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IMPACT OF AMBIENT AIR ON PHYSIOLOGY, POLLEN TUBE GROWTH, POLLEN GERMINATION AND YIELD IN PEPPER (*CAPSICUM ANNUUM* L.)

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Abstract

Pepper (*Capsicum annuum* L.) plants were exposed in open top-chambers to non-filtered ambient air (NFAA) and to charcoal filtered air (FA) to study the effect of ambient air on physiological parameters, growth, yield, as well as pollen tube and pollen growth of plants. NFAA caused reductions in net photosynthetic rates (19%), stomatal conductance (26%), yield components (29% and 25% losses in fresh weight and number of fruits/plant, respectively) as well as degradation of epicuticular wax of its leaves. Moreover, pollen collected from plants grown in NFAA showed lower germination rates and reductions in pollen tube length (41 and 10%, respectively). Our results showed that detrimental effects of O3 on reproductive growth and development are compromising current crop yields and the fitness. Fresh weight of pods was reduced by 29% due to exposure to NFAA. The significance of pollutant-induced impairment of pollen germination and growth for reproductive development are discussed. To the best of our knowledge, this is the first report demonstrating the marked reduction in pollen germination rates and pollen tube length and their significance to crop physiology and yield from the environment in the developing world.

Introduction

Chronic exposure to O_3 reduces photosynthesis and stomatal conductance in leaves, leading to reduced biomass production and reproductive output in crops (Wang & Mauzerall 2004; Ashmore 2005; Ainsworth, 2008; Emberson *et al*., 2009; Feng & Kobayashi, 2009, Hassan, 2006; 2010; Feng *et al*., 2008, 2010; Leisner & Ainsworth, 2012). Tropospheric O_3 concentrations are also important, where significant variations in global climate are projected in the near future (Leisner & Ainsworth, 2012). Richards *et al*., (1958) first demonstrated that tropospheric O_3 is phytotoxic. Since then, concentrations of tropospheric O_3 have shown an increasing trend (Vingarzan, 2004; Ohara *et al*., 2007).

Reproductive development is a critical phase in the life cycle of most plants (Boascc *et al*., 1993, 1994, 1998). Impairment of any step in the developmental sequences involving pollination, fertilization and seed development would have potentially serous implications for both seed quality and quantity in agricultural crops, and competition and species diversity in natural and semi-natural communities, particularly if germinability or seeding vigour were to be similarly affected (Black *et al*., 2000; Kabir *et al*., 2012).

The effect of O_3 on plant reproductive development was reviewed more than a decade ago (Black *et al*., 2000), but recent quantitative assessments have focused on the influence of O_3 on vegetative growth and development or crop yield (Morgan *et al*., 2003; Ainsworth, 2008; Feng *et al*., 2008; Feng & Kobayashi, 2009; Wittig *et al*., 2009; Feng *et al*., 2010). The complex nature of the effects of O_3 on vegetative and reproductive structures, the range of compensatory mechanisms available to plants with different reproductive growth habits, the dependence of plant developmental stage on the level of sensitivity and the consequences of additional environmental stresses make it difficult to generalize the effects of O_3 on reproductive development (Black *et al*., 2000). Yet, understanding the effects of O_3 on reproductive development has significant agronomic and ecological consequences, including securing future food (Leisner & Ainsworth, 2012).

Pollen germination and pollen tube growth are crucial phases in reproductive development since if these processes are impaired, fertilization may fail and, as a consequence, seed set and crop yield may be reduced (Rao *et al*., 1997). Reductions in pollen germination and pollen tube growth have been reported previously following exposure to O_3 and SO_2 in some species but not pepper (Masaru *et al*., 1976; Feder *et al*., 1982; Dubay & Murday, 1983). However, neither the degree of damage nor the sites and modes of action have been fully elucidated, although previous studies suggested that specific environmental and pollution regimes used may influence the responses observed (Varshney & Varshney, 1981; Cox, 1984).

The susceptibility of reproductive processes to environmental stresses such as drought and high temperatures is well established (*e.g*. Saini & Aspinall, 1982, Rao *et al*., 1997), but the impact of air pollutant(s) has only been recognized relatively recently (Dubay & Murdy, 1983, Cox, 1984). Most previous studies have involved the simultaneous exposure of both vegetative and reproductive components of the plant to pollutants, making it extremely difficult to separate the direct effects on reproductive structures from indirect effects mediated by injury to the vegetative organs. In contrast, the present study has focused on the direct effects of air pollution on reproductive development, yield, and fruit quality in pepper (*Capsicum annuum* L.). Pepper was chosen for the study because it is a major crop in rural areas, whose main period of flowering frequently coincides with air pollution episodes. It is phenotypically well suited to experimental manipulation and selective exposure of its reproductive structures to pollution since their inflorescences are held well above the vegetative parts of the plants. Furthermore, each inflorescence includes a range of floral stages extending from mature buds to fertilized flowers, thereby enabling a range of

developmental stages to be exposed to any one pollution event. The effect of air pollution on pollen, have not been reported in developing countries although many studies were carried out in the developed world (e.g. Feder, 1986; Harrison & Feder, 1974; Bosac *et al*., 1993; 1998; Leisner & Ainsworth, 2012)

The aim of the present investigation was to assess the impact of ambient air pollution on some physiological parameters, growth, yield, as well as pollen tube and pollen growth of pepper plants (*Capsicum annuum* L. cv Masri) *In vivo*.

Materials and Methods

Plant material: Seeds of *C.* annuum cv. Masri were sown directly in the field at Al Montazah Botanical garden under open top fumigation chambers (OTCs) on 20/6/2011 and lasted for 83 days (details of exposure and design of the chambers discussed elsewhere, Hassan, 1999b; 2006). Plants were thinned to one seedling per lot after emergence of first true foliage leaf (10 days after sowing). There was no fertilizers or pesticides applied.

Anthesis within individual plants was taken as occurring on the day on which the first flower opened fully. This took place *c*. 11 weeks after sowing.
Pollen was collected by holding the flowers above a ceramic plate and flicking all anthers gently with a steel needle.

Experimental design: Plants were exposed to charcoal-filtered air (FA) or non-filtered air (NFAA) in 6 open-top chambers (OTCs) in a split plot design: three chambers received charcoal-filtered air (FA) and the other three received NFAA (8h d^{-1} between 09:00 and 17:00 for 75d) (Hassan, 1999*a*).

Major air pollutants were monitored regularly over the entire experimental period, and it was found that the major pollutant prevailing at the experimental site is O_3 while other gaseous pollutants (NO_x and SO_2) are present at concentrations lower than the critical threshold (Hassan, 1999*b*).

Visible injury symptoms and destructive harvest: Foliar injury symptoms were assessed carefully, 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') (Taylor *et al*., 1990).

Physiological parameters: Net Photosynthetic rates (A) (μmol m^{-2} s^{-1}) and stomatal conductance (g_s) (mmol m^{-2} s^{-1}) were measured twice a week throughout the experimental period (12 weeks) using a portable infrared gas analyser (IRGA) Model Licor-6200.

Pollen germination and pollen tubes growth: Measurements were made using both light microscope and SEM. Counts of percentage germination were made on 240 grains from all OTCs. Pollen were collected as described by Harrison and Feder (1974) and inoculated into 5 replicates. 100 μl droplets of culture medium (with 17% sucrose) per treatment dispensed into the walls of 15 cm diameter cavity slides (Brewbaker & Kwack, 1963). The slides were then placed in humid culture chambers comprising petri dishes containing a moistured square of tissue paper. These were placed in a growth cabinet at 20°C and incubated overnight before examining the pollen 19 h later (Bosac, 1992, Bosac *et al*., 1993). The lengths of six most central pollen tubes in each of six random fields of view were measured.

Samples were examined using Philips CM11 Scanning electron microscope (Philips, Eindhoven, The Netherlands) operating at 15 KV.

Scanning electron microscopy (SEM): Leaves and flowers were collected from both treatments at the end of the experiment (12 weeks). Ten leaves were prepared for examining with SEM according to Hassan *et al*., (1994), while anthers of twenty flowers were prepared according to Harrison & Feder (1974).

Statistical analysis: One – way ANOVA was applied to log – transferred data. A student's *t* test was used to test for significant treatment effects.

Results

Air quality at the experimental site was characterised by relatively low concentrations of SO_2 and NO_x, where the mean 6-h concentrations of these gases through the four growing seasons were 19 and 14 nl l^{-1}, respectively (Table 1). The mean 8-hour concentrations of ambient O_3 through the growing seasons averaged 78 nl l^{-1} (Table 1). The AOT40 (accumulated ozone concentrations over 40 ppb) was 29600 nll^{-1}. Table 1 also, showed that filtration efficiency was about 80% as O_3 was reduced to 15 nl l^{-1}.

Table 1. Seasonal 8-h (09:00 – 17:00 h Egyptian Local Time) daily average of O_3, SO_2 and NO_x concentrations ±1 SE.

Treatment	O_3 (nl l^{-1})	AOT40	SO_2	NO_x
Control (charcoal filtered) FA	15 ± 3	00	18 ±2	11 ± 2
Ambient (non-filtered) NFAA	78 ± 8	29600 ± 42	19 ± 3	14 ± 2

Exposure of plants to NFAA caused overall reductions in net photosynthetic rates (A) and stomatal conductance (g_s) over the entire period of the experiment (19 and 26%, respectively) (Fig. 1). These reduction in physiological parameters were reflected on reductions in fresh weight of fruits, their number and their length (29and 25%, respectively) (Table 2).

Table 2. Effect of filtration on growth and yield of pepper (*Capsicum annuum* L.) means not followed by the same letter are significantly different from each other at p≤0.05.

Parameter	FA	NFAA
Leaf length (mm)	82.5^a	75.0^a
Leaf width (mm)	35.0^a	32.0^a
Petiole length (mm)	44.5^a	42.5^a
Number of injured leaves	1.8^a	7.6^b
Degree of injury	19^a	34^b
Fruit length (mm)	94.5^b	54.8^a
Fruit weight (gm)	11.9^b	8.5^a
Number of fruits/plant	53^b	38^a
Fruit colour	Bright green	Dark green

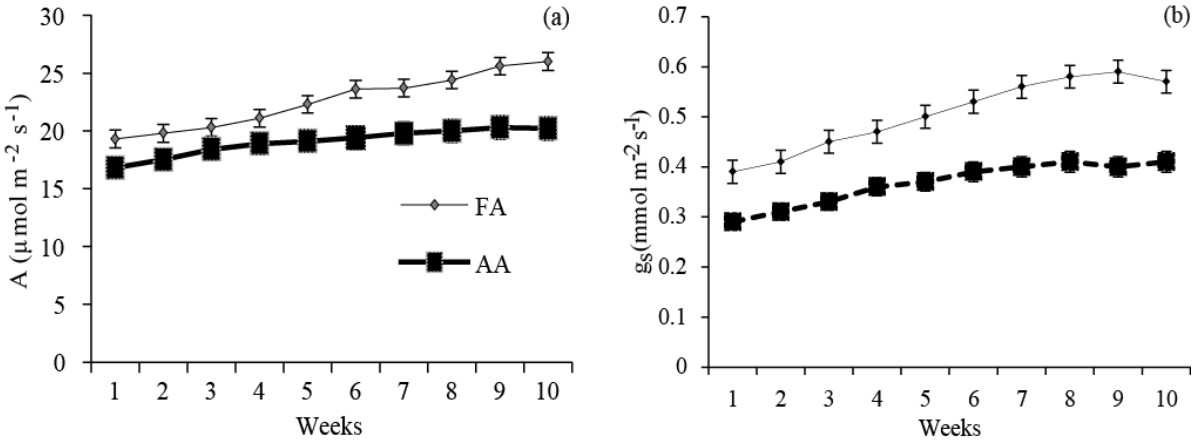

Fig. 1. Effects of air filtration on photosynthetic rates (A) and stomatal conductance (g_s). (n = 5 ± 1 SE bars).

Visible injury was observed on the leaf adaxial surface of plants grown in NFAA as small chlorotic and necrotic spots 25 days after sowing. NFAA caused increase in number of injured leaves by 4-folds, while degree of injury was increased by 78% due to exposure to NFAA (Table 2). Moreover, exposure to NFAA in *vivo* caused a reduction in pollen germination and pollen length by 41 and 10%, respectively (Table 3).

Table 3. *In vivo* pollen germination and pollen tube length (values are means ± 1 SE). FA = filtered air, AA = ambient non-filtered air.

Parameter	FA	NFAA
Pollen germination (%)	20.2 ± 2.18	11.8 ± 2.24
Pollen tube length (µm)	36.9 ± 3.12	33.1 ± 2.78

SEM revealed that O_3 caused degradation of epicuticular wax (Fig. 2), while anthers collected from plants grown in chambers supplied with AA had longer lengths (60 mm) compared to those collected from plants grown in chambers supplied with FA (43mm) (Fig. 3) However, Fig. 4 showed that pollen grains collected from plants exposed to NFAA had denser exine and delayed germination, while those collected from plants grown in chambers supplied with FA had smoother exine and pollen grains germinated more readily.

Discussion

The concentrations of O_3 recorded in the present study further a support for previous results (Hassan *et al*., 1995) that suggested, it is unlikely to have SO_2 and NO_x in a rural site in Egypt, which has been supported recently (Hassan, 1999a, 2006). Moreover, the results of the present investigation are in agreement with the results of Anjea *et al*., (1992) who reported relatively high levels of O_3 in a rural site in the USA and Schenone & Lorenzini (1992), who had similar results in Italy.

It seems very reasonable to conclude that ambient O_3 played a major role in causing the observed alterations in physiological, morphological and reproductive parameters examined in plants exposed to non-filtered ambient air (Prather *et al*., 2001; Meehle *et al*., 2007).

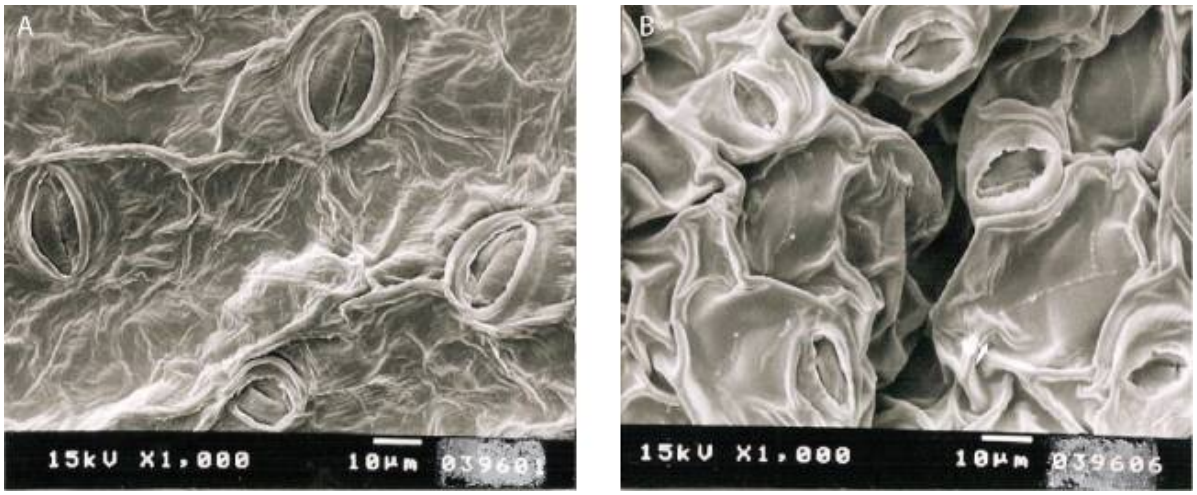

Fig. 2. Scanning electron micrograph of epidermal leaves. (a) Epidermal cells collected from plants grown in NFAA, showing changes and damage to surface morphology and degradation of epicuticular wax, (b) Epidermal cells collected form plants grown in FA (X1000).

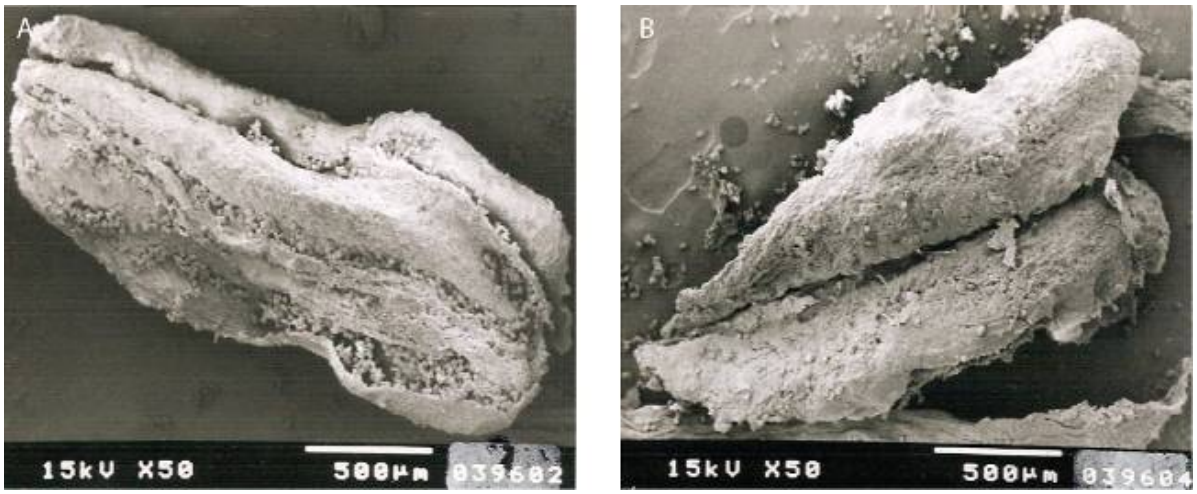

Fig. 3. Scanning electron micrograph of anthers. Anthers collected from NFAA (a) and those collected from FA (b) (X50).

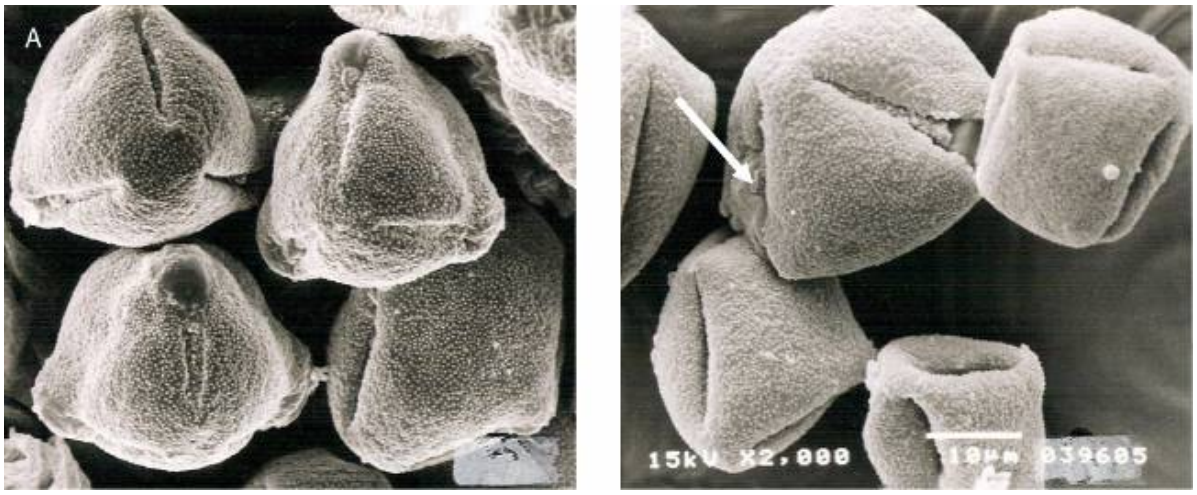

Fig. 4. Scanning electron micrograph of pollen grains. Pollens collected from NFAA had more granular and denser surface (a) while those collected from FA (b) have relatively smoothers surface (X2000). An arrow in (b) showed starting of germination in pollens of plants grown in FA.

Experimental site is characterized by having high concentrations of ambient O_3, while other gaseous pollutants are below the known levels to cause any harm effects. The results clearly demonstrate an inhibitory effect of exposure to ambient O_3 on the germination of pollen grains and pollen tube extension was also reduced, and this indicated sensitivity of the plant to ozone and this is in agreement with the results of Krug (1990), who stated that exposure of Picea plants to SO_2 caused similar effects on pollen grains. These reductions in pollen germination were reflected in lower yield as demonstrated by a reduction in both fresh weight and number of fruits. Reduction in stomatal conductance would reduce CO_2 influx and hence reduced photosynthetic rates that are also reflected in reductions in growth and yield (Mukhtar *et al*., 2013). In their thorough review (Black *et al*., 2000), they stated that O_3 has direct effects on reproductive structures, namely stylar and stigmatal surfaces, pollen, and anthers. Recently (Leisner & Ainsworth, 2012) provided a quantitative and comprehensive assessment of O_3 effects on reproductive growth and development using meta-analysis for analysis, and the log response ratio to estimate the effect of O_3 on reproductive growth of different crops. They supported the earlier findings of Black *et al*., (2000).

While the response of pollens grown in culture cannot be directly extrapolated to those occurring *In vivo* on the plant, it can be postulated that under dry conditions and with the protective influence of the anthers and other floral parts, pollen grains may be shielded from the full impact of O_3 or other pollutant. It is also possible that concentrations or in combination with other gaseous pollutants or environmental stresses which predispose the plant to injury. Benoit *et al*., (1983) indicated that moist pollens of *Pinus strobus* were more sensitive to O_3 than dry ones, as presence of moisture brings pollens into contact with the dissolved products of pollutants. However, O_3 is very much less soluble in water than other pollutants (Tingey & Taylor, 1982) and the presence of moisture might act as a barrier to O_3, reducing its access

to pollen or preventing the formation of toxic secondary products and thereby minimizing or eliminating their adverse effects (Wolters & Martens, 1987). In contrast, under dry conditions significant fluxes of O_3 may reach sensitive sites and promote deleterious effects on a time of day when pollen is maturing and being shed.

Nevertheless, the collected data must be treated with cautions as the development of pollens from immature to mature stage occurs over a period of several hours within individual flowers. Even though, care must be taken to ensure the pollens were collected only from flowers which had recently begun to shed pollen, the pooled sample of pollen collected from several flowers at any one time may nevertheless have contained individual grains of differing maturity and hence potential sensitivity (Perveen & Ali, 2011).

Williams *et al*., (1986) have shown that yields of oilseed rape were increased when plants were shaken to stimulate wind pollination, indicating that insufficient pollen was transferred to the stigmas for maximum seed set in still air. Thus, the quantity of pollen available for fertilization may be limiting, at least under some conditions. Any additional pollutant-induced impairment of pollen viability might be critical for fertilization and seed set, particularly in synchronously flowering species where all flowers would be exposed simultaneously and at a uniform stage of development to specific pollutant event (Khattak & Khattak, 2011). Further work *in vitro* is urgently needed to test weather plants exhibit different sensitivity due to different treatments.

Conclusions

Ambient O_3, in the present study, negatively affected almost all the response variables studied, indicating its detrimental effects on vegetative growth and development. This study highlighted significant gaps in our knowledge. Namely, little is known about changes in carbon allocation to fruiting and flowering structures under ambient O_3, nor is there much information on changes in assimilate partitioning in maternal reproductive structures, or the mechanisms by which this occurs (for vegetative carbon allocation). This warrants further investigation. Such studies are critical to understanding the compound effects that O_3 can have on complex traits, such as seed weight and yield.

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