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Genotype-Dependent Interactions Between Biostimulants and Defense Inducers in Durum Wheat: Implications for Sustainable Crop Management

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Abstract

The intensive use of synthetic pesticides and fertilizers has raised environmental concerns. Sustainable alternatives, such as plant biostimulants and plant resistance inducers, offer promising solutions by enhancing growth, yield, and stress tolerance or by activating defense responses against pathogens. However, the physiological impacts and combined effects of these products remain poorly understood, limiting evidence-based application strategies. Here, we evaluated the effects of a biostimulant and a plant defense inducer on durum wheat (*Triticum turgidum* ssp. durum), a key cereal crop in the Mediterranean Basin. Using controlled experiments, we assessed plant growth, chlorophyll content, defense gene expression and resistance to *Zymoseptoria tritici*, while considering potential trade-offs between growth promotion and defense activation. As expected, our results indicate that the biostimulant improved photosynthetic performance (19 to 45%), whereas the plant resistance inducer enhanced protection against *Z. tritici* (25% reduction in pycnidia). However, the combination of these two treatments can induce moderated interaction effects influenced by the varietal genetic background. This study provides novel insights into the interactions between plant growth promotion and defense induction in durum wheat. Understanding these multifactorial effects (in particular genotype effect) enables the identification of optimal treatment strategies, supporting the development of sustainable crop management practices that reduce chemical inputs while maintaining productivity and resilience under biotic stress.

Keywords: wheat; septoria; biostimulant; plant resistance inducer; growth/defense trade-off

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1. Introduction

The widespread use of synthetic pesticides and fertilizers has led to significant environmental concerns, including water pollution, soil degradation, and harm to beneficial organisms [1]. As a result, there is a growing demand for sustainable alternatives to reduce agrochemical reliance. Two promising tools to achieve this goal are plant biostimulants and plant resistance inducers, both acting through an elicitation of a plant's physiology. Plant biostimulants are valuable tools, enhancing plant growth, food quality, yield, and

stress tolerance [2–5]. Plant defense inducers are substances that trigger resistance mechanisms in plants against biotic stress, such as pathogens and pests [6,7]. These inducers can activate various signaling pathways and defense responses, enhancing the plant's ability to withstand attacks [8]. In Europe, while biostimulants are considered as fertilizers, defense inducers are classified as plant protection products [9].

Despite the increasing use of biostimulation products in agriculture (+12% worldwide growth rate over the last 5 years), scientific understanding of their physiological impacts remains limited [10]. Furthermore, scientific studies have shown that some products can act both to improve tolerance to abiotic stress and resistance to biotic stress, making the binary categorization of this product a scientific challenge [11–13]. Indeed, certain biostimulants can also modify tolerance to biotic stress [5]. Deciphering the mechanisms underlying the action of these products—whether they act as growth promoters, stress mitigators, or immune primers—is not only essential for optimizing their agronomic potential but also for improving their regulatory categorization [14].

While controlled experiments have demonstrated the potential of these biosolutions, their efficacy under field conditions can be variable and unpredictable [15–17]. This inconsistency may be attributed to the complex interplay of multiple factors, such as variety [18–20], formulation [21], fertilization [22], abiotic stresses such as drought [23], and the multiplicity of biotic stresses [24]. Previous studies on this topic have primarily focused on individual factors, neglecting the interactive effects that may significantly influence treatment performance [25,26]. Moreover, while both types of stimulation products target the plant to produce their potential effects, very little knowledge is available about their combined impacts on the plant [25,27,28], and even less concerning the genetics of the response to these stimulation products [29].

In plants, investment in defense against pathogens often comes at the expense of growth and reproduction, as it involves a significant energetic cost, a phenomenon described as the "trade-off" between growth and defense [30–32]. These crosstalks are largely mediated by hormonal regulation and its effects on global gene expression [33]. Furthermore, the redirection of metabolic resources toward the synthesis of defense-related compounds frequently compromises photosynthetic performance and disrupts nutrient partitioning within the plant [32]. Additionally, the overproduction of defense-associated hormones can further inhibit key developmental processes, leading to systemic growth suppression. At the organismal scale, these physiological trade-offs culminate in reduced biomass accumulation, altered interactions with root-associated microbiota, and ultimately diminished yield potential. This trade-off raises concerns about the potential detrimental effects of a biostimulant on a plant defense inducer and vice versa [25,34,35]. For example, analogs to salicylic acid (SA) are known to have a contrasting impact on plant growth [32,36,37]. However, in the field, the apparent trade-off observed in controlled conditions may be mitigated by environmental variability and complex plant-environment interactions [38].

To address this knowledge gap, this study aims to evaluate the combined effects of multiple factors on the efficacy of biostimulants in durum wheat. Durum wheat (*Triticum turgidum* ssp. durum) is a cereal of significance, particularly in the Mediterranean Basin and several regions globally, as it is a cornerstone in human nutrition and a driver of economic value for numerous countries [39]. Annually, it accounts for roughly 8–10% of the world's wheat cultivation area and supports millions of livelihoods from production to processing. However, the productivity and profitability of durum wheat are sharply constrained by multiple biotic stresses, in particular septoria leaf blotch, principally caused by *Zymoseptoria tritici*, standing out as one of the most devastating diseases [40,41]. In wheat, several studies have demonstrated the value of treatments with analogs of salicylic

acid to control fungal diseases. These compounds have demonstrated significant protection against fusarium diseases [42,43], leaf rust [24,44], Septoria tritici blotch—for which disease severity can be reduced by up to 60% [45,46]—and powdery mildew [24].

We investigated the impact of a combination of a biostimulant and a plant defense inducer on plant growth and disease resistance under controlled conditions. By systematically analyzing the putative physiological interactions between these two distinct classes of stimulating products, we aim to elucidate the underlying mechanisms that govern their combined effects on plant performance. Through this approach, we seek to identify optimal application strategies that maximize the benefits of these products while minimizing potential trade-offs. Ultimately, our findings will contribute to the development of more effective and sustainable crop management practices, tailored to the specific needs of durum wheat and other cereal crops facing similar challenges.

2. Materials and Methods

2.1. Experimental Design

Four independent experiments were conducted on seven elite durum wheat varieties showing low-to-intermediate resistance levels to the pathogen strains used [47] (Table 1 and Figure 1).

Table 1. Information about the selected varieties used in this study. The technical institut Arvalis notations in field experiments. A level of 1 is resistant and 9 is susceptible.

Variety	Breeder Companies	Resistance Level to STB
Anvergur	RAGT	7
Cultur	RAGT	5
Obelix	Italian Landrace	na
Pescadou	Florimond-Desprez	4.5
Sculptur	RAGT	5
Surmesur	RAGT	5.5
Voilur	RAGT	6.5

Plants were sown in pots (9 cm diameter) with eight seeds per pot in a substrate containing 50% of topsoil (Richeterre) and 50% of N2 Neuhaus soil (ID 4020.20) supplemented with 100 g/100 L of inputs (TOP-PHOS). The experiment took place in a growth chamber with a controlled temperature (24 °C for 12 h of daylight and 20 °C for 12 h of nightlight) with artificial light of 35,000 lumen. The plants were watered manually every day according to their needs.

Fifty milliliters of the root growth-promoting biostimulant Osiryl® (Frayssinet SAS, groupe Ethicae, 81240 Rouairoux, France) were soil-applied the day of sowing, at a final concentration of 2 $mL{\cdot}L^{-1}$ per pot, corresponding to the field-relevant dose provided by the supplier. The plant defense inducer Bion® (benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester, Syngenta France SAS; no longer approved by the EU) was foliar-applied as a spray at 0.8 $g{\cdot}L^{-1}$ per pot, corresponding to the field-relevant dose, 48 h prior to fungal inoculation (2 dpi on Figure 1). Control plants were sprayed with water only. Three weeks after sowing, at 0 dpi, plants were inoculated with *Zymoseptoria tritici* strain P1a. STB strain P1a was isolated in 2015 in Montpellier (France) by INRAE from durum wheat Pescadou [48]. Inoculation was performed using a spore suspension of 10^6 $spores{\cdot}mL^{-1}$, which was applied evenly to the leaves of each plant using a paintbrush. The conidiospores were counted using the Malassez counting chamber. STB inoculation involved dipping a paintbrush into a leaf spore suspension, made by adding one drop of Tween 20 per 10 mL of inoculum containing conidial spores, and applying it to the last ligulated leaf with six strokes of the brush. After inoculation, seedlings were covered with

plastic bags for three days to maintain high humidity and promote fungal infection and development. Symptom severity was evaluated twenty-one days after inoculation by eye expert evaluation as a percentage area covered by necrosis or pycnidia.

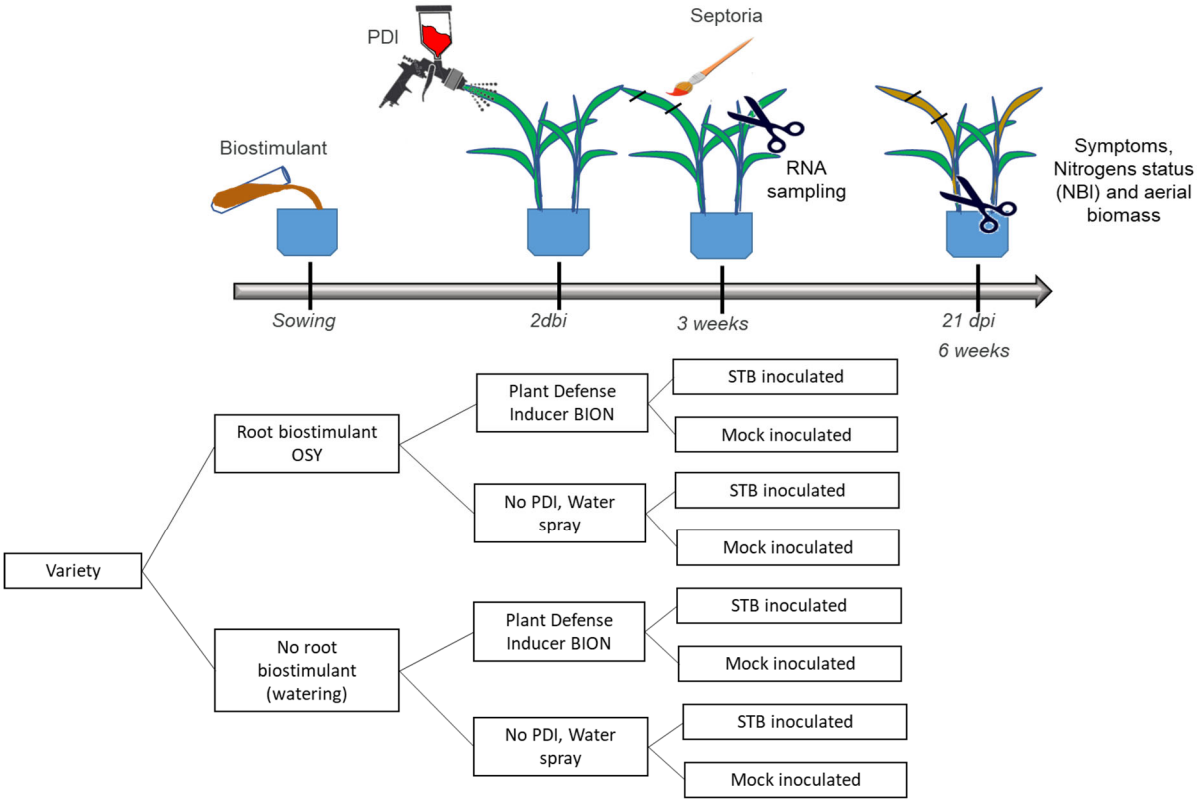


Figure 1. Experimental design showing the combination of a biostimulant and a PDI (plant defense inducer) under *Zymoseptoria tritici* inoculation. The biostimulant was added at sowing. The plant defense inducer was applied as a foliar spray at 2 dbi either forty-eight hours prior to fungal inoculation, almost three weeks (nineteen days) after the sowing. Control plants were sprayed with water only. Three weeks after sowing, plants were inoculated with *Zymoseptoria tritici*. Leaves (for RNA extraction) were sampled just before inoculation (forty-eight hours after the pulverization of the PDI). Symptoms assessment (STB: Septoria tritici blotch), Nitrogen Balance Index (NBI; indicator of nitrogen status) estimation, and aerial biomass were measured 6 weeks after sowing.

The experiment was set up as a randomized complete block design with three pots replicated for each factor in three independent experiments. For each combination of factors, different variables were measured: gene expression (four genes), Septoria tritici blotch (STB) symptoms (percentage of necrosis and percentage of pycnidia), above-ground dry biomass (grams), and Nitrogen Balance Index on 24 plants. Leaves were collected to assess molecular responses. For RNA extraction, the last fully expanded leaf was sampled 48 h after biocontrol agent treatment (0 dpi in Figure 1), three weeks after sowing, prior to fungal inoculation. Phenotypic measurements were taken 6 weeks after sowing at 21 days post-inoculation with STB (21 dpi in Figure 1). Nitrogen Balance Index (NBI), NBI reflects the balance between nitrogen assimilation (chlorophyll) and stress-related flavonol accumulation, serving as an indicator of plant nitrogen status, was measured with a Dualex [49] on the last elongated leaf. Above-ground dry biomass was determined after sampling and drying for 24 h at 50 °C. Symptoms, including necrosis (%) and pycnidia (%), were assessed visually at 21 days post-inoculation.

2.2. Molecular Experiment

The expression of genes (Chitinase 1, PR4, PAL and Noduline) was measured before inoculation, forty-eight hours after the plant defense inducer treatment. These four genes that we examined are proxies for basal immunity in wheat [48]. The protocol described previously by [50] was used to evaluate these gene expressions. Frozen leaf tissues were ground in liquid nitrogen, and ~500 mg of powder was treated with 1 mL of TRIzol (Invitrogen, Waltham, MA, USA). RNA samples (5 µg) were denatured for 5 min at 65 °C with oligo (dT) 18 (3.5 mM) and deoxynucleoside triphosphate (dNTP; 1.5 mM). They were then subjected to reverse transcription for 60 min at 37 °C with 200 U of reverse transcriptase M-MLV (Promega, Madison, WI, USA) in the appropriate buffer. The cDNA (5 µg, dilution 1:10) was then used for reverse-transcription quantitative (RT-q)PCR. The RT-qPCR mixtures contained PCR buffer, dNTP (0.25 mM), MgCl2 (2.5 mM), forward and reverse primers (final concentrations of 150, 300, or 600 nM), 1 U of HotGoldStar polymerase, and SYBR Green PCR mix according to the manufacturer's recommendations (Eurogentec, Seraing, Belgium). The sequences of primers used are given in Appendix A.1.

Amplification was performed as follows: 95 °C for 10 min; 40 cycles of 95 °C for 15 s, 62 °C for 1 min, and 72 °C for 30 s; 95 °C for 1 min and 55 °C for 30 s. RT-qPCR was performed using a LightCycler480 instrument (Roche, Basel, Switzerland), and data were extracted using the accompanying software (LightCycler480 SW 1.5.1). The durum wheat ubiquitin gene (CD921597) was used as an internal control, house-keeping gene, and the expression level did not vary significantly between treatments. The calculation of gene expression was performed using the measured efficiency for each primer pair.

2.3. Statistical Analysis

In order to identify the significance of the effect of the different factors and their variability among varieties, linear mixed models were used. For all the linear models presented, the reference used as the intercept was the average of all the varieties in the absence of Bion® and Osiryl® from one experiment (Table 2).

Table 2. Intercept is estimated by the mean value of one experiment without Bion® or Osiryl® for each variable studied.

Above-Ground Dry Biomass	NBI	Necrosis	Pycnidia/Necrosis	Chitinase 1	Noduline	PR4	Pal
0.2879	18.413	0.6848	0.2417	−1.0847	−0.0831	−4.4064	−4.3564

For the construction of the mixed linear models, the following strategy was chosen. The variety and all interactions with varieties were set as random effects. This allowed for the estimation of the variety variance for each effect. The factor experimentation was set up as a fixed factor, as the four experiments were all carried out under controlled conditions and environments. The aim was to adjust the existing difference from one experiment to another on the average level of each variable tested. Similarly, the effects of Osiryl® and Bion® and their interaction were set as fixed factors in order to study the average response of each variable to the different biosolution products. All the models that were used are summarized in Table 3, and the detailed information of each model is described in Appendix A.2. All data were analyzed using R studio software (version 2024.04.2). The package lme4 and its function glmer.nb were used to estimate the parameters of the model (version 1.1-35.3; [51]). The impact of the factors could not be assessed on Necrosis and pycnidia because GLMM models were used and these tests are not currently available with R for these models. For the genes, the effect of Bion® and Osiryl® alone could not be tested because the model did not converge.

Table 3. Statistical models used and variables tested for each variable and according to data type, where O stands for Osiryl®, B for Bion®, ":" for interaction, Exp for experimentation and V for variety.

Response Variable	Type of Data	Tested Effects	Statistical Model	Statistical Law
Above-ground dry biomass	Quantitative continue	O, B, O:B, Exp, V, O:B, B:V, O:B:V	Linear mixed with random effects	Normal (0, σ^2)
NBI	Quantitative continue	O, B, O:B, Exp, V, O:B, B:V, O:B:V	Linear mixed with random effects	Normal (0, σ^2)
Necrosis	Surface (%)	O, B, O:B, Exp, V, O:B, B:V, O:B:V	Generalized linear with random effects	Beta-binomial **BB** (n, α, β)
Pycnidia-to-necrosis ratio	Counting	O, B, O:B, Exp, V, O:B, B:V, O:B:V	Generalized linear with random effects	Negative Binomial **BN** (n, p)
Genes	Quantitative continue	O, B, O:B, Exp, V, O:B:V	Linear mixed with random effects	Normal (0, σ2)

In addition to linear modeling, we used random forest. All data were analyzed using R studio software (version 2024.04.2) with the vip and randomForest packages (ntree = 1000, and mtry = 3). Random forest is a nonlinear approach used to rank the importance of all the possible predictors (VIP) included in the factorial design. The importance measure considered for the analysis is the mean decrease in accuracy computed via the Random forest Classification Algorithm. The predictors are the dummy variables corresponding to the single and interaction effects between all factors at all available orders. Random Forest also gives an idea of the percentage of variance explained by this full factorial design.

3. Results

3.1. Salicylic Acid Analog Enhances Resistance but Reduces Growth in Durum Wheat

Our multifactorial experiments revealed a clear growth–defense trade-off in durum wheat challenged by septoria leaf blotch. Treatments with the defense-stimulating compound Bion® resulted in improved plant resistance, as evidenced by a significant reduction of 25% in the probability of new pycnidia formation on necrotic leaf surfaces (Figure 2 and Appendix B.1). However, this increased defensive capacity came at the cost of reduced plant growth, with Bion®-treated plants exhibiting an average significant decrease of 8% in above-ground dry biomass (Appendix B.1). Notably, plants treated with the root growth-promoting biostimulant Osiryl® did not show any significant improvement regarding their growth; Figure 2b shows that the plants that were treated had less above-ground dry biomass. Nevertheless, when applied individually, it significantly reduced by 45% the probability of new pycnidia formation on necrotic leaf surfaces (Figure 2 and Appendix B.1). However, neither product significantly altered the overall necrotic surface area. Importantly, the simultaneous application of Osiryl® and Bion® did not produce a synergistic or antagonistic effect on biomass, indicating that the physiological costs associated with induced resistance were not further amplified or mitigated by their combination (Figure 2b). These findings regarding the defense-stimulating compound Bion® are in accordance with the expected trade-offs between resistance to disease and growth in durum wheat. This emphasizes the need during the evaluation of these products to always consider both growth and resistance together.

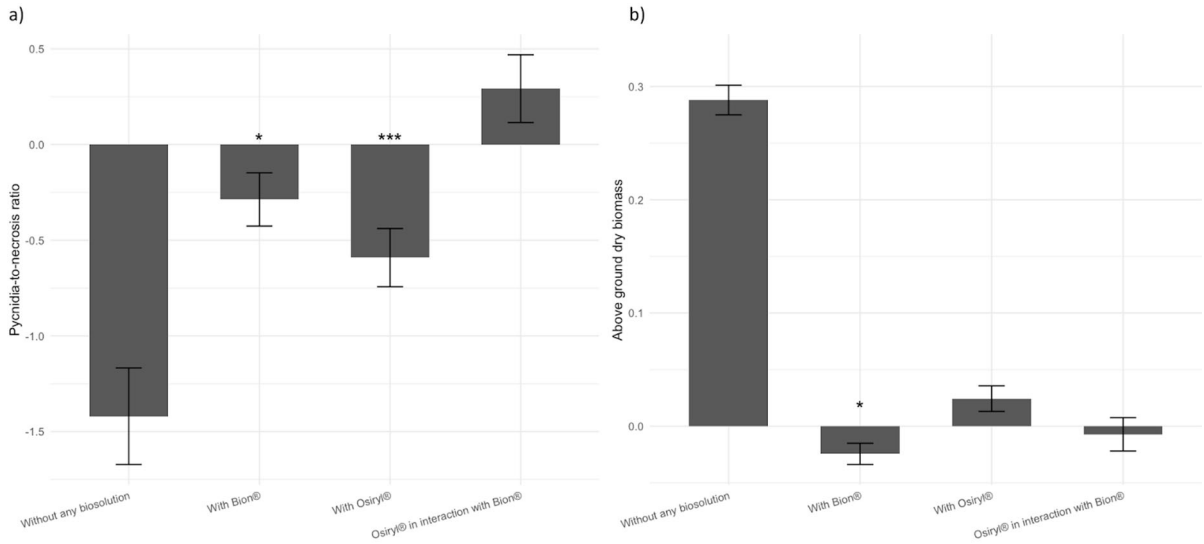


Figure 2. (**a**) Pycnidia-to-necrosis ratio without any biosolution (−1.420), with Bion® (−0.287), with Osiryl® (−0.591) and when Osiryl interacts with Bion® (0.292). (**b**) Above-ground dry biomass without any biosolution (0.288), with Bion® (−0.0244), with Osiryl® (0.0244) and when interacts with Bion® (−0.0072). * and *** represent significant differences at the 0.05 and 0.001 levels. Values are means ± standard error (SE). No error bars were produced for the probabilities in (**a**).

Surprisingly, no significant effect of Bion® was observed on necrosis or NBI. One hypothesis could be that this lack of effect could be due to the fact that this analysis was performed by pooling data of seven varieties, whereas genetic background can impact these phenotypes.

As the study of the different factors shows a clear interaction between biosolution products and the increase in the resistance. It is important to verify this interaction at the molecular level on some defense marker genes.

3.2. Four Defense-Related Genes Expression Induced by Salicylic Acid Analog Can Be Modulated by the Biostimulant

To investigate the mechanism of action of Bion® as a plant defense inducer, the relative expression of three defense-related genes (chitinase 1, PAL, and PR4) and one stress marker gene in wheat (nodulin) was evaluated. The results confirmed Bion®'s role as a plant defense inducer: it significantly increased the expression of three out of the four studied genes by an average of 22% (Figure 3 and Appendix B.2). Notably, the relative expression of chitinase 1 rose by 38%. In contrast, Osiryl® exhibited no significant effect on the expression of any of the four genes, reinforcing its classification as a biostimulant without plant defense inducer activity. The observation that increased resistance was observed even though the expression of defense genes was unchanged is a fairly common in experiments and suggests that the protective effect of Osiryl® is due to resistance mechanisms other than those linked to basal immunity.

The plant defense induction activity of Bion® (applied 48 h before by leaf spray) was counteracted when used in combination with Osiryl® (applied three weekends before by drenching at sowing), as none of the marker genes were induced. Rather, this combination significantly reduced by 11% the expression of the PAL gene (Figure 3 and Appendix B.2). These results show that two products that act differently on the expression of defense marker genes can have their effects canceled out when combined, making it difficult to predict this type of mechanism of action when products are combined.

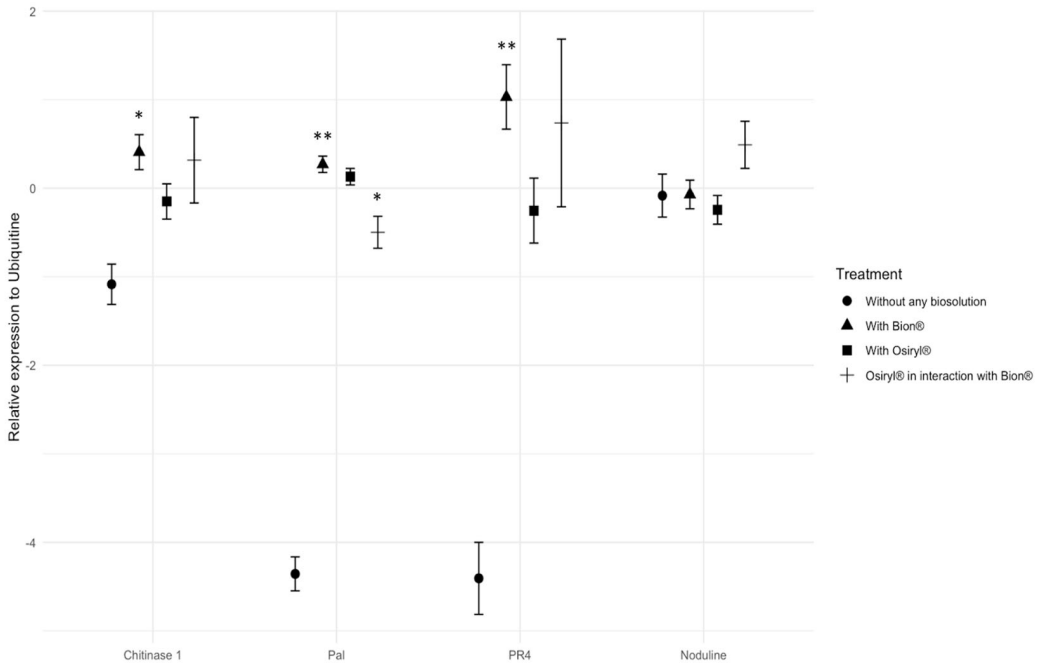


Figure 3. Impact of Bion®, Osiryl® and the combination of both on the expression of the three defense genes (Chitinase 1, Pal and PRA) and one defense marker gene (such as Noduline) in comparison with the expression of genes without any biosolution. * and ** represent significant differences at the 0.05 and 0.01 levels. Values are means ± standard error (SE).

3.3. Variety Dictates Resistance, Defense and Growth Outcomes in Durum Wheat

The absence of a significant effect of the Osiryl® root biostimulant on above-ground biomass and nitrogen status was unexpected. Given that genotype-dependent responses of this type of product are well documented, we therefore examined whether treatment effects varied among the seven genotypes tested. First, variety had a strong main effect—independent of treatment—on all phenotypic traits and on the expression of the four genes analyzed (Table 4), confirming that our experimental system robustly captures genetic background effects across all measured variables. Our analysis revealed that the effect of Osiryl® on above-ground dry biomass was significantly genotype-dependent (Table 4), whereas no such varietal dependence was observed for Bion®. Indeed, the varietal variability associated with Bion® effects on above-ground biomass was approximately half that observed for Osiryl®, indicating a more genotype-sensitive response to the latter product. Notably, the interaction between the two products also varied significantly among varieties, affecting both aerial biomass and the three defense-related genes previously identified as responsive (Chitinase 1, PR4, and PAL) (Table 4).

Overall, our results highlight a pronounced varietal dependence in treatment efficacy, influencing both growth and defense induction-related traits. This strong genotype-specific variability raises a key subsequent question: which genotypes drive these differential responses? Identifying the varieties that contribute most strongly to the observed treatment effects will be essential to translating these findings into practical recommendations, as it will allow us to determine which genetic backgrounds are most likely to benefit from the use of these products.

Table 4. Impact of genetic background and treatments on key variables. Estimates of the random effects standard error were calculated from the mixed model. *, ** and *** represent significant differences at the 0.05, 0.01 and 0.001 levels, respectively. The impact of these factors could not be assessed on necrosis and pycnidia because GLMM models were used and these tests are not currently available with R for these models. For the genes, the effect of Bion® and Osiryl® alone could not be tested because the model did not converge.

	Variety	Osiryl®:Variety	Bion®:Variety	Osiryl®:Bion®:Variety	Residual
Above-ground dry biomass	0.0318 (***)	0.0239 (**)	0.0181	0.0299 (**)	0.0894
NBI	2.939 (***)	3.033 (***)	1.54 (**)	1.436	4.661
Chitinase 1	0.4674 (***)			1.0264 (***)	0.8319
Noduline	0.5627 (***)			0.3332	0.6831
PR4	0.8146 (***)			2.0719 (***)	1.5333
Pal	0.4748 (***)			0.3164 (*)	0.389

3.4. The Interaction Between Factors Is Variable Among Varieties

To characterize the contribution of each variety to treatment responses across traits, confidence intervals (CIs) were estimated for each mixed model. This analysis confirmed that several genotypes displayed robust, treatment-specific effects. Although applications of Osiryl® and Bion® consistently influenced the different traits, the magnitude of these effects varied markedly among durum wheat varieties (Figure 4). As previously reported, Bion® treatment led to an overall reduction in above-ground dry biomass (−8%), an effect that was largely independent of genotype. Nevertheless, CI-based comparisons revealed that only the variety RGT Cultur differed significantly from the reference (Figure 4a).

Although no significant main effect of biosolutions on the Nitrogen Balance Index (NBI) was detected by the model, clear varietal differences in treatment responses were evident in this analysis (Figure 4b). Osiryl® increased NBI significantly for two varieties (by 19% for Obelix and 45% for RGT Surmesur), whereas it reduced NBI by 0.4–5% for the other varieties, except for RGT Anvergur, which seemed unresponsive to the product. In contrast, Bion® increased NBI significantly by 20% in RGT Anvergur but decreased it by 3% in Obelix and RGT Voilur. Importantly, the combined treatment of Osiryl® and Bion® produced effects that differed from those observed with either product alone, underscoring the complexity of their interaction. For example, Pescadou, which exhibited the highest NBI under control conditions, showed an increase in this index following Bion® application but a decrease in response to Osiryl®, with additional modulation arising from the interaction between the two products (Figure 4b). This contrasting pattern illustrates the difficulty of predicting the relevance and reliability of such indicators for monitoring the effects of combined treatments, particularly when strong genotype-dependent responses are involved.

The overall analysis did not reveal any effect of the treatments on the necrotic surface (Table 3). This new analysis allows us to observe a genotype-dependent pattern for Bion® (Figrure 4c). Bion appeared to confer significant protection on RGT Anvergur, while it increased necrosis on Obelix and RGT Surmesur. However, the effects of Osyril® or the interaction between products remained non-significant depending on the variety. On the contrary, although the model indicated that Osiryl® and Bion® applied individually tended to reduce the pycnidia-to-necrosis (P/N) ratio, this response was also strongly variety-dependent (Figure 4d). For instance, Bion® reduced the P/N ratio in RGT Surmesur but increased it in Pescadou and RGT Cultur. The positive effect observed for Osiryl® treatments was observed only for the Obelix variety. Notably, this positive effect could have been counteracted by the negative impact of the interaction between products, further illustrating the genotype-specific nature of combined treatment responses.

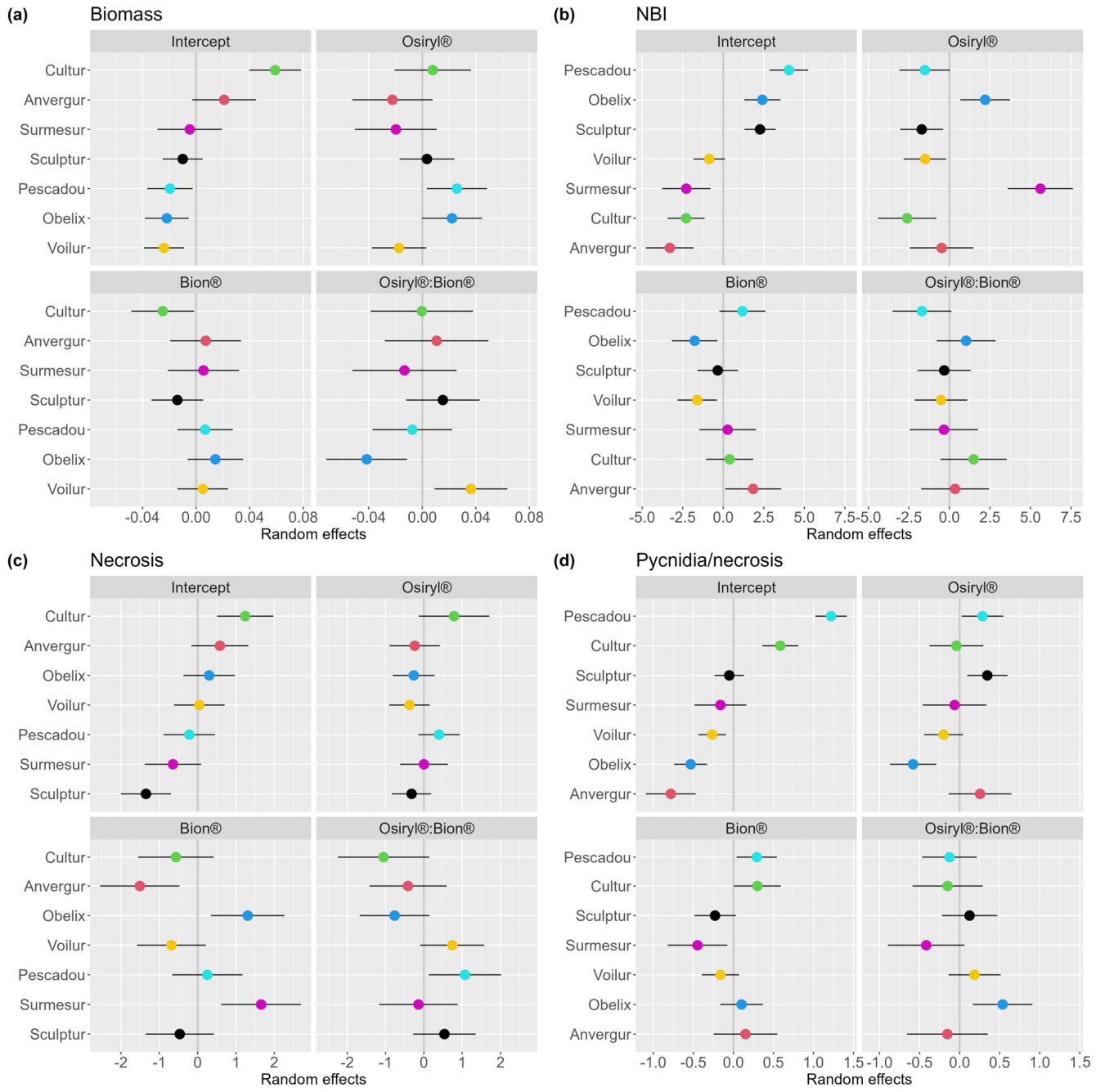


Figure 4. Effect of the variety (intercept) and the effect of Bion® in combination with Osiryl® (Osy) according to the variety on (**a**) the above-ground dry biomass, (**b**) the NBI, (**c**) the necrosis area and (**d**) the pycnidia-to-necrosis ratio (Pycnidia/necrosis). A different color has been chosen for each variety to make reading easier. The variety can be considered to have a different reaction from the reference (one of the experiments without products) when the error bar does not pass through 0. To facilitate the interpretation of this type of graph, a reading grid is provided in the Appendix B.3.

A similar approach was applied to the expression of the four genes; however, limitations in the dataset restricted the analysis to the interaction between variety and the combined Osiryl®–Bion® treatment. As shown in Appendix B.4, the effect of the interaction between products was significantly variety-dependent for all genes except Nodulin, with either positive or negative modulation depending on the genotype. These results indicate that it will be difficult to predict whether these defense marker genes will be consistently

induced by the defense stimulator Bion® when used in combination with Osiryl®, thereby limiting the practical applicability of marker-gene-based approaches.

The observation of trade-off phenomena is inherently difficult to interpret due to these contrasting genotype-dependent responses. At the global scale, our analysis showed that Bion® negatively affected above-ground biomass while reducing pycnidia formation, without a corresponding reduction in necrosis. However, a more detailed, genotype-specific analysis revealed that the negative impact of Bion® on aerial biomass was mainly driven by the RGT Cultur and RGT Sculptur varieties. Notably, in RGT Sculptur, Bion® also reduced pycnidia, suggesting that a growth–defense trade-off may be expressed in this genotype, whereas such a trade-off appears not evident in RGT Cultur, where Bion® treatment was associated with increased pycnidia. Similarly, Bion® had a negative effect on the Nitrogen Balance Index (NBI) in Obelix and RGT Voilur, yet these physiological changes were associated with contrasting disease outcomes: Bion® reduced necrosis in RGT Voilur but increased it in Obelix. Together, these results indicate that the difficulty in experimentally detecting growth–defense trade-offs may stem from the frequent omission of genotype-specific effects. Each variety appears to deploy distinct strategies in response to the different treatments, either engaging or avoiding trade-offs depending on its genetic background.

Together, these results underscore that the genetic background of durum wheat is a major determinant of treatment efficacy, shaping the magnitude of the effect of the different treatments. However, while these multifactorial models are well suited to detect significant effects and interactions, they do not readily allow the relative importance of factors and their combinations to be ranked. This limitation motivated the use of complementary analytical approaches, presented in the following section, aimed at better disentangling and prioritizing the respective contributions of genotype, treatments, and their interactions to durum wheat responses to septoria disease.

3.5. Beyond Main Effects: Interacting Factors Govern Phenotypic Variance

This analysis reveals that, although simple effects are often the most significant—with the effect of both treatments alone consistently dominating—several second- and third-order interactions also show meaningful effect sizes across variables (Figure 5). Despite the random forest model, accuracy is middling (20.4% for aerial biomass, 22.5% for NBI, 25.6% for necrosis and 17.8% for P/N); it allows a classification of the effect of the different factors. For example, it was observed that biomass was influenced by both treatments (Osiryl® and Bion®), interactions with inoculation and interactions between variety and Osiryl®. In contrast, the necrotic lesion area was predominantly determined by genotype, reflecting intrinsic resistance. Notably, the effect of Bion® alone is of similar magnitude to its interaction with variety, which may explain why treatment effects sometimes fail to appear—the direct effect of Bion® can be masked or canceled out by its genotype-specific interactions. These results demonstrated that predicting treatment outcomes requires accounting for both genotype and multifactorial interactions, not just main effects.

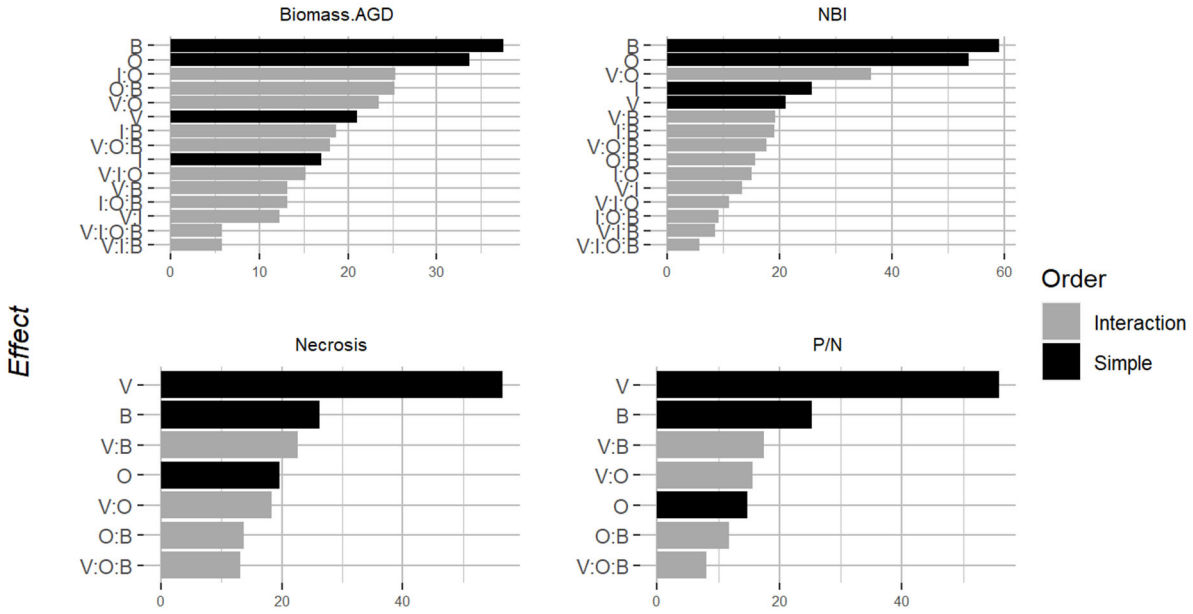


Figure 5. Random forest variable importance plot. The variables are ranked in order of relevance in predicting above-ground biomass, NBI, necrosis surface and pycnidia-to-necrosis ratio. The importance measure considered for the analysis is the mean decrease in accuracy computed via the Random Forest Classification Algorithm. The random forest model accuracy is equal to 20.4% for aerial biomass, 22.5% for NBI, 25.6% for necrosis and 17.8% for P/N, achieved with 1000 trees and 3 mtry. First-order variables are represented in black, and all the interactions between variables are represented in gray.

4. Discussion

The systematic investigation of durum wheat's response to the plant resistance inducer Bion® and the root biostimulant Osiryl® has revealed the complex and interactive nature of disease resistance and plant growth dynamics, consistent with findings across multiple pathosystems [5,52,53]. Our results confirm that defense-inducing compounds such as Bion® significantly enhance disease resistance by reducing pycnidia formation and upregulating defense-related gene expression. These findings align with previous studies demonstrating the efficacy of such products in crop protection [32]. Our results also highlight the growth–defense trade-off, a fundamental constraint in plant defense strategies, where enhanced defense often comes at the cost of aerial biomass [54,55].

Importantly, the efficacy of these treatments is not uniform but varies substantially depending on the genetic background of the durum wheat cultivar. This genotype-dependent variation echoes reports in wheat, Arabidopsis, and other crops, where differences in defense pathway induction and regulation dictate the extent and cost of resistance [29,56–58]. Genotypic variation thus emerges as a major determinant of treatment outcomes and must be carefully considered in cultivar selection for integrated disease management.

When applied in combination, treatments such as Osiryl® and Bion® did not consistently exhibit additive, synergistic, or antagonistic effects. Instead, their combined impacts varied significantly depending on the specific trait being measured—sometimes additive, sometimes antagonistic. This variability in interaction patterns makes the overall effects difficult to predict, as the physiological responses are context-dependent rather than following a uniform trend. Such complexity reflects the general principles in chemical ecology, where multiple elicitors are often assumed to exert unpredictable interactions [59]. It also underscores the need for comprehensive phenotypic assessment beyond disease symptomatology alone [60]. Indeed, the difficulty in predicting treatment effects under field conditions may stem from overlooked factors or unmeasured interactions. For instance,

genotype × product interactions can mask the effects of individual treatments if genotypic variability is not accounted for, emphasizing the importance of experimental designs and analyses that incorporate multiple explanatory factors.

Our multifactorial analyses further revealed that plant response variance arises not only from individual factors such as genotype or treatment but is predominantly shaped by their interactions. This complexity likely underlies the inconsistent field efficacy reported for many biosolutions, highlighting the limitations of extrapolating controlled environment results directly to agricultural contexts. A major trade-off between growth and defense has previously been shown to disappear in field conditions in Arabidopsis [61]. The physiological and agronomic impacts of biosolutions are inherently multifactorial, influenced by genotype, environment, treatment, and their complex interplay. While single-factor analyses can identify significant main effects, our multifactorial framework demonstrated that much of the variance in disease susceptibility and growth is explained by combinations of factors rather than isolated effects. This insight is particularly critical for field applications, where the effectiveness of biosolutions is modulated by the dynamic interplay of genetic, environmental, and agronomic variables [4,7,62].

The multifactorial nature of durum wheat responses to Osiryl® and Bion® offers actionable insights for optimizing biosolution deployment under real-world agricultural conditions. Our findings demonstrate that genotype-specific responses are not merely a source of variability but a critical lever for tailoring treatment strategies to maximize efficacy and sustainability. By systematically mapping these interactions and using tools such as models, farmers and agronomists we may be able to prioritize cultivars that align with specific biosolution goals (e.g., disease resistance vs. yield stability) and environmental constraints (e.g., drought vs. disease pressure) [63,64]. Future strategies should integrate multifactorial analyses and leverage sophisticated statistical tools to dissect and prioritize the contributions of genetic, environmental, and agronomic variables [65]. Moreover, the lack of consistent effects when combining Osiryl® and Bion® underscores the need for dynamic treatment optimization. Rather than relying on fixed application protocols, our results suggest that real-time monitoring of plant physiological states could enable adaptive management strategies. For example, if early-season monitoring detects high stress levels in a particular cultivar (e.g., elevated reactive oxygen species or defense gene expression), farmers could adjust biosolution dosages or timing to mitigate trade-offs between growth and defense. Conversely, in cultivars with robust baseline resistance, treatments could be optimized for growth promotion without compromising disease control. Such precision approaches would not only improve treatment efficacy but also reduce unnecessary inputs, aligning with the EU Green Deal's goals of sustainable intensification. This may require the development of specific tools based on hyperspectral imaging or portable metabolomic sensors [66,67].

Successful septoria management in durum wheat cannot rely on single-factor interventions [68]. The results of this study were obtained in the laboratory, but management aligned with plant development stages is required to apply biosolutions as close as possible to the application timing recommended by the provider for field conditions. This needs to be reproduced in the field in order to clarify if our results are agronomically significant. To operationalize a strategy, we propose a framework for field implementation. The first step is to conduct pre-season trials to identify cultivars with compatible physiological responses to target biosolutions. This could involve high-throughput phenotyping of key traits to generate cultivar × biosolution compatibility matrices [15]. This information could be available in decision-support tools [69]. A second step is to integrate local climatic and soil data with genotype-specific response profiles to predict optimal treatment windows. For instance, under high disease pressure, cultivars with strong induced resistance might benefit from

early-season Bion® applications, while those prone to growth-defense trade-offs could require staggered or reduced dosages to balance productivity and resilience. This will require proposing new models for wheat growth including trade-off mitigation information [63]. Finally, it is necessary in the future to deploy digital decision-support tools that recommend real-time adjustments to biosolution applications based on in-season plant physiological data. Such systems could leverage AI-driven models trained on multifactorial datasets to forecast trade-off risks and synergistic opportunities [69].

Finally, the regulatory and agronomic implications of these findings extend beyond durum wheat. By adopting a multifactorial, genotype-aware approach, stakeholders can develop standardized protocols for biosolution evaluation that account for interactive effects rather than isolated factors. This shift would not only improve field-level predictability but also support policy frameworks that incentivize precision agriculture practices. For example, EU certification schemes could require genotype-specific efficacy data for biosolution registration, ensuring that product labels reflect real-world performance across diverse cultivars and environments. Similarly, breeding programs could prioritize cultivars that are better suited for integrated biosolution strategies.

In summary, our multifactorial analysis provides a blueprint for translating controlled-environment insights into field-ready strategies, emphasizing that the future of sustainable crop management lies in harnessing genotype × treatment × environment interactions through data-driven, adaptive approaches.

5. Conclusions

In summary, our results support the trade-off hypothesis that induced resistance often comes at a cost in growth but also highlight that this cost is strongly mediated by complex genotype × environment × management interactions. These findings underscore the need to move beyond single-factor analyses in crop protection research and to develop predictive models that explicitly account for these interactions.

Our results emphasize that the development of effective septoria management strategies in durum wheat must move beyond a "one-size-fits-all" approach. The interplay between genotype, environment, and treatment creates a landscape of outcomes that cannot be reliably predicted without accounting for multifactorial interactions. Therefore, we advocate for context-specific, cultivar-driven application of biosolutions, with continuous evaluation of both disease control and yield performance. Leveraging advanced statistical and bioinformatic tools will be indispensable for optimizing treatment protocols and accelerating breeding for cultivars that can sustain both productivity and resilience in the face of evolving biotic pressures.

Future biosolution-based strategies should follow an intelligent, integrated screening approach, incorporating the impacts of varietal and environmental factors whenever possible, while reserving less sustainable agrichemicals for conditions that cannot be effectively managed otherwise.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author to allow for commercialization of research findings.

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Conflicts of Interest: Author Marie-Emmanuelle Saint-Macary was employed by the company: Groupe Ethicae (FRAYSSINET SAS). The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

PDI Plant Defense Inducer
STB Septoria tritici blotch
NBI Nitrogen Balance Index

Appendix A

Appendix A comprises two appendices providing additional information on the methodologies used in this article.

Appendix A.1

Table A1. List of primers. ChitinaseI, PR4 and PAL are defense genes and Noduline is a defense marker gene for wheat. Ubiquitine was used as the house-keeping gene.

Gene	Primer Forward	Primer Reverse	Efficiency
Ubiquitine	CTGGCGAGGATATGTTCCAT	TCGGATGGAAGACCTTTGTC	2.000
Chitinase 1	TGCGCTACTGCAGCATACTC	ACCATACGAATCAACTGATCACA	1.836
Noduline	GCCGGTTGCACGAGTAAA	TTACGAAAAGCGGAAGAGGA	1.848
PR4	ATAAAAGGCTAGCTAAAAGTAAGTTGA	TTTGAATAGATAACCCGTTTGG	1.883
Pal	CCCCCATTGGTGTCTCCAT	ACTGCGCGAACATCAGCTT	2.024

Appendix A.2

This appendix describes the methodology to choose models for statistical analysis.

Models used for above-ground dry biomass, NBI and five defense genes

For the variables NBI, above-ground dry biomass and the expression of five defense genes (Chitinase 1, Noduline, PR4, Pal and beta-glutamase), a linear mixed model with random effects was used (cf Equation (A1)). The model was simplified for the defense genes to fit the data (cf Equation (A2)). The lme4 package and its function lmer were used to estimate the parameters of it (version: 1.1-35.5; [51]).

$$h\,(E[Yijkl]) = \beta 0 + ml + \alpha i + ail + \beta j + bjl + \gamma ij + cijl + \delta k \quad (A1)$$

where

Yijkl~N (μ, $\sigma 2$); ml~N (0, $\sigma 2m$); ail~N (0, $\sigma 2a$); bjl~N (0, $\sigma 2b$) et cijl~N (0, $\sigma 2c$)
h: link function: $h(\mu) = \mu$ | i: Bion®, j: Osiryl®, l: Variety, k: experiment
E: Conditional expectation | m, a, b, c: random effects
Yijkl: Variable of interest | α, β, γ, δ: Fixed effects

$$h\,(E[Yijkl]) = \beta 0 + ml + \alpha i + \beta j + \gamma ij + cijl + \delta k \quad (A2)$$

where:

h: link function: h(μ) = μ | i: Bion®, j: Osiryl®, l: Variety, k: experiment

E: Conditional expectation | m, c: random effects

Yijkl: Variable of interest | α, β, γ, δ: Fix effects

Models used for necrosis surface and pycnidia-to-necrosis ratio.

As necrosis is a measure of necrotic area relative to leaf size (in %), a generalized linear random effects model was used (cf Equation (A3)). Percentage data were converted into binary 0, 1 data. To do this, each percentage was reduced to a double vector, the sum of which is 100%. Example: if the necrotic area represents 70% of the total leaf area, the vector will be [30,70]. To apply this method, we assumed that all leaves had the same size. In order to manage the overdispersion of the data, a beta-binomial distribution was used [70,71].

$$h\,(E[Yijkl]) = \beta 0 + ml + \alpha i + ail + \beta j + bjl + \gamma ij + cijl + \delta k \quad \text{(A3)}$$

Yijkl~BB (n, α, β); Yijkl/p~Binomial (n, p) et p~Beta (α, β)

ml~N (0, σ2m); ail~N (0, σ2a); bjl~N (0, σ2b) et cijl~N (0, σ2c)

h: Link function: logit: h(p) = ln(p/1 − p) | i: Bion®, j: Osiryl®, l: Variety, k: experiment

E: Conditional expectation | m, a, b, c: random effects

Yijkl: Variable of interest | α, β, γ, δ: Fix effects

The package glmmTMB and its function of the same name were used to estimate the parameters of the model (version 1.1.9; [72]).

The pycnid variable is assimilated to a count data (pycnidia) on a surface data (necrosis). A generalized linear random effect model was used (cf Equation (A4)). Necrosis is offset in the model to take account of the pycnidia-to-necrosis ratio. A negative binomial distribution was used to correct for overdispersion of the data [70].

$$h\,(E[Yijkl]) = \beta 0 + ml + \alpha i + ail + \beta j + bjl + \gamma ij + cijl + \delta k + \ln(n + 1) \quad \text{(A4)}$$

Yijkl~BN (n, p)

ml~N (0, σ2m); ail~N (0, σ2a); bjl~N (0, σ2b) et cijl~N (0, σ2c)

h: Link function: logarithme: h(p) = ln(p) | i: Bion®, j: Osiryl®, l: Variety, k: experiment

E: Conditional expectation | m, a, b, c: random effects

Yijkl: Variable of interest | α, β, γ, δ: Fix effects

LD: Linear dichroism.

Appendix B

Appendix B contains three appendices presenting additional results, as well as an appendix that may assist in interpreting the results in Figure 4.

Appendix B.1

Table A2. Results of the fixed effects obtained with the statistical models for the above-ground dry biomass, the NBI, the necrosis, and the pycnidia-to-necrosis ratio. Std. error stands for standard error. Significance levels: *p*. value < 0.05.

Fixed Effects				
Variables	**Coefficients**	**Estimate**	**Std. Error**	***p*. Value**
Above-ground dry biomass	Intercept	0.288	0.0131	2.98×10^{-7}
	Osiryl®	0.0244	0.0113	0.0724
	Bion®	−0.0244	0.0094	0.0399
	Osiryl®:Bion®	−0.0072	0.0147	6.38×10^{-1}

Table A2. *Cont.*

Fixed Effects				
Variables	**Coefficients**	**Estimate**	**Std. Error**	***p*. Value**
NBI	Intercept	18.42	1.148	3.10×10^{-6}
	Osiryl®	1.786	1.211	1.91×10^{-1}
	Bion®	0.145	0.692	8.40×10^{-1}
	Osiryl®:Bion®	−1.56	0.765	8.61×10^{-2}
Necrosis	Intercept	0.7758	0.3171	1.44×10^{-2}
	Osiryl®	0.1375	0.2192	5.31×10^{-1}
	Bion®	0.0474	0.4312	9.12×10^{-1}
	Osiryl®:Bion®	0.2996	0.3636	4.10×10^{-1}
Pycnidia-to-necrosis ratio	Intercept	−1.42	0.252	1.80×10^{-8}
	Osiryl®	−0.591	0.152	0.000105
	Bion®	−0.287	0.139	0.0387
	Osiryl®:Bion®	0.292	0.177	0.0985

Appendix B.2

Table A3. Results of the fixed effects obtained with the statistical model for the three defense genes (Chitinase A, Pal and PR4) and the defense marker gene (Noduline). Std. error stands for standard error. Significance levels: *p*. value < 0.05.

Fixed Effects				
Genes	**Coefficients**	**Estimate**	**Std. Error**	***p*. Value**
Chitinase 1	Intercept	−1.0847	0.228	0.00052
	Osiryl	−0.149	0.1991	0.4557
	Bion	0.408	0.1975	0.041
	Osiryl:Bion	0.3164	0.4833	0.52705
Noduline	Intercept	−0.08314	0.24333	0.741
	Osiryl	−0.24449	0.16348	0.1374
	Bion	−0.07036	0.1622	0.6652
	Osiryl:Bion	0.49009	0.26633	0.0779
PR4	Intercept	−4.4064	0.4068	2.16×10^{-7}
	Osiryl	−0.2528	0.367	0.49226
	Bion	1.0302	0.3641	0.00546
	Osiryl:Bion	0.7379	0.9469	0.45562
Pal	Intercept	−4.35642	0.19171	7.12×10^{-8}
	Osiryl	0.13048	0.09312	0.1637
	Bion	0.26984	0.09238	0.00416
	Osiryl:Bion	−0.49797	0.17991	0.0157

Appendix B.3

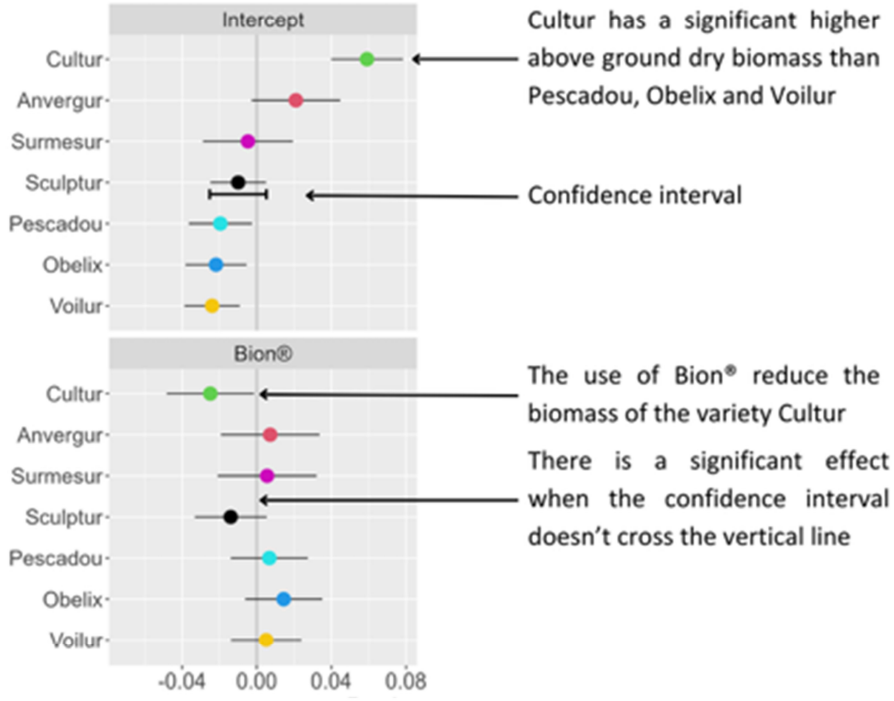


Figure A1. Guide to interpreting Figures 4 and A2.

Appendix B.4

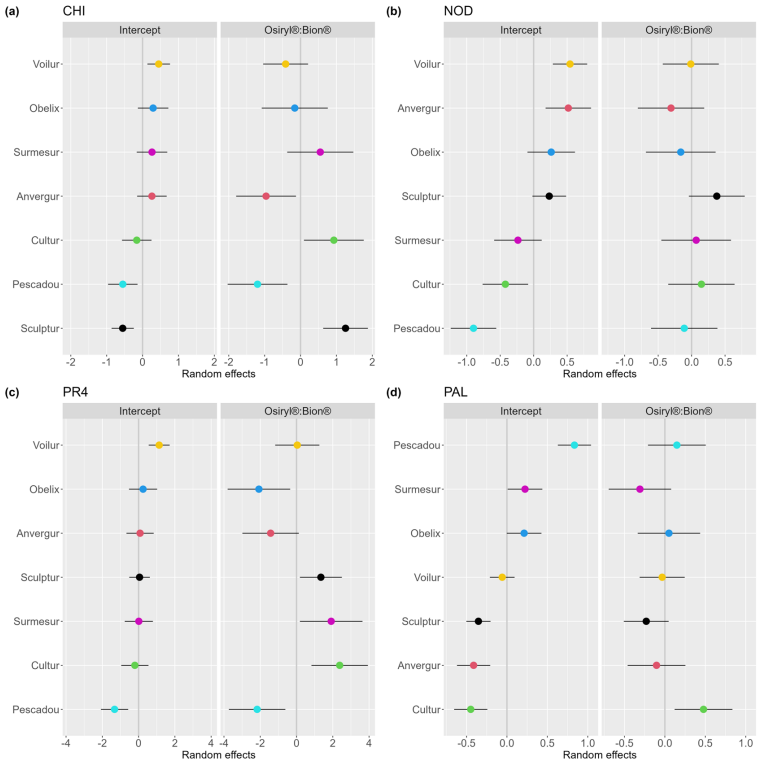


Figure A2. Effect of the variety (intercept) and the effect of Bion® in combination with Osiryl® (Osy) according to the variety for the four defense genes (**a**) Chitinase 1 (CHI), (**b**) Noduline (NOD), (**c**) PR4 and (**d**) Pal. The seven varieties are RGT Voilur, Obelix, RGT Anvergur, RGT Sculptur, RGT Surmesur, RGT Cultur and Pescadou. A different color was chosen for each variety to make reading easier. We can consider that the variety has a different reaction from the reference (one of the experiments without any product) when the error bar does not cross zero.

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