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Note

Prolonged exposure to atmospheric nitrogen dioxide increases fruit yield of tomato plants

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Abstract Prolonged exposure of plants to a sufficient level of atmospheric nitrogen dioxide (NO_2) activates the uptake and metabolism of nutrients that fuel plant growth and development, a phenomenon termed the plant vitalization effect of NO_2. This study examined the effect of NO_2 on the fruit yield of tomato (*Solanum lycopersicum* L. cv. Micro-Tom). Double exposure chambers, designated $\pm NO_2$ chambers, were placed in a confined greenhouse. The air entering the $-NO_2$ chamber was scrubbed of nitrogen oxides including NO_2, and the air entering the $+NO_2$ chamber was supplemented with NO_2 (50 ± 10 ppb). Two-week-old tomato seedlings that had been grown in the $-NO_2$ chamber after sowing were then grown in a $+NO_2$ or $-NO_2$ chamber for the remainder of the growth period until 96 days after sowing. Growth in the $+NO_2$ chamber led to a significant increase in fruit yield of approximately 40% as compared to growth in the $-NO_2$ chamber. This increase in fruit yield was accompanied by acceleration of flowering time by 3.2 days and an increase in flower number per plant of up to 60%. These results show that exposure to NO_2 increases fruit yield via stimulation of flowering in tomato.

Key words: Flowering, fruit yield, Micro-Tom, nitrogen dioxide, tomato.

The prolonged exposure of plants to an adequate level of atmospheric nitrogen dioxide (NO_2) triggers the uptake and metabolism of nutrients that support plant growth and development, a phenomenon called the plant vitalization effect of NO_2 (Adam et al. 2008; Takahashi et al. 2005, 2008). This study investigated whether exposure to NO_2 can also stimulate fruit production using tomato (*Solanum lycopersicum* L. cv. Micro-Tom) as a model plant species.

Besides being an important commercial crop, tomato constitutes a model species for the study of plant developmental processes (Lozano et al. 2009). Tomato has been the third most common target for plant functional genomic studies, following *Arabidopsis* and rice. Various tomato genome initiatives are currently under way in conjunction with an international consortium called the International Solanaceae Genome Project (SOL) (Sun et al. 2006). Micro-Tom, a dwarf tomato cultivar having a short life cycle (70–90 days from sowing to fruit ripening), has been used as a model system for studying tomato genetics (Meissner et al. 1997).

Exposure to NO_2 has been considered either detrimental or slightly beneficial to the vegetative growth of tomato plants (Okano et al. 1988; Wellburn 1990). The fruit yield of tomato plants has been reported to be greatly inhibited by NO_2 (Spierings 1971). However, in these previous studies, tomato plants were exposed to relatively high concentrations (250–500 ppb) of NO_2 (Okano et al. 1988; Wellburn 1990), including the study by Spierings (1971). In contrast, at concentrations as low as 50 ppb, NO_2 has been reported to benefit the growth of some crop species (Adam et al. 2008). Thus, determining whether NO_2 at a lower concentration is beneficial to the fruit production of tomato warrants investigation. In the present study, we grew Micro-Tom tomato plants in air with or without 50 ppb NO_2 for the entire growth period until harvest and determined the fruit yield.

Two glass-walled NO_2 exposure chambers ($1.5\times1.0\times0.7$ m in width, height, and depth, respectively; model NOx-1130-SC; Nippon Medical & Chemical Instruments Co., Ltd., Osaka, Japan), designated $\pm NO_2$ chambers, were placed in a confined greenhouse ($6.9\times2.4\times3.0$ m in width, height, and depth, respectively; model BTH-P1-TH; Nippon Medical & Chemical Instruments Co., Ltd.), as described previously (Adam et al. 2008). The air entering the greenhouse (at a rate of 4 m^3 min^{-1}) from outside was scrubbed of NO, NO_2, and O_3 using

Abbreviation: NO_2, nitrogen dioxide
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activated charcoal and $NaMnO_4$ (PureliteE30; Nippon Puretec Co., Tokyo, Japan). The NO_2 and O_3 concentrations in the greenhouse were <5 ppb. The temperature and relative humidity were controlled at 22 ± 0.3°C and 70 ± 4%, respectively, while the CO_2 concentration was kept at 360 ± 80 ppm using a climate-control system (CTH-G 100; Nippon Medical & Chemical Instruments Co., Ltd.). The air entering the $+NO_2$ chamber at a rate of $1\,l\,min^{-1}$ was mixed with NO_2 and kept at a final concentration of 50 ± 10 ppb NO_2, which was the lowest controllable limit for the $+NO_2$ chamber. The air entering the $-NO_2$ chamber at the same rate as that of the $+NO_2$ chamber was not supplemented with NO_2 and kept at <5 ppb NO_2. The temperature, CO_2 concentration, and relative humidity in each of the chambers were controlled by an independent climate-control system (CTH-C 70; Nippon Medical & Chemical Instruments Co., Ltd.) at the same values as those for the greenhouse.

Micro-Tom (TOMJPF00001) seeds, provided by the University of Tsukuba Gene Research Center through the National Bio-Resource Project of the Ministry of Education, Culture, Sports, Science and Technology, Japan, were sown on compost (Jiffy Mix; Sakata, Yokohama, Japan) in two cell trays (12 cells, each with a volume of 40.5 ml) on 25 February 2009, which was designated day 0. Trays were placed in the $-NO_2$ chamber and watered daily with tap water. On day 14 after sowing, one of the trays was transferred into the $+NO_2$ chamber. On day 16 after sowing, ten seedlings of uniform size from each chamber were transplanted into individual 15-cm pots containing moist compost. From day 28 after sowing, the seedlings were supplied every 2 days with a mixed nutrient solution of Otsuka House No. 1: Otsuka House No. 2 (6 : 4) (Otsuka Chemical Co., Osaka, Japan; N, P, K, Ca, and Mg=18.6, 5.1, 8.6, 8.2, and 3.0 mEq/l, respectively). Harvesting of red, ripe fruits started on day 80 after sowing and all fruits were harvested on day 96 after sowing (1 June 2009).

Nitrogen dioxide (50 ± 10 ppb) appeared to stimulate tomato flowering. The number of days to anthesis of the first flower was 32.3 ± 2.5 days (mean of ten plants ± SD) under $+NO_2$ conditions and 35.5 ± 1.2 days under $-NO_2$ conditions. This difference was significant according to Student's *t*-test ($P<0.002$). Furthermore, exposure to NO_2 increased flower number per plant by a maximum of 60% ($P<0.05$), as shown in Figure 1.

Nitrogen dioxide markedly increased the tomato fruit yield (Table 1, Figure 2), and the total yield of red fruit and total yield of all fruit increased by approximately 40% ($P<0.01$). The numbers of red fruit and of all fruit increased significantly by approximately 60% and 70%, respectively, upon growth under $+NO_2$ ($P<0.01$) (Table 1). Average weight of red fruits and that of all fruits did not significantly differ between exposed and non-exposed plants (Table 1).

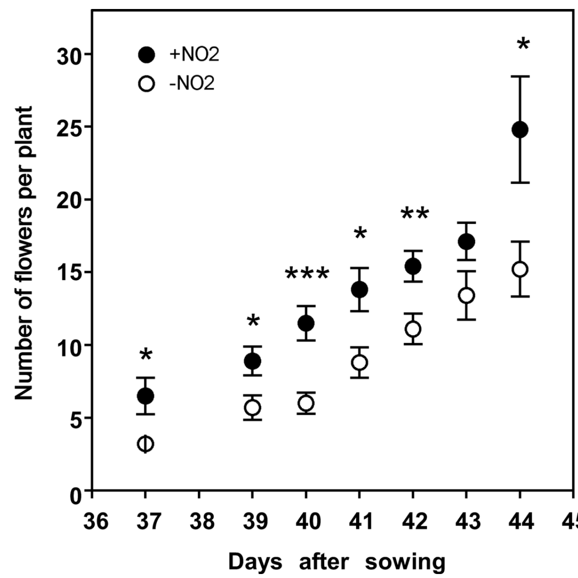

Figure 1. The number of flowers per plant plotted against days after sowing. Plants were grown in air containing ($+NO_2$) or not containing ($-NO_2$) NO_2. The number of flowers per plant was counted from day 37 to 44 after sowing. Data represent means of ten plants ± SD. Asterisks indicate statistical significance (* $P<0.05$, ** $P<0.01$, and *** $P<0.001$) assessed by Student's *t*-test.

Our finding that the fruit yield of Micro-Tom tomatoes increased by 40% upon exposure to NO_2 at 50 ± 10 ppb for 82 days (from 14 to 96 days after sowing) is in sharp contrast to Spierings's (1971) report stating that exposure of Moneymaker tomato plants with NO_2 at 250 ppb during the entire growth period of approximately 4 months caused a 22% decrease in fruit yield. We evaluated the growth of Moneymaker tomato plants in the presence and absence of 50 ppb NO_2 using double exposure chambers. However, the height of the chambers (1.0 m) was too low to allow for growth of Moneymaker tomato plants. Thus, no clear results showing positive or negative effects of NO_2 on the fruit yield of this cultivar were obtained. Further studies using exposure chambers suited to this cultivar are required to clarify whether Moneymaker plants have an increased fruit yield at 50 ppb NO_2 or experience no effect. The increase in biomass in response to NO_2 exposure significantly differed between *Arabidopsis thaliana* ecotype C24 (approximately 2.3 times) and *A. thaliana* ecotype Columbia (1.6 times) (Takahashi et al. unpublished data). Therefore, the response to prolonged exposure to NO_2 likely differs among different tomato cultivars.

Our present study showed that the NO_2-induced increase in fruit yield of Micro-Tom was accompanied by an increase in the number of fruits (but not in fruit weight), an increase in number of flowers per plant, and an acceleration of flowering time. This strongly suggests that NO_2 increases fruit yield via stimulation of flowering in Micro-Tom tomato plants. To our

Table 1. Fruit yield of tomato (cv. Micro-Tom) grown in air containing ($+NO_2$) or not containing ($-NO_2$) NO_2

	Total yield of red fruit (g)	Total yield of all fruit (g)	No. of red fruit	No. of all fruit	Average weight of red fruit (g)	Average weight of all fruit (g)
$+NO_2$	70.0 ± 14.6**	80.3 ± 13.0**	23.1 ± 6.0**	37.9 ± 7.7**	3.0 ± 0.9	2.1 ± 1.4
$-NO_2$	50.9 ± 8.7	59.3 ± 7.1	14.9 ± 3.4	22.9 ± 4.0	3.4 ± 1.4	2.6 ± 1.7

Mean of ten plants ± SD. Statistical significance was assessed by Student's *t*-test. ** $P<0.01$.

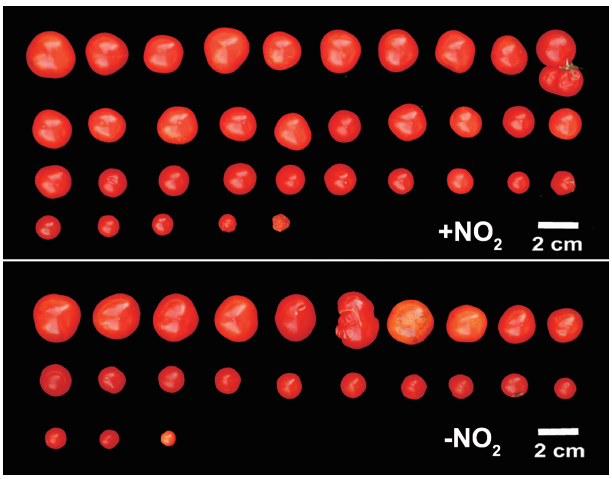

Figure 2. Typical photographs of ripe, red tomato fruits harvested from plants grown in air containing ($+NO_2$) or not containing ($-NO_2$) NO_2.

knowledge, this is the first report on the stimulation of flowering and fruit production by atmospheric NO_2. More research is required to determine if this effect can be generalized to other tomato cultivars or crop species.

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