

Evaluation of biostimulant effectiveness in grapevine 'bois noir' management

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This work, carried out from 2017 to 2020, aimed to evaluate the effectiveness of four commercial products (Delfan plus, Phylgreen, Phylgreen Kuma, Vegenergy), which use as biostimulants is allowed in organic farming, in the management of 'bois noir' (BN), a grapevine disease associated with '*Candidatus* Phytoplasma solani'. The field trials were carried out in a Chardonnay BN-affected organic vineyard located in Gussago (Franciacorta, North Italy), monitored since 2012. In September 2017, the vineyard was monitored for grapevine yellows symptoms and a map reporting the phytosanitary state of each plant was created. Based on this map, four treated blocks (each one treated with one biostimulant), and one untreated (control) block were arranged. The activities, conducted from 2018 to 2020, included: (i) applications with the biostimulants from mid-April (10 cm long grapevine shoots) to the beginning of August (every two weeks (7 treatments/year); (ii) mapping and sampling of symptomatic and asymptomatic vines in September; (iii) extraction of total nucleic acids and molecular identification of '*Ca. P. 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 to evaluate any differences in the curative and preventive effect on BN-symptoms. The statistical analysis, conducted on the increase of symptomatic grapevines from 2017 (pre-treatment) to 2020 (cumulative effect of the treatments conducted from 2018 to 20), showed a significant decrease in symptomatic vines in the Delfan plus block (-4.5 percentage points) in comparison to the other blocks. The curative effect (percentage of recovered grapevines) was calculated considering the health status of symptomatic plants in 2017 over the following three years. Percentage of recovered plants in the Delfan plus block was higher than in all the other blocks, although the difference observed towards the block treated with Vegenergy and the untreated one was not statistically significant. The preventive effect was calculated considering the percentage of new symptomatic plants (grapevines showing symptoms for the first time) in the three-year period 2018-20. Statistical analyses did not reveal significant differences on the preventive effect observed in the different blocks. However, the percentage of new symptomatic grapevines in the block treated with Delfan plus was lower than in the other blocks. 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 biostimulants and not to possible differences in the '*Ca. P. solani*' strain virulence. Based on the obtained results, it emerged that a reduction in the percentage of symptomatic vines was observed exclusively in the block treated with Delfan plus. Further open field trials carry out in different grape growing areas and on different cultivars are necessary to confirm the effectiveness of biostimulants BN control.

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