Effects of a fungal-bacterial consortium along with their individual components on the resistance to the english grain aphid on wheat under water deficit

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JUSTINE VAN MERHAEGHE

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CO-PROMOTEURS: PR. FREDERIC FRANCIS AND PR. CLAUDIO RAMÍREZ

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Abstract

Microorganisms can live in the rhizosphere and interact with the roots of plants. Nowadays, they are studied as biological alternatives to chemicals in pest control and to be beneficial for growth promotion. They are also able to mitigate the negative effect of decreasing water availability for the plant. Henceforth, the present study investigates the effects of microorganisms on both plant growth and induced resistance in wheat plants (*Triticum aestivum* L.) against the grain aphid (*Sitobion avenae*) under different water availability. The two microorganisms studied were *Bacillus subtilis* and *Trichoderma virens*. Their consortium was also studied and compared to non-inoculated plants. The resistance of plants against aphids and their feeding behaviour were studied as well as plant response parameters. Aphid probing behaviour was recorded using electropenetrography. Results reveal reduced aphid resistance on plants inoculated with microorganisms. However, the inoculation of *T. virens* has a negative impact on the duration of aphid feeding in the phloem. The opposite trend was obtained for the ones feeding on plants inoculated with *B. subtilis*. The consortium's effects are nuanced. Water availability influences the results. Microorganisms' inoculation promote plant growth, namely shoot height. To conclude, the use of microbials is discussed as a tool in crop protection.

Keywords: *Triticum aestivum*, microorganisms, *Bacillus subtilis*, *Trichoderma virens*, *Sitobion avenae*, water availability, induce systemic resistance, electropenetrography.

Résumé

Les microorganismes peuvent vivre dans la rhizosphère et d'interagir avec les racines des plantes. De nos jours, ils sont étudiés comme alternatives biologique aux produits chimiques dans les méthodes de lutte contre les ravageurs et pour favoriser la croissance des plantes. Ils sont également capables atténuer les effets négatifs de la diminution de la disponibilité en eau pour la plante. Dès lors, la présente étude vise à déterminer les effets des microorganismes à la fois sur la croissance des plantes et sur la résistance induite des plants de blé (*Triticum aestivum* L.) vis-à-vis du puceron des céréales (*Sitobion avenae*) sous des conditions de disponibilité en eau différentes. Les deux microorganismes étudiés étaient *Bacillus subtilis* et *Trichoderma virens*. Leur consortium a également été étudié et comparé à des plantes non inoculées. La résistance des plantes aux pucerons et leur comportement alimentaire ont été étudiés, ainsi que les paramètres de réponse des plantes. Le comportement alimentaire des pucerons a été enregistré grâce à l'électropénétrographie. Les résultats révèlent une résistance réduite des pucerons sur les plantes inoculées avec des microorganismes. Toutefois, l'inoculation de *T. virens* a un impact négatif sur la durée d'alimentation des pucerons dans le phloème. La tendance inverse a été observée pour ceux se nourrissant sur les plantes inoculées par *B. subtilis*. Les effets du consortium sont nuancés. La disponibilité en eau influence les résultats. L'inoculation de microorganismes favorise la croissance des plantes, notamment la hauteur de la partie aérienne. Pour conclure, l'utilisation de microorganismes est discutée comme un outil dans la protection des plantes.

Mots-clés : *Triticum aestivum*, microorganismes, *Bacillus subtilis*, *Trichoderma virens*, *Sitobion avenae*, disponibilité en eau, résistance systémique induite, électropénétrographie.

Table of contents

Acknowledgments I
Abstract II
Résumé III
Table of contents IV
Tables of figures V
Table of tables VI
List of abbreviations and acronyms VII
I. General introduction 1
II. Context of study 2
A. Rhizosphere and beneficial microbials 2
a. Bacteria 2
b. Fungi 3
c. Combination Bacteria and fungi 5
B. Wheat 5
C. Aphids 6
a. Classification 6
b. Morphology 6
c. Life cycle 7
d. Aphid host selection, acceptance and probing behaviour 8
e. Damages 9
D. Pesticide use in wheat 10
a. Pest control 10
b. Alternatives in pest control 10
E. Plant defence mechanisms 11
III. Objectives and hypothesis 13
IV. Materials and Methods 14
A. Materials 14
a. Aphids 14
b. Microorganisms 14
c. Plants 14
d. Substrate 14
B. Materials and methods 15

a. Pre-experiment 15
b. Main experiment 16
C. Data collection 19
a. Plants resistance 19
b. Plant responses 19
c. Description and impact of aphids' probing 21
D. Statistical analysis of data 23
V. Results 24
A. Plant resistance 24
B. Aphid probing behaviour 25
C. Plant performance 27
a. Increase of height after introduction of aphids 27
VI. Discussion 36
A. Plant resistance 36
B. Aphid probing behaviour 37
C. Plant performance 39
VII. Personal contributions 40
VIII. Conclusions and perspectives 41
IX. Literature reference 43
X. Appendices 51
A. Varietal datasheet provided by ANASAC for the *Triticum aestivum* variety 'Matylda' 51
B. Statistical analysis 52

Tables of figures

Figure 1 - Aphid morphology (Dixon, 2012) A : proboscis, B: antennae, C : ocular tubercle, D : two tarsal segments, E : longitudinal vein, F : pair of siphunculi. 7
Figure 2 - Sitobion avenae (Leclant, 1999). 7
Figure 3 - Life cycle of nonhost-alternating, holocyclic Sitobion avenae (Wu et al., 2022)..... 8
Figure 4 – Variation in resistance against the aphid Sitobion avenae of the fourteen cultivars of Triticum aestivum plants under laboratory conditions. 16
Figure 5 - Culture room of the Instituto de Ciencias Biológicas. 17
Figure 6 - Pots isolated by systems. 18
Figure 7 - Illustration of EPG technique. ① gold wire ② electrode connected to the aphid ③ electrode into the soil ④ ground ⑤ EPG recording (computer) (Tjallingii, 2006). 22
Figure 8 - Illustration of EPG waveforms (Garzo et al., 2020). 23
Figure 9 - Comparison of predicted values of resistance (mean ± SE) of wheat plants inoculated with different microorganisms ('C', 'B', 'BT' and 'T') and submitted or not to water deficit against Sitobion avenae. Different letters above error standard bars indicate significantly different mean values ($p<0.05$, Tukey's test). 25
Figure 10 – Means ± SE of the increase of the height of the plants from the introduction of Sitobion avenae until the end of the experiment. A. Based on the 'Aphids' factor. B. Based on the 'Water availability' factor. Different letters above bars indicate significantly different mean values ($p<0.05$, Tukey's test). 28
Figure 11 - Ratio root/shoot means ± SE comparisons of the plants inoculated by different microorganisms and submitted to the 'Water availability' factor. Different letters above bars indicate significantly different mean values ($p<0.05$, Tukey's test). 29
Figure 12 - Comparison of the means ± SE of dry weight of the leaves of Triticum aestivum under pressure from Sitobion avenae or not, water deficit of not and inoculated with different microorganisms. Different letters above bars indicate significantly different mean values ($p<0.05$, Tukey's test). 30
Figure 13 - Comparison of the means ± SE of dry weight of the roots of Triticum aestivum under pressure of Sitobion avenae or not, water deficit or not and inoculated with different microorganisms. Different letters above bars indicate significantly different mean values ($p<0.05$, Tukey's test). 31
Figure 14 - Predicted values ± SE of the dry weight ratio root/shoot of plants inoculated with different microorganisms, submitted to the pressure of Sitobion avenae or not and submitted to different water availability. Different letters above bars indicate significantly different mean values ($p<0.05$, Tukey's test). 32
Figure 15 - Comparison of the means ± SE of dry weight of the roots of Triticum aestivum under pressure from Sitobion avenae or not, water deficit or not and inoculated with different microorganisms. Different letters above bars indicate significantly different mean values ($p<0.05$, Tukey's test). 33
Figure 16 - Predicted values ± SE of Fv/Fm of plants inoculated with different microorganisms, submitted to the pressure of Sitobion avenae or not and submitted to different water availability. Different letters above bars indicate significantly different mean values ($p<0.05$, Tukey's test). 34
Figure 17 - PI_{abs} means comparisons between plants inoculated with different microorganisms, submitted to Sitobion avenae pressure or not and to water deficit or not. A. Based on the 'Aphid' factor. B. Based on the 'Water availability' factor. Different letters above bars indicate significantly different mean values ($p<0.05$, Tukey's test). 35

Table of tables

Table 1 – Percentage of germination of the cultivars 14 days after sowing under laboratory conditions. 15
Table 2 – 2x2x4 experimental design. The number of replicates is indicated in each cell. 17
Table 3 – Mean ± SE value for different EPG analysis of the plants inoculated with different microorganisms and submitted to different water availability. The results are based on the feeding behaviour of Sitobion avenae. Means within rows followed by different letters are significantly different $p<0.05$, Tukey's test). "*" indicates significant differences of means. 27
Table 4 - General Linear Model results of the resistance of the plants submitted to aphids pressure... 52
Table 5 - Statistical analysis of EPG number of waveforms results of aphids feeding on plants inoculated with different microorganisms and under water deficit or not. The results are based on the feeding behaviour of Sitobion avenae for each factor studied independently. P-values are those followed comparisons among treatments within each factor. 52
Table 6 - Statistical analysis of EPG duration of waveforms results of aphids feeding on plants inoculated with different microorganisms and under water deficit or not. The results are based on the feeding behaviour of Sitobion avenae for each factor studied independently. P-values are those followed comparisons among treatments within each factor. 53
Table 7 - Variance analysis of plants increase of height after the introduction of aphids. 54
Table 8 - Variance analysis of the root/shoot length ratio. 54
Table 9 - Variance analysis of the dry weight of the shoots 54
Table 10 - Variance analysis of the dry weight of the shoots based on the 'Aphids' factor. 55
Table 11 - Variance analysis of the dry weight of the shoots based on the 'Water availability' factor. 55
Table 12 - Variance analysis of the dry weight of the shoots based on the 'Microorganisms' factor... 56
Table 13 - Variance analysis of the dry weight of the roots. 56
Table 14 - Variance analysis of the dry weight of the roots based on the 'Aphids' factor. 57
Table 15 - Variance analysis of the dry weight of the roots based on the 'Water availability' factor. . 57
Table 16 - Variance analysis of the dry weight of the roots based on the 'Microorganisms' factor. 58
Table 17 - General Linear Model results of the root/shoot dry weight ratio. 58
Table 18 - General Linear Model results of the maximal photochemical yield (Fv/Fm). 61
Table 19 - Variance analysis of the Performance Index. 61

List of abbreviations and acronyms

A+	Plants under pressure of *Sitobion avenae*
A-	Plants without any aphid pressure
W+	Plants well-watered
W-	Plants under water deficit
C	Plants without any inoculated microorganisms
B	Plants inoculated with *Bacillus subtilis*
T	Plants inoculated with *Trichoderma virens*
BT	Plants inoculated with the consortium *B. subtilis and T. virens*
EPG	Electrical Penetration Graph
GLM	General Linear Model
Np	Non-probing behaviour waveform
C	Stylet in pathways activities waveform
Pd	Intracellular puncture waveform
E1	Salivation into the phloem sieve elements waveform
E2	Phloem sap uptake from the sieve elements waveform

I. General introduction

Bacteria and fungi are microorganisms that can be found in the rhizosphere and are able to increase rates of plant growth. These microorganisms also play a crucial role in biological control strategies against insect pests by inducing systemic resistance within plants. Notably, *Bacillus subtilis* and *Trichoderma virens*, classified as bacteria and fungi respectively, are known as promoters of plant growth and resistance (Lastochkina et al., 2017; Coppola et al., 2019). Furthermore, their potential to mitigate adverse impacts of water availability fluctuations on crops has been emphasized (Mastouri et al., 2012; Sood et al., 2020).

Currently, wheat (*Triticum aestivum*) is the most important source of food around the world (Igrejas et al., 2020). Nevertheless, the cultivation of this vital crop is subject to the pressure of numerous biotic and abiotic stresses. One of the most significant biotic stresses is the aphid *Sitobion avenae*. By feeding on the plant, this aphid induces direct damage in the plant and may serve as a vector for transmitting viruses (Sorensen, 2009). In recent years, in response to the environmental challenges posed by pest management, an increasing number of chemical insecticides used to combat pest insects have been banned. This has led to the exploration of alternative biological agents.

Moreover, the impeding effects of climate change, characterised by altered precipitation patterns, pose new challenges for crop cultivation. These variations are projected to increase the frequency of drought events, thereby necessitating adaptative strategies for crop resilience (Rebetzke et al., 2009).

The achievement of this master thesis is to investigate the effects of the fungal-bacterial consortium comprising *B. subtilis* and *T. virens,* along with their individual components, on the growth and aphid resistance of *T. aestivum* in the face of *S. avenae* infestation under contrasting water availability. It is expected that these microorganisms will not only enhance plant growth and recovery from water deficits but also exert a modulating influence on the feeding behaviour of insects.

This master thesis is a result of the collaboration between the Department of Functional and Evolutionary Entomology (Gembloux Agro-Bio Tech - University of Liège, Belgium) and the Instituto de Ciencias Biológicas (Universidad de Talca, Chile).

II. Context of study

The general framework of this master's thesis is related to the effects generated by the relevant microorganisms of the rhizosphere on wheat plants, both in terms of growth parameters and physiological response, as well as the induced systemic resistance to aphids, all under contrasting conditions of water availability. For this reason, the literature on microorganisms of the rhizosphere is reviewed first, both in their individual effects and in the form of a consortium on plants, followed by those corresponding to wheat, and then ending with a review of aphids with a focus on the English grain aphid *Sitobion avenae*.

A. Rhizosphere and beneficial microbials

The rhizosphere is defined by Hiltner as "the soil compartment influenced by the root". The concept currently considers the influence of root growth and activity on the physical, chemical and biological properties of the soil surrounding a root. In this compartment, roots may live in a symbiotic association with microbes, which are able to colonise plants internally. Those microbes are called "endophytes" and protect the plant against phytopathogens or/and promote its growth in exchange for nutrients and shelter (Malfanova et al., 2012; Sivasakthi et al., 2014; Dreischhoff et al., 2020). Bacteria and fungi are main components of the rhizosphere.

a. Bacteria

Bacteria are the most abundant microorganisms in the rhizosphere. Some of those bacteria are free-living, soil-borne bacteria and are called Plant Growth Promoting Rhizobacteria (PGPR). They are so called because of their ability to promote plant growth and yield enhancements. Their presence significantly increases plant height, root length and dry matter production of shoots and roots of plants. They are also known to have a high colonisation rate of the rhizosphere and to suppress soil-borne pathogens at the root surface. Paul and Nair (2008) also suggest that they can prevent deleterious effects of stresses from the environment, namely saline stresses. Some of them are able to produce auxin IAAs, which increases the level of this hormone in the plant and provides adaptative responses to biotic and abiotic stresses, namely aphid behaviour (Sivasakthi et al., 2014; Lastochkina et al., 2017; Serteyn et al., 2020; Sood et al., 2020).

Moreover, PGPR can stimulate inducible defence mechanisms of the plants, through manipulation of host plant's physical and biochemical properties. Those defence mechanisms are termed as "induced systemic resistance" (ISR) (Ongena et al., 2007; Sivasakthi et al., 2014; Lastochkina et al.,

2017; Dreischhoff et al., 2020). Studies showed that PGPR improve plants defence against insect pests by inducing physiological changes within plants which result in a significantly reduced aphid population. It can for example increase the accumulation of phenolic compounds and phytoalexins, modulate the ethylene-modulated signal transduction pathway or increase the lignification (Naeem et al., 2018).

Finally, PGPR is proven to alleviate drought stress by altering some physiological and biochemical processes, namely ethylene formation. They are also able to form biofilms which help to mediate plant drought tolerance. The alleviation of drought stress consequently improved the growth of the plants (Lastochkina et al., 2017; Sood et al., 2020).

Bacillus, a Gram-positive bacterium, is the most abundant genus of PGPR in the rhizosphere, naturally present in the immediate vicinity of plant roots. It is proven to increase water-holding capacity in leaf tissues, to increase significantly plant growth and biomass and to be efficient as elicitor for induction of ISR (Sivasakthi et al., 2014; Lastochkina et al., 2017; Meena et al., 2022). It explains why it is currently highly employed as a biopesticide (Ongena et al., 2007).

The presence of the PGPR is recognised by the plant root cells through the elicitors emitted by the bacteria. An elicitor is a low weight molecular compound which is able to initiate plant immune response by activating signal cascade (Patel et al., 2020). There are two types of elicitors; endogenous elicitors, which are released by the plant in response to pathogen attacks, and exogenous elicitors, which are molecules produced by pathogens. Those elicitors can be biological, chemical or physical of biotic or abiotic nature. After the recognition of exogenous elicitors, pathways regulated by ethylene (ET), jasmonic acid (JA) and salicylic acid (SA) are activated in the plant and a cascade of protective mechanisms is activated (Abdul Malik et al., 2020; Dreischhoff et al., 2020; Meena et al., 2022).

It should be noted that herbicide application has an adverse effect on metabolic activities of the PGPR (Sivasakthi et al., 2014).

b. Fungi

Soil fungus presence in the rhizosphere can lead to a beneficial interaction with the plant roots and the creation of a new organ; the mycorrhiza. Two major groups of mycorrhiza-forming fungi are known : arbuscular mycorrhizal fungi (AMF) and ectomycorrhizal fungi (EMF) (Dreischhoff et al., 2020). The mutualism between plants and mycorrhizal fungi is known as “mycorrhizal symbiosis”, occurring when photosynthetic products are exchanged for soil-derived mineral nutrients (Cameron et al., 2013).

It is proven that mycorrhizal symbiosis enhances the performance of plants. Indeed, as for some beneficial bacteria, fungi can be Plant Growth Promotors (PGP) (Stewart et al., 2014).

Fungi of the genus Trichoderma, a soil-borne ascomycete, is present in nearly all soils and colonises root surface or cortex (Schuster et al., 2010; Mahato et al., 2018). It is well known as a PGP for a large number of different groups of plants. For those, it is proven to increase, among others, root and shoot length, root and shoot dry weight, leaf area, emergence rates, chlorophyll contents and flower production. The ability of Trichoderma to be a PGP is dependent for each crop involved and has certain limitations. Several mechanisms explain the ability of Trichoderma to increase plant growth, such as its influence on the balance of hormones, on the synthesis of phytohormones, the production of vitamins, the enhance of solubilisation of soil nutrients, the increase in nutrient uptake efficiency and the increase in the rate of plant defence mechanisms. Each strain of Trichoderma uses one of several of those mechanisms. It should be noted that Trichoderma improves the ability of plants to protect themselves from oxidative damage (Mastouri et al., 2012) and that secondary metabolites have also been reported to have a role in plant growth promotion (Bae et al., 2009; Stewart et al., 2014; Mahato et al., 2018; Coppola et al., 2019).

Moreover, the presence of Trichoderma in the roots enhance whole-plant tolerance to biotic and abiotic stresses, namely water deficit (Bae et al., 2009; Mastouri et al., 2012). Indeed, besides the fact that Trichoderma can increase root length, some strains have been found to increase the number of deep roots, which can ease the water uptake for crops and improve their resistance to drought (Stewart et al., 2014).

Trichoderma is also proven to enhance resistance of plants (Bae et al., 2009; Schuster et al., 2010; Coppola et al., 2019). To do so, it must first colonise the roots of its host plant. Then, in order to ease roots invasion, Trichoderma must tolerate or suppress plant defence mechanisms. The colonisation implies a reprogramming of the plant gene expression. Several classes of compounds are released by Trichoderma, such as proteins and oligosaccharides, and play the role of elicitors. Hence, those elicitors are recognised by the plant and initiate JA and ET signals transmission. The fungus is therefore able to induce systemic resistance (Shoresh et al., 2010; Saldajeno et al., 2014).

By dint of all those qualities, products based on Trichoderma are commonly used to enhance yield and reduce plant diseases (Schuster et al., 2010; Mastouri et al., 2012; Fingu-Mabola et al., 2021).

c. Combination Bacteria and fungi

Some authors tested the combination of Bacillus genus and Trichoderma genus as growth promotors on several arable plants. The results show that the combination of the two gave better results than each organism alone. The mixture of the two microorganisms was postulated to increase siderophore production, mineral uptake and plant growth promoter substances (Stewart et al., 2014).

B. Wheat

Wheat belongs to the family of *Poacea* and has been classified as *Triticum* for the first time by Linnaeus in 1753 (Goncharov et al., 2009). At the present time, two main classifications are used by the scientific community: Mac Key's classification and its revision by Goncharov on the basis of comparative-genetic analysis (Goncharov et al., 2009; Goncharov, 2011; Goriewa-Duba et al., 2018). The existence of diploid and polyploid species complicates the classification.

Wheat is considered as a primary product, namely "product sold for the purpose of production or consumption in a natural or standardised form (Eurostat, 2021)". Nowadays it is the most important source of food around the world, notably due to its adaptability (Ahmad et al., 2018; Igrejas et al., 2020). In 2021, 907.8 million tonnes of wheat were produced worldwide (Food and Agriculture Organization of the United Nations, 2023). The global average yield is about 2.8 tonnes ha^{-1}. This low performance is due to water and nutrient deficiencies as well as the incidence of pests and pathogens (Shewry, 2009). As a matter of fact, plants alter their gene expression and protein production in response to environmental stress. This leads to various physiological responses, including a reduction in plant height (Nezhadahmadi et al., 2013) and in grain yield (Slatyer, 1973; Rebetzke et al., 2009).

Climate change, due to global warming, is predicted to increase rainfall variability and causes considerable global losses in agriculture. Drought affects wheat production in high-income and low-income countries, around 60% and 32% of 99 million hectares respectively. Of all water withdrawals, agriculture currently accounts for a range from 70 to 85%. However, with climate change and a growing world population, they are expected to increase in order to meet the demand for food. These withdrawals are also in direct competition with domestic water needs. Alternatives to water lacking are therefore needed (Rebetzke et al., 2009; Ahmad et al., 2018)

Tolerance to water stress, including drought avoidance and dehydration tolerance, can be one of the solutions (Nezhadahmadi et al., 2013; Sood et al., 2020). It is known that plants with an extensive root system are advantaged when growing under drought conditions (Hurd, 1974). Passioura (1996) defined drought tolerance "in terms of yield in relation to a limiting water supply", Fleury et al. (2010) proposed the definition as "the ability of a plant to live, grow, and reproduce satisfactorily with limited

water supply or under periodic conditions of water deficit". Drought tolerance is a quantitative trait along with complex phenotype. Until now, breeding has focused almost exclusively on physiological and molecular selection. However, given the limited success of this approach, there is a need to rethink strategies for understanding and selecting for drought tolerance (Fleury et al., 2010).

C. Aphids

a. Classification

Aphids belong to the order of Hemiptera. This order includes six sub-orders; namely Paleorrhyncha, Sternorrhyncha, Fulgoromorpha, Cicadomorpha, Coleorrhyncha and Heteroptera and contains over 110.000 species classified. The suborder Sternorrhyncha contains four infra-orders ; namely Aphidomorpha, Coccidomorpha, Aleyrodomorpha and Psyllodea containing the superfamilies Aphidoidea (aphids), Coccoidea (scale insects and mealybugs), Aleyrodoidea (whiteflies) and Psylloidea (jumping plant lice) respectively (Sorensen, 2009; Drohojowska et al., 2020).

The Aphidoidea superfamily is composed of three families: Adelgidae (adelgids), Phylloxeridae (phylloxerids) and Aphididae (Sorensen, 2009; Dixon, 2012). Insects belonging to the Adelgidae and Phylloxeridae families have an ovipositor and reproduce by means of ovipary. They are considered as "primitive aphids" and consist approximatively of 50 species each. Aphididae contains more than 4000 species and set oneself apart from the other ones by lack of ovipositor and viviparous parthenogenetic reproduction, bearing live young (Remaudière et al., 1997; Sorensen, 2009).

The *Sitobion avenae* (Fabricius, 1775) aphid belongs to the Aphididae family, the Aphidinae sub-family, the Macrosiphini tribe and the Sitobion gender (Inventaire national du patrimoine naturel, 2023).

b. Morphology

The thorax consists of three segments: the prothorax, the mesothorax (sclerotized in the alatae) and the metathorax. It carries three pairs of legs and two pairs of wings in alatae (Ighil et al., 2011). Aphids possess two antennae that play an important role in the odorant-binding proteins in food and host searching and mating (Wu et al., 2022). For *S. avenae*, those antennae are inserted on the front tubers and are not exceeding the length of the body (Fig. 1) (Leclant, 1999; Ighil et al., 2011).

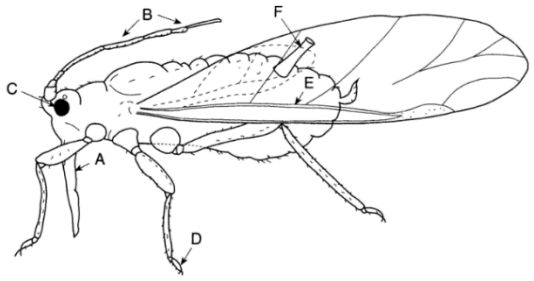

Figure 1 - Aphid morphology (Dixon, 2012) A : proboscis, B: antennae, C : ocular tubercle, D : two tarsal segments, E : longitudinal vein, F : pair of siphunculi.

The abdomen consists of ten segments, the fifth and the sixth carry a pair of cornicles. These are organs of secretion of alarm pheromones emitted when aphids feel in danger. For *S. avenae*, the cornicles are dark brown or black and crosslinked at the endpoint (Fig. 2). The last segment of the abdomen represents the cauda (Leclant, 1999; Ighil et al., 2011).

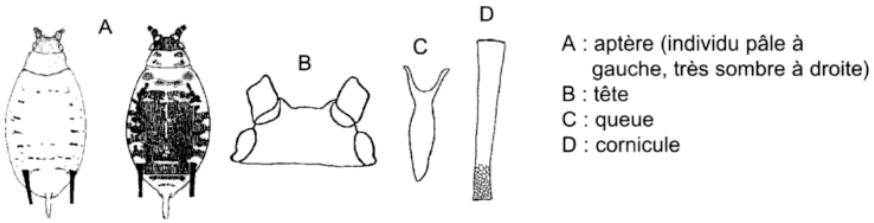

Figure 2 - Sitobion avenae (Leclant, 1999).

However, environmental factors have a great effect on aphid size and morphology (Helden et al., 2013).

c. Life cycle

Sitobion avenae is a monoecious holocyclic species (Choe et al., 2006; Sorensen, 2009; Helden et al., 2013; Wu et al., 2022). It means that asexual periods of reproduction alternate with sexual reproduction, only on one host plant species (nonhost-alternating) (Fig. 3). This cyclical parthenogenesis occurs under spring and summer conditions and alternates with sexual reproduction in autumn in response to decreasing day length (Sorensen, 2009; Ighil et al., 2011; Byrne et al., 2022).

During asexual period of reproduction, egg development begins directly after ovulation. Mature fundatrix emerges from the egg and gives parthenogenetically birth to nymphs that become viviparae carrying embryos which themselves carry embryos, creating a “telescoping” of generations. This feature allows aphids to reproduce extremely rapidly in a short period of time. Viviparae can be apterae

(wingless) or alatae (winged) (Dixon, 1985, 2012; Sorensen, 2009). Apterae are optimised for reproduction, alatae are optimised for dispersion, they invest their major resources in their flight system (Sorensen, 2009).

During the switching between the two types of reproduction mode, seven morphs are produced: virginoparae alate and apterous, sexuparae alate and apterous, male alate, oviparae apterous and apterous fundatrices (Wu et al., 2022). The existence among a species of distinct forms and morphs is known as polyphenism (Dixon, 2012).

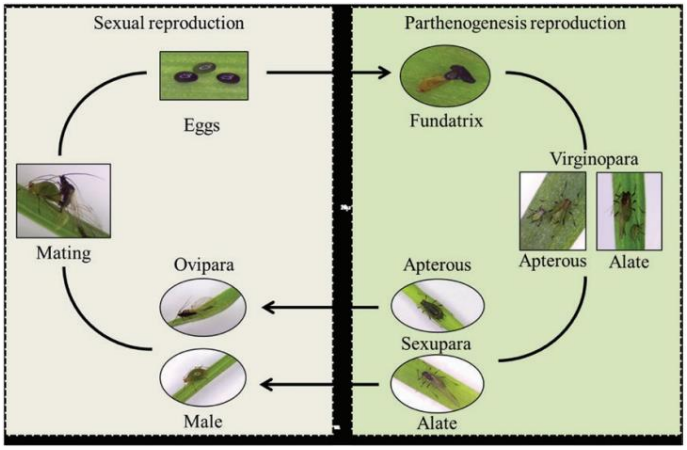

Figure 3 - Life cycle of nonhost-alternating, holocyclic Sitobion avenae (Wu et al., 2022).

d. Aphid host selection, acceptance and probing behaviour

Flying aphids face three major challenges in finding a suitable host: (a) most of the species are highly specialised in their feeding preferences; (b) they are susceptible to desiccation, this influences the amount of time they can survive without feeding; and (c) they are relatively weak flyers, they can only move easily at low wind speeds (Powell et al., 2006). Their susceptibility to dehydration compels aphids to access xylem or phloem bundles while avoiding or sabotaging plant defence responses and to withdraw phloem sap while keeping those cells alive (Giordanengo et al., 2010). To feed themselves, aphids must overcome phloem-related plant properties and reactions, such as coagulation proteins in the sieve elements of the plant and aphid stylet. Aphids overcome this issue by injection of watery saliva (Tjallingii, 2006a). Indeed, their saliva has been proved to contain proteins able to bind calcium and prevent calcium-induced sieve elements occlusion (Will et al., 2008).

Two different terms must be distinguished: aphid host finding and host selection. Both of them are influenced by naturally occurring chemical compounds (Döring, 2014). Host selection includes several processes; host habitat location, host location, host acceptance, host suitability and host regulation (Thompson et al., 1999). The process of host finding initiates with the pre-contact phase and ends up

when the aphid recognises the plant as a host. Aphids make use of visual and olfactory stimuli to find their way around. This phase is carried out at distance and is not considered specific, the tactile stimulus is required for more specificity. Test bites are used by aphids to recognise suitable host plants. Those bites may initiate the defence systems of the plants (Fingu-Mabola et al., 2021).

Following arrival to a host plants, aphids can either consume xylem sap to compensate for water losses occurring during starvation or feed directly on phloem of plants, ingesting sugars, nitrogen compounds and other nutrients (Dinant et al., 2010; Pompon et al., 2010). These sucking insects use their stylet, the capillary food canal, to feed themselves, by penetrating between plant cells and reaching the phloem sieve tubes (Tjallingii, 2006a; Sorensen, 2009). In order to alleviate the osmotic effects of ingested phloem sap, aphids need to feed themselves with xylem sap regularly (Will et al., 2006). Different mechanisms are put into place to find a host species, bypass the defences of the plant and manage to feed.

In order to avoid and bypass the plant defences set up during their feeding, aphids established several defence mechanisms: (a) behavioural to avoid predators, pathogens and environmental stressors; (b) protective cuticle and gut pH inhospitable; (c) immunological defence activating a signalling cascade and responses (Gerardo et al., 2010).

e. Damages

Sitobion avenae is one of the most important pests of temperate agriculture, particularly in wheat. The consequences of the feeding of this aphid on the plants are multiple: removing photo-assimilates, transmitting plant viruses (in particular the Barley Yellow Dwarf Virus, BYDV) and altering plant growth and development by removing enough sap which leads to a reduction in crop yield, which can even end in yellowing and death. Their proliferation in the period from the beginning of the heading to the filling of the grains can reduce the yield by about 10% (Ighil et al., 2011). Moreover, aphid honeydew can become a growth medium for sooty molds that interfere with photosynthesis and promote fungal diseases (Sorensen, 2009; Byrne et al., 2022; Wu et al., 2022).

Also, aphids' salivary secretions can be phytotoxic and cause leaf deformation, gall formation and stunting (Sorensen, 2009). Several studies have proven that the presence of aphids in wheat fields reduces the flag leaf area duration of the crop and reducing thus the assimilate supply for the ear (Wratten, 1975). Moreover, aphids may affect plant hormone balance by changing host metabolism to their advantage (Sorensen, 2009).

D. Pesticide use in wheat

a. Pest control

Pesticides are used in wheat crops because of their significant effect on grain yield (Jolánkai et al., 2008; Hossard et al., 2014). A French study has shown that a 50% reduction in the amount of pesticides, usually applied to wheat plots, resulted in a loss of grain yield. The latter would be between 5 and 13% of the yield obtained with the current pesticide use level, while yield loss estimates for zero-pesticide systems range from 24 to 33% (Hossard et al., 2014).

Nevertheless, even if pesticides are useful to farmers to ensure the best yield possible, their application has a significant impact on the surrounding environment. Pesticides induce indeed pronounced negative effects on the regional biodiversity of stream invertebrates in Europe and Australia (Beketov et al., 2013), interrupt natural ecological nutrient cycling (Sivasakthi et al., 2014), harm water air and soil quality (Hossard et al., 2014; Tongur et al., 2020) and is correlated with the prevalence of Parkinson's disease in exposed workers (Elbaz et al., 2009).

Moreover, the effectiveness of commonly used pesticides is jeopardised by the apparition and evolution of resistant pathogens, insects and weeds pests. Three potential evolutionary origins of insecticide resistance alleles can be highlighted; *de novo* mutation, standing genetic variation and adaptive introgression (Hawkins et al., 2019).

As the world's population continues to grow, it increases the need for food which inevitably increases the use of pesticides (Tongur et al., 2020). This tendency in pesticide use is not sustainable in the long term. Moreover, since 2013, the European Union banned several chemicals and insecticides after researchers highlighted their harmful effect, particularly on bees and other pollinators. The case of the neonicotinoids has been particularly studied. It is a class of synthetic and systemic insecticide, which is taken by the plant in soluble form and distributed to all tissues. Neonicotinoids are harmful for the insects as it interacts directly with their central nervous system, which can lead to their death and possibly to accumulate and persist in the soil. The use of this product is now forbidden in Europe. Nevertheless, aphids are resistant to alternative insecticides to neonicotinoids (Bass et al., 2018; Butler, 2018; Jactel et al., 2019). Hence, alternative methods to control aphids and associated virus transmission must be considered and studied more closely.

b. Alternatives in pest control

Alternatives to neonicotinoids for pest management exist. The main use is another chemical insecticide. However, Jactel et al. (2019) mention that in 78% of cases, a non-chemical alternative

method could have been used. One of the most promising substitutable alternative methods studied is biological control with the use of microorganisms which is effective against leaf and sap feeders.

Even if those alternatives exist, several criteria need to be taken into account before their use becomes widespread in the agricultural world. The first one is that the alternative method should be less harmful to the environment and less toxic than the chemical product. The second one is that the efficiency, applicability, durability and/or practicability of the alternative method should correspond to those of the neonicotinoid, which is not necessarily the case currently. Indeed, for some microorganisms there is still a high variability across sites and seasons (Stewart et al., 2014). The third one is that the price of the alternative method should match in a close range the one of the chemical product. The fourth one is that the registration and authorisation processes of those alternative methods should be accelerated in order to be available in a short time for the farmers (Jactel et al., 2019). Therefore, before a replacement of chemicals by alternative methods can be observed, further, much more research still needs to be done.

E. Plant defence mechanisms

Plants possess two defence strategies : constitutive (passive) and inducible (active) defence systems (Zhao et al., 2009; Abdul Malik et al., 2020; Patel et al., 2020; Serteyn et al., 2020). Both are based on the recognition by a receptor of an effector molecule (Abdul Malik et al., 2020).

Different molecules produced by microorganisms and insects can be recognised by the plant when it is attacked ; Pathogen-Associated Molecular Patterns (PAMPs), Microbe-Associated Molecular Patterns (MAMPs), Herbivore-Associated Molecular Patterns (HAMPs), and Damage-Associated Molecular Patterns (DAMPs). PAMPs, HAMPs and DAMPs are referred to as MAMPs (Abdul Malik et al., 2020).

The activation of plant innate (constitutive) immunity is based on two methods. Firstly, on the perception and recognition by patterns recognition receptors (PRRs) of molecular patterns produced by attackers. Those patterns are molecular signatures that are used by the innate immune system to identify self from non-self (Weber, 2014). This recognition leads to pathogen-associated molecular patterns (PAMP)-triggered immunity (PTI), which has the role to stop the colonisation of pathogens, followed by effector-triggered immunity (ETI), which perceives and protects the plant from harmful pathogens. Secondly, on the identification of pathogens by a resistance (-R) protein-mediated process against race-specific effector molecules (Abdul Malik et al., 2020).

The constitutive immunity comprises several structural, chemical and protein-based defences to protect the plant against pathogen attack and invasion. Structural defences comprise the topography of

leaf surface, degree of stomatal opening, thickness of the cuticle, etc. Chemical defences comprises biochemical substances having inhibitory action (Patel et al., 2020).

The active defence system is induced in the second phase of innate immunity, when pathogens and insects breach the innate immunity defences (Ongena et al., 2007; Abdul Malik et al., 2020). This active defence is based on two main mechanisms : systemic acquired resistance (SAR) and induced systemic resistance (ISR). SAR is mainly triggered by local infection, correlated with the activation of PR genes and requires the implication of the salicylic acid molecule. It provides long-term systemic resistance to the whole plant against subsequent pathogen attack. ISR results from the colonisation of roots by specific non-pathogenic rhizobacteria or antagonistic fungi. Unlike SAR, it is not dependent on SA but requires components of the jasmonic acid (JA) and ethylene signalling pathways (Shoresh et al., 2010; Hartman et al., 2016; Patel et al., 2020).

Plant induced immunity is based on the activation of defence responses, due to signalling molecules, to protect plant tissues from additive damages from biotic and abiotic stresses. In this case it is no longer PAMPs that are recognised by the plant, but virulence factors secreted by the pathogens, into the plant, that act as effector molecules and are identified by R proteins. After recognition, the immune responses of the plants are activated through the induction of ETI (Ongena et al., 2007; Abdul Malik et al., 2020).

The induced immunity comprises substances present in insignificant amounts or either absent before the infection that are activated or synthesised after infection. Those substances can be gums and vascular gels, tyloses, callose, phenolic compounds, phytoalexins and defensive proteins. The plant can also form abscission layers and synthetise secondary cell walls (Patel, 2020).

III. Objectives and hypothesis

The aim of this master's thesis is to study the effects of the fungal-bacterial consortium *Bacillus subtilis* and *Trichoderma virens* and its individual components on the resistance of the wheat *Triticum aestivum* to the aphid *Sitobion avenae* and on plant growth under and without water deficit.

It includes three research objectives :

1. **Objective 1** : Study of biological and population parameters of one clone of *S. avenae* feeding on *T. aestivum* inoculated with *B. subtilis*, *T. virens* and the consortium of both previous under and without water deficit.

   Hypothesis 1 : Plants under water deficit are less vigorous, aphids develop then poorly on those plants.
   Prediction : Higher plant resistance to aphids in wheat plants under water deficit.

   Hypothesis 2 : Plants inoculated with microorganisms can recover from water deficit but microorganisms also induce resistance to aphids.
   Prediction : Higher plant resistance to aphids in wheat plants under water deficit and treated with microorganisms.

   Hypothesis 3 : The feeding behaviour of aphids is negatively influenced by the presence of microorganisms inoculated on *T. aestivum*.
   Prediction : Lower feeding in plants under water deficit and treated with microorganisms.

2. **Objective 2** : Measurement of *T. aestivum* response to the inoculation of *B. subtilis*, *T. virens* and the consortium of the previous under and without water deficit, with and without the pressure of *S. avenae*.

   Hypothesis 4 : Microorganisms promote plant growth and allow plants to recover from water deficit.
   Prediction : Weaker plants for those under water stress and stronger plants for those inoculated with microorganisms.

IV. Materials and Methods

A. Materials

a. Aphids

The *Sitobion avenae* aphids used in this experiment belong to one and the same clone. This clone was collected from a wheat plantation in the location of Gorbea in the Araucanía region in Chile on the 7th January 2017. The last PCRs of March 2023 for facultative endosymbiont detection showed no infection. This clone is the most widely distributed in cereal crops of the central-south region of Chile. Individuals of this genotype were kindly provided by Dr. Francisca Zepeda-Paulo. This genotype was characterised with eight microsatellite loci as described by Figueroa et al. (2005). It was denoted as genotype G1 in a previous study and found to be infected and uninfected with the secondary endosymbiont *R.insecticola* (see more details in Zepeda-Paulo et al.(2017)). Here the variant uninfected of secondary endosymbiont was used. The rearing of these aphids took place in a culture room with controlled environmental conditions (21°C ± 1°C, 60% Relative Humidity and a photoperiod of 16h day/8h night) in the *Laboratorio de Interacciones Insecto-Planta* (Universidad de Talca, Chile). The clonal aphid population was reared on oat plants.

b. Microorganisms

The strains of *Bacillus subtilis* and *Trichoderma virens* come from the Bio Insumos Nativa SPA biotech company (Chile). The microorganism powder was kept in a dry place in the *Laboratorio de Interacciones Insecto-Planta* (Universidad de Talca, Chile), free from contamination and protected from light.

c. Plants

The cultivar Matylda of *Triticum aestivum* was employed for all the experiments. Firstly, because *Triticum aestivum* is one of the hosts of *Sitobion avenae*, a globally widespread crop pest (Ighil et al., 2011). Secondly, because *Triticum aestivum* is the most important source of food around the world nowadays (Igrejas et al., 2020). The seeds used were purchased from the company ANASAC in 2020 (Appendice 1).

d. Substrate

The substrate used is an autoclaved mixture composed of 25% sand, 25% perlite and 50% sifted compost. The choice to use a substrate composed of sand for a study based on a difference in water

treatment is based on the ease of handling the roots once the experiment is over. It is important to note that the choice of pots used as well as the substrate may have a significant impact on the results based on water treatment (Turner, 2019).

B. Materials and methods

a. Pre-experiment

In order to determine which cultivar of *Triticum aestivum* was the most suitable for the main experiment, a short experiment based on fourteen wheat cultivars was conducted. The aim of this experiment was to make a preliminary screening of resistance of the cultivars against *Sitobion avenae*. The seeds used were purchased from the company ANASAC in 2020.

The fourteen wheat cultivars studied were Millan, Don Feña, Don Manuel, Matylda, Maxenses, Swindy, Bicentenano, Halcón, Pantera, Patras, Lasana, Queltehue, Maxwell and Gorrión. Fourteen days after being sewed, the germinate rate of each cultivar was calculated (Table 1). The same day, for each cultivar, five plants were subjected to the pressure of two synchronised *Sitobion avenae* which lasted for ten days. Ten days later, the number of aphids on each plant was counted. With that information, the resistance of each one of the cultivars was calculated following this equation : resistance = $\frac{1}{Number\ of\ aphids\ alive}$ (Fig. 4).

The statistical analysis of 'resistance of cultivars after ten days of aphid introduction' was made using the software RStudio. The homogeneity of variances being respected but not normality, different mathematical transformations were tested but did not lead to normality. Henceforth, the General Linear Model in Poisson regression was used.

Table 1 – Percentage of germination of the cultivars 14 days after sowing under laboratory conditions.

Cultivar	% germination	Cultivar	% germination
Millan	68.3	Halcón	65.0
Don Feña	83.3	Pantera	55.0
Don Manuel	66.7	Patras	21.7
Matylda	56.7	Lasana	88.3
Maxenses	21.7	Queltehue	58.3
Swindy	66.7	Maxwell	66.7
Bicentenano	55	Gorrión	65.0

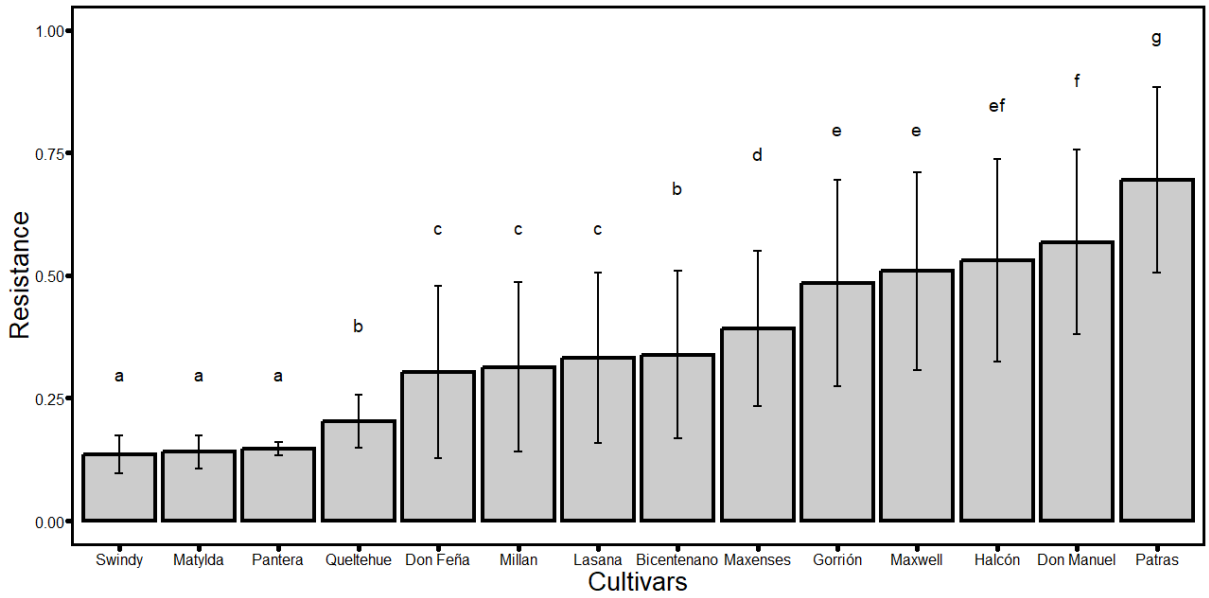

Figure 4 – Variation in resistance against the aphid Sitobion avenae of the fourteen cultivars of Triticum aestivum plants under laboratory conditions.

The choice of the cultivar was based on several factors. Firstly, it was based on the number of seeds owned. Secondly, on the value of the resistance of the cultivars against the pressure of *Sitobion avenae*. Thirdly, on the amount of 2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one (DIMBOA) in the plant. Those benzoxazolinones are naturally present in plants as glucosides (Flamini, 2012). Its presence in the plant is effective in conferring resistance against herbivores and pathogens, thus also against phloem feeding aphids (Bohidar et al., 1986; Niemeyer et al., 1989; Nicol et al., 1997; Morant et al., 2008).

In order to observe the efficiency of several microorganisms inoculation (see 'Main experiment') with the least possible bias of defence of the plant, a cultivar containing the least amount of DIMBOA was selected. In the view of a study of Gonzalez-Gonzalez on the DIMBOA content in each of the fourteen cultivars studied, 'Pantera' and 'Matylda' are those that contain the least (results non-published). Moreover, in order to see if the inoculation of microorganisms and the presence of aphids will change the resistance of the plants, a cultivar with a low resistance was preferred. Then, the cultivar 'Matylda' was chosen for all the subsequent experiments.

b. Main experiment

The main experiment was based on 16 treatments; plants subject to water deficit (W-) or well-watered plants (W+), subject to aphid pressure (A+) or not (A-) and inoculate with *Bacillus subtilis* (B) or with *Trichoderma virens* (T) or a consortium between *B. subtilis* and *T. virens* (BT) or without addition of microorganisms (C) (Table 2).

Table 2 – 2x2x4 experimental design. The number of replicates is indicated in each cell.

	Plants under water deficit		Plants well-watered	
	With aphids	Without aphids	With aphids	Without aphids
B. subtilis (1)	15	15	15	15
T. virens (2)	15	15	15	15
Consortium (1+2)	15	15	15	15
Control	15	15	15	15
Total plants	60	60	60	60

i. Seeds pregermination, seeding and growing

The seeds were cleaned twice with distilled water and then left for 15 hours in distilled water. They were then pre-seeded in plastic containers at a rate of 5 seeds per container (4x4 cm). This choice was made because of the poor germination power of the seeds used, as determined by a previous germination experiment (Table germination). Seeds were grown in a culture room of the Instituto de Ciencias Biológicas (Universidad de Talca, Chile) (Fig. 5) with controlled environmental conditions (22°C ± 1°C, 50% relative humidity and a photoperiod of 12h light per day).

Ten days later, the seedlings were transferred to pots of 8 cm in diameter. Each pot was watered with 45 mL of water. Since the experiment is based on the use of aphids on part of the plants, for logistical reasons, each pot was isolated using a system (Fig. 6). It is composed of a transparent plastic tube of 30 cm high, covered by a square of tulle held by an elastic band, placed on the pot and hermetically sealed with parafilm. Systems were placed in a culture room of the Instituto de Ciencias Biológicas (Universidad de Talca, Chile) with controlled environmental conditions (21°C ± 2°C, 60% Relative Humidity and a photoperiod of 16h light per day).

Figure 5 - Culture room of the Instituto de Ciencias Biológicas.

Figure 6 - Pots isolated by systems.

ii. Synchronisation of aphids

In order to obtain aphids of the same age, a synchronisation was carried out. It consists of placing a certain number of adult aphids on plants, letting them reproduce for a certain number of hours (22 hours in this case) and then removing all of the adults, while letting the new nymphs produced by the adults growing on the plants.

iii. Inoculation

Twelve days after their sowing, the plants were inoculated with the microorganisms, 15 mL of bacterial culture at $5*10^8$ Colony Forming Units (CFU). The solution of microorganisms was applied with a lab pipette 10 mL on the tulle and against the wall of the plastic tube so as not to undo the systems. The same amount of water was used for the control plants.

In order to verify that the inoculated microorganisms have successfully colonised the plant roots, the latter were analysed. The roots of two plants for each of the sixteen treatments were studied. Firstly, they were cleaned using distilled water to remove excess substrate. Then, they were cleaned a second time in water. Afterwards, they were sterilised. For that, they were immersed in 70% ethanol, then in 2% hypochlorite, and finally in distilled water (for respectively two minutes, one minute, and two minutes). Finally, in a sterile environment, they were cut using a cutter and placed in a sterile Eppendorf tube containing 200 µL of distilled water. This preparation was crushed and the maceration was finally placed in Petri dishes and spread using a rake. The Petri dishes were then placed in ovens at different temperatures. Those used to observe the presence of *Trichoderma virens* were kept at 27.5°C for 5 days, and those used to observe the presence of *Bacillus subtilis* were kept at 37°C for 24 hours. Afterwards, all the Petri dishes were stored in a refrigerator at 7°C.

iv. Aphid infestation

Two days after inoculation of the microorganisms, synchronised 6-day-old *S. avenae* were placed on plants corresponding to code A+, at the rate of three aphids per plant. Aphids were gently placed on the leaves of each plant using a paint brush.

v. Water-deficit treatment

The field capacity was determined by applying 75 grams of water to the substrate and allowing drainage for 24 hours after weighing (Heitholt, 1989), determining that the field capacity weight of the pots was 45 grams. It was measured with the aim of keeping the "well-watered" plants at a field capacity ranging between 70 and 90%, and the "under water deficit" plants at a field capacity ranging between 20 and 50% all along the experiment.

C. Data collection

a. Plants resistance

The plant resistance to aphids was assessed as antibiosis (Leimu et al., 2006). Butt et al. (2022) defined antibiosis as an “antagonistic association between two organisms […] in which one is adversely affected”.

Hence, the plant resistance was assessed by the reproductive success of *Sitobion avenae* in the plants following this equation : resistance = $\frac{1}{Number\ of\ aphids}$. The aphids were collected and counted at the end of the experiment.

b. Plant responses

Due to the complexity of the systems used, data collection was carried out only once at the end of the experiment, 12 days after the introduction of aphids on the plants. After their survey and counting, the measurement of the plant parameters could be carried out the following two days.

i. Photochemical efficiency of photosystem II (Fv/Fm) and plant vitality (PI_{abs})

Biotic and abiotic stresses are able to affect photosynthetic performance and then affect the intensity of the chlorophyll fluorescence emission. The isolation of the leaf in the dark and the sudden illumination with a high-intensity light source is referred to as the Kautsky Induction. This induction allows the study of fluorescence emission changes (Hansatech Instruments Ltd, 2017).

The maximum quantum efficiency of photosystem II photochemistry (Fv/Fm) is measured according to this equation : $\frac{Fv}{Fm} = (Fm - F0)/Fm$, where Fm is the maximum fluorescence value obtained for a continuous light intensity and $F0$ is thought to represent emission by excited chlorophyll a molecules in the antennae structure of photosystem II. (Fv/Fm) is able to indicate plant stress before the apparition of the symptoms on the leaves (Zivcak et al., 2008; Hansatech Instruments Ltd, 2017).

The Performance Index reflects the functionality of both photosystems I and II. It is able to illustrate the photosynthetic performance of the plant according to this equation : $PI_{abs} = \frac{1-(F0/Fm)}{M0/Vj} \times \frac{Fm-F0}{F0} \times \frac{1-Vj}{Vj}$, where $M0$ is the initial slope of fluorescence kinetics and Vj is the relative variable fluorescence at 2 ms. This Index includes three independent parameters including efficiency of electron movement, density of fully active reaction centers (RCs) and probability for an absorbed photon to be trapped by RCs. This Index will give information about the effect of water deficit on the plants' vitality (Zivcak et al., 2008; Hansatech Instruments Ltd, 2017).

(Fv/Fm) and PI_{abs} were measured at the end of the experiment with a chlorophyll fluorimeter (Hansatech Pocket PEA) on each plant after the removal of the aphids. A light-with-holding clip was put on the tallest leaf of each plant of the experiment in order to obscure them for 15 minutes and the measure was then taken.

ii. Plants phenotypical parameters

Several plant parameters were measured at the end of the experiment. Those parameters were taken 13 days after the introduction of aphids, the day after the removal of aphids. Data collection was conducted systematically to minimise bias. Hence, the data from the 16 treatments were collected by block, one replicate after another. Shoot height was measured at the surface of the soil, above the roots. Then, roots and shoots lengths were measured after the cleaning of the roots with water to remove excess of compost entangled in it. Roots and shoots were placed in separate paper bags and put to dry in an oven (Memmert Beschickung-Loading Modell 100-800) for five days at 60 degrees. The roots and shoots weight were weighted with an analytical balance (Radwag as 220/c/2).

c. Description and impact of aphids' probing

i. Aphids and plants

The aphids studied come from the same *S. avenae* strain as those used in the previous experiment.

The plants used for this experiment are plants that were sown on the same day as those used for the previous experiment and have been subject to the same conditions. They were also equipped with a system even if they were not subjected to aphid pressure, in order to maintain homogeneity between all the treatments. Only eight treatments were studied, corresponding to the factors 'Microorganisms' (B, T, BT and C) and 'Water availability' (W+ and W-).

ii. Electropenetrography (EPG) technique

This technique was developed by McLean and Kinsey "to record electronically some phases of aphid feeding and salivation within a plant or other electrically conductive substrate (McLean et al., 1964)". Indeed, when the mouth parts of the insects, the stylet, is inside the plant, it is invisible and thus impossible to study without another technique than simple eye observations (Backus et al., 2020).

The system is an electrical circuit composed as follows : two electrodes, one connected to the aphid and the other placed in the substrate of the plant, an input resistor with a value of 1GΩ (Ri), an amplifier connected to a recording device and a DC voltage source (Fig. 7). Aphids were taken from the culture room right before their preparation for the EPG recording. The connection of the aphid to the electrode is carried out by means of a thin (25µm) gold wire, connected to the aphid and the electrode using a small droplet of conductive silver-glue (Colloidal Silver Liquid). In order to connect the gold wire to the aphid, it is immobilised by a vacuum device. It is then left for 30 minutes in a damp petri dish in order for it to acclimatise to its new condition. Thereafter, the aphid is carefully placed onto the leaf on a wheat plant. Thanks to the wire, the aphid remains free to move on the leaf even if it is connected to the electrode. The system is placed in a Faraday cage to isolate it from the external environment (Tjallingii, 1978, 2006; Sarria et al., 2009; Seo et al., 2009; Garzo et al., 2020).

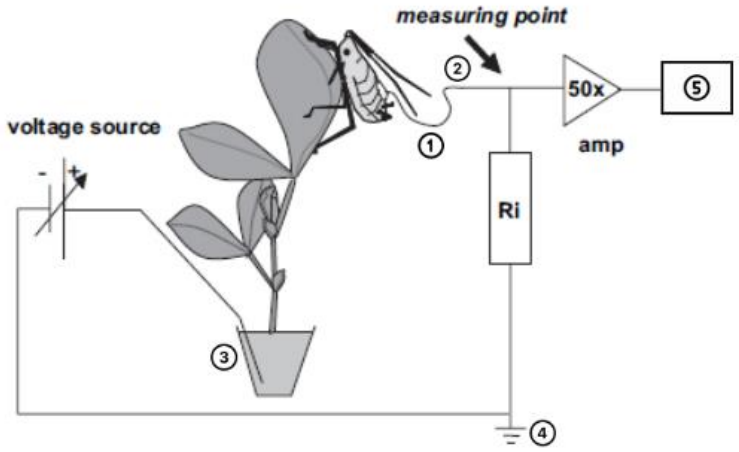

Figure 7 - Illustration of EPG technique. ① gold wire ② electrode connected to the aphid ③ electrode into the soil ④ ground ⑤ EPG recording (computer) (Tjallingii, 2006).

When the aphid inserts its stylet into the plant, the circuit is completed and a fluctuation voltage, the EPG signal, occurs at the measuring point. This signal is amplified 50 times and recorded on a computer. The various patterns of voltage fluctuation are called "waveforms". EPG signals were recorded during four hours by means of the use of the software Stylet+d. A 4-chanel and a 8-chanel amplifier (models Giga-4 and Giga-8) were used to carry out five recordings of each combination of treatments (Water availability* Microorganisms).

Each waveform corresponds to a specific stylet tip position and aphid feeding behaviour. When the aphid stylet is not in contact with the leaf tissue, the waveform is called "Np" (Nonprobing behaviour) and the voltage level remains at nearly zero on the graph (Fig. 8). Waveform "C" encompass the forms A, B and C and is observed when the stylet gets through the intercellular apoplastic, the aphid shows a cyclic activity of mechanical stylet penetration and secretion of saliva. During stylet penetration, waveforms "Pd" can be found. It reflects intracellular punctures of plant cells and can be associated with cytosolic content ingestion. Those waveforms are performed by the aphid to decide to feed or leave before reaching the phloem. A drop of potential appears on the EPG graph. Waveform "F" reflects derailed stylet mechanisms. Furthermore, the waveform "G" reflects active intake of xylem sap. Lastly, waveforms "E1" and "E2" are related to phloem activity. "E1" always comes first and reflects salivation into phloem sieve elements. This is performed by aphids in order to prevent sieve tube plugging. "E2" reflects phloem sap uptake from the sieve elements (Tjallingii, 2006; Seo et al., 2009; Cao et al., 2014; Chen et al., 2018; Garzo et al., 2020).

Since only a 4-chanel and an 8-chanel of recording were available, the EPG recording last for seven days. The plants studied were randomly chosen in order to not input bias in the results. Indeed, the moment of the day, the temperature during the day, the amount of light, etc. could influence the aphid behaviour.

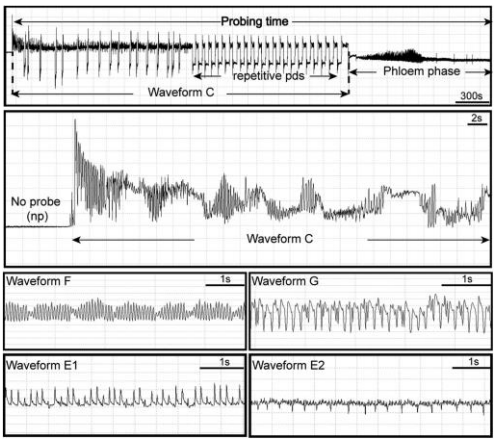

Figure 8 - Illustration of EPG waveforms (Garzo et al., 2020).

The recording graphs were analysed by the software A2EPG (Adasme-Carreño et al., 2015). All recordings were then analysed to correct by hand waveforms errors. After rectification, the forty recordings information was imported in the Excel workbook of Sarria *et al.* (2009). This workbook automatically calculates a large number of relevant parameters needed to interpret insect probing and ingestion behaviour. Selected of the obtained parameters were statistically analysed.

It is worth noting that the EPG recordings were taken again when aphids were disconnected from the plants for a moment during the four hours of recording.

D. Statistical analysis of data

Statistical analyses were made using the software RStudio (Verzani, 2011). When the normality of the population was verified, an ANOVA test was performed. When an interaction was found between two or three factors, the ANOVA was decomposed in an ANOVA with one of the factors fixed. When no interaction is found, the factors can be studied independently directly. When the normality was not verified, mathematical transformations were tested. When these did not give results, the General Linear Model in Poisson was used (McCullagh et al., 1989). Three conditions of existence must be respected to be able to carry out this model : independence of answers, distribution of answers according to a Poisson distribution and absence of overdispersion. When the CE of 'absence of overdispersion' was not respected, a GLM in quasi-Poisson error structure was performed. This model differs from the simple Poisson regression in that the dispersion parameter is not fixed at one. It should be noticed that an outlier is an observation that is distant from other observations made on the same phenomenon. 'Microorganisms' and 'Water availability' were used as factors for the study of the plant resistance and EPG. 'Microorganisms' ('B', 'T', 'BT' and 'C'), 'Water availability' ('W+' and 'W-') and 'Aphids' ('A+' and 'A-') were used as factors for the study of the plant response.

V. Results

Statistical analysis tables are located in Appendices B.

A. Plant resistance

There was a very highly significant interaction between the 'Microorganisms' and 'Water availability' factors ($p<2.2e-16$***).

When the aphids are feeding on well-watered plants, the value of resistance is lower on all the inoculated plants in comparison to the non-inoculated plants. Indeed, the resistance expected on plants inoculated with 'B', 'T' and 'BT' is respectively 87%, 92% and 82% of the value of the resistance expected on non-inoculated plants.

When the aphids are feeding on plants under water deficit, the value of resistance is lower for plants inoculated with 'B' and 'T' and higher for the ones inoculated with 'BT' in comparison to the non-inoculated plants. Indeed, the resistance expected on plants inoculated with 'B', 'T' and 'BT' is respectively 27%, 63% and 113% of the value of resistance expected on non-inoculated plants. It should be noticed that the value of the resistance of plants inoculated with 'BT' is higher than the control one under water deficit.

It should be noticed that for all the 'Microorganisms' treatments, the value of resistance is higher for the plants under water deficit than the ones well-watered. However, the plants inoculated with 'B' do not follow the same trend, the value of resistance is higher for plants well-watered (Fig. 9).

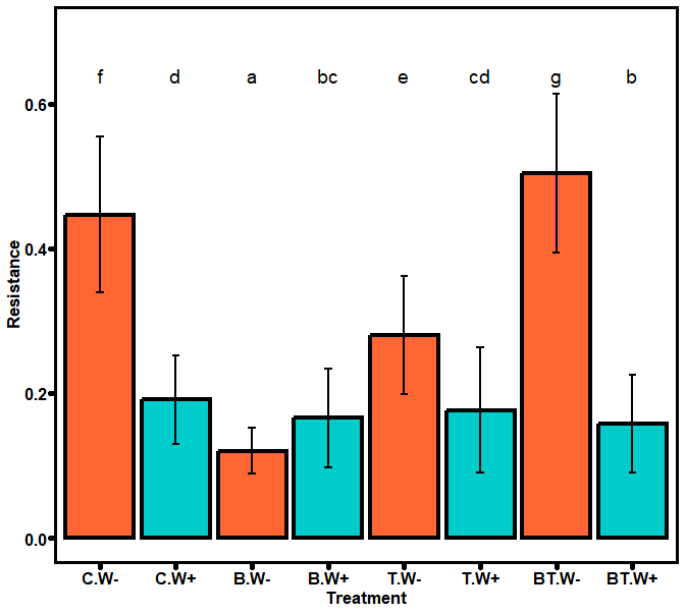

Figure 9 - Comparison of predicted values of resistance (mean ± SE) of wheat plants inoculated with different microorganisms ('C', 'B', 'BT' and 'T') and submitted or not to water deficit against Sitobion avenae. Different letters above error standard bars indicate significantly different mean values (p<0.05, Tukey's test).

B. Aphid probing behaviour

Some EPG parameters obtained using the Sarria et al. (2009) workbook were analysed in order to understand the feeding behaviour of aphids in different plants under all the combinations of the 'Microorganisms' and 'Water availability' factors.

Firstly, the presentation of the results is discussed regarding the feeding behaviour of aphids for each microorganism inoculated on plants under the two modalities of 'Water availability' factor.

The results highlighted a significant interaction between the 'Microorganisms' and 'Water availability' factors for the number of Np (non-probing), C (stylet in pathways activities) and Pd (intracellular punctures) waveforms (p=8.138e-05***, p=0.002** and p=4.286e-15***, respectively). No interaction was found between the factors for the number of E1 (salivation into phloem sieve elements) and E2 (phloem sap uptake from the sieve elements) waveforms. Regarding the duration of each waveform, for each of them, there is a significant interaction between the two factors (p<2.2e-16***) (Table 3).

As for the number of the two waveforms Np and C, a significant difference of means is highlighted for the plants inoculated with 'T'. For both of them, the value is higher for the plants well-watered. For the amount of Pd, a significant difference of means is brought out for the 'B' and 'T' inoculated plants. For 'T', as for the Np and C waveforms, the value is higher for the plants well-watered. The opposite

trend is observed for ‘B’; aphids perform more Pd on plants under water deficit. No significant difference of means is highlighted for any of the other waveforms for any of the other modalities of the ‘Microorganisms’ treatment (Table 3).

A significant interaction between the ‘Microorganisms’ and ‘Water availability’ factors was highlighted for the total duration of each waveforms ($p<2.2e-16$***). As for the total duration of the aphids in each waveform, for almost all of them, a significant difference of means is highlighted for all the modalities of the ‘Microorganisms’ factor (Table 3). However, a general trend cannot be discerned.

For the duration of the Np waveform, the value is higher for the well-watered plants for ‘C’, ‘T’ and ‘BT’. The opposite trend is found for ‘B’; the value is higher for the plants under water deficit. For the duration of the C waveform, the value is higher for the well-watered plants for ‘C’. The opposite trend is found for ‘B’, ‘T’ and ‘BT’; the value is higher for the plants under water deficit. For the duration of the ‘Pd’ waveform, the value is higher for the well-watered plants for ‘C’ and ‘T’ and lower for ‘B’. No significant difference of means is highlighted for the ‘BT’ plants. For the total duration in the phloem, the duration of E1 is higher for the well-watered plants for ‘C’, ‘B’ and ‘T’ and lower for ‘BT’. For the duration of E2, the value is higher for the well-watered plants for ‘B’ and lower for ‘C’, ‘T’ and ‘BT’ (Table 3).

Secondly, presentation of the results regarding the feeding behaviour of aphids for each microorganisms inoculated in comparison to non-inoculated plants.

For the number of Np, C, E1 and E2 waveforms, no significant difference of means is highlighted between the inoculated plants and the non-inoculated ones. However, for the ‘Pd’ waveform, a significant difference is observed for ‘B’ plants; the number of Pd is lower. For the duration of each waveform, a significant difference of means is highlighted between all the plants inoculated with microorganisms and those non-inoculated, except for the waveforms E2 and Pd for ‘BT’ (Table 3).

For the total duration of Np waveform, the value is lower for all the plants inoculated. For the total duration in C, the value is lower for ‘B’ and ‘BT’ but higher for ‘T’. For the total duration of E1, the value is lower for ‘BT’ and higher for ‘B’ and ‘T’. For the total duration of E2, the value is lower for ‘T’ and higher for ‘B’. On the contrary, for the total duration of Pd, the value is lower for ‘B’ and higher for ‘T’ (Table 3).

Table 3 – Mean ± SE value for different EPG analysis of the plants inoculated with different microorganisms and submitted to different water availability. The results are based on the feeding behaviour of Sitobion avenae. Means within rows followed by different letters are significantly different p<0.05, Tukey's test). "" indicates significant differences of means.*

EPG parameter	CW+	CW-	BW+	BW-	TW+	TW-	BTW+	BTW-	Pr(>Chisq)
Number Np	8.4 ± 1.4 b	6.6 ± 1.69 ab	4.2 ± 1.24 ab	8 ± 1.95 b	8.6 ± 2.54 b	2.8 ± 0.58 a	9.4 ± 2.46 b	6 ± 0.89 ab	**8.138e-05** ***
Number C	10.6 ± 2.32 ab	10.2 ± 2.73 ab	8.2 ± 2.75 a	9.6 ± 1.89 ab	15.4 ± 3.54 b	6.2 ± 1.96 a	12.2 ± 2.31 ab	8.6 ± 1.44 ab	**0.001557** **
Number E1	1.8 ± 0.58 a	1.6 ± 0.51 a	1.8 ± 0.58 a	1.6 ± 1.12 a	3.2 ± 1.16 a	3 ± 0.71 a	2.4 ± 0.51 a	2.4 ± 0.4 a	0.9972
Number E2	0.6 ± 0.24 a	0.8 ± 0.37 a	0.4 ± 0.25 a	0.2 ± 0.2 a	0.2 ± 0.2 a	1 ± 0.45 a	0.8 ± 0.37 a	1.4 ± 0.51 a	0.4960
Number Pd	72.8 ± 18.30 bc	60.8 ± 11.53 b	44.2 ± 12 .91 a	62.8 ± 21 .04 b	79.6 ± 21.44 c	42.0 ± 8.46 a	67.4 ± 7.70 bc	65.6 ± 8.93 bc	**4.286e-15** ***
Total duration Np	3720.6 ± 973.25 f	2899.8 ± 1490.65 d	1529.2 ± 600.07 a	4481.8 ± 2343.96 g	2270.4 ± 309.71 c	1521.8 ± 505.63 a	3285.6 ± 967.18 e	1680.0 ± 291.03 b	**< 2.2e-16** ***
Total duration C	7356 ± 1812.63 f	6030.2 ± 1304.82 d	4100.4 ± 1284 .59 b	5609.4 ± 1671.96 c	8263.6 ± 786.79 g	3788.8 ± 919.90 a	6227.8 ± 1135.59 e	8126 ± 1294.54 g	**< 2.2e-16** ***
Total duration E1	2129.2 ± 665.19 d	1347.2 ± 643.16 c	2854.4 ± 1005.59 g	918.8 ± 659.82 a	2525.8 ± 1015.51 f	2234.2 ± 945.73 e	1136 ± 299.43 b	3364.8 ± 1152.8 h	**< 2.2e-16** ***
Total duration E2	1496.2 ± 674.23 c	2250.2 ± 1803.97 d	2765.4 ± 2034.65 e	232.4 ± 232.4 a	346.0 ± 346.0 b	5885.8 ± 2417.25 g	1533.4 ± 889.79 c	3272 ± 1015.11 f	**< 2.2e-16** ***
Total duration Pd	393.6 ± 90.88 c	326.2 ± 58.27 b	271.2 ± 91.05 a	328.8 ± 114.01 b	445.6 ± 108.51 d	260.2 ± 50.15 a	368.8 ± 58.52 c	366.6 ± 37.62 c	**< 2.2e-16** ***

C. Plant performance

Significant differences of means can be highlighted by the statistical ANOVA or GLM analysis (view 'Tables') but cannot be clearly visible on the results of the Tukey post-hoc test. This difference could be explained by the sensibility of this test to the sample size, the multiplicity of comparisons and the small size of the differences.

a. Increase of height after introduction of aphids

A three-factor ANOVA was performed in view of the residues graph even if the normality was not respected. Neither triple nor double interaction was shown. There was no interaction for the factors studied two by two. Nevertheless, each factor studied separately presents a difference of means. There is a very high difference of means for the 'Aphids' and 'Water availability' factors (p=3.36e-05*** and p=3.81e-08***, respectively) and a significant difference of means for the 'Microorganisms' factor (p=0.043*).

For the 'Aphids' factor, there is a difference of means between the plants for the plants inoculated by 'B' (Figure 9.A.). For the 'Water availability' factor, the means of the increase in height is higher for the plants well-watered (W+), for each modality of the 'Microorganisms' factor (Fig. 10).

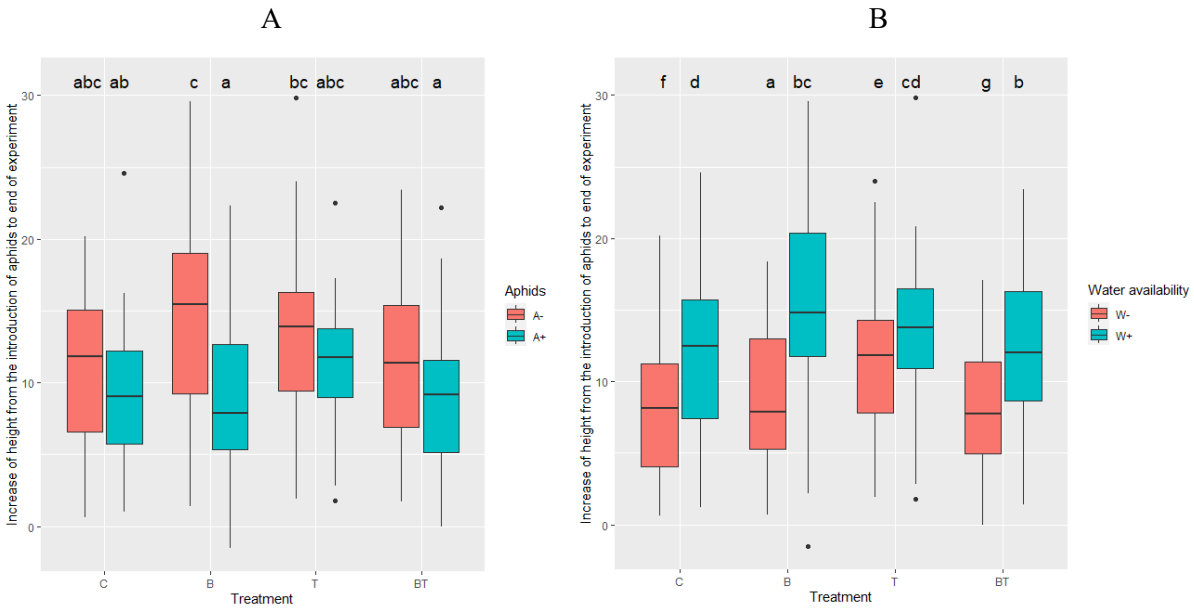

Figure 10 – Means ± SE of the increase of the height of the plants from the introduction of Sitobion avenae until the end of the experiment. A. Based on the 'Aphids' factor. B. Based on the 'Water availability' factor. Different letters above bars indicate significantly different mean values ($p<0.05$, Tukey's test).

i. Root/shoot length ratio

After the removal of outliers, in view of the residues graph, the normality will be assumed. Henceforth, a 3-factor ANOVA was performed on the dataset.

The results highlight that there is no interaction between the three factors (p=0.6975). There is no interaction for the factors studied two by two and neither significant difference of means was highlighted for the 'Microorganisms' and 'Aphids' factors. For the 'Water availability' factor, a significant difference of means was highlighted (p=0.0130*). Hence, the ratio root/shoot is consequently influenced by the amount of water available for the plants. This ratio has a higher value for the plants under water deficit, and this for all the modalities of the 'Microorganisms' factor (Fig. 11).

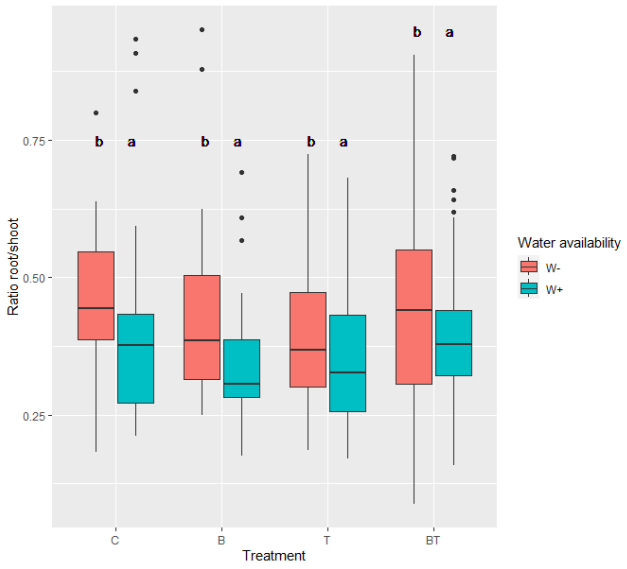

Figure 11 - Ratio root/shoot means ± SE comparisons of the plants inoculated by different microorganisms and submitted to the 'Water availability' factor. Different letters above bars indicate significantly different mean values (p<0.05, Tukey's test).

ii. Dry weight shoots

The results highlight that there is an interaction between the three factors (p=0.012379*).

Results of the AV2 based on the factor 'Aphids' show that there is no interaction for 'A+' but there is a significant interaction for 'A-' (p=0.0381*). For 'A-' and 'A+', there is a significant difference highlighted between the means for the 'Water availability' factor (p=0.024* and p=0.0317*, respectively) but none for the 'Microorganisms' factor (Fig. 12).

Results of the AV2 based on the factor 'Water availability' show that there is no interaction for 'W-' but there is a significant interaction for 'W+' (p=0.01367*). For 'W-' as for 'W+', there is a highly significant difference between the means for the 'Aphids' factor (p=0.00746*** and p=0.00668**, respectively) but none for the 'Microorganisms' factor. The only significant difference of means highlighted by the results is for the 'B' treatment.

Results of the AV2 based on the factor 'Microorganisms' show that there is no significant interaction between the factors and no difference of means for factors studied individually. However there is a significant interaction for 'B' (p=0.010*). It highlighted that there is a highly significant difference between the means for the 'Aphids' factor (p=0.001**) and a significant difference of means for the 'Water availability' factor (p=0.018*).

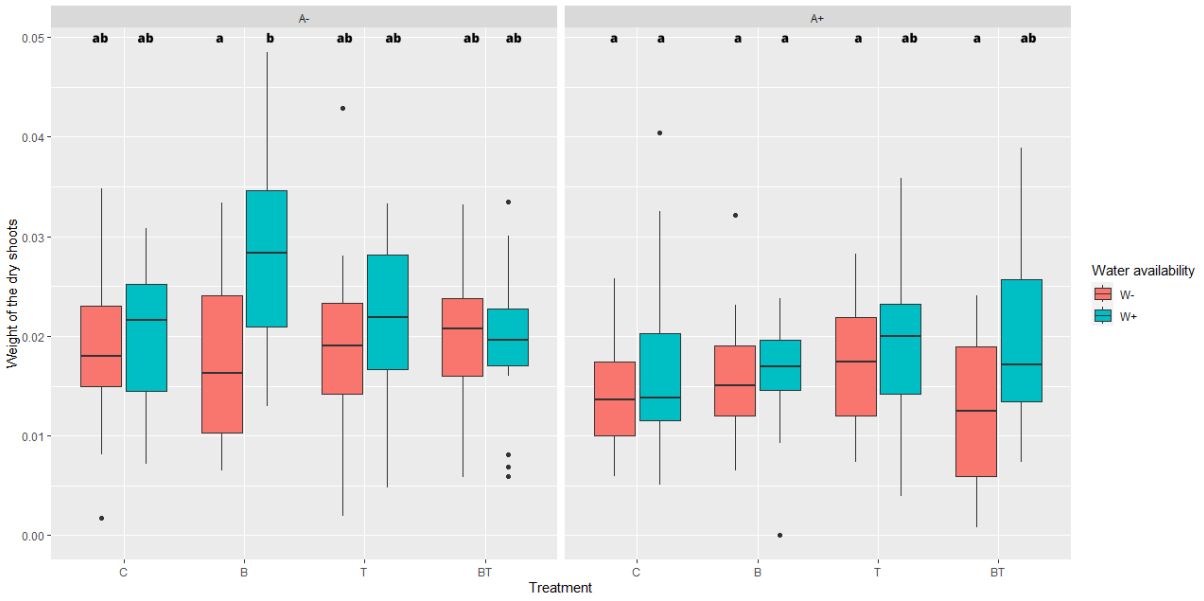

Figure 12 - Comparison of the means ± SE of dry weight of the leaves of Triticum aestivum under pressure from Sitobion avenae or not, water deficit of not and inoculated with different microorganisms. Different letters above bars indicate significantly different mean values (p<0.05, Tukey's test).

iii. Dry weight roots

After the discarding of three outliers, the normality of the population is respected and a 3-factor ANOVA (AV3) was performed on the dataset.

There is an interaction between the three factors (p=0.036*).

Following AV2 based tests on the factor 'Aphids', there is no interaction between the factors, neither for 'A+' and for 'A-'. When the factors are studied separately, no significant difference of means is highlighted for none of them.

After AV2 based on the factor 'Water availability', there is a highly significant interaction for 'W-' (p=0.007**) but no interaction for 'W+'. For both modalities, no interaction was highlighted between the factors and no significant difference of means was shown for almost none of them studied alone. Indeed, only a slight difference of means is highlighted for the 'Aphids' factor (p=0.043*) when the plants are well-watered.

According to AV2 based on the factor 'Microorganisms', there is no interaction between the factors for the modalities 'C, 'B' and 'BT' and neither for any of the factors studied individually. However there is a highly significant interaction for 'T' (p=0.006**). No significant difference of means is highlighted, neither for the 'Aphids' factor or for the 'Water availability' factor.

In conclusion, no significant difference of means has been highlighted for any of the modalities of the factors (Fig. 13).

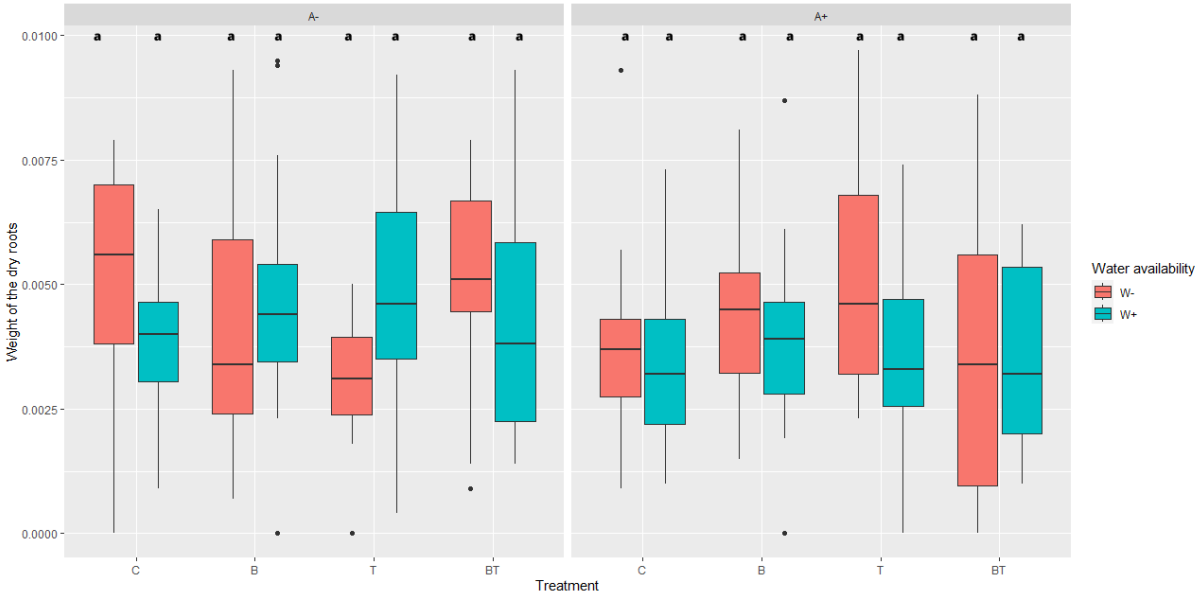

Figure 13 - Comparison of the means ± SE of dry weight of the roots of Triticum aestivum under pressure of Sitobion avenae or not, water deficit or not and inoculated with different microorganisms. Different letters above bars indicate significantly different mean values ($p<0.05$, Tukey's test).

iv. Root/shoot dry weight ratio

The results highlight that there is a very highly significant interaction between the three factors ($p<2.2e-16$***).

When the factors studied are combined, the value of the root/shoot dry weight ratio is higher for plants under water deficit (W-) for the 'Microorganisms' and 'Aphids' factor. However, there exists an exception for 'T', the value is higher for the plants well-watered when they are not under pressure of aphids (Fig. 14).

For the plants non submitted to aphid pressure (A-) and under water deficit (W-), the value of the ratio is lower for all the inoculated modalities of the 'Microorganisms' factor compared to the non-inoculated plants. Indeed, the values expected for 'B', 'T' and 'BT' are 70%, 52% and 74% of the value of 'C'. For the 'W+' plants, the value of the ratio is lower for 'B' and higher for 'T' and 'BT' compared to the non-inoculated plants. Indeed, the values expected for 'B', 'T' and 'BT' are 77%, 104%, 106% of the value of 'C'.

For the plants subjected to aphid pressure (A+) and under water deficit (W-), the value of the ratio is lower for 'BT' and higher for 'B' and 'T' compared to the non-inoculated plants. The values expected for 'B', 'T' and 'BT' are 101%, 115%, 88% of the value of 'C'. For the 'W+' plants, the value

of the ratio is the same for 'B' and lower for 'T' and 'BT' in comparison to non-inoculated plants. The values expected for 'B', 'T' and 'BT' are 100%, 80% and 81% of the value of 'C'.

In conclusion, there is a significative difference of means for almost all the modalities of every factor.

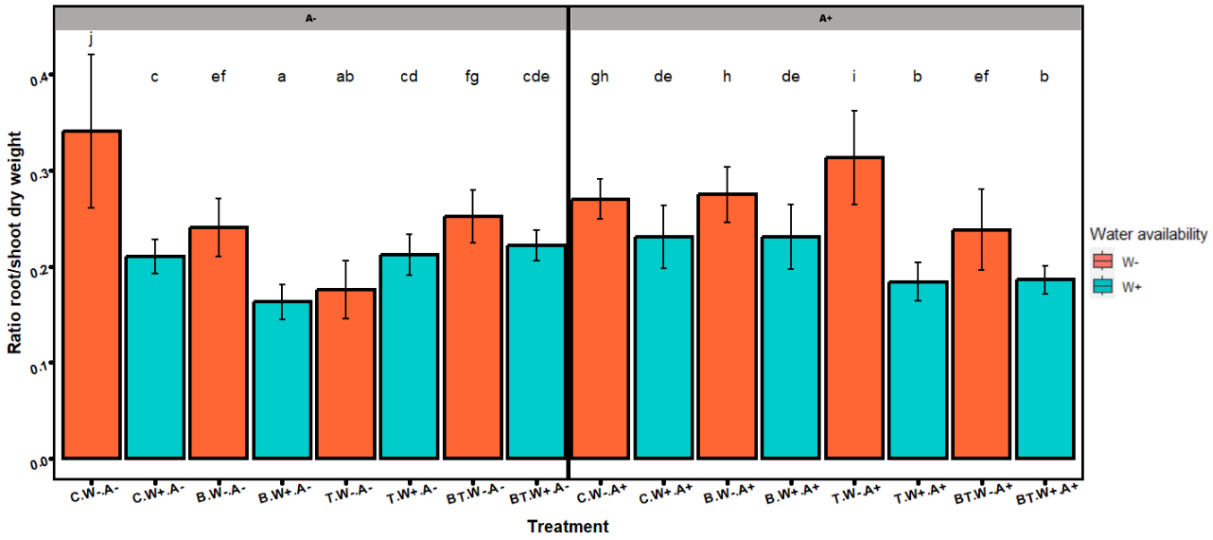

Figure 14 - Predicted values ± SE of the dry weight ratio root/shoot of plants inoculated with different microorganisms, submitted to the pressure of Sitobion avenae or not and submitted to different water availability. Different letters above bars indicate significantly different mean values (p<0.05, Tukey's test).

v. Dry weight total biomass

There is an interaction between the three factors (p=0.020*).

Following AV2 tests on the factor 'Aphids', there is no interaction between the factors. There is no significant difference of means for each factor studied individually, except for the 'Water availability' factor for plants without pressure of aphids (p=0.035*).

Results of the AV2 based on 'Water availability' factor show that there is interaction for 'W+' but none for 'W-' (p=0.049* and p=0.155). For 'W+' and 'W-', a significant difference of means was highlighted for the 'Aphids' factor (p=0.006** and p=0.016*, respectively) but none for the 'Microorganisms' factor.

Following AV2 tests on the 'Microorganisms' factor, there is no interaction between the two factors for 'C', 'T' and 'BT' and neither for the factors individually. However, there is a significant interaction between the factors for 'B' (p=0.020*) and a significant difference of means for the 'Aphids' and 'Water availability' factors (p=0.003** and 0.033*, respectively).

The only significant difference of means highlighted is for the plants inoculated with ‘B’, the dry weight of the total biomass is higher for the plants well-watered (Fig. 15).

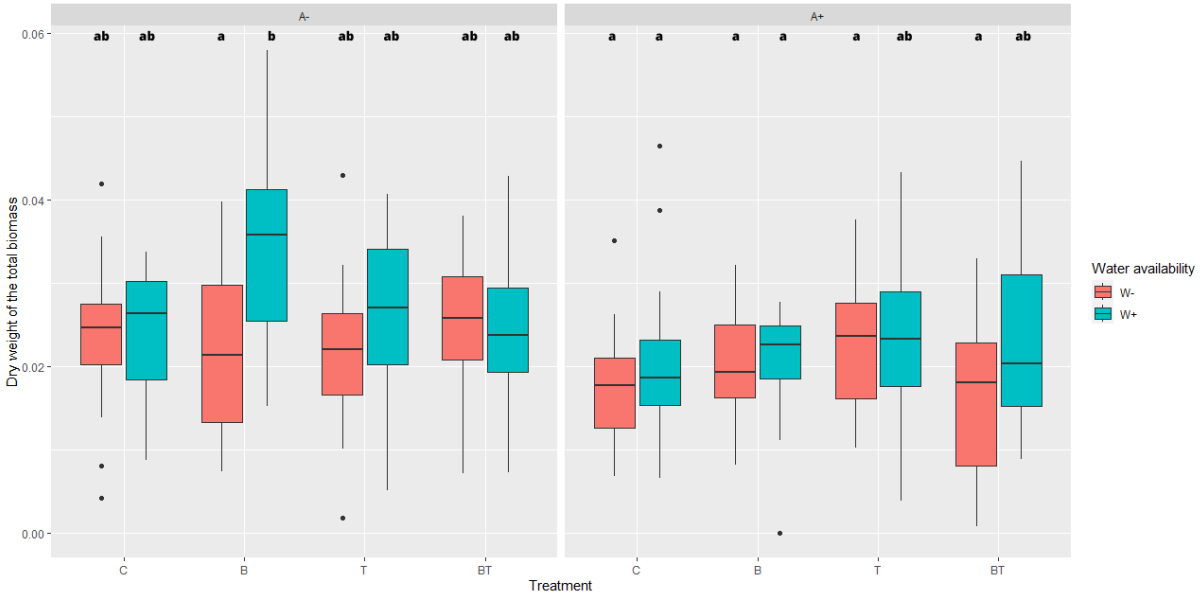

Figure 15 - Comparison of the means ± SE of dry weight of the roots of Triticum aestivum under pressure from Sitobion avenae or not, water deficit or not and inoculated with different microorganisms. Different letters above bars indicate significantly different mean values (p<0.05, Tukey’s test).

vi. Fv/Fm

There is a very highly significant interaction between the ‘Microorganisms’, ‘Water availability’ and ‘Aphids’ factors (p=1.407e-07***).

For the three factors studied independently, the means of the photochemical efficiency of the photosystem II (Fv/Fm) was very highly significant (p<2.2e-16***) different.

When the factors studied are combined, the value of photochemical efficiency of the photosystem II is lower for plants under water deficit (W-), and this whatever the ‘Microorganisms’ and ‘Aphids’ factors studied (Fig. 16).

For the plants non submitted to aphid pressure (A-), the value of Fv/Fm is lower for all the inoculated modalities of the ‘Microorganisms’ factor for the ‘W-‘ plants. Indeed, the values expected for ‘B’, ‘T’ and ‘BT’ are 98%, 96% and 88% of the value of ‘C’. On the contrary, for the ‘W+’ plants, the value of Fv/Fm is higher for all the inoculated modalities of the ‘Microorganisms’ factor. Indeed, the values expected for ‘B’, ‘T’ and ‘BT’ are 103%, 104% and 103% of the value of ‘C’.

For the plants submitted to aphid pressure (A+), for the ‘W-‘ plants, the value of Fv/Fm is higher for the ‘B’ and ‘T’ and lower for the ‘BT’ inoculated modalities of the ‘Microorganisms’ factor. Indeed,

the values expected for ‘B’, ‘T’ and ‘BT’ are 108%, 106% and 92% of the value of ‘C’. For the ‘W+’ plants, the value of Fv/Fm is slightly higher for the ‘B’ and ‘BT’ and equivalent for the ‘T’ inoculated modalities of the ‘Microorganisms’ factor compared to the non-inoculated plants. Indeed, the values expected for ‘B’, ‘T’ and ‘BT’ are 101%, 101% and 100% of the value of ‘C’.

In conclusion, there is almost no difference of means for the plants exempt from the pressure of aphids, for each modality of the ‘Microorganisms’ and ‘Water availability’ factors. Indeed, only the well-watered plants inoculated with ‘T’ are significantly different from the others. For the plants with pressure of aphids, there is no difference of means between the microorganisms, for any of the well-watered plants. However, for the plants under water deficit, there is a significant difference for all of them.

Studies have proven that the mean Fv/Fm value of wheat plants kept in the laboratory at 20°C and in the dark for several minutes is about 0.8 (Groom et al., 1992).

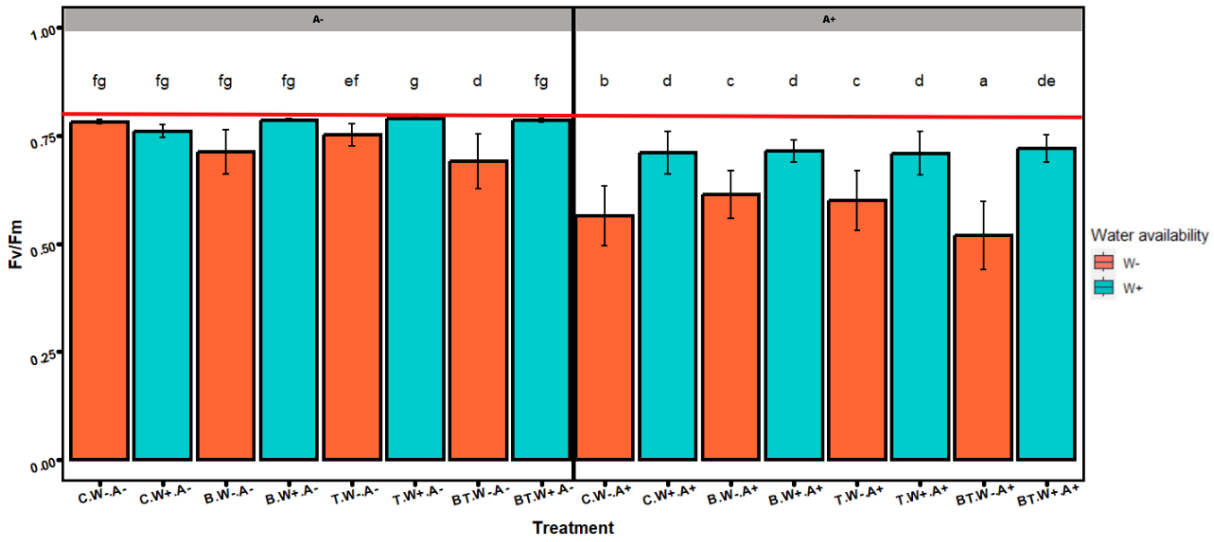

Figure 16 - Predicted values ± SE of Fv/Fm of plants inoculated with different microorganisms, submitted to the pressure of Sitobion avenae or not and submitted to different water availability. Different letters above bars indicate significantly different mean values (p<0.05, Tukey’s test).

vii. PI_abs

In view of the normality of the residues, the population will be considered as normal. A 3-factor ANOVA test was therefore performed on the dataset.

There is no interaction between the three factors (p=0.102), neither for the factors studied two by two nor for the ‘Microorganisms’ factor. However, there is a significant difference for the different modalities of the ‘Water availability’ factor (p=0.026*) and a very highly significant difference for the ‘Aphids’ factor (p<2e-16***). Hence, the Performance Index of the plant is influenced by the amount

of water available as well as the presence or absence of aphids but not by the administered microorganisms. The plant vitality is greater when the plant is well-watered (W+) and when it is not subject to the pressure of aphids (A-) (Fig. 17).

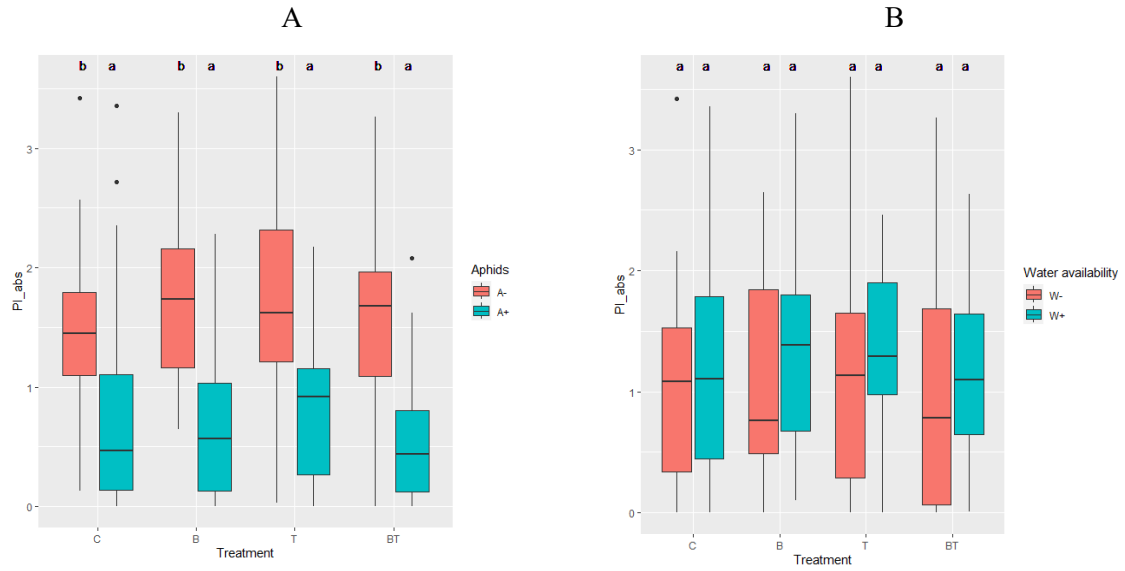

Figure 17 - PI_{abs} means comparisons between plants inoculated with different microorganisms, submitted to Sitobion avenae pressure or not and to water deficit or not. A. Based on the 'Aphid' factor. B. Based on the 'Water availability' factor. Different letters above bars indicate significantly different mean values ($p<0.05$, Tukey's test).

VI. Discussion

T. aestivum is the most important source of food around the world (Igrejas et al., 2020). Unfortunately, this cereal is submitted to the pressure of *S. avenae* aphids which can have a negative impact on the crop yield (Ighil et al., 2011). Since more and more pesticides are being banned, alternatives to those chemicals are being closely studied (Jactel et al., 2019). This is the case of *B. subtilis* and *T. virens,* which are well-known microorganisms used as Plant Growth Promotors. They are proven to induce defence in plants and to protect them against biotic cues, including pests such as aphids. Moreover, they are also proven to alleviate drought stress (Bae et al., 2009; Lastochkina et al., 2017; Sood et al., 2020).

A. Plant resistance

As plant resistance is a function corresponding to the inverse of the quantity of living *S. avenae*, water deficit is expected to increase plant resistance. Indeed, it has been reported that water deficit has a negative impact on *S. avenae* fitness, directly by decreasing their fecundity (Liu et al., 2018). Therefore, the plant resistance to aphids should increase when water availability decreases (Ramírez et al., 2023). This hypothesis is supported by the obtained results for the non-inoculated plants. For the **Hypothesis 1** which is "Plants under water deficit are less vigorous, aphids develop thus poorly on those plants" is corroborated.

Given that PGPR are known to induce systemic resistance to aphids, the resistance of plants inoculated with microorganisms should have been higher than the resistance of the non-inoculated plants. Indeed, Coppola et al. (2019) proved that the inoculation of a strain of *Trichoderma* is used to negatively influence the survival rate of *M. euphorbiae*. Moreover, *B. subtilis* and *T. virens* are microorganisms well-known to induce systemic resistance in the plants (Ongena et al., 2007; Shoresh et al., 2010). However, the results do not follow that prediction when plants were well-watered. Indeed, for plants inoculated with microorganisms, the value of resistance is lower for all of them. This could be explained by the value of root/shoot dry weight ratio, which is lower for all the inoculated plants. It is thus assumed that microorganisms inoculation improve plants conditions which could improve aphids conditions. This could also be explained by the ability of aphids to secrete evolutionarily conserved effectors which are capable of suppressing plant immune responses (Coppola et al., 2019).

In comparison, for the plants under water deficit, the resistance is increased for all of the plants, except for plants inoculated with *B. subtilis*. However, numerous studies tend to prove that colonisation of plant roots by *B. subtilis* strains induces ISR and enhances hydrogen peroxide production, callose deposition and cell death which reduces consumption rates and phloem-feeding performances

(Shakarami et al., 2021). This could be explained by plant traits. Indeed, plants inoculated with *B. subtilis* and well-watered showed the lowest value of root/shoot dry weight ratio, which suggests that these plants were in better growth condition than the other plants. As for plants under water deficit and inoculated with the consortium, they showed the highest value of resistance to aphids and the lowest value of root/shoot dry weight ratio. It can be assumed that those plants are less impacted by both water and aphids stress. Hence, the **Hypothesis 2** which is "Plants inoculated with microorganisms can recover from water deficit but microorganisms also induce resistance to aphids" is not fully corroborated by the results.

Increase resistance under water deficit can be explained by the fact that when plants are under water deficit usually stomata are closed, photosynthesis and growth is reduced, and thus plants use storage carbons and soluble sugar to increase the circulation within the plants. This implies that plants accumulate circulating non-structural carbohydrates when they are under stress, namely drought (Fajardo et al., 2012). Henceforth, the water deficit could have an impact on stored carbohydrates and then have an impact on aphids. Interestingly, a high concentration of non-structural carbohydrates may have an impact on the osmotic homeostasis of aphids (Sadras et al., 2020). Studies have shown that artificial diet containing a high content of sucrose involves that aphids are not able to survive or reproduce (Alkhedir et al., 2013). So, water-deficit may increase resistance to aphids, which was even increased a bit more when plants were treated with both microorganism.

To support the assumption that those results have something to do with the concentration of non-structural carbohydrates, an analysis of the roots and leaves will be performed, the results weren't available in time to discuss them in this thesis.

B. Aphid probing behaviour

EPG data demonstrate the actual effects of microorganisms inoculation of plants and their water availability on aphid feeding behaviour of *S. avenae*.

Despite the difference in resistance against aphids obtained for well-watered and under water deficit plants, the behaviour of aphids was very similar for the time in each waveform. For the inoculated plants, the same trend is not followed. The time spent by aphids feeding in the phloem of plants is the highest for plants inoculated with *T. virens* whenever those plants are under water deficit and the lowest whenever they are well-watered. The opposite trend is observed for the time spent in the stylet pathways. It has been proved that prolonged E1 salivation and reduced E2 feeding would indicate a reduced ability of aphids to suppress phloem wound responses. It is assumed that *T. virens* inoculation could counterbalance the suppression mechanism of sieve-tube occlusion due to aphid saliva (Will et al.,

2008). In the view of the results, it can also be assumed that *B. subtilis* is more efficient to reduce phloem feeding on plants under water deficit than those well-watered.

Globally, in the view of the results, aphids seems to spend more time feeding in the phloem when inoculated plants are under water deficit, with an exception for those inoculated with *B. subtilis*. This trend is also followed by the resistance of plants against aphids; the resistance is higher for the inoculated plants except for those with *B. subtilis*. Resistance under water deficit in inoculated plants seems thus associated with longer time in E2. It could be explained by the increase of sugar as a response of the plants to water deficit. Those sugars, needed by aphids for sustained feeding, might act as phagostimulant (Douglas et al., 2006). The exception of *B. subtilis* could be explained by the time spent by aphids salivating in the phloem, which helps to prevent protein clogging inside a sieve element (Tjallingii, 2006).

For the plants inoculated with *B. subtilis* and *T. virens*, in term of time spent navigating through the plant tissues, the consortium seems to follow the trend of aphids feeding on plants inoculated with *B. subtilis*. In term of time spent walking around exploring the best feeding position and feeding in the phloem, the consortium seems to follow the trend of aphids feeding on plants inoculated with *T. virens*. Inoculating plants with the consortium of the two microorganisms appear to mitigate the opposing effects of the two microorganisms inoculated separately on the feeding behaviour of aphids.

The results of EPG have shown that *B. subtilis*, *T. virens* and the consortium between the two of them affect the settling, probing and ingestion behaviours of *S. avenae*. However, the impact of inoculated microorganisms does not follow a clear trend for the three of them. Hence, the **Hypothesis 3** which is " The feeding behaviour of aphids is negatively influenced by the presence of microorganisms inoculated on *T. aestivum*" is not corroborated by the results of the EPG.

Differences are seen between the results of the plant resistance study and the study of the feeding behaviour of insects. Those can be attributed to several factors. Firstly, it has been proven that the study of the ability of plants to resist aphids under conditions of water restrictions must consider that the results might be affected by plant genotypic variation in tolerance of drought (Ramírez et al., 2023). Indeed, the response of aphids feeding on drought-stressed plants is associated with reduced plant vigour and increased of chemical defence (Leybourne et al., 2021). Secondly, the presence of microorganisms has proved to enhance plant performance under water stress conditions (Sood et al., 2020). Their presence could reduce the adverse effect of carbohydrates presence on the performance of aphids (Sadras et al., 2020). Thirdly, for the plant resistance experiment, some modalities could have been under pressure from more winged aphids than the others and Wratten (1977) has proven that apterae "are consistently more fecund than alatae of comparable weight, producing about three more nymphs on average in any 5-day period". Thus, the presence of winged aphids could have an important impact on the aphids count and a result on the plant resistance.

C. Plant performance

The impact of water deficit on plant performance is marked on the increase of shoots height. Indeed, as shown by Raza (2012), drought has a negative impact and significantly reduces plant height. The reduction of plant growth observed when plants are under water deficit can be explained by the reduction in nutrient uptake, transport and cell elongation (Pons et al., 2020). The presence of aphids has also a negative impact on the value of plant height (Kieckhefer et al., 1992).

In regard to the root/shoot length ratio, the values are higher for the plants under water deficit. It is explained by the necessity of the plants under water deficit to increase their root system in order to enhance water absorption (Ahmad et al., 2018). The presence of aphids did not influence this ratio.

Regarding the dry weight shoots and the dry weight total biomass, the only significant difference of means spotted is for the plants inoculated with *B. subtilis*. The values are higher for the plants well-watered. The results for the dry weight shoots for *B. subtilis* are corroborated by the study of Heitholt (1989), where the water stress significantly reduce it. The results of the biomass are in line with those from Estrada-Campuzano et al. (2012), where the weight is significantly diminished by water stress. Regarding the increase of height, under optimal conditions, *B. subtilis* inoculated on plants showed the highest values. When the same plants are subjected to aphid pressure, no more differences are observed.

The root/shoot dry weight ratio is higher for plants under water deficit. It corroborates the studies proven that the ratio tend to increase whenever the plants are grown in stressful environment (McMichael et al., 1991). The inoculated plants showed a lower ratio than non-inoculated plants. Thus, it could be assumed that the microorganisms play a role in the plant capacity to face drought stress. *T. virens* treatment showed the lowest root/shoot dry weight ratio. Moreover, plants inoculated with *T. virens* or the consortium, under well-watered conditions and attacked by aphids, showed lower root/shoot dry weight ratio than non-inoculated plants. It could thus be assumed that *T. virens* inoculation plays a role in the reducing of both water and aphid pressure stress.

The Fv/Fm ratio reflects the photochemical efficiency of photosystem II and was significantly reduced whenever plants are under pressure of *Sitobion avenae*. Those values were even more reduced whenever those plants are under water deficit. Indeed, this ratio tend to decrease with days after water deficit treatment (Ibaraki et al., 2007). However, the inoculated plants with the consortium and under water deficit present the lowest value of Fv/Fm but the highest value for the resistance of plants against aphids. Those results are opposed to the results obtained by Gantner et al. (2010) for the pressure of *Myzocallis coryli* Goetze on *Corylus* L. and those of Kmieć et al. (2018) for the pressure of galling aphids on *Ulmus* sp. As for the Performance Index (PI_{abs}), it reflects the functionality of photosystems I and II (Zivcak et al., 2008). When plants are under aphid pressure, the values of the index are significantly lower (Miller, 2019).

In the view of all the results, **Hypothesis 4** which is “Microorganisms promote plant growth and allow plants to recover from water deficit” is not accepted.

It should be noted that a posteriori study of the presence of microorganisms within the plant was conducted, and the microorganisms were only detected on the relevant plants in 90% of the cases. This could have influenced the obtained results.

VII. Personal contributions

All laboratory manipulations, data collection, statistical analysis, interpretation of results, and writing were carried out by the author of this study. The author received occasional assistance from other individuals, namely for laboratory manipulations. They are acknowledged in the acknowledgments section.

Personal contributions and insights that were implemented during this experimentation are attributed to the author.

Through literature review, the choice of the degree of water deficit and the quantity of inoculated microorganisms were determined.

Based on a prior experimentation, it was known that the roots would be cleaned at the end for weighing. However, cleaning proved challenging when using compost, as it contained numerous non-decomposed and rigid organic residues. To streamline the process, the substrate composition was adjusted and preliminary, compost sieving was performed. Considering that data collection from this same experiment was lengthy and somewhat disorganised, the implementation of data collection for the experiment discussed in this article was thoughtfully designed and adjusted to maximise efficiency and minimise potential biases in the results.

This experimentation allowed me to apply the knowledge accumulated over my five years at university. It provided an opportunity to execute and manage a bioengineering project within an integrated and holistic framework, which is one of the many skills to be acquired upon completing a bioengineering education.

VIII. Conclusions and perspectives

In the current context of chemical pesticides limitation and global climate change, the use of biocontrol agents is studied to regulate crop pests and ensure yield. The present research focuses on the effects of *Bacillus subtilis* and *Trichoderma virens*, alone and in consortium, on the wheat growth and resistance against *Sitobion avenae* infestation, under varied water availability.

Using the electropenetrography, it has been shown that the microorganisms infection affects the settling, probing and ingestion behaviour of *S. avenae*. Despite the difference in resistance against aphids obtained for well-watered and under water deficit plants, the behaviour of aphids was very similar for the time in each waveform. Globally, aphids seem to spend more time feeding in the phloem when inoculated plants are under water deficit, with an exception for those inoculated with *B. subtilis*. The resistance of aphids is higher for all the inoculated plants, except for those inoculated with *B. subtilis*. Regarding plants with the consortium of the two microorganisms, it appears to mitigate the opposing effects of the two microorganisms inoculated separately on the feeding behaviour of aphids. Hence, the impact of inoculated microorganisms on aphid feeding does not follow a clear trend. Since sugars had been studied as potential phagostimulant, it would be relevant to study the concentration in non-structural carbohydrates in the plants.

Since the electropenetrography recording was only four hours long, in future experiment it would be interesting to perform longer recordings to focus more on the feeding behaviour of the aphids in the phloem. Moreover, a hormone profile analysis could help understanding the obtained results.

As for the resistance of plants against aphids, the value was lower for all the plants inoculated. The value of the root/shoot dry weight ratio follows more or less the same trend: the value is lower for *B. subtilis* and equivalent for the plants inoculated with the other microorganisms. It is thus assumed that microorganisms inoculation improves plants conditions, which could improve aphids conditions.

In regard to the water availability, the value of resistance was higher whenever plants were under water deficit, except for those inoculated with *B. subtilis*. The time spent by aphids in the phloem follows the same trend. It is assumed that the increasing time spent by aphids in the phloem is correlated with the increase of resistance. This could explain the different trend for *B. subtilis*, since the time spent in the phloem decrease whenever plants are under water deficit in comparison to well-watered plants. The consortium presents the highest value of resistance under water deficit and an intermediate value of phloem feeding. It also presents the lowest value for the root/shoot dry weight ratio. It is assumed that the behaviour of aphids on those plants presents a mitigation of the effects for each microorganism

studied individually. It should be worth inoculating a more higher concentration of microorganisms and to conduct the experiment on a longer period of time in order to see if similar results are obtained.

To conclude, in view of all the results, the consortium between *Bacillus subtilis* and *Trichoderma virens* appears to be the best product of microorganism to inoculate in order to enhance the wheat resistance against *Sitobion avenae* when the crop is subjected to water deficit. This product could be used at large scale as biological control against aphids and help to cope with the rain variabilities expected in the future.

IX. Literature reference

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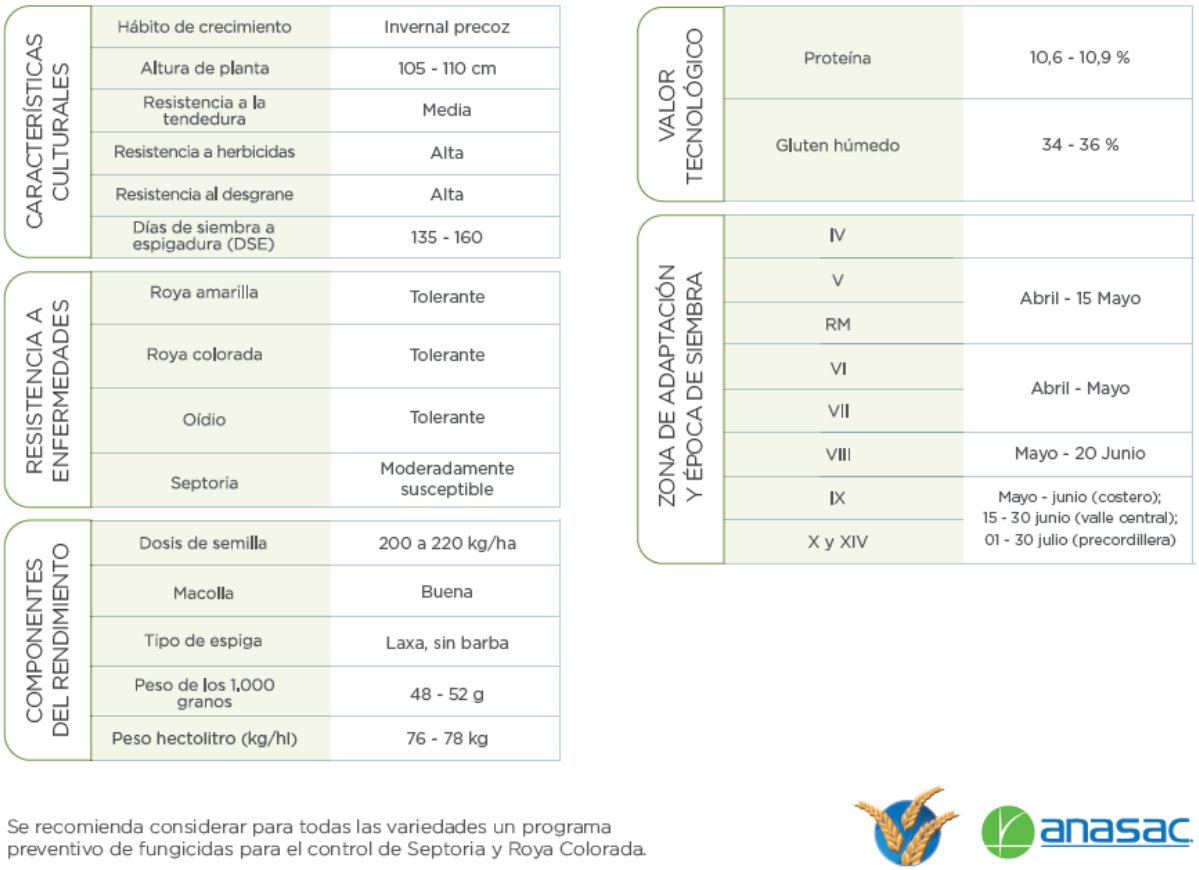
X. Appendices

A. Varietal datasheet provided by ANASAC for the *Triticum aestivum* variety 'Matylda'.



CARACTERÍSTICAS CULTURALES	
Hábito de crecimiento	Invernal precoz
Altura de planta	105 - 110 cm
Resistencia a la tendedura	Media
Resistencia a herbicidas	Alta
Resistencia al desgrane	Alta
Días de siembra a espigadura (DSE)	135 - 160

RESISTENCIA A ENFERMEDADES	
Roya amarilla	Tolerante
Roya colorada	Tolerante
Oídio	Tolerante
Septoria	Moderadamente susceptible

COMPONENTES DEL RENDIMIENTO	
Dosis de semilla	200 a 220 kg/ha
Macolla	Buena
Tipo de espiga	Laxa, sin barba
Peso de los 1.000 granos	48 - 52 g
Peso hectolitro (kg/hl)	76 - 78 kg

VALOR TECNOLÓGICO	
Proteína	10,6 - 10,9 %
Gluten húmedo	34 - 36 %

ZONA DE ADAPTACIÓN Y ÉPOCA DE SIEMBRA	
IV	
V	Abril - 15 Mayo
RM	
VI	Abril - Mayo
VII	
VIII	Mayo - 20 Junio
IX	Mayo - junio (costero); 15 - 30 junio (valle central); 01 - 30 julio (precordillera)
X y XIV	

Se recomienda considerar para todas las variedades un programa preventivo de fungicidas para el control de Septoria y Roya Colorada.

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B. Statistical analysis

Table 4 - General Linear Model results of the resistance of the plants submitted to aphids pressure.

Resistance	DF	LR Chisq	P-Value	Significance
Microorganisms	3	2907.50	<2.2e-16	***
Water availability	1	3273.30	<2.2e-16	***
Microorganisms*Water availability	3	1646.60	<2.2e-16	***

Table 5 - Statistical analysis of EPG number of waveforms results of aphids feeding on plants inoculated with different microorganisms and under water deficit or not. The results are based on the feeding behaviour of Sitobion avenae for each factor studied independently. P-values are those followed comparisons among treatments within each factor.

Number of Np	DF	LR Chisq	P-Value	Significance
Microorganisms	3	4.4548	0.21636	NS
Water availability	1	4.8143	0.02822	*
Microorganisms*Water availability	3	21.5380	8.138e-05	***
Number of C	**DF**	**LR Chisq**	**P-Value**	**Significance**
Microorganisms	3	2.1341	0.545037	NS

Water availability	1	8.6257	0.003314	**
Microorganisms*Water availability	3	15.3277	0.001557	**
Number of E1	**DF**	**LR Chisq**	**P-Value**	**Significance**
Microorganisms	3	5.8954	0.1168	NS
Water availability	1	0.1011	0.7505	NS
Microorganisms*Water availability	3	0.0488	0.9972	NS
Number of E2	**DF**	**LR Chisq**	**P-Value**	**Significance**
Microorganisms	3	4.9739	0.1737	NS
Water availability	1	1.8357	0.1755	NS
Microorganisms*Water availability	3	2.3871	0.4960	NS
Number of Pd	**DF**	**LR Chisq**	**P-Value**	**Significance**
Microorganisms	3	19.2670	0.0002408	***
Water availability	1	10.8710	0.0009770	***
Microorganisms*Water availability	3	69.9920	4.286e-15	***

Table 6 - Statistical analysis of EPG duration of waveforms results of aphids feeding on plants inoculated with different microorganisms and under water deficit or not. The results are based on the feeding behaviour of Sitobion avenae for each factor studied independently. P-values are those followed comparisons among treatments within each factor.

Duration of Np	**DF**	**LR Chisq**	**P-Value**	**Significance**
Microorganisms	3	4462.5	<2.2e-16	***
Water availability	1	11.6	0.0006729	***
Microorganisms*Water availability	3	11461.0	<2.2e-16	***
Duration of C	**DF**	**LR Chisq**	**P-Value**	**Significance**
Microorganisms	3	5050.3	<2.2e-16	***
Water availability	1	578.8	<2.2e-16	***
Microorganisms*Water availability	3	11024.2	<2.2e-16	***
Duration of E1	**DF**	**LR Chisq**	**P-Value**	**Significance**
Microorganisms	3	1325.1	<2.2e-16	***
Water availability	1	184.5	<2.2e-16	***
Microorganisms*Water availability	3	11770.8	<2.2e-16	***
Duration of E2	**DF**	**LR Chisq**	**P-Value**	**Significance**
Microorganisms	3	6575.0	<2.2e-16	***
Water availability	1	8645.0	<2.2e-16	***
Microorganisms*Water availability	3	37772.0	<2.2e-16	***
Duration of Pd	**DF**	**LR Chisq**	**P-Value**	**Significance**
Microorganisms	3	84.185	<2.2e-16	***
Water availability	1	70.627	<2.2e-16	***

Microorganisms*Water availability	3	235.084	<2.2e-16	***

Table 7 - Variance analysis of plants increase of height after the introduction of aphids.

Increase of height	DF	F-Value	P-Value	Significance
Microorganisms	3	2.752	0.0435	*
Water availability	1	32.456	3.81e-08	***
Aphids	1	17.923	3.36e-05	***
Microorganisms*Water availability	3	0.995	0.3690	NS
Microorganisms*Aphids	3	1.482	0.2202	NS
Water availability*Aphids	1	0.560	0.4549	NS
Microorganisms*Water availability*Aphids	3	2.123	0.0981	NS

Table 8 - Variance analysis of the root/shoot length ratio.

Root/shoot length ratio	DF	F-Value	P-Value	Significance
Microorganisms	3	2.547	0.0568	NS
Water availability	1	6.278	0.0130	*
Aphids	1	2.070	0.1516	NS
Microorganisms*Water availability	3	0.408	0.7473	NS
Microorganisms*Aphids	3	0.481	0.6961	NS
Water availability*Aphids	1	0.056	0.8124	NS
Microorganisms*Water availability*Aphids	3	0.479	0.6975	NS

Table 9 - Variance analysis of the dry weight of the shoots.

Dry weight shoots	DF	F-Value	P-Value	Significance
Microorganisms	3	1.094	0.3524	NS
Water availability	1	10.297	0.0015	**
Aphids	1	15.625	0.0001	***
Microorganisms*Water availability	3	0.642	0.5887	NS
Microorganisms*Aphids	3	1.327	0.2665	NS
Water availability*Aphids	1	0.082	0.7749	NS
Microorganisms*Water availability*Aphids	3	3.709	0.0124	*

Table 10 - Variance analysis of the dry weight of the shoots based on the 'Aphids' factor.

Dry weight shoots AV2 on 'Aphids' factor modality 'A+'	**DF**	**F-Value**	**P-Value**	**Significance**
Microorganisms	3	0.749	0.5254	NS
Water availability	1	4.735	0.0317	*
Microorganisms*Water availability	3	1.290	0.2812	NS
Dry weight shoots AV2 on 'Aphids' factor modality 'A-'	**DF**	**F-Value**	**P-Value**	**Significance**
Microorganisms	3	1.590	0.1959	NS
Water availability	1	5.562	0.0201	*
Microorganisms*Water availability	3	2.902	0.0381	*
Dry weight shoots AV1 on 'Aphids' factor modality 'A-'	**DF**	**F-Value**	**P-Value**	**Significance**
Water availability	1	5.231	0.024	*
Dry weight shoots AV1 on 'Aphids' factor modality 'A-'	**DF**	**F-Value**	**P-Value**	**Significance**
Microorganisms	3	1.46	0.229	NS

Table 11 - Variance analysis of the dry weight of the shoots based on the 'Water availability' factor.

Dry weight shoots AV2 on 'Water availability' factor modality 'W-'	**DF**	**F-Value**	**P-Value**	**Significance**
Aphids	1	7.427	0.0075	**
Microorganisms	3	0.359	0.7830	NS
Aphids*Microorganisms	3	1.071	0.3643	NS
Dry weight shoots AV2 on 'Water availability' factor modality 'W+'	**DF**	**F-Value**	**P-Value**	**Significance**
Aphids	1	8.206	0.0050	**
Microorganisms	3	1.289	0.2816	NS
Aphids*Microorganisms	3	3.713	0.0137	*
Dry weight shoots AV1 on 'Water availability' factor modality 'W+'	**DF**	**F-Value**	**P-Value**	**Significance**
Microorganisms	3	1.139	0.337	NS
Dry weight shoots AV1 on 'Warter availability' factor modality 'W+'	**DF**	**F-Value**	**P-Value**	**Significance**
Aphids	1	7.624	0.0067	**

Table 12 - Variance analysis of the dry weight of the shoots based on the 'Microorganisms' factor.

Dry weight shoots AV2 on 'Microorganisms' factor modality 'C'	**DF**	**F-Value**	**P-Value**	**Significance**
Aphids	1	3.151	0.0813	NS
Water availability	1	1.019	0.3170	NS
Aphids*Water availability	1	0.196	0.6601	NS
Dry weight shoots AV2 on 'Microorganisms' factor modality 'B'	**DF**	**F-Value**	**P-Value**	**Significance**
Aphids	1	13.594	0.0005	***
Water availability	1	7.800	0.0071	**
Aphids*Water availability	1	7.098	0.0101	*
Dry weight shoots AV1 on 'Microorganisms' factor modality 'B'	**DF**	**F-Value**	**P-Value**	**Significance**
Aphids	1	11.12	0.0015	**
Dry weight shoots AV1 on 'Microorganisms' factor modality 'B'	**DF**	**F-Value**	**P-Value**	**Significance**
Water availability	1	5.899	0.0183	*
Dry weight shoots AV2 on 'Microorganisms' factor modality 'T'	**DF**	**F-Value**	**P-Value**	**Significance**
Aphids	1	0.802	0.374	NS
Water availability	1	1.136	0.291	NS
Aphids*Water availability	1	0.155	0.696	NS
Dry weight shoots AV2 on 'Microorganisms' factor modality 'BT'	**DF**	**F-Value**	**P-Value**	**Significance**
Aphids	1	2.594	0.1129	NS
Water availability	1	2.482	0.1208	NS
Aphids*Water availability	1	3.923	0.0526	NS

Table 13 - Variance analysis of the dry weight of the roots.

Dry weight roots	**DF**	**F-Value**	**P-Value**	**Significance**

Microorganisms	3	0.102	0.9587	NS
Water availability	1	0.452	0.5021	NS
Aphids	1	3.681	0.0563	NS
Microorganisms*Water availability	3	0.470	0.7036	NS
Microorganisms*Aphids	3	1.569	0.1978	NS
Water availability*Aphids	1	0.880	0.3491	NS
Microorganisms*Water availability*Aphids	3	2.903	0.0357	*

Table 14 - Variance analysis of the dry weight of the roots based on the 'Aphids' factor.

Dry weight roots AV2 on 'Aphids' factor modality 'A+'	**DF**	**F-Value**	**P-Value**	**Significance**
Microorganisms	3	0.969	0.4100	NS
Water availability	1	1.409	0.2380	NS
Microorganisms*Water availability	3	0.811	0.4900	NS
Dry weight roots AV2 on 'Aphids' factor modality 'A-'	**DF**	**F-Value**	**P-Value**	**Significance**
Microorganisms	3	0.704	0.5514	NS
Water availability	1	0.032	0.8578	NS
Microorganisms*Water availability	3	2.451	0.0672	NS

Table 15 - Variance analysis of the dry weight of the roots based on the 'Water availability' factor.

Dry weight roots AV2 on 'Water availability' factor modality 'W+'	**DF**	**F-Value**	**P-Value**	**Significance**
Aphids	1	4.198	0.0428	*
Microorganisms	3	0.436	0.7276	NS
Aphids*Microorganisms	3	0.124	0.9455	NS
Dry weight shoots AV2 on 'Water availability' factor modality 'W-'	**DF**	**F-Value**	**P-Value**	**Significance**
Aphids	1	0.466	0.4964	NS
Microorganisms	3	0.141	0.9350	NS
Aphids*Microorganisms	3	4.235	0.0071	**
Dry weight shoots AV1 on 'Water availability' factor modality 'W-'	**DF**	**F-Value**	**P-Value**	**Significance**
Microorganisms	3	0.134	0.9390	NS
Dry weight shoots AV1 on 'Water availability factor modality 'W-'	**DF**	**F-Value**	**P-Value**	**Significance**

Aphids	1	0.438	0.5090	NS

Table 16 - Variance analysis of the dry weight of the roots based on the 'Microorganisms' factor.

Dry weight roots AV2 on 'Microorganisms' factor modality 'C'	**DF**	**F-Value**	**P-Value**	**Significance**
Aphids	1	3.173	0.0803	NS
Water availability	1	2.106	0.1523	NS
Aphids*Water availability	1	0.692	0.4089	NS
Dry weight roots AV2 on 'Microorganisms' factor modality 'B'	**DF**	**F-Value**	**P-Value**	**Significance**
Aphids	1	0.534	0.468	NS
Water availability	1	0.015	0.903	NS
Aphids*Water availability	1	0.598	0.443	NS
Dry weight roots AV2 on 'Microorganisms' factor modality 'T'	**DF**	**F-Value**	**P-Value**	**Significance**
Aphids	1	0.554	0.4597	NS
Water availability	1	0.065	0.7996	NS
Aphids*Water availability	1	8.344	0.0055	**
Dry weight roots AV1 on 'Microorganisms' factor modality 'T'	**DF**	**F-Value**	**P-Value**	**Significance**
Aphids	1	0.498	0.4830	NS
Dry weight roots AV1 on 'Microorganisms' factor modality 'T'	**DF**	**F-Value**	**P-Value**	**Significance**
Water availability	1	0.052	0.820	NS
Dry weight roots AV2 on 'Microorganisms' factor modality 'BT'	**DF**	**F-Value**	**P-Value**	**Significance**
Aphids	1	3.976	0.0511	NS
Water availability	1	0.163	0.6882	NS
Aphids*Water availability	1	0.733	0.3958	NS

Table 17 - General Linear Model results of the root/shoot dry weight ratio.

Root/shoot dry weight ratio	**DF**	**LR Chisq**	**P-Value**	**Significance**
Microorganisms	3	280.49	<2.2e-16	***
Water availability	1	826.53	<2.2e-16	***
Aphids	1	40.46	2.008e-10	***

Microorganisms*Water availability	3	49.67	9.394e-11	***
Microorganisms*Aphids	3	374.38	<2.2e-16	***
Water availability*Aphids	1	12.23	0.0004702	***
Microorganisms*Water availability*Aphids	3	585.18	<2.2e-16	***

Total biomass	**DF**	**F-Value**	**P-Value**	**Significance**
Microorganisms	3	0.860	0.4624	NS
Water availability	1	7.068	0.0084	**
Aphids	1	14.134	0.0002	***
Microorganisms*Water availability	3	0.620	0.6028	NS
Microorganisms*Aphids	3	1.254	0.2911	NS
Water availability*Aphids	1	0.237	0.6270	NS
Microorganisms*Water availability*Aphids	3	3.331	0.0204	*

Total biomass AV2 on 'Aphids' factor modality 'A+'	**DF**	**F-Value**	**P-Value**	**Significance**
Microorganisms	3	0.840	0.4750	NS
Water availability	1	2.584	0.1110	NS
Microorganisms*Water availability	3	1.191	0.3170	NS
Total biomass AV2 on 'Aphids' factor modality 'A-'	**DF**	**F-Value**	**P-Value**	**Significance**
Microorganisms	3	1.239	0.2989	NS
Water availability	1	4.549	0.0351	*
Microorganisms*Water availability	3	2.634	0.0533	NS

Total biomass AV2 on 'Water availability' factor modality 'W-'	**DF**	**F-Value**	**P-Value**	**Significance**
Aphids	1	6.041	0.0155	*
Microorganisms	3	0.184	0.9072	NS
Aphids*Microorganisms	3	1.782	0.1547	NS
Total biomass AV2 on 'Water availability' factor modality 'W+'	**DF**	**F-Value**	**P-Value**	**Significance**
Aphids	1	8.097	0.0053	**
Microorganisms	3	1.183	0.3195	NS
Aphids*Microorganisms	3	2.699	0.0492	*

Total biomass AV1 on 'Water availability' factor modality 'W+'	**DF**	**F-Value**	**P-Value**	**Significance**
Microorganisms	3	1.071	0.3640	NS
Total biomass AV1 on 'Water availability factor modality 'W+'	**DF**	**F-Value**	**P-Value**	**Significance**
Aphids	1	7.727	0.0063	**

Total biomass AV2 on 'Microorganisms' factor modality 'C'	**DF**	**F-Value**	**P-Value**	**Significance**
Aphids	1	3.565	0.0642	NS
Water availability	1	0.322	0.5729	NS
Aphids*Water availability	1	0.303	0.5844	NS
Total biomass AV2 on 'Microorganisms' factor modality 'B'	**DF**	**F-Value**	**P-Value**	**Significance**
Aphids	1	11.286	0.0014	**
Water availability	1	6.010	0.0174	*
Aphids*Water availability	1	5.699	0.0204	*
Total biomass AV1 on 'Microorganisms' factor modality 'B'	**DF**	**F-Value**	**P-Value**	**Significance**
Aphids	1	9.668	0.0029	**
Total biomass AV1 on 'Microorganisms' factor modality 'B'	**DF**	**F-Value**	**P-Value**	**Significance**
Water availability	1	4.776	0.0329	*
Total biomass AV2 on 'Microorganisms' factor modality 'T'	**DF**	**F-Value**	**P-Value**	**Significance**
Aphids	1	0.319	0.574	NS
Water availability	1	1.080	0.303	NS
Aphids*Water availability	1	1.029	0.315	NS
Total biomass AV2 on 'Microorganisms' factor modality 'BT'	**DF**	**F-Value**	**P-Value**	**Significance**
Aphids	1	3.018	0.0879	NS
Water availability	1	1.589	0.2127	NS
Aphids*Water availability	1	3.109	0.0833	NS

Table 18 - General Linear Model results of the maximal photochemical yield (Fv/Fm).

Fv/Fm	DF	LR Chisq	P-Value	Significance
Microorganisms	3	83.84	<2.2e-16	***
Water availability	1	637.56	<2.2e-16	***
Aphids	1	1227.52	<2.2e-16	***
Microorganisms*Water availability	3	126.68	<2.2e-16	***
Microorganisms*Aphids	3	7.84	0.0494	*
Water availability*Aphids	1	313.95	<2.2e-16	***
Microorganisms*Water availability*Aphids	3	34.70	1.407e-07	***

Table 19 - Variance analysis of the Performance Index.

Performance Index	DF	F-Value	P-Value	Significance
Microorganisms	3	1.189	0.3147	NS
Water availability	1	5.009	0.0262	*
Aphids	1	99.968	<2e-16	***
Microorganisms*Water availability	3	0.059	0.9813	NS
Microorganisms*Aphids	3	0.545	0.6521	NS
Water availability*Aphids	1	1.821	0.1786	NS
Microorganisms*Water availability*Aphids	3	2.094	0.1019	NS