The International Journal of Plant Reproductive Biology 8(2) July 2016, pp.186-196
DOI 10.14787/ijprb.2016 8.2.186-196

Impact of tropospheric O_3 on reproductive growth and development of plants-A Review

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Received : 02.09.2015; Revised: 10.09.2015; Accepted and Published on line: 01.07.2016

ABSTRACT

Tropospheric ozone (O_3) is a harmful secondary air pollutant which negatively affects plant growth and development. Present O_3 concentrations cause reductions in crop yields. Numerous studies have shown indirect effects of O_3 to the vegetative organs and associated changes in the supply of assimilates and other essential resources to support reproductive growth, which is a crucial stage in the life cycle. However, there are limited studies reporting on direct effects of O_3 on reproductive development. So the present review focuses on quantification of impact of O_3 on reproductive growth of crops, trees and native species. The studies have shown changes in anther development, reductions in pollen viability and pollen tube germination. Reductions in number of floral sites, number of flowers and inflorescence, number of ovules per capsule and increase in numbers of abortive reproductive sites leading to reductions in yield attributes and crop yield. Reproductive development of both C_3 and C_4 plants is found to be sensitive to O_3. Indeterminate crops have more ability to compensate against O_3 stress compared to determinate crops and thus maintain their yield. It is difficult to generalize the effects of O_3 on reproductive development due to the complexity in the nature of the effects of O_3 on vegetative and reproductive structures. Compensatory mechanisms in plants with different reproductive growth habits and the dependence of plant developmental stages modify the level of sensitivity.

Keywords : Ozone, anthers, ovules, seeds, C_3 & C_4, crops

Tropospheric O_3 formation is a natural phenomenon, but its concentrations in the troposphere have substantially increased from 10- 15 ppb before industrial revolution to present day values of 30- 40 ppb, with steepest rise after 1950 (Vingarzan 2004, Parrish *et al*. 2012). O_3 concentrations are rising at a rate of 0.3 ppb $year^{-1}$ (IPCC, 2013). Current O_3 concentrations are considerably higher in the Northern hemisphere than the Southern hemisphere, with background monthly mean O_3 in the former hemisphere ranging from 35-50 ppb (Ainsworth *et al*. 2012).

It is a well known fact that O_3 causes foliar injury, reductions in plant growth, productivity and biomass accumulation and changes in crop quality (Krupa and Kickert 1989, Morgan *et al*. 2003, Ashmore 2005, Fiscus *et al*. 2005, Emberson *et al*. 2009, Broberg *et al*. 2015). Numerous studies have shown that ambient levels of O_3 are sufficient enough to reduce crop yield (Burney and Ramnathan 2014). The impact of O_3 varied between different crops and their cultivars. The sensitivity to O_3 may differ not only between species, but also between cultivars and population of individual species. The impact of O_3 exposure is highly dependent on its concentration duration and timing of exposure. It has been documented that frequency of occurrence of higher O_3 concentrations coincides mostly with flowering time of most of the crops causes more negative on yield (Rai and Agrawal 2008, Black *et al*. 2012, Leisner and Ainsworth 2012).

Sexual reproductive development is a crucial stage in the life cycle of higher plants as any impairment of the processes involved might have significant implications for the productivity of crop plants and the survival of native species. Numerous studies have shown indirect effect of O_3 to the vegetative organs and associated changes in the supply of assimilate and other essential resources to support reproductive growth, but there are limited studies reporting on direct effect of O_3 on reproductive development. However, it remains unclear, what proportion of the decrease in reproductive output is caused by direct damage to reproductive processes, such as flower initiation, pollen and ovary development, and seed abortion, as opposed to damage to vegetative tissues that subsequently reduces assimilate availability to reproductive development. The present review focuses on evaluation of impact of O_3 on reproductive growth of crops, trees and native species.

Variations and future projections of O_3 concentrations— Tropospheric O_3 is a secondary air pollutant formed from the photochemical oxidation of nitrogen dioxides, methane, carbon monoxide and volatile organic compounds (VOCs) in the presence of sunlight. These reactions are principally controlled by sunlight and temperature. O_3 concentration peaks during the late morning and early afternoon hours. In the ambient air, O_3 precursors play important role during long range transport downwind from the sources. Polluted air masses from urban and industrial areas can affect suburban and rural areas even reaching to remote rural areas for considerable distances (Ainsworth *et al*. 2012).

In Northern America and Europe, higher O_3 concentrations occur in the summer, with high peak in the late afternoon. Very high concentrations of O_3 episodes occur in metropolitan areas or in more remote areas during heat waves, where O_3 levels range between 200- 400 ppb (Ainsworth *et al*.

2012) and even O_3 concentrations frequently exceed 60 ppb for several days. O_3 concentration above 90 ppb were experienced in countries such as the UK, Belgium, Netherlands, France, Germany, Switzerland and Italy, with the highest one hour value recorded as 180 ppb in Italy (EEA 2007). In 2011, O_3 concentrations exceeded EU's threshold of 90 ppb in 16 member states, whilst the alert threshold of 120 ppb was exceeded in Bulgaria, France, Greece, Italy, Portugal and Spain (ICP 2014).

In the present scenario, global photochemical modeling studies have shown reductions in peak surface O_3 concentrations in North America and Europe due to effective controls on NOx and VOCs over the past decades in response to the Clean Air Act in the United States and the Long Range Transboundary Air Pollution Convention and European Union targets in Europe. Secondary global photchemical model predicts that O_3 levels in Asia are going in an upward trend owing to continued rapid industrialization across the region. In rural areas of Indo- Gangetic plain tropospheric O_3 levels were 40- 50 ppb (Rai *et al*. 2015, Ghude *et al*. 2014). In China, O_3 concentration frequently exceeds 50- 60 ppb (Yuan *et al*. 2015).

Mechanism of action of O_3 toxicity—Tropospheric O_3 is a strong oxidant and causes oxidative stress within plants (Chutteng *et al*. 2015). O_3 movement into the intercellular spaces of the mesophyll is controlled mostly by stomatal aperture. The rate of O_3 penetration into the leaf and its capacity to tolerate O_3 induced reactive oxygen species (ROS) are the major control points of the downstream effects of O_3 on growth and yield. Within plant cells, O_3 reacts rapidly in the apoplast with a number of potential molecules to produce other ROS, including H_2O_2, superoxide radicals and NO (Ainsworth *et al*. 2012). After O_3 exposure, there is a need to tune the level of ROS produced to achieve a positive cell reaction through the signalling cascade without inducing uncontrolled cell death. As ROS are physiologically generated from various sources during cell metabolism, plants have evolved very efficient enzymatic and non- enzymatic antioxidant defence system, capable of detoxifying substantial amount of these reactive oxygen species (Chutteng *et al*. 2015). The antioxidant defence system plays a fundamental role in determining the cell fate, not only by keeping ROS level under control, but also acting as a central component of the cell redox balance and of the signalling modulation (Overmeyer *et al*. 2005).

The first line of defense against O_3 derived ROS is the apoplast, where ascorbate (ASC) is believed to provide important protection from the oxidative injury. The O_3 induced changes in apoplast ascorbate and redox state were first reported in 1996 (Ranieri *et al*. 2000). The antioxidant role played by ascorbate (ASC) depends mainly on the cell ability to maintain it in a reduced state and it occurs at the cost of reduced glutathione (GSH) by monodehydroascorbate reductase (MDHAR) or dehydroascorbate reductase (DHAR). Glutathione is generated by glutathione reductase (GR) at the expense of NADPH oxidation in Halliwell- Asada cycle. Among the tobacco cultivars Bel B and Bel W3 known for their differential sensitivity to O_3, reduction in the chloroplastic GR mRNA was recorded in Bel W3 at an exposure of 150 ppb O_3 for 5 h (Pasqualini *et al*. 2001). Ascorbate may act as reducing substrate for ascorbate peroxidase (APX), which is one of the most efficient ROS scavenging systems.

In the chloroplast, O_3-induced responses could directly or indirectly impair the light and dark reactions of photosynthesis (Fiscus *et al*. 2005). Different studies indicate that O_3 damages the photosynthetic machinery leading to a progressive loss in the amount as well as activity of ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO) (Agrawal *et al*. 2002, Cho *et al*. 2008). Exposure of Arabidopsis to acute O_3 leads to rapid transient decrease in stomatal conductance accompanied by a burst of ROS in the guard cells followed by a slower recovery to initial rates of stomatal conductance (Ainsworth *et al*. 2012). Long term chronic O_3 exposure at lower concentration also leads to irreversible decrease in stomatal conductance and impairment of photosynthetic rate and increased internal CO_2, which in turn lower stomatal conductance (Mishra *et al*. 2015). Early symptoms of O_3 exposure are decrease in rate of photosynthesis and RuBisco activity (Long and Naidu 2002).

Several studies have indicated that an early or primary response to O_3 in leaves is an interference with photosynthesis, carbohydrate metabolism, partitioning of photosynthetic products between mobile and stored pools in the leaf, and/or the translocation of photosynthate within the plants. Molecular studies have shown proteomics changes in RuBisCo content and other components of the photosynthetic machinery and Calvin Benson enzymes like RuBisCo activase, ATP synthase, the oxygen evolving subunit of photosystem II aldolase, phosphoglycerate kinase and NADP-glyceraldehydes 3-phosphate dehydrogenase, which affect primary metabolism and reduce photostynthate assimilation in wheat and rice (Sarkar *et al*. 2010; Cho *et al*. 2008). O_3 stressed plants have higher mitochondrial respiration in crops and trees (Gillespie *et al*. 2012).These effects are followed by accelerated senescence and decrease in leaf area. Decrease in carbon assimilation and alteration in carbon partitioning due to O_3 stress- induced metabolic pathways result in altered allocation and lower total biomass accumulation in plants (Cooley and Manning 1987, Gorissen and vonVeen 1988, McCrady and Andersen 2000).

Carbon availability reduces under O_3- stress in plants due to changes in primary metabolism, plant carbon balance affected by indirect costs associated with the detoxification of ROS generated by O_3 and the rate of regeneration of the reduced compounds. The metabolic changes can alter the source –sink reactions with decrease in root and reproductive biomass under chronic O_3 exposure (Black *et al*. 2007, Cooley and Manning 1988).

Effect of O_3 on reproductive processes—Tropospheric O_3 exposure induces a spectrum of direct effects on reproductive development and there are several points of interaction between O_3 and reproductive process (Fig. 1). These include modulation of pollen or ovule maturation, changes in the timing, rate or number of flowers produced and effects on seed and fruit development, yield, seed germinability and seedling vigour.

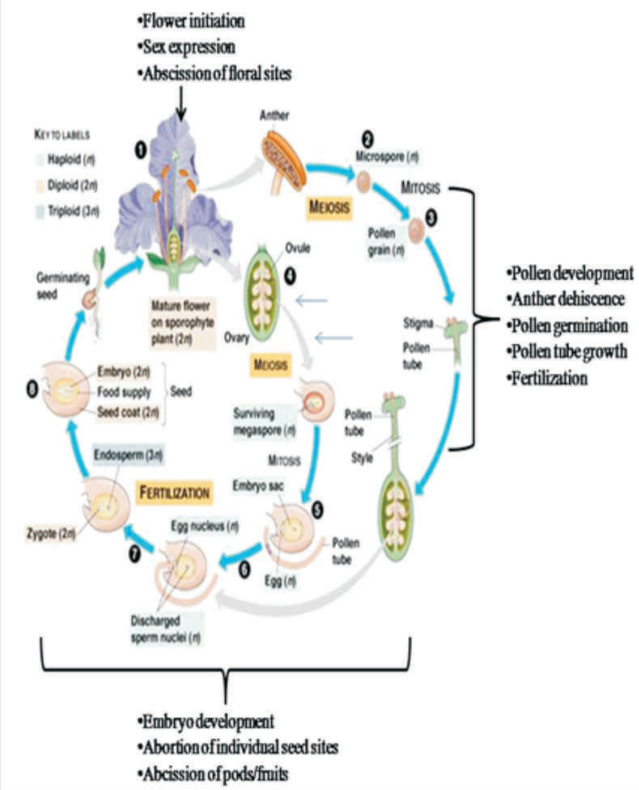

Fig. 1—Impact of ozone on processes affecting sexual reproduction in higher plants (Modified from Black *et al.* 2000).

Pollen germination and pollen tube growth—The availability of viable pollen and sufficient numbers of germinating pollen grains and the successful growth of the pollen tube to the ovule are of fundamental importance for sexual reproduction. O_3 induces reductions in number of viable pollen and florets in different crops (Fig. 2). Sarkar and Agrawal (2012, 2010) reported that total number of viable pollens and florets were affected significantly in wheat and rice cultivars under O_3 exposure in an open top chamber study. Similar findings were observed by Tripathi and Agrawal (2013) in mustard and Singh *et al*. (2014) in maize cultivars (Table 1). Ambient O_3 played a major role in causing the alteration in reproductive structure like anther length (Tai *et al*. 2013). Tai *et al*. (2013) observed longer length of anthers and denser exine of pollen of pepper (*Capsicum annum* L.) grown in open top chambers receiving 78 ppb of O_3 for 8 h d^{-1} compared to control. Differential sensitivity to O_3 with respect to pollen characteristics not only varied between species, but also within the species. Singh *et al*. (2014) reported lower number of male flowers in maize cultivars (HQPM and DHM 117) exposed to ambient (53.5 ppb) and elevated O_3 (68.5 and 83.5 ppb) from germination to maturity in open top chambers. Stewart (1998) found that a 6 h exposure of 120 ppb O_3 in *in-vitro* decreased pollen germination in *Plantago major* populations, Lullington and Sibton but no significant effect on germination of Penicuik.

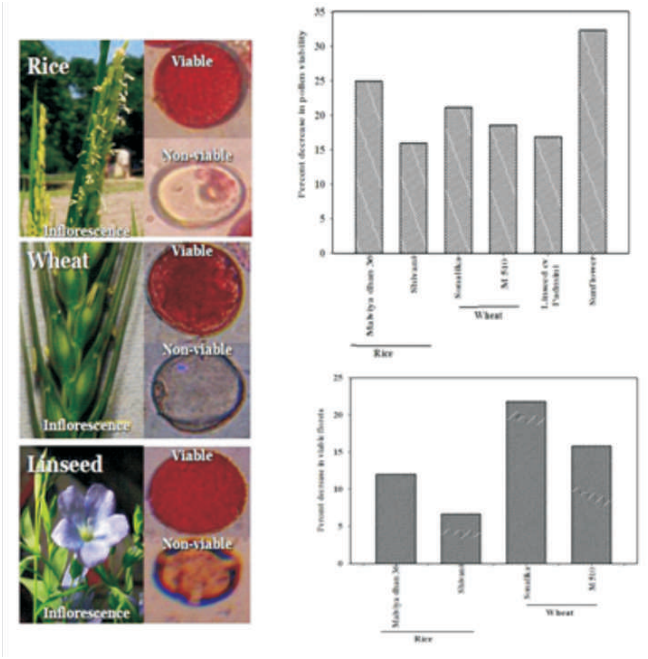

Fig. 2—Impact of O_3 on pollen viability and viable florets in wheat, rice and linseed (Source: Sarkar and Agrawal 2010, 2012, Tripathi and Agrawal 2013).

Exposure of O_3 affects pollen germination and pollen tube growth. The sensitivity of pollen to O_3 depends on O_3 exposure concentration and environmental conditions. Reductions in pollen germination and tube growth were observed in pollen of apple (*Malus domestica* L.), apricot (*Prunus americana* L.) and almond (*Prunus dulcis* L.) *in vitro* at 20, 40 and 60 ppb O_3 for 4 h. Feder (1981) showed similar response in tobacco and Petunia and suggested that pollen from these species might be used as marker (bioassay) in areas experiencing higher O_3 concentrations. However, *Brassica campestris* L. exposed to 100 ppb O_3 for 6 h *in vitro* showed no effect on pollen tube

Table 1—Effects of ozone on different reproductive processes of plants

Parameters	Plant	Effect	References
1. Pollen germination or Pollen tube growth	*Plantago major* L.	Decreased	Stewart (1998)
	Brassica campestris L	Decreased	Stewart (1998)
	Lolium perrene L.	Decreased	Schenone *et al,* (2004)
	Capsicum annum L.	Decreased	Tai *et al.* (2013)
Number of viable pollens	*Triticum aestivum L. cv* M 510 and Sonalika	Decreased	Sarkar *et al.* (2010)
	Oryza sativa L. cv Malviya dhan 36 and Shivani	Decreased	Sarkar *et al.* (2012)
	Linseed cv Padmini and T 39	Decreased	Tripathi and Agrawal (2013)
Number of viable florets	*Triticum aestivum L. cv* M 510 and Sonalika	Decreased	Sarkar *et al.* (2010)
	Oryza sativa L. cv Malviya dhan 36 and Shivani	Decreased	Sarkar *et al,* (2012)
Number of reproductive sites	*Brassica campestris* L.	Increased	Black *et al.* (2007)
Number of aborted reproductive sites	*Brassica campestris* L.	Increased	Black *et al.* (2007), Black *et al.* (2012)
Number of female flowers	*Zea mays* L.	Decreased	Singh *et al.* (2014)
Number of buds	*Brassica campestris* L.	Decreased	Black *et a*l. (2012)
Number of male flowers	*Zea mays* L	Decreased	Singh *et al.* (2014)
Number of flowers	*Brassica campestis* L.	Decreased	Singh *et al.* (2009)
	Glycine max L.	Decreased	Rai *et al.* (2015)

growth, but significant reduction was observed at 120 ppb, suggesting that the threshold concentration for damage may vary between different species.

Pollen germination and tube growth are interdependent processes, but exhibit differential responses to O_3 with respect to the pollen germination (Benoit *et al*.1983) or pollen tube growth (Riley and Feder 1974). Feder (1968) reported that mature tobacco pollen is particularly sensitive to ozone. Pollen germination and pollen tube elongation were reduced by 40-50% after exposure to 100 ppb of O_3 for 5.5 h in tobacco. Similar results were obtained from exposure experiments with mature pollen of other plant species such as corn (Mumford *et al*. 1972), oat (Myhre *et al*. 1988), fruit and nut tree species (Hormaza *et al*. 1996). The possible cause for germination failure may be the reduction in starch, which is a major energy source for pollen germination and pollen tube elongation in grasses (Baker and Baker 1979).

Secondly, the threshold might differ for the two processes as pollen germination occurs on the stigmatic surface, whereas tube growth occurs largely within the stylar tissue, where it is protected from direct contact of tropospheric O_3 (Black *et al*. 2000).

The mechanism responsible for the observed effects of O_3 on pollen germination and tube growth is still not clear. The possible mechanism predicted by Feder and Sullivan (1969) is inactivation of chemical regulator of pollen tube growth due to the oxidizing effect of O_3. Harrison and Feder (1974) demonstrated that exposure of *Petunia* pollen grains to 500 ppb O_3 for 3 h reduced 21% germination in O_3 sensitive cultivar white bountiful and 15% in tolerant cultivar Blue Lagoon. The result of detailed microscopic study showed that in white bountiful 50% of the fumigated pollen had a peripheral band of cytoplasm free of all organelles, while in Blue lagoon fewer pollen grains showed similar cytoplasmic changes, suggesting organelles might have shifted away from the plasma membrane in response to O_3 in O_3-sensitive cultivar affecting its germination and cell- wall development within the pollen tube.

Biochemical analyses of pollen from maize exposed to 120 ppb O_3 for 5 h d^{-1} showed increase in free amino acid and reductions in reducing sugar and sugar neutral contents (Mumford *et al*. 1972). The carbohydrate content of mature pollen is known to decrease when plants are grown under low light levels probably because of associated reductions in photosynthesis (Fuhrer & Booker, 2003). As carbohydrates provide metabolic substrates required as an energy source in germinating pollen, therefore any reduction in carbohydrate content might adversely affect germination (Fuhrer and Booker 2003).

Perennial ryegrass plants (*Lolium perenne* L.) were grown in closed top chambers under ambient (65 ppb for 8 h) and elevated (110 ppb for 4 h) ozone concentrations along with control as charcoal filtered ambient air to study the effects of ozone on the development of pollen under different treatments the results showed that O_3 affected the maturation of pollen by inhibiting starch accumulation in pollen (Schoene *et al*. 2004). Affected pollen persisted in the vacuolated state while normal pollen in the same anthers was filled with amyloplasts. Underdeveloped grains were lying in the lumen of the locule and not with their pores adjacent to the tapetum at the periphery as it is normally found. Higher numbers of underdeveloped pollen without amyloplasts were found under O_3 exposure (Fig. 3). Serial sections through one pollen layer were analysed to find out the stage at which underdeveloped

pollen had stopped growing and the result showed presence of two cells with one nucleus each in one pollen grain. This observation suggests that development of damaged pollen ceased after the first haploid mitosis. The amount of cytoplasm had not increased and amyloplasts were not yet differentiated in O_3 exposed pollens. Normal pollen grains were densely packed with starch containing amyloplasts and showed a peripheral orientation in the locule with their pores adjacent to the tapetum. Higher numbers of smaller pollen grains were observed in O_3 exposed grasses compared to its control, due to its disturbed nutrient translocation and supply of assimilate.

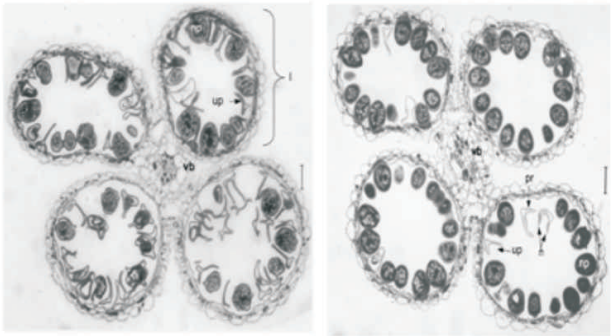

Fig. 3—Impact of O_3 on pollen development in perennial ryegrass; up: undeveloped pollen; np: normal pollen; vb: vascular bundle. A. Treated and B. Control (Schoene *et al.* 2004).

There are limited studies on direct impact of O_3 on stigmatic structure and function as any alteration to the stigmatic topography or the chemical composition of the germination medium provided by the stigma may affect the ability of pollen to germinate and penetrate the stigmatic surface or stylar tissues and may affect ovule fertilization and seed yield.

Floral initiation and development—Higher levels of tropospheric O_3 coincide with timing of flowering may have potentially important implications for seed set (Rai and Agrawal 2008, Rai *et al*. 2011). Higher concentrations of O_3 were recorded during reproductive stage of rice, wheat and soybean leading to reductions in their respective yields.

Amundson *et al*. (1987) found that exposure of winter wheat before anthesis reduced numbers of grain presumably by decreasing the number of floral sites produced, whereas exposure anthesis once the grain primordial had been formed, affected kernel weight. Moreover, exposure of *Brassica campestris* L. to 70 ppb O_3 for 7 h d^{-1} over a 10 d period before flowering reduced the number of reproductive sites whereas no significant effects were observed when inflorescence was exposed once all floral initials were initiated (Stewart 1998).

Ozone induced losses of potential reproductive sites and hence the numbers of pods were not observed following exposure of the inflorescence of *Brassica campestris* L. to 100 ppb O_3 for 6 h d^{-1} between 17 and 20 d after sowing (Stewart 1998). Results of metanalysis study showed that numbers of flower and inflorescence were affected differently under O_3 exposure (Leisner and Ainsworth 2012). Number of flower and weight of flower increased under elevated O_3 compared to ambient O_3. These results suggest that accelerating vegetative development and increasing reproductive output are a general features to maintain fitness in response to stress (Bazzaz *et al*. 1987) and compensatory responses have been documented in *Brassica* species (Black *et al*. 2000, Black *et al*. 2007). However, increase in number of flower at elevated O_3 was unable to compensate the decrease in number of fruits and seed size. Higher yield reductions under O_3 exposure may be due to the maintenance of increased flower numbers at elevated O_3 which may have caused a proportionally greater decrease in individual seed mass as plants attempt to fill more seeds with less available carbon.

Hayes *et al.* (2012) found that number of flowers decreased by 50% after exposure to O_3 in mesocosm having seven species mixtures representing Calcareous grassland community. Reduction in length of terminal raceme of *Brassica campestris* under 70 ppb O_3 for 7 h d^{-1} for 10 days was suggested to be a compensatory response to O_3 (Black *et al*. 2007). Number of aborted reproductive sites on terminal raceme was maintained by increase in reproductive sites. Ozone exposure led to abortion of flowers, ovules and seeds. Linseed cultivars Padmini and T-397 exposed to 50 ppb O_3 showed reductions of 31.5 and 26.3% in number of ovules per capsule (Tripathi and Agrawal 2013) (Fig. 4). The potential for O_3 to directly impact reproductive structures may result from direct uptake of O_3 through stomata on petals or sepals. Floral stomata are located mainly on the surface of the outer sepals of the mature flower (Smyth *et al*. 1999). Stewart (1998) reported that multiple exposures of inflorescence of *Brassica campestris* L. to 100 ppb O_3 for 6 h d^{-1} on consecutive days during flowering resulted in development of compensatory responses like retaining more number of seeds against O_3 induced seed abortion in the apical pod. Exposure of flowers to elevated O_3 led to increase in flower abortion, which negatively affected seed set, fruit number and fruit weight (Leisner and Ainsworth 2012).

Effects on assimilate translocation and its consequences on yield attributes—The grain yield of a crop is a function of a number of factors that are under genetic and environmental controls. The potential ear density is mainly dependent on the extent of tillering before ear initiation (Evans *et al*. 1975) in combination with the sowing density. The number of spikelet initials per spikelets is also determined early during plant development. Numerous studies have shown that ambient O_3 frequently reduces yield in grain crops by decreasing ear and pod numbers, seed numbers per ear, spikelet or pod (and hence seed number per plant) and individual seed weight (Rai *et al*.

2015, Rai *et al*. 2010, Singh *et al*. 2014, Yuan *et al*. 2015) (Table 2).

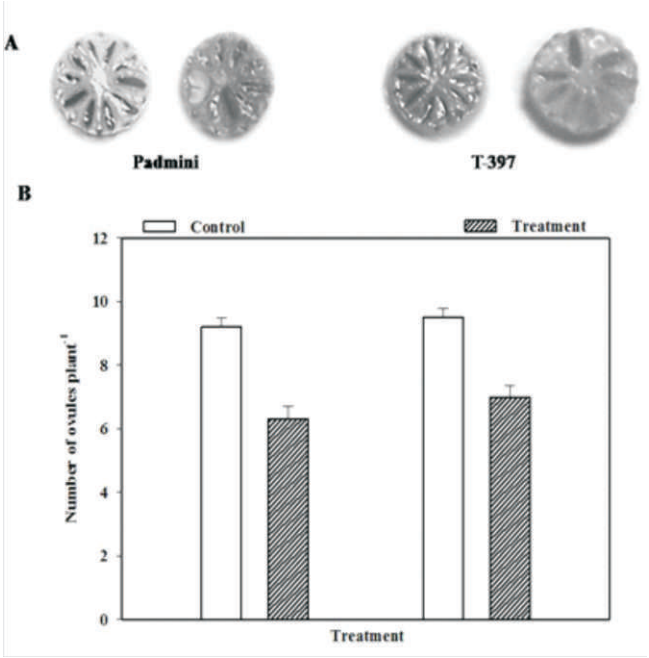

Fig. 4 — (A) Variations in T.S of capsule of linseed cultivars grown in O_3 exposed fumigated and control treatment (B) Changes in number of ovules plant^{-1} in linseed cultivars O_3 fumigated and control treatment (Source: Tripathi & Agrawal 2013)

Meta analysis conducted by Feng and Kobayashi (2009) showed that response of different yield components varied between crop species and O_3 treatment. It is noteworthy that reduced weight of individual grain or seed is often the major cause of the yield loss due to O_3 exposure in cereals (Leisner and Ainsworth 2012). This yield component is under genetic control and usually most conservative among the yield components against the environmental stresses. It is affected by occurrence of stresses during the grain filling stage (Gelang *et al*. 2000). Zhu *et al*. (2011) reported reductions in reduced grain mass by 19.2% and grain number per ear by 3.5% in wheat cultivar exposed to elevated O_3 (61.3 ppb) compared to ambient O_3 (45.7 ppb). Shi *et al*. (2009) found reductions in yield by 12% in rice cultivars grown at elevated O_3 (90 ppb) compared to ambient O_3 (45 ppb).

Shi *et al*. (2009) reported that number of panicles per unit area appeared to be the least affected by elevated O_3 among the yield components. However, the number of spikelets per panicle reduced by 9.2%. Number of spikelets per panicle is the difference between the number of differentiated spikelets and of degenerated spikelets. Results showed that elevated O_3 caused reductions in number of differentiated spikelets and increase in undifferentiated spikelets. Spikelet number per unit area is the product of panicle number per unit area and spikelet number per panicle. which also decreased under elevated O_3 (Shi *et al*. 2009). The main cause attributed to reduction in spikelet number per panicle was due to the suppression of spikelet differentiation under O_3 exposure.

Singh *et al*. (2014) found reductions in various yield attributes in maize like number of rows of kernels and number of husk leaves in maize exposed to ambient and elevated O_3 in open top chambers. Reductions in thousand kernel weight were observed at elevated O_3 doses in both the cultivars indicating that a reduction in the size of individual kernel was due to less availability of photosynthates. Number of kernel cob^{-1} and weight of kernels reduced however, weight of cob plant^{-1} increased in both the maize cultivars under ambient (53.5 ppb) and elevated (68.5 and 83.5 ppb) O_3. Increase in weight of cob with increasing concentration of O_3 shows more translocation of photosyntheates towards the husk leaves during O_3 stress just to provide protection and safeguard the female reproductive organs (Singh *et al*. 2014).

The floret initiation starts when the ear is at 1 cm measured in wheat from the attachment of the lowest leaves (Tottman and Broad 1987). After anthesis, both the number of ears per unit area, apart from late tillers contributes little to the yield. Then, the rate and the duration of the grain filling processes are the dominant factors influencing the final grain yield. The maximum grain size is related to the number of endosperm cells in the developing grains, which is determined before starch storage begins of 1-2 weeks anthesis (Evans *et al*. 1975, Gelang *et al*. 2000). Ozone has been shown to be more important for yield reductions when the exposure is conducted during and after anthesis (Pleijel *et al*. 1998). Thus the rate and the duration of grain filling seem to be the key processes to study in order to understand the mechanism behind the O_3 induced reduction of grain yield in wheat.

O_3 reduces net photosynthesis (Lehnher *et al*. 1988) and duration of the green leaf area (the period during which the leaves have a positive carbon balance) of wheat (Meyer *et al*. 2000). Grandjean and Fuhrer (1989) showed that O_3 induces early senescence of the flag leaves, which is expressed as a loss of chlorophyll and soluble proteins and as an earlier peak in the activity of the enzyme glutamate dehydrogenase which is involved in the redistribution of amino acids from leaf proteins to the grains during senescence. Furthermore, Pleijel *et al*. (1997) presented a strong correlation between an ozone induced reduction of the flag leaf duration expressed as chloroplast breakdown and grain yield loss in wheat. This indicated that the effect of O_3 on the duration of assimilate production, a grain filling process was a key factor behind the yield reduction.

***Effects of O_3 under different reproductive growth habits*—**The results of the studies conducted to compare the effects of O_3 was compared across plants having C_3 and C_4 photosynthetic pathways and results indicated that yield, seeds per fruiting structure and individual seed weight of C_4

Table 2—Effects of ozone on yield attributes of different crops and their cultivars.

Parameters	Plant	Response	References
Number of ears plant^{-1}	Wheat cv M 234, M 533, PBW 343, Sonalika, M 510	Decreased	Rai *et al.* (2007), Rai and Agrawal (2014) and Sarkar *and Agarwal* (2010)
Number of spikes plant^{-1}	Rice cv NDR 91, Saurabh 950, Malviya dhan 36, Shivani	Decreased	Rai and Agrawal (2008), Sarkar and Agrawal (2012)
	Rice cv WJ15, YD6, SY63, LYPJ	Decreased	Shi *et al.* (2009)
Number of pods plant^{1}	Mustard	Decreased	Singh *et al.* (2009)
	Soybean	Decreased	Rai *et al.* (2015)
	Mung bean	Decreased	Chauwdhary *and Agarwal* (2015)
Number of seeds pod^{-1}	Mustard	Decreased	Singh *et al.* (2011)
	Mustard	Decreased	Black *et al.* (2007)
Number of seeds per pod	Mustard	Decreased	Black *et al.* (2007)
Number of aborted seeds pod	Mustard	Decreased	Black *et al.* (2007)
Number of grains per ear	Wheat cv. Y2, Y19, Y 15 and Y 16	Decreased	Feng *et al.* (2011)
	Wheat cv M 234	Decreased	Zhu *et al.* (2010)
Wt. of pods	Soybean	Decreased	Rai *et al.* (2015)
	Mustard	Decreased	Black *et al.* (2012)
Wt. of ears	Wheat cv M 234, PBW 343, M 533, Sonalika and M 510	Decreased	Rai *et al.* (2007), Sarkar and Agrawal (2010), Rai and Agrawal (2014)
	Wheat cv Sufi and Bijoy	Decreased	Akhtar *et al.* (2010)
Filled grain per ear	Wheat cv Sufi and Bijoy	Decreased	Akhtar *et al,* (2010)
Filled grain per spikes	Rice cv WJ15, YD6, SY63, LYPJ	Decreased	Shi *et al.* (2009)
Unfilled grain per ear	Wheat cv Sufi and Bijoy	Increased	Akhtar *et al.* (2010)

plants are equally sensitive to elevated O_3 as those variables in case of C_3 plants (Leisner and Ainsworth 2012).

Determinate plants produce only a set number of flowers and/or floral initials in their inflorescences, which may limit compensation during reproductive development under O_3-induced loss of reproductive sites, or failure/inhibition of pollination and syngamy (Black *et al*. 2000). Indeterminate plants do not produce a set number of flower and/or floral initiations in there inflorescences, which could lead to an increase in capacity to compensate for seed loss in stressful environments. Black *et al*. (2007) reported reductions in number of reproductive sites on terminal racemes, number of buds, number of seeds per pod, total number of seed per plant, but yield did not reduce significantly in *Brassica campestris* exposed to 70 ppb O_3 for 7 h from germination to maturity. This response suggests that an effective compensatory mechanism operated during the flowering and seed development stages. Because of its indeterminate reproductive habit, *B. campestris* normally produces more floral sites than can be sustained to maturity, a strategy that maximizes the number of seeds set in environments where pollination of all flowers cannot be guaranteed. This capability may ensure that reductions in the number of floral sites due to abiotic stress factors such as O_3 do not translate into decrease in pod number and seed yield. Excess floral sites were aborted in control plants, whereas O_3-treated plants retained sites that would normally have been aborted, with the result that pod number at maturity did not differ significantly from control plants. Rai *et al*. (2010) reported reductions of 10-14% in yield of rice cultivars grown under 12-h mean O_3 concentration of 35 ppb of from germination to maturity because of due to its determinate reproductive habit, which limited the capacity to compensate for any losses of reproductive sites or impairment of pollination and fertilization under higher occurrence of O_3 concentrations above 40 ppb during the reproductive phase. While, Leisner and Ainsworth (2012) in a metanalyses study found that 52% reductions in fruit weight of indeterminate plants, but it was not affected in determinate plants under elevated O_3. It is possible that the indeterminate plants continue to produce floral sites despite increasing O_3 stress and then competition for assimilate reduces the plant's ability to fill the developing fruit. This result suggests that the reproductive growth habit is not the sole factor which is involved in determining O_3 tolerance (Pleijel *et al*. 1997, Mulholland *et al*. 1997), but other important factors are also important like the length of the reproductive phase and anatomical differences in reproductive structures (Black *et al*. 2000).

Conclusions and future research—It is difficult to generalize the effects of O_3 on reproductive development due to the complexity in the nature of the effects of O_3 on vegetative and reproductive structures, also compensatory mechanisms in plants with different reproductive growth habits and the dependence of plant developmental stage on the levels of sensitivity. The projected levels of O_3 are critically alarming for crops, trees and native species. Scientific evidences clearly show the detrimental effect of O_3 directly to the reproductive organs and on reproductive growth stages or processes including pollen germination and tube growth and the abscission or abortion of flowers, pods and individual ovules or seeds. Most of the studies conducted to assess direct impact of O_3 on plant reproductive processes are conducted in modified environment such as fumigation based and closed chambers. Limited studies are available from near natural field

based studies to understand the clear mechanism of detrimental effects of O_3 on reproductive processes of plants for developing realistic threshold for protection of crops and natural flora against O_3. Even, little is known about changes in carbon allocation to fruiting and flowering structures and changes in assimilate partitioning in maternal reproductive structures under elevated O_3. In view of the intrinsic importance of C_4 plant species in their natural communities. Therefore, more research is needed to understand the effects of O_3 on C_4 plants, in both natural communities and agricultural systems. So, understanding the effects of O_3 on reproductive development has significant agronomic and ecological consequences, including securing future food resources and ensuring the fecundity and species composition of native flora.

Acknowledgements—The authors are thankful to the Head of the Department of Botany for all the laboratory facilities and to the Department of Science and Technology, New Delhi and Council of Scientific and Industrial Research, New Delhi for providing financial support to the work. Richa Rai is grateful to the Department of Science and Technology, New Delhi for Fast Track Young Scientist project.

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