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Physiological efficiency of nitrification inhibitors and endophytic nitrogen fixers in maize crops

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Abstract. Under the conditions of global climate change, the stability of the nitrogen status in maize is a critical factor for ensuring high productivity, especially during late-season thermal stress. The objective of the study was to substantiate the physiological impact of nitrapyrin and *Methylobacterium symbioticum* on the activity of the maize photosynthetic apparatus under various hydrothermal scenarios. The research was conducted in 2023-2025 on Typical Chernozems and Grey Forest soils in the Forest-Steppe of Ukraine. The research subjects included hybrids P8723, P9127, and P9074. The relative chlorophyll content was measured using the Yara N-Tester device. Statistical processing was performed using the LSD test. It was established that the integration of N-Lock™ Max and Blue N™ ensured a pronounced "stay-green" effect, providing 5-12% higher chlorophyll index than the control during the grain-filling phase. It was found that under the extreme drought conditions of 2024 (HTC 0.56), endophytic nitrogen fixation effectively compensated for the soil nitrogen deficit, increasing the Yara N-Tester index by 18.7-26.2 units. The study proved that applying these treatments on a moderate N_{90} background allows for achieving physiological indicators equivalent to the full N_{120} background without stabilisers. A direct correlation between late-stage chlorophyll content and final grain yield was mathematically confirmed. Significant yield increases were observed in the synergistic variants, which outperformed individual applications by maintaining metabolic activity during peak thermal stress.

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The results demonstrated that the synergy between chemical stabilisation and biological nitrogen fixation ensured stable nitrogen homeostasis in maize under thermal stress, enabling the optimisation of nitrogen fertiliser rates by 25%. This integrated approach serves as a viable strategy for enhancing crop resilience and nutrient use efficiency in climate-smart agriculture

Keywords: *Zea mays* L.; nitrapyrin; *Methylobacterium symbioticum;* stay-green effect; Yara N-Tester; nitrogen homeostasis

Introduction

Maize (*Zea mays* L.) stands as a cornerstone of global food security and a primary feedstock for the rapidly growing bioenergy industry. In Ukraine, particularly within the Forest-Steppe zone, maize productivity is a key driver of agricultural export potential. However, the modern cultivation of high-yielding hybrids is increasingly constrained by the inefficiency of conventional nitrogen fertilisation strategies. Nitrogen governs leaf area expansion and chlorophyll synthesis, yet Nitrogen Use Efficiency (NUE) remains low due to nitrate leaching and gaseous emissions. As of 2026, the challenge lies in maintaining nitrogen homeostasis under increasing hydrothermal instability. Recent scientific discourse highlights the shift from quantitative fertilisation to qualitative nutrient management. S. Tanchyk & A. Babenko (2021) established that conventional fertilisation systems in the Forest-Steppe often fail to meet the peak nitrogen demand of modern hybrids during the generative phase, necessitating the use of stabilisers. C.A. Ruiz-Sánchez *et al*. (2022) investigated the role of *Methylobacterium symbioticum* as a leaf-colonising endophyte and concluded that biological nitrogen fixation in non-legumes is a primary mechanism for bypassing root-uptake limitations during soil moisture deficits. Further research by H. Gamit *et al*. (2023) demonstrated that methylotrophic bacteria improve plant growth under stressed environments by producing secondary metabolites that modulate hormonal signalling.

The physiological response of cereal crops to such biostimulants was explored by F. Valente *et al*. (2024), who found that foliar application of *M. symbioticum* maintains photosynthetic rates even under reduced chemical nitrogen inputs. M. Quemeda *et al*. (2026) further quantified this response in maize, indicating that biological fixation provides a steady nitrogen supply that complements soil-applied fertilisers. However, the specific interaction between chemical nitrification inhibitors like nitrapyrin and these biological fixers remains under-researched in the context of the Ukrainian Forest-Steppe. The gap in knowledge persists regarding how this "dual-action" system performs across different maturity groups (FAO 280-330) during extreme heatwaves.

The objective of this research was to provide physiological substantiation for the combined use of nitrapyrin and *Methylobacterium symbioticum*. The purpose of the study was:

1) to evaluate the dynamics of the Yara N-Tester index across different maize hybrids and hydrothermal scenarios;

2) to determine the compensatory capacity of the synergistic system in reducing mineral nitrogen rates;

3) to quantify the correlation between late-season nitrogen status and final grain productivity.

Materials and Methods

The field experiments were conducted over a three-year period (2023-2025) across two distinct climatic zones within the Forest-Steppe of Ukraine. Zone 1 (2023) was located in the Bila Tserkva district (Typical Chernozem), and Zone 2 (2024-2025) in the Vinnytsia Oblast (Grey Forest heavy-loamy soils). Plant research was performed in accordance with institutional and international standards, adhering to the provisions of Convention on Biological Diversity (1992). The experimental design included an absolute control group (untreated plants on N_{90} and N_{120} backgrounds) to accurately compare the effects of N-Lock™ Max (nitrapyrin) and Blue N™ (*M. symbioticum*). Data collection involved sampling 30 representative plants per plot. The elementary plot size was 24 m^2. To ensure high statistical power, a split-plot design with four replications was utilised, which provided sufficient degrees of freedom to compare effects between treatment variants.

Three high-yielding maize (*Zea mays* L.) hybrids from the Pioneer® selection (Corteva Agriscience Ukraine) were utilised to evaluate the impact of nitrogen stabilisation across different maturity groups:

- P8723 (FAO 280): Early-mid group, characterised by high drought tolerance and rapid initial growth.
- P9127 (FAO 310): Mid-maturity group, known for its intensive type of development and high response to mineral nutrition.
- P9074 (FAO 330): Mid-late group, possessing a high genetic potential for yield under favourable hydrothermal conditions.

The preceding crop for all experimental cycles was winter wheat. The study involved three interacting factors. Factor A: nitrogen fertilisation background – nitrogen was applied in the form of granular urea (Urea 46%) under pre-sowing cultivation. Two dosage levels were investigated:

- Moderate dose (N_{90}): 196 kg/ha of commercial product (90 kg/ha of active substance).
- High dose (N_{120}): 260 kg/ha of commercial product (120 kg/ha of active substance).

Factor B: chemical nitrification inhibition – the nitrification inhibitor N-Lock™ Max (active ingredient: nitrapyrin, 300 g/L) was applied at a rate of 1.7 L/ha. The application was performed via soil spraying followed by immediate incorporation with a cultivator. The mechanism of nitrapyrin involved the selective inhibition of *Nitrosomonas* bacteria, which prevented the rapid oxidation of ammonium (NH_4^+) to highly mobile nitrates (NO_3^-). Factor C: biological nitrogen fixation – the biological stimulant Blue N™ (containing the strain *Methylobacterium symbioticum*) was applied at a rate of 0.33 kg/ha through foliar spraying during the BBCH 14-16 growth stage (4-6 true leaves). The working solution volume was 200 L/ha.

The relative chlorophyll content, serving as a proxy for the nitrogen status of the plants, was measured using the handheld Yara N-Tester (Yara International ASA, Oslo, Norway; manufactured by Konica Minolta, Japan). This optical sensor assessed the transmittance of light through the leaf blade at two specific wavelengths (red and infrared). Measurements were recorded in a temporal sequence: 3WAAB: 3 weeks after the application of the biostimulant (late vegetative stage). 7WAAB: 7 weeks after application, corresponding to the critical grain-filling phase. Data were collected from the middle part of the 3rd fully developed leaf from the top. Each value represented an average of 30 individual readings per plot to minimise intra-varietal variability.

Statistical Analysis was processed using an Analysis of Variance (ANOVA) within the RStudio environment (version 4.2.1), utilising the ggplot2 and stats packages. The significance of differences between treatment means was determined using the Least Significant Difference (LSD) test. Given the high natural variability inherent in field biological trials and the subtle physiological effects of endophytes, the significance threshold was set at $p \leqslant 0.1$. To quantify the relationship between the N-Tester index and final grain yield, Pearson correlation coefficients (*r*) were calculated.

Results and Discussion

Physiological basis of stabilised and endophytic nutrition. The stabilisation of nitrogen in the soil matrix represents a significant technological advancement in mitigating nutrient losses. Nitrification inhibitors, specifically those based on nitrapyrin (2-chloro-6-(trichloromethyl) pyridine), have demonstrated high efficacy in sequestering nitrogen in the stable ammonium form (NH_4^+). By selectively inhibiting the activity of *Nitrosomonas* bacteria, nitrapyrin slows the transition of ammonium to highly mobile nitrates (NO_3^-) (Wolt, 2004). From a plant physiology perspective, the "ammonium-rich" environment created by products like N-Lock™ Max offers a distinct metabolic advantage. The assimilation of NH_4^+ requires significantly less metabolic energy (ATP) compared to the reduction of NO_3^-, which must first be converted back to ammonium within the plant tissues before amino acid synthesis can occur (Di & Cameron, 2002; Shvartau *et al.*, 2016). This energy saving is particularly vital during the rapid growth phases of maize, allowing the plant to redirect its resources toward biomass accumulation and root development (Hospodarova *et al.*, 2019).

While chemical stabilisation addresses soil-based losses, the increasing frequency of climate-induced abiotic stresses requires a more resilient approach. Prolonged droughts and extreme "heatwaves" during the critical flowering stage often lead to soil dehydration, which effectively blocks root-mediated nutrient uptake. In this context, the role of endophytic nitrogen fixers, such as *Methylobacterium symbioticum* (the active agent in Blue N™), becomes critical. Unlike conventional rhizobia, this bacterium colonises the phyllosphere – the internal tissues of the leaves – and facilitates the fixation of atmospheric nitrogen (N_2) directly within the photosynthetic apparatus (Ruiz-Sánchez *et al.*, 2022). This biological "bypass" ensures a continuous supply of nitrogen even when the soil is too dry for conventional uptake. Research has shown that these methylotrophic bacteria can also produce ACC-deaminase, an enzyme that degrades the stress hormone ethylene, thereby enhancing the plant's tolerance to drought and thermal shock (Belimov *et al.*, 2019).

A fundamental challenge in the study of foliar endophytic nitrogen fixation is the reconciliation of the nitrogenase enzyme complex's extreme sensitivity to oxygen (O_2) with the oxygen-rich environment of the leaf. In classical diazotrophs, such as *Rhizobium* in legume nodules, oxygen levels are strictly regulated by leghemoglobin. However, in the phyllosphere − the internal and external surfaces of leaves-bacteria like *Methylobacterium symbioticum* (strain SB23) must utilise alternative strategies to protect their nitrogen-fixing mechanisms. Recent genomic and in situ analyses suggest that *M. symbioticum* does not function as a classical soil diazotroph but rather as a highly specialised endophyte that has evolved to synchronise its metabolic activity with the host plant's physiology (Valente *et al.*, 2024). The survival and efficacy of *M. symbioticum* within the leaf are driven by its unique methylotrophic nature. These bacteria utilise methanol, a byproduct of pectin methylesterase activity released during plant cell wall expansion and division, as their primary carbon and energy source (Iguchi *et al.*, 2015). This metabolic pathway is crucial for oxygen management. The rapid aerobic consumption of methanol and other single-carbon molecules by the bacteria creates a localised "micro-respiratory" environment. This high rate of oxygen consumption at the site of bacterial colonisation helps to maintain an internal O_2 tension low enough to protect the nitrogenase complex while allowing the bacteria to remain active in an otherwise aerobic tissue.

In conclusion, the integration of chemical and biological tools creates a multi-layered defence system for maize nutrition. By establishing a stable endophytic population, the plant gains a physiological "bypass" for nitrogen acquisition that is particularly effective during peak photosynthetic activity. This direct delivery system minimises energy losses associated with long-distance transport and nitrate reduction. Ultimately, these mechanisms fundamentally improve the overall Nutrient Use Efficiency (NUE) of the maize crop across diverse hydrothermal conditions.

Spatiotemporal dynamics of relative chlorophyll content. The ultimate expression of a plant's nitrogen status is its photosynthetic capacity, typically measured through relative chlorophyll content. The use of portable optical sensors, such as the Yara N-Tester, provides a non-destructive and accurate assessment of the "nitrogen-chlorophyll" relationship (Uddling *et al.*, 2007). In maize, maintaining high chlorophyll levels during the post-anthesis period is defined as the "stay-green" trait. This physiological characteristic is highly correlated with prolonged grain-filling duration and protection against premature leaf senescence (Tollenaar & Daynard, 2002; Thomas & Ougham, 2014). For modern hybrids with a high FAO index, the ability to maintain active photosynthesis under late-season thermal stress is the determining factor for reaching maximum kernel weight (Stasyk *et al.*, 2011).

The 2023 experimental cycle in the Kyiv Oblast (Bila Tserkva) was characterised by a favourable distribution of precipitation, providing an ideal baseline for evaluating the maximum potential of nitrogen management tools. At the initial monitoring stage (3WAAB), all treatments exhibited a statistically insignificant difference in their baseline nitrogen status ($p > 0.1$). However, as the plants transitioned toward the grain-filling stage (7WAAB), a rapid upward trend was observed. The combined application of N-Lock™ Max and Blue N™ on the N_{90} background resulted in a significant index increase to 775-786 units (Fig. 1).

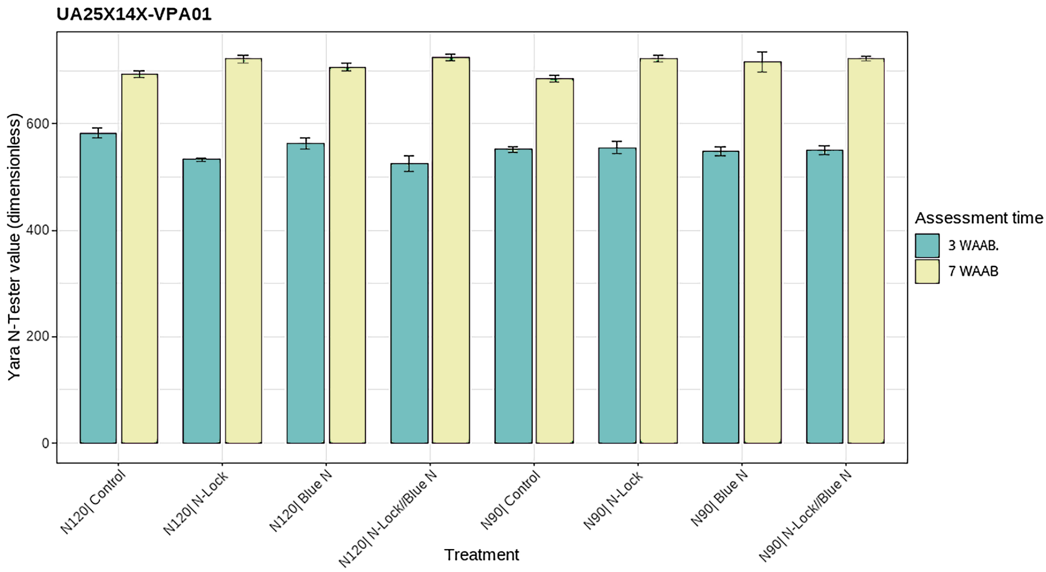

***Figure 1.** Dynamics of leaf chlorophyll index (Yara N-Tester, dimensionless units) in maize hybrid P8723 (FAO 280) depending on nitrogen nutrition systems (Kyiv Oblast, 2023)*

Note: vertical bars represent the standard error of the mean (±SE). Yara N-Tester values are expressed in dimensionless units reflecting relative chlorophyll content

Source: developed by authors based on own research

The data indicates that under sufficient hydrothermal conditions, nitrogen stabilisation leads to a substantial extension of photosynthetic activity. The physiological surge observed between Week 3 and Week 7 of monitoring confirms that the synergistic interaction prevents the premature loss of functional leaf area. While the control variants exhibited a plateau in the Yara N-Tester index during late maturity, the treated plots maintained a significant upward trend. This suggests that chemical inhibition of nitrification, combined with endophytic fixation, provides a steady nitrogen gradient necessary for biomass accumulation. Consequently, the maintenance of high chlorophyll density during these stages serves as a critical foundation for achieving maximum productivity levels.

Physiological adaptation to extreme hydrothermal stress. The results from the 2024 growing season in the Sharhorod district provide evidence for the adaptive role of the investigated technology under extreme conditions. This period was marked by a severe "heat dome" effect, with 19 consecutive days where maximum temperatures exceeded +33.4°C, peaking at +38.1°C. Such pressure typically triggers a sharp decline in chlorophyll due to photo-oxidation and nitrogen deficiency caused by the cessation of root-mediated uptake in desiccated soil (HTC 0.56). Under such stress, plants experience a surge in ethylene, which triggers the breakdown of light-harvesting complexes. In the control plots (N_{90} without treatments), the Yara N-Tester index plummeted to its seasonal minimum of 510.3 ± 5.4 units (Fig. 2).

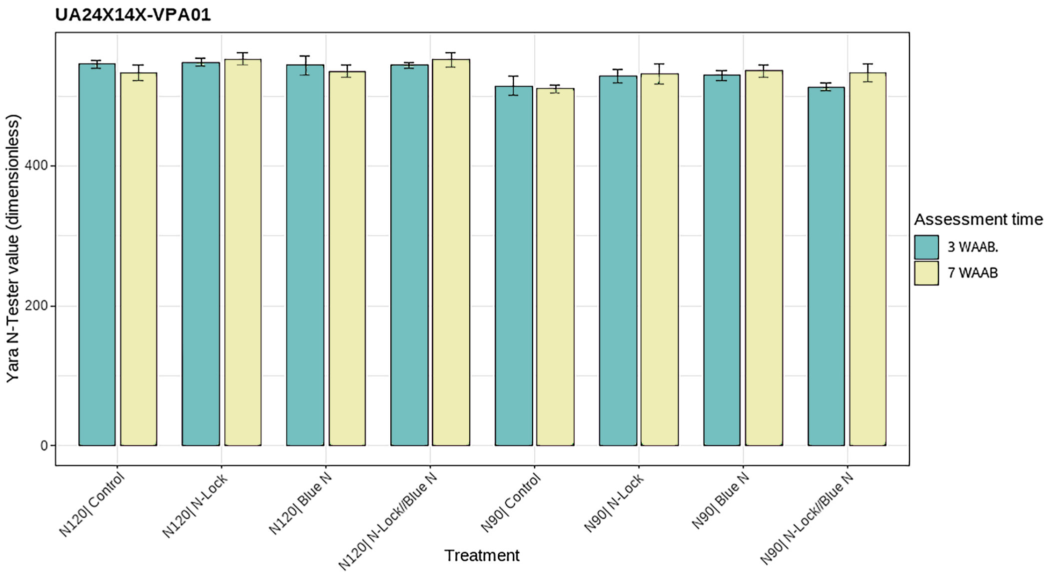

***Figure 2.** Dynamics of leaf chlorophyll index (Yara N-Tester, dimensionless units) in hybrid P9127 (FAO 310) as an indicator of physiological adaptation to drought conditions (Vinnytsia Oblast, 2024)*

Source: developed by authors based on own research

As illustrated in Figure 2, the variants treated with Blue N™ maintained a significantly higher nitrogen status, reaching 539.5 ± 7.4 units. This relative increase of 18.7 to 26.2 units over the control proved that *M. symbioticum* acts as a metabolic "bypass". While the root system's ability to absorb nitrogen was restricted by low soil water potential, the endophytic bacteria continued to fix N_2 directly within the leaf tissues. Furthermore, data from over 2,500 field trials indicate that strain SB23 promotes the regulation of key phytohormones, including auxins and cytokinins, which stimulate aerial biomass accumulation (Quemeda *et al.*, 2026). The production of ACC-deaminase by the bacteria likely mitigated the negative effects of abiotic stress, preventing "premature yellowing".

This physiological resilience highlights the importance of biological nitrogen sources when environmental factors paralyse conventional soil-based uptake. By preserving the integrity of chloroplast membranes from reactive oxygen species generated during thermal peaks, the technology ensured that the leaves remained active during the late stages of maturity. The fact that the N_{90} synergistic variant outperformed the non-stabilised N_{120} control proves that biological stress mitigation is more critical for resilience than high chemical loads. Ultimately, these results support the conclusion that foliar biological pathways serve as a crucial supplement to maintain nitrogen homeostasis during extreme climatic variability.

Furthermore, the obtained results are consistent with the findings of M. Farooq *et al.*, (2009) who emphasised that hormonal regulation is a primary defence mechanism against desiccation. The ability of *Methylobacterium* to potentially modulate plant stress signals and enhance antioxidant enzyme activity, as discussed by M.N. Dourado *et al.* (2015) and H. Gamit *et al.* (2023), provides a plausible explanation for the higher Yara N-Tester readings. By preserving the integrity of the chloroplast membranes from reactive oxygen species (ROS) generated during the +38°C peaks, the technology ensured that the "source" (leaves) remained active during the late stages of maturity.

Nutrient optimisation and resource use efficiency. The 2025 experimental cycle in the Tulchyn district focused on the mid-late hybrid P9074 (FAO 330), which has a high demand for nitrogen during late ontogenesis. In this environment, a distinct "equalisation effect" was observed, underscoring the resource-optimising potential of technology. Monitoring of the canopy during the late milk maturity stage (7WAAB) revealed that the synergistic system (N-Lock™ Max + Blue N™) on the moderate N_{90} background yielded a Yara N-Tester index of 722.0 ± 3.8 units (Table 1). This value was statistically equivalent to the N_{120} + Synergy variant (724.5 ± 15.2 units) and significantly outperformed the high-input N_{120} control, which reached only 693.0 ± 6.1 units.

Table 1. *Relative chlorophyll content (Yara N-Tester units) in maize leaves during the grain-filling phase (7WAAB) depending on the treatment and hydrothermal conditions*

Treatment (Code)	Kyiv Oblast (2023)	Sharhorod (2024)	Tulchyn (2025)
Urea N_{120} (Control)	772.0 ± 12.1	533.3 ± 10.3	693.0 ± 6.1
N_{120} + Synergy	761.5 ± 8.5	552.0 ± 10.3	724.5 ± 15.2
Urea N_{90} (Control)	732.0 ± 4.5	510.3 ± 5.4	684.0 ± 8.1
N_{90} + Synergy	775.0 ± 11.2	539.5 ± 7.4	722.0 ± 3.8

Note: Data are presented as Mean ± SE (n = 30 per plot). "Synergy" refers to the combined application of N-Lock™ Max and Blue N™

Source: developed by authors based on own research

This numerical advantage over the high-nitrogen control is of profound significance, demonstrating that qualitative management of the existing nitrogen pool is more effective for sustaining the photosynthetic apparatus than a simple quantitative increase in fertiliser. The success of this strategy in 2025 can be attributed to the synchronisation of nitrogen availability with the plant's peak demand. Nitrapyrin (N-Lock™ Max) effectively regulated the soil nitrogen cycle by slowing the microbial conversion of ammonium to nitrate. This ensured that a reservoir of stable nitrogen remained available in the root zone during the late reproductive stages, precisely when mid-late hybrids like P9074 reach their maximum sink-strength. Simultaneously, the stay-green effect was highly evident across the experimental plots. While the control leaves on the N_{120} and N_{90} plots showed early symptoms of chlorophyll degradation and nitrogen remobilisation (senescence), the synergistic treatments maintained a high density of active chloroplasts, reflected in the dark green colour of the upper canopy (Fig. 3).

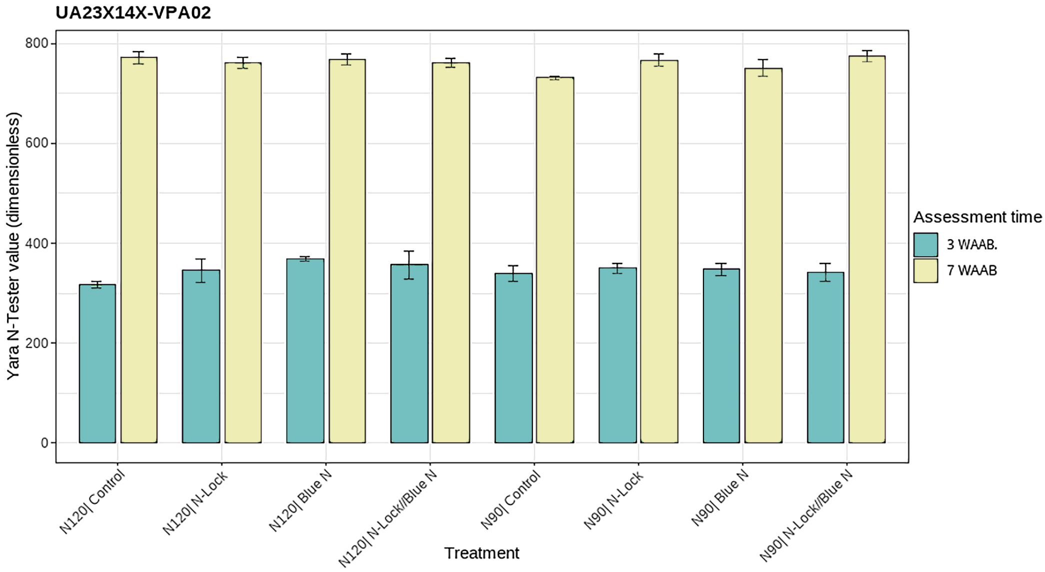

Figure 3. *Dynamics of leaf chlorophyll index (Yara N-Tester, dimensionless units) as an indicator of the stay-green effect in hybrid P9074 (FAO 330) (Vinnytsia Oblast, 2025)*

Source: developed by authors based on own research

The graphical representation confirms high technological stability for mid-late hybrids. By delaying the transition to the senescence phase, the dual-action system allows the plant to prolong the synthesis of photoassimilates. This phenomenon ensures that the realisation of the hybrid's genetic potential is secured despite the hydrothermal challenges of the growing season. The high degree of chlorophyll preservation in the N_{90} + Synergy variant confirms that a balanced metabolic environment can be maintained even with a 25.0% lower nitrogen input level. This decoupling of yield from high chemical inputs represents a major milestone for sustainable agriculture in the Forest-Steppe of Ukraine.

Correlation between photosynthetic status and productivity. The physiological merit of the investigated nitrogen management technology is determined by its ability to convert solar radiation into harvestable biomass. To quantify this, a regression analysis was performed between the Yara N-Tester index at the grain-filling stage (7WAAB) and the final grain yield (t/ha). The results demonstrate a robust and statistically significant linear correlation across all experimental sites and years (Fig. 4). The coefficients of determination (R^2) ranged from 0.82 to 0.93, confirming that the nitrogen status during the post-anthesis period explains the vast majority of yield variance. The strongest correlation was observed in the 2023 trial (Kyiv Oblast, $R^2 = 0.93$**)**, where optimal moisture conditions allowed the plants to fully translate their enhanced nitrogen status into grain weight. Under these conditions, the regression slope was steepest, indicating that every incremental increase in the N-Tester index yielded a higher marginal return in grain productivity. Conversely, in the drought-stressed 2024 season (Sharhorod), the correlation remained high ($R^2 = 0.82$), although the overall yield levels were lower. This indicates that even when water is the primary limiting factor, the "biological nitrogen buffer" provided by Blue N™ remains a critical survival mechanism, preventing the total collapse of the photosynthetic apparatus. In the 2025 Tulchyn cycle, the correlation reached $R^2 = 0.89$, with the synergistic variants occupying the same space on the regression line as the high-input control, thereby proving the compensatory capacity of the investigated technology.

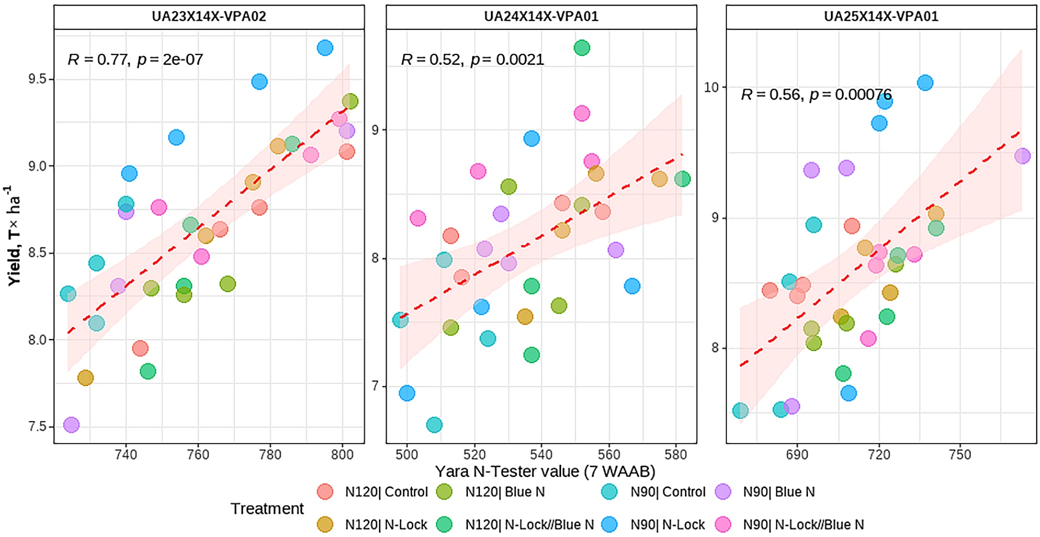

***Figure 4.** Correlation between the Yara N-Tester index (dimensionless units) at the grain-filling stage (7WAAB) and maize grain yield across different experimental environments (2023-2025)*

Note: statistical significance is established at $p \leqslant 0.1$

Source: developed by authors based on own research

The significant yield increases observed in the "Synergy" variants (N-Lock™ Max + Blue N™) can be explained through the source-sink relationship. In maize, approximately 40-60% of the nitrogen found in the grain at maturity is remobilised from the vegetative tissues, particularly the leaves. If the soil nitrogen supply is exhausted early or blocked by drought, the plant accelerates leaf senescence to meet the grain's demand, leading to a premature loss of photosynthetic surface area. Obtained data show that the investigated technology effectively disrupts this cycle of early senescence. By sequestering nitrogen in the stable ammonium form (NH_4^+) in the soil and providing a supplemental foliar N_2-fixation pathway, the system ensures a continuous "source" of nitrogen. This delays the degradation of chlorophyll-protein complexes, a phenomenon known as the functional stay-green effect (Thomas & Ougham, 2014). The prolongation of leaf activity by even days 5-7 during the late milk-wax stage can lead to a substantial increase in kernel depth and test weight, as confirmed by the higher PFP (Partial Factor Productivity) values recorded in the study. This extension of the photosynthetic period aligns with the physiological models of I.A. Ciampitti & T.J. Vyn (2012), who demonstrated that yield stability in modern hybrids is governed by the efficiency of nitrogen remobilisation. By delaying senescence, a process described in detail by P.L. Gregersen *et al.* (2013), the nitrogen stabilisation technology ensures that the grain sink remains active during the final stages of maturity, maximising the accumulation of dry matter.

A critical point of discussion is why the combination of a nitrification inhibitor and a biostimulant consistently outperformed individual applications. Nitrapyrin (N-Lock™ Max) acts as a foundational stabiliser, ensuring that the primary mineral nitrogen dose (N_{90}) remains within the reach of the root system for a longer duration. However, its efficacy is dependent on soil moisture. When the soil surface dries out, as seen in July 2024, the root-mediated uptake of even stabilised nitrogen is compromised. At this precise moment of abiotic stress, the foliar endophytic bacteria (*Methylobacterium symbioticum*) reach their peak population density. By fixing nitrogen directly within the phyllosphere, Blue N™ mitigates the transient nitrogen deficit caused by drought. Furthermore, the bacteria's metabolic by-products may further stimulate the plant's antioxidant defence systems. This "dual-layer" protection ensures that the nitrogen homeostasis of the crop is maintained across a wider range of hydrothermal conditions (HTC 0.56-1.09), providing resilience that single-component systems lack. Beyond the physiological benefits, the ability of the N_{90} + treatments variant to match or exceed the N_{120} control has profound implications for Nitrogen Use Efficiency (NUE). Traditionally, farmers compensate for potential nitrogen losses by increasing total fertiliser rates, which leads to groundwater contamination and high input costs.

Experimental results demonstrate that a 25% reduction in nitrogen application rates is not only feasible but also agronomically viable when integrated with stabilisation tools. Such a reduction significantly lowers the carbon footprint of maize production, as the industrial synthesis and field application of nitrogen fertilisers are among the most energy-intensive processes in contemporary farming. The high PFP value of 98.0 kg grain per 1 kg of applied N achieved in this study reflects a broader trend toward "decoupling" crop yields from excessive chemical inputs. As highlighted by Y. Rouphael & G. Colla (2020), the integration of microbial biostimulants represents a cornerstone of "climate-smart" agriculture and a paradigm shift in nutrient management, enabling substantial fertiliser reductions without compromising the inherent biological potential of the crop. This results confirm a strong and stable relationship between nitrogen status during grain filling and final maize yield, validating the Yara N-Tester as an effective diagnostic tool. The combined use of N-Lock™ Max and Blue N™ ensures sustained nitrogen availability, delays senescence, and stabilises productivity even under drought stress.

Conclusions

The integrated application of the nitrification inhibitor nitrapyrin (N-Lock™ Max) and the endophytic nitrogen fixer *Methylobacterium symbioticum* (Blue N™) fundamentally alters the nitrogen homeostasis and senescence dynamics in maize. At the critical grain-filling stage (7 weeks after application), the synergistic treatments maintained a significantly higher relative chlorophyll content, increasing the Yara N-Tester index by 14 to 42 units compared to the control, thereby inducing a functional stay-green effect. This effect is driven by the synchronisation of soil-based ammonium stabilisation with a direct foliar biological supply, ensuring that the photosynthetic "source" remains active during peak demand.

The foliar endophytic nitrogen fixation pathway served as a vital physiological buffer during extreme abiotic stress. In the drought-stressed 2024 season (Sharhorod), characterised by 19 days of temperatures above +33°C, the use of Blue N™ compensated for the lack of root-mediated nutrient uptake, maintaining the photosynthetic capacity at a level 6-8% higher than the control. Furthermore, the metabolic advantage of direct ammonium assimilation provided the plants with significant energy savings (ATP), which were redirected toward grain filling and biomass accumulation. A robust compensatory capacity of the dual-action nitrogen stabilisation system has been demonstrated. The application of N-Lock™ Max and Blue N™ on a moderate background (N_{90}) allowed for a 25% reduction in total nitrogen fertiliser rates without a reduction in photosynthetic potential or grain yield, matching the performance of the non-stabilised N_{120} high-input control. This demonstrates that qualitative management of the nitrogen pool is superior to a mere quantitative increase in chemical fertilisation. Statistical analysis confirmed that the Yara

N-Tester index measured during the late stages of vegetation is a highly reliable predictor of maize productivity (R_2 = 0.82-0.93, p ≤ 0.1). This validates the effectiveness of optical nitrogen monitoring as a diagnostic tool for evaluating the physiological status of crops under varying hydrothermal conditions in the Forest-Steppe of Ukraine.

Future research should focus on the impact of these nitrogen stabilisation tools on the qualitative characteristics of the grain (amino acid profile and protein fractions) and the potential reduction of the carbon footprint in industrial maize production systems.

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Conflict of Interest

None.

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Фізіологічна ефективність інгібіторів нітрифікації та ендофітних азотфіксаторів у посівах кукурудзи

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Анотація. В умовах глобальних кліматичних змін стабільність азотного статусу кукурудзи є критичним чинником забезпечення високої продуктивності, особливо під час термічного стресу на пізніх етапах вегетації. Мета дослідження – обґрунтувати фізіологічний вплив нітрапірину та *Methylobacterium symbioticum* на активність фотосинтетичного апарату кукурудзи за різних гідротермічних сценаріїв. Дослідження проводили у 2023-2025 рр. на чорноземах типових та сірих лісових ґрунтах у Лісостепу України. Об'єктами дослідження були гібриди P8723, P9127 та P9074. Відносний вміст хлорофілу визначали за допомогою приладу Yara N-Tester. Статистичну обробку методом LSD-тесту. Встановлено, що інтеграція N-Lock™ Max та Blue N™ забезпечує виражений «Stay-green» ефект, що зумовлює на 5-12 % вищий індекс хлорофілу порівняно з контролем у фазу наливу зерна. З'ясовано, що за умов екстремальної посухи 2024 р. (ГТК 0,56) ендофітна азотфіксація ефективно компенсувала дефіцит ґрунтового азоту, підвищивши індекс Yara N-Tester на 18,7-26,2 од. Доведено, що застосування цих препаратів на помірному фоні N_{90} дозволяє досягти фізіологічних показників, еквівалентних повному фону N_{120} без використання стабілізаторів. Математично підтверджено пряму кореляцію між вмістом хлорофілу на пізніх стадіях вегетації та кінцевою врожайністю зерна. У синергічних варіантах спостерігалося суттєве зростання врожайності, що перевищувало показники за роздільного застосування препаратів завдяки підтримці метаболічної активності в період пікового термічного стресу. Результати демонструють, що синергія хімічної стабілізації та біологічної фіксації азоту забезпечує стабільний азотний гомеостаз кукурудзи в умовах термічного стресу, що дозволяє оптимізувати норми азотних добрив на 25 %. Такий інтегрований підхід виступає дієвою стратегією підвищення стійкості агроценозів та ефективності використання ресурсів у кліматично-орієнтованому землеробстві

Ключові слова: *Zea mays* L.; нітрапірин; *Methylobacterium symbioticum*; stay-green ефект; Yara N-Tester; азотний гомеостаз