MASTER II Biologie Végétale
« Plant in Sustainable AgroEcosystems »
2024-2025

INTERNSHIP REPORT PRESENTED BY:

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SUBJECT:

EVALUATION DES PRATIQUES AGROECOLOGIQUES INNOVANTES EN VITICULTURE ET ARBORICULTURE :

Impact des Biostimulants, Biocontrôles et Paillis sur la Réduction des Stress et l'Amélioration de la Qualité

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ABSTRACT (French)

Cette étude a évalué l'impact de biostimulants (FRAY7, Nutribio 5.4.2 + Si et ACT'IN, Frayssinet) et d'un paillage à base d'*Inule visqueuse* (*Dittrichia viscosa*) sur l'état hydrique, la vigueur, la santé et la qualité des fruits dans deux vignobles et un verger de pêchers du Roussillon (Pyrénées-Orientales, France). Les résultats indiquent que le paillage améliore la conservation de l'humidité du sol et réduit le développement des adventices. Les biostimulants ont montré des effets positifs sur la vigueur des plantes, un retard partiel des symptômes de maladies fongiques (mildiou, cloque) et une amélioration de la qualité des fruits en verger. Ces pratiques apparaissent comme des leviers agroécologiques intéressants pour renforcer la résilience des cultures méditerranéennes face au stress hydrique, bien que leur efficacité dépende fortement des conditions locales et de leur intégration dans une gestion globale de l'agrosystème.

ABSTRACT (English)

This study evaluated the impact of biostimulants (FRAY7, Nutribio 5.4.2 + Si, and ACT'IN, Frayssinet) and a mulch based on *Dittrichia viscosa* (sticky inula) on water status, vigor, plant health, and fruit quality in two vineyards and one peach orchard in Roussillon (Pyrénées-Orientales, France). Results showed that mulching improved soil water conservation and reduced weed development. Biostimulants enhanced plant vigor, partially delayed fungal disease symptoms (downy mildew, leaf curl), and improved fruit quality in the orchard. These practices appear to be promising agroecological tools to strengthen the resilience of Mediterranean crops facing water stress, although their effectiveness strongly depends on local conditions and their integration into comprehensive agroecosystem management strategies.

Acknowledgements

Firstly, I would like to thank Professor Cédric Bertrand and Mr Rémi Espert for their guidance throughout my internship. I am grateful to them for the trust they placed in me to carry out this project management and coordination internship. I thank them for their numerous pieces of advice and for proofreading my report. I would like to thank the staff at Akinao, who helped me during my internship. I would also like to thank Dr Marie-Emmanuelle Saint Macary from Frayssinet, with whom I had the opportunity to go out into the field and exchange ideas.

LIST OF ABBREVIATIONS

- **ANOVA** – Analysis of Variance
- **B** – Control
- **BP** – Control+ Mulch (Paillage)
- **CS** – Leaf pressure chamber (Chambre à pression de Scholander)
- **FRAY7** – Biostimulant product from Frayssinet
- **I** – Biostimulant FRAY7 1 + Nutribio 5.4.2+ Si
- **IP** – Biostimulant FRAY7 1 + Nutribio 5.4.2+ Si + Mulch (Paillage)
- **N** – Biostimulant FRAY7 2 + Nutribio 5.4.2+ Si
- **NP** – Biostimulant FRAY7 2 + Nutribio 5.4.2+ Si + Mulch (Paillage)
- **Si** – Silicon
- **SPAD** – Soil Plant Analysis Development (index for leaf chlorophyll content)
- **WUE** – Water Use Efficiency

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INTRODUCTION

1. Climatic context and drought in the Pyrénées-Orientales

Over the last decade, the Pyrénées-Orientales department has experienced a marked intensification of climatic hazards, mainly linked to rising temperatures and decreased water availability. Located at the southern edge of France, this Mediterranean region is naturally characterized by a hot, dry climate, with historically low annual rainfall (on average 500–600 mm/year, versus nearly 900 mm at the national level). However, data from Météo-France (2023) and Meteociel (2024) indicate a significant worsening of the situation since 2010: mean summer temperatures have increased by approximately 1.2 °C, and prolonged drought episodes have become more frequent and more severe.

The year 2022 is a particularly striking example: the department recorded a precipitation deficit exceeding 40% compared with seasonal normals, coupled with several summer heat waves exceeding 38 °C. This combination caused exceptional soil desiccation and a marked weakening of groundwater tables, which were already stressed by recurring rainfall deficits. In 2023, the Pyrénées-Orientales ranked among the French departments most affected by drought, with strict water restrictions imposed as early as spring. These conditions have directly impacted agricultural production, reducing yields and jeopardizing the long-term viability of some crops.

The trend observed in the department illustrates more broadly the vulnerability of Mediterranean regions to climate change. The IPCC projections (2021) confirm that southern Europe will be one of the areas most exposed to water and heat stresses in the coming decades. This situation places the Pyrénées-Orientales on alert regarding the adaptation of its agricultural systems.

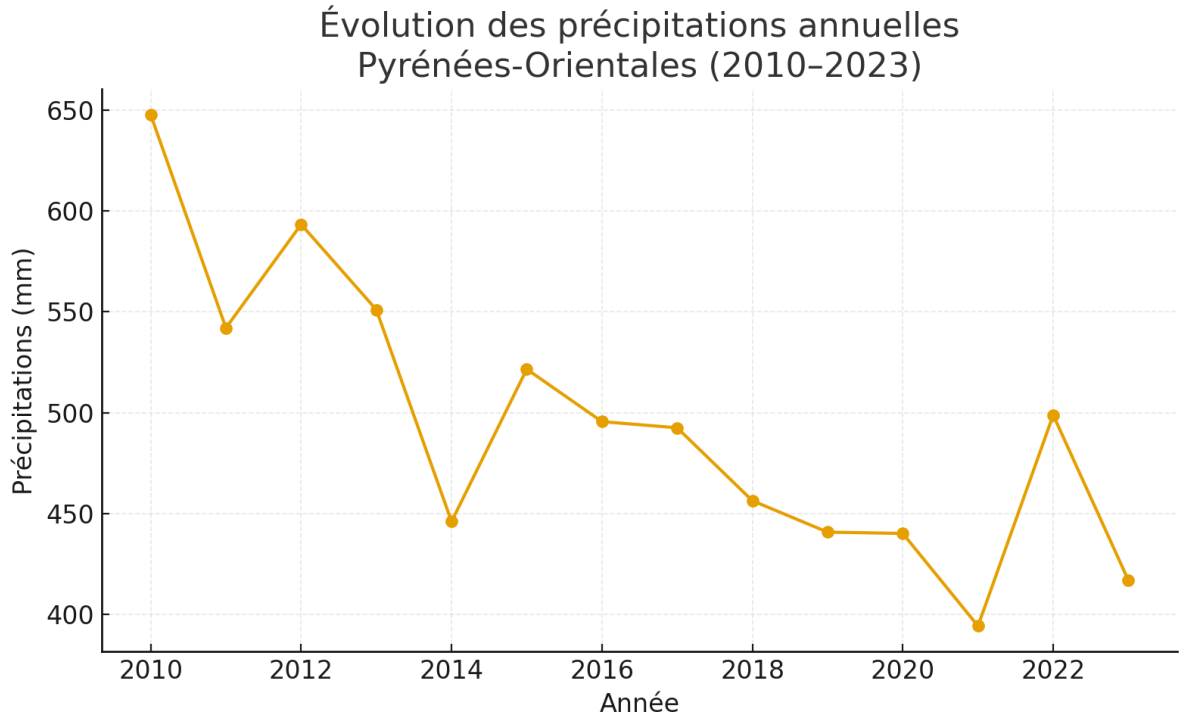


Figure 1. *Évolution des précipitations annuelles dans les Pyrénées-Orientales (2010–2023), données Meteociel/Météo-France.*

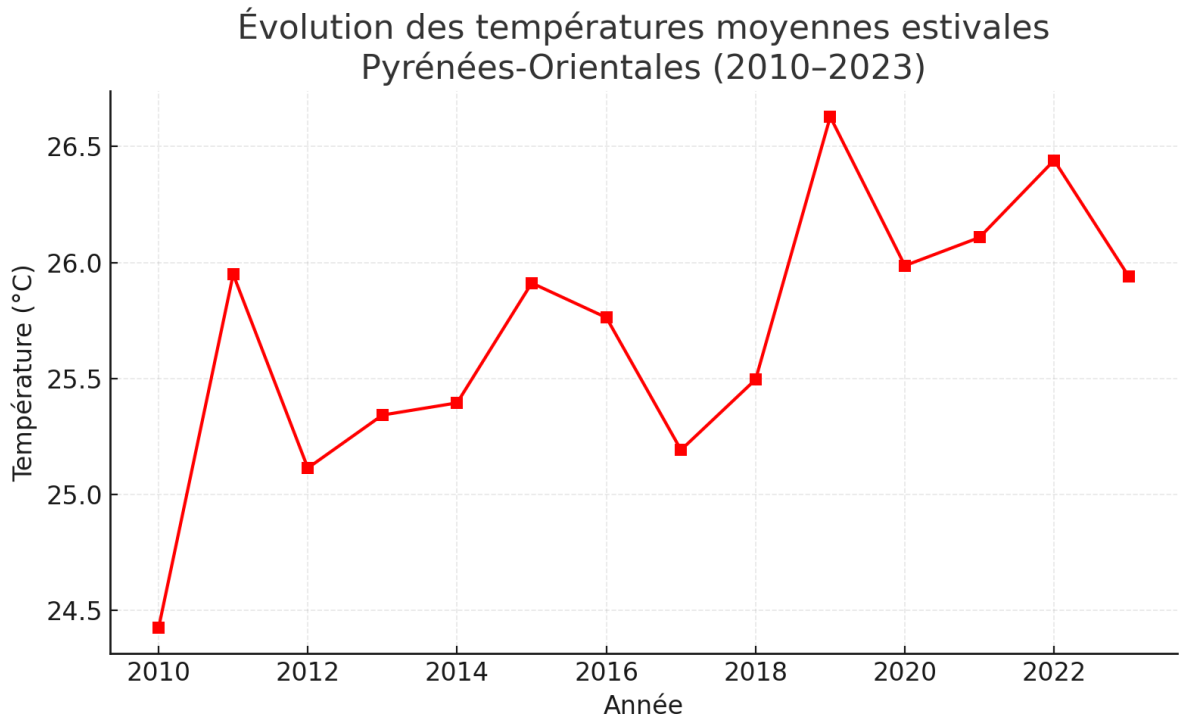


Figure 2. *Évolution des températures moyennes estivales dans les Pyrénées-Orientales (2010–2023), données Meteociel/Météo-France.*

2. Importance of viticulture and tree fruit production

Viticulture and fruit tree production represent two major sectors for the Pyrénées-Orientales department, both economically and culturally. Vine occupies around 20,000 hectares, i.e. more than half of the utilized agricultural area (FranceAgriMer, 2022). Local appellations (Banyuls, Maury, Rivesaltes, Côtes-du-Roussillon) give the territory a strong identity and generate significant added value, notably through wine exports. At the national level, France remains one of the leading wine exporters worldwide, which makes the wine sector strategically important for the agricultural economy.

Arboriculture focuses mainly on stone fruits, in particular peaches and nectarines. With nearly 7,000 hectares of orchards, the Roussillon area is one of the main fruit-producing basins in France (Agreste, 2023). These productions contribute to the region's gastronomic identity and reputation, while also representing an important source of seasonal employment.

However, both sectors are particularly sensitive to climate change. Grapevine, although relatively tolerant to drought thanks to deep rooting, suffers reduced productivity and altered grape quality during prolonged drought episodes (Gutiérrez-Gamboa et al., 2024). Excessive water stress leads to a reduction in leaf area, decreased photosynthesis and changes in the aromatic and phenolic composition of grapes, with direct consequences on wine quality.

Peach trees, for their part, show even greater sensitivity. This species requires regular and substantial water supply for fruit growth. Water deficit reduces fruit size, firmness and taste potential, and increases susceptibility to physiological disorders (Li et al., 2025). Over time, the accumulation of dry years can undermine orchard longevity and jeopardize the economic viability of farms.

3. Innovative agroecological practices

To address climate change challenges, Mediterranean agriculture must evolve toward more resilient and sustainable systems. Agroecology is a promising approach based on optimizing biological processes and ecosystem interactions (Altieri, 2018). Its key principles include crop diversification, sustainable soil management, reduction of synthetic inputs and valorization of biodiversity.

In viticulture and arboriculture, several agroecological levers are particularly relevant:

- Mulching and ground cover: reduce evaporation, improve soil structure and foster biological activity.
- Biocontrol and biostimulation practices: offer alternatives to chemical inputs by strengthening plant natural defenses and tolerance to abiotic stress.
- Species diversification (intercrops, agroforestry): create more favorable microclimates and reduce sanitary risk.

These levers align with current public policies, notably the European Green Deal and the "Farm to Fork" strategy, which aim, among other goals, to reduce pesticide use by 50% by 2030 and to promote sustainable agricultural systems. In this framework, experimenting with innovative solutions in Mediterranean viticulture and arboriculture is a scientific and economic priority.

4. Biostimulants: definitions, mechanisms and experimental results

Definition and regulatory framework

Biostimulants are defined under EU Regulation (2019/1009) as products intended to "stimulate plant nutrition processes independently of the product's nutrient content," with the aim of improving one or more of the following: nutrient use efficiency, tolerance to abiotic stress, crop quality traits, or the availability of nutrients in the soil or rhizosphere.

Mechanisms of action

Biostimulants encompass a wide variety of substances and microorganisms (amino acids, seaweed extracts, humic and fulvic acids, mycorrhizal fungi, plant growth-promoting rhizobacteria, silicon, etc.). Their modes of action include:

- stimulation of cellular signalling and hormonal pathways (auxins, cytokinin, abscisic acid);
- improvement of photosynthesis and nutrient assimilation.
- activation of antioxidant defences to limit oxidative damage.
- enhancement of water use efficiency through stomatal regulation.

Experimental results

Numerous studies support the effectiveness of biostimulants under stress conditions. In grapevine, applications have been associated with improved stomatal regulation, enhanced photosynthesis and increased synthesis of phenolic compounds, while mitigating the effects of water and heat stress (Gutiérrez-Gamboa et al., 2024; Petoumenou et al., 2025). In peach, foliar silicon and protein hydrolysates have been reported to improve drought tolerance, reduce physiological fruit drop and enhance final fruit quality (Asgari-Targhi et al., 2021; Li et al., 2025).

5. Case study: Frayssinet biostimulants

Frayssinet, a major French company in plant nutrition and biostimulation, develops several innovative product lines tested in this internship:

- FRAY7: a biostimulant combining three technologies (IP TECH, PRO-T10 and BIOTECH C2A). Multi-year trials have shown an average increase of 14% in vigor, 20% in yield, and a 31% reduction in powdery mildew pressure in vine (Frayssinet, 2023).
- Nutribio 5.4.2+Si: a liquid formulation combining NPK, silicon, polyphenols, amino acids and Osyr®. Its action aims to strengthen photosynthetic efficiency and tolerance to oxidative stress.
- ACT'IN: an organic biostimulant based on amino acids and organic compounds, intended to stimulate fruit metabolism under stress, with particular focus on fruit quality.

6. Mulching and the interest of inula

Mulching is a complementary agroecological lever, especially in Mediterranean contexts. It reduces soil water evaporation, improves structure, limits weed growth and creates a favorable microclimate for root development. Mulch also stimulates soil biological activity and biodiversity (Tejada *et al.,* 2008).

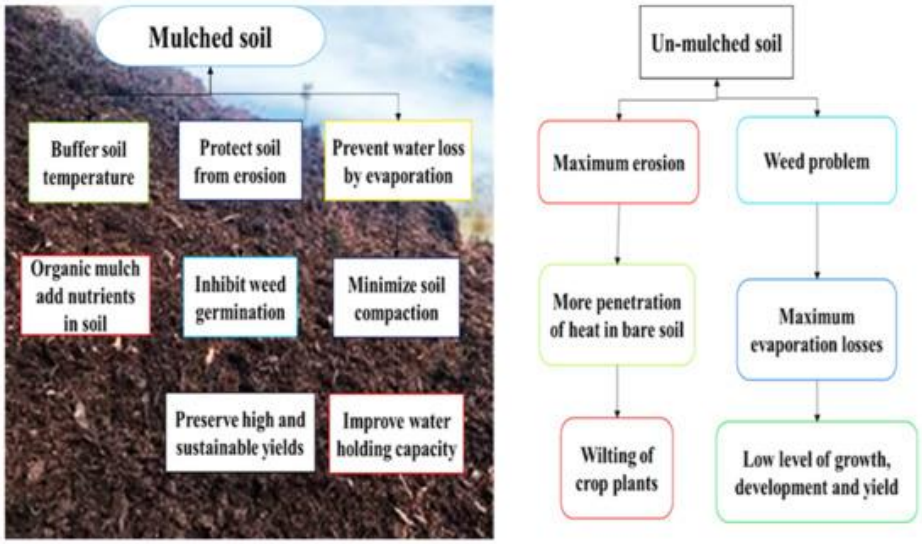


Figure 3. *Comparative approach to the mulched and un-mulched soil/crops. (Hossam S. El-Beltagi, et al. 2022)*

The inula mulch (*Dittrichia viscosa (L.) Greuter 1973*), a perennial Mediterranean plant, has specific interest. Its residues contribute to soil cover and release secondary compounds that may limit the development of certain weeds via allelopathy. Although scientifically less documented, its use appears promising in Mediterranean orchards and vineyards to enhance hydric resilience.

The combined use of biostimulants and mulching forms the core of the experimental approach presented here.

Climate change requires a profound reorganization of Mediterranean agricultural systems. In the Pyrénées-Orientales, viticulture and tree fruit production — key sectors of the territory — are threatened by the increased frequency of droughts and the depletion of water resources. In response, innovative agroecological practices represent promising solutions.

Biostimulants, by stimulating plant physiology and stress tolerance, and mulching, by improving soil water management, are two complementary levers. This internship evaluates the effectiveness of several Frayssinet biostimulants (FRAY7, Nutribio 5.4.2+Si, ACT'IN) and an inula mulch, in order to generate scientific data under real cropping conditions in a Mediterranean context.

7. Internship organisation

This master's internship was carried out within a project supported by the UPVD Foundation and its AgroLab BioMed Chair. The foundation, created in 2008, aims to foster links between academic and socio-economic actors in the Pyrénées-Orientales territory. It supports research and the dissemination of scientific culture, promotes quality education and equal opportunities, and encourages innovative projects.

The UPVD Foundation oversees several chairs, which are programs of innovation and excellence designed to develop teaching and research expertise in innovative thematic areas. Chair topics address economic, societal or scientific issues. The AgroLab BioMed Chair aims to foster innovation in agriculture focused on reducing synthetic inputs. It supports large-scale experimental projects addressing local challenges in the Pyrénées-Orientales, in viticulture, arboriculture, market gardening and urban permaculture. The chair works to develop future solutions for sustainable agriculture according to three axes: (1) in-field experimentation at realistic scale in organic farming, (2) stakeholder exchanges (biocontrol industry, institutions, researchers, farmers and consumers) through conferences, videos and workshops, and (3) dissemination of results and new practices to a broad audience.

It is a research organization based on cooperation between private companies, local producers, administrative bodies and the University of Perpignan. Its main objectives are to reduce synthetic inputs, support the adoption of bio-based solutions, and implement a Living Lab. The AgroLab BioMed Living Lab is a user-driven research system where innovation is designed with the users; it aims to engage end-users to achieve concrete improvements to existing systems. The Living Lab receives project ideas and problem statements from farmers that the Lab and its partners work to realize.

8. Internship objectives

This internship aims to study the combined effects of biostimulant products and a mulch on grapevines and a peach orchard under drought conditions.

Objectives:

- Mulch: observe effects on soil water status and surface microclimate.
- Biostimulants: evaluate effects on stress tolerance and on disease incidence by analyzing each plot separately.
- Plants: record initial indicators of production and quality.

Our hypotheses propose that treated plants will show improved yield and fruit quality compared with untreated plants under drought.

MATERIALS AND METHODS

1. Experimental plots

The study was carried out on three different plots, located about five kilometers apart between Ponteilla and Thuir, southwest of Perpignan. They included two vineyard plots and one orchard plot. In the vineyards, the experimental unit was the row, while in the orchard, the unit was the tree, with vines and/or leaves considered as sub-samples.

The first vineyard plot, hereafter referred to as Vigne Akinao, belongs to the company Akinao and is located in Ponteilla. This vineyard is nearly eighty years old, planted with a cousin of *Grenache noir*. The plot was recently acquired by Akinao and pruned in February, before the start of this internship. In March, weeds were mowed. It is organically farmed and not irrigated.

The second vineyard plot, referred to as Vigne Saint-Joseph, is located on the Saint-Joseph wine estate in Thuir. It consists of *Chardonnay* vines, organically cultivated and irrigated by drip starting in June. The experiment was conducted on a section of the vineyard comprising thirteen rows, each with between twenty-five and sixty-five vines.

The third plot, the **orchard**, is a peach orchard located in Ponteilla. It consisted of six rows with approximately thirteen to fourteen trees each. Rows and inter-rows were mowed by the farmer before and during the experiment. The trees were irrigated with drip irrigation throughout the experiment (starting in early June).

2. Tested products

On these plots, three different biostimulants and one mulch were tested under drought conditions.

Mulch

The mulch consisted of *Dittrichia viscosa* (L.) Greuter 1973 (sticky inula), harvested and shredded. It was intended to be tested on all three plots. It was first applied in Vigne Akinao in mid-April 2025 on preselected rows. However, due to technical constraints, mulch was only applied in the orchard at the end of June 2025 and could not be spread in Vigne Saint-Joseph. Therefore, mulch effects were only evaluated in the rows of Vigne Akinao. Expected effects include soil water conservation and reduction of weeds at the plant base.

Mulch was applied in mid-April 2025 along the length of a row, with a width of 80 cm and thickness of 30 cm. For modalities B and N, only one row was mulched per modality, and two rows were mulched for modality I.



Figure 4. *Picture of two vine rows, one with mulch and one without mulch. Akinao vineyard.*

Biostimulants

The biostimulants tested on the three plots were developed by Dr. Marie-Emmanuelle Saint-Macary at FRAYSSINET.

- **FRAY7** is a root fertilizer with an added biostimulant. On the plots, it was tested in three versions:
    - **Base B**: without biostimulant,
    - **I**: with one dose of biostimulant,
    - **N**: with two doses of biostimulant.

The biostimulant is a plant extract from the pharmacopoeia with high energizing potential, developed on the concept of nutritional immunology. It is designed to optimize plant metabolism and resource allocation for optimal growth. Expected effects include catalyzing plant health mechanisms, increasing vine vigor by about 14%, reducing powdery mildew pressure by approximately 31%, and increasing average yield over three years by nearly 20%.

- **Nutribio 5.4.2 + Si** is a foliar fertilizer sprayed onto leaves, containing a biostimulant. In this experiment, it was combined with FRAY7 in the vineyards only. It targets leaf physiology and is expected to indirectly impact yield by boosting plant vigor and triggering immune defenses against powdery mildew (*Erysiphe necator*) and downy mildew (*Plasmopara viticola*). Anticipated effects include delaying the most severe disease symptoms by 1 to 2 weeks and increasing leaf cuticle thickness, thus enhancing drought stress tolerance. More specifically, Nutribio 5.4.2 + Si stimulates cellular metabolism, optimizes antioxidant metabolism, and corrects plant nutritional deficiencies by improving amino acid assimilation, water uptake, and photosynthesis.

- **ACT'IN** is a fruit-targeted fertilizer containing a biostimulant. The first spraying is performed at the end of flowering–early fruit set. In this study, it was combined with FRAY7 in the orchard only. The biostimulant is designed to regulate calcium and manage water fluxes within the fruit through a reverse action on vacuoles: it reverses polarity and rebalances calcium (Ca) and potassium (K) within the fruit. Expected effects include delaying brown rot (*Monilinia* spp.), preventing fruit cracking, reducing leaf curl disease, and improving both plant vigor and yield (fruit number and size).

3. Application of biostimulants and mulch in vineyards and orchard

Biostimulants and mulch were applied in different ways across plots to study their combinatorial effects.

Akinao vineyard:

Rows were divided into three modalities.

- B (blue): control modality with FRAY7 base (no biostimulant) and foliar water applications instead of Nutribio 5.4.2 + Si. One of these rows was mulched.
- I (pink): 13 rows, of which 2 were mulched, treated with FRAY7 (1 dose of biostimulant) and 5 applications of Nutribio 5.4.2 + Si from the 5-leaf stage onwards.
- N (yellow): 3 rows, of which 1 was mulched, treated with FRAY7 (2 doses of biostimulant) and the same Nutribio 5.4.2 + Si applications as I.
  Untreated buffer rows (3–4 rows on the edges and 1 between modalities) were maintained to minimize border effects.

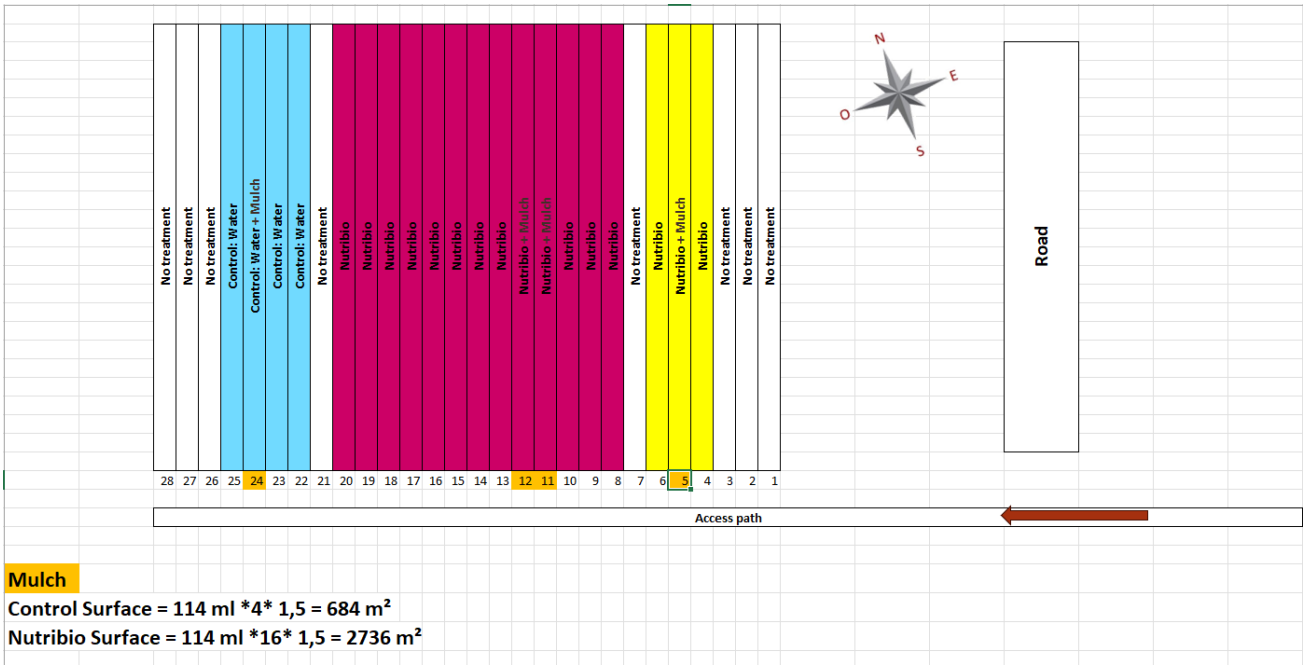
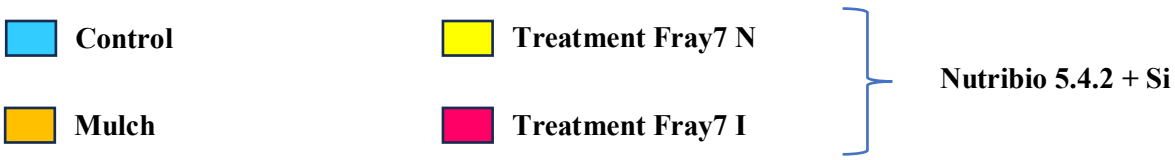


Figure 5. *Plan of the modality treatments in the Akinao vineyard.*



Saint-Joseph vineyard:

The first external row was left untreated to avoid border effects.

- B (blue): 4 rows as control, sprayed with water (instead of Nutribio 5.4.2 + Si). One row was intended for mulching but mulch was not applied due to technical issues.
- I (pink): 8 rows treated with 5 Nutribio 5.4.2 + Si applications starting from the 5-leaf stage.

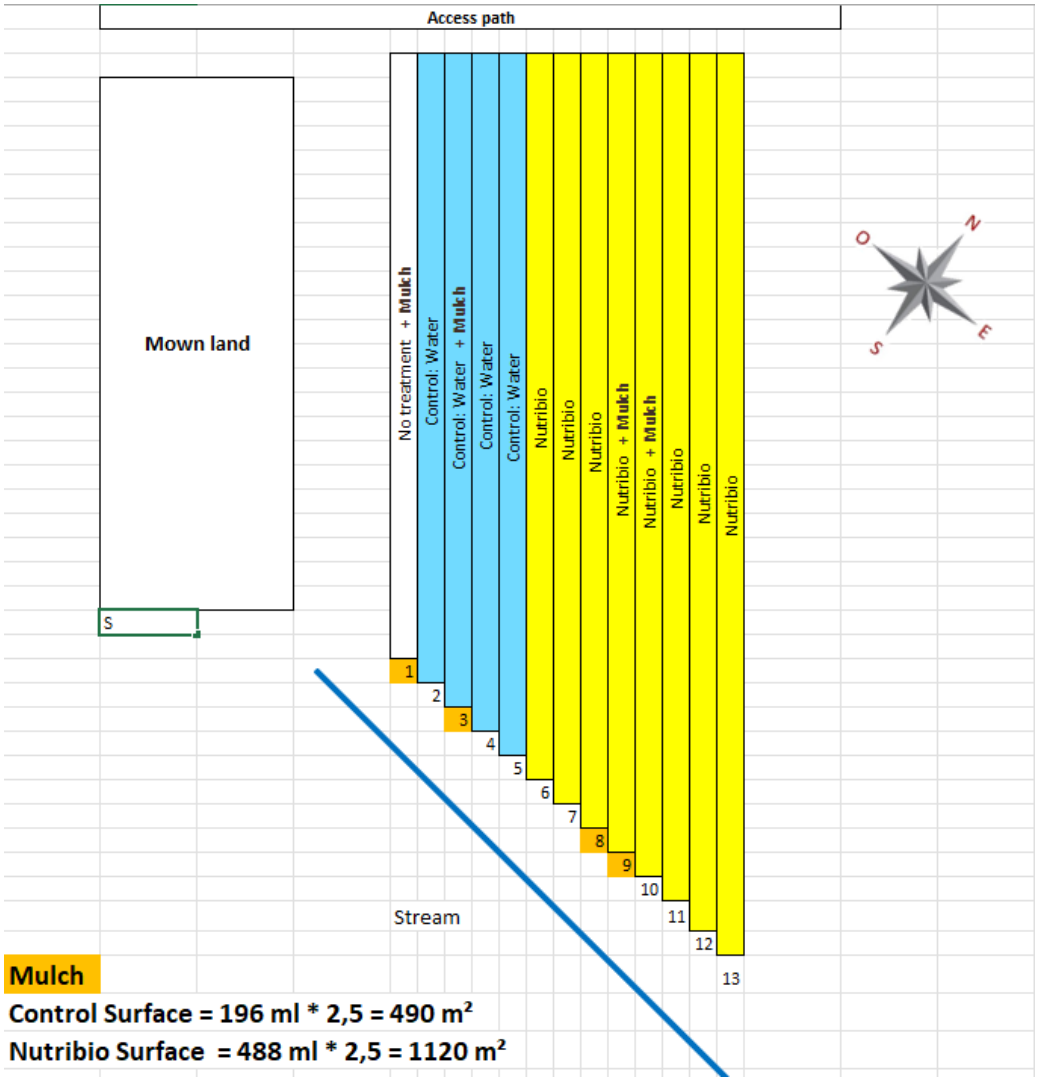


Figure 6. Plan of the St Joseph vineyard and the treatments realised on the different rows.



Orchard:

- B (blue): 3 rows treated with FRAY7 base (no biostimulant) and sprayed with water.
- I (pink): 3 rows treated with FRAY7 (1 dose of biostimulant) and 5 applications of ACT'IN, every 10 days from end of flowering–early fruit set.

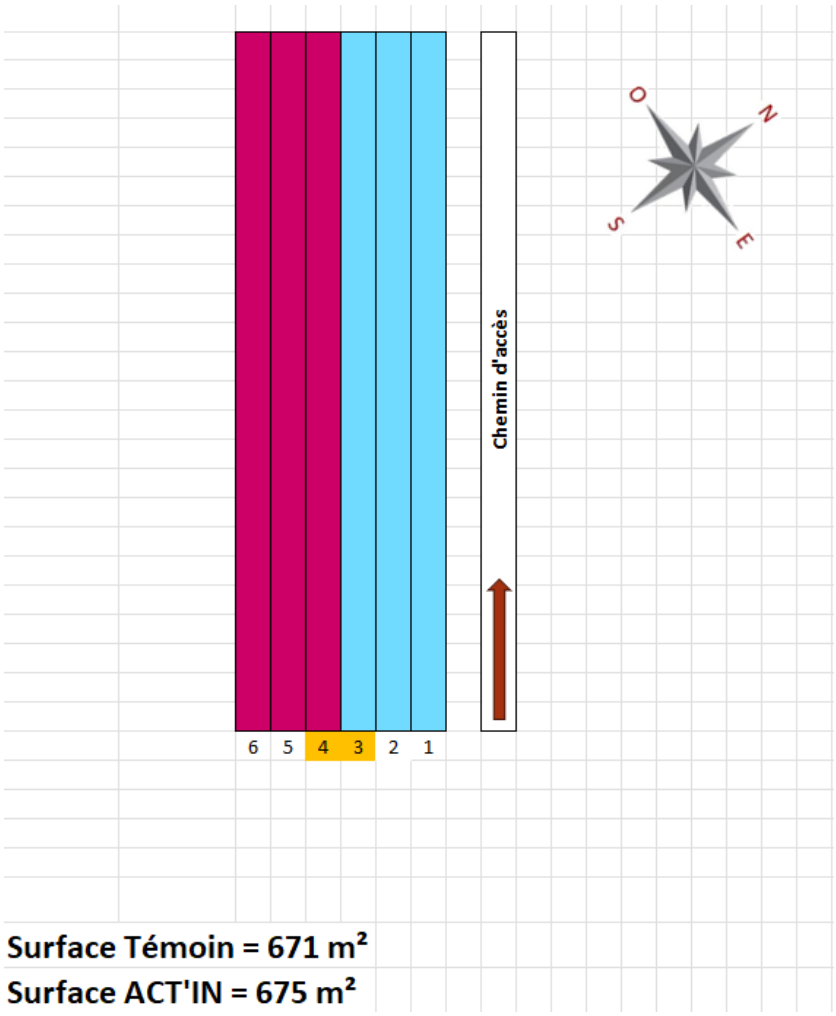


Figure 8. Plan of the Orchard and the treatments applied to the tree rows.



4. Measurements of biostimulant and mulch effects on plants

In order to study the impact of biostimulants and mulch on vines and orchards, measurements were taken of plant vigour, changes in their water status and diseases.

Scholander chamber

To measure plant water stress, readings of leaf water status were taken using a Scholander chamber. Three readings were taken at approximately 15-day intervals between June and July.

For each plot and on each date, one row was selected at random for each treatment (B, BP, I, IP, N, or NP), and five different plants or trees were chosen at random, from which three leaves were taken to record the leaf water potential Ψ (bar).

Scholander's pressure chamber allows the water potential of vines to be measured using a neutral gas (nitrogen). The lower the amount of water in the plant, the greater the gas pressure required to extract water from the plant (Deloire et al, 2004). This technique does not allow continuous measurement and is invasive. To take the measurement, the leaf is separated from the branch manually at the point where it is attached to the node. The petiole is then cut cleanly with a blade. The freshly picked vine leaf is placed in the pressure chamber, which is connected to a bottle of pressurised nitrogen. The leaf petiole exits the chamber through an orifice. The pressure inside the chamber is gradually increased, causing a meniscus of sap to emerge from the tip of the petiole. When the meniscus appears, the pressure corresponding to the water potential is recorded (Chone et al, 2000; Van Leeuwen et al, 2003). Therefore, the higher the pressure, the greater the water deficit to which the vine is subjected.



Figure 9. *Pictures of the Scholander pressure chamber and its use on the vine leaves in Akinao vineyard.*

Soil tensiometry

In order to assess the soil's water status, tensiometric probes were planted in the Akinao Vineyard plot. These Watermark brand probes are installed in pairs: one deep probe and one surface probe. The deep probe is buried approximately 60 cm into the soil and the surface probe approximately 30 cm. Three pairs of probes were buried in the Akinao vineyard, each in the middle of the plot. The first pair was placed at the top of the plot, in an unmulched row, the second pair in the middle of the plot, in a mulched row of type I, and the last pair at the bottom of the plot in an unmulched row.

These probes provide instantaneous measurements of soil water tension and soil temperature. Readings are taken using a box equipped with crocodile clips that are connected to the connector wires of each probe. The data is then read on the box. Tensiometer measurements are in cbar or kPa, and temperature measurements are in °C. Readings were taken three times a week from 28 April to 25 July. These measurements allow us, among other things, to assess the impact of mulch on soil water conditions.



Figure 10. *Hydric tension measuring probes and the measuring unit in a mulched row, Akinao vineyard.*

The soil moisture measurements can be interpreted as follows:

From 0 to 10 cbar: the soil is waterlogged. The environment is suffocating and unfavourable for the root development of most cultivated plants.

From 10 to 30 cbar: the soil is moist and well-drained, with plenty of water available. This is ideal for roots.

From 30 to 120 cbar: the soil is gradually drying out. Water is becoming less and less available.

From 120 to 200 cbar: water reserves at the measured depth are at a critical level. In addition, a break in the capillary link between the sensor and the soil may occur, making it difficult to re-moisten the sensor.

Pathogens

In order to assess plant vigour and the effect of biostimulants and mulch on plant health, measurements of disease progression were taken in the plots.

In the orchard, the progress of blister rust on tree branches was mainly noted using reference stage photos. The aim was to note the tip of a branch with an initial blister rust outbreak in order to assess any impact of ACT'IN on plant health. As shown in Figure 11, at stage 1, the leaf is considered healthy; at stage 2, the leaf begins to curl widthwise, indicating the onset of blight; and finally, at stage 3, the leaf is considered to be showing signs of a new outbreak on the branch. Five branches on twelve trees selected at random in each category were observed.



Figure 11. *Leaf curl reference stages for measuring in the orchard. From left to right: stage 1 the leaf is sane ; stage 2 the leaf starts to curl, the pathogen is growing ; stage 3 a new infection site is confirmed.*

In the vineyards, particularly the Akinao vineyard, the first symptoms of mildew and powdery mildew appear in mid-April. Treatments with Nutribio 5.4.2 + Si and water begin at the same time, to which sulphur and copper mixture are added.

In order to establish observations on the effectiveness of Nutribio 5.4.2 + Si in activating the plant's immune resources, the progression of mildew symptoms was assessed on the vines.

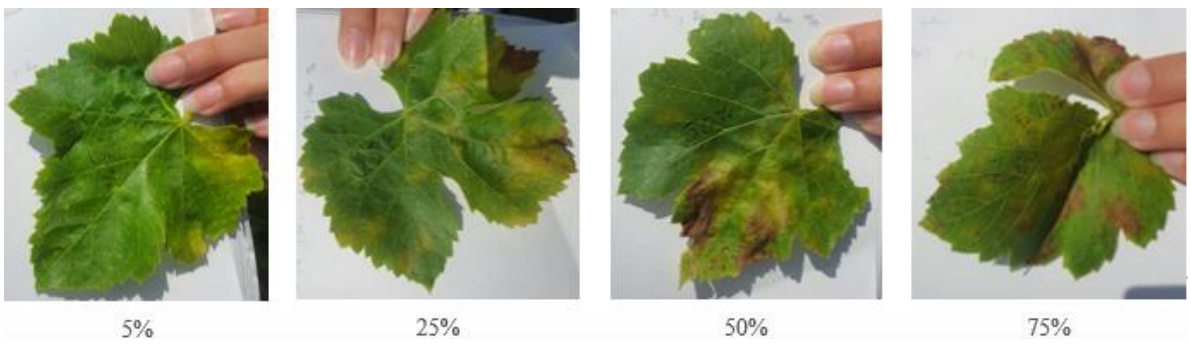


Figure 12. *Pictures of the different percentages of Mildew expansion on the vine leaf, serving as a reference to measure the advance of the pathogen in the vineyard.*

Based on a reference showing the different stages of fungal growth on vine leaves, the proportion of mildew in the vineyard was assessed according to the different methods. In **Figure 12**, the percentage represents the portion of the leaf covered by the fungus. For each method, 100 leaves taken at random, 1 per vine, were observed for the severity of mildew on the leaf and the sporulation of the pathogen. In addition, for each leaf observed and noted, the sporulation of mildew was also noted.

Measurements on fruit, yield and quality

In the orchard, in order to get an idea of the effects of FRAY7 and ACT'IN on fruit yield and quality, the peaches were measured using a calliper. Two diameter measurements were taken per fruit, on three fruits per tree. Three trees were selected per row for each row of each treatment (I and B).

In addition, to get an idea of the impact on yield, all the fruit borne by the trees were counted for each treatment and divided into two categories: the number of ripe fruit per tree and the number of aborted fruit per tree.



Figure 13. *Picture of the method used to measure an estimated volume of the peaches in the orchard. Two measures (in mm) were taken per fruit, three fruits measured per tree and three trees per row, three rows per modality treatment (B and I).*

At the Akinao vineyard, the grapes were harvested on 9 September 2025. The plot was divided transversally into four blocks (B1, B2, B3 and B4) so that each block contained all the different treatment methods for the vines. Six vines per block and per treatment (i.e. 124 vines) were marked so that they could be harvested separately and the bunches counted and weighed. See **Figure 14**.

In this cluster survey design, treatment B (control), treatment I (FRAY7 1 dose of biostimulant + Nutribio 5.4.2 + Si) and treatment N (FRAY7 2 doses of biostimulant + Nutribio 5.4.2 + Si) were renamed M1, M2 and M3 respectively.

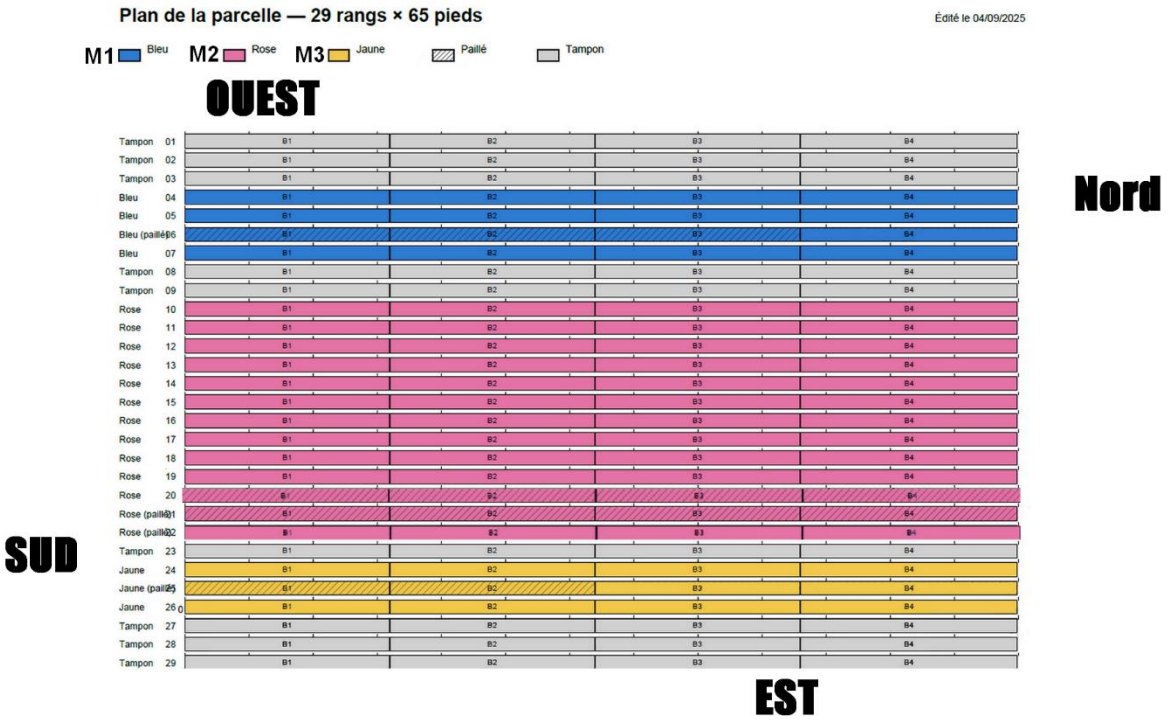


Figure 14. *Plan of the Akinao vineyard for the grape harvest. The vineyard was divided in 4 transversal blocks to assess more data about the fruits and the effect of the biostimulants and mulch on the production.*

Statistical analyses

The data from each set of measurements were formatted and analysed using RStudio. The pressure data obtained with the Scholander chamber were formatted and analysed using ANOVA and a Tuckey HSD test.

The soil tensiometry measurements were analysed using a mixed linear model (Appendix 1).

Data on the progression of blister stages in the orchard were analysed using a Wilcoxon statistical test (comparison test of modalities B and I).

Measurements of mildew expansion on vine leaves were analysed using ANOVA and a Tuckey HSD test, while mildew sporulation on leaves was analysed using a matrix and a Chi2 and Fisher test.

The number of ripe fruits per tree and the number of aborted fruits per tree in the orchard were analysed using ANOVA and a Tuckey HSD test.

RESULTS

1. Tensiometer probes – Akinao vineyard

Data from probes planted in the soil of the Akinao vineyard show a significant difference between tensiometer measurements taken by pairs of probes outside mulch and pairs of probes planted in a mulched row. In fact, the tensiometry measurements observed in the mulch are significantly lower than those obtained outside the mulch. The difference is early 200 cbar in the spring and almost 100 cbar at the beginning of summer, at the start of the dry season.

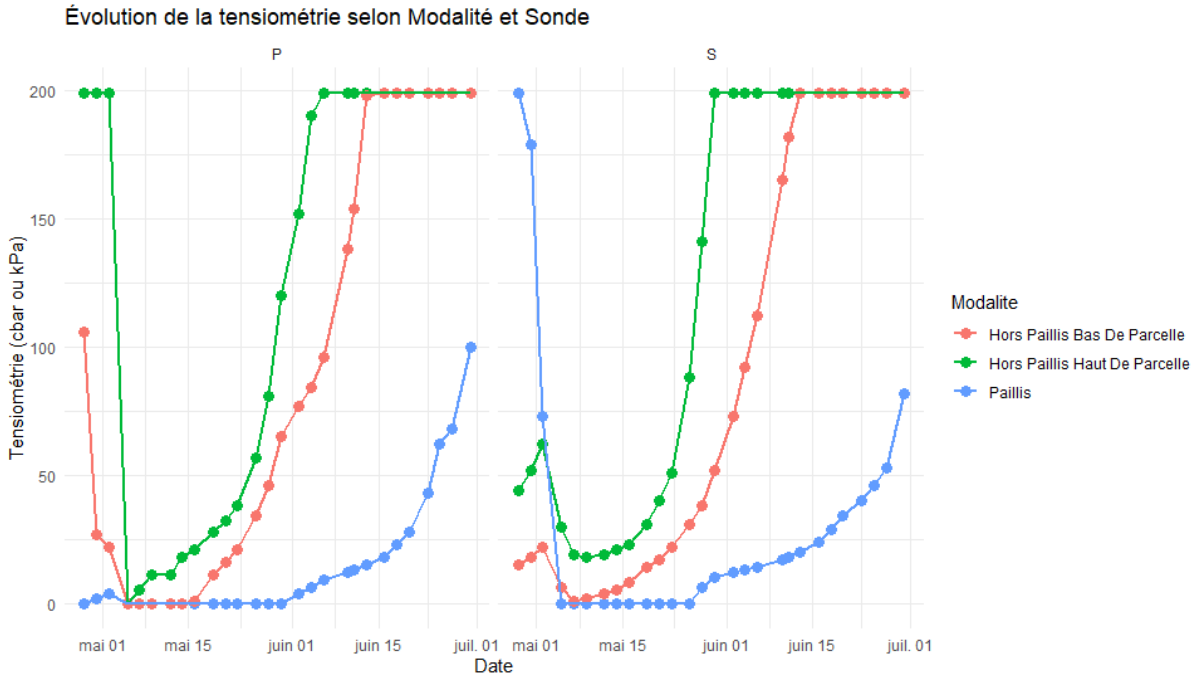


Figure 15. *Line graph of the evolution of the soil hydric tension measures (cbar or kPa) for both the deep (P) and superficial (S) probes in mulch or without mulch in Akinao vineyard.*

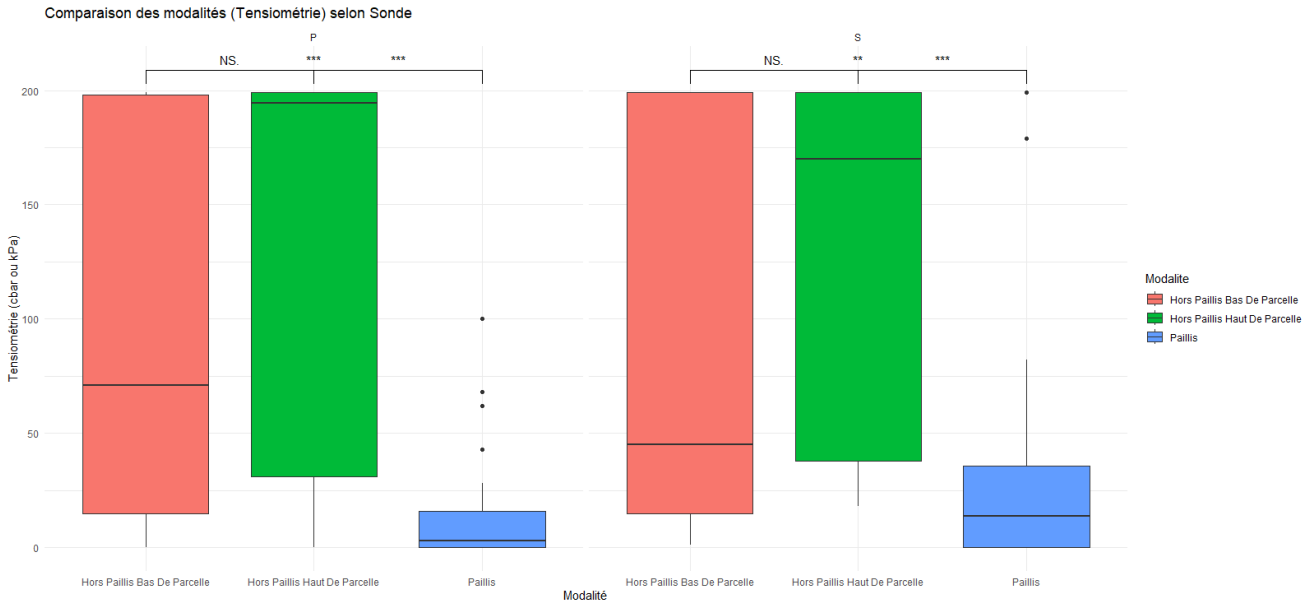


Figure 16. *Boxplots of the in mulch and outside mulch soil hydric tension measures (cbar or kPa) in Akinao vineyard.*

2. Mildew – Akinao vineyard

With regard to the collection of data on the presence and spread of mildew on vine leaves, no significant differences were observed between the different methods.

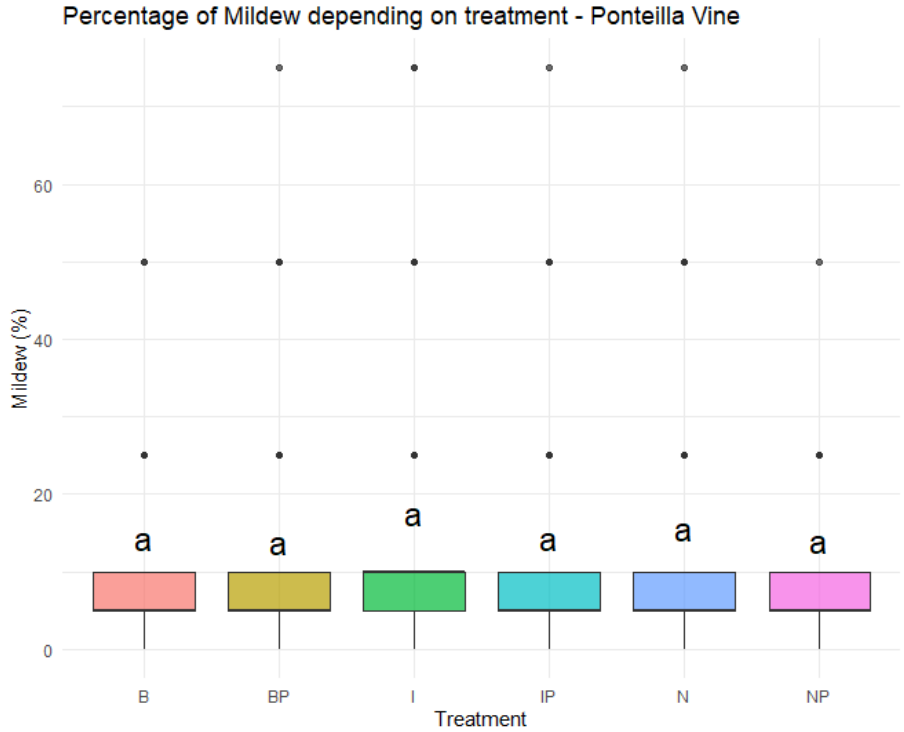
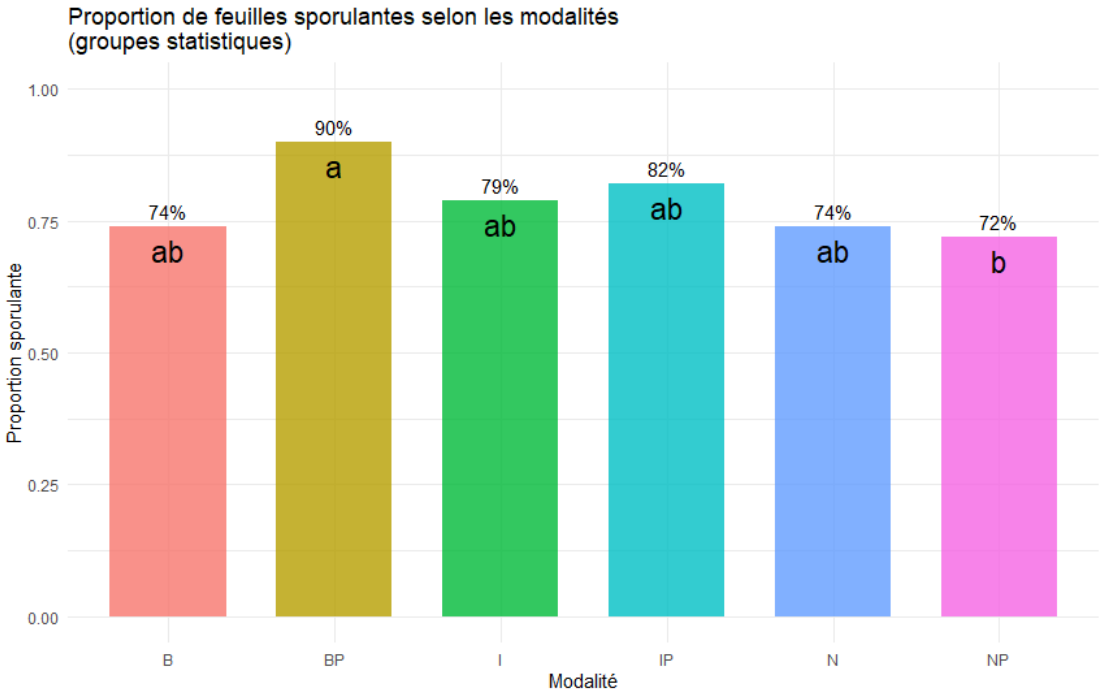


Figure 17. *Boxplots of the percentage of Mildew recorded for each treatment in Akinao vineyard.*

However, a significant difference can be observed between the BP (mulched control) and NP (double dose of mulched biostimulant) treatments in terms of the sporulation of



mildew on the leaves. There is also a slight tendency towards greater sporulation of the pathogenic fungus on the mulched treatments.

Figure 18. *Graph of the proportions of sporulating Mildew for the different treatments in Akinao vineyard.*

3. Leaf curl – Orchard

The number of new blister spots forming on already infected branches in the orchard shows no difference between the control and treated areas.

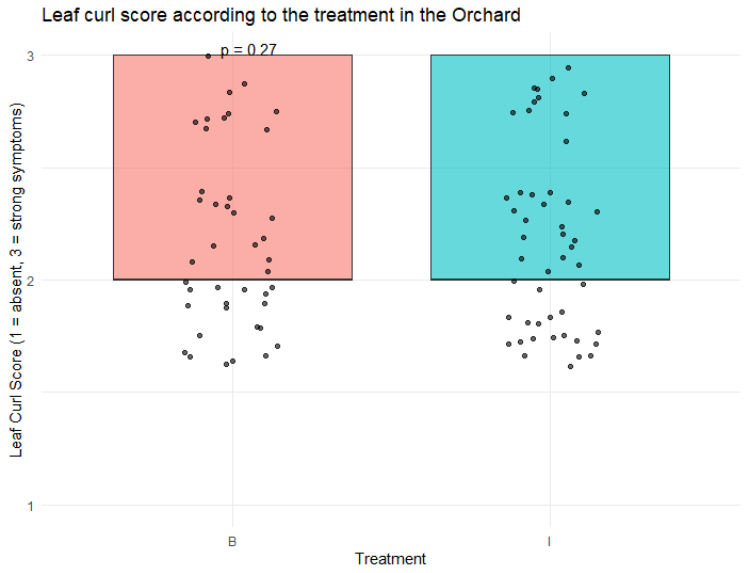


Figure 19. *Boxplots of the leaf curl score for each modality treatment in the Orchard.*

4. Estimated fruit volume – Orchard

In the orchard, measurements taken of peaches to estimate their volume and compare them between treatments did not reveal any significant differences between treatments, but there was a tendency for larger fruits in treatment I (biostimulants), as observed in the medians for each data set for the different measurement dates.

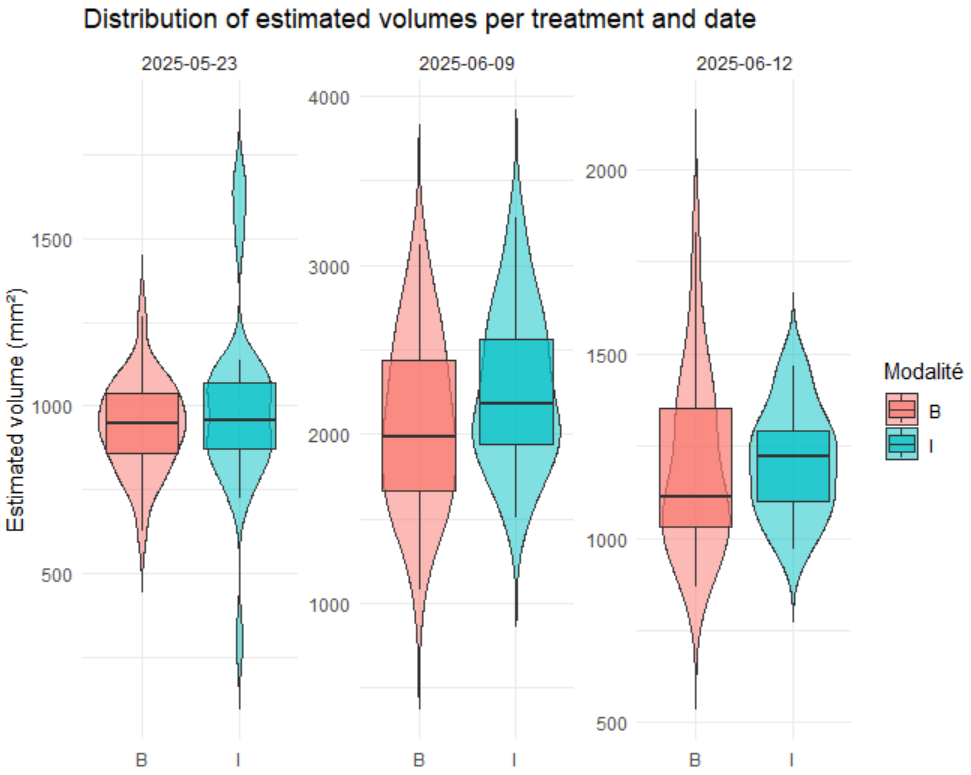


Figure 20. *Violin plots of the estimated volumes of the peach fruits per treatment and date in the Orchard.*

5. Number of fruits and aborted fruits – Orchard

The count of the number of fruits per tree per method in the orchard shows no significant difference. However, there is a significant difference between the two methods in terms of the number of aborted fruits per method, with a much lower number of aborted fruits, a difference of approximately 5 fruits. The treated method (I.) had fewer aborted fruits than the control method (B).

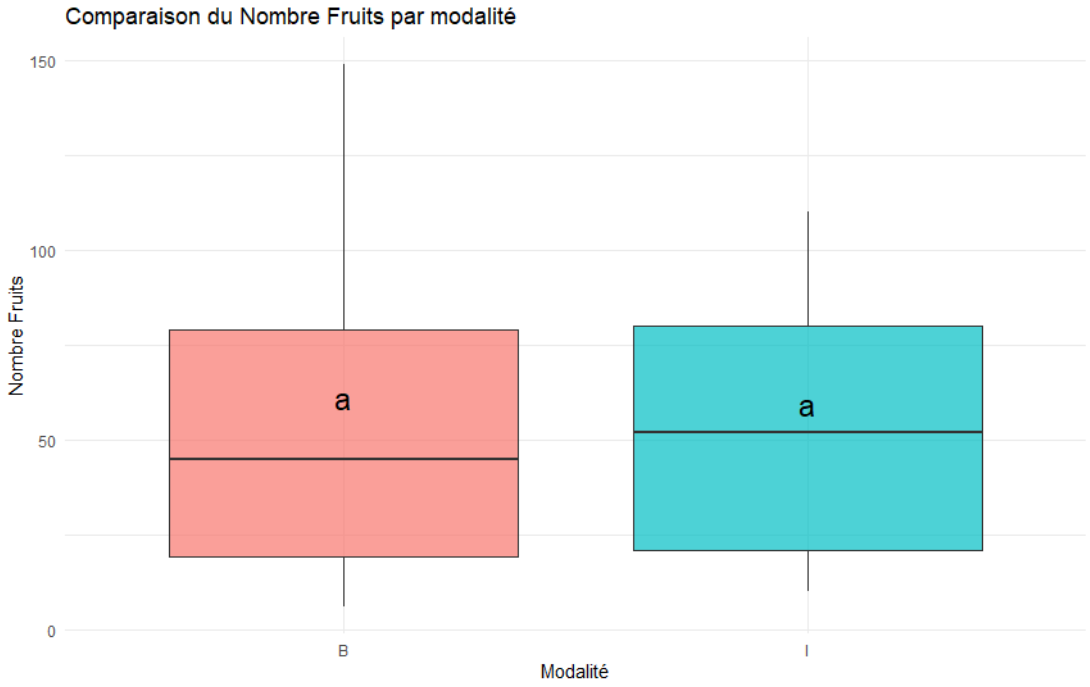


Figure 21. *Boxplots of the number of fruits per modality of treatment in the Orchard.*

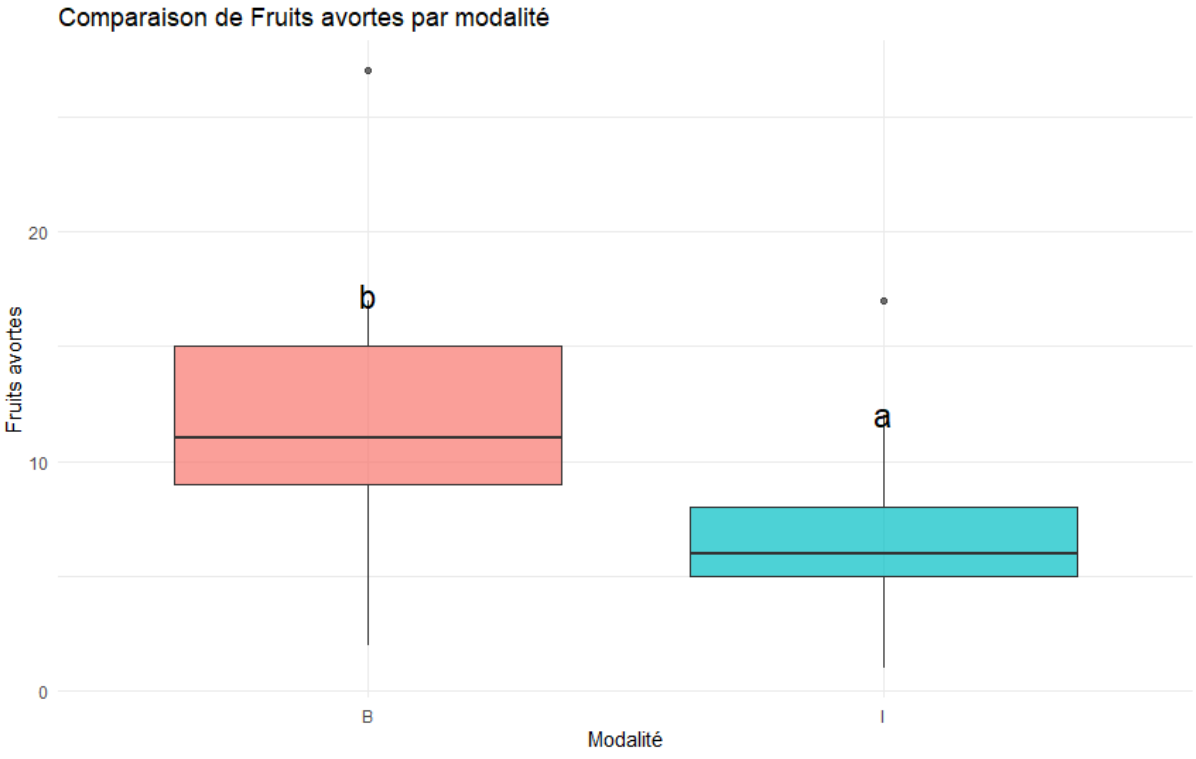


Figure 22. *Boxplots of the number of aborted fruits per modality of treatment in the Orchard.*

6. Scholander pressure chamber – Akinao vineyard

Pressure measurements taken with the Scholander chamber in the Ponteilla vineyard show a significant difference in leaf water potential (Ψ, Bar) recorded on 12 June between the IP (one dose of mulched biostimulant) and N (two doses of unmulched biostimulant) treatments and the other treatments. However, there was no significant difference between the treatments in the data taken on 26 June.

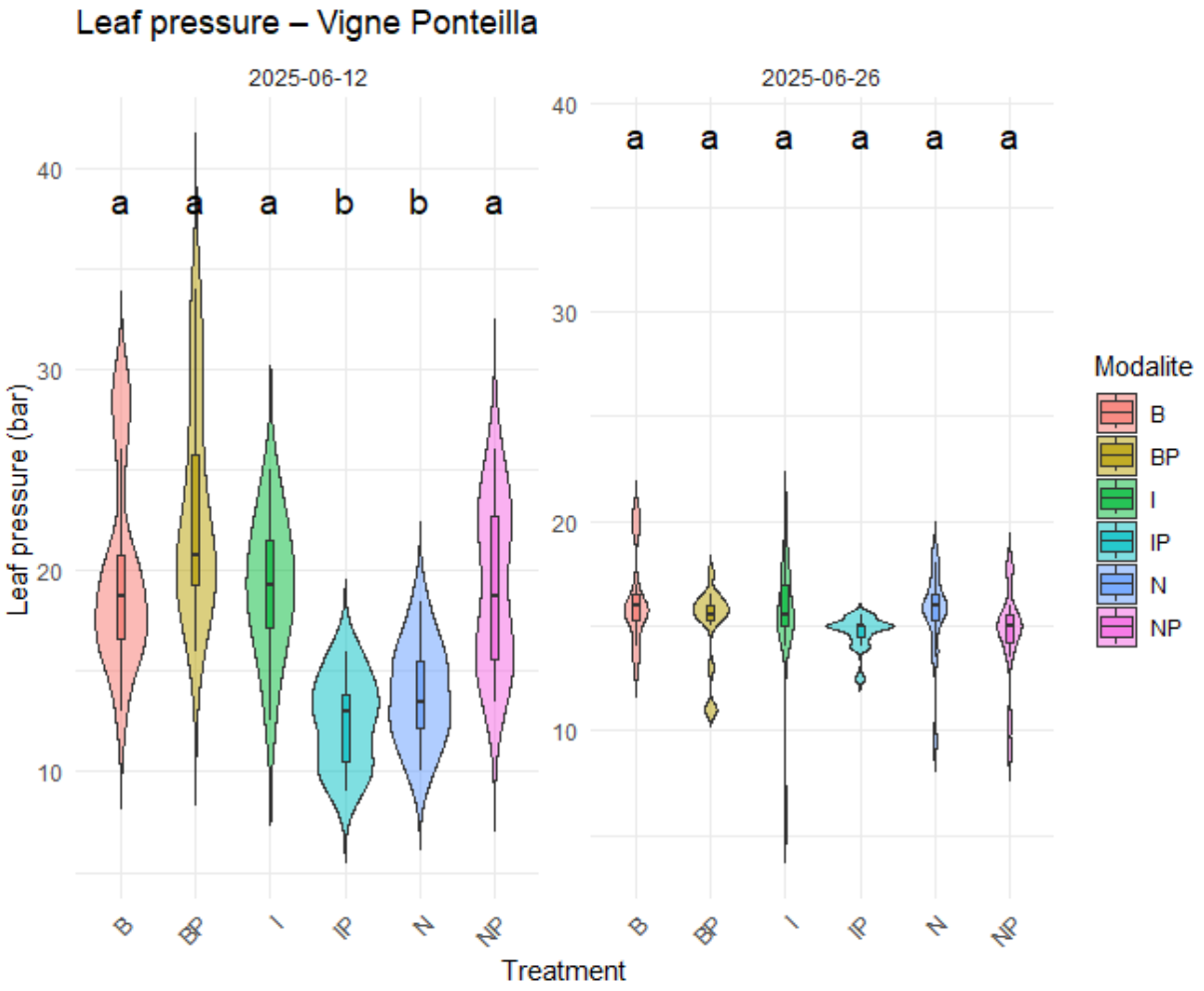


Figure 23. *Violin plots of the leaf pressure (bar) recorded for the different treatment modalities and per date in Akinao vineyard.*

7. Scholander pressure chamber – St Joseph vineyard

For measurements of leaf water potential (Ψ, Bar) in St Joseph vine leaves, no significant difference was observed between the two conditions (treated or control) or between the two measurement dates.

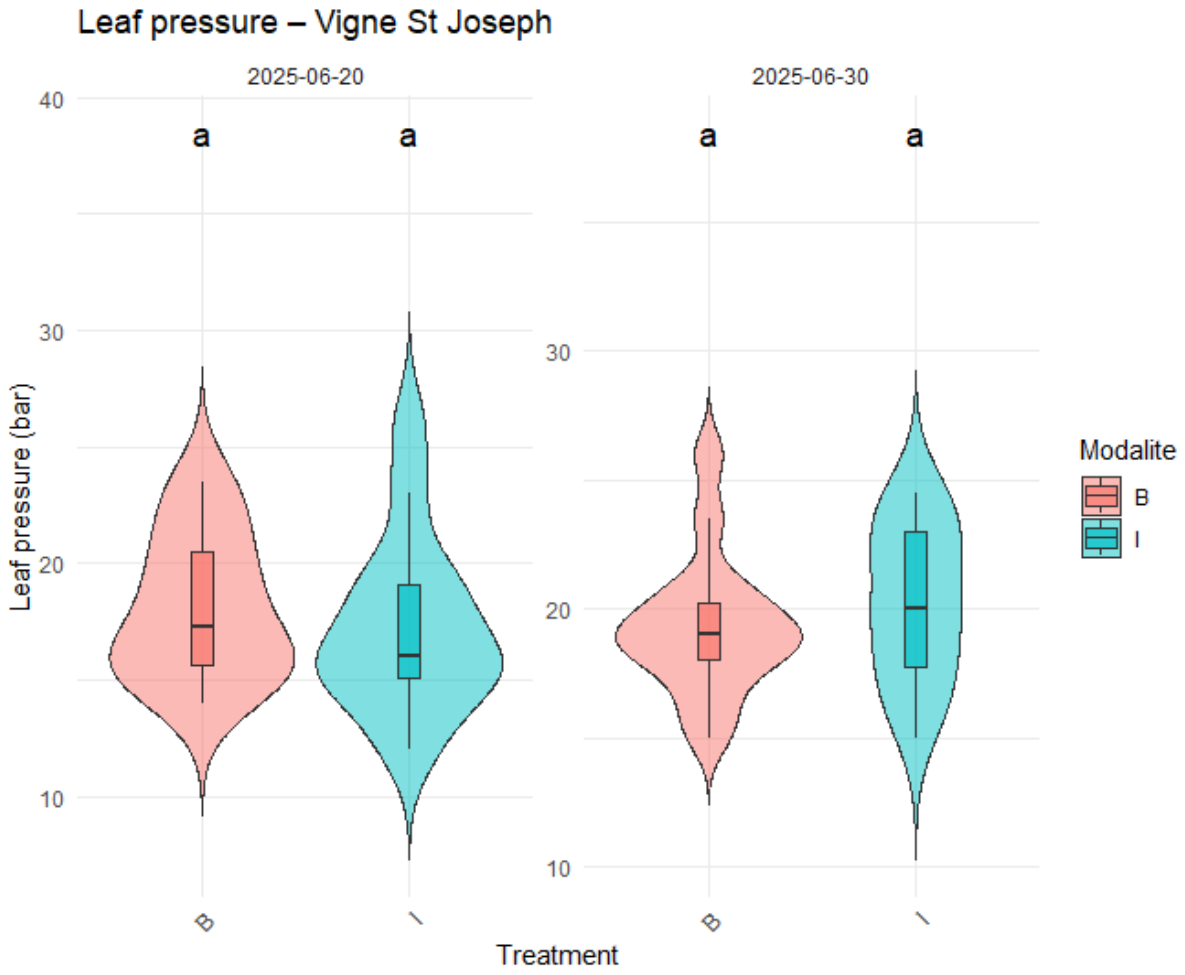


Figure 24. *Violin plots of the leaf pressure (bar) recorded for the different treatment modalities and per date in St Joseph vineyard.*

8. Scholander pressure chamber – Orchard

As with the St Joseph vine, measurements of leaf water potential (Ψ, Bar) show no significant difference between the Treated (I) and Control (B) conditions on any of the survey dates. Nor is there any difference in the values obtained for the conditions between the two different dates.

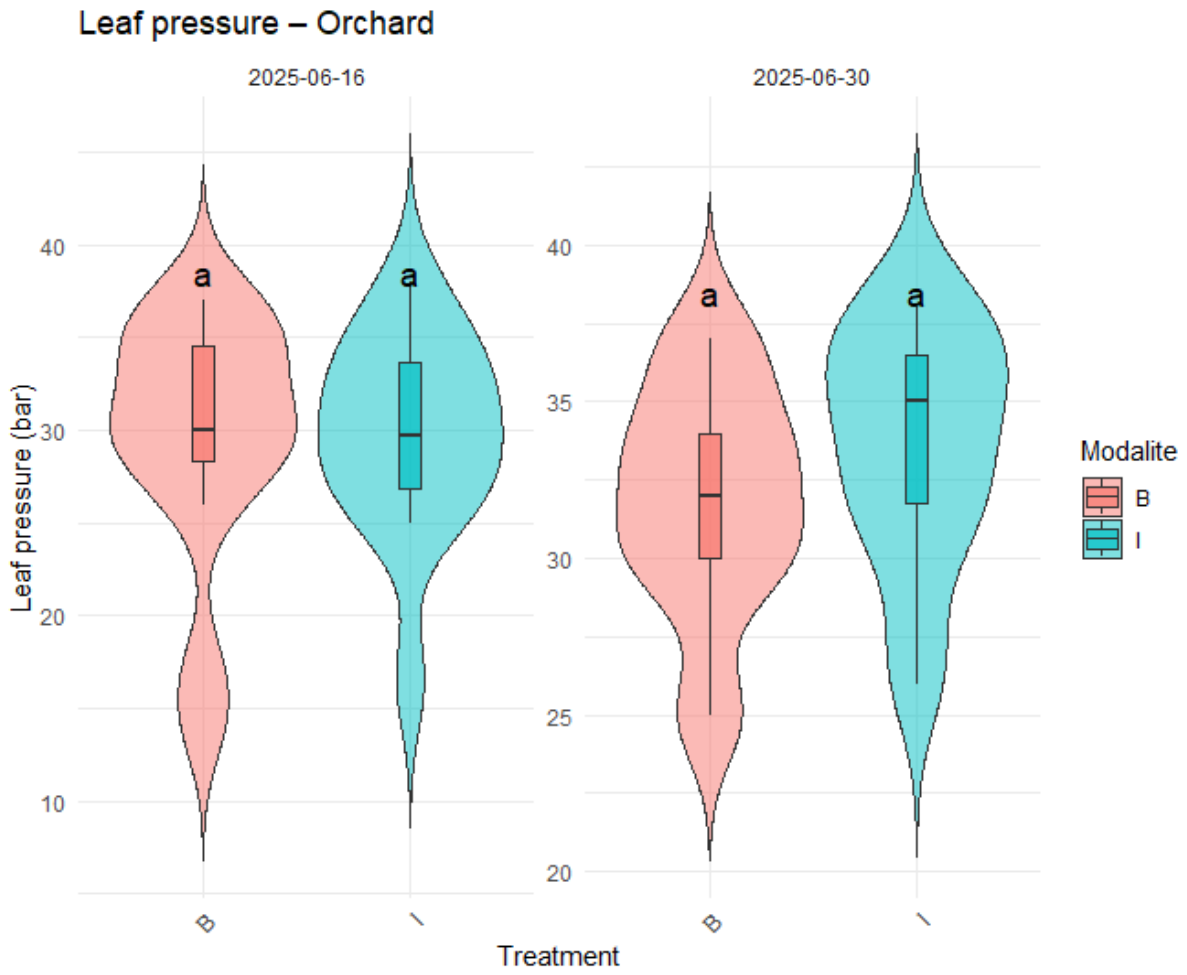


Figure 25. *Violin plots of the leaf pressure (bar) recorded for the different treatment modalities and per date in the Orchard.*

9. Number of bunch of grapes – Akinao vineyard

Boxplots of the number of clusters according to treatment or block show a slight tendency towards a greater number of clusters on vines treated with biostimulants (I, IP, N and NP) compared to control vines (B and BP) (**Figure 26**).

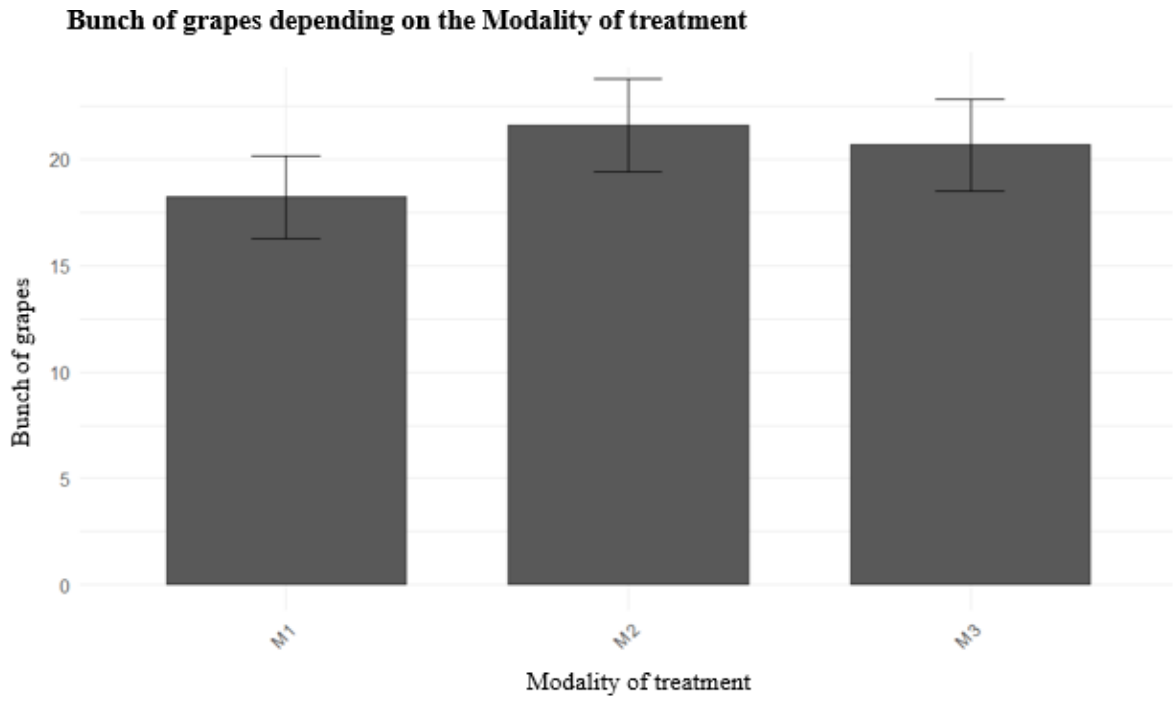


Figure 26. *Boxplots of the number of bunch of grapes observed for the different treatment modalities at harvest in Akinao vineyard.*

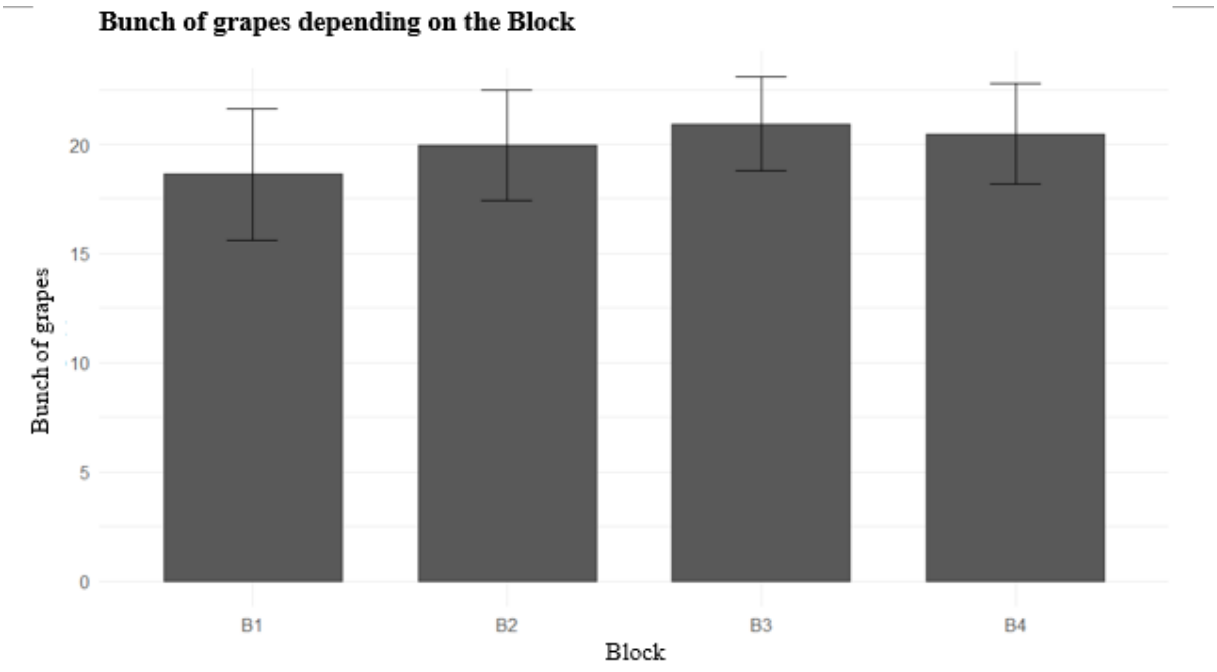


Figure 27. *Boxplots of the number of bunch of grapes observed for the different Blocks at harvest in Akinao vineyard.*

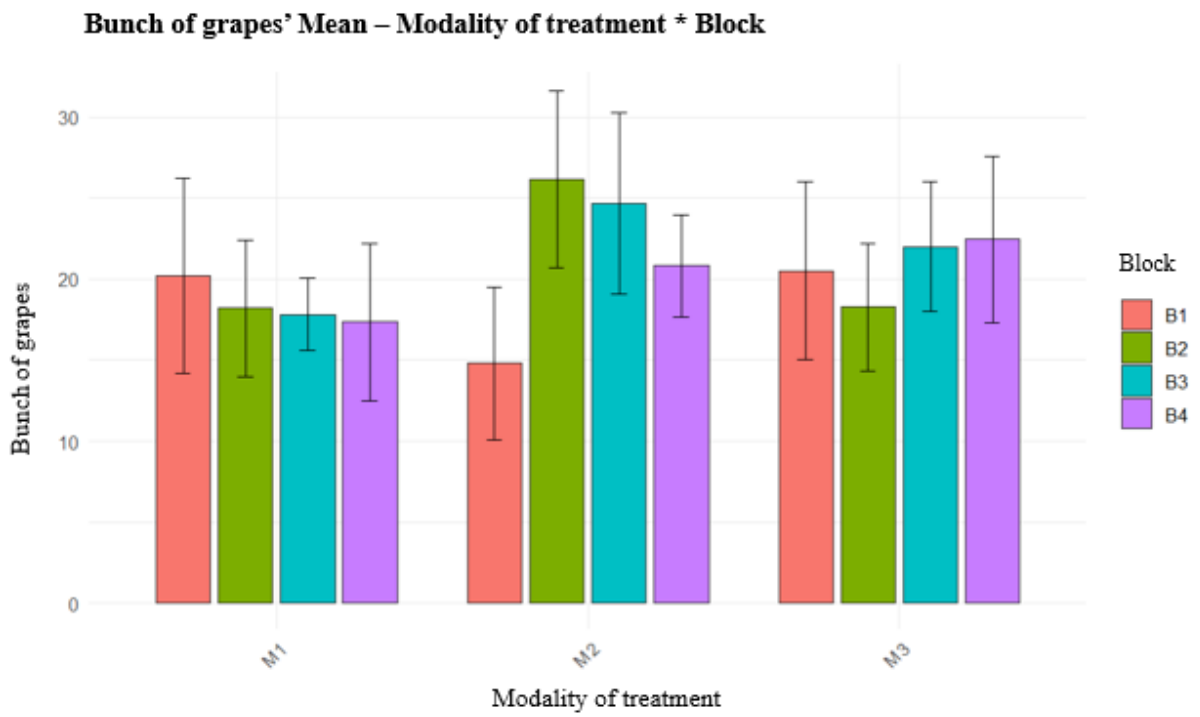


Figure 28. *Boxplots of the mean number of bunch of grapes observed for the different treatment modalities per Block at harvest in Akinao vineyard.*

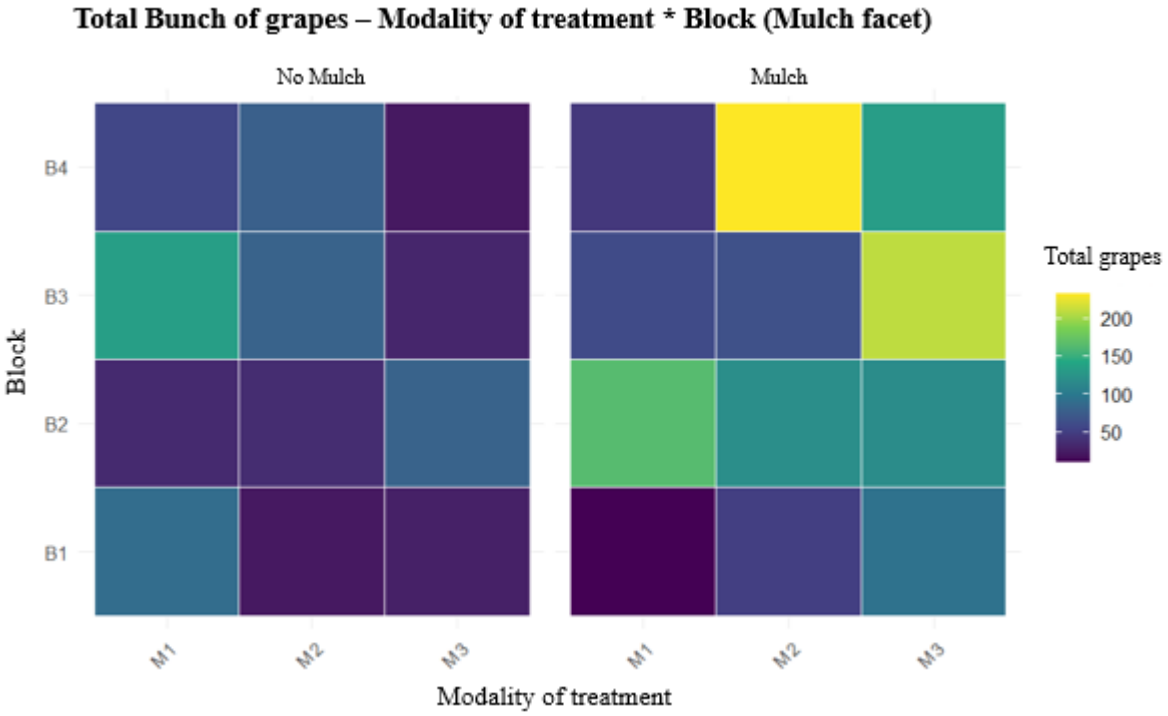


Figure 29. *Facet graph of the number of bunch of grapes observed for the mulched and un-mulched per modalities of treatment at harvest in Akinao vineyard.*

There was also a tendency for a greater number of grape clusters per vine in the mulched treatments (BP, IP and NP), as shown in Figure 29. Figure 30 seems to contradict this observation, but it is important to take into account the design of the experiment, with only one mulched row for treatments B and N, compared to two mulched rows for treatment I.

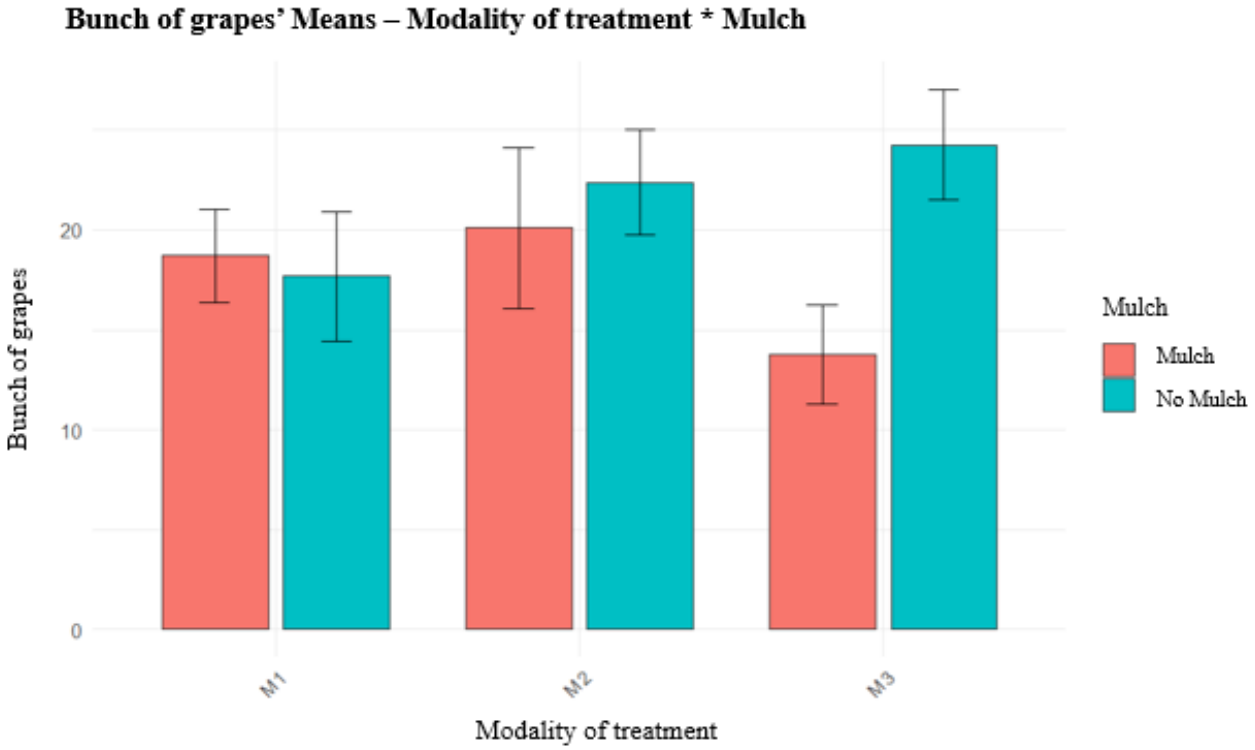


Figure 30. *Boxplots of the mean number of bunch of grapes observed for the different treatment modalities mulched or not at harvest in Akinao vineyard.*

10. Grapes weight – Akinao vineyard

However, the boxplots presented in Figures 31 and 32 do not show any significant difference between the treatments or the mulched treatments in terms of grape cluster weight per vine.

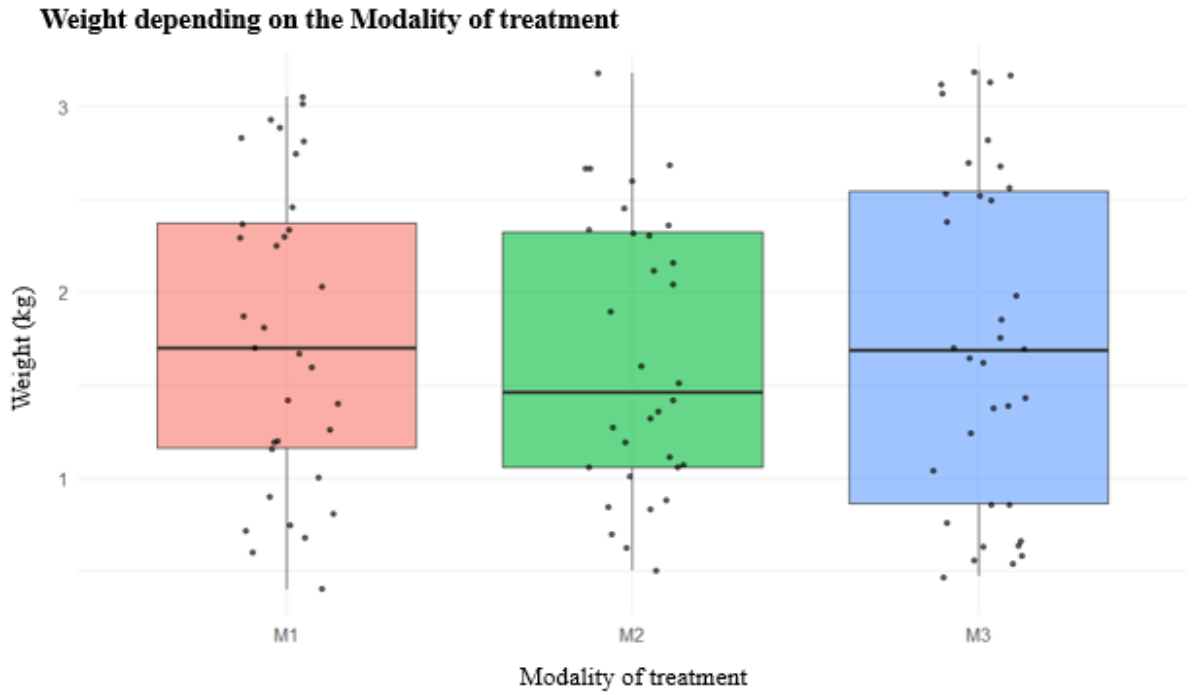


Figure 31. *Boxplots of the grapes weight depending on the modality of treatment at harvest in Akinao vineyard.*

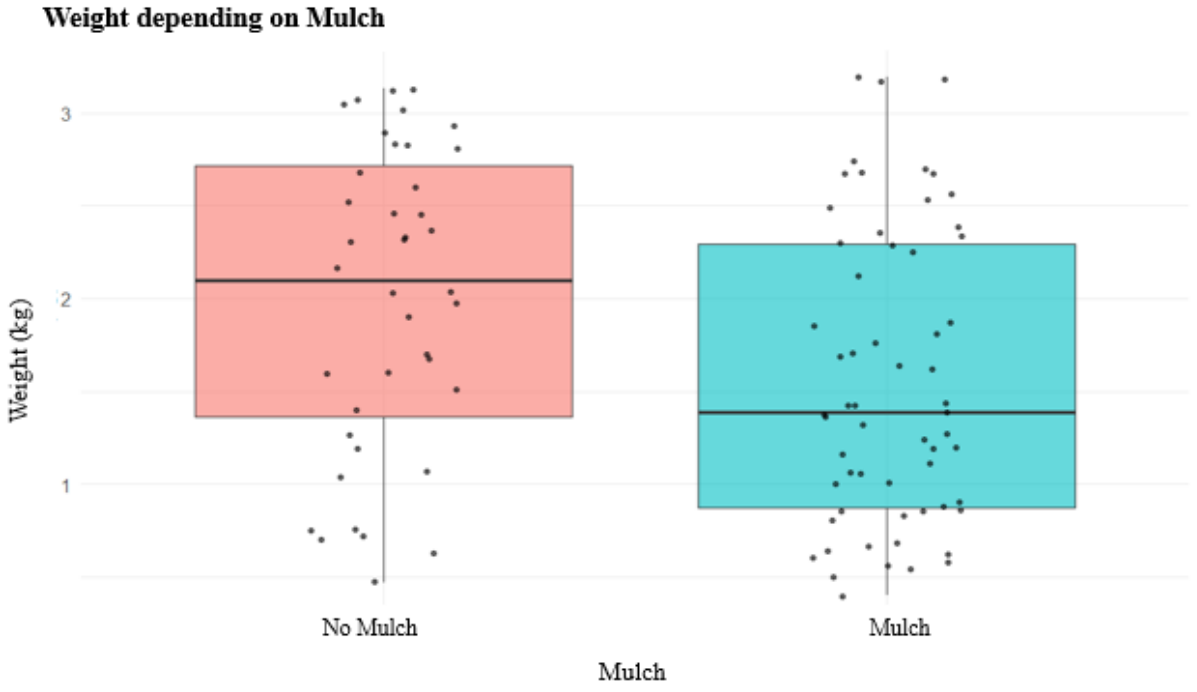


Figure 32. *Boxplots of the grapes weight depending on the mulch modality at harvest in Akinao vineyard.*

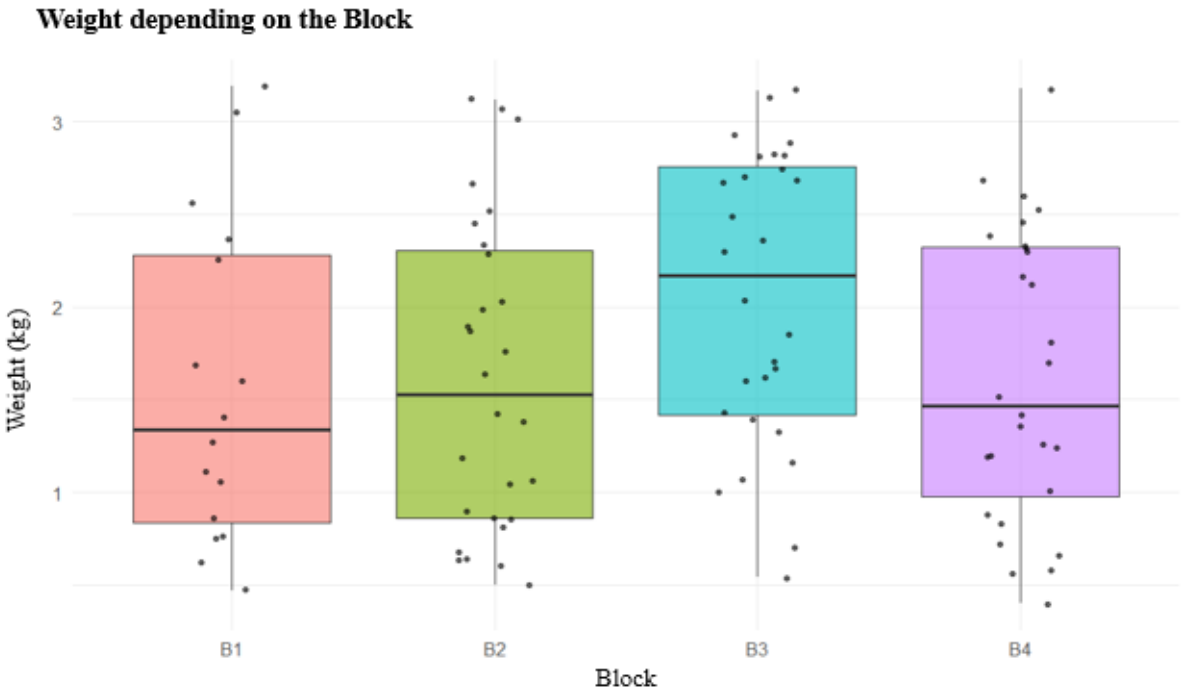


Figure 33. *Boxplots of the grapes weight depending on the modality of Block at harvest in Akinao vineyard.*

DISCUSSION

The results obtained in this study highlight the potential benefits of biostimulants and mulching as agroecological levers for coping with the growing constraints imposed by climate change in the Mediterranean region.

Firstly, the application of Inula viscosa mulch had a beneficial effect on soil water status, reducing evaporation and maintaining more favourable moisture levels in the root zone. Although limited to the Akinao vineyard, this practice made it possible to differentiate the water profiles between mulched and unmulched rows, confirming the results reported in the literature (Brito et al., 2019; Montanaro et al., 2017). However, the technical implementation remains challenging, particularly due to the volume of biomass required and the difficulty of achieving uniform distribution on a large scale.

With regard to biostimulants, the products from the FRAYSSINET range that were tested (FRAY7, Nutribio 5.4.2 + Si and ACT'IN) contributed overall to improving plant vigour, particularly in combinations of Nutribio in vineyards and ACT'IN in orchards. The expected effects, such as improved tolerance to water stress and reduced severity of fungal diseases, were partially confirmed. In vineyards, the treated treatments tended to delay the onset and intensity of mildew symptoms, although the results were not always significant. In orchards, the use of ACT'IN appeared to slightly reduce the incidence of leaf curl and improve

the distribution of calcium and potassium flows, resulting in better quality fruit (size, absence of cracks).

These observations are consistent with recent studies showing that biostimulants act mainly by stimulating the natural defences of plants and optimising their metabolism (Calvo et al., 2014; du Jardin, 2015). However, their effectiveness can be strongly influenced by soil and climate characteristics, as well as by the phenological stage of the plant at the time of application (Rouphael & Colla, 2020).

Finally, from an agronomic perspective, although the yields observed did not show a dramatic increase, the positive trends observed in terms of fruit quality in orchards and vine vigour suggest potential economic benefits. In a Mediterranean context marked by water scarcity, these solutions may be an interesting complement to traditional farming practices, even if they cannot entirely replace rational irrigation and fertilisation management.

CONCLUSION

This study tested the effectiveness of biostimulants and mulching with Inula viscosa in two vineyards and a peach orchard in Roussillon. The results indicate that:

- mulching contributes to better soil moisture conservation,
- biostimulants improve plant vigour and delay certain disease symptoms,
- positive effects on fruit quality (size, absence of physiological defects) were observed in the orchard.

Although sometimes limited or statistically insignificant, these results confirm the value of these practices as complementary tools in an agroecological strategy aimed at strengthening crop resilience to water stress and pathogen pressure.

Nevertheless, their large-scale implementation remains to be evaluated, particularly in terms of cost, logistics and variability in effectiveness depending on soil and climate conditions. These avenues deserve further exploration in order to optimise application protocols and assess the agronomic and economic sustainability of these practices.

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APPENDICES

1. R script of the statistical analyse for the soil hydric tension measures.

```
# Packages nécessaires
library(readxl)
library(dplyr)
library(lubridate)
library(lme4)
library(emmeans)
library(ggplot2)
library(ggsignif)

# Chemin fichier et feuille Excel
fichier <- "C:/Users/rouss/OneDrive/Documents/A ranger/Scripts Stage M2/Data Stage M2.xlsx"
feuille <- "Sondes Waterm'AK"

# Importer les tableaux selon plages
haut <- read_excel(fichier, sheet = feuille, range = "A2:F58") %>%
  mutate(Modalite = "Hors Paillis Haut De Parcelle")

paillis <- read_excel(fichier, sheet = feuille, range = "H2:M58") %>%
  mutate(Modalite = "Paillis")

bas <- read_excel(fichier, sheet = feuille, range = "O2:T58") %>%
  mutate(Modalite = "Hors Paillis Bas De Parcelle")

# Harmoniser noms colonnes (supposés identiques dans chaque tableau)
noms_colonnes <- c("Date", "Sonde", "Tensiometrie", "T_sol", "T_ext", "Meteo", "Modalite")

colnames(haut) <- noms_colonnes[1:7]
colnames(paillis) <- noms_colonnes[1:7]
colnames(bas) <- noms_colonnes[1:7]

# Fusionner les tableaux
data <- bind_rows(haut, paillis, bas)

# Nettoyer et préparer les variables
```

```
data <- data %>%
  filter(!is.na(Date), !is.na(Tensiometrie)) %>%  # supprimer lignes vides ou en-têtes éventuels
  mutate(
    # Convertir en Date (si Excel a gardé en texte)
    Date = as.Date(Date, origin = "1899-12-30"), # origine standard Excel
    Sonde = factor(Sonde),
    Modalite = factor(Modalite),
    Meteo = factor(Meteo),
    Tensiometrie = as.numeric(Tensiometrie),
    T_sol = as.numeric(T_sol),
    T_ext = as.numeric(T_ext)
  )

data

# Modèle linéaire mixte
mod <- lmer(Tensiometrie ~ Modalite * Sonde + T_ext + (1 | Date), data = data)

# Résumé du modèle
summary(mod)

# Comparaisons post-hoc
emm <- emmeans(mod, pairwise ~ Modalite | Sonde, adjust = "tukey")

# Tableau des moyennes estimées
emm_df <- as.data.frame(emm$emmeans)

# Exemple de graphiques boxplot avec comparaisons significatives affichées (ggsignif)
ggplot(data, aes(x = Modalite, y = Tensiometrie, fill = Modalite)) +
  geom_boxplot() +
  facet_wrap(~ Sonde) +
  geom_signif(
    comparisons = list(
      c("Hors Paillis Haut De Parcelle", "Paillis"),
      c("Hors Paillis Bas De Parcelle", "Paillis"),
      c("Hors Paillis Haut De Parcelle", "Hors Paillis Bas De Parcelle")
    ),
    map_signif_level = TRUE,
    test = "t.test"
```

```
) +
theme_minimal() +
labs(title = "Comparison of Mulch and No Mulch for the probes",
    y = "Soil hydric tension (cbar or kPa)",
    x = "Mulch")
```