



Ozone injury to leafy sweet potato and spinach in northern Taiwan

E. J. Sun

Department of Plant Pathology and Entomology, National Taiwan University,
Taipei, Taiwan, Republic of China

(Received September 25, 1993; Accepted March 17, 1994)

Abstract. Leafy sweet potato plants with bleaching symptoms on their adaxial leaf surface have been noticed in the Taipei area in the past few years. Recently, severe bleaching has also been found on spinach leaves in suburban farms. The monitoring of ozone concentrations at the experimental farm of National Taiwan University, one of the affected areas, showed that when leafy sweet potato and spinach were damaged, the ozone concentration was at the phytotoxic level, with a peak of 230 ppb. Bel-W3 tobacco, an indicator of ozone injury, showed the typical symptoms of weather flecks on upper leaf surfaces on days of high ozone concentrations. Fumigation of spinach and young leafy sweet potato seedlings with ozone at a concentration of 200 ppb (v/v) for 2 h in continuously stirred tank reactors (CSTRs) in a greenhouse can reproduce the symptoms found in the field, whereas fumigation with peroxyacetyl nitrate did not produce similar symptoms on the leaves. This shows that the ozone concentration in northern Taiwan occasionally reaches levels high enough to cause injury to plants.

Keywords: Ozone; Spinach; Sweet potato; Taiwan

Introduction

Injury to vegetation caused by photochemical smog was first reported by Middleton and his coworkers in 1944 in the Los Angeles basin (Middleton et al., 1950), but the first disease proved to be caused by ozone was leaf stipple on grape plants, reported by Richards et al. (1958). In 1959, Heggstad and Middleton (1959) reported that ozone was the cause of weather fleck on tobacco in the eastern United States. Subsequently, various investigators showed that ozone was responsible for injury to many crops (Heagle, 1989; Heagle et al., 1983; Heck et al., 1986; Hill et al., 1961; Jacobson and Hill, 1970; Reinert et al., 1972) and deciduous and evergreen trees (Jacobson and Hill, 1970; Shafer and Heagle, 1989; Long and Davis, 1991).

In Taiwan, the first case of ozone injury to vegetation was revealed in the early 1970s by Street et al. (1971) using Bel-W3 tobacco from Maryland as a bioindicator. They studied the weather fleck problem on tobacco plants at the Taiwan Tobacco Research Institute at Taichung, and found that the symptoms on Bel-W3 tobacco were identical to those found in the USA. Sung et al. (1973), of the same institute, found that a charcoal filter could protect tobacco from injury, indicating that ozone was responsible for this disease. Later studies, however, showed that tobacco vein-banding mosaic virus and phosphorus deficiency could produce similar fleck symptoms on tobacco leaves (Wu and Sung, 1973). This uncertainty postponed further study of the problem for about 20 years.

The recent rapid industrialization and economic growth in Taiwan has made the pollution situation more serious than at anytime before. Since 1989, several peroxyacetyl nitrate (PAN) types of plant injury, such as bronzing and silvering of native lettuce and silvering of spinach, have been found in fields in the Taipei, Taichung, and Koahsiung metropolitan areas (Sun, 1992, 1993). Monitoring data from the Environmental Protection Administration of Taiwan (Taiwan EPA) showed that ambient ozone occasionally reached the phytotoxic level (Liu et al., 1990). Since sensitive plant species, such as spinach, peanut, soybean, bean, and tobacco, are commonly cultivated, it was assumed that ozone must have caused some injury problems on the island. This report elucidates the role of ozone in some cases of vegetation injury in Taiwan.

Materials and Methods

Field investigation

After an ambient ozone monitor was set up at the experimental farm of National Taiwan University in 1991, a field investigation was conducted after each occurrence of high ozone concentrations. We surveyed Hsin-Chuang, Hsin-Tien, San-Shia, Yin-Ko, Shu-Lin, San-Chung, Pan-Chiao in Taipei County, Pei-Tou, Shih-Lin, Mu-Cha, and NTU in Taipei City, Taoyuan City, and Hsinchu City.

Measurement of ozone in the atmosphere

Since 1991, an ozone monitor (Model 49 UV photometric ozone monitor made by Thermo Environment Instrument Co., USA.) has been used at the experimental farm of National Taiwan University to monitor ambient and experimental ozone concentrations. This instrument is regularly calibrated by the supplier with a standard ozone generator.

Indicator plant tests

Plants specific for indicating ozone injury were used to identify the ozone problem. The Bel-W3 tobacco, developed by Heggstad (1991), was introduced and grown in pots containing soil, peat moss, and vermiculite in a ratio (by volume) of 3:2:1 in a greenhouse. The typical injury symptoms of weather fleck described by Heggstad and Middleton (1959) were used to show when phytotoxic concentrations of ozone occurred in the atmosphere.

Exposure tests in chambers

Six continuously stirred tank reactors (CSTRs), similar to the design of Rogers et al. (1977), were set up in the greenhouse and used for plant exposure tests with ozone or PAN. The cylindrical CSTRs (1.2 m diameter and 1.8 m high) were made of transparent plexiglas. We hung a 700 W high intensity lamp above each chamber. There was a stirring fan beneath the roof of the chamber to mix the incoming air. A ventilation system consisting of a charcoal filter, an outlet pipe, and a suction pump was connected to the bottom of the chamber. The negative pressure produced by the pump drew the air in through the charcoal filter, through the chamber, and out the exhaust system. The air exchange rate was determined by measuring the air flow rate in the outlet pipe, and was adjusted with a valve. A diagram of the chamber system was presented in a previous report (Sun, 1990).

For ozone exposure tests, the ozone produced by an electrostatic discharge in air was introduced into the exposure chambers through the inlet pipe. The ozone output was adjusted by a voltage controller. In the PAN studies, pure PAN in *n*-tridecane, synthesized by the method of Gaffney et al. (1984), was applied with a constant evaporating system developed by Sun and Su (1985), producing a constant concentration of PAN in the CSTR. During the exposure period, ozone concentrations in the exposure chambers were monitored by a UV photometric ozone monitor as previously described. PAN concentrations in the chambers were determined by a Varian 3400 gas chromatograph (Katz, 1977) which was calibrated, using the procedure described by Joos and Landolt (1986), with a chemiluminescence nitrogen oxide analyzer (Model 42) of Thermo Environment Instrument Co., USA.

Seedlings of sweet potato (*Ipomoea batatas* (L.) Lam. cv. Changhua) were grown in a medium containing soil, peat moss, and vermiculite in a ratio by volume of 3:2:1. Number 43 fertilizer from Taiwan Fertilizer Co., con-

taining N, P₂O₅, and K₂O in a ratio of 15:15:15, was added at the rate of 60 g m⁻² of pots. For the spinach (*Spinacia oleracea* L. cv. Chummy F1 Hybrid), chicken manure was added to the medium mentioned above. Plants were grown in the greenhouse and exposed to ozone or PAN in CSTRs at a temperature of 23–28 °C, relative humidity of 50–80% and light intensity of 10,000–50,000 Lux. After the exposure, they were moved back to the greenhouse for further observation in the following days or weeks.

Results

Field investigation

Symptoms of bleaching injury to leaves of leafy sweet potato (Figure 1) have occurred in fields in the Taipei area since 1991. The necrotic lesions usually appear only on the upper surface of sweet potato leaves.

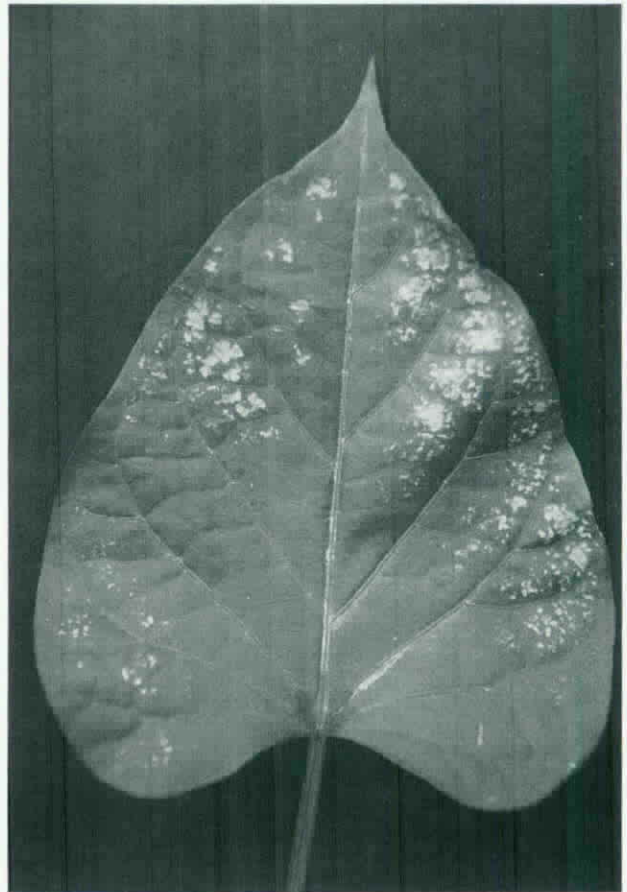


Figure 1. Bleaching and necrotic symptoms on leafy sweet potato in the field, caused by ozone in the Taipei area.

These symptoms are similar to those caused by ozone but different from those caused by PAN (Jacobson and Hill, 1970). Among several cultivars of sweet potato inspected, only Changhua leafy cultivar showed symptoms in the field, but the frequency of injury was not as high as that of the PAN injury to lettuce described previously (Sun, 1992, 1993).



Figure 2. Bleaching and necrotic symptoms on spinach leaf in the field, caused by ozone in the Taipei area.

Visible necrotic symptoms also occurred on spinach leaves in fields in the same area. The bleaching spots or flecks (Figure 2) are usually located on upper leaf surfaces. Some of the spots coalesce to form larger lesions and cause early leaf senescence. These symptoms are similar to those caused by ozone (Daines et al., 1960; Hill et al., 1961; Manning et al., 1972), but different from the silvering on the lower leaf surface caused by PAN (Jacobson and Hill, 1970).

After an ozone monitor was set up in late 1991 and used to intermittently monitor the ambient ozone concentrations, the appearance of injury symptoms was found to be closely associated with high ozone concentrations. For example, the morning after a day of extremely high ozone (November 18, 1992), all sweet potato and spinach plants showed severe bleaching and necrotic symptoms on the upper leaf surfaces. All the symptoms were those of ozone, not PAN. The monitoring data showed that the ozone concentrations at noon of that day reached a peak of 220 ppb, with an average of 200 ppb for 3 h near noon. These conditions exceeded the phytotoxic level of various plant species. On the same day, the peak PAN concentrations were only about 5–6 ppb, which was not harmful to most of the plant species (Temple and Taylor, 1983).

A preliminary survey during the past two years indicated that ozone could have adverse influence on sweet potato and spinach in the Taipei Basin (encompassed by

Shih-Lin to the north, Mu-Cha to the east, Yeong-Ho to the south and Hsin-Chuang to the west. The frequency of ozone injury episodes was very low in these areas, indicating the lower prevalence of high levels of ozone in the basin.

Ambient ozone concentrations

The monitoring data collected intermittently during 1992 showed that ozone concentrations in the National Taiwan University area fluctuated and were high only on some sunny days. On rainy, cloudy, or very windy days, ambient ozone was very low—ranging from 0 to 20 ppb. Ozone concentration increased significantly only during some clear but windless days, and was usually higher in the warm season than in the cool season. It was initially found that ambient ozone concentration is highly correlated with that of PAN. As the intermittent data from only one station are not enough for analyses, the patterns of long-term changes of air quality can not yet be elucidated and need further study.

The highest ozone concentration recorded in the National Taiwan University area was 230 ppb at noon on November 18, 1992. There were only two ozone injury episodes in that month, however, and none in December. On November 18, 1992, the ozone concentration started to rise at about 10 am and peaked at 12:40 pm. The level

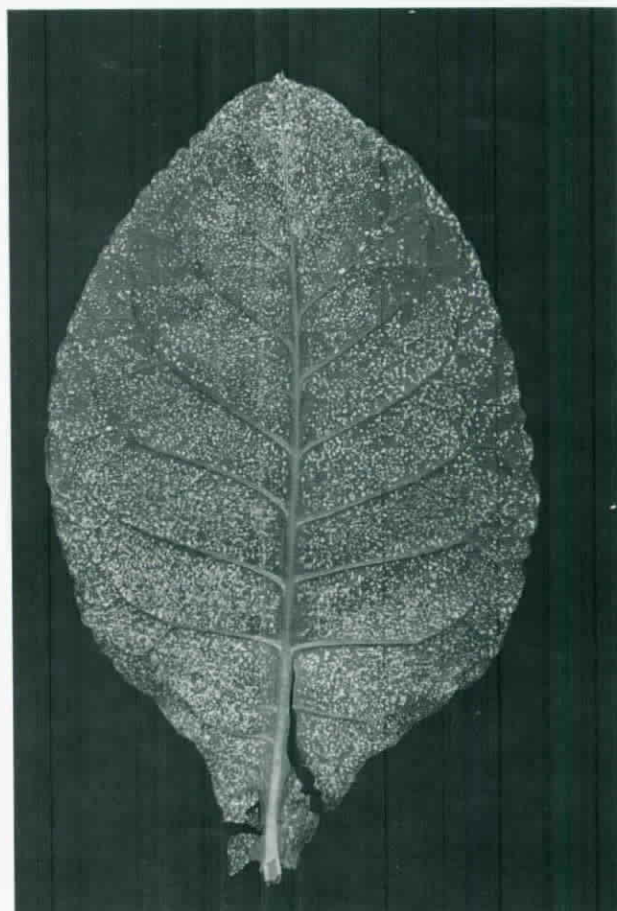


Figure 3. Typical symptoms of weather fleck on Bel-W3 tobacco leaf caused by ozone in the Taipei area.

dropped for a short while as clouds covered the sky, then rebounded and maintained at over 100 ppb till 2:30 pm. During this episode, all the plants received an average dose of 200 ppb for 2 h plus 120 ppb for 1 h and most sensitive species, such as sweet potato, spinach, Bel-W3 tobacco, and tomato, were severely damaged.

Indicator plant tests

The investigation was conducted for two months. In November 1992, the Bel-W3 tobacco plants expressed the typical symptoms of ozone injury (Figure 3) twice, each time when the monitored ambient ozone was higher than the phytotoxic level. In December 1992, the test plants did not show any symptoms, as the ambient ozone was low for the whole month.

Fumigation tests in CSTRs

Fumigation tests in CSTRs conducted in December 1992 and January 1993 showed that leafy sweet potato of the cultivar Changhua was sensitive to ozone at levels higher than 180 ppb for 2 h. The spinach plants were more sensitive to ozone than were sweet potato; they were affected at 100 ppb for 2 h. At higher doses these two crops showed severe injury symptoms on their leaves.

The same symptoms as those found in the field were produced in the CSTRs when leafy sweet potato or spinach plants were exposed to 200 ppb for 2 h in the daytime. These results confirmed that ozone was the factor causing the injuries in this area.

Fumigation tests with PAN in CSTRs did not produce symptoms typical of ozone injury to leafy sweet potato or spinach. A silvering of the lower surface of spinach was found after treatment with over 28 ppb PAN for 3 h, but sweet potato was not affected by the same dose. Therefore, the symptoms of bleaching necrosis found on spinach and sweet potato in the field were not caused by PAN, although the PAN was detected in the same area.

Discussion

Bleaching and necrotic symptoms on spinach plants in the field were similar to the ozone injury symptoms reported by other authors (Daines et al., 1960; Hill et al., 1961; Manning et al., 1972). Exposure of spinach plants to ozone in the CSTRs produced the same symptoms, indicating that ozone is the cause of the bleaching injury to spinach leaves in the Taipei area. The results from measurements of ambient ozone and of the bioassay with Bel-W3 tobacco indicator plants further confirmed the causal relationship between ozone and plant injury.

The results of exposure tests with spinach and sweet potato plants suggest that PAN is not the cause of the bleaching injury to either species, although PAN frequently induced leaf injury to lettuce and spinach in the same area (Sun, 1992, 1993). The interaction between PAN and ozone remains to be investigated.

The ambient ozone concentrations were monitored intermittently, at only one station. Thus the data were not sufficient for us to determine the trend for ozone levels in northern Taiwan in 1992. The generally low frequency of ozone injury to Bel-W3 tobacco plants, and the low frequency of high ozone concentration in this area indicate that ozone injury to vegetation in Taipei does not happen as frequently as is reported in other countries (Heck et al., 1986; Krupa and Manning, 1988). Liu et al. (1990) reported that in 1987 there were only 23 days with ozone concentrations (peak hour mean) higher than 120 ppb in the Taipei area.

The present study shows that among the available cultivars of sweet potato, only the leafy cultivar is sensitive to the ambient level of ozone in this area; the relative sensitivity of all cultivars needs to be further analyzed.

In addition to the acute symptoms described in this study, ozone has been reported to affect the yield and quality of crops even with no visible symptoms (Jacobson and Hill, 1970; Heck et al., 1986). The chronic and invisible influence of ozone on vegetation in Taiwan, including the quantitative dose-response relationship, the yield loss of crops, and the physiological mechanisms involved in injury, deserve further comprehensive study.

Acknowledgements. The work was supported by a grant from the Council of Agriculture, Republic of China. The author is grateful to Dr. W. H. Ko of University of Hawaii for his critical review of the manuscript and to the experimental farm of National Taiwan University for the experimental materials.

Literature Cited

- Daines, R. H., I. A. Leone, E. Brennan, and J. T. Middleton. 1960. Damage to spinach and other vegetation in New Jersey from ozone and other airborne oxidants. *Phytopathology* **50**: 570. (Abstract)
- Gaffney, J. S., R. Fajer, and G. I. Senum. 1984. An improved procedure for high purity gaseous peroxyacetyl nitrate production: Use of heavy lipid solvents. *Atmosph. Environ.* **18**: 215–218.
- Heagle, A. S. 1989. Ozone and crop yield. *Annu. Rev. Phytopathol.* **27**: 397–423.
- Heagle, A. S., M. B. Letchworth, and C. A. Mitchell. 1983. Injury and yield responses of peanuts to chronic doses of ozone in open-top field chambers. *Phytopathology* **73**: 551–555.
- Heck, W. W., A. S. Heagle, and D. S. Shriner. 1986. Effects on vegetation: Native, crops, forests. *In* A. C. Stern (ed.), *Air Pollution*. Vol. 6. Academic Press, pp. 247–350.
- Heggstad, H. E. 1991. Origin of Bel-W3, Bel-C and Bel-B tobacco varieties and their use as indicators of ozone. *Environ. Pollut.* **74**: 264–292.
- Heggstad, H. E. and J. T. Middleton. 1959. Ozone in high concentrations as a cause of tobacco leaf injury. *Science* **129**: 208–210.

- 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.
- Jacobson, J. S. and A. C. Hill. 1970. Recognition of Air Pollution Injury to Vegetation: A Pictorial Atlas. Air Pollut. Control Assoc., Pittsburgh, USA.
- Joos, L. F. and W. F. Landolt. 1986. Calibration of peroxyacetyl nitrate measurements with an NO_x analyzer. *Environ. Sci. & Technol.* **20**: 1269–1273.
- Katz, M. 1977. *Methods of Air Sampling and Analysis*, 2nd edn. Amer. Publ. Health Assoc., Washington, D. C., 984 pp.
- Krupa, S. V. and W. J. Manning. 1988. Atmospheric ozone: Formation and effects on vegetation. *Environ. Pollut.* **50**: 101–137.
- Liu, C. M., S. C. Liu, and S. H. Shen. 1990. A study of Taipei ozone problem. *Atmosph. Environ.* **24A**: 1461–1472.
- Long, R. P. and D. D. Davis. 1991. Black cherry growth response to ambient ozone and EDU. *Environ. Pollut.* **70**: 241–254.
- Manning, W. J., W. A. Feder, and I. Perkins. 1972. Sensitivity of spinach cultivars to ozone. *Plant Dis. Repr.* **56**: 832–833.
- Middleton, J. T., J. B. Kendrick, Jr., and H. W. Schwalm. 1950. Injury to herbaceous plants by smog or air pollution. *Plant Dis. Repr.* **34**: 245–252.
- Reinert, R. A., D. T. Tingey, and H. B. Carter. 1972. Ozone induced foliar injury in lettuce and radish cultivars. *J. Amer. Soc. Hort. Sci.* **97**: 711–714.
- Richards, B. L., J. T. Middleton, and W. B. Hewitt. 1958. Air pollution with relation to agronomic crops: V. Oxidant stipple to grape. *Agron. J.* **50**: 559–561.
- Rogers, H. H., H. E. Jeffries, E. P. Stahel, W. W. Heck, L. A. Ripperton, and A. M. Witherspoon. 1977. Measuring air pollutant uptake by plants: A direct kinetic technique. *J. Air Pollut. Control Assoc.* **27**: 1192–1197.
- Shafer, S. R. and A. S. Heagle. 1989. Growth response of field-grown loblolly pine to chronic doses of ozone during multiple growing seasons. *Can. J. For. Res.* **19**: 821–831.
- Street, O. E., C. H. Sung, H. Y. Wu, and H. A. Menser, Jr. 1971. Studies on weather fleck of tobacco in Taiwan. *Tobacco Science* **15**: 128–131.
- Sun, E. J. 1990. Sorption of three major air pollutants by bean plants. *J. Environ. Prot. Soc. R. O. C.* **13**: 39–50.
- Sun, E. J. 1992. Major air pollutants and their effects on vegetation in Taiwan. *In Proceedings of the 1992 Joint International Symposium on Air Pollution, Soil Microbiology and Biotechnology of Forestry, Taipei, Taiwan*, pp. 51–76.
- Sun, E. J. 1993. Effects of peroxyacetyl nitrate on lettuce plants in Taiwan. *Plant Pathol. Bull.* **2**: 33–42.
- Sung, C. H., H. H. Chen, and J. K. Wu. 1973. Air pollution related to tobacco weather fleck. *In Annual Report Tobacco Research Inst. Taiwan Tob. Wine Monopoly Bureau 1973, Taichung, Taiwan*. pp. 38–41. (in Chinese)
- Sun, E. J. and H. J. Su. 1985. Fluoride injury to rice plants caused by air pollution emitted from ceramic and brick factories. *Environ. Pollut. (Ser. A)* **37**: 335–342.
- Temple, P. J. and O. C. Taylor. 1983. World wide ambient measurement of peroxyacetyl nitrate (PAN) and implications for plant injury. *Atmosph. Environ.* **17**: 1583–1587.
- Wu, J. K. and C. H. Sung. 1973. The study on environmental factors in relation to the occurrence of weather fleck in tobacco. *In Annual Report of Tobacco Res. Inst., Taiwan Tobacco and Wine Monopoly Bureau 1973, Taichung, Taiwan*, pp. 42–50.

台灣北部地區臭氧對葉用甘藷及菠菜之影響

孫岩章

國立台灣大學植物病蟲害學系

台北地區過去數年中偶而會發現葉用甘藷葉片上表面出現漂白病徵，而在郊區之菠菜也曾出現嚴重漂白。在台灣大學試驗農場利用臭氧監測儀監測的結果，發現當葉用甘藷及菠菜受害時，大氣臭氧濃度皆升至有害程度，且測過之最高值達 230 ppb。而此高臭氧污染狀況亦可從 Bel-W3 煙草指標植物葉上表面出現氣候斑 (weather fleck) 得到驗證。人工以臭氧 0.2 ppm 在連續攪拌熏氣箱 (continuously stirred tank reactor) 內對甘藷及菠菜熏氣發現皆可令其產生與田間所見相同的病徵，但以過氧硝酸乙醯酯熏氣則否，此些結果皆證明台灣北部地區大氣中之臭氧在某些時候已達損害作物或植物之程度。

關鍵詞：臭氧；甘藷；菠菜；台灣。