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Effects of Air Filtration on Egyptian Clover (*Trifolium alexandrinum* L. Cv. Messkawy) Grown in Open-Top Chambers in a Rural Site in Egypt

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Abstract: An Egyptian variety of clover plants (Trifolium alexandrinum L. cv. Messkawy) were either untreated (control) or treated with soil drench of Ethylenediurea (EDU) at 50, 100, 150, or 200 ppm or benomyl at 250, 350, 450 or 550 ppm for the whole growing season and sown in the soil either under Egyptian field conditions at a rural site in northern Egypt or in open top chambers receiving either charcoal filtered or no-filtered air. It was noticed that both EDU and benomyl caused an increase in growth parameters and provided some protection against O_3 visible injury symptoms, with EDU having better protection than benomyl. Moreover, photosynthetic rates were increased in treated plants while stomatal conductance did not show any significant difference from control plants under OTCs conditions. It is clear from the results of the present investigation that benomyl and EDU would be useful tools to assess the effects of ambient O_3 on plants under Egyptian field conditions. The implications to Egyptian agriculture were discussed.

Key words: Open top chambers, rular sites, Egyptian clovers, air filtration, ozone

INTRODUCTION

Ozone (O_3) is the most important and prevalent phytotoxic gaseous pollutant in many parts of the world causing severe damage to a variety of susceptible plants (Krupa and Manning, 1988; Chappelka and Chevone, 1992; Hassan *et al*., 1994, 1995, 1999; Hassan and Anttonen, 1999; Wahid *et al*., 2001; Barbo *et al*., 2002; Pihl Karlsson, 2003). Tropospheric concentrations of O_3 are currently increasing globally at a rate of 1-2% annually (IPCC, 1992; Thompson, 1992; Oksanen, 2001). Its effects on agricultural crops and horticultural plants range from overt injury to subtle modification of cellular biochemistry and whole-plant physiology.

Although concentrations of ambient O_3 recorded in rural, urban and suburban sites in Egypt (100-200, 55-70, 55-78, 55-67 and 76-99 ppb in the studies of Nasralla and Shakour (1981), WHO/UNEP (1992), Farag *et al.* (1993), Hassan *et al.* (1995) and Hassan (1999), respectively are potentially high enough to cause damage to natural ecosystems and yield reductions of sensitive agricultural crops, there are very few studies have attempted to assess its effects on crops and studies investigating its effects under field conditions are even rarer (Hassan *et al*., 1995).

Numerous assessments have been carried out to evaluate effects of air pollution on vegetation (Krupa and Manning, 1988; Weigel and Jäger, 1988; Manning and Krupa, 1992; Brunschön-Harti *et al*., 1994; Hassan *et al*., 1995, 1999; Wahid *et al*., 2001). Open-Top Chambers (OTCs) provide a controlled field environment for studies on crop response to O_3.

An alternative method to assess crop losses due to O_3 is to use the antiozonant chemicals such as the antioxidant Ethylenediurea (EDU) (N-(2-(oxo-1-imidazolidinyl)ethyl - N- phenylurea) and the fungicide benomyl (Methyl -1- butyl - carbamyl - 2 - benzidmidazole carbamate) (Manning *et al*., 1974; Carnaham *et al*., 1978; Foster *et al*., 1983; Manning, 1988; Bambwale, 1998; Varshney and Rout, 1998; Kuchler and Flagler, 1999; Manning *et al*., 2003).

In his thorough study, Manning (2000) reported that the antioxidant EDU and the fungicide benomyle were extensively used as very powerful tools for verifying incidence of phytotoxic concentrations of ambient O_3 and detecting losses in yield and growth of different plant species under natural conditions.

International Cooperation Project on crops (ICP-Crops) was established in late 1980´s to investigate the effects of air pollutants including O_3 by UN - ECE working groups on effects (Mortenson, 1992; Mortenson *et al*., 1996). In 1990´s clover plants have been introduced into the programme (Pihl Karlsoon *et al*., 1995a, b; 2002, 2003; Velissariou and Kyrrazi, 1996).

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Many clover species are known to be sensitive to O_3, as they produce visible leaf injury and reductions in growth and yield after exposure to O_3 (Becker *et al.*, 1989). In Egypt, the information on the effects of O_3 on plants is extremely scanty and fragmentary (El-Khatib, 2003). The present work was conducted to fill the above-mentioned gap of knowledge, using the O_3 protectant chemicals EDU and benomyl. Egyptian variety of clover (*Trifolium alexandrinum* L. cv. Messkawy) has been used in this experiment. Clover was used in the present study for its compact size and rapid growth as well as it is important feeder crop. For sound interpretation of the field study controlled experiments using Open-Top Chambers (OTCs) were carried out to determine whether these chemicals had effects in the absence of O_3 and also whether they provide protection against adverse effects of O_3 on the species chosen for the present study.

MATERIALS AND METHODS

Field work

Cultural method and air quality monitoring: The field experiment was conducted over the period 10 October 2002-5 December 2002 at Abbis village (rural site), located 35 km to the south of the Nile Delta, this being the main agricultural are of the country. The experimental area was about 2 km from the nearest traffic road and was surrounded by *Eucalyptus* and *Casuarina* trees (Hassan *et al.*, 1995).

Four plots (each 6.5×2 m) were chosen in the experimental area. Each plot was divided into five equal subplots (0.9×1 m) each with three rows, with 0.5 m distance between subplots. The between-row distance was 0.3 m.

Seeds of clover (*Trifolium alexandrinum* L. cv. Messkawy) obtained from Department of Crop Science of Alexandria University were planted on 10/10/2002, 10 cm apart. Five seeds were sown 5 cm deep at each position. After expansion of first true foliage leaf, seedlings were thinned to one per lot. Thus there were 20 plants/row and 60/subplot.

Two plots were used for EDU application and the others were for benomyl application. One subplot within first and second plot was assigned for control treatment (irrigated with tap water without any chemicals added), while the other four subplots were assigned randomly for EDU treatments (one received 50 ppm, second, third and fourth were receiving 100, 150 and 200 ppm EDU, respectively).

The 3rd and 4th plot was divided as first one, however, subplots were assigned for 0, 250, 350, 450 and 550 ppm benomyl.

No fertilisers or pesticides were applied.

Monitoring of air quality (concentrations of O_3, SO_2 and NO_2), were performed over the entire period of the experiment (Hassan, 1999).

EDU and Benomyle application: Four different concentrations of EDU (50, 100, 150 and 200 ppm) and four different concentrations of benomyl were used (250, 350, 450 and 550 ppm).

(In Egypt, benomyl was applied to agricultural fields in a concentration of 550 ppm, so it was worth to investigate the effect of lower concentrations).

Each plant received 200 mL EDU or benomyl as soil drench, (as it gives a better protection than foliar spray according to Velissariou and Kyriazi, 1996), of the corresponding concentration. In each case, the remaining subplot not being treated with either EDU or benomyl received the same amount of tap water (150 mL).

Four applications of chemicals were applied to the plants. The first was applied on 21st October 2002 when the first true foliage flag leaf was fully expanded (11 days after seeding), while the second, third and fourth were applied 20, 30 and 40 days after seeding.

Gas exchange measurements: Photosynthetic rates (A) and stomatal conductance (g_s) were measured on the first true leaf on each plant between 10:00 and 15:00 h (Egyptian local time) every fourth day for 52 days using a steady-state portable IRGA (LI-COR 6200, LICOR, Lincoln, Nebraska, USA).

Sampling procedure: Leaf injury symptoms were assessed on 1st December 2002 (51 days after seeding) by counting the number of injured leaves and estimating the percentage of each leaf's area showing injury (on a score of 0 `no injury` to 5 `100% injury`).

Plants were sampled destructively on 2 December (52 days after seeding). The plants were divided into different organs, dried in an oven at 105°C for one week and weighed to assess crop growth.

Open-Top Chamber (OTCs): Seeds of the same variety of clover plants used in the field experiment were grown in clay pots of loamy clay soil in a glasshouse, with 25/15°C day/night temperature, there was no additional lighting. 15 days after seeding (when plants were at 3 leaf stage), plants were transferred to four OTCs of the design of Ashmore *et al.* (1986) housed in Abbis village (35 km south of Alexandria city). There were 54 pots per chamber. OTCs were used in a split plot design: Two chambers received charcoal Filtered Air (FA) and the others received Non-Filtered Air (NFA) for 51 days (09:00-17:00 Egyptian local time). Concentrations of different major air pollutants were measured (Hassan, 1999).

EDU and Benomyl were dissolved in warm tap water and then diluted with tap water to final concentrations of 50, 100, 150 and 200 mg EDU L^{-1} and 250, 350, 450 and 550 mg benomyl L^{-1}. The chemicals were applied as soil drench (150 mL/pot). There were 54 pots in each chamber; 24 pots in each chamber received EDU (6 pots for each concentration) and 24 pots in each chamber received benomyl (6 pots for each concentration). Pots did not receive benomyl or EDU received an equal volume of water.

The OTC experiment was running simultaneously with the field experiment and OTCs were adjacent to plots of the field experiment.

Gas exchange measurements: Photosynthetic rates (A) and stomatal conductance (g_s) were measured on the first true leaf on each plant between 10:00 and 15:00 h (Egyptian local time) every third day for 51 days using a steady-state portable IRGA (LI-COR 6200, LICOR, Lincoln, Nebraska).

Visible injury and destructive harvest: Number of injured leaves and degree of injury were assessed on each leaf on each plant after 50 days of filtration prior to the destructive harvest.

Plants growth was assessed destructively at the end of the filtration (51 days after seeding). All plants were divided into main organs, dried in an oven at 105°C for one week and weighed to assess crop growth.

Data analysis: Data were subjected to three-way ANOVA based on chamber means in the case of OTCs experiment, using O3, EDU and benomyl as factors and two-way ANOVA based on subplot means in the case of field experiment, using EDU and benomyl as factors. Light intensity (PPFD) was used as a covariate in ANOVA of gas exchange measurements, while no covariates were used in ANOVA of leaf injury and growth parameters. Data were log-transformed prior to analysis to ensure that they were normally distributed.

RESULTS

Air quality: Air quality at the experimental site was characterised by very low concentrations of SO_2 and NO_x, where the mean 6-h concentration of these gases were 11.5 nl L^{-1} each (Table 1).

O_3 concentration was averaged to be 88 nl L^{-1} (8 h d^{-1}), with hourly maximum ozone values reached 105 nl L^{-1} at least twice during the experimental duration in the hottest sunny days.

Filtration efficiency was 86% for O_3 and 74 for SO_2 and NO_3.

Antioxidant field study: Treating plants with 150 ppm EDU or 550 ppm benomyl caused an increase in photosynthetic rates (A) of clover plants by 27 and 13%, respectively (Fig. 1). On the other hand, stomatal conductance (g_s) showed an increase in non-EDU and non-benomyl plants by 34% (Fig. 1).

There was no significant ($p \geq 0.05$) differences between effects of different concentrations of EDU and benomyl.

As the concentration of EDU used increased a better protection against O_3 damage to growth parameters was observed; a concentration of 150 ppm EDU caused increases in RDW, SDW, TDW and RSR by one fold,

Table 1: Effects of EDU or benomyl on growth parameters under Egyptian field conditions

Treatment	SDW (g)	RDW (g)	RSR (g)	TDW (g)
Control	0.35a	0.07a	0.20a	0.42a
50 ppm EDU	0.40b	0.11b	0.27b	0.51b
100 ppm EDU	0.57c	0.15c	0.26b	0.72c
150 ppm EDU	0.61d	0.20d	0.33c	0.81d
200 ppm EDU	0.61d	0.21d	0.34c	0.82d
250 ppm benomyl	0.39b	0.10a	0.26b	0.49b
350 ppm benomyl	0.46b	0.12b	0.26b	0.58c
450 ppm benomyl	0.63c	0.17c	0.27b	0.80d
550 ppm benomyl	0.62c	0.18c	0.29b	0.80d

Means not followed by the same letter are significantly different from each other at $p \leq 0.05$

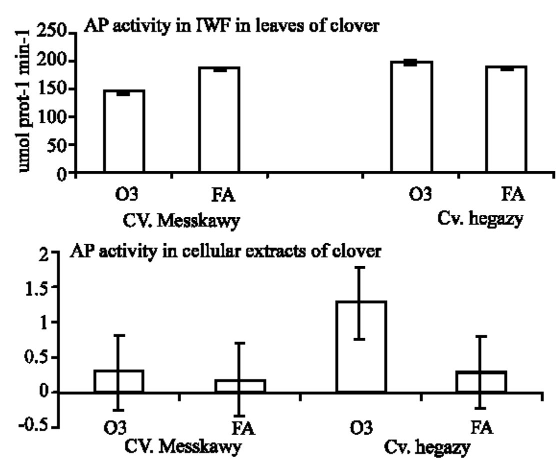

Fig.1: Increase in photosynthetic rates (A) of clover plants

Table 2: Effects of EDU or benomyl on visible injury symptoms under egyptian field conditions

Treatment	No. of injured leaves	Degree of injury
Control	1.98d	1.67d
50 ppm EDU	1.56c	1.33c
100 ppm EDU	1.02b	0.93b
150 ppm EDU	0.45a	0.37a
200 ppm EDU	0.42a	0.40a
250 ppm benomyl	1.85d	1.55d
350 ppm benomyl	1.47d	1.20c
450 ppm benomyl	1.18c	0.95b
550 ppm benomyl	0.79ac	0.83b

Table 3: Effects of EDU and benomyl on visible injury symptoms in OTCs

Treatment	Number of injured leaves	Degree of injury
FA-antioxidant	0.36a	0.39a
FA+50 ppm EDU	0.39a	0.38a
FA+100 ppm EDU	0.35a	0.36a
FA+150 ppm EDU	0.31a	0.32a
FA+200 ppm EDU	0.38a	0.32a
FA+250 ppm benomyl	0.37a	0.40a
FA+350 ppm benomyl	0.36a	0.38a
FA+450 ppm benomyl	0.34a	0.38a
FA+550 ppm benomyl	0.33a	0.37a
NFA-antioxidant	1.89d	2.01c
NFA+50 ppm EDU	1.21c	1.83c
NFA+100 ppm EDU	0.91b	1.05b
NFA+150 ppm EDU	0.38a	0.45a
NFA+200 ppm EDU	0.39a	0.42a
NFA+250 ppm benomyl	1.61d	1.89d
NFA+350 ppm benomyl	1.30c	1.38c
NFA+450 ppm benomyl	1.00c	1.02b
NFA+550 ppm benomyl	0.65b	0.75b

45, 29 and 62%, respectively (Table 2). However, treating plants with 200 ppm EDU did not cause any significant change compared to 150 ppm and the effects of both concentrations were comparable.

Furthermore, Table 2 shows that treating clover plants with benomyl gave a protection against damaging effects of O_3 and again as the concentration of antioxidant chemical used increased the degree of the protection increased. Nevertheless, applying benomyl at a concentration of 550 ppm (the actual concentration used by farmers in agricultural practice in Egypt) gave better protection than the other concentrations of the same chemical used in the present study. It caused increases in RDW, SDW, TDW and RSR by 75, 47, 25 and 60%, respectively (Table 2). However, EDU gave better protection than benomyl.

Visible injury symptoms appeared as flecking on the upper surfaces of clover leaves.

Exposure to ambient air caused increases in number of injured leaves and degree of injury 4 and 5 fold, respectively (Table 3).

The extent of injury was significantly reduced by application of EDU or benomyl. Again, as in growth parameters, as the concentration of the antiozonant chemical used increased the degree of protection against

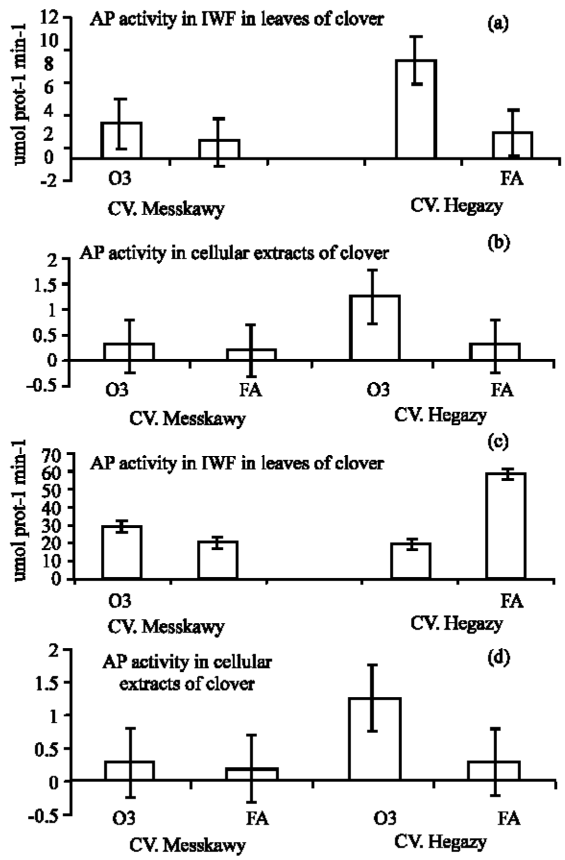

Fig. 2: Increase in g_s of clover plants

protection against foliar injury while 550 ppm benomyle decreased degree of injury and number of injured leaves by 62 and 34%, respectively (Table 3).

Filtration study: Exposure to NFA caused increase in g_s of clover plants by 29%, while it caused a reduction in A by 30% (Fig. 2).

Moreover, plants treated with either EDU or benomyl, either with or without filtration showed no significant differences in A and g_s from the control plants (Fig. 2).

The number of injured leaves and the degree of injury were decreased by 4 and 5 fold, respectively due to treatment with 150 ppm EDU, while application of 550 ppm EDU caused increase by 2 fold in both parameters (Table 3).

As in the field, 200 ppm EDU gave the same magnitude of protection against O_3 as 150 ppm EDU.

Table 3 shows that plants treated with either EDU or benomyl caused a protection against detrimental effects of O_3, as plants treated with wither EDU or benomyl showed an increase in growth parameters measured in the O_3 damage increased with 200 ppm EDU giving full

Table 4: Effect of EDU or benomyl on growth parameters in OTCS

Treatment	SDW (g)	RDW (g)	RSR (g)	TDW (g)
FA-antioxidant	0.85d	0.24c	0.28a	1.09d
FA+50 ppm EDU	0.91d	0.25c	0.27a	1.16d
FA+100 ppm EDU	0.89d	0.25c	0.28a	1.14d
FA+150 ppm EDU	0.89d	0.27c	0.30a	1.16d
FA+200 ppm EDU	0.90d	0.26c	0.29a	1.16d
FA+250 ppm benomyl	0.83d	0.24b	0.29a	1.07d
FA+350 ppm benomyl	0.86d	0.23b	0.27a	1.09d
FA+450 ppm benomyl	0.85d	0.26c	0.30a	1.12d
FA+550 ppm benomyl	0.88d	0.26c	0.29a	1.12ad
NFA - antioxidant	0.28a	0.09a	0.32a	0.37a
NFA+ 50 ppm EDU	0.35a	0.11a	0.31a	0.46a
NFA+100 ppm EDU	0.45b	0.16b	0.36b	0.61b
NFA+150 ppm EDU	0.61c	0.22c	0.36b	0.83c
NFA+200 ppm EDU	0.62c	0.19c	0.30a	0.81c
NFA+250 ppm benomyl	0.30a	0.10a	0.33a	0.31a
NFA+350 ppm benomyl	0.39a	0.13a	0.33a	0.52a
NFA+450 ppm benomyl	0.44a	0.15a	0.34a	0.59b
NFA+550 ppm benomyl	0.58b	0.21b	0.36b	0.79c

present study with EDU having better protection than EDU. Moreover, as the concentration of applied antiozonant chemical increased. The degree of protection increased.

One hundred and fifty ppm EDU caused significant increases in RDW, SDW, TDW and R SR by 2 fold, 74, 65 and 93%, respectively (Table 4). However, growth parameters increased due to application of 50 and 100 ppm EDU but at a lower extent than 150 ppm. There was no significant difference between the effects of 150 ppm and 200 ppm EDU on these parameters (Table 4).

Five hundred and fifty ppm EDU caused increases in RDW, SDW, TDW and RSR by 2 fold, 71, 50 and 82%, respectively and this is consistent with visible injury symptoms.

DISCUSSION

Filtration efficiency is comparable to the results of Weigel *et al.* (1987) who reported that filtration efficiency for O_3, in their study on impact of filtration of air on wheat, was 87 and it was 86% in our study. However, the filtration efficiency in our study was higher than that reported by Zhang *et al.* (2001) who reported ca. 50% filtration of air on native species.

The measurements in the present study were carried out during winter season (October-December 2003), ozone levels are likely to be higher during summers when daylight periods are longer, with higher temperatures and irradiance. There is evidence that the levels of ambient O_3 in Egypt are higher during summer times than during winters. Thus the adverse effects of O_3 on crops in Egypt may be greater in the summer seasons. However, during summer periods, crops may experience drought stress, an important environmental factor altering response of plants to O_3 (Hassan *et al.*, 1999). However, this crop grows from October to December and this is the winter season in Egypt and there is no possibility that this plant may suffer drought stress.

However, low concentrations of NO_x and SO2 were recorded in the present study and this furthers our early suggestion that it is unlikely that SO_2 and NO_2 were present in any significant concentration at this rural site (Hassan *et al.*, 1995).

The use of OTCs allowed an assessment to be made of the effect of the antiozonant chemicals (EDU and benomyl) on the Egyptian clover in the presence of filtered and non-filtered air. OTCs experiment clearly indicated that EDU and benomyl provided protection against visible injury against O_3, as suggested, in a variety of plants, by Manning *et al.* (1973, 1974), Hassan *et al.* (1995), Varshney and Rout (1998). Moreover, OTCs clearly indicated that EDU and benomyl had no toxic effects on growth and physiology of clover plant when applied to plants grown in clean air, this finding is in agreement with our previous research, which has reported an absence of EDU-phytotoxicity in radish and turnip plants when fumigated with 80 nl L^{-1} O_3 in OTCs (Hassan *et al.*, 1995). Moreover many other studies have reported absence of EDU and benomyl toxicity in many crop species including clover (Manning *et al.*, 1973, 1974; Toivonen *et al.*, 1982; Clark *et al.*, 1984; Brennan *et al.*, 1990; Velissariou and Kyrazi, 1996).

Our results, especially field experiment, are similar to that of Velissariou and Kyrazi (1996) who reported that EDU caused an increase by about 90% in biomass of an Egyptian variety of clover (*Trifolium alexandrinm* L. cv. Lito), which is of the same size of the effect of EDU in our study, while benomyl in their study gave a better protection that it did in the present study. This may be due to the cultivars used, O_3 concentration (was 64 nl L^{-1} in their study with AOT40 = 18010, while O_3 concentration in the present study was 88 nl L^{-1}, with AOT40 = 19968). EDU and benomyl did not affect stomatal conductance as treated plants had the same gs as control and this indicated that induced tolerance due to theses chemicals is biochemical in nature (through mediation of some antioxidative systems inside plant tissues) and not biophysical (due to stomatal closure) and this in agreement with the results of Ommo *et al.* (1994). Hassan *et al.* (1995) who reported that EDU did not alter the stomatal conductance (gs) in white clover and radish plant, respectively.

The results of OTCs support, confidently, that the differences between antiozonant treated-plants and control plants in field can be ascribed to effect of ambient O_3.

Both OTC and field experiment showed that Egyptian clover is very sensitive to visible injury for O_3. Growth parameters measured in both experiments showed the same trend as visible injury, whether due to non-filtered air in OTCs experiment or a lack of EDU or benomyl in the filed experiment.

A short-term critical level (AOT40) for injury development of 700 ppb-h O_3 has been suggested by Fuhrer and Achermann (1994). The maximal AOT40 for three consecutive days prior to the first observation of injury in the present study was 1152 ppb-h. Thus, our results clearly indicated that this short-term critical level does not always protect clover plants against O3-induced injuries and this in agreement with the results of Tonneijk and Van Dijk (1996) and Pihl Karlsson *et al.* (2003) on subterranean clover.

Photosynthetic rates (A) were found to decrease in OTCs due to NFA and in the filed due to untreating with antiozonant chemical. This provides further confidence that effects observed in the filed were due to O_3. Moreover, the percentage reduction in A and growth parameters were higher in the present study than other studies which indicated that Egyptian clover may be more sensitive than western cultivars that used in ICP-programme Therefore, it is worth in a future study to investigate the variation among different cultivars of clover, including Egyptian one, to identify the most sensitive cultivar to be used as a bio indicator worldwide. One possible explanation of the greater sensitivity of Egyptian clover is provided by the observation that O_3 was found to increase the stomatal conductance (g_s) which is considered detrimental as it would increase the O_3 flux into the leaf, with subsequent disturbance to physiology and biochemistry, which ultimately reflected in growth and yield.

The present study provided further confidence that EDU and benomyl suppress the detrimental effects of O_3 and can be used to assess the impact of O_3 on crops under remote field sites, where there are technical problems and limitation of controlled exposures and OTCs (Manning, 2000). Moreover, these chemicals can be used without interfering with plant growth itself.

Further work is urgently needed to quantify the impact of O_3 on other important crops in Egypt. While the construction of OTCs would provide an ideal approach to this, this study has demonstrated that EDU and benomyl provide valuable tools to estimate the impact of O_3 on crops in Egypt.

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