

Plasma-fixed Nitrogen Improves Lettuce Field Holding Potential

Benjamin Wang¹, Qiyang Hu¹, Bruno Felix Castillo¹, Christina Simley¹, Andrew Yates¹, Brian Sharbono², Kyle Brasier³, and Mark A. Cappelli¹

KEYWORDS. agronomic quality, bolting prevention, marketable yield, nitrogen efficiency, romaine lettuce, stem quality

ABSTRACT. This study shows that plasma-fixed nitrogen applied as an inorganic biostimulant can improve marketable lettuce (*Lactuca sativa* var. *longifolia*) yield following delayed harvest. Using just one-tenth of the conventional nitrogen, plasma-fixed nitrogen—which is generated by a dielectric barrier discharge over water—was field-tested against traditional fertilization methods. Although no statistically significant differences were observed in total weight of heads among treatments, plasma-fixed nitrogen-treated plants had significantly increased marketable yields of 250% compared to those grown conventionally, despite reducing applied nitrogen fertilizer.

One of the challenges facing agriculture is the reliance on nitrogen (N) fertilizers. Its overuse can lead to environmental degradation, eutrophication of water bodies, and nitrous oxide emissions. The energy-intensive fertilizer synthesis and its transportation contribute to resource depletion and carbon emissions. The emergence of plasma technology enabling the decentralized synthesis of plasma-fixed nitrogen (PFN) from potentially renewable electricity presents a revolutionary shift toward sustainable farming.

Low-temperature plasmas have the capability to activate water and fixate atmospheric N, creating a reactive solution rich in nitrates and other

compounds beneficial for plant growth (Bradru et al. 2020). PFN harnesses the efficient ionization and bond-breaking capability of plasmas, infusing water with nitrogenous compounds, hydroxyl radicals, and hydrogen peroxide, all known to enhance seed germination, root development, and overall plant vitality (Thirumdas et al. 2018). The current Haber–Bosch process has an energy consumption of $\sim 0.48 \text{ MJ} \cdot \text{mol}^{-1}$ ammonia produced, whereas nonthermal plasmas have a theoretical limitation of $0.2 \text{ MJ} \cdot \text{mol}^{-1}$ for nitrogen oxide synthesis (Li et al. 2018). Simultaneously, the PFNs offer a novel approach to using the N in the atmosphere, transforming it into a form readily used by plants.

We describe a field study in which PFN is used as a biostimulant for romaine lettuce (*Lactuca sativa* var. *longifolia*) grown in the Salinas Valley, CA, USA, a major production region. Here, groundwater has high nitrate (NO_3) concentrations ($>20 \text{ ppm NO}_3\text{-N}$) resulting from chemical-intensive agricultural practices. Lettuce growers typically apply between 120 to 200 lb/acre N, which often surpasses crop needs, to hedge against underfertilization (Cahn et al. 2016). Studies have investigated the role of nitrates generated by plasmas as a viable substitute for traditional N sources, with their investigations centered around direct nutrient supplementation (Ruamrungsri et al. 2023; Subramanian et al. 2021). Previous research has demonstrated that

PFN serves as an efficacious source of NO_3 for turfgrass growth, with promising outcomes in growth rate and overall plant health (Sze et al. 2021).

Materials and methods

Air plasmas were generated using an atmospheric dielectric barrier discharge (DBD) reactor operating at a 23-kHz driving frequency and with a sinusoidal peak-to-peak voltage of 7 kV. The discharge draws 1.8 kW of input power. Industrial water in a recirculating channel generates a free-surface flow close to the surface of the DBD and is treated until the PFN solution reaches desired pH (1.5) and $\text{NO}_3\text{-N}$ (492 ppm) levels, with minor reactive oxygen and N species (Armenise et al. 2023).

Beds were established on loam soils as a randomized complete block design on standard two-row plots measuring 40 inches wide and 25 feet long. Plants were on 10-inch in-row spacing. Transplants were planted 17 Aug 2023 in Salinas, CA, USA (lat. $36^\circ 37' \text{ N}$, long. $121^\circ 33' \text{ W}$) and harvested 14 Oct 2023. The study used drip irrigation, kept constant between treatments, to meet water demands and followed a mixed grass–legume cover crop. Seeds of the hearting variety (Stokes Seed, Holland, MI, USA) were used because of their popularity in organic agriculture.

Lettuce plots were subjected to three treatments: a zero-fertilizer control, conventional fertilizer (calcium ammonium nitrate, 17N–0P–0K; Simplot, Boise, ID, USA), and PFN, with four replications per treatment. No added fertilizer was applied to the control treatment, whereas the conventional treatment received 75 lb/acre N and the PFN treatment received 8 lb/acre N split over three applications between 21 Sep and 5 Oct 2023. Irrigation water contained 40 ppm N, whereas soils contained 41 ppm N at the time of transplanting.

Phenotypic traits (heart fresh weight, percentage marketability quality, and height) were measured 7 d after the optimal harvest date for romaine hearts to observe field holding capacity. Six randomly selected plants were chosen from each plot and chopped for hearts, which was defined as the inner portion of the lettuce plant where the leaves form an enclosed structure.

Received for publication 4 Dec 2023. Accepted for publication 26 Jan 2024.

Published online 6 Mar 2024.

¹Stanford University Department of Mechanical Engineering, Stanford University, Stanford, CA 94305, USA

²Stanford University Woods Institute for the Environment, Stanford University, Stanford, CA 94305, USA

³Vilmorin-Mikado USA Inc., Salinas, CA 93901, USA

This project was funded by the Stanford Sustainability Accelerator, Stanford High Impact Technology Fund, Stanford Woods Institute Realizing Environmental Innovation Program, and the Stanford TomKat Innovation Transfer Grant.

B.W. is the corresponding author. E-mail: bwang17@stanford.edu.

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<https://doi.org/10.21273/HORTTECH05369-23>

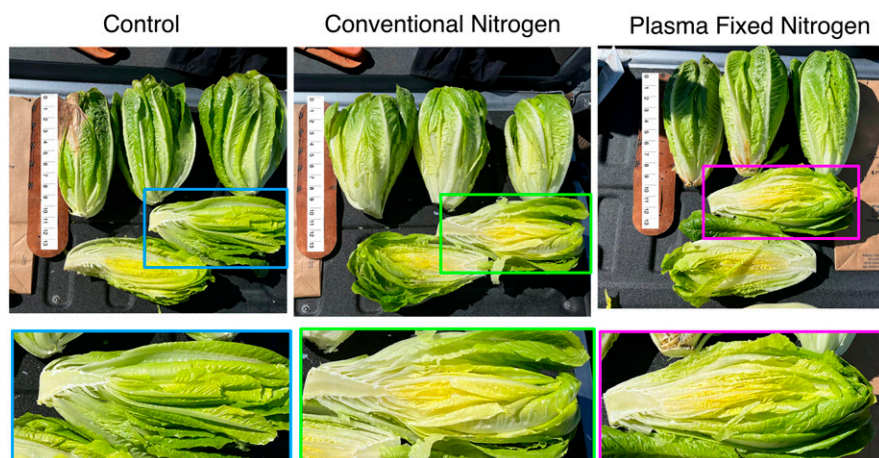


Fig. 1. Representative images of romaine hearts (*Lactuca sativa* var. *longifolia*) grown under a no-fertilizer control (left column), conventional nitrogen fertilizer (middle column), and plasma-fixed nitrogen (right column) treatments.

Marketability was determined by a commercial harvester and was defined as hearts without core elongation, spiraling, or physical damage (illustrated by the halved hearts in Fig. 1, where the PFN and control groups pass and do not pass marketability requirements, respectively). R Statistical Software v. 4.31 (R Foundation for Statistical Computing, Vienna, Austria) was used to perform analyses of variance on the data for statistical significance of measurements.

Results and discussion

Figure 1 shows harvested lettuce samples with longitudinal cross sections. The control group exhibited signs of

suboptimal quality derived from core elongation (i.e., early bolting with increased spacing between leaves), which reduced marketable quality of the lettuce. In contrast, these issues were less pronounced in the lettuce from the conventional N and PFN treatments. Lettuce grown under the PFN treatment exhibited greater heart quality, with minimal signs of bolting or leaf gaps. The leaves were particularly turgid and high in density. In an evaluation of N-use efficiency across different treatment trials, the PFN treatment produced greater marketable yield per unit of applied N compared with the conventional conditions, possibly because of the high concentrations of N in the water and soil being made available by the PFN.

Figure 2A illustrates the average total heart weight for the plot ($n = 6$ hearts) in which the PFN treatment averaged (2.83 kg/6 heads = 476 g/head), which was not significantly different from the control treatment (2.75 kg/6 heads = 458 g/head) or no-fertilizer control treatment (2.45 kg/6 heads = 408 g/head). This finding is consistent with previous experiments in the Salinas Valley, where lettuce yields do not differ significantly when more than 100 lb/acre N is available from water, soil, and fertilizer (Hoque et al. 2010). Figure 2B shows no statistical difference in the average length of the hearts across treatments. Figure 2C plots the marketable yield across the three treatments, in which the PFN treatment demonstrated the greatest marketable heart yield 7 d after the optimal harvest date (42%), followed by the conventional (17%) and control (4%) treatments. The high marketable heart yield of the PFN treatment, linked primarily to delayed core elongation, provides a crucial nongenetic control that has the potential to improve grower harvest flexibility and reduce in-field loss (Hayes and Simko 2016).

Conclusion

The interaction of plasma-activated species, such as plasma-fixed nitrates, with plant roots can stimulate root growth and density, increasing the surface area available for nutrient uptake (Ka et al. 2021). As a result, plants treated with PFN may not only have access to N fertilizer, but also have an

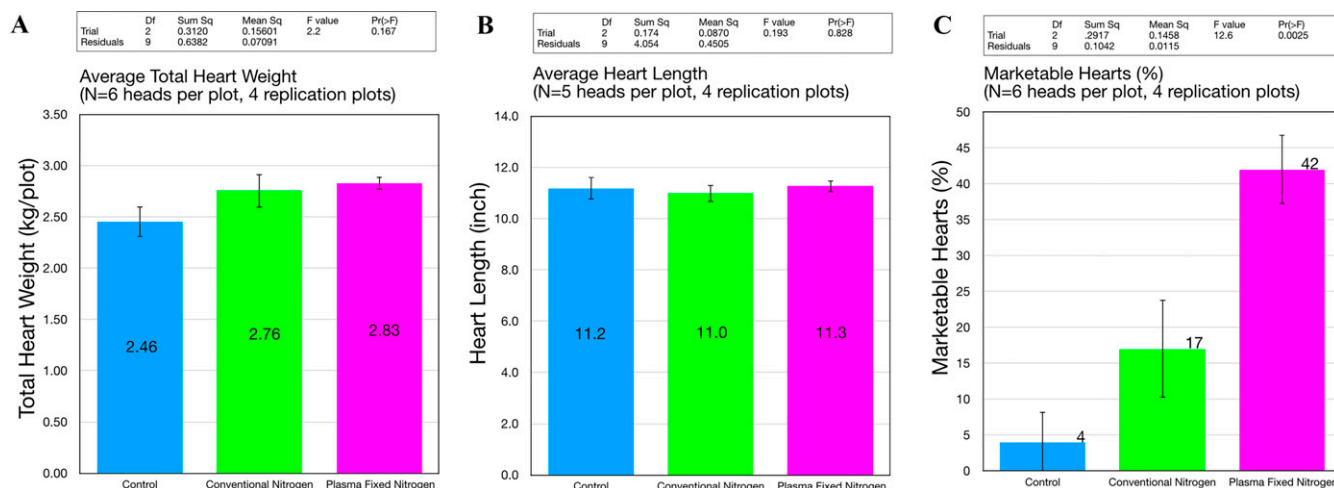


Fig. 2. Plots showing (A) the average plot heart weight ($n = 6$ lettuce hearts), (B) heart length for plot ($n = 5$), and (C) percentage of marketable romaine hearts (*Lactuca sativa* var. *longifolia*) for the control (no fertilizer), conventional nitrogen, and plasma-fixed nitrogen treatment groups. All error bars represent the standard error of the mean.

enhanced capability to access N from the soil. Nitrates derived from plasmas lack associated mineral cations, which paves the way for novel chemical mixtures that stimulate novel biological pathways in plants. This field study demonstrates the ability of PFN to increase the field holding capacity of lettuce. The complex interplay between PFN and plant physiology, especially in the realm of root growth and nutrient uptake, necessitates further research to integrate these insights into a comprehensive model for maximizing crop yield and quality.

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