Foliar spraying with zineb increases fruit productivity
and alleviates oxidative stress in two tomato cultivars

Á. CALATAYUD and E. BARRENO

Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Facultat de Ciències Biològiques, Universitat de València,
 c/Dr. Moliner 50, 46100 Burjassot (València), Spain

Abstract

The effects of foliar spraying of the dithiocarbamate zineb on two cultivars of tomato grown in the field in a site with high ozone concentrations were studied by means of biomass assessment, antioxidant enzyme assays, lipid peroxidation, and chlorophyll fluorescence measurements. Zineb prevented the peroxidation of membrane lipids and decreased the activity of scavenging enzymes, which suggests that plants sprayed with zineb are subjected to lower oxidative stress than controls. The beneficial effects of zineb protection is the utilization of a larger fraction of absorbed radiant energy in photosynthesis and a larger fruit yield in plants of both cultivars.

Additional key words: antioxidant enzymes; chlorophyll fluorescence; lipid peroxidation; Lycopersicon esculentum; ozone; production.

Introduction

Tropospheric O₃ is a major regional air pollutant over wide areas downwind of urban and industrialized regions of the world. The adverse effects of O₃ on growth and yield of agricultural crops have been well documented (Lefohn 1992, Davison and Barnes 1998). The need to prevent the adverse effects of O₃ on plant growth and productivity prompted the search of chemical compounds effective in counteracting O₃-induced phytotoxicity. The assayed compounds include some antioxidants but also antisenescence agents, antitranspirants, dusts, growth regulators, growth retardants, fungicides, and pesticides (Kendrick et al. 1962, Dass and Weaver 1968, Cathey and Heggestad 1972, Tomlinson and Rich 1973, Carnahan et al. 1978, Ormrod and Beckerson 1986). One of such compounds has been the fungicide zinc ethylene-bis-zinc-dithiocarbamate (zineb) (Kendrick et al. 1954, 1962). While the efectiveness of this compound in preventing O₃-induced leaf injury in Pinto beans is well established there is a paucity of knowledge of its effects on plant productivity as well as the type of protection to plants afforded by this substance. The effects of zineb were studied in tomato, a crop of paramount importance to the agricultural economy of the Valencia region (East Spain). The objective of the present study was twofold. Firstly, we wanted to determine whether tomato plants experienced oxidative stress in the Valencia area and, at the same time, to study the effectiveness of zineb in preventing O₃-induced losses in productivity. Secondly, we intended to explore the type of protection afforded by zineb to plants.

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Fax: 34-6-3864082, e-mail: angeles.calatayud@uv.es

Abbreviations: AP, ascorbate peroxidase; AsA, ascorbate; CAT, catalase; DHA, dehydroascorbate; Chl, chlorophyll; Fₘ, maximal fluorescence in dark-adapted samples; Fₘ, maximal fluorescence in irradiated samples; Fₗ, minimal fluorescence in dark-adapted samples; Fₗ, level of modulated fluorescence during a brief interruption of actinic irradiation in the presence of far-red radiation; Fₘ, chlorophyll fluorescence during irradiation; Fₗ, variable fluorescence (Fₘ - Fₗ) in dark-adapted leaves; GR, glutathione reductase; MDA, malondialdehyde; NPQ, non-photochemical quenching from Stern-Volmer equation; POD, peroxidase; PS, photosystem; qₑ, photochemical quenching coefficient; SOD, superoxide dismutase; TAA, total ascorbate; zineb, ethylene-bis-zinc dithiocarbamate; Φₚₘ, quantum efficiency of PS2.

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Materials and methods

Plants: Tomato (*Lycopersicon esculentum* Mill.) cultivars Claudia and Maresme were germinated in a greenhouse using a commercial soil mixture (*Terraplant, BASF, Germany*) at the Carcaixent Experimental Station (Valencia, Spain). Three-week-old seedlings were transplanted to large containers and transferred to the field. The growth period had a duration of 104 d and extended from the end of June to the beginning of October for both cvs. Total plant number for each cultivar was 20. Only fully expanded adult leaves were used in the experiments. During the growth period plants were watered daily.

Zineb treatment: Ethylene-bis-zinc-dithiocarbamate (zineb, ARGOS) was applied as foliar spray at the dose prescribed by the maker (2.5 kg m$^{-2}$) to one half of the plants of each cultivar every ten days. Plants that did not receive zineb were sprayed with an equal volume of water.

Biomass production was assessed at the end of the growing season. The fresh mass of the shoot, roots, and fruits was determined gravimetrically in both cultivars and for each treatment.

Chlorophyll (Chl) a fluorescence induction kinetics at ambient temperature was measured with a portable pulse-modulated fluorometer (*PAM-2000 Walz*, Effeltrich, Germany). Leaves were darkened for 30 min prior to the measurements. The minimum (dark) fluorescence yield $F_0$ was obtained upon excitation of leaves with a weak measuring beam [14 μmol(photon) m$^{-2}$ s$^{-1}$] from a light-emitting diode. The maximum Chl fluorescence yield ($F_{m}$) was determined with a 600 ms saturating pulse of "white light". Variable fluorescence ($F_{v}$) was calculated as $F_{m} - F_0$. Following 2 min for dark readaptation, actinic "white light" [500 μmol(photon) m$^{-2}$ s$^{-1}$] was switched on and 600 ms saturating pulses [8 000 μmol(photon) m$^{-2}$ s$^{-1}$] were applied at 60 s intervals for determination of the maximum Chl fluorescence yield during actinic irradiation ($F_{m}^{'}$), the level of modulated Chl fluorescence during a brief interruption of actinic irradiation in the presence of far red radiation ($F_{r}$), and the Chl fluorescence yield during actinic irradiation ($F_{c}$). Calculation of quenching due to non-photochemical dissipation of absorbed radiant energy (NPQ) was determined at each saturating pulse according to the equation $NPQ = (F_{m} - F_{m}^{'})/F_{m}^{'}$ (Bilger and Björkman 1991). The coefficient for photochemical quenching, $q_{p}$, represents the fraction of open PS2 reaction centres and was calculated as $(F_{m}^{'} - F_{c})/(F_{m}^{'} - F_{r})$ (Schreiber et al. 1986). The quantum efficiency of PS2 photochemistry, $\Phi_{PS2}$, reflects the fraction of radiation absorbed by PS2 that is utilized in photochemistry (Demming-Adams et al. 1996), and was estimated as $(F_{m}^{'} - F_{r})/(F_{m}^{'} - F_{0})$ (Genty et al. 1989).

Enzyme analytical methods: Leaf material (2 g), without the main midrib, was homogenized in 10 cm$^{3}$ of 100 mM potassium phosphate buffer, pH 7.5 containing 2 mM EDTA and 2 % PVP. The slurry was centrifuged at 15 000×g for 20 min. The supernatant was filtered (*Millipore, Mitex 0.5 μm*) and utilised for enzyme analysis. All operations (until analysis) were carried out at 3-5 °C. The enzymatic assays were performed after the plants had remained 10, 50, and 100 d under field conditions.

Peroxidase (POD) activity was analyzed as described by Astorino et al. (1995) with slight modifications. The assay was performed in a 3 cm$^{3}$ cuvette containing 100 mM potassium phosphate buffer, 1 % guaiacol, and 6 mM H$_2$O$_2$. Reaction assays were started by the addition of an appropriate volume of the leaf extract. Activity was determined by the increase in absorbance at 470 nm due to guaiacol oxidation.

NADPH-dependent glutathione reductase (GR) activity was determined by following the oxidation of NADPH at 340 nm as described by Rao (1992) and Rao et al. (1996). The reaction mixture contained 100 mM potassium phosphate buffer, 0.2 mM NADPH, 0.5 mM GSSG, and the appropriate amount of leaf extract. The assays were initiated by the addition of NADPH at room temperature. Corrections were made for NADPH oxidation in the absence of GSSG.

Superoxide dismutase (SOD) activity was measured as described by Beyer and Fridovich (1987). The reaction mixture was composed of 50 mM potassium phosphate buffer, 9.9 mM methionine, 57 μM nitroblue tetrazolium (NBT), 0.9 μM riboflavin, 0.025 % (m/v) Triton X-100, and the appropriate amount of leaf extract. The A$_{550}$ was recorded after a 7 min irradiation period. In this assay, 1 unit of SOD is defined as the amount required to inhibit the photoreduction of NBT by 50 %.

Lipid peroxidation: The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) in leaves, according to the protocol proposed by Heath and Packer (1968), with the modifications made by Dhindsa et al. (1981).

Determination of atmospheric pollutant levels: During the experiments, O$_3$, SO$_2$, NO, and NO$_2$ concentrations were continuously monitored at the experimental site using EPA-approved monitors specific for each pollutant (DASIBI models 1008, 4108, and 2108).
Results

During each day of the growth period, O₃ concentration at the Carcaixent Experimental Estation exceeded the EC Ambient Air Quality Standards, set at a daily mean of 33.16 mm³ m⁻³ (Table 1). The concentrations of SO₂ and NO₂ were far below Ambient Air Quality Standards set at daily means of 30.0 and 104.6 mm³ m⁻³, respectively, by the Spanish Ministry of Environment. Therefore, O₃ could be considered the main environmental pollutant affecting plant development.

Table 1. Monthly means of the air pollutants O₃, SO₂, NO, and NO₂ [mm³ m⁻³] recorded in the Carcaixent Experimental Estation during the growth period.

<table>
<thead>
<tr>
<th>Month</th>
<th>O₃</th>
<th>SO₂</th>
<th>NO</th>
<th>NO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>June</td>
<td>41.1±4.5</td>
<td>1.7±0.5</td>
<td>3.2±0.6</td>
<td>7.1±3.1</td>
</tr>
<tr>
<td>July</td>
<td>47.3±7.1</td>
<td>2.0±0.3</td>
<td>5.3±2.2</td>
<td>9.3±3.3</td>
</tr>
<tr>
<td>Aug</td>
<td>46.6±5.3</td>
<td>1.8±0.3</td>
<td>5.5±1.4</td>
<td>9.6±4.2</td>
</tr>
<tr>
<td>Sept</td>
<td>38.6±6.5</td>
<td>2.0±0.6</td>
<td>4.3±1.3</td>
<td>7.8±3.5</td>
</tr>
<tr>
<td>Oct</td>
<td>35.8±5.2</td>
<td>2.1±1.1</td>
<td>3.2±0.4</td>
<td>7.5±3.1</td>
</tr>
</tbody>
</table>

As a result of zineb spraying (+zineb), cv. Claudia showed a lower shoot growth and a similar root development (Table 2). Similarly, cv. Maresme experienced a lower shoot growth, although in this cv. root growth was enhanced. These changes were translated into a lower shoot/root ratio in both species by 14.00 and 36.87 % in cvs. Claudia and Maresme, respectively. Finally, fruit production was increased by 23 and 16 % in +zineb Claudia and Maresme cultivars.

In +zineb leaves, the parameter Fv/Fm, indicating the efficiency of excitation capture of PS2 of a dark-adapted leaf, was higher than in -zineb leaves of both cvs., with increases of 7.6 and 8.6 % in cvs. Claudia and Maresme, respectively (Table 3). The quantum efficiency of the PS2-mediated electron transport showed an increase of 23 and 24 % in +zineb leaves of both cvs. Additionally, foliar spraying with zineb also affected the photochemical quenching coefficient qP, with increases of 18 and 14 % in +zineb plants. Finally, non-photochemical Chl fluorescence quenching (NPQ) was

Table 2. Biomass production [g(fresh mass)] of shoot and roots as well as the shoot/root ratio and fruit mass [g] by plant at harvesting in -zineb and +zineb Claudia and Maresme tomato cultivars. Means of n = 10 plants. Asterisks indicate significative differences (Student’s t-test: * = p<0.05; ** = p<0.01; NS = p>0.05).

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Treatment</th>
<th>Shoot</th>
<th>Root</th>
<th>Shoot/root</th>
<th>Fruit mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Claudia</td>
<td>-zineb</td>
<td>637</td>
<td>131.3</td>
<td>5.23</td>
<td>1424.9</td>
</tr>
<tr>
<td>Claudia</td>
<td>+zineb</td>
<td>601</td>
<td>130.5</td>
<td>4.50</td>
<td>1749.2</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Maresme</td>
<td>-zineb</td>
<td>1290</td>
<td>142.5</td>
<td>9.03</td>
<td>1268.2</td>
</tr>
<tr>
<td>Maresme</td>
<td>+zineb</td>
<td>915</td>
<td>175.1</td>
<td>5.70</td>
<td>1948.0</td>
</tr>
<tr>
<td>p</td>
<td>*</td>
<td>NS</td>
<td>**</td>
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![Graph showing Malondialdehyde (MDA) content measured at days 10, 50, and 100 following transfer of +zineb (hatched bars) and -zineb (hollow bars) tomato plant cvs. (A) Claudia and (B) Maresme. Asterisks indicate significant differences (Student’s t-test: * = p<0.05; ** = p<0.01). Values are means±SD for n = 5.](image)

**Fig. 2.** Malondialdehyde (MDA) content measured at days 10, 50, and 100 following transfer of +zineb (hatched bars) and -zineb (hollow bars) tomato plant cvs. (A) Claudia and (B) Maresme. Asterisks indicate significant differences (Student’s t-test: * = p<0.05; ** = p<0.01). Values are means±SD for n = 5.

The activity of all the antioxidant enzymes (Fig. 1) assayed during the growing season tended to increase with increasing permanence time of plants in the field both in +zineb and -zineb plants of both cultivars. Nevertheless, zineb-treatment induced a significant decline in the activity of all enzymes at day 100 in cvs. Maresme and Claudia, except for SOD in the latter cv. Claudia.

Lipid peroxidation (Fig. 2) was measured during the growing season as the concentration of MDA. Lipid peroxidation increased markedly with exposure of plants to field conditions in both tomato cultivars. Additionally, the +zineb plant leaves experienced a significantly lower amount of lipid peroxidation than their untreated counterparts.

**Discussion**

Our results are in accordance with previous findings by Kendrick et al. (1954, 1962) that zineb is effective in preventing ozone-induced plant damage. This was seen as decreases in fruit yield in -zineb plants in addition to increased oxidative stress as probed by MDA content and the activity of antioxidant enzymes. On the other hand, +zineb plants showed increased fruit yield in addition to a reduced shoot/root ratio in both cvs. This ratio increased on exposure to O₃, indicating changes in the partitioning of photosynthates. This shift of assimilate partitioning is one of the most sensitive effects of O₃ on higher plants and is associated with a compensatory process that maintains leaf biomass at the expense of roots (Cooley and Manning 1988, Kostka-Rick and Manning 1992, Pell et al. 1994). Therefore, the fact that -zineb plants showed increased shoot/root ratios suggests that tomato plants were experiencing O₃-induced oxidative stress and that zineb treatment afforded partial protection against this oxidant.

Some studies suggest that the need to reduce the oxidizing environment created when plants are exposed to O₃ results in an enhancement of SOD and GR activities (Conklin and Least 1995, reviewed by Kangasjärvi et al. 1994, Sharma and Davis 1994, Rao and Ormrod 1995, Sharma et al. 1996). In the present study, +zineb plants showed reduced activities of these antioxidant enzymes, and thus zineb might be reducing the susceptibility of tomato plants to oxidative stress. In turn, this was seen in the lower content of MDA in +zineb compared to -zineb plants. The MDA content represents the state of peroxidation of membrane lipids and is positively correlated with the degree of O₃ exposure in higher plants (Price et al. 1990, Yoshida et al. 1994, Ranieri et al. 1996) and lichens (Fisser et al. 1994). Therefore we suggest that zineb may protect plants by decreasing pollutant-induced oxidative stress.

Most stress factors, even if they do not directly affect the composition of the photosynthetic apparatus, induce a decline in the proportion of absorbed photons used in photosynthesis, which in turn results in an increase of heat and Chl fluorescence emission (Lichtenenthaler 1996). Analysis of Chl fluorescence parameters in tomato plants showed various differences, which suggested a higher stress on PS2 in -zineb plants. For instance, -zineb plants utilized a lower fraction of absorbed photons in photochemistry, as indicated by the lower values of the Chl fluorescence parameter ΦPS2 (Demmig-Adams et al. 1996). In accordance with this, -zineb plants exhibited higher levels of thermal energy dissipation activity as probed by NPQ (Demmig-Adams et al. 1996). This would be consistent with -zineb plants experiencing a higher relative level of absorbed photons as excessive compared to +zineb plants due to their lower utilization in photochemistry. Finally, the decline in the F/Fₐ ratio could be due to photodamage of PS2 centres but also to the observed increase in protective non-radiative energy dissipation, or both (see Osmond 1994). Therefore, our results do not allow to conclude that the Chl fluorescence characteristics of -zineb plants were due to damage to the photosystem or to a downregulation of PS2 activity.

To summarize, our study partially explains the mechanisms underlying zineb protection to tomato plants. Zineb somehow prevents the peroxidation of
membrane lipids and decreases the activity of scavenging enzymes, which suggests that +zineb plants might be subjected to lower oxidative stress than -zineb plants. The beneficial effects of zineb protection ultimately can be seen in a higher fraction of absorbed photons going into photosynthesis and in a greater fruit yield in +zineb plants of both cultivars.

References


