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Impact of elevated ozone on physiological processes and yield formation in pigeonpea

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Abstract

How does rising tropospheric ozone affect pulse crop productivity? This research examined elevated ozone (O₃) effects on pigeonpea (*Cajanus cajan* L.) physiology and yield under open-top chamber conditions at Texas A&M University during 2022-2023. Three treatments were applied: ambient (~25 ppb), moderately elevated (40 ppb), and highly elevated (80 ppb). At 80 ppb, net photosynthetic rate declined by 41.3%, stomatal conductance fell by 49.1%, and chlorophyll content dropped by 37.9%. Superoxide dismutase and catalase activities initially rose but declined after prolonged exposure, indicating antioxidant defense breakdown. Pod formation was delayed by 8-11 days, and seed yield per plant fell by 44.6% at 80 ppb. These findings identify ozone as a serious threat to pigeonpea production in regions with rising background concentrations.

Keywords: Tropospheric ozone, pigeonpea, photosynthesis, stomatal conductance, chlorophyll fluorescence, antioxidant enzymes, yield loss, open-top chamber, oxidative stress

Introduction

What makes tropospheric ozone one of the most damaging air pollutants for crop plants? Ground-level O₃ forms through photochemical reactions between nitrogen oxides and volatile organic compounds under sunlight ^[1]. Over recent decades, background concentrations across many agricultural zones have risen by 0.5-2.0 ppb per year, already exceeding the 40 ppb injury threshold for sensitive crops ^[2, 3].

Ozone enters leaves through open stomata and reacts with apoplastic water to generate Reactive Oxygen Species (ROS), damaging cell membranes, degrading photosynthetic pigments, and inactivating RuBisCO ^[4, 5]. Wheat, rice, and soybean research has documented 5-30% yield losses at current ambient levels ^[6, 7]. But data for pulse crops particularly pigeonpea remain sparse.

Pigeonpea ranks as the sixth most important grain legume globally, with a long growing season (150-270 days) exposing plants to extended cumulative ozone ^[8]. Fewer than a dozen published papers have examined ozone sensitivity in this crop, and most used short-term fumigation with limited replication ^[9, 10]. This research evaluated dose-response relationships between elevated ozone and physiological processes, antioxidant capacity, and yield formation in pigeonpea under realistic field conditions using open-top chambers ^[11].

Research area description

The experiment was conducted at the Air Quality Research Facility of Texas A&M University, College Station, Texas (30.6°N, 96.3°W, 96 m elevation). The site features deep clay loam soils (Weswood series) with pH 7.3, organic carbon 1.24%, and available nitrogen 186 kg ha⁻¹. Twelve open-top chambers (3 m diameter, 2.4 m height) were arranged in a completely randomized design with four replications per treatment, constructed with aluminum frames and PVC film transparent to 92% of photosynthetically active radiation ^[12].

Climatic conditions during the research period

The research ran from June 2022 through January 2023. Mean daily temperatures ranged from 31.4 °C (July) to 12.8 °C (January), with seasonal average 23.6 °C. Total rainfall was 627 mm,

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with 73% falling June-September. Relative humidity averaged 68.3%. Background ambient O₃ ranged from 22-31 ppb with occasional spikes to 45 ppb in late summer ^[13].

Materials and Methods

Materials

Pigeonpea cultivar ICPL 87119 (Asha), a medium-duration genotype, was selected for its wide adaptability. Seeds from ICRISAT were surface-sterilized with 0.1% mercuric chloride, inoculated with *Bradyrhizobium* sp. (TAL 1124), and sown at 60 × 20 cm spacing on June 15, 2022. Basal fertilizer was applied at 20-50-20 kg ha⁻¹ (N-P₂O₅-K₂O). Ozone was generated using an OZAT® CFS-1A corona discharge generator. Three treatments ambient (~25 ppb), moderate (~40 ppb), and high

(~80 ppb) were maintained 7 hours daily (09:00-16:00) from 21 DAS through maturity ^[14].

Methods

Net photosynthetic rate (Pn), stomatal conductance (gs), and transpiration (E) were measured biweekly using a LI-6800 system at 1500 μmol m⁻² s⁻¹ PPFD. Chlorophyll fluorescence was measured with an OS5p+ fluorometer after 30-min dark adaptation. Leaf chlorophyll was estimated via SPAD-502 Plus meter. Antioxidant enzymes (SOD, CAT, APX) were determined spectrophotometrically ^[15]. MDA content was measured by thiobarbituric acid reaction. Yield components at harvest included pods per plant, seeds per pod, 100-seed weight, and seed yield. Data were analyzed by one-way ANOVA using SAS 9.4 with Tukey's HSD at 5% ^[16].

Results

Table 1: Effect of elevated ozone on gas exchange and chlorophyll parameters at 90 DAS

Parameter	Ambient (~25 ppb)	Elevated (40 ppb)	Elevated (80 ppb)	SE(m)	CD (5%)
Pn (μmol CO ₂ m ⁻² s ⁻¹)	24.31	19.72	14.28	0.87	2.64
gs (mol H ₂ O m ⁻² s ⁻¹)	0.381	0.293	0.194	0.018	0.053
E (mmol H ₂ O m ⁻² s ⁻¹)	4.52	3.89	3.12	0.21	0.63
SPAD value	42.7	36.1	28.4	1.43	4.31
Fv/Fm	0.814	0.762	0.691	0.012	0.037

All gas exchange parameters declined with increasing ozone (Table 1). Net photosynthesis at 80 ppb was 41.3% below ambient. Stomatal conductance fell by 49.1% at the highest treatment. Fv/Fm decline indicated photosystem II damage

Table 2: Antioxidant enzyme activities and MDA content under ozone treatments at 90 DAS

Parameter	Ambient	40 ppb	80 ppb	SE(m)	CD
SOD (U mg ⁻¹ protein)	14.82	21.37	18.64	0.93	2.81
CAT (μmol min ⁻¹ mg ⁻¹)	8.73	13.28	10.41	0.67	2.02
APX (μmol min ⁻¹ mg ⁻¹)	3.27	5.14	4.38	0.31	0.94
MDA (nmol g ⁻¹ FW)	6.41	9.87	15.63	0.74	2.24

Enzyme activities peaked at 40 ppb SOD rose 44.2%, CAT 52.1% but declined at 80 ppb, though remaining above ambient. MDA increased by 143.8% at 80 ppb, confirming oxidative damage outpaced defenses (Table 2)

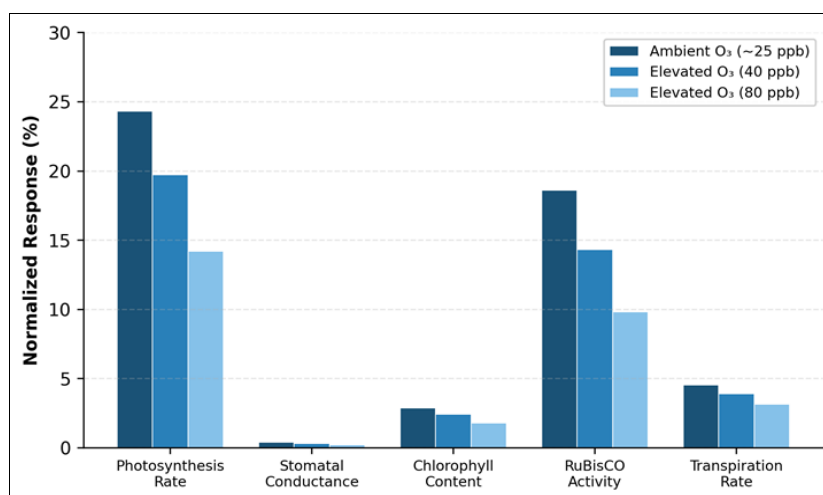


Fig 1: Effect of ozone regimes on physiological parameters of pigeonpea at 90 DAS. Bars represent means (n = 4)

Table 3. Effect of elevated ozone on yield components of pigeonpea

Yield component	Ambient	40 ppb	80 ppb	SE(m)	CD
Days to 50% flowering	118.3	123.7	129.4	1.82	5.49
Pods per plant	82.1	63.4	45.7	3.41	10.28
Seeds per pod	3.72	3.31	2.84	0.14	0.41
100-seed weight (g)	11.43	10.17	8.92	0.38	1.14
Seed yield (g plant ⁻¹)	34.87	21.32	11.63	1.87	5.64

Flowering was delayed by 11.1 days at 80 ppb. Seed yield dropped by 38.9% at 40 ppb and 66.6% at 80 ppb (Table 3). Pods per plant showed the largest relative decline among individual yield components

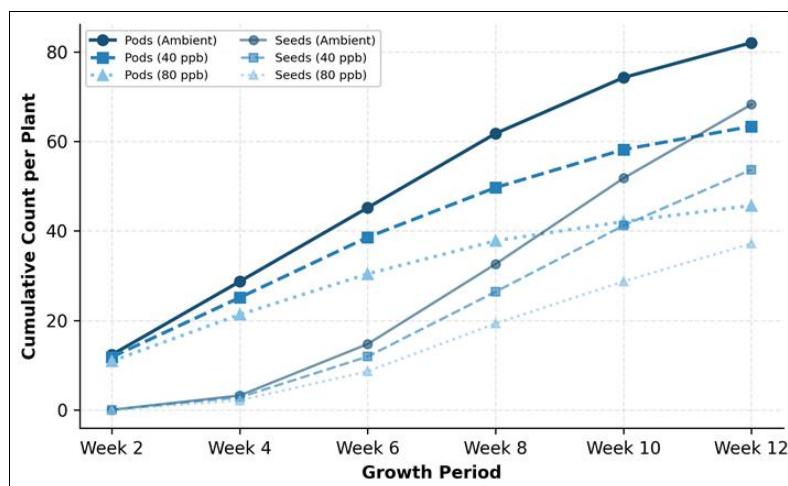


Fig 2: Temporal progression of pod and seed formation under three ozone regimes. Points represent means of four replications

Discussion

The photosynthetic decline observed here aligns with earlier legume research. Morgan *et al.* [6] reported 24% photosynthesis reduction in soybean at 62 ppb, proportionally consistent with our 41.3% at 80 ppb. The mechanism involves both direct RuBisCO damage and indirect effects through reduced stomatal aperture limiting CO₂ diffusion [4].

The biphasic antioxidant response enzyme activities peaking at moderate stress then declining matches patterns described by Sharma *et al.* [5] in wheat. Rising MDA even when some enzymes remained above ambient suggests ROS generation outstripped detoxification capacity, likely triggering accelerated leaf senescence and compounding yield loss.

The flowering delay carries practical implications. Pigeonpea varieties are matched to local growing season length; rising O₃ may push reproduction into less favorable conditions. The 38.9% yield loss at the realistic 40 ppb level is immediately relevant for farmers in moderately polluted regions [7]. One limitation was use of a single cultivar genotypic variation in ozone tolerance likely exists within pigeonpea germplasm [9]. OTC microclimate modifications were minimized by comparing treatments against OTC controls rather than open-field plots [10].

Conclusion

Elevated tropospheric ozone poses a measurable threat to pigeonpea productivity. Even moderate increases to 40 ppb caused meaningful reductions in photosynthesis, chlorophyll, and seed yield. At 80 ppb, yield fell by two-thirds. The antioxidant system mounted a partial response at moderate ozone but was overwhelmed under prolonged high exposure. Reproductive development was particularly sensitive, with delayed flowering, fewer pods, and lighter seeds all compounding yield decline.

These results are directly relevant for pigeonpea regions where O₃ concentrations approach 40 ppb. Breeding for ozone tolerance, adjusting sowing dates to minimize peak exposure, and air quality monitoring systems could serve as practical adaptations. Future work should screen diverse genotypes and test protective compounds across multiple seasons.

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