

Evaluation of biostimulant effectiveness in “Bois noir” control in vineyards

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INTRODUCTION

Bois noir (BN) infection dynamics in vineyards are determined by two main driving forces: new infections and recovery (Mori *et al.*, 2015). Several studies showed that recovery can be induced by stressing the plants through uprooting followed by immediate transplanting, pruning or pollarding, treating the plants with resistance inducers, and grafting the plants with recovered shoots (Pierro *et al.*, 2024). Several studies demonstrated that treatment of grapevine canopy by biostimulants can prevent diseases (Gutierrez-Gamboa *et al.*, 2019). Recently, small-scale field trials showed that treatments with high concentration of L- α amino acids (commercial formulation Delfan plus, Tradecorp Italia, Saronno (VA), Italy) reduced the percentage of BN-infected symptomatic vines increasing the recovery rate (Moussa *et al.*, 2021). The aim of this study was to evaluate the preventive and curative effects of this biostimulant in large-scale field trials.

MATERIALS AND METHODS

Field trials were carried out from 2021 to 2023 in four Chardonnay BN-affected vineyards (size ranging from 0.7 to 1.2 Ha) located in Franciacorta (North Italy). In September 2021, the vineyards were monitored for grapevine yellows symptoms. Each vineyard was divided in two blocks: one treated with the L- α amino acids (commercial formulation Delfan plus) (T), and one untreated (NT). In each year, the activities included: (i) applications with the biostimulant every two weeks, from mid-April (10 cm long grapevine shoots) to the beginning of August (7 treatments/year); (ii) mapping and sampling of symptomatic and asymptomatic vines in September; (iii) extraction of total nucleic acids and molecular identification of '*Candidatus* Phytoplasma solani' by nested PCR-based amplification of *stamp* gene; (iv) molecular characterization of phytoplasma strains by means of *stamp* gene nucleotide sequence analysis; (v) statistical analysis (Mann-Whitney U Test in SPSS software) to evaluate any differences in the curative and preventive effect based on BN-symptom observations. The curative effect (percentage of recovered grapevines) was calculated considering the health status of symptomatic plants in 2021 over the following two years. The preventive effect was calculated considering the percentage of new symptomatic plants (grapevines showing symptoms for the first time) in the two-year period 2022-23.

RESULTS AND DISCUSSION

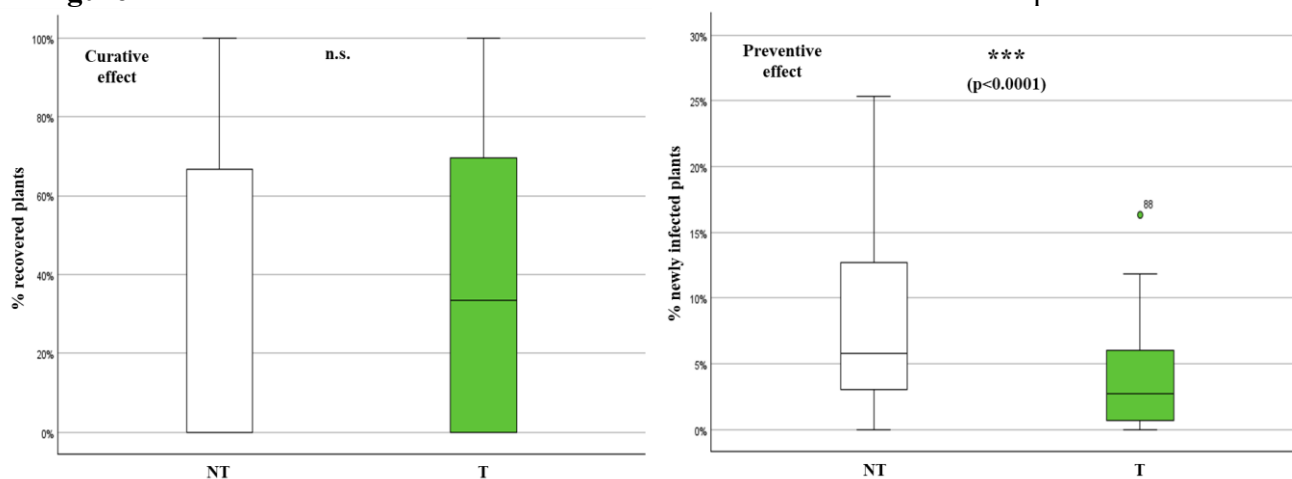
Statistical analyses showed that: (i) curative effect was higher in T block in vineyard 4 and in NT block in vineyard 2, while no differences were found between the blocks in vineyards 1 and 3; (ii) preventive effect was higher in T block in vineyards 3 and 4, while no differences were found in T and NT blocks in vineyards 1 and 2 (Table 1). Analyses of overall data highlighted no statistically significant differences in curative effect, while preventive effect was found higher in T blocks (Figure 1). Molecular and bioinformatics analyses showed the presence of eight distinct '*Ca. P. solani*' strains carrying different *stamp* gene variants (St1, St5, St8, St10, St16, St18, St19, St30). The statistical analysis showed a uniform distribution of such phytoplasma strains within the vineyard, reinforcing the evidence that the effect on BN incidence in the blocks is due to the action of the biostimulant and not to possible differences in the '*Ca. P. solani*' strain virulence. Based on the obtained results and in

accordance with previous study (Minuz *et al.*, 2020), it is reasonable to hypothesize that the L- α amino acids preventive effect can be related to differences in volatiles emitted by the treated plants, affecting the attractiveness to the main vector *Hyalesthes obsoletus*. Symptom observation and molecular analyses in 2024 are necessary to confirm the effectiveness of the biostimulant in BN control.

Table 1. Curative and preventive effects of L- α amino acids treatments

Vineyard	Block	No. symptomatic plants (2021)	% recovered plants (2022/23)	No. symptomless plants (2021)	% newly infected plants (2022/23)
1	T	86	31 a	1247	12 a
	NT	98	41 a	2256	14 a
2	T	344	58 a	3574	3 a
	NT	69	84 b	3164	4 a
3	T	293	44 a	2807	8 a
	NT	320	28 a	2530	12 b
4	T	46	93 b	5757	1 a
	NT	39	46 a	4323	3 b

Figure 1. Overall data statistical differences in L- α amino acids curative and preventive effect



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