when individual bulblets were separated and transferred to media containing the basal constituents with 0.1 mg/liter NAA (Fig. 3). Bulblets of 'Delft Blue' had a higher percentage of root initiation than did those of 'Anna Marie'.

Ninety to 100% of the bulblets of both cultivars grown on medium with 0.1 mg/liter NAA had developed sufficiently to be transplanted to a soil mixture (Table 2). All of the transplanted bulblets had produced new growth after 3 weeks.

Using this technique, it is possible that more than 1,000 plantlets can be obtained from a single inflorescence in a period of 4 to 6 months. Further multiplication could be obtained by inducing bulblet formation from the *in vitro* generated bulblets.

## Literature Cited

1. Bajaj, Y. P. S. and R. L. M. Pierik. 1974. Vegeta-

tive propagation of freesia through callus culture. Neth. J. Agr. Sci. 22:153-159.

- Hosoki, T. and T. Asahira. 1980. *In vitro* propagation of *Narcissus*, HortScience 15:602–603.
- Hussey, G. 1975. Totipotency in tissue explants and callus of some members of Liliaceae. Iridaceae, and Amaryllidaceae. J. Expt. Bot. 26:253–262.
- Hussey, G. 1975. Propagation of hyacinths by tissue culture. Sci. Hort. 3:21–28.
- Hussey, G. 1977. *In vitro* propagation of some Liliaceae, Iridaceae and Amarylliceae. Acta Hort. 78:303–309.
- Murashige, T. 1976. Manipulation of organ initiation in plant tissue culture. Bot. Bul. Academia Sinica. 18:1-24.
- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15:473–497.
- Nishiuchi, Y. 1979. Studies on vegetative propagation of tulip. II. Formation and development of adventitious buds in the excised bulb-scale cultured *in vitro*. J. Jap. Soc. Hort. Sci. 48:99–105.
- Papachatzi, M., P. A. Hammer, and P. M. Hasegawa. 1980. *In vitro* propagation of *Hosta plantaginea*. HortScience 15:506–507.
- 10. Pierik, R. L. M. and A. J. M. Post. 1975. Rapid veg-

etative propagation of *Hyacinthus orientalis* L. *in vitro*. Sci. Hort. 3:293–297.

- Pierik, R. L., M. and H. H. M. Steegmans. 1975. Effect of auxin, cytokinins, gibberellins, abscisic acid and ethephon on regeneration and growth of bulblets on excised bulb scale segments of hyacinth. Physiol. Plant. 34:14–17.
- Saniewski, M. 1975. The effect of gibberellic acid and benzyladenine on bulblets differentiation in scooped out and scored hyacinth (Hyacinthus orientalis L.) bulbs. Bul. Acad. Pol. Sci. 23:49–52.
- Saniewski, M. 1975. Root and bulblet regeneration by flower buds of *Hyacinthus orientalis* L. in tissue culture. Bul. Acad. Pol. Sci. 23:121–123.
- Saniewski, M., J. Nowak, and R. Rudnicki. 1974. Studies on the physiology of hyacinth bulbs (*Hyacin-thus orientalis* L.). IV. Hormonal regulation of induction of roots and bulblets in *Hyacinthus orientalis* L. grown in culture. Plant Sci. Lett. 2:373–376.
- 15. Sheridan, W. F. 1968. Tissue culture of the monocot *Lilium*. Plants 82:189–192.
- Wochok, Z. S. and C. J. Sluis. 1980. Gibberellic acid promotes *Atriplex* shoot multiplication and elongation. Plant Sci. Lett. 17:363–369.

## HortScience 16(5):647–648. 1981. Sensitivity of Ginseng to Ozone and Sulfur Dioxide<sup>1</sup>

## J. T. A. Proctor and D. P. Ormrod

Department of Horticultural Science, University of Guelph, Guelph, Ontario, Canada NIG 2W1

Additional index words. Panax quinquefolius, air pollution, oxidant

Abstract. American ginseng (Panax quinquefolius L.), was injured by exposure to 20 pphm ozone and/or 50 pphm (v/v) sulfur dioxide for 6 hr daily for 4 days. Ozone induced upper surface leaflet stippling along the veins and interveinally, and sulfur dioxide induced mild chlorosis to irregular necrotic areas. Ginseng was less sensitive to ozone and as sensitive to sulfur dioxide as 'Cherry Belle' radish (Raphanus sativus L.) and 'Bel W-3' tobacco (Nicotiana tabacum L.).

Ginseng (Panax sp.) grows wild and is cultivated in countries known to have air pollution problems. For instance, ginseng production areas of southern Ontario, Canada (8) frequently experience phytotoxic levels of air pollutants, specially ozone (Air monitoring data, Air Resources Branch, Ontario Ministry of the Environment, Toronto). Injury to ginseng by ambient air pollutants in this area has not been documented (7) and to our knowledge has not been reported for any other ginseng growing area (6). The purpose of this study was to establish, under controlled conditions, the sensitivity of American ginseng to 2 common air pollutants, ozone and sulfur dioxide, singly and in combination.

Three-year-old field grown roots of American ginseng were transplanted, 1 root per pot, to 15 cm diameter plastic pots containing 1 sphagnum peat:1 perlite (v/v) on November 1, 1979. Pots were placed in storage at  $7^{\circ} \pm$  $2^{\circ}$ C and  $50 \pm 5\%$  relative humidity to satisfy the dormancy requirements of the roots. On January 8, 1980, pots were moved to the greenhouse  $(23^\circ \pm 3^\circ \text{ day}, 18^\circ \pm 2^\circ \text{ night})$  and placed under a nylon mesh shade cloth which permitted 30% of full sunlight to reach the top of the pots. By February 11 shoot development had started and by March 5 the plants had reached maximum height and the leaves were fully developed. The plants were then moved to controlled environment chambers (Controlled Environment Model EY8M) maintained at day/night temperature of 20°/  $15^{\circ} \pm 2^{\circ}$ , photosynthetically active irradiance at the top of the plants of  $155 \pm \mu \text{Em}^{-2}\text{s}^{-1}$  provided by 73% input wattage Cool White fluorescent and 27% incandescent lamps, a photoperiod of 12 hr, and relative humidity of 45  $\pm$  5%. The plants were irrigated alternately with deionized water and half-strength complete nutrient solution (3). Pots were sequentially removed at monthly intervals from cold storage to the greenhouse and then to the controlled environment chambers to establish 3 replications. Also periodically 'Cherry Belle' radish and 'Bel W-3' tobacco were seeded in 5 cm diameter plastic pots and grown in the controlled environment chambers with the ginseng. These plants, which have known sensitivity to  $O_3$  and  $SO_2$ , were included for comparison with ginseng.

Ginseng, radish, and tobacco plants were moved to the exposure chambers one day before beginning the pollutant treatments. Exposure conditons were 350-400 µEM<sup>-2</sup>s<sup>-1</sup> high pressure sodium light, 12 hr photoperiod,  $22 \pm 2^{\circ}$ C temperature and  $50 \pm 5\%$ relative humidity. The plexiglass exposure chambers were 70 cm cubes and the exposure system included a Grade Model LG-2-L2 ozone generator with a Dasibi Model 1003AH ozone monitor, sulfur dioxide from a gas cylinder and a Beckman Model 953 sulfur dioxide analyzer. Six-hour daily exposures in the middle of the photoperiod for 4 days were given. Treatments were (a) control; (b) 15 pphm ozone; (c) 50 pphm sulfur dioxide, and (d) a mixture of (b) and (c). There were 5 plants per treatment, the experiment was repeated 3 times and the combined data are presented.

Pollutant injury severity as percent of leaf area injured was determined on the 4th day after the end of exposure. The rating was based on the Horsefall and Barratt (4) scheme.

Tobacco was more severely injured by ozone than either radish or ginseng (Table 1). Characteristic upper surface stippling (2) was obvious by day 2 of the exposure, and by day 4 a high proportion of the leaf had bifacial necrosis. The ozone sensitive 'Cherry Belle' radish (9) had the expected necrotic fleck and bifacial necrosis on leaves and cotyledons. Ginseng leaflets had upper surface stippling along the veins and interveinally, which was more pronounced near the base of the leaflets (Fig. 1A). Injury was similar on all 5 leaflets.

Ginseng was less sensitive to ozone as the

<sup>&</sup>lt;sup>1</sup>Received for publication April 8, 1981. This research was supported in part by grants No. A6697 and A5951 of the Natural Sciences and Engineering Research Council of Canada, and by the Ontario Ministry of Agriculture and Food. We thank Glenna Rebick, Larry Pyear and Dean Louttit for technical assistance, and Walter Hellyer of Waterford, Ontario for supplying the ginseng roots.

The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

Table 1. Effect of ozone (O<sub>3</sub>), sulfur dioxide (SO<sub>2</sub>), and their combination on ginseng, 'Bel W-3' tobacco, and 'Cherry Belle' radish as assessed by % leaf area injured. Three-year-old fully developed ginseng plants and young tobacco and radish plants were exposed for 6 hr daily for 4 days. Data for leaf number and plant height are included to show stage of plant growth.

Pollutant treatment	Concn (pphm)	Leaf area injured (%)			No. of leaves			Plant height (cm)	
		Ginseng	Tobacco	Radish	Ginseng	Tobacco	Radish	Ginseng	Tobacco
Control	0	0 <sup>2</sup>	0	0	$3.3 \pm 0.5$	$5.0 \pm 1.0$	$2.7 \pm 1.0$	$19.2 \pm 4.7$	$10.7 \pm 1.1$
0,	20	$2.1 \pm 2.1$	$7.3 \pm 0.6$	$4.0 \pm 1.4$	$3.5 \pm 0.5$	$5.0 \pm 0.0$	$2.5 \pm 1.2$	$20.6 \pm 5.3$	$10.5 \pm 0.9$
SO <sub>2</sub>	50	$1.1 \pm 1.9$	$4.0 \pm 1.0$	0.0	$3.2 \pm 0.4$	$5.0 \pm 0.0$	$3.3 \pm 1.0$	$18.4 \pm 5.4$	$12.3 \pm 2.1$
$O_3 + SO_2$	20 + 50	$2.7 \pm 2.5$	$9.6 \pm 2.0$	$6.0 \pm 0.9$	$3.4 \pm 0.6$	$6.3 \pm 0.6$	$3.0 \pm 0.9$	$20.0\pm4.7$	$14.2 \pm 0.7$

<sup>a</sup>Rating based on Horsfall and Barratt scheme (4): 0 = 0% injured leaf area, 1 = 1-3%, 2 = 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-78%, 8 = 78-94%, 9 = 94-97%, 10 = 97-99%, 11 = 99-100%, and 12 = 100%. Mean of 3 runs of 5 replicate plants each  $\pm$  sp.

mean foliar injury to the 2 ozone sensitive species was about 50%, whereas it was about 5% for ginseng (Table 1). The concentration but not the duration of ozone used here may be approached or exceeded in ambient air. For instance, Anlauf et al. (1) have reported periods in August for Toronto, Ontario, when 20 pphm ozone concentration was exceeded. Ozone at concentrations which caused ginseng injury in controlled environments may occur in the crop producing areas of southern Ontario (7) and may also occur in the ginseng producing areas of the United States and Asia.

Ginseng was more sensitive than radish, and less sensitive than tobacco, to sulfur dioxide injury (Table 1). Since radish is considered to be relatively sensitive to sulfur dioxide (5), ginseng should also be classified as sensitive. Injury to ginseng leaflets ranged from a mild chlorosis to irregular necrotic areas which sometimes fell out giving a ragged appearance to the leaflets.

Injury to plants in the combined treatment was greater for all plants than when the gases were given singly (Table 1). Characteristic ozone stippling was accompanied by irregular bleached patches particularly towards the base of the leaflets; typical bleaching is shown in Fig. 1B, in this instance occurring, as it sometimes did, at the tip of the leaflets.

Documentation of leaf injury on ginseng is sparse and old. For instance, the North American standard for ginseng diseases and their control by Whetzel et al. (10) was published in 1930. Advances in the recognition of air pollution injury to crops in the last 20 years allow possible relationships between monitored phytotoxic levels of local pollutants and associated plant injury to be established. This report, establishing symptoms of ozone and  $SO_2$  injury and classifying ginseng as moderately sensitive to ozone and sensitive to sulfur dioxide, will be of use in disease assessment in the field.

## Literature Cited

- Anlauf, K. G., M. A. Lusis, H. A. Wiebe, and R. D. S. Stevens. 1975. High ozone concentrations measured in the vicinity of Toronto, Canada. Atmos. Environ. 9:1137–1139.
- Heggestad, H. E. and H. A. Menser. 1962. Leaf spot-sensitive tobacco strain Bel W-3, a biological indicator of the air pollutant ozone. Phytopathology 52:735. (Abstr.)
- Hoagland, D. R. and D. I. Arnon. 1950. Water culture method for growing plants without soil. Calif. Agr. Expt. Sta. Cir. 347.
- Horsfall, J. G. and R. W. Barratt. 1945. An improved grading system for measuring plant diseases. Phytopathology 35:655.
- 5. Jacobson, J. S. and A. C. Hill (eds). 1970. Recognition of air pollution injury to vegetation: a pictorial



Fig. 1A. Ozone-induced stippling of the upper surface of a ginseng leaflet. The stippling was photographed 4 days after an exposure of 20 pphm for 6 hr daily for 4 days.



Fig. 1B. Ginseng leaf tip chlorophyll bleaching induced by an exposure of 20 pphm ozone plus 50 pphm sulfur dioxide for 6 hr daily for 4 days. Photograph taken 4 days after the exposure.

- atlas. Air pollution Control Assoc., Pittsburg, Pa.
  Ormrod, D. P. 1978. Pollution in horticulture. Elsevier. New York.
- Ormrod, D. P., J. T. A. Proctor, G. Hofstra, and M. L. Phillips. 1980. Air pollution effects on agricultural crops in Ontario: a review. Can. J. Plant Sci. 60:1023-1030.
- Proctor, J. T. A. 1980. Some aspects of the Canadian culture of ginseng (*Panax quinquefolius* L.) particularly the growing environment. p. 39–47. In:

Proc. of the Third Intern. Ginseng Symp., Korean Ginseng Research Inst., Seoul, Korea.

- 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.
- Whetzel, H. H., J. Rosenbaum, J. W. Brann, and J. A. McClintock. 1930. Ginseng diseases and their control. U.S. Dept. Agr. Farmers' Bul. 736.