Internship thesis

European Master Erasmus + Plant Health

On the research topic of

Effect of plant defense stimulators on the evolution of *Venturia inaequalis* populations - consequences for sustainability

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List of Abbreviations

ASM : Acibenzolar -S-methyl

AUDPC : Area Under Disease Progress Curve

CTIFL: Interprofessional Technical Center for Fruit and Vegetable

Dpi: Day post inoculation

EcoFun: Evolutionary Ecology of Fungal Pathosystems

GIS Fruit: Scientific Interest Group on Fruits

IFPC: French Institute of Cider Production

INA: 2,6 -dichloro-isonicotinic acid

INRA : National Institute of Agronomic Research

IPM: Integrated Pest management

IRHS : Research Institute for Horticulture and Seeds

Light IPM: IPM with use of fungicides only in case of high risks of scab infection

PDS : Plant defense stimulators

ResPom: Resistance in Apple and Pear

SAR: Systemic acquired resistance

SMaCH : Sustainable Management of Crop Health

1. Introduction-Scientific and socio-economic context

Apple trees encounter several microbial pathogens, herbivores, parasitic plants in their life span. Among all, apple scab, caused by the fungus *Venturia inaequalis* is the foremost critical disease in terms of financial cost for apple growers all over the world (Bowen et al., 2011). Because any scab contamination diminishes the quality and makes the fruits of apple commercially less marketable, apple becomes one of the highest pesticide consuming fruit crops including at least 30 treatments per year in France in conventional and biological apple production systems (Didelot et al., 2016). Besides a high risk for nature, the presence of residues in the food and consumers concern, frequent fungicides application significantly increases the risk of pathogen resistance (Ortega et al., 1998). Using apple cultivars that are resistant to scab is a useful instrument for implementing in integrated crop management. However, host resistance is often pathogen-specific and ephemeral; many of the cultivars failed to keep up the resistance over a long period because of pathogens capability to adapt. It also takes a lot of research and time to introduce resistance gene into such cultivars (Ortega et al., 1998). Among the approaches taken for pesticide reduction, the use of plant defense stimulators (PDS), also called elicitors appears as a prospective choice to address the phytosanitary problems of standard agricultural methods (Walters et al., 2013). A range of chemical or biological stimulators are capable of activating plant defenses by exogenous application to a wide array of pathogens. This induction of resistance in host plants leads to ample disease delay (Marolleau et al., 2017). However, we don't know whether the repeated application of plant defense stimulators in orchard exert selection pressure on fungus population through the defense metabolites produced by the plant and could thus lead to losses of efficacy during time as so often described with pesticides or resistant varieties. The success of induced resistance to control apple scab has been achieved so far in controlled conditions with one strain of *V. inaequalis* (Marolleau et al., 2017). But, in practical field conditions plants are encountered with different strains of a single pathogen (Ortega et al., 1998), and we don’t know if the efficacy of plant defense simulators varies strain by strain.

To address this question of sustainability of PDS for the control of apple scab, a project called TavInnov has been developed which is funded by the Metaprogram SMACH (Sustainable management of Crop Health, 2018-2021) of INRA. This project involves two teams of IRHS

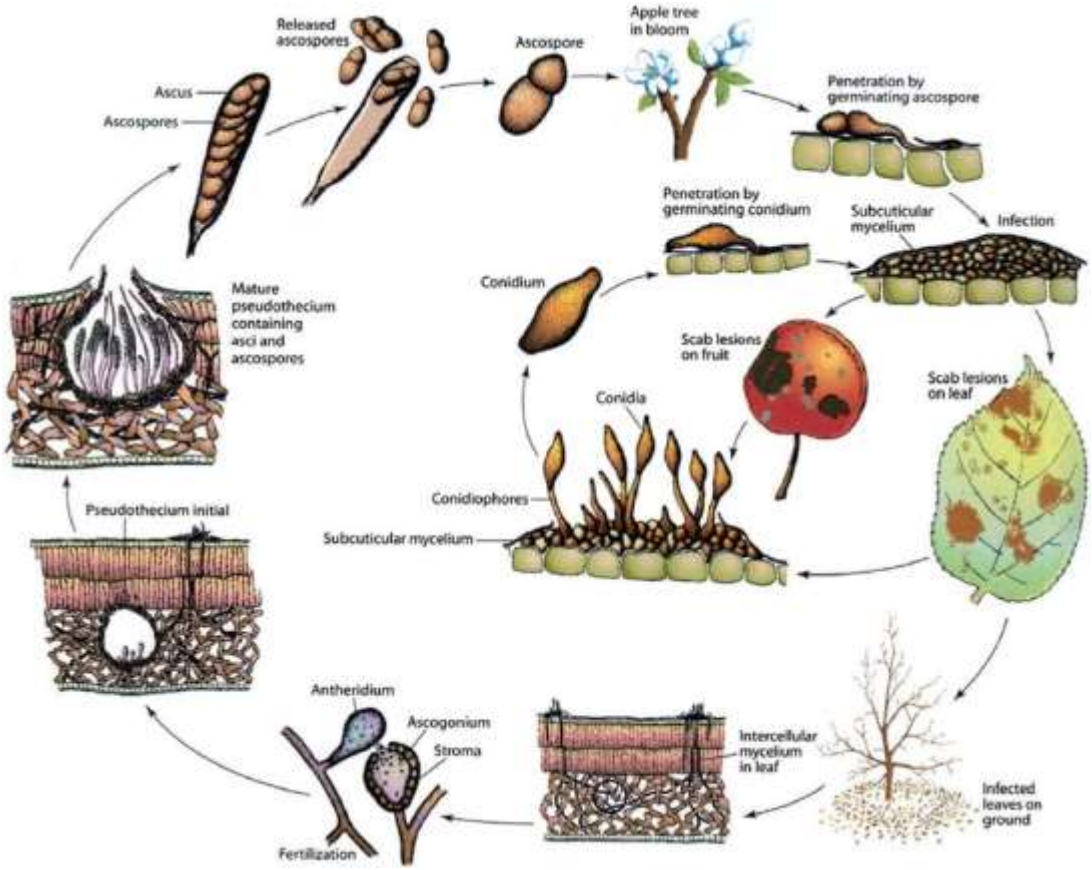


Figure 1: The life cycle of *Venturia inaequalis* (Agrios, 2005)

(EcoFun, Evolutionary Ecology of Fungal Pathosystems and ResPom, Resistance in Apple and Pear) and the Horticultural Experimental Unit (UEH) from Angers (Annex I)

The aim of the TavInnov project is to combine different methods of scab management into Integrated Fruit Production (IFP) system, which will finally help the orchard farmers to have better production. This project has two parts of work going on:

1- Analyze the combined effects of varietal resistance, nitrogen stress, mechanical stress and PDS on apple scab.

2- Analyze the defense mechanisms of the apple tree involved and analyze their impact on pathogenic populations.

My internship was funded by the Gis-Fruit and was done in the EcoFun team of IRHS. My research is included in the second part of the TavInnov project. The aim is to evaluate the efficacy of PDS towards a large set of strains of *V. inaequalis* and to define if the use of PDS in orchard is able to select for strains that are less controlled by PDS. IFPC (French Institute of Cider Production) and CTIFL (Interprofessional Technical Center for Fruit and Vegetable) are involved in this project for the sampling of *V. inaequalis.*

2. Literature review

2.1. Apple (*Malus x domestica* Borkh.)

The cultivated apple (*Malus x domestica*) belongs to the division: Angiosperms, class: Dicotyledons and family: Rosaceae and sub-family: Spiraeoideae (Potter et al., 2007) which is a major fruit species commonly grown in the temperate parts of the globe (Harris et al. 2002). It is the 2nd largest producing (around 83 million metric tons) fruit after banana (93 Million metric tons). In France, about 17 million tons of apple are produced every year ("FAOSTAT", 2019). As apple is an industrial fruit in France, any damage caused by pathogens may incur significant financial losses to the growers. More than 70 infectious diseases have been reported in apple. Among many other diseases apple scab [*Venturia inaequalis* (Cooke) Wint.] is considered as the main disease of apple in Europe.

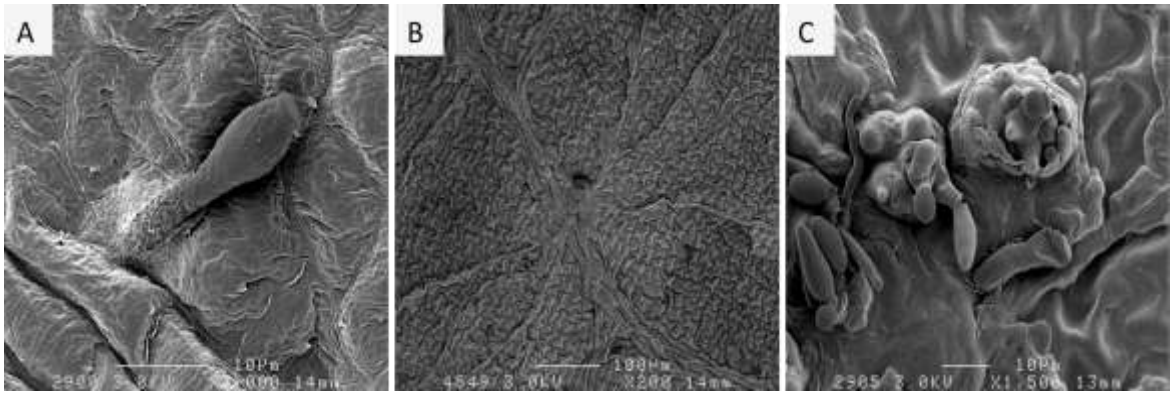


Figure 2: Process of infection by *Venturia inaequalis* on apple leaf surface seen by scanning electron microscopy. (A) a germinated *V. inaequalis* spore forms an appressorium and adheres to the surface of the leaf, (scale bar = 10 µm). (B) The fungus gradually invades the leaf forming a subcuticular mycelium, (scale bar = 100 µm). (C) The conidiophores bud on the leaf surface and sporulation starts, (scale bar = 10 µm). (Le Cam, 2011)

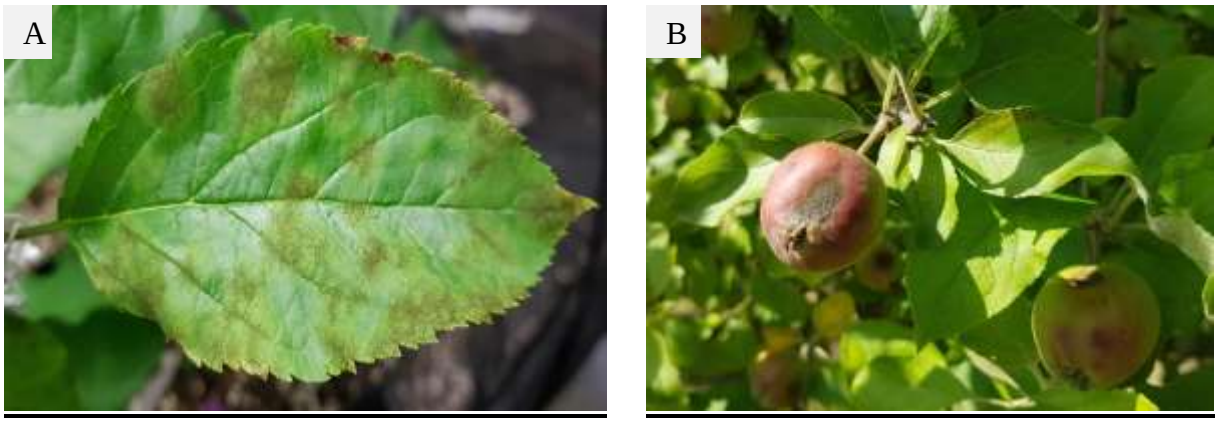


Figure 3: Scab symptom on (A) leaf and (B) fruits (personal photo taken from experiment and INRA orchard)

2.2. Scab (*Venturia inaequalis*)

2.2.1. Taxonomy and life cycle of *V. inaequalis*

Venturia inaequalis is a fungus under Phylum Ascomycota (thallus partitioned filamentous); Subphylum Euascomycota (sexual reproduction via Asci containing ascospores) class Dothideomycetes, order Pleosporales, family Venturiaceae (Bowen et al., 2011). It infects several species of Rosaceae, particularly domestic apple of which it is the most studied pathogen. The life cycle of *V. inaequalis* has two phases (Figure 1): a parasitic stage in the spring and summer on the leaves and fruits, and a saprophytic phase in dead fallen leaves during autumn and winter. During the saprophytic phase sexual reproduction takes place inside pseudothecia. The success of sexual reproduction depends on the presence of the two opposite mating types on the same leave (Bowen et al., 2011).

2.2.2. Process of infection

In spring from March to June, ascospores are ejected from the pseudothecia during rain. In the presence of free water on leaf surface the ascospores germinate and form an appressorium (Figure 2, A). With the pressure of the appressorium the fungus breaks the cuticle layer and penetrates into the leaf. Then the primary hyphae develop between the cuticle and the epidermal cells followed by development of sub-cuticular primary stroma. From this stroma, hyphae progress under the cuticle. These stromal cords then differentiate secondary stromal beds followed by protruding new hyphae and so on (Figure 2, B). The sub-cuticular colonization by the fungus is the disease lag phase. Massive stroma or mycelial strands differentiated conidiophores that pierce the cuticle and give rise to conidia (Figure 2, C). The conidium leaves a scar flange or bead at the tip of conidiophore. This phase corresponds to the primary infection with the onset of symptom after 10-20 days of germination of ascospores depending on the temperature (Chevalier et al., 1991).

Conidia disperse from the lesions though wind and rain and are responsible for secondary infections in orchards throughout the development period of the leaves and fruits. These asexual spores are responsible for an increase of the disease during the apple growing season, when there is a moist condition for a sufficient time depending on temperature (Mills, 1951).

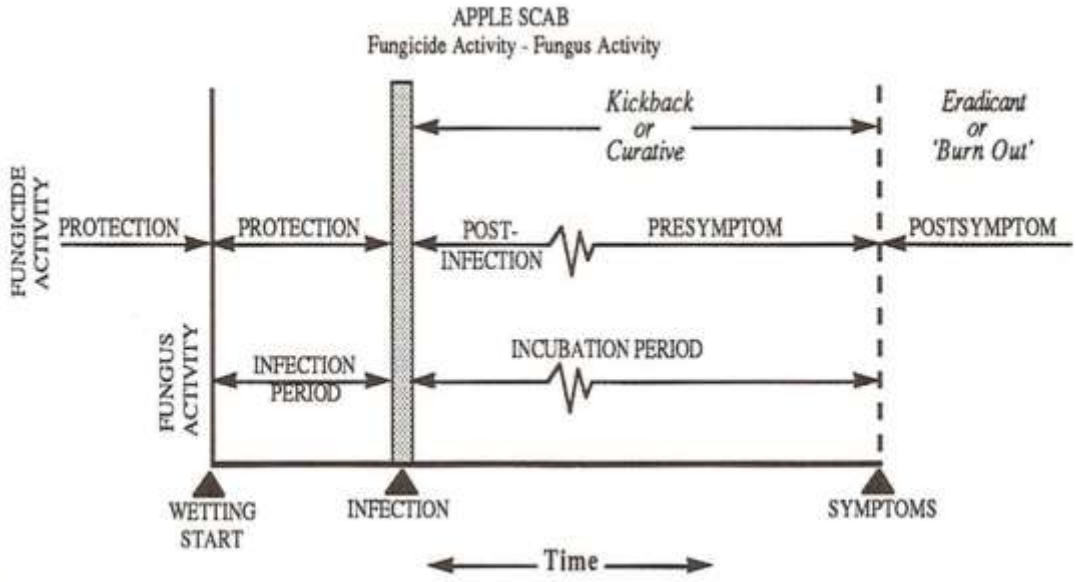


Figure 4. The three-effective times of fungicide applications to control *V. inaequalis* are infection, incubation, and post-symptom periods (Szkolnik, 1978)

2.2.3. Symptomatology

The symptoms of apple scab appear on petioles, sepals, flowers, buds and fruits. The lesions develop faster on young leaves than older leaves because of the existence of an ontogenetic resistance on older leaves. Afterwards, the lesions enlarge and become dark brown and take a velvety appearance with plenty of conidiogenous cells (Figure 3, A). Eventually, the lesions may be dispersed over the entire leaves resulting in defoliation. Likewise, the lesion on fruit (Figure 3, B) appear at the juvenile stage and spread faster in younger fruit than ripe fruit. In case of severe attack deformation takes place and dropping of fruit can occur. At later stage, the infection remains latent and symptoms appear during storage of fruits (MacHardy, 1996).

2.3. Control methods

2.3.1. Fungicide treatments

Fungicides are used to reduce the primary infections of *V. inaequalis*. The 3 effective times of fungicide application are protection, incubation and post-symptom (Figure 4; Szkolnik, 1978). Some active ingredients, for instance mancozeb, act before infection; other, for instance trifloxystobin and muclobutanil act until 3 days after infection.

Fungicide resistance has already been acquired to the main chemical groups Sterol Biosynthesis Inhibitors, Anilinopyrimidine fungicides (Köller et al., 2005) and Strobilurin fungicides (Remuson et al., 2007). These resistances appear as a result of the selection pressure exerted on the pathogenic agents by the repeated application of the same fungicides. To limit this pressure, it is imperative to limit the number of chemical applications, alternating chemical groups and also diversifying the methods of control.

2.3.2. Prophylactic measures

Scab overwinters on fallen dead leaves and this is the main source of primary inoculum for contamination. So, reduction of the scabbed leaves masses and prevention of the pathogen development in the litter are two main ways to reduce primary inoculum. Several studies have shown effective sanitary practices against apple scab in orchard. A study over 5 years' time showed that leaf litter removal in autumn together with minimal use of fungicides

specially where the risk of disease was high can reduce the scab infection in a sustainable way. In this study fungicide use was reduced to 60% (Didelot et al., 2016).

2.3.3. Biological Control

Biological control with the use of antagonist fungi could also reduce scab infection. Autumn application of *Microsphaeropsis ochracea and Athelia bombacina* reduced spring ascospore production to 85% and 81 % respectively (Vincent et al., 2004). But to be efficient, repeated applications are necessary since those fungus do not survive for long time in the leaves; thus, cost is high and biological control is not used by apple growers. In controlled condition, on apple plantlets Fiss et al. (2003) successfully used *Auerobasidium botrytis, Cladosporium spp* and several epiphytic yeast strains to inhibit *V. inaequalis* germination and mycelial growth (up to 80%).

2.4. Plant resistance

Two main types of resistance have been defined.

2.4.1. Qualitative resistance or total resistance (Gene for gene theory):

The qualitative resistance results from the interaction between a major resistance gene (R) in the host and an avirulence gene (Avr) in the pathogen, this relationship is thus called "gene for gene". Knowing that virulence is the ability of a pathogen to infect an organism and avirulence its inability to infect the host. When plants' R gene alleles recognize the pathogen Avr gene alleles, a series of signaling cascade takes place in the plant to achieve complete resistance against the pathogen and stop the process of infection. If the host plant does not have the R gene or if the avirulence allele is no more functional in pathogens, then recognition of the pathogen by the plant is no more effective and the plant becomes sensitive. Till date, 17 genes of major resistance to apple scab have been identified (Bus et al., 2011).

2.4.2. Quantitative resistance or Partial resistance:

Quantitative plant resistance impacts the aggressiveness of pathogens, the aggressiveness of the strain corresponding to the quantitative component of the pathogenicity of the fungus. It reduces the progress of the pathogen; however, it cannot prevent disease completely. This type of resistance acts on the defenses of the plant with shielding alerts or the production of chemical substances. Quantitative resistance is controlled by several genes, called QTL (Quantitative Trait Loci) or QRL (Quantitative Resistance Loci) (Gessler et al., 2006). This

type of resistance is dependent on environmental factors and strains aggressiveness unlike qualitative resistance (William and Kuć,1969).

2.4.3 Resistance durability:

The typical life span of an apple plant in orchard is around 12-20 years. Breeding for resistance takes at least 25 years to obtain a resistant cultivar, while overcoming of resistance by pathogen may take only a few years in some cases (Parisi et al., 2004). The major gene of resistance *Vf (=Rvi6)* introduced from *Malus floribunda* has been widely used in plant breeding (Laurens, 1996), but it resulted in the emergence of virulent strains (Parisi et al., 2004) that exist now in most European countries. The other major genes of resistance have not yet been released in commercial cultivars. However, a monitoring of virulences done in orchards planted in different countries worldwide (Vinquest, 2019) show that only two of these major resistance genes are not yet overcome. Actually, it is well known that a mutation event (SNP, deletion, transposon insertion etc.) in Avr gene may be sufficient to impede plant to recognize the pathogen that lead to disease. Once overcome, symptoms intensity will depend on the aggressiveness of the overcoming strains and on the putative presence of quantitative resistance in the plant.

Due to a less selective pressure exerted on pathogens, quantitative resistances are regarded to be more enduring than qualitative resistance (Parlevliet, 2002). However, a complete erosion of QTL has been observed in a serial passage experiment to the virus PVY in pepper (Montarry et al., 2012) and to powdery mildew in barley (Villaréal and Lannou, 2000). Mundt et al., (2002) found that a resistant cultivar of wheat showed complete erosion against septoria blotch in wheat while experimenting over 10 years period of time in comparison with a complete susceptible variety. Caffier et al., (2014, 2016) also showed that QTL of resistance to apple scab can lose their effectiveness in orchards over time.

2.5. Plant defense stimulators

2.5.1 Biotic and abiotic inducers

Induced systemic resistance is the process where resistance in plants against diseases is systematically induced by local infection or therapy with microbial elements or by a broad group of inorganic or organic compounds (Kuć, 2001). Both biotic and abiotic agents can work as plant defense stimulators and can induce resistance in plants. Pre-treatment on

susceptible plants with an avirulent pathogen (biotic inducer) or abiotic agents can increase resistance to subsequent attack, not only at the site of treatment but also in tissue distant from the area of the attack. This phenomenon is called systemic acquired resistance (SAR). For example, among

Table 1: List of studies done to control apple scab by using different defense stimulator agents

Nature of agents	Name of the product	Conditions of experiment	Results	References
Chemical compounds	3,5-dichlorosalicylic acid (INA)	Fluorescence microscopy during infection	Number and length of runner hyphae were reduced	Ortéga et al., 1998
		Evaluation of scab symptoms in controlled conditions	56% of disease reduction on leaves of 'Golden delicious" cultivar	
	Acibenzolar-S-methyl (ASM)	Histopathology	Stages of the infection process (pre-penetration, penetration and post-penetration events) were reduced significantly	Bengtsson et al., 2009
	Acibenzolar-S-methyl (ASM)	Evaluation of scab symptoms in controlled conditions	50% of disease reduction on 'Golden delicious' cultivar with strain 104 of *V. inaequalis*	Marolleau et al., 2017
		Evaluation of scab symptoms in orchard	35-40% of disease reduction on 'Golden delicious'	
Chemical And Natural compounds	Rigel (a.i. Salicylic acid derivative), Phoenix (a.i. Potassium phosphite and Messenger (a.i. Harpin protein from *Erwinia amylovora* bacteria), and)	Evaluation of scab symptoms in orchard	little difference in the magnitude of scab protection conferred by each SDP agent compared to the fungicide penconazole.	Percival et al., 2009
Natural compounds	*Laminaria digitata*	Evaluation of scab symptoms in controlled conditions	77% of disease reduction on 'Golden delicious"	Creemers, 2001
		Evaluation of scab symptoms in orchard	50% of disease reduction	
	Extract of *Yucca schidigera*	Microscopy on seedlings in controlled conditions	Germination of spores are reduced	Bengtsson et al., 2009

	Chitinase of *Trichoderma atroviride*	Molecular study	Significant resistance in chitinase transgenic lines of Ariane and Galaxy cultivars	Faize et al., 2003

abiotic agents, most studied elicitors are acibenzolar-S-methyl (ASM) and 2,6 -dichloro-isonicotinic acid (INA), both are functional analogue of Salicylic acid and can instigate SAR. Both induce systemic resistance and resistance is linked to the fast accumulation of the same structurally unrelated putative defense compounds such as phytoalexin (Lateur, 2002). Several studies have shown that induced resistance is efficient against several plant diseases for instances, late leaf spot of peanut and bean rust with INA, white rust of spinach, downy mildew of maize and powdery mildew of wheat with ASM (Vallad and Goodman, 2004). In the case of apple plant, use of defense inducer is relatively new. Very few studies have been done so far, and they are listed in Table 1. Nonetheless, PDS holds significant potential in the near future for a better, environmentally friendly means of combating pome fruit diseases.

2.5.2. Factors effecting plant defense stimulators performance in the field:

PDS don't have direct microbial effect but the effect of PDS in the field can be affected by several factors including host genotype, crop species, abiotic environment, frequency of application, prior induction, Crop stages, nutrition (Walters et al., 2013). Among them plant genotype has found to be the most influencing factors. For instance, a study with BABA (β-aminobutyric acid) -induced resistance against *Phytophthora infestans*, Sharma et al., (2010) showed that expression of resistance in tomato genotypes was considerably diversified. Also, the effect significantly differed among the pathogen isolates although only two isolates were compared. Herman et al., (2007) has observed that while ASM caused defense gene expression in tomatoes after first application; then after second application a considerably higher amount of gene expression has been noted. So, frequency is crucial for efficiency of PDS. Effect of PDS differs among crop species too. For example, ASM has been found to be successful in reducing diseases in arabidopsis, cucumber, cabbage, tomato and many other crops (Vallad and Goodman, 2004). On the other hand, ASM failed to provide significant control in a field trial study against *barley yellow dwarf virus* (Huth and Balke, 2002) and leaf pathogen Xan*thomonas axonopodis* on sweet orange (Graham and Leite, 2004).

Unfortunately, in peanut ASM was even found to spread infection in case of the late leaf spot pathogen, *Cercosporidium personatum* (Zhang et al., 2001). So, use and efficacy of elicitors can't be generalized for all crops.

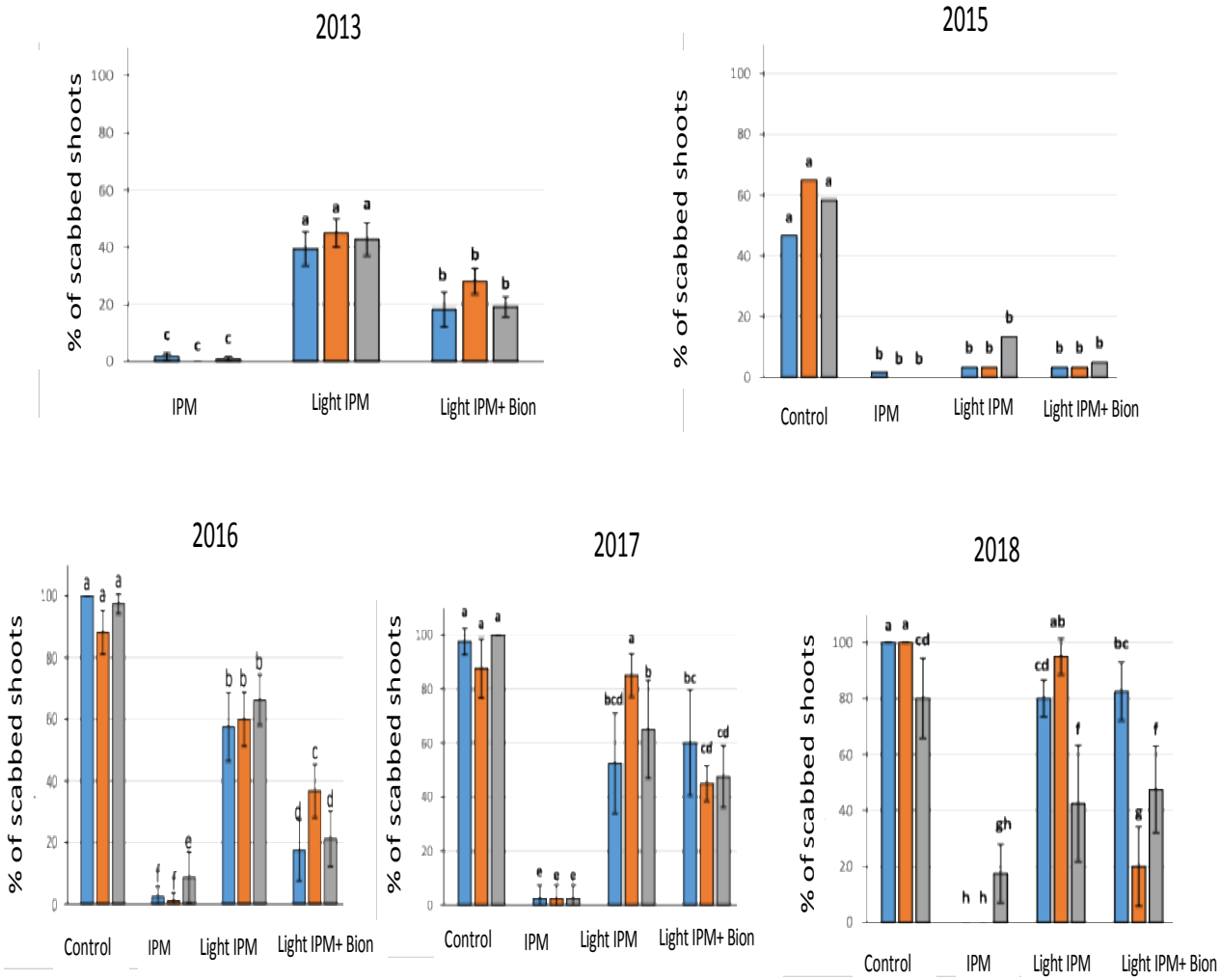


Figure 5 : Several years field study on efficiency of ASM treatments. Comparison of 4 modalities Control (= No treatment), IPM, Light IPM, Light IPM+ Bion treatment, with three blocks in a single orchard (blue, orange, grey) (Gaucher et al., unpublished, personal communication)

2.5.3. Mode of action and use of ASM in the control of fungal diseases in plants

Among abiotic defense stimulator agents, ASM is the best studied for a broad range of plant diseases. ASM acts downstream of SA (salicylic acid), which is the best-known endogenous signal of SAR. SAR genes codes for the proteins that are called as pathogenesis-related (PR) proteins. ASM can induce cell wall degrading enzymes such as b-1,3 glucanases and chitinases (Suo and Leung, 2001) and also catalase (CAT), antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), correlated with reduced leaf spot severity (Cavalcanti et al., 2007). Commercially this agent is marketed by Syngenta and named as Bion. Regarding this agent, most studies have been done in non-timber dicotyls including tomato, arabidopsis, melon, cucumber, potato and monocotyls such as corn, wheat, rice and barley (Lateur, 2002). It is only very recently that researchers have taken it into interest in woody fruit plants like apple, kiwi, nashi etc. The research done for controlling apple scab is listed in Table 1. In most cases, disease control was not complete (45%-66%). Recently Marolleau et al., (2017) conducted an experiment under controlled conditions on apple seedlings with a strain of *V. iinaequalis* (strain 104). This study showed 50% reduction of apple scab symptom. On the other hand, in a one-year experiment Marolleau et al., (2017) compared 3 different pest management strategies (standard IPM Integrated Pest management, light IPM, ASM+light IPM) under natural contamination during primary infection period. In standard IPM where all risks of scab infection were controlled by fungicides, disease severity on leaves was 11%. In light IPM orchard where only severe risks of scab infection were controlled by fungicides disease severity was 43%. By integrating ASM into light IPM orchard, disease severity was 25%. In case of fruits, ASM+light IPM disease incidence was 34% whereas in light IPM orchard disease incidence was 69%.

Then this study continued for next 5 years and showed that application of ASM increased significantly the control of apple scab in a light IPM system. However, the control of apple scab was fluctuating from year to year. In 2013, ASM was significantly effective on the three plots of the experiment, whereas in 2018, ASM was significantly effective on only one of the three plots (Gaucher et al., personal communication, Figure 5). Fluctuation in the climatic

conditions (temperature, humidity) and physiological stages of the trees at times of ASM application in the orchard may be responsible for the variation in the effectiveness of SDP over years. On the other hand, the sustainability of this control method is not known yet. There is no information about the risk that plant defense stimulator like ASM might select strains insensitive to defense

mechanisms activated by its application, which could result in a decrease of its efficiency. It should be known for the use of ASM in the field successfully.

3. Objectives :

Until now, the efficacy tests of plant defense stimulator on apple scab have been carried out under controlled conditions only with the strain of *V. inaequalis* called 104 by using Acibenzolar-S-methyl, commercially known as Bion (Marolleau et al., 2017). In the first year of TavInnov project, Sarah Fauvre (2018) tested the efficiency of ASM on 14 strains coming from different orchards that were not treated against scab. Her results showed a good reproducibility of the experiment and the existence of one strain that had a low sensitivity to ASM. To continue the TavInnov project, my research questions are:

* Is ASM effective against a range of diverse strains of *V. inaequalis?* For this purpose, the efficiency of ASM has been tested in controlled condition on 20 additional strains that were sampled in untreated orchards.

* Does repeated applications of ASM in orchard select for less sensitive strains, which could result in a decrease in the efficiency of ASM to control apple scab? For this purpose, the efficiency of ASM has been tested in controlled condition on 30 strains collected from an orchard with a light IPM and 30 strains collected from an orchard with a light IPM and ASM treatments. A comparison of the efficiency of ASM has been performed according to the origin of the strains.

These questions arise from the perspective of sustainability of plant defense stimulators so that they can be subsequently used in orchards. The results obtained from my internship experiments will help to define next step for the TavInnov project.

4. Materials and methods

4.1 Selection of Plant material

The apple cultivar "Golden delicious" was selected for the experiment, because of its high susceptibility to apple scab. The gene of resistance Rvi1(Vg) is present in this cultivar (Bus et al., 2011), but the majority of *V. inaequalis* strains present in Europe are virulent towards this resistance gene. This cultivar was used in a previous study where ASM was shown to control apple scab in controlled conditions and in orchard (Marolleau et al., 2017).

4.2 Selection of defense stimulator

Different chemical resistance inducers are known to be effective against apple scab. Among them, Acibenzolar-S-methyl effectiveness is well known. Its trade name is Bion 50 WG (Syngenta) in Europe (Annex II). It contains water dispersible granule form containing 50% active ingredient. Unlike other simulators, the effectiveness of ASM has been proven both in controlled and orchard conditions (Marolleau et al., 2017).

4.3 Selection of the strains

For this experiment, 94 different strains of *V. inaequalis* were selected (Annex III).

-Among the strains,34 strains were collected from different orchards of Europe that had never been treated by Bion.

-14 strains were collected between 1978 and 2009 from different orchards in Europe. These strains were tested in 2018 by a graduate student named Sarah Fauvre but are included in the analysis of the present manuscript.

- And 20 strains were collected between 2006 and 2009 from 3 different orchards of France (Angers 49, Lanxade 24, Villeneuve d'ascq 59).

- Other 60 strains were collected in 2018 from a single orchard of the Experimental Horticultural Unit in Angers (France). This orchard was conducted with light IPM with use of fungicide treatments in case of severe risks of scab infection. 30 of these strains were collected from plots (Light IPM+ASM) that were treated by ASM 6 to 11 times per year since 2013 (one treatment per week during the primary scab infection period) and rest of 30 strains were collected from plots (Light IPM) that never received ASM treatment.

Due to technical constraints, unavailability of space in climate chamber and inoculum suspension calibration, this experiment was carried away into 5 different series. Using the same protocol each series comprised 20 strains (except the experiment of 2018 with 14 strains).

Figure 6: Youngest actively grown leaf at time of inoculation labelled and named F0.

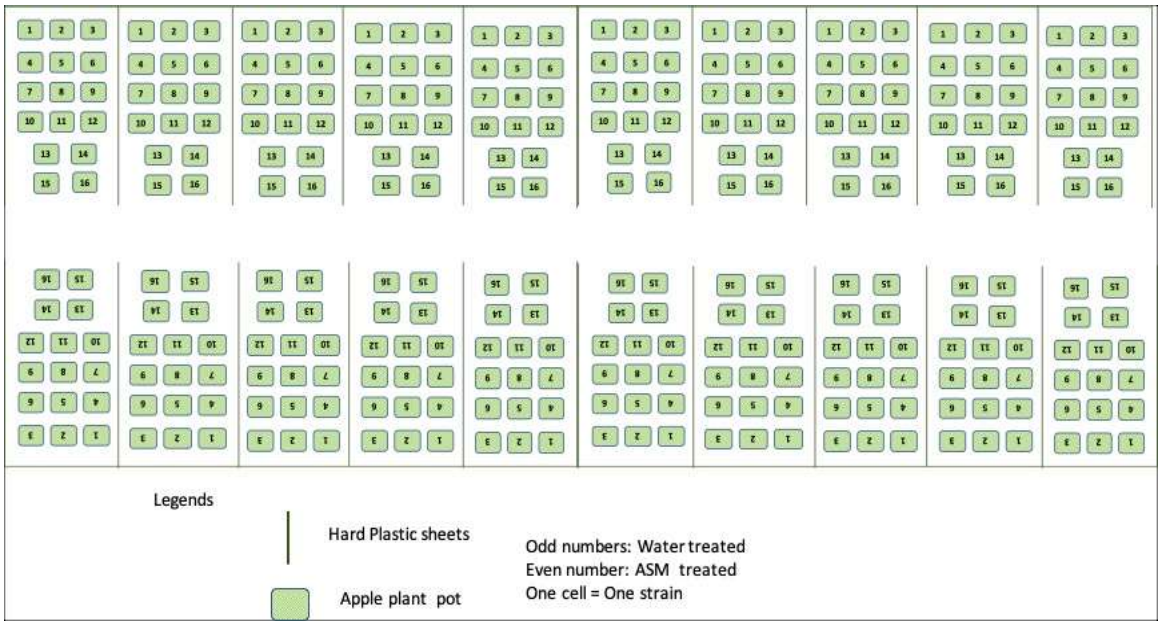


Figure 7: Placement of plants in the growth chamber for artificial inoculation with 20 strains of *Venturia inaequalis.*

4.4. Preparation and placement of the plants

4.4.1. Grafting and growing of the plants

Two batches of 640 apple trees were grafted on the MM106 rootstock in January (week 2) and February (week 7). This rootstock was chosen because it favors the vegetation growth. After grafting, plants were re-potted in pots containing a mixture of compost (60%), peat (30%), and disinfected soil (10%). Plants were used after 7 to 8 weeks of growth in the glasshouse. Each batch of plants was used several times. At the end of an experiment, plants are cut back and can be used again 3 to 4 weeks later.

4.4.2. Defense stimulator treatment

ASM was used for inducing resistance to grafted apple plants. It was dissolved in water (0.4 g/l) and sprayed on to half of plants. Other half of plants were treated with water with same method. ASM treated plants were marked with a red color. Four days after treatment plants were transferred to climate chamber for further experiment. For clarity, these two modalities of treatment will be named as **ASM** plants and ***water*** plants, respectively.

4.4.3. Placement of the plants in the climate chamber

Before transferring the plants into the climate chamber, plants were chosen according to their active growth. The youngest actively grown leaf was labelled with a white sticker label and named as F0 (Figure 6).

In the climate chamber, a total of 320 plants were transferred. Since 20 strains were used for each experiment, 20 compartments were prepared with solid plastic sheets and plants were placed there (Figure 7). In each compartment, there were 16 plants. Among these plants, 8 were ASM treated, and 8 were water treated.

4.5. Preparation of the strains

4.5.1. Isolation and multiplication

Single spore strains were obtained from the scabbed leaves sampled in the orchards. Each strain was multiplied on cellophane sheets overspread onto malt agar (10 g L^{-1}cristomalt,15 g L^{-1}agar) in Petri dishes (Caffier et al., 2010). After 7-10 days of incubation in a climate room at 17^0c with 16 h of light; cellophane sheets were removed from the Petri dishes. Then those cellophane sheets were dried for 7 days and then kept in the freezer at -20°C until use.

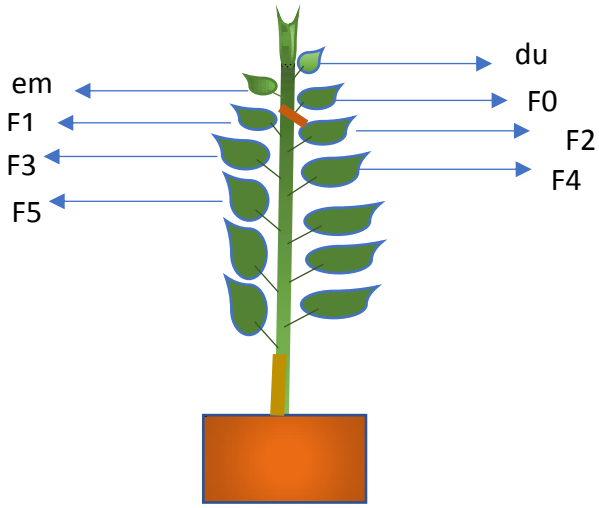


Figure 8: Leaves counted for disease scoring

Figure 9: Disease scoring scale with 10% increments in 100% of sporulating scabbed leaf area (Laloi, 2016)

4.5.2 Preparation of the inoculum suspension

For each strain, cellophane sheets were shaken into 50 ml of water. Then the suspension was filtered using the medical gaze. The concentration of spores in the suspension was measured using a particle counter (Beckman Coulter Counter, model Z2, no of series BA 29466), and adjusted to a concentration of 100,000 spores/ml for the inoculation. Dilution was done where necessary (Annex IV).

4.6. Germination rate count of the inocula

During preparing inoculum suspension, three drops of 20µl of suspension were put in a Petri dish containing malt agar media (10 g L^{-1}cristomalt,15 g L^{-1}agar) for each strain. These Petri dishes were placed in an incubation chamber at 17 ° C with 16h of light to allow germination of the spores. After 1 day of incubation, the germination percentage was counted under the light microscope (manufactured by scop-pro, series no 208524). Thus, it was possible to count the number of germinated spores and the number of non-germinated spores out of a total of one hundred spores for each strain (Annex IV).

4.7. Inoculation

In the climate chamber, each compartment was inoculated with 50 ml of suspension. These 50 ml were sprayed with glass chromatography sprayers using a compressed air pump. During the time of inoculation, to prevent contaminations among strains, separate tube was used for each strain. Also, disinfection of the pump hose and disinfection of the hands and wrists of the person who sprayed was done. Plants were placed in a solid plastic compartment to ensure no contamination with different strains. Each compartment was covered with a plastic sheet to maintain the wetness of leaves for 48 hours after inoculation.

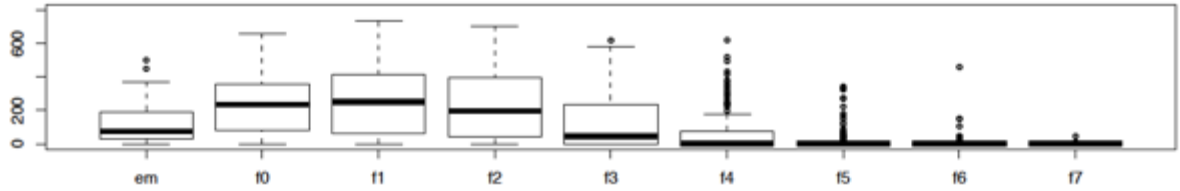
4.8. Incubation

The temperature was 17°-18°C during the experiment. After the inoculation, a period of two days in the dark with 100% hygrometry and minimum airflow allowed the plants under the plastic sheets to retain moisture and thus allow the germination of spores and the beginning of the development of mycelial stroma. Then the plastic sheets that covered the plants were removed, and the relative humidity was reduced to 80% hygrometry. This period (5 hours) allows the leaves to dry before putting the light back and avoid burning them. Then the plants were incubated until the end of the test with the following climatic conditions (Annex V):

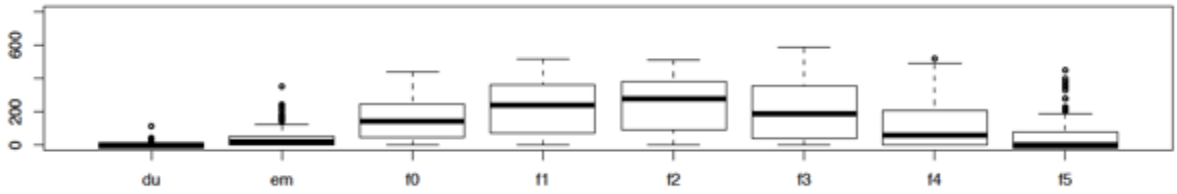
• 16h of day, 17-18 ° C, 80% hygrometry

• 8h night, 17-18 ° C, 90% hygrometry (For Series O3 and C2 80% hygrometry)

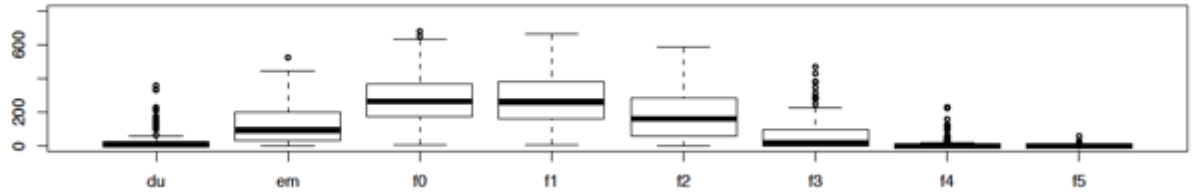
Series C1



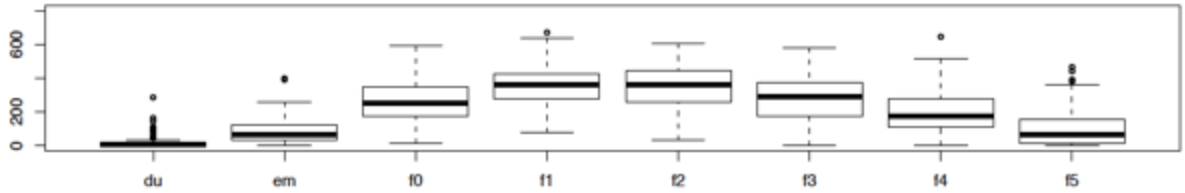
Series C2



Series O1



Series O2



Series O3

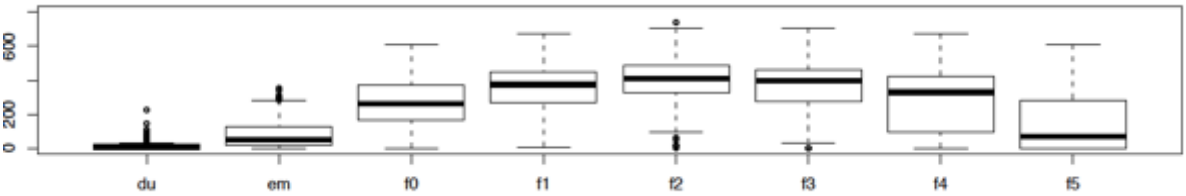


Figure 10: Kinetics of disease development on leaves of apple plants on *water* plants for each experiment

4.9. Randomization:

All the 20 strains were randomized into 4 blocks in the climate chamber for each experiment to avoid any bias. Every single block contained 2 *water* plants and two ASM plants for each strain with ASM treated and water treated plant in a pair side by side.

4.10. Disease scoring

Disease severity was scored as one quantitative component of pathogenicity in this experiment. For each series of experiment, disease scoring was performed at 7, 9, 13 and 16 dpi (dpi: day post-inoculation). Each scoring consists of evaluating the % of sporulating scabbed leaf area on upper 8 leaves of each plant (Figure 9) using a scale with 10% increments in 100%.

4.11. Data analysis

In this report, the experiments with strains sampled from the same orchard with two different origins (light IPM+ASM treated plots and light IPM treated plots) were named as series O1, O2 and O3 for better understanding. The experiments with strains from untreated orchards were named as series C1 and C2. Experiment C1 had been performed in 2018 by an intern student named Fauvre Sarah, and I further analyzed her data in my internship.

4.11.1. Calculation of AUDPC for each plant

On each scored leaf, the AUDPC (Area Under Disease Progress Curve) was calculated as a variable summarizing the kinetics of disease development. This AUDPC is the sum of the areas under the curve between each disease severity scoring date (Annex VI). In this study, AUDPC represents the aggressiveness of the strains. For each experiment, AUDPC was analyzed through boxplots on the different leaves (du-f5) of the *water* plants, taken into account all strains together. For each experiment, we decided to remove severity count of leaves that had a median close to zero (Figure 10). An averaged AUDPC was then calculated for each plant. All the analyses presented below are done on the average AUDPC per plant.

4.11.2. Effect of germination rate on AUDPC

Pearson correlation test was performed to see if there was a correlation between the germination % and AUDPC.

4.11.3. Effect of ASM treatment on AUDPC

To observe the overall effect of "Treatment" (ASM plants and *Water* plants) on AUDPC, analysis of variance was performed by taking two factors into account, "Treatment" and "Strains", and their interaction. "Upper leaves", "under leaves" and "blocks", were used into the model as co-variables. Since the interaction between "Treatment" and "Strains" was significant, the effect of "Treatment" on AUDPC was performed using one-way ANOVA strain by strain, with "upper leaves", "under leaves" and "blocks" as co-variables. No visual deviation of residuals was observed.

4.11.4. Effect of the origin of the strains on AUDPC (only for experiments O1, O2, O3)

For each treatment, the effect of origin (light IPM and light IPM+ASM) of the stains on AUDPC was tested using linear mixed effect model (LME). Here, "Origin" was considered as a fixed factor and "Strains" was considered as a random factor nested into the "Origin". Residues did not show any apparent deviations from homoscedasticity or normality. P-values were obtained by likelihood ratio tests of the full model with "origin" factor in question against the model without "origin" factor in question.

4.11.5. Relationship between efficiency of ASM and AUDPC

Efficiency of ASM was calculated as follow:

Efficiency% = ((AUDPC on *water* plants -AUDPC on ASM plants)/ AUDPC on *water* plants) *100

Pearson correlation test was performed to see if there was a correlation between efficiency % of ASM and AUDPC on *water* plants.

All statistical tests were done using R studio version 3.5.1 (2018-07-02). For ANOVA and LME analyses, Shapiro test and Barlett tests were performed to check for deviations from normality and homoscedasticity, respectively, as well as visual inspection of residual plots.

Avirulent strain (06VIL226) Virulent strain (06LAN67)

Figure 11: Observation on the cultivar Golden delicious 16 days after inoculation by two strains of *Venturia inaequalis*: reaction of resistance (necrosis) with an avirulent strain and symptoms of disease (sporulation) with a virulent strain (Personal photography)

5. Results :

In each experiment, all the strains showed virulence on *Rvi1*, the resistance gene found in Golden delicious variety, except one strain 06VIL226 showing complete avirulence in series C2 (Figure 11). In each experiment, all the strains showed virulence on *Rvi1*, the resistance gene found in Golden delicious variety, except one strain 06VIL226 showing complete avirulence in series C2 (Figure 11). This strain wasn't taken into account for the data analysis.

5.1. AUDPC on the different leaves

Scab developed differently on the different leaves among the different experiments. Therefore, the decision concerning the choice of the leaves that were kept for calculating the averaged AUDPC per plant was specific to each series and the number of analyzed leaves varied from four to seven depending on the experiment:

C1 → em, f0, f1, f2, f3

C2 → f0, f1, f2, f3, f4

O1 → em, f0, f1, f2

O2 → em, f0, f1, f2, f3, f4, f5

O3 → em, f0, f1, f2, f3, f4, f5

5.2. Analysis of the relationship between the germination percentage of the strains and AUDPC

There was no correlation found between the germination percentage of the strains and the aggressiveness of the strains observed in any of the series of experiments (Figure 12). So, the aggressiveness of strains was not depended or effected by germination percentage of strains.

5.3. Effect of ASM on AUDPC:

5.3.1. Overall effect of ASM on AUDPC:

In all experiments AUDPC was significantly less on ASM plants than on *water* plants ($P < 0.05$) (Figure 13). Efficiency percentage varied depending on the experiments: C1 (33%), C2 (45%), O1 (53%), O2 (41%), O3 (27%). There was effect of blocks in each experiment. Since there was interaction between “Treatment” and “Strains”, strain by strain analysis was performed.

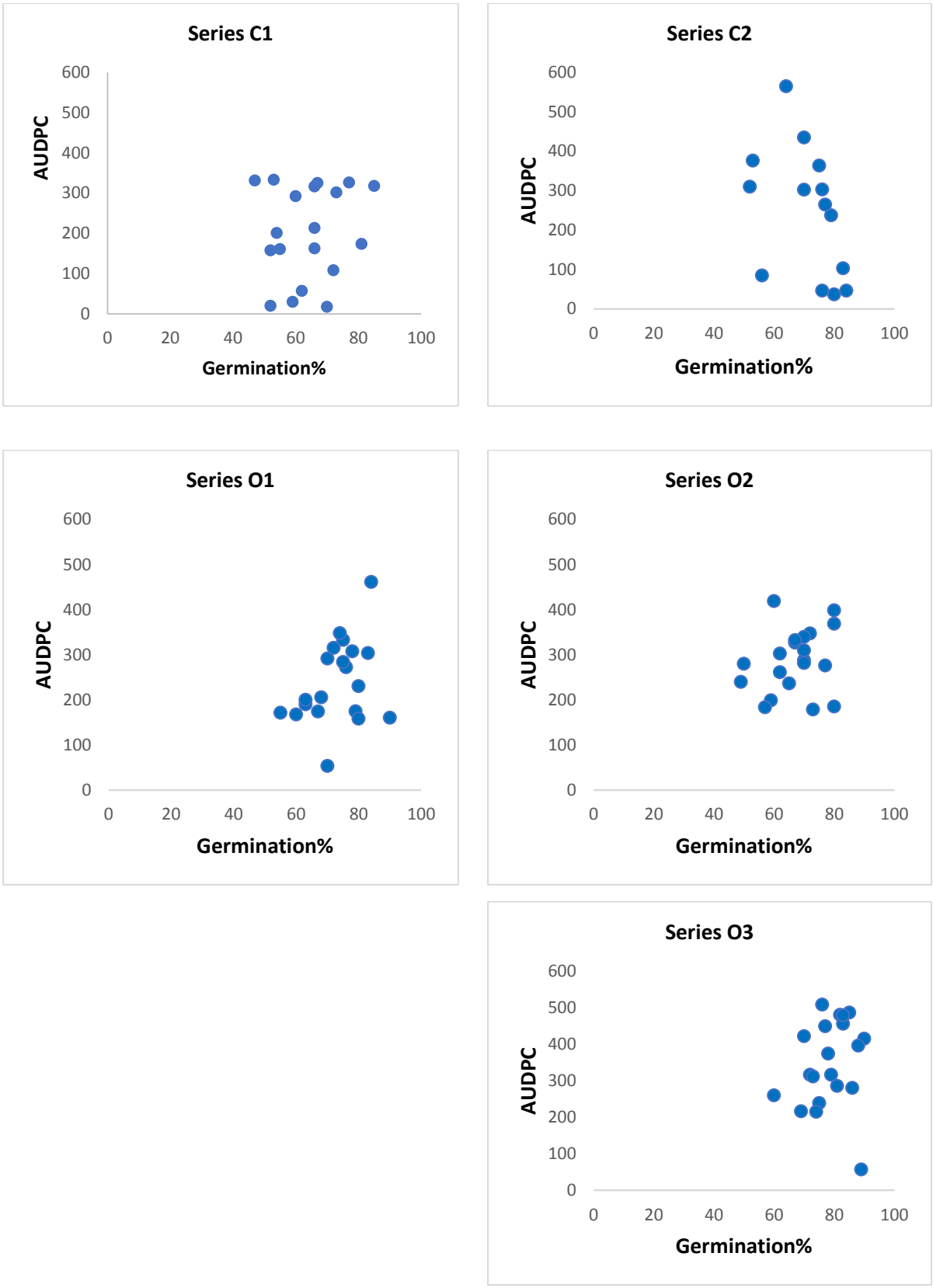


Figure 12: Relation between germination % and disease severity (AUDPC) of strains of *Venturia inaequalis* inoculated on *water* apple plants. Each point indicates one strain.

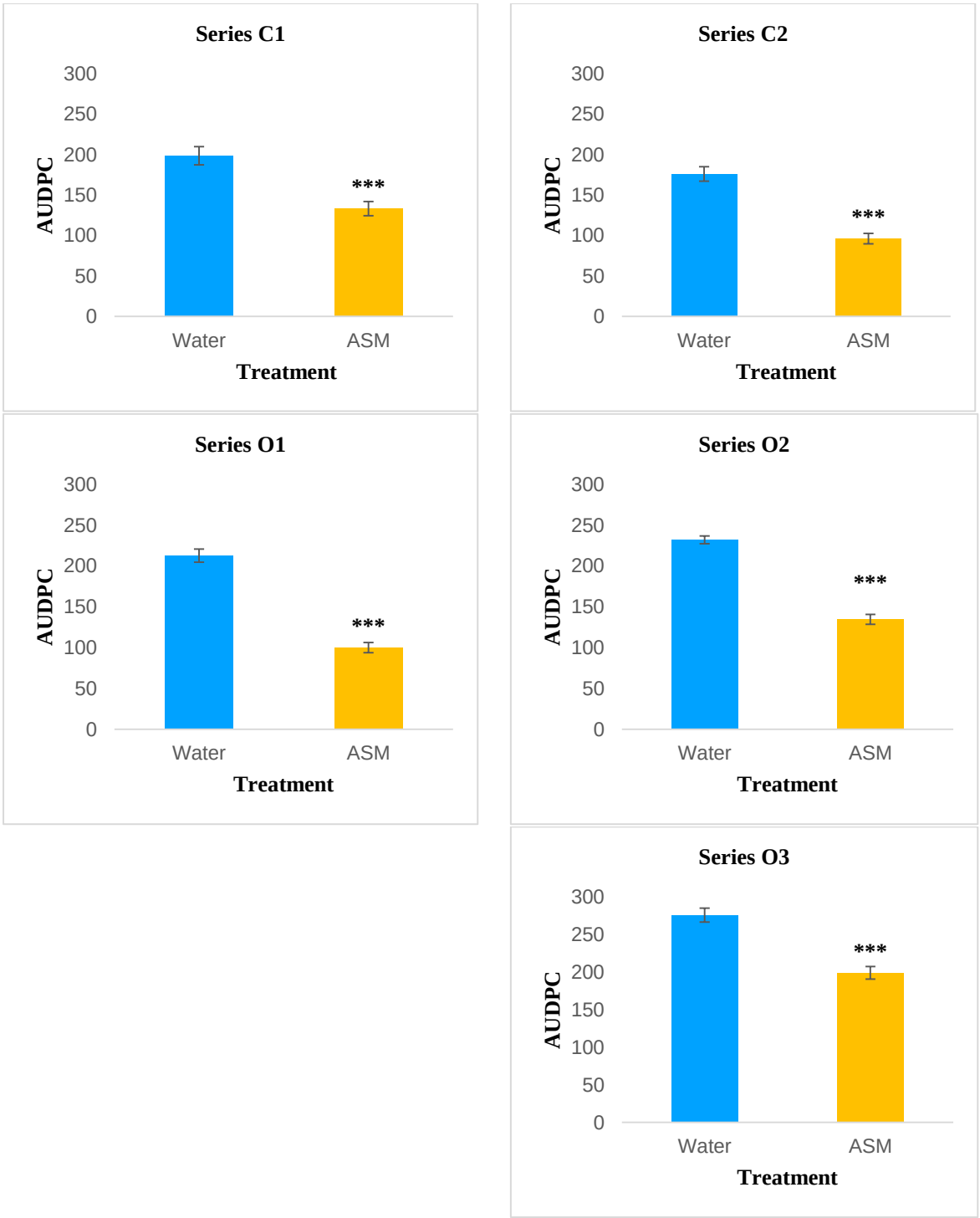


Figure 13. Overall effect of ASM treatment on disease severity (AUDPC) on apple plants inoculated by strains of *Venturia inaequalis*. Bar graph represents the mean AUDPC (±SE) where n= 160 plants per treatment for each experiment except Series C1 (n=168)
(P values ***<0.001, ANOVA analysis)

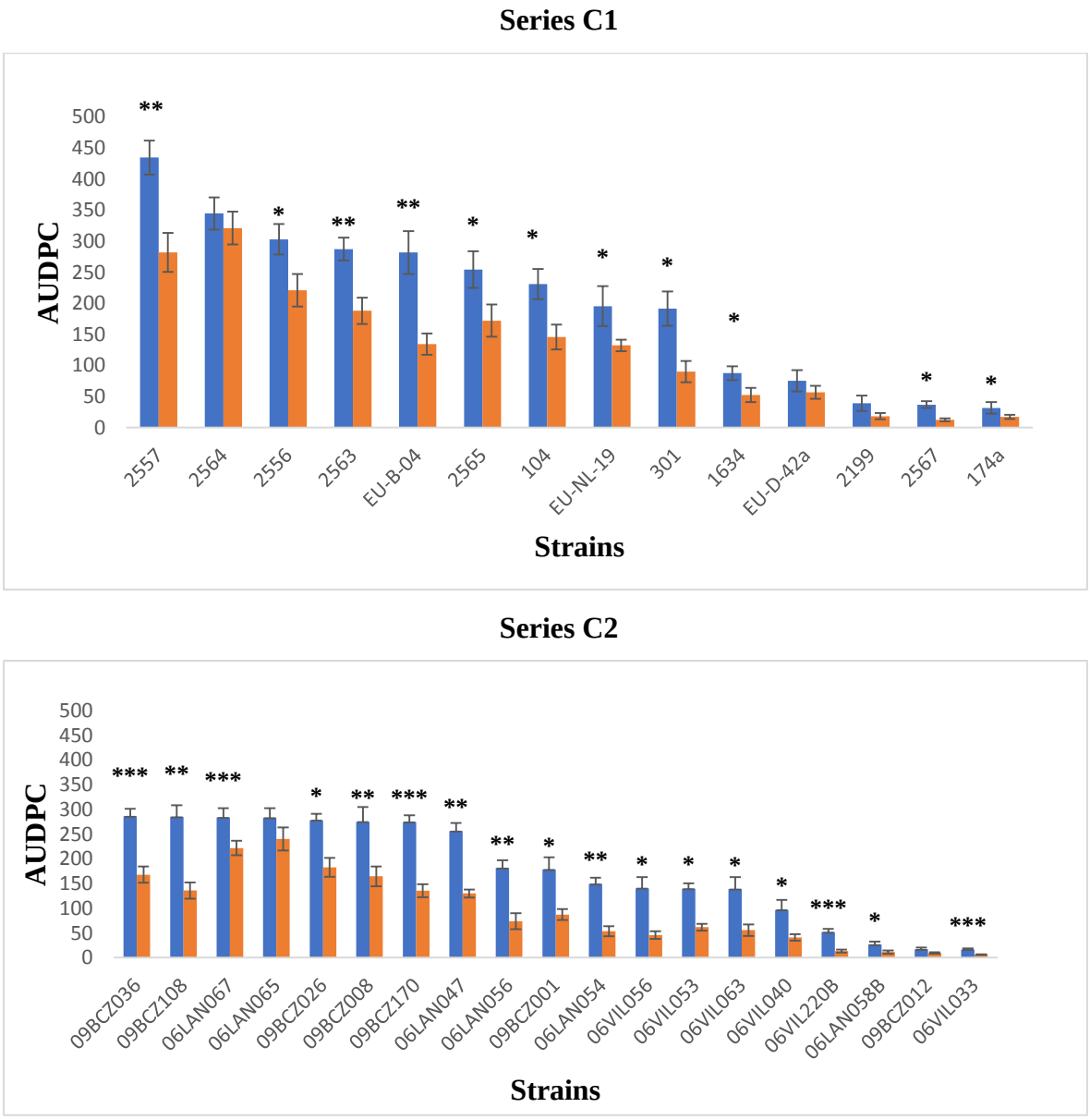
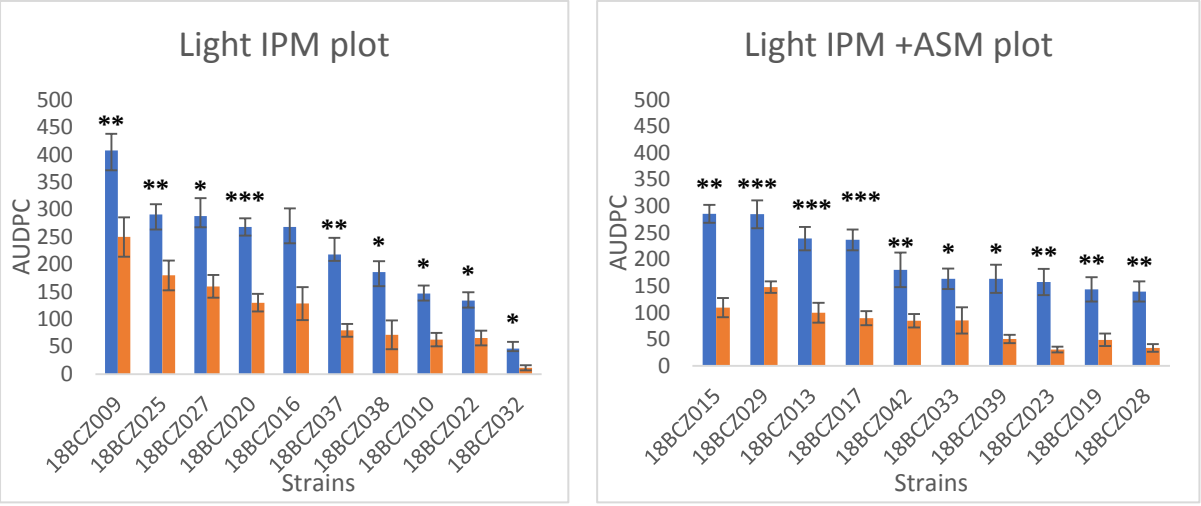


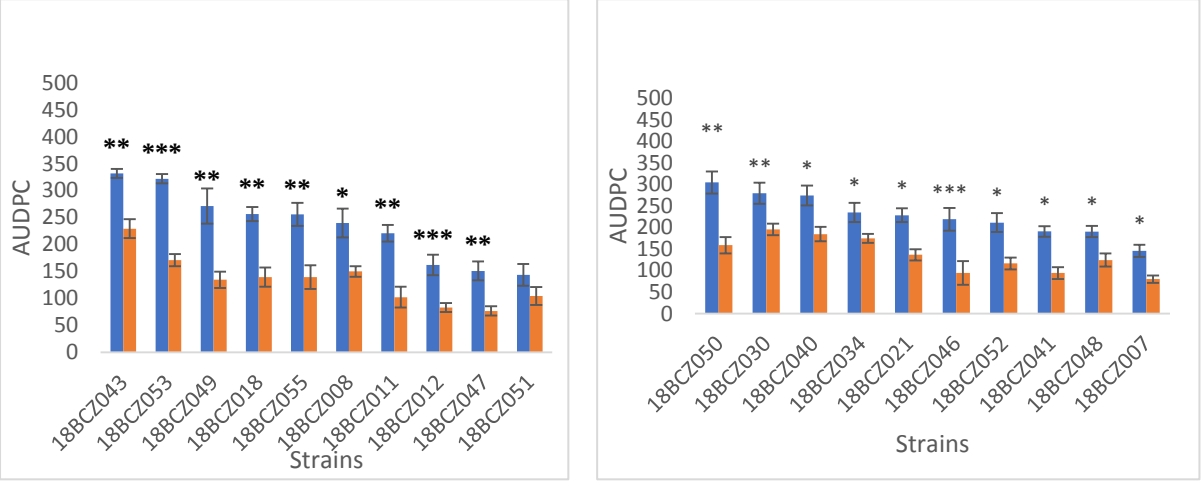
Figure 14 : Effect of ASM treatment on disease severity (AUDPC) on apple plants for each *Venturia inaequalis* strain . Bar represents the mean AUDPC (± SE) where n=12 and n=8 plants per treatment in series C1 and series C2 respectively.
(P values '***' < 0.001, '**' <0.01, '*' <0.05, ANOVA analysis)

Legends ASM Water

Series O1



Series O2



Series O3

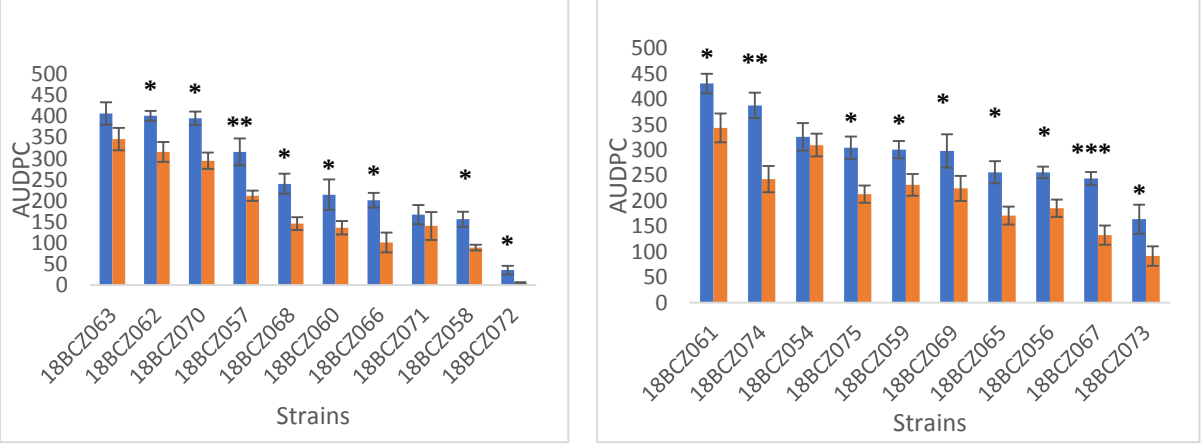


Figure 14 (continuation): Effect of ASM treatment on disease severity (AUDPC) on apple plants for each *Venturia inaequalis* strain. Bar represents mean AUDPC (± SE) where n=8 plants per treatment.Left side graph represents light IPM plot and right side represents light IPM+ASM plots. (P values '***' < 0.001, '**' <0.01, '*' <0.05, ANOVA analysis)

Legends ASM Water

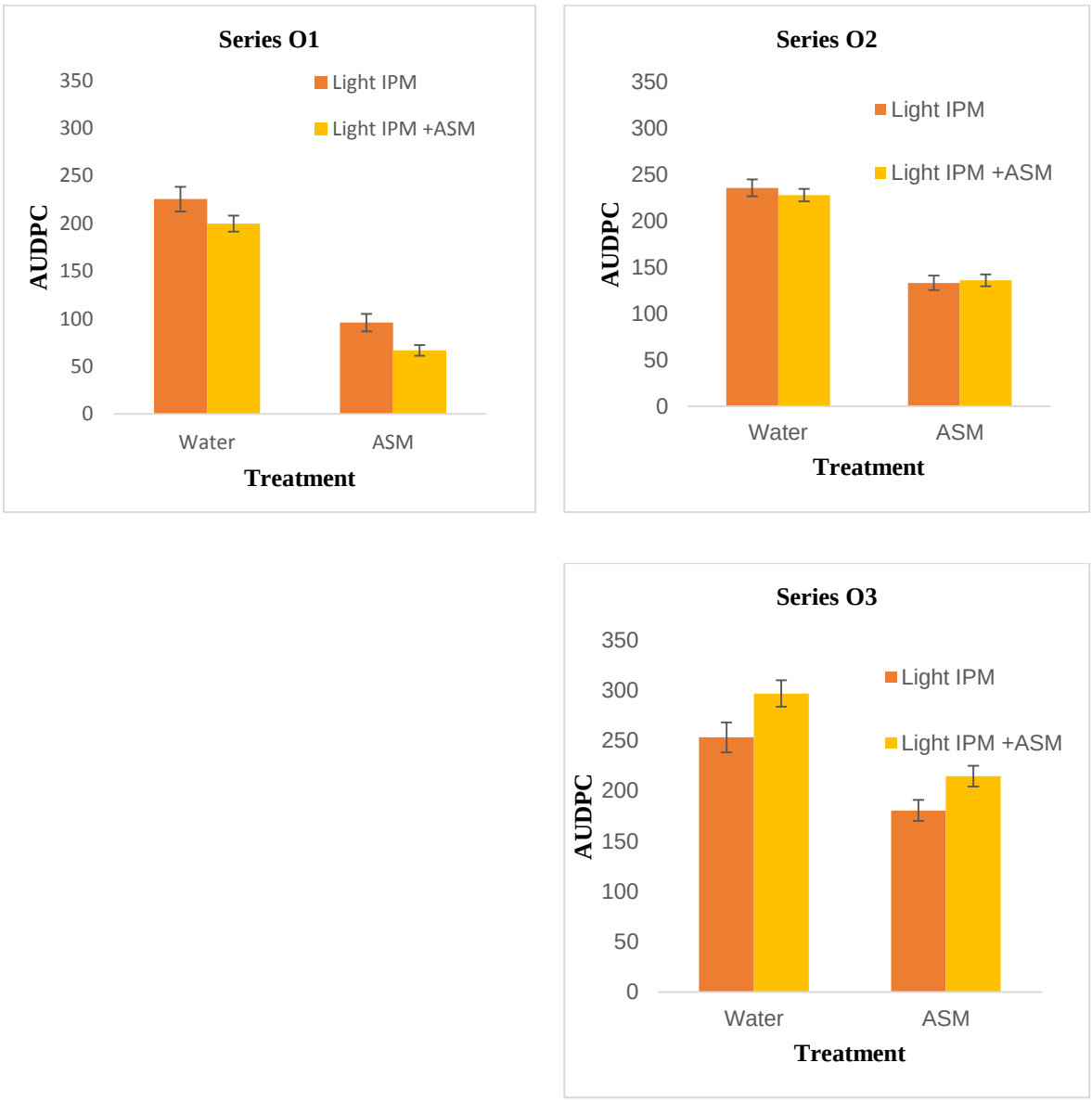


Figure 15: Effect of origin of the *Venturia inaequalis* strains (Light IPM and Light IPM + ASM plot) on disease severity (AUDPC) on ASM apple plants and on *water* apple plants. Bar shows mean AUDPC (± SE) where n=80.
(There was no significant effect of the origin of the strains; for each treatment P value >0.05, LME analysis)

5.3.2. Effect of ASM on AUDPC strain by strain:

For each series of experiment high variability of aggressiveness was observed on *water* plants (Figure 14). ASM significantly reduced AUDPC for most strains. The effect of ASM on AUDPC was not significant for only 3 strains in C1, 2 strains in C2, 1 strain in O1, 1 strain O2 and 3 strains in O3. Three of these strains had a low aggressiveness on *water* plants: 2199 and EU-D42a in series C1, 09BCZ012 in series C2. One strain presented a high variability from plant to plant: 18BCZ016 in series O1. Six strains had an intermediate to high aggressiveness on non-treated plants: 2564 in series C1, 06LAN065 in series C2, 18BCZ051 in series O2, 18BCZ063, 18BCZ071 and 18BCZ054 in series O3. Only one of these strains came from an orchard that was treated by ASM (18BCZ054).

5.4. Analysis of effect of origin of the strains on AUDPC (series O1, O2, O3):

For each series of experiment LME analysis was performed separately on ASM plants and *water* plants. There was no significant effect of the origin of strains on AUDPC on *water* plants; there was also no significant effect of the origin of strains on ASM plants (Figure 15).

5.5. Analysis of the relation between the % of efficiency of ASM and the aggressiveness of the strains on *water* plants

In each series of experiment the efficiency of ASM was very variable depending on the strain, from less than 10% to 80% (Figure 16). In three series of experiment, there was a significant correlation between efficiency of ASM and aggressiveness of the strains in *water* plants (Figure 16), with a Pearson' correlation of 0.50, 0.28 and 0.41 respectively for series O3, C1 and C2. In series O3, one strain (18BCZ072) was far from all the strains with a low aggressiveness and a high efficiency of ASM, but the correlation was still significant even without this strain.

So, it can be interpreted that in these three experiments the lower the aggressiveness of the strain the greater is the effectiveness of the ASM. On the other hand, in series O1 and O2 no significant correlation was observed. Moreover, the efficiency of ASM can also differ for strains having similar levels of aggressiveness. In series C2 for instance, the efficiency of ASM varied from 10 to 50 % for strains having an AUDPC of 300.

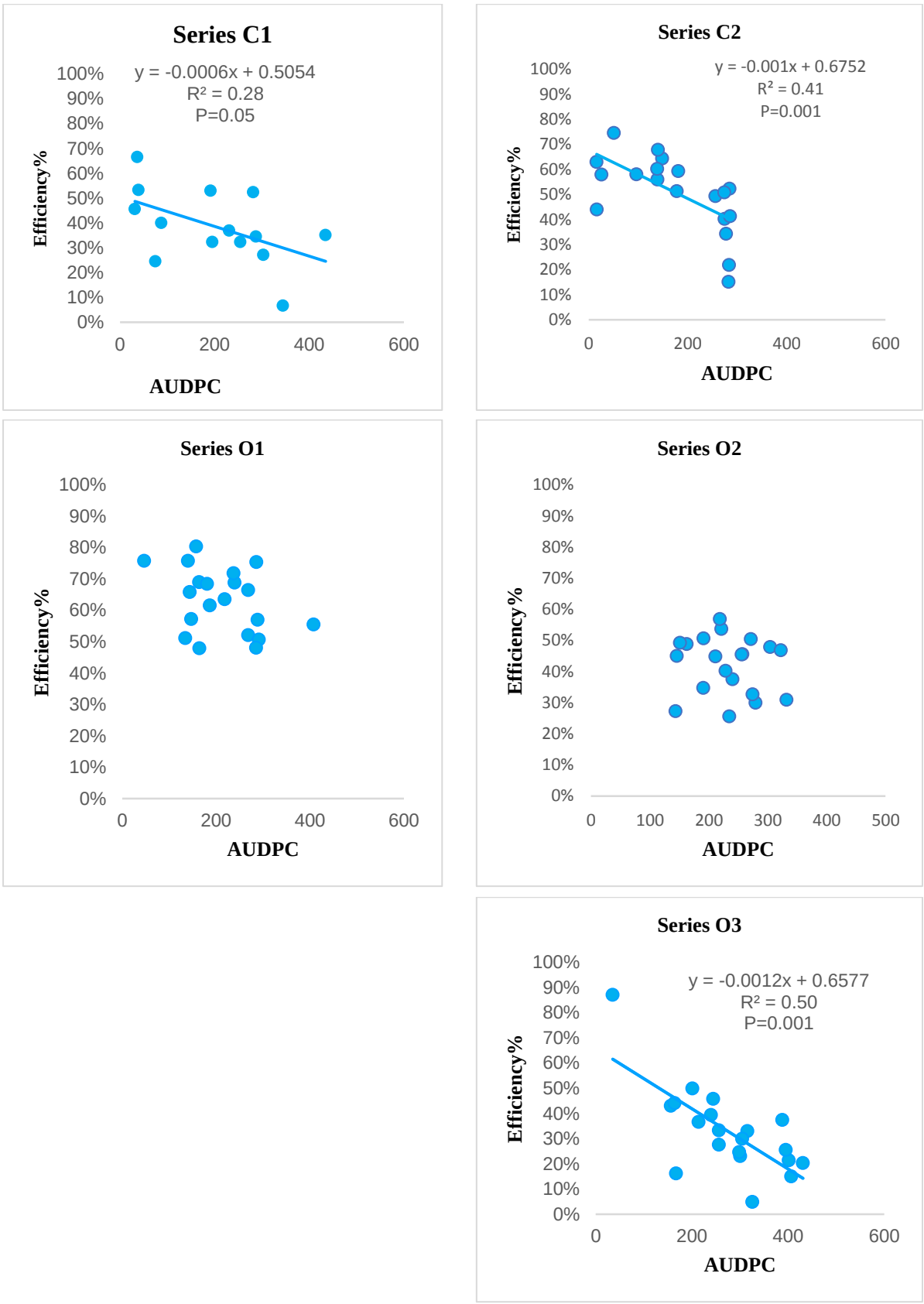


Figure 16: Relation between the % of efficiency of ASM and the aggressiveness (AUDPC) of the *Venturia inaequalis* strains on *water* apple plants. Each point indicates one strain.

6. Discussion :

ASM is the most studied inducer of SAR and numerous data have confirmed its efficiency against foliar pathogens on various crops (Vallad and goodman., 2004). However, it is not known if this efficiency can be lost over time because of selection of strains that would be not well controlled by ASM.

6.1. Overall effect of ASM in the reduction of scab

A study previously done in apple scab has shown around 50% efficiency in controlled condition with the strain 104 of *V. inaequalis* (Marolleau et al., 2017). The present study confirmed that ASM is significantly effective to reduce apple scab in controlled condition. This efficiency was observed in each of the 5 series of experiment, with overall values of efficiency that varied between 25 and 63%. This variation in efficiency may be due to environmental conditions during the test, because the climatic chamber was not exactly similar for temperature and relative humidity for each experiment (Annex V). This variation also could be due to different physiological status of the apple plants that were produced in the glasshouse with fluctuating climatic conditions, to different climatic conditions after the spraying of ASM in glasshouse and also to the use of different strains in each experiment. Therefore, each experiment needed to be analyzed separately, and it was not possible to compare the efficiency of ASM for strains that were tested in different experiments.

6.2. Variability in the efficiency of ASM according to strains

Here, in this study after comparing 94 strains of *V. inaequalis*, it can be stated that most of the strains are significantly sensitive to ASM. However, the efficiency of ASM is variable according to the strain (from 5 to 87%). Such a variability was also observed with another SDP, BABA, on tomato inoculated by two different strains of *Phytophthora infestans* (Sharma et al., 2010). Here, we confirm this result over a large range of strains. In three out of five series of experiments, there was a significant negative correlation between efficiency of ASM on aggressiveness of the strains on *water* plants. So, there is a tendency that the more aggressive the strains are, the less effective the efficiency of ASM is. 10 strains were found to be non-significantly affected by ASM. For one of this strain no conclusion could be drawn because of a high variability from plant to plant. Three of these strains had a low aggressiveness on the *water* plants and their low sensitivity to ASM may have no impact on the efficiency of scab control in orchard, because these strains will give a low amount of

disease. The six other strains had an intermediate to high aggressiveness on the *water* plants and their low sensitivity to ASM

may have consequences on the efficiency of scab control by ASM in orchard if these strains increase in frequency in orchard under the selection pressure of ASM treatments.

6.3. Efficiency of ASM according to the origin of the strains

The 94 strains tested in this study came from different origins: 34 from various orchards not treated against scab, 30 from an orchard managed with light IPM, 30 from an orchard with light IPM + ASM. The strains which are not sensitive to ASM were not related to their origin. Two of the six strains with intermediate to high aggressiveness on *water* plants and low sensitivity to ASM were sampled from orchards that were not treated against scab (06LAN065 from Lanxade, France, and 2564 from Angers, France). Three strains came from the orchard with light IPM. Only one strain came from the orchard with light IPM + ASM. In addition, we did not find significant difference in the efficiency of ASM between the 30 strains from the orchard with light IPM and the 30 strains from the orchard with light IPM + ASM. This result suggests that the repeated use of ASM integrated within an IPM strategy did not select for less sensitive strains over a 6-years period. Practically it denotes that ASM doesn't lose its capabilities over time. However, like fungicide insensitivities or breakdown of plant resistance any inefficiency is not easy to recognize in short time experiments specially for perennial crop like apple and should be confirmed over large scale of time and space.

6.4. Integration of ASM into IPM strategy and sustainability

Bousset and Pons-Kühnemann (2003) studied the selection pressure of ASM alone or the combination of ASM with the fungicide ethirimol on a laboratory population of barley powdery mildew for 10 generations in controlled condition. In both cases (ASM alone or ASM with ethirimol), they did not observe any evolution of the population towards a less sensitivity to ASM, but they indicated that a limit of their study may have been a low diversity in the sensitivity of the strains to ASM in the initial laboratory population. Field pathogen populations are larger and may have wider variation in aggressiveness. So, more variation of effectiveness of ASM may be observed in populations from field than in populations from laboratory. There was a large diversity in the sensitivity of strains to ASM in our study. However, similarly to Bousset and Pons-Kühnemann (2003), we observed no evolution of the population towards a less sensitivity to ASM when ASM was integrated into a light IPM. Recent study conducted in field concluded to use ASM with low input fungicide

to get highest effectiveness against scab (Marolleau et al., 2017). The present study suggests that this strategy could also increase sustainability. However, because we did not test the effect of selection pressure by ASM alone,

we cannot conclude if it is necessary to integrate ASM into IPM to get sustainability or if a control method based on ASM alone would be also sustainable.

7. Conclusion and perspectives :

Compounds used as elicitors of plant defense stimulators offer new avenues for controlling pome fruit tree diseases. This new method of plant protection for pome fruit tree diseases has a great potential to reduce fungicide application in orchards. The present study gives first insight into the question of sustainability of ASM. Strains with a reduced sensitivity to ASM have been found in orchards that were not treated with ASM, but there was no evolution towards a reduced sensitivity of the strains to ASM due to repeated applications of ASM in an IPM strategy over a 6-years period in orchard. However, observation of the evolution of pathogenicity over an extended period of time is important, and also over a larger range of scab populations. For that last purpose, new sampling has been carried out in July 2019 in a cider orchard in Sées (IFPC, 61, France) and in an orchard in Lanxade (CTIFL, 24, France).

This study was performed on the scab susceptible variety Golden delicious. It could be enlarged in future on partially resistant varieties, that could improve efficiency of ASM control. With partially resistant varieties, it may be possible to control scab with ASM alone without other fungicides. So, there is also a need to evaluate the sustainability of ASM when applied alone on partially resistant varieties. The results obtained in this study on ASM cannot be generalized to other elicitors, because the underlying mechanisms of defense simulation are different according to the elicitors used. So, this study should be performed on more elicitors (for instance: BABA (β-aminobutyric acid), INA (2,6 -dichloro-isonicotinic acid) etc) to have a better knowledge on the potential sustainability of elicitors to control apple scab.

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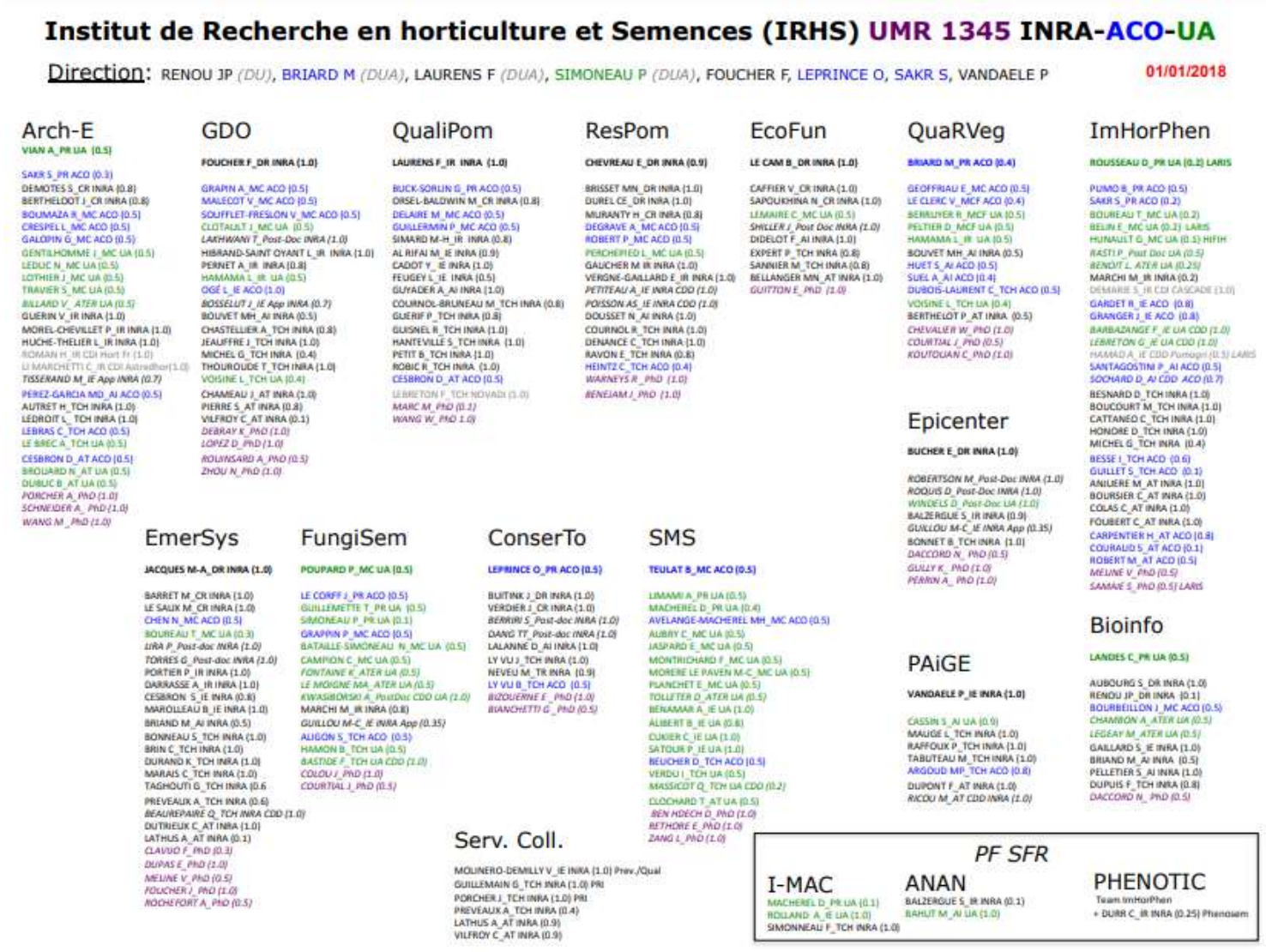
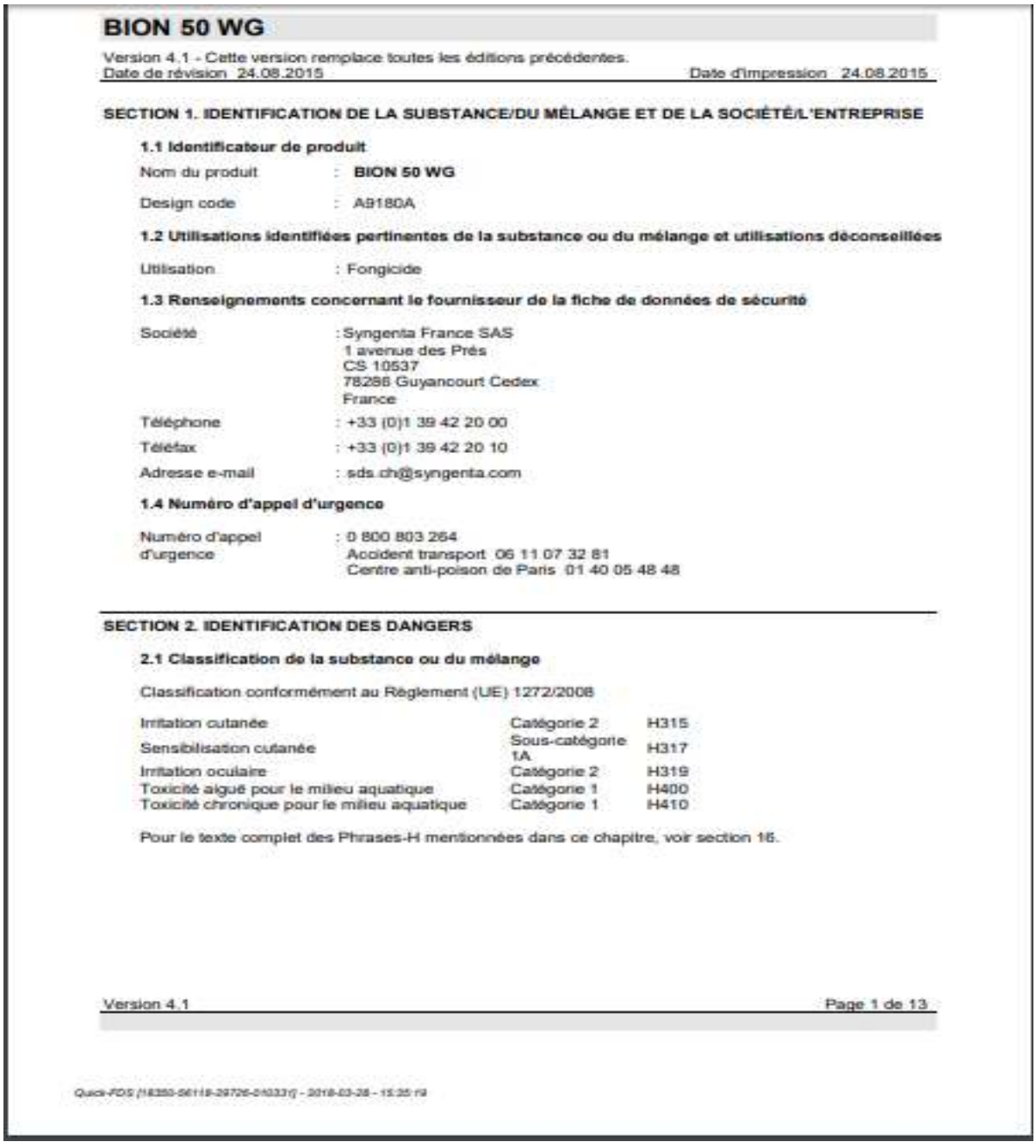
Annex I: Organisation of IRHS



Annex II: Security information of Bion



BION 50 WG

Version 4.1 - Cette version remplace toutes les éditions précédentes.
Date de révision 24.08.2015 Date d'impression 24.08.2015

SECTION 1. IDENTIFICATION DE LA SUBSTANCE/DU MÉLANGE ET DE LA SOCIÉTÉ/L'ENTREPRISE

1.1 Identificateur de produit

Nom du produit : **BION 50 WG**

Design code : A9180A

1.2 Utilisations identifiées pertinentes de la substance ou du mélange et utilisations déconseillées

Utilisation : Fongicide

1.3 Renseignements concernant le fournisseur de la fiche de données de sécurité

Société : Syngenta France SAS
1 avenue des Prés
CS 10537
78286 Guyancourt Cedex
France

Téléphone : +33 (0)1 39 42 20 00

Téléfax : +33 (0)1 39 42 20 10

Adresse e-mail : sds.ch@syngenta.com

1.4 Numéro d'appel d'urgence

Numéro d'appel d'urgence : 0 800 803 264
Accident transport 06 11 07 32 81
Centre anti-poison de Paris 01 40 05 48 48

SECTION 2. IDENTIFICATION DES DANGERS

2.1 Classification de la substance ou du mélange

Classification conformément au Règlement (UE) 1272/2008

Irritation cutanée	Catégorie 2	H315
Sensibilisation cutanée	Sous-catégorie 1A	H317
Irritation oculaire	Catégorie 2	H319
Toxicité aiguë pour le milieu aquatique	Catégorie 1	H400
Toxicité chronique pour le milieu aquatique	Catégorie 1	H410

Pour le texte complet des Phrases-H mentionnées dans ce chapitre, voir section 16.

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2.2 Éléments d'étiquetage

Étiquetage: Règlement (CE) No. 1272/2008

Pictogrammes de danger

Mention d'avertissement	:	Attention	
Mentions de danger	:	H315	Provoque une irritation cutanée.
		H317	Peut provoquer une allergie cutanée.
		H319	Provoque une sévère irritation des yeux.
		H410	Très toxique pour les organismes aquatiques, entraîne des effets néfastes à long terme.
Conseils de prudence	:	P102	Tenir hors de portée des enfants.
		P270	Ne pas manger, boire ou fumer en manipulant ce produit.
		P273	Éviter le rejet dans l'environnement.
		P280	Porter des gants de protection/ des vêtements de protection/ un équipement de protection des yeux/ du visage *pendant toutes les étapes de manipulation du produit.*
		P302 + P352	EN CAS DE CONTACT AVEC LA PEAU: Laver abondamment à l'eau.
		P305 + P351 + P338	EN CAS DE CONTACT AVEC LES YEUX: rincer avec précaution à l'eau pendant plusieurs minutes. Enlever les lentilles de contact si la victime en porte et si elles peuvent être facilement enlevées. Continuer à rincer.
		P333 + P313	En cas d'irritation ou d'éruption cutanée: consulter un médecin.
		P391	Recueillir le produit répandu.
		P501	Éliminer le contenu/récipient dans une installation d'élimination des déchets agréée.
Information supplémentaire	:	EUH401	Respectez les instructions d'utilisation pour éviter les risques pour la santé humaine et l'environnement.
Etiquetage supplémentaire	:	SP 1 Ne pas polluer l'eau avec le produit ou son emballage. (Ne pas nettoyer le matériel d'application près des eaux de surface./Éviter la contamination via les systèmes d'évacuation des eaux à partir des cours de ferme ou des routes.).	
		SPe 3 Pour protéger les organismes aquatiques, respecter une zone non traitée de 5 mètres par rapport aux points d'eau.	
		Délai de rentrée sur les parcelles traitées : 48 heures.	

Composants dangereux qui doivent être listés sur l'étiquette:

Annex III: Information of the strains of *Venturia inaequalis* (series C1 and C2)

Experiment	Strain	Country	Commune	Apple genotype	Sampling date	Scab managment of the orchard
C1*	0104	France	St Lézin	Golden delicious	1978	non treated
C1	0174	France	Beaucouzé	9A2T128	1986	non treated
C1	0301	Germany	Ahrensburg	81/19-53	1988	non treated
C1	1634	France	Beaucouzé	TSR33T239	2001	non treated
C1	2199	Danemark	Arslev	Florina	2003	non treated
C1	2556	France	Lanxade	J108	2006	non treated
C1	2557	France	Beaucouzé	E063	2009	non treated
C1	2563	France	Beaucouzé	J119	2007	non treated
C1	2564	France	Beaucouzé	J63	2007	non treated
C1	2565	France	Beaucouzé	J80	2007	non treated
C1	2567	France	Beaucouzé	E074	2009	non treated
C1	EU-B-04	Belgium	Unknown	Golden delicious	1998	non treated
C1	EU-D-42a	Germany	Ahrensburg	Prima	1999	non treated
C1	EU-NL-19	The Netherlands	Elst	Golden delicious	1998	non treated
C2	06LAN047	France	Lanxade	J153	2006	non treated
C2	06LAN054	France	Lanxade	J61	2006	non treated
C2	06LAN056	France	Lanxade	J160	2006	non treated
C2	06LAN058B	France	Lanxade	J115	2006	non treated
C2	06LAN065	France	Lanxade	J99	2006	non treated
C2	06LAN067	France	Lanxade	J99	2006	non treated
C2	06VIL033	France	Villeneuve d'Ascq	J80	2006	non treated
C2	06VIL040	France	Villeneuve d'Ascq	J66	2006	non treated
C2	06VIL053	France	Villeneuve d'Ascq	J153	2006	non treated
C2	06VIL056	France	Villeneuve d'Ascq	J160	2006	non treated
C2	06VIL063	France	Villeneuve d'Ascq	J119	2006	non treated
C2	06VIL220B	France	Villeneuve d'Ascq	J66	2006	non treated
C2	06VIL226	France	Villeneuve d'Ascq	J99	2006	non treated
C2	09BCZ001	France	Beaucouzé	E012	2009	non treated
C2	09BCZ008	France	Beaucouzé	E035	2009	non treated
C2	09BCZ012	France	Beaucouzé	E053	2009	non treated
C2	09BCZ026	France	Beaucouzé	E125	2009	non treated
C2	09BCZ036	France	Beaucouzé	E169	2009	non treated
C2	09BCZ108	France	Beaucouzé	E157	2009	non treated
C2	09BCZ170	France	Beaucouzé	E224	2009	non treated

Series C1 was conducted by Sarah Fauvre in 2018

Annex III (continuation): Information of the strains of *Venturia inaequalis* (Series O1, O2,O3)

O1	18BCZ009	France	Beaucouzé	Golden delicious	2018	light IPM
O1	18BCZ010	France	Beaucouzé	Golden delicious	2018	light IPM
O1	18BCZ016	France	Beaucouzé	Golden delicious	2018	light IPM
O1	18BCZ020	France	Beaucouzé	Golden delicious	2018	light IPM
O1	18BCZ022	France	Beaucouzé	Golden delicious	2018	light IPM
O1	18BCZ025	France	Beaucouzé	Golden delicious	2018	light IPM
O1	18BCZ027	France	Beaucouzé	Golden delicious	2018	light IPM
O1	18BCZ032	France	Beaucouzé	Golden delicious	2018	light IPM
O1	18BCZ037	France	Beaucouzé	Golden delicious	2018	light IPM
O1	18BCZ038	France	Beaucouzé	Golden delicious	2018	light IPM
O1	18BCZ013	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O1	18BCZ015	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O1	18BCZ017	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O1	18BCZ019	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O1	18BCZ023	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O1	18BCZ028	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O1	18BCZ029	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O1	18BCZ033	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O1	18BCZ039	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O1	18BCZ042	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O2	18BCZ008	France	Beaucouzé	Golden delicious	2018	light IPM
O2	18BCZ011	France	Beaucouzé	Golden delicious	2018	light IPM
O2	18BCZ012	France	Beaucouzé	Golden delicious	2018	light IPM
O2	18BCZ018	France	Beaucouzé	Golden delicious	2018	light IPM
O2	18BCZ043	France	Beaucouzé	Golden delicious	2018	light IPM
O2	18BCZ047	France	Beaucouzé	Golden delicious	2018	light IPM
O2	18BCZ049	France	Beaucouzé	Golden delicious	2018	light IPM
O2	18BCZ051	France	Beaucouzé	Golden delicious	2018	light IPM
O2	18BCZ053	France	Beaucouzé	Golden delicious	2018	light IPM
O2	18BCZ055	France	Beaucouzé	Golden delicious	2018	light IPM
O2	18BCZ007	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O2	18BCZ021	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O2	18BCZ030	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O2	18BCZ034	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O2	18BCZ040	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O2	18BCZ041	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O2	18BCZ046	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O2	18BCZ048	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O2	18BCZ050	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O2	18BCZ052	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O3	18BCZ057	France	Beaucouzé	Golden delicious	2018	light IPM
O3	18BCZ058	France	Beaucouzé	Golden delicious	2018	light IPM
O3	18BCZ060	France	Beaucouzé	Golden delicious	2018	light IPM
O3	18BCZ062	France	Beaucouzé	Golden delicious	2018	light IPM
O3	18BCZ063	France	Beaucouzé	Golden delicious	2018	light IPM
O3	18BCZ066	France	Beaucouzé	Golden delicious	2018	light IPM
O3	18BCZ068	France	Beaucouzé	Golden delicious	2018	light IPM
O3	18BCZ070	France	Beaucouzé	Golden delicious	2018	light IPM
O3	18BCZ071	France	Beaucouzé	Golden delicious	2018	light IPM
O3	18BCZ072	France	Beaucouzé	Golden delicious	2018	light IPM
O3	18BCZ054	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O3	18BCZ056	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O3	18BCZ059	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O3	18BCZ061	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O3	18BCZ065	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O3	18BCZ067	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O3	18BCZ069	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O3	18BCZ073	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O3	18BCZ074	France	Beaucouzé	Golden delicious	2018	light IPM + Bion
O3	18BCZ075	France	Beaucouzé	Golden delicious	2018	light IPM + Bion

Annex IV: Table of preparation of strains inoculum of *Venturia inaequalis* for inoculation (50 ml - 100000 sp/ml)

Series C1

Strain	Color	comptage1	comptage2	comptage3	comptage4	moyenne	D	vol initial	vol eau à ajouter	vol FINAL	[exacte]	% Germination
104	blanc	108 800	103 000	114 600	110 800	109 300	1,09	80,00	7,4	87,44	100000	52%
0174a	blanc_1	103 400	101 000	105 000	103 200	103 150	1,03	80,00	2,5	82,52	100000	76%
301	bleu	169 400	173 200	165 800	162 000	167 600	1,68	80,00	54,1	134,08	100000	79%
1634	bleu_1	157 200	202 800	170 200	156 400	171 650	1,72	80,00	57,3	137,32	100000	83%
2199	jaune	132 600	124 600	133 000	125 800	129 000	1,29	80,00	23,2	103,20	100000	80%
EU-B-04	jaune_1	119 400	117 000	111 400	114 800	115 650	1,16	80,00	12,5	92,52	100000	70%
EU-D-42a	orange	152 200	149 000	179 400	161 000	160 400	1,60	80,00	48,3	128,32	100000	56%
EU-NL-19	orange_1	167 200	167 800	162 200	164 200	165 350	1,65	80,00	52,3	132,28	100000	77%
2556	rose	104 800	105 800	117 800	110 000	109 600	1,10	80,00	7,7	87,68	100000	53%
2557	rouge	113 400	109 800	119 600	115 600	114 600	1,15	80,00	11,7	91,68	100000	64%
2563	vert	168 800	153 600	149 400	153 200	156 250	1,56	80,00	45,0	125,00	100000	75%
2567	vert_1	333 000	328 200	352 400	336 800	337 600	3,38	80,00	190,1	270,08	100000	84%
2564	violet	223 000	196 800	213 600	216 200	212 400	2,12	80,00	89,9	169,92	100000	70%
2565	violet_1	292 200	288 200	276 400	265 000	280 450	2,80	80,00	144,4	224,36	100000	76%

Series C2

strain	name	color	nb cellos	date cello	time	comptage1	comptage2	comptage3	comptage4	moyenne	D	vol initial	vol eau à ajouter	% Germination
06VIL033	2440	white	3	14/3/11	8.28	625,800	622,400	668,000	652,400	642,150	6.42	50.00	271.1	70%
09BCZ001	2639	white_1H	3	19/3/13	8.29	242,400	259,600	253,800	242,800	249,650	2.50	45.00	67.3	54%
06LAN047	2422	white_2H	3	10/4/14	8.53	299,200	295,200	301,600	304,200	300,050	3.00	50.00	100.0	60%
06VIL040	2436	blue	3	26/3/10	8.57	212,800	210,000	226,600	201,600	212,750	2.13	50.00	56.4	72%
09BCZ008	2641	blue_1H	3	25/3/13	9.04	136,600	134,600	137,200	132,200	135,150	1.35	50.00	17.6	66%
06LAN054	2417	yellow	3	10/4/14	9.10	125,200	122,200	113,600	111,000	118,000	1.18	50.00	9.0	81%
06VIL053	2437	yellow_1H	4	29/3/10	9.20	134,200	123,400	132,200	137,800	131,900	1.32	45.00	14.4	52%
09BCZ012	2644	yellow_2H	3	14/3/11	9.30	373,200	382,400	392,800	386,400	383,700	3.84	50.00	141.9	52%
06LAN056	2419	orange	3	29/3/10	9.40	173,400	175,600	161,200	166,200	169,100	1.69	50.00	34.6	66%
06VIL056	2433	orange_1H	3	1/4/10	9.50	404,000	437,000	402,600	411,400	413,750	4.14	50.00	156.9	55%
09BCZ036	2656	orange_2H	6	3/2/11	12.20	172,000	170,800	173,400	172,000	172,050	1.72	50.00	36.0	77%
B	2426	pink	3	14/6/10	12.25	355,000	340,000	364,000	353,800	353,200	3.53	50.00	126.6	59%
06VIL063	2431	pink_1H	3	2/5/14	13.40	116,600	119,600	116,000	116,800	117,250	1.17	50.00	8.6	66%
09BCZ026	2568	red	3	27/3/13	14.26	102,200	99,800	102,400	118,400	105,700	1.06	50.00	2.9	67%
06LAN065	2420	red_1H	6	29/3/10	14.49	177,600	161,000	183,600	175,800	174,500	1.75	50.00	37.3	47%
06VIL220B	2438	green	4	19/1/11	14.15	252,400	239,800	224,000	237,800	238,500	2.39	50.00	69.3	62%
09BCZ108	2569	green_1H	4	28/5/15	14.25	543,200	557,400	537,000	546,600	546,050	5.46	50.00	223.0	85%
06LAN067	2421	violet	4	1/4/10	14.45	121,800	116,800	127,000	138,000	125,900	1.26	50.00	13.0	53%
06VIL226	2435	violet_1H	3	25-Mar	14.54	403,600	393,400	372,400	388,200	389,400	3.89	50.00	144.7	65%
09BCZ170	2703	violet_2H	3	22/3/10	15.10	316,800	317,600	317,800	320,000	318,050	3.18	50.00	109.0	73%

Annex IV (Continuation): Table of preparation of strains inoculum of *Venturia inaequalis* for inoculation (50 ml - 100000 sp/ml)

Series O1

strain	Orchard treatment	color	nb cellos	time	comptage1	comptage2	comptage3	comptage4	moyenne	D	Vol initial	Vol eau à ajouter	% Germination
18BCZ042	Bion+Light IPM	white	3	9	101,400	101,600	108,000	114,400	106,350	1.06	50.00	3.2	63%
18BCZ009	Light IPM	white_1H	3	9	198,600	196,000	200,600	206,200	200,350	2.00	50.00	50.2	84%
18BCZ013	Bion+Light IPM	white_2H	3	9.15	121,600	112,000	107,000	108,800	112,350	1.12	50.00	6.2	70%
18BCZ016	Light IPM	blue	2	9.3	106,400	109,200	111,800	106,800	108,550	1.09	50.00	4.3	76%
18BCZ015	Bion+Light IPM	blue_1H	2	10	152,800	163,200	160,600	160,800	159,350	1.59	50.00	29.7	75%
18BCZ020	Light IPM	yellow	2	10.10	113,800	107,400	105,000	105,400	107,900	1.08	50.00	4.0	72%
18BCZ017	Bion+Light IPM	yellow_1H	4	10.15	122,000	136,000	122,200	111,400	122,900	1.23	50.00	11.5	75%
18BCZ022	Light IPM	yellow_2H	2	10.20	177,400	162,000	178,200	175,800	173,350	1.73	50.00	36.7	60%
18BCZ019	Bion+Light IPM	orange	2	10.40	113,400	115,600	113,200	123,400	116,400	1.16	45.00	7.4	55%
18BCZ025	Light IPM	orange_1H	2	11	90,200	98,000	96,600	100,000	96,200	0.96	50.00	-1.9	78%
18BCZ023	Bion+Light IPM	orange_2H	2	11.10	115,000	106,800	114,200	114,000	112,500	1.13	50.00	6.3	79%
18BCZ027	Light IPM	pink	3	13	117,000	124,000	123,800	128,600	123,350	1.23	50.00	11.7	74%
18BCZ029	Bion+Light IPM	pink_1H	2	13.05	179,200	180,000	196,600	186,400	185,550	1.86	50.00	42.8	83%
18BCZ032	Light IPM	red	2	13.2	135,000	132,600	124,600	125,000	129,300	1.29	50.00	14.7	70%
18BCZ033	Bion+Light IPM	red_1H	3	13.3	117,600	123,200	105,600	116,000	115,600	1.16	50.00	7.8	67%
18BCZ038	Light IPM	green	3	13.45	112,200	112,200	100,000	114,200	109,650	1.10	50.00	4.8	63%
18BCZ039	Bion+Light IPM	green_1H	3	14.25	117,200	120,600	122,400	121,200	120,350	1.20	50.00	10.2	68%
18BCZ010	Light IPM	violet	3	14	130,400	124,000	124,400	115,200	123,500	1.24	50.00	11.8	90%
18BCZ028	Bion+ Light IPM	violet_1H	3	14.3	130,800	125,000	131,000	136,200	130,750	1.31	50.00	15.4	80%
18BCZ037	Light IPM	violet_2H	3	14.55	111,800	105,000	101,200	106,000	106,000	1.06	50.00	3.0	80%

Series O2

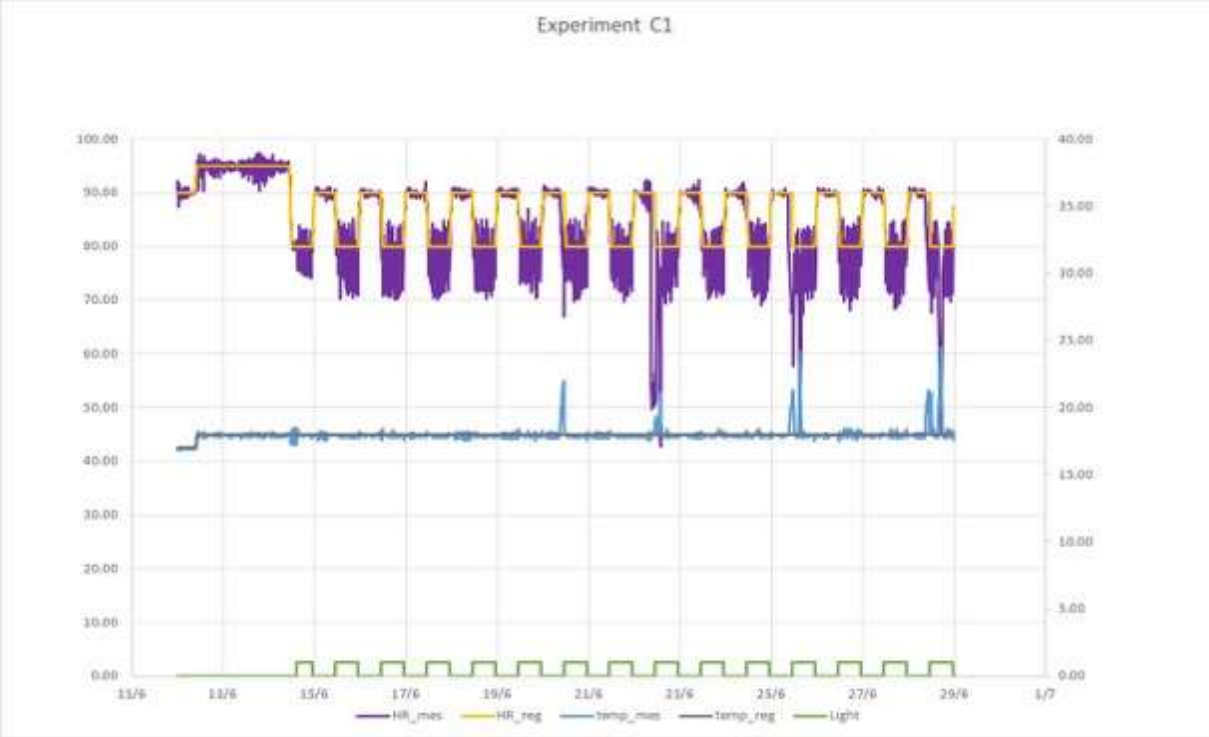
strain	Orchard treatment	color	nb cellos	time	comptage1	comptage2	comptage3	comptage4	moyenne	D	vol initial	eau à ajouter	% Germination
18BCZ021	Bion+Light IPM	white	4	8.59	120,400	118,000	113,000	109,000	115,100	1.15	50.00	7.6	70%
18BCZ008	Light IPM	white_1H	4	9.30	129,000	118,000	125,000	122,000	123,500	1.24	50.00	11.8	62%
18BCZ030	Bion+Light IPM	white_2H	4	9.48	177,000	185,000	197,000	191,000	187,500	1.88	50.00	43.8	72%
18BCZ011	Light IPM	blue	3	9.35	110,600	122,200	123,400	111,600	116,950	1.17	50.00	8.5	50%
18BCZ034	Bion+Light IPM	blue_1H	4	10	105,000	106,000	112,000	102,000	106,250	1.06	50.00	3.1	70%
18BCZ012	Light IPM	yellow	3	9.5	182,000	172,600	191,200	182,200	182,000	1.82	50.00	41.0	59%
18BCZ040	Bion+Light IPM	yellow_1H	3	9.55	134,600	130,600	143,800	137,200	136,550	1.37	50.00	18.3	70%
18BCZ018	Light IPM	yellow_2H	3	10.30	134,000	130,000	129,000		131,000	1.31	50.00	15.5	67%
18BCZ041	Bion+Light IPM	orange	4	10.51	114,000	95,800	112,800	96,600	104,800	1.05	45.00	2.2	49%
18BCZ043	Light IPM	orange_1H	3	10.4	147,800	131,800	147,200	143,600	142,600	1.43	50.00	21.3	60%
18BCZ007	Bion+Light IPM	orange_2H	3	10.4	331,800	333,600	290,600	303,400	314,850	3.15	40.00	85.9	80%
18BCZ047	Light IPM	pink	5	11.50	154,000	154,400	145,000	143,200	149,150	1.49	50.00	24.6	57%
18BCZ046	Bion+Light IPM	pink_1H	3	13.	131,600	137,200	140,600	142,600	138,000	1.38	50.00	19.0	62%
18BCZ049	Light IPM	red	3	13.10	101,400	106,000	103,200	107,000	104,400	1.04	50.00	2.2	67%
18BCZ048	Bion+Light IPM	red_1H	3	13.20	168,800	169,000	173,800	163,200	168,700	1.69	50.00	34.4	65%
18BCZ051	Light IPM	green	3	13.25	155,000	156,000	166,000	156,000	158,250	1.58	50.00	29.1	73%
18BCZ050	Bion+Light IPM	green_1H	3	13.40	128,000	120,600	125,600	125,800	125,000	1.25	50.00	12.5	80%
18BCZ055	Light IPM	violet	3	13.5	132,800	124,800	133,000	128,600	129,800	1.30	50.00	14.9	70%
18BCZ052	Bion+Light IPM	violet_1H	3	14	198,000	212,000	197,600	212,800	205,100	2.05	50.00	52.6	77%
18BCZ053	Light IPM	violet_2H	5	14.35	206,600	198,000	190,400	202,000	199,250	1.99	50.00	49.6	80%

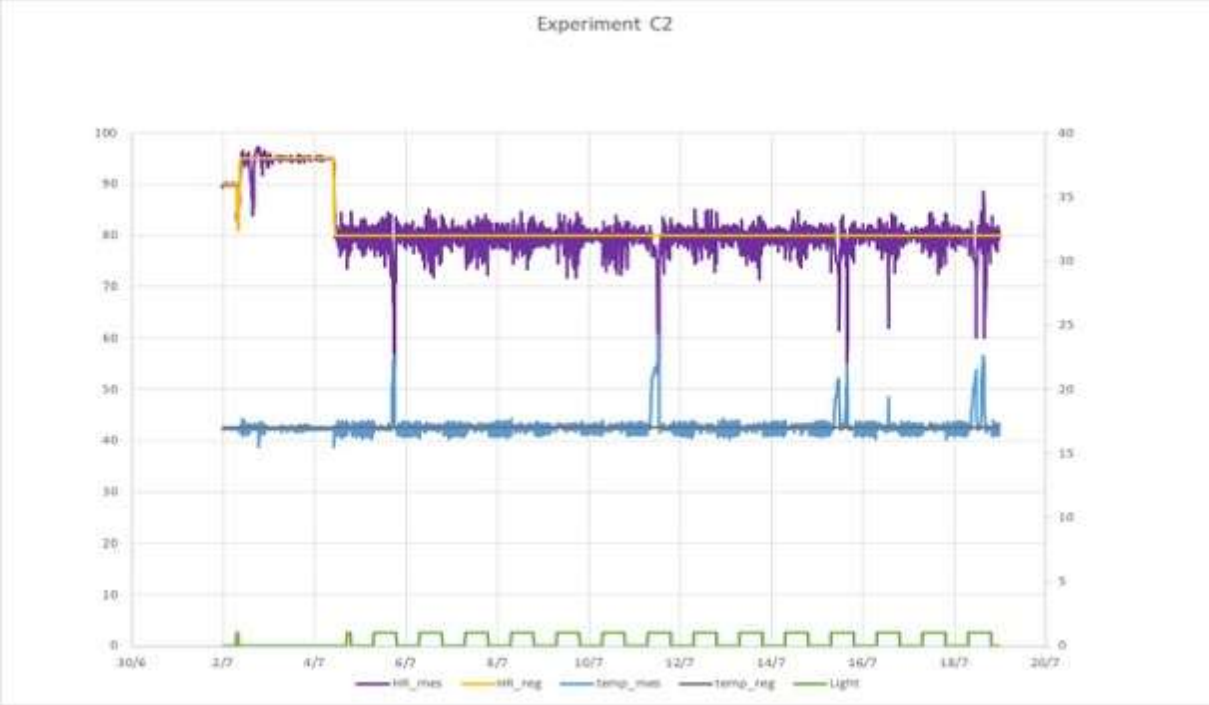
Annex IV (Continuation): Table of preparation of strains inoculum of *Venturia inaequalis* for inoculation

(Series O3)

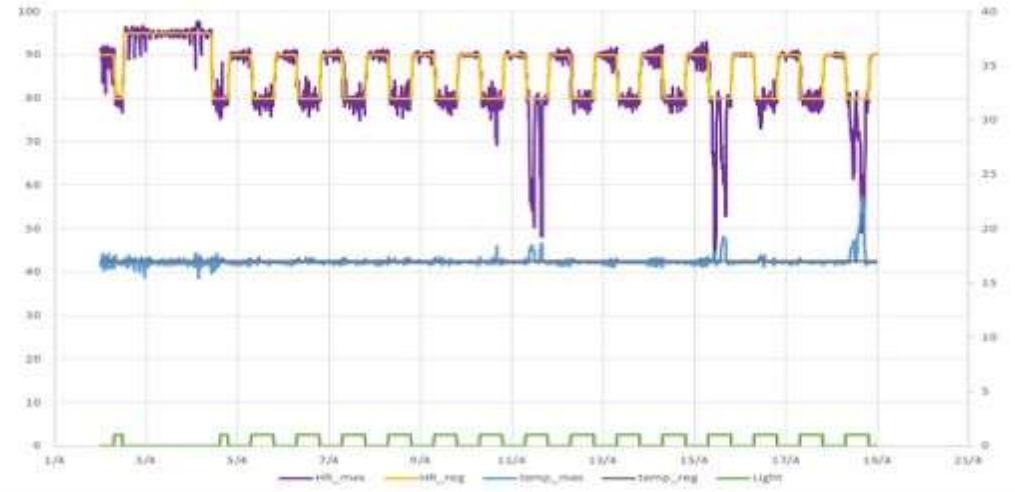
strain	Orchard treatment	color	nb cellos	time	comptage 1	comptage 2	comptage3	comptage4	D	vol initial	à ajouter	% Germination
18BCZ054		white	3	8.4	146,200	146,000	130,200	143,800	1.42	50.00	20.8	77%
18BCZ057	Light IPM	white_1H	5	9.04	169,800	156,600	150,400	159,600	1.59	50.00	29.6	90%
18BCZ056	Bion+Light IPM	white_2H	4	9.00	212,200	225,600	222,000	218,000	2.19	45.00	53.8	72%
18BCZ058	Light IPM	blue	5	9.47	159,800	176,000	167,000	170,600	1.68	50.00	34.2	69%
18BCZ061	Bion+Light IPM	blue_1H	5	9.30	187,200	198,000	201,000	183,400	1.92	50.00	46.2	76%
18BCZ062	Light IPM	yellow	5	9.57	231,200	240,600	235,000	216,000	2.31	50.00	65.4	83%
18BCZ065	Bion+Light IPM	yellow_1H	3	9.45	112,600	105,200	110,800	109,400	1.10	47.00	4.5	79%
18BCZ063	Light IPM	yellow_2H	5	10.05	331,200	329,800	359,200	369,200	3.47	45.00	111.3	82%
18BCZ067	Bion+Light IPM	orange	3	10.22	192,600	194,400	204,600	203,400	1.99	45.00	44.4	81%
18BCZ070	Light IPM	orange_1H	3	10.47	226,200	228,800	228,800	225,800	2.27	50.00	63.7	85%
18BCZ069	Bion+Light IPM	orange_2H	3	12.40	237,200	239,600	247,800	237,000	2.40	50.00	70.2	78%
18BCZ071	Light IPM	pink	3	12.4	137,600	135,000	144,800	146,800	1.41	45.00	18.5	75%
18BCZ073	Bion+Light IPM	pink_1H	4	13.00	188,800	186,600	194,800	209,600	1.95	50.00	47.5	74%
18BCZ072	Light IPM	red	4	13.05	190,400	186,200	179,000	188,800	1.86	50.00	43.1	89%
18BCZ074	Bion+Light IPM	red_1H	4	13.20	127,800	129,200	129,600	126,800	1.28	50.00	14.2	83%
18BCZ060	Light IPM	green	3	13.2	144,600	143,800	145,800	140,400	1.44	50.00	21.8	60%
18BCZ075	Bion+Light IPM	green_1H	4	1301	171,600	177,000	162,000	168,400	1.70	50.00	34.9	88%
18BCZ066	Light IPM	violet	3	13.45	119,400	118,600	104,400	117,000	1.15	50.00	7.4	86%
18BCZ071	Bion+ Light IPM	violet_1H	5	1356	207,400	219,000	195,200	194,200	2.04	50.00	52.0	70%
18BCZ068	Light IPM	violet_2H	4	13.54	183,400	170,000	179,800	172,800	1.77	45.00	34.4	73%

Annex V: Climatic conditions during the five series of experiments

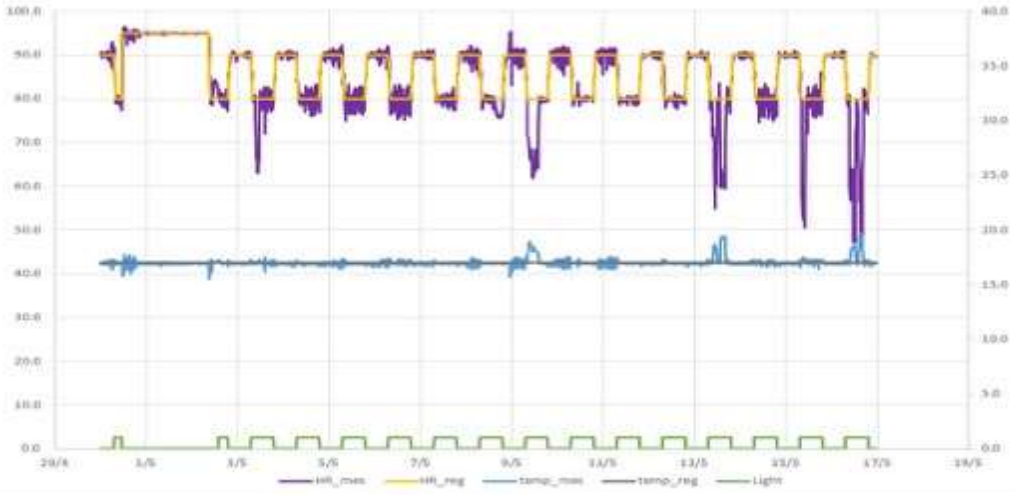




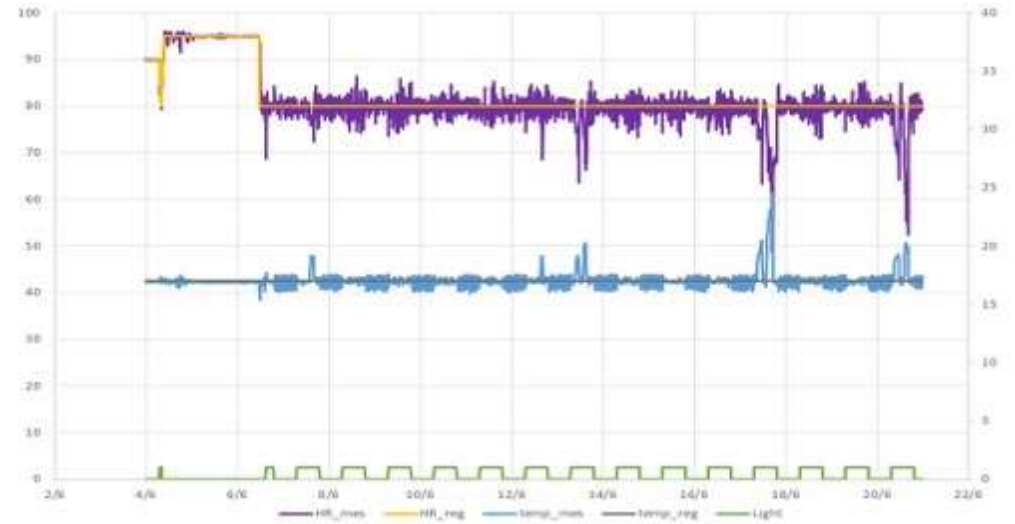
Experiment O1



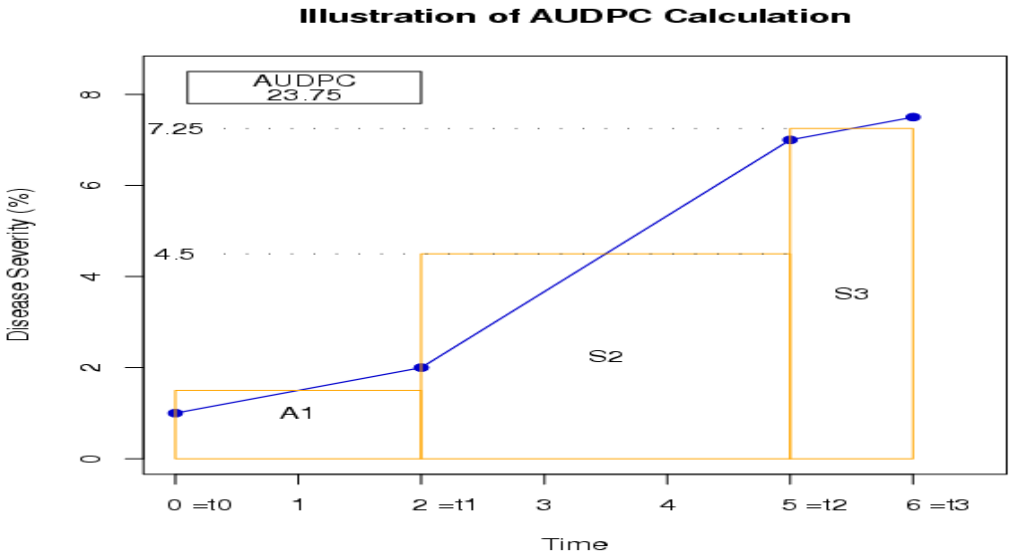
Experiment 02



Experiment O3



Annex VI : An illustration of Area Under Disease Progress Curve(Photo : APS publication website)
(https://www.apsnet.org/edcenter/disimpactmngmnt/topc/EcologyAndEpidemiologyInR/DiseaseProgress/Pages/audpc.aspx)



Nom et Prénom de l'auteur: Jenny Farhana
Titre du mémoire: Effet des stimulateurs de défenses des plantes sur l'évolution des populations de *Venturia inaequalis* – conséquences sur la durabilité
Ecole d'inscription: Agroparistech
Lieu du stage : IRHS, INRA, 42 rue Georges Morel – CS 60057, 49071 Beaucouzé cedex – France.

Résumé:
La tavelure du pommier est une maladie importante sur le plan commercial et pour lutter contre cette maladie, beaucoup de fongicides sont utilisés dans les vergers. Les gens s'inquiètent maintenant de l'utilisation de fongicides et de ses effets sur la santé humaine et l'environnement. Les inducteurs de la défense des plantes peuvent constituer une solution pour réduire l'utilisation de fongicides; en particulier l'Acibenzolar-S-méthyl (matière active du Bion). Cependant, aucune étude n'a été réalisée pour évaluer les effets de l'ASM sur une gamme de souches et définir si l'utilisation répétée d'ASM entraîne la sélection de souches moins sensibles à l'ASM. Pour répondre à cette question de durabilité, des expériences ont été menées en conditions contrôlées sur 84 souches de *Venturia inaequalis*: 34 provenant de vergers non traités, 30 souches provenant d'un verger géré en Protection Fruitière Intrégrée (PFI) allégée et 30 souches provenant d'un verger combinant PFI allégée et traitements au l'ASM. Chaque souche a été inoculée sur 8 plantes traitées au l'ASM et 8 plantes traitées à l'eau. La gravité de la maladie a été observée sur chaque plante de 7 à 16 jours après l'inoculation. Une large gamme de variation de la gravité de la maladie, autrement dit de l'agressivité des souches, a été constatée. L'ASM est efficace pour la plupart des souches. Cependant, cette efficacité est rès variable en fonction de la souche, et quelques souches ont montré un effet non significatif du l'ASM. La plupart d'entre elles ont été échantillonnées dans des vergers non traités au l'ASM, une seule souche a été échantillonnée dans un verger traité à l'ASM. Ce résultat suggère que malgré l'existence de souches peu sensibles à l'ASM, ces souches n'ont pas été sélectionnées par une utilisation répétée de l'ASM dans un verger géré en PFI allégée. Davantage d'études et de données sont nécessaires pour comprendre ce processus.

Mots-clés: Tavelure du pommier, *Venturia inaequalis*, Acibenzolar-S-methyl, Durabilité, Stimulateurs de défense

Summary:
Apple scab is a commercially important disease and to control this disease a large amount of fungicides is used in orchards. People are now concern against fungicide use and its effect on human health and environment. In addition to other control methods, plant defense inducers may be a solution for reducing fungicide use; particularly Acibenzolar-S-Methyl (active ingredient of Bion product). However, no study has been done to see effects of ASM to a vast range of strains and if repeated use of ASM results in selection of strains that are less sensitive to ASM. To answer this question of sustainability experiments were conducted in controlled condition on 94 strains of *Venturia inaequalis*: 34 from untreated orchards, 30 strains from an orchard with light Integrated Pest Management (IPM) and 30 strains from an orchard with light IPM and ASM treatments. Each strain was inoculated on 8 ASM and 8 water- treated plants. Disease severity was observed on each plant from 7 to 16 days after inoculation. Wide range of variation of disease severity in other words aggressiveness of strains was found. ASM was effective to most of the strains. However, the efficiency of ASM was very variable depending of the strain, and a few strains showed non-significant effect of ASM. Most of them were sampled from non ASM treated orchards, only one strain was sampled from ASM treated orchard. This result suggests that despite the existence of strains that have a low sensitivity to ASM, these strains were not selected by repeated use of ASM in an orchard managed in light IPM. More study and data are needed to understand this process.

Keywords: Apple scab, *Venturia inaequalis, Acibenzolar*-S-methyl, Sustainability, plant defense stimulaotrs