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Screening agrochemicals as potential protectants of plants against ozone phytotoxicity

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ABSTRACT

We tested seven contemporary agrochemicals as potential plant protectants against ozone phytotoxicity. In nine experiments, Bel-W3 tobacco plants were experienced weekly exposures to a) 80 nmol mol^{-1} of ozone-enriched or ozone-free air in controlled environment chambers, b) an urban air polluted area, and c) an agricultural-remote area. Ozone caused severe leaf injury, reduced chlorophylls' and total carotenoids' content, and negatively affected photosynthesis and stomatal conductance. Penconazole, (35% ± 8) hexaconazole (28% ± 5) and kresoxim-methyl (28% ± 15) showed higher plants' protection (expressed as percentage; mean ± s.e.) against ozone, although the latter exhibited a high variability. Azoxystrobin (21% ± 15) showed lower protection efficacy and Benomyl (15% ± 9) even lower. Trifloxystrobin (7% ± 11) did not protect the plants at all. Acibenzolar-S-methyl + metalaxyl-M (Bion MX) (−6% ± 17) exhibited the higher variability and contrasting results: in some experiments it showed some protection while in others it intensified the ozone injury by causing phytotoxic symptoms on leaves, even in control plants.

1. Introduction

Ambient ozone level has risen over the last four decades, it is still being gradually increasing, and it is expected to be a major menace for cultivated plants and natural ecosystems in the near future (Fishman et al., 2010). The nowadays occurring ambient ozone levels are high enough to negatively affect wild plant species (Bermejo et al., 2003; Feng et al., 2014; Agathokleous et al., 2014a) and field-growing cultivated plants (Booker et al., 2009; Avnery et al., 2011), especially in the Mediterranean countries (Saitanis and Karandinos, 2001a; Saitanis, 2003). Retardation of plants' growth and severe yields' losses in crop plants, due to increased ozone levels, have been reported (Booker et al., 2009). Fishman et al. (2010) estimated the global economic loss to the farming community to exceed $10 billion annually.

The main goal, nowadays, is the reduction of ambient ozone levels through the reductions of its precursors (NOx, VOCs). This, however, is considered a long-term goal. Thus, methods for protection of plants against ozone should be urgently developed. Many substances have been tested as potential protectants of plants against ozone phytotoxicity, among which agrochemicals, such as fungicides, herbicides, insecticides, and plant growth regulators (Manning, 2000).

Substances that cause stomatal closure, such as phenylmercuric acetate and monoethyl esters of decenylsuccinic acid have been tested many years ago (Rich, 1964; Seidman et al., 1965), and they have been found to protect plants. Similar protective efficacy has also been obtained by abscisic acid (ABA), which also induces stomata closure (Lin et al., 2001). In a recent study, Francini et al. (2011) found that the antitranspirant di-1-p-menthene significantly protected Pinto bean plants from acute ozone injury, but this substance did not work on tobacco plants (Agathokleous et al., 2014b). However, the stomatal closure induced by such substances does not only impede ozone entry to leaf tissues, but simultaneously impedes CO_2 uptake, which, in turn, may lead to undesirable yield loss due to reduced CO_2 assimilation.

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A well-known promising antioxidant is EDU (N-[2-(2-oxo-1-imidazolidinyl)ethyl]-N'-phenylurea; abbreviated EDU for ethylenediurea) (Carnahan et al., 1978; Feng et al., 2010; Manning et al., 2011). However, the major disadvantage of EDU is that it is not commercially available (only very few laboratories and only for research purpose can prepare it).

A short review of the available publications shows that many biocides or some antioxidant substances, available in commerce, may offer protection of plants against ozone. Since 1972, benomyl (a fungicide now being banned) was tested as plant-protectant against ozone (Reinert and Spurr, 1972; Manning et al., 1973a, b, c) with positive results. When benomyl was applied as a foliar spray to field growing bean plants resulted to a 70–80% suppression of oxidant injury (Manning et al., 1973b), while when applied in Bel-W3 plants mitigated leaf injury up to ~60% (Reinert and Spurr, 1972). Azoxystrobin and epoxiconazole fungicides have also been reported possessing antiozonate properties (Wu and Von Tiedemann, 2002). Although numerous substances have been tested and proposed as potential antiozonants over the years, there is a lack of understanding of their antiozonate mechanisms for all of them. Even for EDU, which is the most studied antiozonate substance, the actual underline mechanism has not been uncovered yet (Agathokleous et al., 2014c).

In the framework of this investigation, we tested seven contemporary agrochemicals as potential plant-protectants against ozone phytotoxicity. We focused on agrochemicals because they are commercially available and they are extensively used in intensive agriculture. The knowledge that some agrochemicals exhibit – as a side effect – antiozonate property, gives to them an advantage, when compared with other homologous agrochemicals, so they would be preferable in agricultural practice. Besides, such agrochemicals would also be used by researchers in setting up control group plants in studies aiming to assess crop yield loss by ozone under field conditions.

2. Material and methods

2.1. Plant materials

As experimental plant, the Bel W3 tobacco (*Nicotiana tabacum* L.) variety was used. This variety is known to be hypersensitive to ozone (Saitanis and Karandinos, 2001b) with a threshold of sensitivity of about 40 nmol mol^{-1} of ozone for few hours. Thus it is not cultivated and often the results coming from experiments based on this variety – especially those dealing with yield – are not representative to what really happens in cultivations. However, because of its high sensitivity to ozone, it is widely used as a useful tool in laboratory and field experiments to reveal the mechanisms of plants' response to ozone.

Seeds of Bel-W3 variety were planted in peat. After germination, seedlings were transferred to 12 cm d plastic pots (one seedling per pot) filled with commercial soil (Floran, STEDIP corp.). When plants reached the fifth leaf stage of growth, they were selected for uniformity and divided equally between treatments' groups. The number of plants used per agrochemical treatment (subgroup) differed among experiments (8-20 plants per agrochemical per ozone treatment per experiment), with a total of about 800 plants. Within each chamber the positions of the plants were randomly rotated, at least once daily, in order to minimize any chamber edge effects.

2.2. Experiments

A total of nine (laboratory and field) experiments were conducted.

2.2.1. Laboratory experiments

In seven experiments, sets of Bel-W3 tobacco potted plants were transferred to two identical walk-in chambers (230 × 190 × 170 cm Model 60R - CDR corp.) under the same conditions: 14:10 (L:D) h photoperiod, 65 ± 3% relative humidity and 28 ± 0.5 °C temperature. Both chambers were supplied with purified air, by passing it through dry purafil ($KMnO_4$) and activated charcoal filters, in order to minimize contamination by the ambient air pollutants. In one of the chambers the filtered air was enriched with ozone. Ozone was produced by an electric generator (Air-Zone® XT-6000) that uses a new patented technology producing no NOx byproducts. Teflon lines led sample air from the chamber to a UV ozone analyser (Environnement S.A O3 42M). The ozone concentration within the chamber was stabilized via a feedback controller (CDR corp.). The wind speed within the chambers, above the plants' canopy, was about 2 m s^{-1}.

In each experiment, the plants of the "ozone chamber" were exposed to 80 nmol mol^{-1} of ozone for 8 h per day for 7 days (AOT40: 2240 nmol mol^{-1} h); this level is very close to the ambient ozone levels occurring in rural - agricultural areas nowadays (Saitanis, 2003). Those plants constituted the "ozone exposed" main group (hereafter: OZ^+) while the plants of the chamber provided with filtered air constituted the "ozone-free" – control main group (hereafter: OZ^-). In each experiment, the plants of each chamber were further subdivided to eight subgroups. The plants of each of the seven subgroups were sprayed with one of the agrochemicals, as described below, while those of the eighth subgroup were sprayed with distilled water and constituted the "control subgroup".

2.2.2. Field experiments

Two field experiments were additionally conducted to test the potential protective role of the used agrochemicals under ambient conditions.

One ambient experiment was conducted in the urban (polluted) campus of Agricultural University of Athens (AUA). In this experiment 9–12 plants per treatment were used.

The second ambient experiment (six plants per treatment) was conducted at the agricultural area of Aliartos, located about 70 km away (NW) from Athens.

In the overall data analysis, the plants exposed to ambient urban (AUA) or rural (Aliartos) environment were considered exposed to ozone enriched air (OZ^+).

2.3. Agrochemicals tested

In each experiment, different subgroups of plants were sprayed with different water solutions of the following seven agrochemicals (in brackets their abbreviation): azoxystrobin [AZOX], benomyl [BENML], hexaconazole [HEX], kresoxim-methyl [KRM], penconazole [PENC], trifloxystrobin [TRIFL] and the mixture acibenzolar-S-methyl + metalaxyl-M [BION]. The active constituents, the trade names and the used dose rates of the used agrochemicals are shown in Table 1. The spray solution of the applied dose of each agrochemical was prepared according to the instructions indicated on packaging. One more subgroup of plants, per experiment, was sprayed only with water [WATER] and served as control. All the leaves of the treated plants were sprayed on both surfaces until the applied agrochemical run off.

2.4. Parameters measured

After the end of each experiment, the leaf visible injury was estimated and the following parameters were measured in the three middle (fully expanded) leaves of each plant.