discharged to open air.

Experimental procedure: Three varieties of tobacco (*Nicotiana tabacum* L), H-mutant[20], H-2[20] and Bel-W3[7] were used. Their seedlings were transplanted into 12 cm clay pot and grown to the expected stage in a greenhouse (20°～25°C) free from natural air pollution.

In Experiment 1, detached leaves (9～12th) of H-mutant at flowering stage were used. Leaves, of which basal parts were put in a sack of vinyl film and immersed, were hung at sixty-four equidistant locations in the plant exposure area (cf. table 2) to examine the effects of variability of chamber environment on the responses of plants to ozone. Sixty-four leaves used for one fumigation treatment arose all at the same insertion on the stalks. Fumigation treatments were conducted at 13°C[18] for 2 hours at 25 pphm ozone level. After fumigation the leaves were placed in total darkness at 13°C.[18] The amount of flecks was examined 48 hours after fumigation[18].

In Experiment 2, plants (H-2 and Bel-W3) with 17 leaves were used. Fumigation treatments were conducted at 13°C and in full light (23 Klx) for 1, 2, 3 and 4 hours at 10, 20, 30 and 40 pphm ozone levels respectively. Five plants were used for one treatment and these treatments were repeated three times in order to avoid the effect of fumigation time of day[19]. After fumigation the plants were transferred to a temperature-controlled glasshouse (13°C). The amount of flecks were examined 48 hours after fumigation.

In Experiment 3, plants (var. Bel-W3) with 20 leaves were used. Two days prior to fumigation

Table 1 Schedule of fumigation treatments in Experiment 3

Time of day	Day				
	1st	2nd	3rd	4th	5th
7.00～ 8.00	5	2	3	4	5
8.15～ 9.15	3	5	1	5	4
9.30～10.30	4	4	5	2	3

Each number shows plot.

they were transferred to the condition of 12-h light (7.00～19.00, 33 Klx) and 12-h dark (19.00～7.00) at 23°C[18]. According to time of fumigation 5 plots were designed. In each plot total dosage (pphm × h) of ozone was the same, but differred in ozone level and exposure time at fumigation (13°C, 23 Klx). Schedule of fumigation treatments of each plot (containing 9 plants) is shown in table 1. Ozone levels in plot 1, 2, 3, 4 and 5 were 40.0, 20.0, 13.4, 10.0 and 8.0 pphm respectively. After fumigation they were brought to the conditions mentioned above. The amount of flecks was examined 62 hours after the completion of all treatments.

The amount of flecks for each leaf was visually rated to 0 (healthy), 0.1,...0.9, and 1.0 (mild to severe) corresponding to flecked portion of each leaf, and the severity of ozone damage was represented as injury index, which was average rate per leaf (in the experiment 1) or per plant (in the experiments 2 and 3).

RESULTS AND DISCUSSION

1. Effects of variability of micro-environment in the fumigation chamber on the severity of injury.

Vertical and horizontal-distributions of the amount

Table 2 Difference in ozone injury of leaves situated at different positions in the fumigation chamber

2-a. Vertical position

Height cm	Injury index*				Light intensity
75	0.58	0.65	0.61	0.56	24 Klx
55	0.62	0.65	0.73	0.64	18
35	0.60	0.62	0.65	0.72	12
15	0.60	0.61	0.62	0.72	7
Frontage cm	10	30	50	70	

2-b. Horizontal position

Depth cm	Injury index*			
70	0.58	0.61	0.65	0.68
50	0.60	0.61	0.65	0.65
30	0.59	0.64	0.63	0.63
10	0.62	0.66	0.66	0.68
Frontage cm	10	30	50	70

* Each figure is the average of 16 leaves in 4 treatments.

of flecks appeared on the leaves hunged in the fumigation chamber are presented in table 2-a and 2-b respectively. There was a band where higher values of injury indices were observed from the lower parts lying leeward obliquely to the upper parts (table 2-a). In the direction of depth little difference in injury index was observed among four horizontal planes, however, there was a slight gradiant of injury index from the wind side to the leeward (table 2-b).

The maximum difference in temperature in the fumigation chamber was less than 1.5°C. The polluted air was blown slantwise into the plant fumigation area passing through the crevices of the fumigation chamber (cf. fig. 1). Ozone concentration in the rear space (leeward) was higher (ca. 9%) than that in the front space. Then these differences in injury would be mainly due to the variability of ozone concentration induced by the lack of uniformity in velocity of polluted air moving around in the fumigation area[3,18]. However, these disadvantages could be covered by carefull attention to the position where the plants were placed in the fumigation chamber.

In this experiment each leaf was exposed to different intensities of light, within such a range of light intensities (7～24 Klx) it was confirmed to have no effect on the severity of ozone damage[19].

Taylor et al.[21] reported that leaf turgidity influenced the fleck outbreaks. In this experiment several leaves wilted somewhat after 2-hours' exposure, but no significant difference in injury was observed.

2. Relation between dosage of ozone and amount of flecks.

Results are shown in table 3. H-2 was less sensitive than Bel W3, a cigar-wrapper tobacco susceptible to ozone[5,6,7,13]. In H-2 flecking was first observed when ozone dose was 30 pphm×h and in Bel W3 20 pphm×h. Flecks similar to those caused by airborn oxidants were observed (see Plate 1 and 2)[17]. Congregated flecks occurred on the lower leaves fumigated at relatively high ozone levels.

There was a tendency that the greater the ozone dose, the more intensive flecking developed. However, no linear relationship between ozone dose and injury was observed in both varieties. That is, a shorter exposure at higher ozone levels was more injurious than a longer exposure at lower levels, even though the total dose was the same.

The same results were also reported on tobacco and pinto bean[2,5].

Kuroda et al.[10] reported that the incidence of oxidants injury to tobacco (var. H-2) became 100% when the peak value of oxidants exceeded 11 pphm or when oxidants dose was over 72.3 pphm×h.

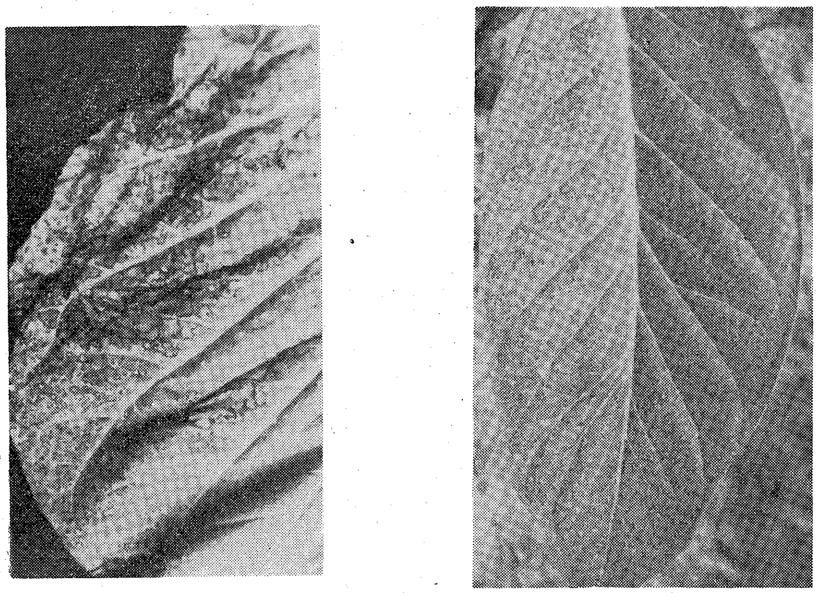
Table 3 Relation between ozone doses and the amount of flecks

Ozone concentration \ Hours exposed to ozone	1	2	3	4
10 pphm	0(0)	0(0.6)	+ (4.4)	+ (4.2)
20	+(0.5)	0.3(5.3)	1.9 (5.9)	3.3 (7.8)
30	2.2(6.5)	2.7(7.9)	3.7 (8.7)	4.6 (9.2)
40	3.2(7.9)	4.9(9.6)	5.6 (9.3)	6.0 (9.4)

Note: Each value shows injury index. Two varieties of tobacco, H-2 and Bel-W3 (in brackets) were used.
+; Some leaves flecked slightly.

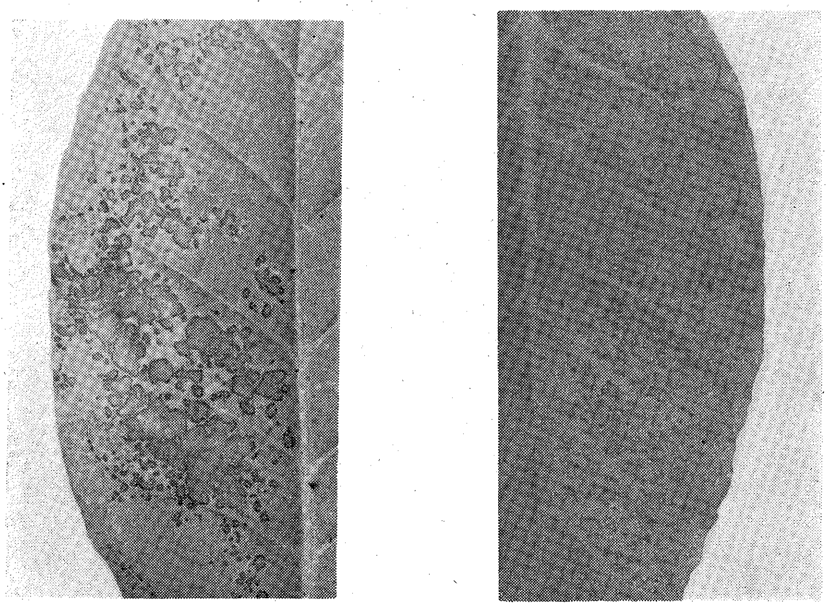
Table 4 Relation between time of ozone treatment and the amount of flecks

Times of ozone treatment	(ozone concentration of one treatment)	Injury index	Number of yellowing leaves
5	(8.0 pphm)	3.02	0.6
4	(10.0)	3.42	1.0
3	(13.4)	3.74	1.0
2	(20.0)	5.20	1.8
1	(40.0)	6.46	3.2



Type II　　　　Type III

Plate 1 Fleck injuries observed in the fields (var. H-2)



30 pphm × 1 h　　　　40 pphm × 1 h

Plate 2 Flecks induced by ozone fumigation (var. H-2)

Considering both the ozone content of air-born oxidants[1,11] and environmental factors (time of day, temperature, humidity, etc.)[15,16], the minimum dose required for visible damage obtained in this experiment may well be considered. MacDowall et al.[12] reported that the threshold doses of air-polluting ozone and experimentally generated ozone were approximately the same. Several reports indicate that the minimum ozone dose enough to cause visible injury to tobacoo is 15–32 pphm × h[7,6,13]. Many factors (variety, plant age, environmental factors)[6,15,16] may affect the susceptibility to ozone, and therefore it is difficult to calculate the threshold dose.

3. Relation between time of ozone fumigation and amount of flecks.

Results of table 4 show that few times of ozone exposure at higher levels was more injurious than frequent times of ozone exposure at lower levels when the total dose was the same. The number of yellowing leaves was correlated to the amount of flecks.

Results obtained in the experiments 2 and 3 suggested that the plants may react to ozone with biological protections[4]. The severity of injury was greatly affected with ozone levels more than with hours exposed to ozone. Therefore, ozone injury cannot be discussed with a simple dose concept, but must be discussed in relation to both hours and concentrations.

Hastening of leaf senescence by the exposure to oxidants was reported.[6,9,16] In this experiment yellowing of leaves was accelerated by ozone fumigation, of which mechanism is left to be solved. In this connection, further studies will be expected to examine an invisible damage caused by long-term and low-level exposure.

The authors are indebted to Mr. S. Kuroda (the chief of Tobacco Leaf Production Division, Shikoku Branch Office, Japan Monopoly Corporation) and Mr. S. Uchida (Okayama Tobacco Experiment Station) for designing the plant fumigation chamber.

Summary

1. A plant fumigation chamber equipped with provisions controllable for light (23 Klx), temperature (5～35°C) and relative humidity (55～75%) was constructed for ozone studies.

Minor environmental fluctuations in the plant exposure area in the chamber were observed, giving an evidence that they were not considered to be detrimental to the experimental procedures.

2. Flecks similar to those induced by air-born oxidants were observed.

3. The minimum dose required for visible damage was 30 pphm×h in H-2 and 20 pphm×h in Bel W3. In both varieties non-linear relationship between ozone dose and injury was observed.

Doses in the shorter exposures were more injurious than equal or even higher doses given for longer periods. And few times of ozone-treatment at higher levels was more injurious than frequent times of ozone-treatment at lower levels when the total dose was the same.

4. Detached leaf could be used for ozone studies when fumigation was carried out a shorter period of time such as 1–2 hours.

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〔和 文 摘 要〕

タバコにおけるオゾン処理と障害との関係

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1) タバコのオゾン障害についての研究を行なうため，光 (23 Klx)，温度 (5°～35°C) および相対湿度 (55～75%) の制御のできるオゾン処理装置を試作した．処理チャンバー (80×90×100 cm) 内においてわずかな環境条件のばらつきがみられたが，実験遂行上大きな支障となるものではなかつた．

2) オゾン処理により圃場で観察されたと同じような病斑症状が発生した．

3) Bel-W3 では 20 pphm×h，H-2 では 30 pphm×h で病斑が現れた．両品種ともオゾン dose (pphm×h) と病斑の発生程度とはかならずしも比例的でなかつた．すなわち dose が同一の場合，短時間で高濃度で処理した方が長時間で低濃度で処理するよりも障害が大で，また dose が前者より後者の方が大なる時でさえ，短時間高濃度の方が障害が大となる傾向がみられた．また同一 dose の場合，低濃度で何回にも分けて処理するよりも，高濃度で処理回数を少なくした方が障害の発生は大となつた．

4) 1～2 時間の短時間処理ならば，切断葉でも着生葉と同様にオゾン反応をみる実験に供することができた．