

## Impact of climate change on plant-aphid interactions

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# **IMPACT OF CLIMATE CHANGE ON PLANT-APHID INTERACTIONS**

**FLORENCE WILLISCOTTE**

**TRAVAIL DE FIN D'ÉTUDES PRÉSENTÉ EN VUE DE L'OBTENTION DU DIPLÔME DE  
MASTER BIOINGÉNIEUR EN SCIENCES AGRONOMIQUES**

**ANNÉE ACADÉMIQUE 2018-2019**

**PROMOTEUR: PR. FRANÇOIS VERHEGGEN**

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## Abstract

As concluded by the Intergovernmental Panel on Climate change (IPCC), the atmospheric carbon dioxide (CO<sub>2</sub>) concentration is predicted to double in the next decades. In addition, the mean surface temperature will rise by two degrees and the rain events will be less frequent. The impact of climate change is mainly investigated at Earth and ecosystems scale, with fewer studies conducted on trophic interactions. By assessing the population dynamics of the English grain aphid, *S. avenae*, on wheat in large chambers (ECOTRON, Terra, ULiege), we found that the aphids infestation levels will likely be lower in 2094 compared to 2015. We also found that elevation in temperature and CO<sub>2</sub> will impact negatively aphid life history traits such as their fitness, size and weight. Host plant selection by apterous parthenogenic females is not affected by an elevation in temperature and CO<sub>2</sub> concentration even if the volatile profile of winter wheat is affected by those increases. In the future, aphids will migrate at an earlier phenological stage of winter wheat which may lead to a greater impact on yield. As aphids will be noticed earlier on wheat ears, it may help farmers to better manage their crops.

**Keywords:** climate change, CO<sub>2</sub> concentration, temperature, ECOTRON, performances, migration, plant selection

## Résumé

Comme l'a conclu le Groupe d'experts intergouvernemental sur l'évolution du climat (GIEC), la concentration atmosphérique du dioxyde de carbone (CO<sub>2</sub>) devrait doubler au cours des prochaines décennies. De plus, la température moyenne en surface augmentera de deux degrés et les épisodes pluvieux seront moins fréquents. L'impact du changement climatique est principalement étudié à l'échelle de la Terre et des écosystèmes, avec peu d'études menées sur les interactions trophiques. En évaluant la dynamique de population du puceron des épis, *S. avenae*, sur le froment dans de larges enceintes (ECOTRON, Terra, Uliege), nous avons constaté que les niveaux d'infestation des pucerons seront probablement inférieurs en 2094 par rapport à 2015. Nous avons également constaté que l'élévation de la température et du CO<sub>2</sub> impacteront négativement les caractéristiques de vie des pucerons telles que leur reproduction, taille et poids. La sélection de plantes hôtes par des femelles parthénogéniques aptères n'est pas impactée par l'augmentation de la température et de la concentration en CO<sub>2</sub> même si le profil volatile du froment d'hiver est modifié par ces augmentations. A l'avenir, les pucerons migreront à un stage phénologique plus précoce du blé d'hiver, ce qui pourrait avoir un impact plus grand sur le rendement. Comme les pucerons seront observés plus tôt sur les épis de froment, cela pourrait aider les agriculteurs à mieux gérer leurs cultures.

**Mots-clés:** changement climatique, concentration en CO<sub>2</sub>, température, ECOTRON, performances, migration, sélection de plantes

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## Abbreviations list

AR5 = Fifth assessment report

BLRV = Bean leaf roll virus

BYDV= Barley yellow dwarf virus

BYMV = Bean yellow mosaic virus

CH<sub>4</sub> = Methane

CNS = Central nervous system

CO<sub>2</sub> = Carbon dioxide

CRA-w = Centre wallon de recherches agronomiques

EPA = Environmental protection agency

FAO = Food and agriculture organisation

GC-MS = Gas chromatography - mass spectrometer

GHG = Greenhouse gases

GIEC = Groupe d'experts intergouvernemental sur l'évolution du climat

GLM = General linear model

GtCO<sub>2</sub> = Gigaton of CO<sub>2</sub>

IPCC = Intergovernmental panel on climate change

LED = Light-emitting diode

MPB = Mountain pine beetle

NO<sub>2</sub> = Nitrous oxide

ORNs = Olfactory receptor neurons

PAR = Photosynthetically active radiation

PCA = Principal component analyses

PPM = Parts per million

PPMV = Parts per million by volume

PPTV = Parts per trillion by volume

RCP = Representative concentration pathways

RH = Relative humidity

RMI = Royal meteorological institute

Si = Silicon

Sqm = Square per meter

TNCS = Total non-structure carbohydrates

UNFCC = United Nations framework convention on climate

VOCs = Volatile organic compounds

W/M<sup>2</sup> = Watts per square meter

WMO = World mondial organisation

# 1. Introduction

## 1.1 Climate change

Since the preindustrial area, economic and demographic growth led to anthropic emissions of greenhouse gases such as methane (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>) and nitrous oxide (NO<sub>2</sub>) (GIEC, 2014). These gases are naturally present in the atmosphere and play a beneficial role in maintaining an adequate temperature, 15°C, for humans and ecosystems on Earth. Indeed, without the greenhouse gas effect, the terrestrial temperature would be -18°C (Guiot, 2017). However, the increase of atmospheric greenhouse gases concentrations is the main source of global warming on Earth (GIEC, 2014) and will lead to long term changes in overall climatic system components. These long term changes will induce severe and irreversible impacts for humankind and the ecosystem stability (Guiot, 2017).

Since 1958, atmospheric carbon dioxide concentration has increased from 320 ppmv (parts per million by volume) to more than 400 ppmv, which means that this concentration has risen up to 40% in one century. Other greenhouse gases are following similar increase. For example, methane concentration went from 650 to 1800 pptv (parts per trillion by volume) since the preindustrial area, while nitrous oxide has known a smaller increase from 270 to 325 ppmv for the same period of study (Guiot, 2017).

### 1.1.1 Definition

Climate change can be defined in many ways. Two definitions are particularly relevant for the work carried out in this study. According to UNFCCC<sup>1</sup>, climate change is “a change of climate which is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and which is in addition to natural climate variability observed over comparable time periods”. A distinction is made by the UNFCCC between two main concepts: “climate change” and “climate variability”. The UNFCCC associates “climate change” with human activities which modify the atmospheric composition, whilst “climate variability” is associated with natural causes (Metz and Davidson, 2007).

The Intergovernmental Panel on Climate change (IPCC<sup>2</sup>) identifies climate change as “a change in the state of the climate that can be identified (e.g., using statistical tests) by changes in the mean and/or the variability of its properties, and that persists for an extended period, typically decades or longer. Climate change may be due to natural internal processes or external forcings, or to persistent anthropogenic changes in the composition of the atmosphere or land use” (GIEC, 2014).

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<sup>1</sup> UNFCCC is a secretariat which provides technical expertise and helps in the analysis and review of climate change information reported by Parties and in the implementation of the Kyoto mechanisms.

<sup>2</sup> IPCC is a governmental organization which provides, based on scientific information, regular assessments of climate change, its impacts and future risks. Moreover, this organization helps to find options for adaptation, mitigation and climate change policies.

According to Jubb, Canadell and Dix, 2013, “ the pathways are characterised by the radiative forcing produced by the end of the 21<sup>st</sup> century”. The radiative forcing is the variation of energetic flow caused by an unbalance between the solar radiation and the greenhouse gases emissions to the atmosphere. The forcing is measured in Watts per square meter ( $W/m^2$ ) (GIEC, 2014).

### 1.1.2 Global forecasts: scenarios

In order to forecast the evolution of climate change, IPCC has established different scenarios of climatic models and socio-economic developments which provide futuristic screenings. These scenarios are called Representative Concentration Pathways (RCP) (Van Vuuren *et al.*, 2011) and are based on chronological series of emissions and concentrations of the overall greenhouse gases (GHG), aerosols, chemically active gases as well as land use (Moss *et al.*, 2010). Each RCP represents the 1850-2100 period and is followed by the forecast radiative forcing. For instance, RCP4.5 is represented by a pathway which forecasts a radiative forcing of  $4.5 W/m^2$ .

**RCP2.6** represents an optimistic and ambitious scenario in which the GHG emissions should be drastically reduced in order to maintain the global temperature below two degrees relative to the preindustrial area (1850-1900) (Guiot, 2017). As Figure 1 shows, the GHG emissions for this pathway may peak at a radiative forcing of  $3 W/m^2$  before 2100 and then may fall due to negative emissions induced by the removal of greenhouse gases especially carbon dioxide from the atmosphere (Jubb, Canadell and Dix, 2013) and will remain constant after 2100 (GIEC, 2014). Cumulative  $CO_2$  emissions for a 2010-2100 period will range from 510 to 1505 Gt $CO_2$  (Gigaton) (IPCC, 2013). Model simulations have projected that global mean surface temperature will be  $1.7^\circ C$  for the 2081-2100 period compared to  $0.3^\circ C$  for the preindustrial period. Upper two degrees, global temperature could negatively impact the survival and adaptation of a high number of living organisms (Bador, 2017).

**RCP4.5** and **RCP6.0** are the most probabilistic scenarios if some governmental actions are taken to reduce GHG emissions. These scenarios are known as intermediate pathways with radiative forcing between  $4.5 W/m^2$  and  $6.0 W/m^2$  after 2100. Cumulative  $CO_2$  emissions will range from 2180 to 3690 Gt $CO_2$  and from 3080 to 4585 Gt $CO_2$  for 2100, respectively (IPCC, 2013). In these two scenarios, GHG concentrations are supposed to be constant after 2150 (Figure1) (GIEC, 2014). Global surface temperature may not exceed  $2^\circ C$  for RCP4.5 at the end of the 21<sup>st</sup> century with an increase from  $1.1$  to  $2.6^\circ C$  between preindustrial area and 2100, according to IPCC. On the contrary, according to the RCP6.0 scenario, the global surface temperature will likely exceed  $2^\circ C$  for the same century and will rise from  $1.4$  to  $3.1^\circ C$ .

**RCP8.5** refers to the worst scenario with a radiative forcing reaching  $8.5 W/m^2$  in 2100. This pathway is known as the “business as usual” pathway as it forecasts the highest warming by considering a continuous increase of GHG emissions after 2100 (Figure 1) and even after 2500 (GIEC, 2014). Cumulative  $CO_2$  emissions for a 2010 - 2100 period will range from 5185



to 7005 GtCO<sub>2</sub> (IPCC, 2013). Global surface temperature could increase from 2.6°C to 4.8°C in this scenario with potential damages similar to a glacial-interglacial transition (Guiot, 2017).

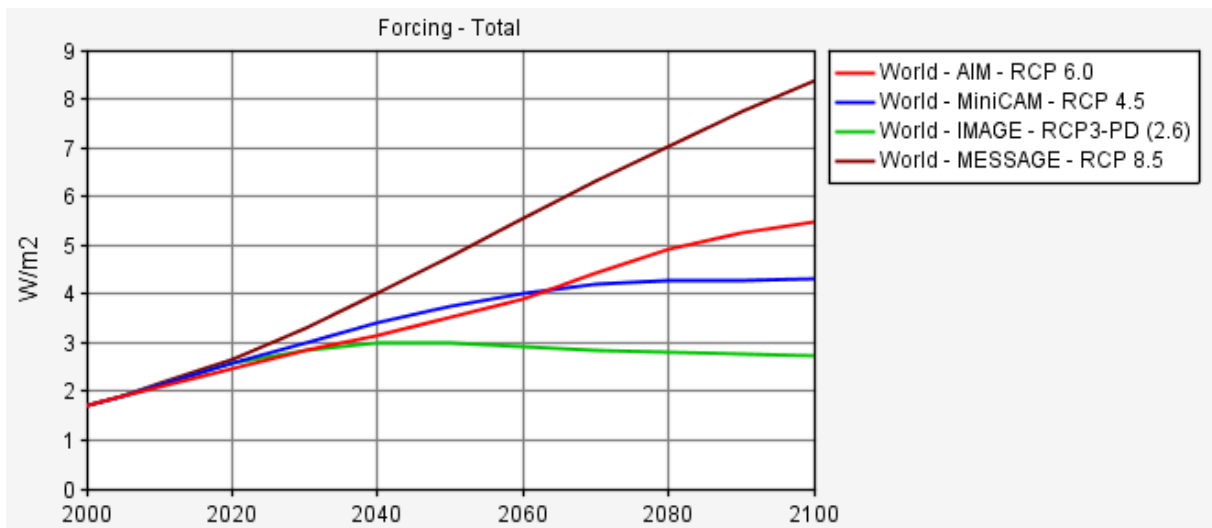


Figure 1 - Radiative forcing of total GHG for the 2000-2100 period (RCP Database, 2008)

## 1.2 Main climatic parameters

In order to better understand how the interaction between insects and ecosystems will evolve, it is interesting to focus on three main climatic parameters which are CO<sub>2</sub> concentration, temperature and precipitation.

### 1.2.1 Carbon dioxide (CO<sub>2</sub>)

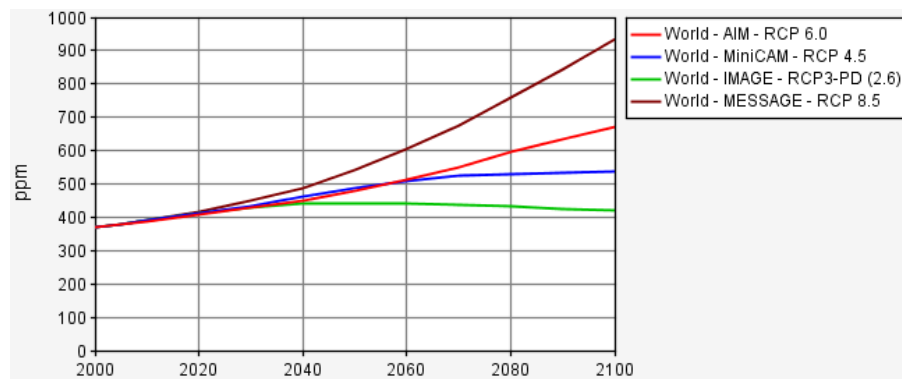
Carbon dioxide (CO<sub>2</sub>) is the main greenhouse gas (GHG) produced by anthropogenic activities (about 90% in 2015). In 2014, the total carbon dioxide emissions per habitant represented about 8 tons of CO<sub>2</sub> in Belgium (Mondial Bank). Increase in GHG (particularly CO<sub>2</sub>) contributes to an increase in global surface temperature, leading to weather patterns instabilities such as precipitation frequency and quantities as well as episodes of extreme weather such as heat waves (Ziska and McConnell, 2016). As discussed below, CO<sub>2</sub> concentration has a huge impact on interactions between insects and plants particularly on phenology, distribution and competition (Bjorkman and Niemela, 2015).

The increase of global atmospheric carbon dioxide (CO<sub>2</sub>) comes mainly from human activities like fossil fuel burning, cement production and the altered landscape patterns (mainly deforestation). The CO<sub>2</sub> anthropogenic emissions had risen of about 2040 GtCO<sub>2</sub> from 1750 to 2011. About 40% of those emissions are stocked in the atmosphere, 30% are stocked in the soil and trapped by the vegetation, and 30% are absorbed by the ocean and contribute to its acidification. Nowadays, CO<sub>2</sub> emissions are still rising due to the demographic and economic growth mainly associated with the use of fossil fuels (GIEC, 2014). According to the

World Meteorological Organization (WMO)<sup>3</sup>, the global average concentration in CO<sub>2</sub> has increased up to 39% from 1750 to 2013 (Hakeem, 2015).

Furthermore, the level of CO<sub>2</sub> is expected to double by the end of the 21<sup>st</sup> century (Sun, Su and Ge, 2010). As Figure 2 below shows, the concentration of carbon dioxide in the atmosphere will increase differently according to the studied scenario.

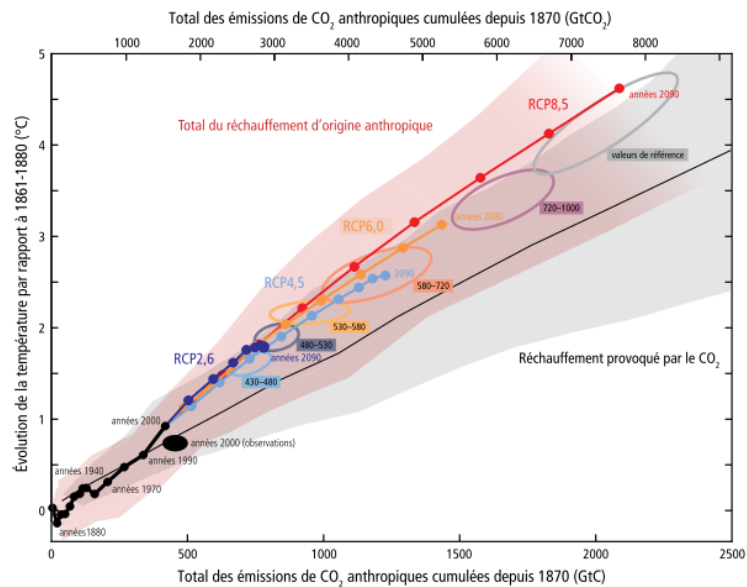
For RCP 2.6, the carbon dioxide concentration will reach around 400 ppm by 2100. The forecasts of RCP 4.5 for the carbon dioxide concentration are around 550 ppm at the end of the 21<sup>st</sup> century. Concerning the scenario RCP 6.0 which is used for the experimentations of the study, the concentration of CO<sub>2</sub> will be around 700 ppm for the same century. Finally, for the worst scenario, RCP 8.5, at the end of the 21<sup>st</sup> century, the CO<sub>2</sub> concentrations will reach around 900 ppm.



[Figure 2 - Evolution of CO<sub>2</sub> concentration for the different models between 2000 and 2100 \(RCP Database, 2008\)](#)

According to the working group III of the IPCC, there is a quasi-linear relation between the cumulated emissions in CO<sub>2</sub> and the change in global mean surface temperature until the 21<sup>st</sup> century (Figure 3) (GIEC, 2014). The rise of atmospheric CO<sub>2</sub> has contributed to the increase of global average surface temperature by 0.6°C over the 20<sup>th</sup> century. Models estimated that the surface air temperature will scale up from 1.1 to 2.9°C in the 'low scenario case' or 2.4-6.4°C if considering the 'high scenario forecasts' at the end of the 21<sup>st</sup> century (Singh, 2009).

<sup>3</sup> WMO is an intergovernmental organization of the United Nations which deals with issues about weather and climate, water distribution and geophysical sciences

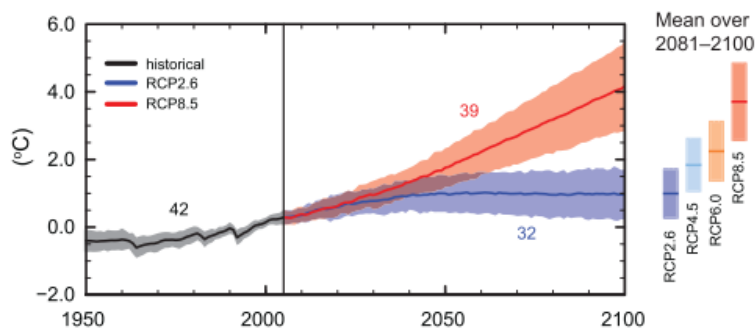


**Figure 3 - Evolution of temperature relative to the period from 1861 to 1880 with the total cumulated CO<sub>2</sub> emissions since 1870 (GtCO<sub>2</sub>) (GIEC, 2014)**

### 1.2.2 Temperature

Global mean temperature at Earth surface has increased by about 0.6°C since the preindustrial area and is predicted to increase more by the end of the 21<sup>st</sup> century. According to Auverlot, 2015, this temperature will increase to more than 1.5°C for three of the fourth IPCC scenarios, rising up from 2 to 6°C on average by 2100, between 0.15 to 0.6°C per decade (Houghton, 2005).

In order to forecast the evolution of global average surface temperature, two of the IPCC scenarios are mainly studied: the optimistic one (RCP 2.5) and the worst one (RCP 8.5). According to Figure 4, global average surface temperature will reach 1.7°C at the end of the 21<sup>st</sup> century in the more optimistic scenario. On the contrary, concerning the RCP 8.5, global average surface temperature will exceed 4°C at the end of the 21<sup>st</sup> century.



**Figure 4 - Global average surface temperature change for RCP 2.6 and RCP 8.5 between 1950 and 2100 (IPCC, 2013)**

Global warming will occur at different scales all over the world. Indeed, It will mainly take place in the Northern Hemisphere (high latitude and continental masses) and will be less important in the ocean than on land because of its higher inertial mass (Figure 5) (Royer, Dufresne and Braconnot, 2006). The reason for this higher warming on the Northern Hemisphere is the decrease of snow cover and sea ice extension. Therefore, there is an essential reduction of the reflection by solar radiation surface which induces a higher quantity of absorbed radiation on land, leading to an increase of temperature. Another

reason of this warming is the high transport of water vapour to those regions by atmospheric circulation (Royer, Dufresne and Braconnot, 2006).

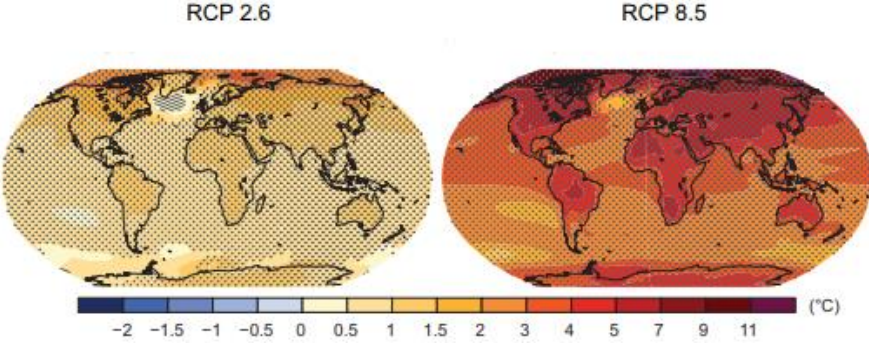


Figure 5 - Change in average temperature (1986-2005 to 2081-2100) for RCP 2.6 and RCP 8.5 (GIEC, 2014)

Moreover, it is nearly certain that both the frequency and duration of heat waves will scale up leading to more droughts and forest fires. Oceans will continue to warm up and acidify. Furthermore, the atmosphere will warm up as well as Earth. Its warming will lead to the glacier melting and then to the rise of the sea level. There will be less extreme cold temperatures and extreme precipitations will be more intense and frequent in a lot of regions (GIEC, 2014).

### 1.2.3 Precipitation

According to the fifth assessment report (AR5) of IPCC, changes in precipitation will not be the same around the world. In case of RCP 8.5 scenario, annual mean precipitation will rise particularly in high latitude and in the equatorial Pacific Ocean as well as in humid regions of mid-latitude (Figure 6) and will decrease in arid subtropical regions and mostly mid-latitude regions until the end of the century. Extreme episodes of precipitation will be more frequent and intense at mid-latitude and for humid tropical regions as the global temperature increases (GIEC, 2014). Concerning Europe, precipitations will be more critical in the North and will be less abundant around the Mediterranean basin which may cause a drying process of this area (Royer, Dufresne and Braconnot, 2006).

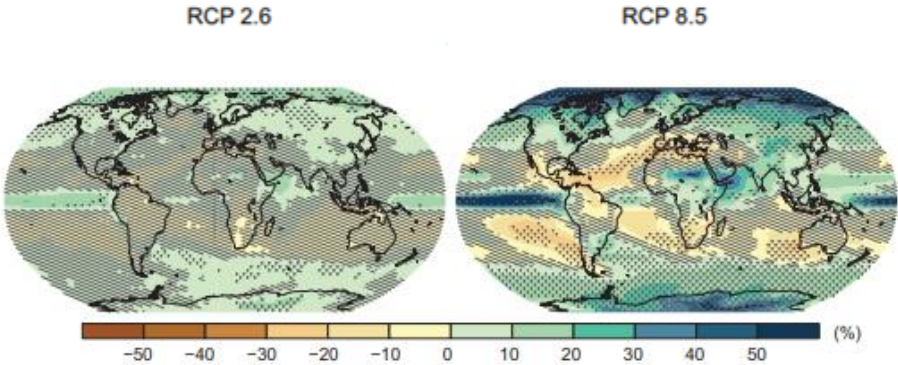


Figure 6 - Change in average precipitation (1986-2005 to 2081-2100) (IPCC, 2013)

### 1.3 Interaction between climate change, crop plants and insect pests

The consequences of global warming on crop production will likely be significant, including impacts on the growth, development and yield. Because of the rise of temperature, the irrigation demands will skyrocket. In addition, a shift in food growing areas will occur as well as changes in cultivars, planting dates and locations (Deutsch, Tewksbury and Tigchelaar, 2018). In warmer areas, crop yields will decrease due to the higher presence of pests, crops diseases and weeds (Hakeem, 2015). In order to decrease the curb of crop yield and maintain it, it may be necessary to introduce new crop rotations or improve management measures such as the rotation schedule, the use of pesticides and insecticides in order to avoid pest resistance (Deutsch, Tewksbury and Tigchelaar, 2018).

According to the Environmental Protection Agency (EPA)<sup>4</sup>, insect distributions are expected to be affected by climate change. Pests are expected to extend their distribution range (Porter, Parry and Carter, 1991), and climate change will disrupt their establishment, competitiveness and impact in crop systems.

Insect development is mainly depending on temperature and the length of winter season (van Asch and Visser, 2006; Jamieson *et al.*, 2012). If the temperature and precipitation are modified, it may be possible that the development rate of insects increases or decreases even if the environmental conditions are near their optimal range (Deutsch, Tewksbury and Tigchelaar, 2018). Higher temperatures lead to faster development rate by reducing the time to reproductive maturity which can increase the pest populations (Jamieson *et al.*, 2012). For example, if the winter temperatures are above the lethal range of a species, the species can survive better and produce more offspring, promoting earlier infestations of crops in spring and leading to substantial economic impacts (Bale *et al.*, 2002). In a warmer climate, insect populations will be more numerous (Porter, Parry and Carter, 1991; Deutsch *et al.* 2018) which will lead to earlier mass migrations and pests establishments at early crop growth stage. Moreover, the insects will be able to colonise a larger range of crops due to temperature increase (Porter and Xie, 2014).

For instance, *Dendroctonus ponderosae*, known as the mountain pine beetle (MPB), is an important forest pest in western North America. A study realised by Mitton and Ferrenberg, 2012 has shown that MPB outbreaks become larger at higher latitude and elevations than before. Other studies have determined that MPB is now able to reproduce twice per year, instead of one, because the temperature conditions are becoming more suitable to its life cycle. Consequently, the pest populations develop faster and have higher expansion range (Bentz *et al.*, 2010). Moreover, due to the increase in temperature, plants are more susceptible to insect infestations. Thus, the plant defensive system can be weakened which

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<sup>4</sup> EPA is an independent agency of the United States government which aims to protect human rights and the environment

enable pest outbreaks to occur more likely as the plant resistance to pest infestation dwindle (Porter, Parry and Carter, 1991).

Higher temperatures can also contribute to more generations of multivoltine<sup>5</sup> species of insects (Porter, Parry and Carter, 1991; Glazaczow, Orwin and Bogdziewicz, 2016). In addition, host plant crops range can be different, which may impact the insect distribution and spread. Thus, the dispersion of a pest in a crop at a new area will depend on some environmental conditions as for example the presence of overwintering sites, soil type and moisture availability. Host plants can also be more or less suitable for pests. For instance, the life cycle of weeds may be accelerated, and their density increased under higher temperatures which will provide a more suitable habitat for pests (Porter, Parry and Carter, 1991; Ziska and McConnell, 2016). Furthermore, higher CO<sub>2</sub> concentration can change the nutritional quality of host plants and then affect insect pest feeding (Porter, Parry and Carter, 1991). Because of the higher number of insects and the acceleration of their metabolic rate, the biomass consumption per time unit could be more important and could lead to decreasing crop yields (Jamieson *et al.*, 2012; Deutsch, Tewksbury and Tigchelaar, 2018). On the contrary, if winter temperatures are near lethal range, the insect proportion will be lower which limits the possibilities of outbreaks (Porter and Xie, 2014).

If global mean surface temperature increases by 2°C, the median increase in yield losses due to pest pressures is estimated at 46% for wheat which, at a total scale, corresponds to 59 metric megatons per year. Because the carrying capacity of temperate populations happens late in the growing season, crop yield losses will be more critical in temperate climate than in tropical regions as population size will increase with temperature (Deutsch, Tewksbury and Tigchelaar, 2018). For other parts of the world, the prognoses are different. In the lowland tropics, pest populations will probably decrease as the pests are already in their optimal temperature. In contrast, growth temperature will be near the optimal range for pests which will likely increase their population, as well as their diapause survival (Deutsch, Tewksbury and Tigchelaar, 2018)

To better understand the correlation between plant-aphid interactions and climate, two insect models, represented by *S. avenae*, the English grain aphid and *A. fabae*, the black bean aphid, are studied. The plant hosts of those insects, characterised by the winter wheat and the swamp bean are also analysed. Aphids are studied as they are economically damaging for cultivated plants.

## 1.4 Aphids

### 1.4.1 Description

Aphids are small herbivorous insects with a worldwide distribution. Those insects are biting-sucking hemipterans which are known as pests in agriculture mainly on cultivated cereal crops. Aphids can cause direct or indirect damages on crops mainly in spring (Honek *et al.*,

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<sup>5</sup> Is a species which is able to produce three or more generations per year

2017). They mainly lead to economic damages by feeding and removing plant sap from phloem sieve elements of cereals which diminish the plant strength and reduce the quality and quantity of grain (Perring *et al.*, 2018). They can also produce honeydew which is an aqueous excretory product mainly composed by sugar and amino acids (Leroy *et al.*, 2008). When honeydew is produced in large quantities on cereals, it can cover leaves which encourages the growth of fungal pathogens such as sooty mold and can decrease the photosynthesis activity (Perring *et al.*, 2018). Those economic damages can reach up to 50 % of yield losses (Ciss *et al.*, 2014).

Aphids can also transmit viruses to plants such as the barley yellow dwarf luteovirus (BYDV) which is an important barley disease, and a secondary one for wheat (Weppeler, 2009). Aphid species are vectors of almost 50% of viruses (Duffy, Fealy and Fealy, 2017).

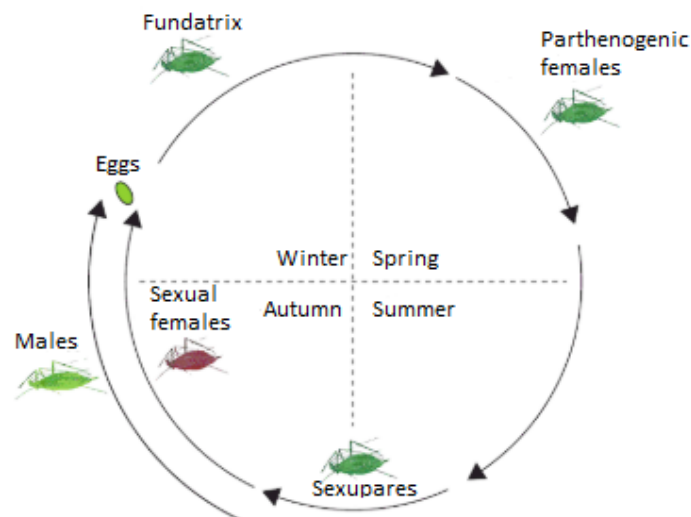
Aphids are dispersed on all parts of cereal shoots, however the most harmful ones are those which live inside the ears of cereals such as *Sitobion avenae* (F.) and *Rhopalosiphum padi* (L.) (Honek *et al.*, 2017). They can also be present on the soil and in the plants roots (Dixon, 1977). The aphid dispersion by winged migrant individuals is characterised by three peaks. Two main peaks occur in spring and summer and are most of the time indistinguishable. The last peak happens in autumn in order to overwinter (Reimer, 2004).

#### 1.4.2 Life cycle

Aphid species differ based on their life cycles. The most common one includes the holocyclic and anholocyclic life cycles. The holocyclic cycle refers to an alternation between asexual phase and sexual phase (Reimer, 2004). During summertime, asexual reproducing females, called “sexuparae”, give birth to sexual reproducing males and females, called oviparae, when perceiving a change in autumn temperature and photoperiod (Lees, 1989; Llewellyn *et al.*, 2003). At the end of autumn, sexual forms mate and lay eggs on host plants which will overwinter (Dedryver *et al.*, 1998; Llewellyn *et al.*, 2003). If the sexual forms lay eggs on the same host plant that the one asexual females feed on through the year, the species is called monoecious. On the contrary, if the sexual forms lay eggs on different host plants from the one asexual females feed on, the species is called heteroecious (Llewellyn *et al.*, 2003). Then, in the beginning of spring, the eggs hatch and give birth to fundatrix. Those fundatrix produce about a dozen of asexual larvae. After two weeks between 15°C and 20°C, asexual larvae reach the adult stage and are able to produce a new line of parthenogenic individuals (Turpeau-Ait Ighli *et al.*, 2011). Between spring and autumn, apterous and winged adult individuals are produced by parthenogenesis. Apterous adult morphs are mainly spread on host plant in situ. In contrast, winged adult individuals are responsible for the dispersion of the species and the exploitation of host plants at a larger scale. The dispersion of the species can occur at small scale by active flight (Llewellyn *et al.*, 2003; Ciss *et al.*, 2014) or at large scale by passive flight which is a flight supported by wind streams (Loxdale *et al.*, 1985; Hardie, 1993; Simon *et al.*, 1999; Ciss *et al.*, 2014). Apterous adult morphs will turn into winged adult morphs when environmental conditions are less favorable for apterous morphs such as the decrease in host plant quality and/or overcrowding (Newton *et al.*, 1987; Ciss *et*

*al.*, 2014). Winged adult morphs give rise to gendered females which in turn give birth to sexual clones, males and oviparous females (Figure 7) (Turpeau-Ait Ighli *et al.*, 2011).

The holocyclic cycle occurs mainly in regions where the winter is cold. Eggs can enter in diapause period and can be more resistant to cold temperature than in an anholocyclic cycle (Llewellyn *et al.*, 2003).



[Figure 7 - Holocyclic cycle of aphids \(Inspired from Turpeau-Ait Ighli \*et al.\*, 2011\)](#)

The anholocyclic cycle, also called obligate parthenogenesis, represents a cycle where clones do not respond to autumn temperatures and photoperiod (Llewellyn *et al.*, 2003) and overwinter in viviparous forms. The maintenance in an active form during winter allows the species to respond rapidly to the temperature increase in the beginning of spring. Thus, aphids can reach maturity and reproduce immediately after optimal temperatures (Duffy, Fealy and Fealy, 2017). This cycle is mainly present in regions where the winter is mild and the overwinter in an egg shape is not necessary (Dedryver *et al.*, 1998; Reimer, 2004).

Aphids are a relevant biologic model to study due to their high ability to produce a lot of clones, their fast reproduction and short life span (Reimer, 2004). Among all aphid species, *Sitobion avenae*, the main pest in winter wheat, is particularly interesting to study at climatic change scale (Turpeau-Ait Ighli *et al.*, 2011) as it causes huge economic damages on cereal crops. In addition, this species is qualified as a 'specialist' aphid. The second model, *A. fabae* is considered a 'generalist' aphid. It is, thus, interesting to study the climate change impact on a 'generalist' and 'specialist' species.



### 1.4.3 Model 1: *Sitobion avenae* (Fabricius)

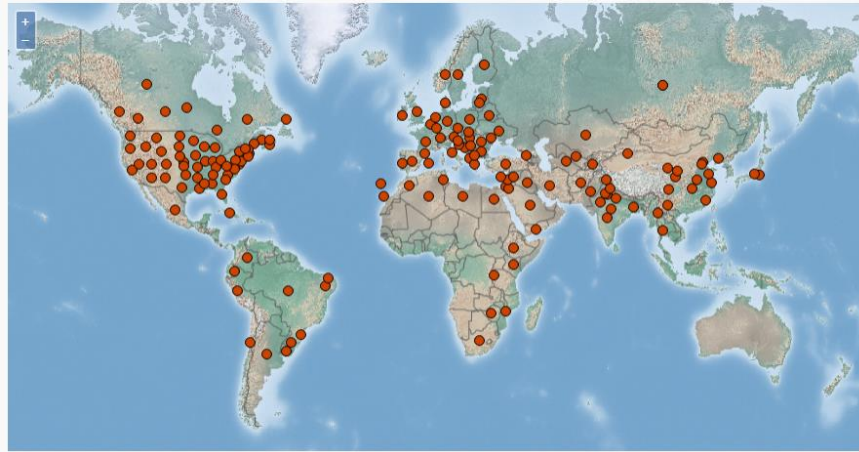
#### ❖ *Characteristics*

The wheat aphid, *S. avenae*, is a major pest in agricultural systems mainly in temperate regions of the Northern and Southern Hemisphere.

*S. avenae* belongs to the Aphididae family and is commonly called the English grain aphid (Reimer, 2004). As Weppler explains in his study in 2009, this species feeds on leaves, stalks and mainly on ears. During outbreaks, wheat yields can be diminished by up to 20-30%. In addition, the English grain aphid can impact the cereal harvest by reducing the number of heads, the number of grains per head and the seed weight. The main yield losses can be observed between ear emergence and flowering (Rautapää, 1966; Kolbe and Linke, 1969; Weppler, 2009). According to Turpeau-Ait Ighli *et al.*, 2011, during high infestation, this aphid can provoke a decline in grain number per ear and weight of 1000 grains. In direct damages, those cumulated effects can contribute to a yield loss of 25 q/acre (quintal/acre). After these growth stages, *S. avenae* is less harmful for wheat. However, it can alter the quality of flour for bread if it appears during grain ripening. Other indirect damages such as the accumulation of honeydew on wheat can be caused by the English grain aphid. This accumulation decreases photosynthesis (Rabbinge *et al.*, 1981), affects the physiological properties and causes chlorotic symptoms in leaves (Rossing and Van De Wiel, 1990). This species is also a vector of BYDV on wheat (Weppler, 2009). Because of the feeding of *S. avenae* on ears, the rachis and the base of spikelets, substantial yield losses may be observed mainly during the boot stage (Larsson, 2005). According to Voss *et al.*, 1997, 21% of yield loss were observed when the population density reached 300 aphids per plant, and 18% of loss when the population density reached 1200 aphids per plant.

#### ❖ *Distribution*

The English grain aphid is present worldwide and mainly in temperate regions. Thus, its presence can be mainly observed in Europe, North Africa, the Middle East and Asia. The aphid is less present in tropical regions and seems to be absent in Australia and New Zealand, as the world map below shows (Figure 8) (Weppler, 2009).



**Figure 8 - World distribution of *Sitobion avenae* (CABI, 2019)**

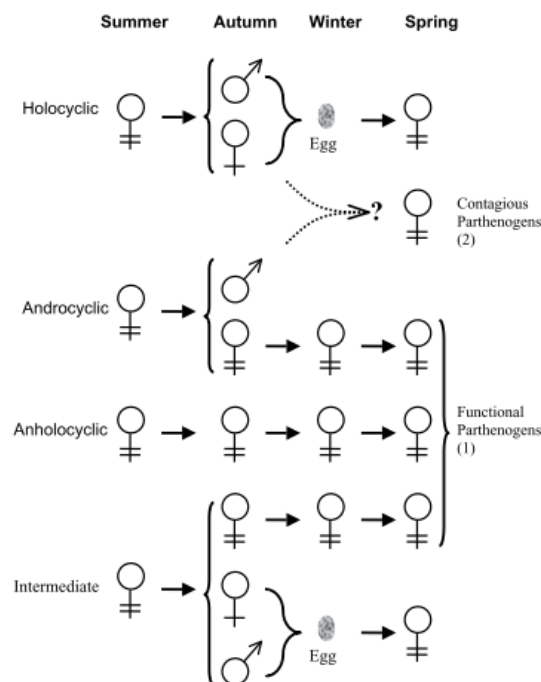
### ❖ *Biology*

*Sitobion avenae* (F.) is a ‘specialist’ species which feeds only on grasses and cereals belonging to the Poaceae family (Llewellyn *et al.*, 2003) such as barley, wheat, maize and rice (Ciss *et al.*, 2014). This species is considered as monoecious as it develops and produces generations of clones on the same host plant species or related host plant species (Llewellyn *et al.*, 2003; Weppler, 2009).

The wheat aphid has mainly a holocyclic reproduction especially after colder winters. Its life cycle starts in early winter when sexual females lay eggs on grasses (Ciss *et al.*, 2014), cereals culms or at the stalk base (Turpeau-Ait Ighli *et al.*, 2011). These eggs overwinter and hatch in early spring. After hatching, fundatrix (asexual females) emerge and produce a succession of instars (Figure 9). Those fourth instars lead to the development of two generations of asexual apterous (Turpeau-Ait Ighli *et al.*, 2011) and then winged adult individuals (Dixon, 1977) depending on the environmental conditions. If the host quality decreases and/or there is overcrowding, winged adult morphs are produced instead of apterous adult nymphs in order to foster the migration to winter wheat (Llewellyn *et al.*, 2003). When the aphid reaches the adult stage, his life span lasts twenty days for a temperature range comprised between 10°-20°C (Duffy, Fealy and Fealy, 2017). In spring (typically between May and June), winged asexual aphids colonise the blade of the upper leaves of winter wheat. Then, just after ear emergence, winged asexual aphids migrate to the ears (Turpeau-Ait Ighli *et al.*, 2011) where they will live and produce many nymphs, for instance, 49 nymphs per adult in the East of Canada (Adams and Drew, 1964). The majority of nymphs give birth to apterous individuals (e.g. about 36 individuals) whose reproductive rate is faster than for winged individuals (Wratten, 1977). At the end of July, beginning of August, when the grain ripening stage is reached, winged asexual females leave winter wheat and produce sexual females due to the change in autumn parameters such as temperature and light. In autumn and winter, sexual females and males mate and lay eggs on grasses (Weppler, 2009).

Depending on the winter temperature and the “plastic life-cycle” adaptation, *S. avenae* can also reproduce anholocyclically. Obligate parthenogenesis allows the aphid to perform a

high reproductive capacity of viviparous clones during favourable days (Figure 9). It can contribute to an early emergence and infestation if the temperatures enable it (Dedryver *et al.*, 1984). Holocyclic cycle makes it possible to bring together interesting genes by promoting the individual recombination. Both life cycles could coexist in a heterogeneous environment where the space (resources availability and winter refuges) and time (development period of host plants and climate change) differ (Reimer, 2004).



[Figure 9 - Reproduction cycles of \*Sitobion avenae\* 1: see Llewellyn \*et al.\* \(2003\), 2: see Delmotte \*et al.\* \(2001,2003\) \(Reimer, 2004\) ♀ = Sexual female ♂ = Parthenogenic female](#)

#### ❖ *Reproduction rate and fecundity*

The fecundity of aphids is mainly influenced by the age or development stage of the host plant (Watt, 1979). The reproductive rate of the English grain aphid decreases with the maturation of leaves and reaches its minimum as the leaves mature. *S. avenae* produces a larger number of young individuals and has a higher fecundity on the ears of the plant than on the leaves, between the start of flowering and the late milky-ripe stage (Watt, 1979; Vereijken, 1979). Then, there is a significant rise in the reproductive potential once ears appear. After the milky-ripe stage, the reproductive rate is the same on leaves and ears. It decreases as the leaves dried up and the ears mature. The highest reproductive rate on ears is observed on a period of 21 days and reaches a maximum at the watery ripe stage (Watt, 1979). Then, the reproductive potential decreases before harvest at the beginning of August (Dean and Luuring, 1970).

#### ❖ *Dispersion and colonisation on winter wheat (Triticum aestivum)*

Two main migrations take place during the aphid life cycle. The first one happens at the beginning of spring and the second one occurs at the end of autumn. An additional migration can occur during the summer if the number of aphids is too high or if the wheat grains are ripening (Turpeau-Ait Ighli *et al.*, 2011). The wheat aphid is highly migratory (Woiwod *et al.*, 1988) because genetic homogeneity was found at large geographic distances (Llewellyn *et al.*, 2003; Reimer, 2004). The speed at which the aphid reproduces on wheat depends on the growth stage of the host plant. For example, the increase rate will be highest at the milk-ripe stage (Weppeler, 2009). The English grain aphid has a rapid ability to colonise ears as they appear and to disperse. About 42% of tillers are infected before the end of heading (N. Carter, I.F.G McLean and A.D. Watt, unpublished results) even if the average population density is low (two aphids per tiller). This rapid colonisation allows the aphid to take advantage of quality food which is richer around flowering (Watt, 1979).

The colonisation and indirectly the migration of aphids on wheat depend on several factors such as temperature, global radiation, duration of sunlight, wind speed, precipitation and relative humidity. An increase in temperature, global radiation and duration of sunlight will positively impact aphid colonisation on wheat. On the contrary, the migration decreases if the precipitation, relative humidity and wind speed increase (Klueken *et al.*, 2009).

#### 1.4.4 Model 2: *Aphis fabae* (Scopoli)

##### ❖ *Characteristics*

*Aphis fabae* (Scopoli), also known as the black bean aphid, is highly polyphagous. It is considered as a 'generalist' aphid. It can feed on a wide variety of host plants from different families such as Fabaceae, Brassicaceae and Solanaceae. This ability allows it to be worldwide distributed (Hullé *et al.*, 1999).

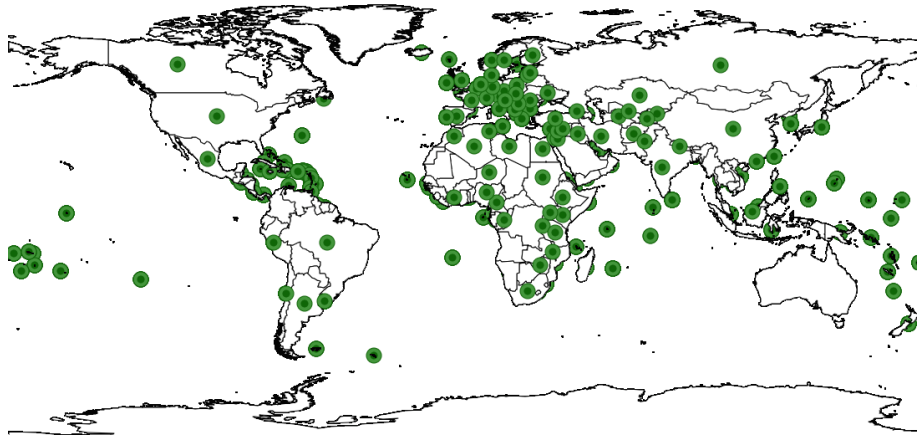
One of the main host plants of this species is the fava bean, *Vicia faba* L. (Basedow, Hua and Aggarwal, 2006). In addition, the black bean aphid is a pest of cultivated, ornamental and herbaceous plants such as sugar beet (*Beta Vulgaris*) (Jones, 1942), tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*), tobacco (*Nicotiana tabacum*) (Akca *et al.*, 2015), guelder-rose (*Viburnum opulus*), mock orange (*Philadelphus coronaries*) (Alford, 2012), lamb's quarters (*Chenopodium album*), bitter dock (*Rumex obtusifolius*) (Jones, 1942), poppy (*Papaver*) and Yucca (*Yucca gigantean*) (Alford, 2012).

*A. fabae* is described as a myrmecophilous species meaning that it has a beneficial relationship with ants. Indeed, this aphid produces honeydew which is appreciated by ants. In return, ants protect aphids from their predators and enhance a better hygiene of their colony by removing honeydew from aphids (Way, 1963; Blanchard *et al.*, 2019). An accumulation of honeydew on plants reduces the photosynthesis and enhances the development of fungi such as sooty molds (Hullé *et al.*, 1999) which weakens plants and aphid colony.

In addition, this species is a vector of a lot of pathogen viruses (Hullé *et al.*, 1999) such as the Bean Leaf Roll Virus (BLRV) and the Bean Yellow Mosaic Virus (BYMV) (Turpeau-Ait Ighli *et al.*, 2011).

#### ❖ *Distribution*

As the world map shows below (Figure 10), *Aphis fabae* (Scop.) is mainly observed in temperate regions such as Western Europe, Asia, North America and Africa, but can also be noticed in tropical regions such as Latin America and Central Africa. However, the species is absent in Australia.

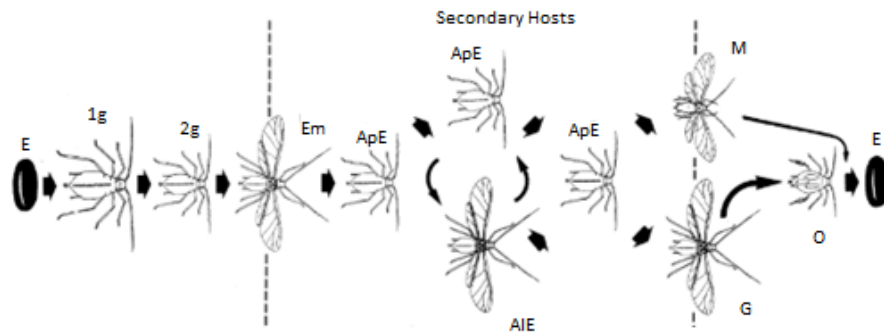


[Figure 10 - Global distribution of \*Aphis fabae\* \(Scop.\) \(Plantwise, 2019\)](#)

#### ❖ *Biology*

This species is defined as holocyclic dioecic because of its overwintering in shape of eggs and development on two different host plants during its cycle. The black bean aphid alternates between its primary host plant, generally the spindle, and its secondary host plants, herbaceous plants from different botanic families.

*A. fabae* lays eggs which overwinter on spindle, *Euonymus europaeus* L., in winter (Dixon and Wellings, 1982). At the beginning of March, eggs hatch and give birth to parthenogenetic individuals (Fundatrix) on spindle (Hullé *et al.*, 1999). Then, parthenogenetic individuals perform two generations of wingless individuals. From this generation succeeds a third generation of winged individuals which produces apterous and winged nymphs (Dixon and Wellings, 1982). In April, first winged individuals are observed and migrate to these secondary host plants (Hullé *et al.*, 1999) such as beans, *Vicia faba* L., sugar beet (*Beta vulgaris* L.) and other vegetal species (Jones, 1942; Way, 1971). Other alate individuals are produced on secondary plants and migrate to other secondary host plants such as clematis, spindle and guilder-rose (Alford, 2012). In autumn, gynopares produce apterous sexual females, oviparae. Those oviparae mate with males and lay eggs on spindle in October (Dixon and Wellings, 1982) (Figure 11). Like *Sitobion avenae*, *A. fabae* does not necessary reproduce with a sexual phase. When winters are mild, aphids can reproduce parthenogenetically on secondary host plants (Hullé *et al.*, 1999).



[Figure 11 - Generalized life cycle of aphids from Aphidinae family \(1g, 2g, 3g represent the first, second and third generations respectively, Em = Emigrants, AlE= Alate exules, ApE = Apterous exules, G= Gynopares, M= Males, O= Oviparae and E= overwintering Eggs\) \(Wellings, Leather and Dixon, 1980\)](#)

#### ❖ *Reproduction rate and fecundity*

According to Akca *et al.*, 2015, development duration for both adult and immature individuals depends on the temperature. For instance, if the temperature goes from 15 to 25°C, the development of immatures stages will take less time. The adult development rates as well as their total longevity seem to decrease when temperatures rise from 15 to 30°C. Up to 30°C, the larval development duration increases again. The fecundity of this aphid scales down when the temperature reaches 30°C. The optimal temperature conditions which ensure good results in terms of survival potential, growth rate, reproduction and longevity are comprised between 20 and 25°C.

Concerning the reproduction rate of the black bean aphid, in case of high intraspecific competition, the survival of aphids can be reduced. Furthermore, because of the low fecundity of *A. fabae* (Dixon and Wratten, 1971), the ability of summer migratory individuals to colonise other vegetal plants decreases (Dixon, 1977). Behrendt has shown by a study conducted during 13 years that the heavier the infestation is, the lower is the number of alates. It means that if the number of aphids colonising plants in spring is important, the number of migratory adults produced in summer per plant will be lower (Behrendt, 1966; Behrendt, 1969). When summer migratory aphids are produced in larger numbers, the higher density of migratory aphids leads to a shortage of suitable host plants which in turn promotes and intensifies intraspecific competition and induce lower populations of autumn migratory aphids (Way and Banks, 1964; Way, 1967; Dixon, 1977).

Way, 1968 found out that black bean aphid from first and second generations are more fertile than individuals from subsequent generations. Apparently, this differential in generation is associated with seasonal differences in the fat content in host plants (Wellings, Leather and Dixon, 1980).

### ❖ *Dispersion and colonisation on swamp bean and spindle (Euonymus europaeus)*

The first generation of black bean aphids develops on spindle, as this vegetal species brings a rich nutritive source. This first generation is characterised by the presence of large gonads and the rapid production of small offspring. As the nutritive source decreases and is less suitable, the second generation presents smaller gonads and produces less offspring, which are larger (Akca *et al.*, 2015).

Winged adult morphs colonise beans when the plants are in active expansion and provide a suitable nutritive food for aphids. Again, when beans cease growing and become less nutritive for aphids, the second generation produces larger offspring in order to compensate the decrease in nutrients and become more resistant to harsh nutritional conditions as they appear to contain more fat than the first generation (Akca *et al.*, 2015).

*A. fabae* is distinguishable on beans by the formation of black colonies, arranged in a sleeve along the stems and mainly at the extremities (Hullé *et al.*, 1999).

## 1.5 Interaction between climate change, aphids and winter wheat

### 1.5.1 Influence of climate change on winter wheat development

#### ❖ *Carbon dioxide*

As the IPCC forecasts, the carbon dioxide (CO<sub>2</sub>) concentration and global mean temperature will rise by the end of the 21<sup>st</sup> century.

Due to increase in atmospheric CO<sub>2</sub> concentrations, C3 plants have a higher photosynthesis activity and are more efficient to use water because of their reduced transpiration in response to the decrease of stomatal conductance (Nogués *et al.*, 1998; Morison, 1998; Long *et al.*, 2004). Carbon dioxide enrichment also promotes a better simulation of plant growth (Singh, 2009) which can favour an higher plant biomass (Lindroth, Arteel and Kinney, 1995; Hughes and Bazzaz, 2001) and early leaves senescence (Heineke *et al.*, 1999)

However, CO<sub>2</sub> rise can also have some negative impacts on plants. Higher CO<sub>2</sub> concentrations can disrupt the C: N balance by altering physical structures of plant tissues (Torbert *et al.*, 2004) and affect development, yield (Kimball, Kobayashi and Bindi, 2002), drought tolerance (Lacuesta *et al.*, 2007), nutritional status and concentration of defensive chemicals (Lindroth, Arteel and Kinney, 1995; Agrell, McDonald and Lindroth, 2000; Hartley *et al.*, 2000; Sun, Su and Ge, 2010; ). The latter results in modification in terms of resistance to pests (Heagle, 2003) and trophic levels interactions. The C: N ratio mainly increases in the foliage due to the accumulation of non-structural carbohydrates (Lindroth, Arteel and Kinney, 1995).

High atmospheric carbon dioxide contributes to a higher number of seeds which, in turn, leads to better individual and total net weight of grain wheat. However, winter wheat under elevated CO<sub>2</sub> seems to allocate less total nitrogen in seeds (Jablonski, Wang and Curtis,

2002). Furthermore, the metabolic content of seeds can be affected by high CO<sub>2</sub> concentrations. According to a study realised by Williams, Shewry and Harwood, 1994, the protein and lipid level are reduced under elevated CO<sub>2</sub> concentrations in grain winter wheat.

#### ❖ *Temperature*

High temperatures could lessen the positive effect expected by a rise of carbon dioxide concentration. If the local average temperature increases over a range of 1-3°C, the crop productivity is expected to increase. But, if the local average temperature exceeds this range, the crop productivity may decrease (IPCC, 2007b). This loss in productivity is mainly due to the shortening of vernalization period (Trnka *et al.*, 2004), early phenological stages (Mitchell *et al.*, 1993), low photosynthesis activity, low accumulated resources during grain formation (Lawlor, 2005) and increased evapotranspiration (Nobel, 2005).

The elevation of temperature can reduce grain yield and number (Polowick and Sawhney, 1988; Young, Wilen and Bonham-smith, 2004; Prasad *et al.*, 2008)), impact reproductive potential (Ferris *et al.*, 1998; Polowick and Sawhney, 1988) such as flowering (Morison and Lawlor, 1999) and reduce plant biomass, due to the reduction in leaf photosynthesis and the increase in transpiration (Nobel 2005; Prasad *et al.*, 2008). Moreover, temperatures above 30°C occurring near the period of seed filling wheat, may drive plants sterile (Ferris *et al.*, 1998; Wheeler *et al.*, 2000). In reaction to temperature stress, those plants tend to develop smaller leaves, deeper and longer root systems to reduce the evapotranspiration and increase the root water withdrawal (Gliessman, 1998). In other cases, winter wheat plants can show signs of overheating by the presence of leaf rolling and changes in leaf orientation (Larcher, 2003; Nobel, 2005).

Elevated temperature can also impact wheat at a molecular scale. Increase in temperature may alter cell metabolism and modify protein synthesis leading to the accumulation of new proteins such as heat shock proteins (Vierling, 1991; Larkindale, Miskhkind and Vierling, 2005; Prasad *et al.*, 2008)

#### ❖ *Drought and precipitations*

As said previously, high temperatures may alter precipitation patterns. Indeed, precipitation may become less frequent in some areas and more intense in other parts of the world. For instance, the equatorial belt and areas at higher latitude (IPCC, 2007a; IPCC, 2007b) may be characterised by heavy downpours during the harvesting period which can affect the yield quality of cereal crops. On the contrary, less precipitation may also occur in mid-latitude, dry tropics and semiarid areas (FAO, 2008) which can reduce the yield quality as well.

A study conducted by Wade *et al.*, 2015 shows that wheat plants in drought conditions contain a higher proportion of essential amino acids such as tryptophan than wheat plants in watering conditions (Bowne *et al.*, 2012). Plants under drought conditions seem to have a lower level of stress than plants submitted to constant precipitation. This phenomenon can be explained by the better penetration of water to the soil under extreme rainfall episodes



which in turn favours a greater bulk soil. Furthermore, because of this improved bulk soil, water can remain longer in the soil which promotes a more in-depth rooting system and increase water intake during drought events (Heisler-White *et al.*, 2009).

The content of plants may also vary depending on the watering. Plants under drought periods contain lower silicon (Si) concentrations but higher nitrogen in leaf and amino acid concentrations compared to plants under normal water conditions. While barley growth was lessened under continuous drought conditions, the plant growth was not affected under changes in precipitation frequency (Wade *et al.*, 2015). Water availability and drought stress for plants also impact levels of defence secondary metabolites such as terpenoids even if these chemicals vary according to species and contexts (Llusià and Peñuelas, 2002; Jamieson *et al.*, 2012). The decrease of defence compounds can reduce the ability of plants to protect themselves against herbivory attacks (Jamieson *et al.*, 2012).

### 1.5.2 Influence of climate change on aphids

#### ❖ Carbon dioxide

Increase in temperature and CO<sub>2</sub> concentration may have strong impacts on the biology and ecology of insect species, by acting directly on the organism physiology or indirectly by modifying their habitat (Cannon, 1998; Root *et al.*, 2003).

The effects of CO<sub>2</sub> enrichment on aphids are difficult to identify. Indeed, depending on the aphid species, the reactions on development and fertility rates are different. It might increase (Awmack, Woodcock and Harrington, 1997), decrease (Hughes and Bazzaz, 2001) or has no effect on aphids (Mondor *et al.*, 2004). Under elevated CO<sub>2</sub> aphid abundance increase, but the mean number of alate aphids would be lower. The number of offspring produced by alate nymphs is more important on plants grown under elevated CO<sub>2</sub> conditions rather than ambient CO<sub>2</sub> conditions (Chen, Wu and Ge, 2004). Moreover, aphids prefer to lay eggs on plants cultivated under elevated CO<sub>2</sub> conditions (Peltonen *et al.*, 2006).

Alate adults present on spring wheat show better population abundance and fecundity under elevated CO<sub>2</sub> while the mean number of winged individuals decreases (Chen, Wu and Ge, 2004). In the case of *Sitobion avenae*, according to Awmack *et al.*, 1996 his fecundity seems to increase when the species is reared on winter wheat grown under elevated CO<sub>2</sub> (Awmack *et al.*, 1996; Sun and Ge, 2011).

Atmospheric CO<sub>2</sub> amount can also impact the migration peak period. A study conducted by Newman, 2003 explained that in presence of elevated CO<sub>2</sub>, aphids reached a smaller population peak which is also later in the season. Aphids can respond differently to CO<sub>2</sub> modification and disperse rapidly. Most plants are not able to spread in new geographical areas which limit the potential of specialist aphids to be adapted and to spread in new territories. Generalist aphids are more adapted to a range of plants which can facilitate their spread (Hullé *et al.*, 2010). *S. avenae*, in presence of 700 ppm of CO<sub>2</sub>, shows earlier

reproduction period and higher number of offspring per adult. Development time was unaffected by the increase in carbon dioxide (Whittaker, 1999).

Finally, elevated CO<sub>2</sub> could enhance the pest status of aphids and their damages on crops, according to a meta-analysis conducted by Robinson, Ryan and Newman, 2012. They showed better performances of aphids in presence of enriched CO<sub>2</sub>.

### ❖ *Temperature*

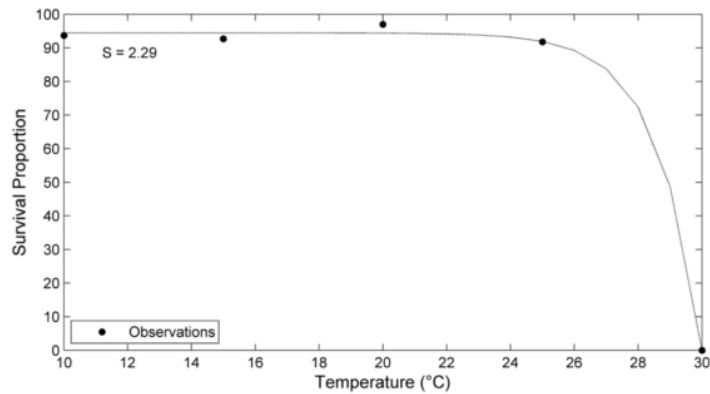
Impacts of elevated temperatures on aphids are easier to identify. Indeed, temperature influences the development rate of aphids. Optimal temperatures and upper limits are between 20 to 30°C. An aphid can reach adulthood if it develops under a certain number of degree-days above the limit temperature (Harrington, Bale and Tatchell, 1995; Hullé *et al.*, 2010).

According to a study conducted by Hullé *et al.*, 2010, changes in global temperature may accelerate the development of aphid populations because of their short generation time and high reproductive capacities (Yamamura and Kiritani, 1998). If the global temperature increases up to 2°C, the number of aphid generations could increase from 18 to 23 in the UK, as well as their population size (Harrington, 1994; Hullé *et al.*, 2010).

The number of alates individuals and their dispersal capacity are dependent on temperature. Higher temperatures may increase the number of winged individuals and their mobility. Indeed, temperature elevation allows an earlier onset of sexual maturity leading to an earlier population increase. A higher proportion of alate individuals induce crowding which in turn lead to a decrease of the population (Duffy, Fealy and Fealy, 2017). However, aphids are not able to fly if the temperatures are lower than the range 13-16°C or upper 31°C (Irwin, Kampmeier and Weisser, 2007). If it was the case, aphids would be able to produce more offspring on plants which may lead to heavier damages. The reduction of food quantity will reduce more rapidly the aphid population.

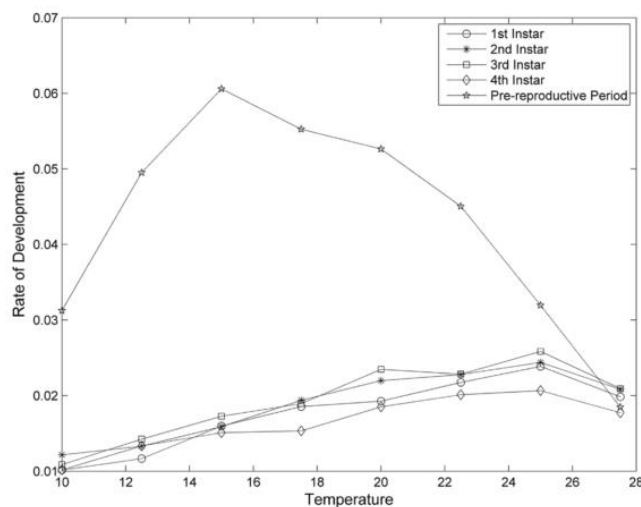
Other biological functions are related to temperature. As explained above, the reproduction of aphids depends mainly on winter temperature. If the winter conditions are mild, aphids reproduce anholocyclically. On the contrary, by cold winter, aphids reproduce holocyclically. The laying of eggs by sexual females is correlated to the night length and autumn temperature. In case of temperature above 20°C, the sexual reproduction might be delayed or avoided (Blackman, 1974). Higher temperatures will promote parthenogenetic reproduction and increase the survival probabilities (Hullé *et al.*, 2010).

Higher temperatures may affect the speed of development, fecundity and survival of the English grain aphid. After 24°C, the survival proportion of nymphs is scaling down (Duffy, Fealy and Fealy, 2017), and above 30°C, all the nymphs will die before finishing their development (Figure 12) (Dean, 1974). To conclude, the elevation of temperature shows a constant relationship with the survival rate until 24°C. After this temperature, the relationship becomes negative (the survival proportion decreases).



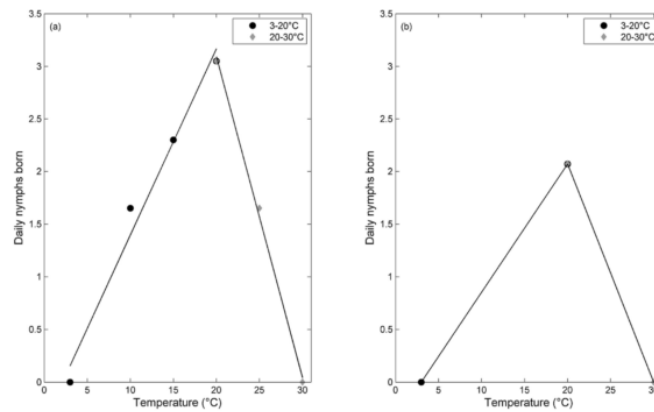
[Figure 12 - Survival proportion of nymphal under different temperatures \(Dean, 1974\)](#)

As Figure 13 shows, temperature also affects the developmental rate of the different instars. The development rate during the pre-reproductive period seems to diminish around 15°C. On the contrary, the developmental rate of the fourth instars seems to rise with increasing temperature for the same temperature. After 25°C, the rate of development decreases for all the instars as the figure below shows (Duffy, Fealy and Fealy, 2017). Because the development rate increases in all instars forms, all the instars are positively affected by the elevation of temperature.



[Figure 13 - Response of \*S. avenae\* for all developmental stages to temperature \(Duffy, Fealy and Fealy, 2017\)](#)

According to Dean, 1974, the fecundity of wingless individuals increases from 3 to 20°C. After 20°C, the number of daily nymphs born decreases and reaches a minimum at 30°C. The number of daily winged nymphs born is lower in the same range of temperature than wingless individuals. The response of alate nymphs is similar to apterous nymphs. In both forms, individuals experience a higher survival rate between 15 to 20°C (their optimal range) (Figure 14).



**Figure 14 - Fecundity of *S. avenae* in response to temperature (a) wingless individuals (b) winged individuals (Duffy, Fealy and Fealy, 2017; Dean, 1974)**

#### ❖ *Drought and precipitations*

Precipitation variability also has an impact on aphids. Indeed, reduction in water quantity can influence the aphid mass. Wade *et al.*, 2015 have shown that aphids are heavier when they are reared on plants grown in drought conditions than the one reared in normal conditions. However, the fecundity of aphids is not affected by the watering conditions of plants.

### 1.5.3 Interaction between aphids and winter wheat under global warming

#### ❖ *Carbon dioxide*

Under CO<sub>2</sub> gain, the plant should increase its contents in starch, sucrose, glucose, total non-structure carbohydrates (TNCs) and the ratio of TNCs: nitrogen and free amino acids. The richer the nutriment content is, the higher the aphid abundance and reproductive activity. On the contrary, a dilution in nitrogen foliar and fructose decrease the aphid fitness (Pritchard, Griffiths and Hunt, 2007; Yin *et al.*, 2009; Sun *et al.*, 2010). Chen, Wu and Ge, 2004 have found that aphids impact the plant traits negatively. Nonetheless, the negative impacts provoked by aphids are lessened by the positive effects given by elevated CO<sub>2</sub> on the plant traits (Chen, Wu and Ge, 2004).

In presence of aphids, ear length and dry weight of grains per ear increase under CO<sub>2</sub> enrichment which compensates the adverse effects of aphids on wheat. Because of the enrichment in different nutritive values, *S. avenae* tends to choose more easily plants developed under higher CO<sub>2</sub> conditions (Chen, Wu and Ge, 2004). However, the content in nitrogen and other trace elements such as iron and zinc tends to decrease (Bloom *et al.*, 2010). As a consequence, the nutritional intake and the plant quality are lower, resulting in a lower aphid growth, development and performance (Holopainen, 2002; Bezemer and Jones, 1998). It means that they feed on more plants and modify their feeding behaviour and metabolism (Sun and Ge, 2011) in order to respond to their nutritive requirements (Lincoln, Fajer and Johnson, 1993). Aphids adopt compensatory feeding behaviors to balance their requirements (Awmack, Woodcock and Harrington, 1997) such as higher phloem-sap

pressure and flow rates, penetration frequency (Watling *et al.*, 2000), alteration in their position on plants and also post-ingestive metabolism (Abisgold, Simpson and Douglas, 1994). Compensatory feeding may play a role as buffer in order to await the impact of CO<sub>2</sub> concentration on host plants quality. By adopting other feeding behaviours, aphids are more suitable to changes in nitrogen concentration as they are able to synthesize amino acids thanks to their symbionts (Hughes and Bazzaz, 2001).

However, because of the stress, leaves may be more difficult to penetrate with a stylet under enriched CO<sub>2</sub> which may decrease the intake, speed rate along with ingestion. Elevated CO<sub>2</sub> may increase the thickness of epidermal layer (Sun and Ge, 2011) and modify the foliage water content (Lindroth, Arteel and Kinney, 1995; Hughes and Bazzaz, 2001). However, aphids are potentially more attracted by wheat plants cultivated in enriched CO<sub>2</sub> because elevated CO<sub>2</sub> increases the amino acid content as well as the fructose concentration of plants tissues (Awmack *et al.*, 1996; Oehme *et al.*, 2013). The richer content in amino acid and fructose affect positively the relative growth rate of *R. padi* (Oehme *et al.*, 2013).

The C:N ratio in plant tissues is also influenced by the increase in CO<sub>2</sub> concentration. High carbon quantity can be allocated to cell walls (Pritchard *et al.*, 1999). Under elevated CO<sub>2</sub>, plants may have some changes in sap flow rate (Girousse and Bournoville, 1994) as well as an increase in leaf surface temperature (Idso *et al.*, 1993) which can impact aphids.

Aphids can also meet their nutritional requirements by recognising and localising host plants in a way to feed, reproduce and grow (Visser, 1983; Visser, 1986; Bruce, Wadhams and Woodcock, 2005). The location of host plants is mainly due to the perception of plant volatiles (Bruce, Wadhams and Woodcock, 2005). Plants volatiles are perceived by numerous sensory inputs within insect central nervous system (CNS) such as olfactory, gustatory and physical information (e.g. plant colour, shape and texture). Thus, when the right information are perceived by the insect, it is attracted to the plant (Bruce, Wadhams and Woodcock, 2005). More precisely, the primary and secondary rhinaria, which are sections of aphid antenna, respond to plant odours (Nottingham *et al.*, 1991; Pickell, Wadhams and Woodcock, 1992; Campbell *et al.*, 1993; Visser and Fu-shun, 1995).

Chemical signs called kairomones play an important function in the recognition of host plants by aphids at a distance (Visser, 1988; Bernays and Chapman, 1994; Pickett, Wadhams and Woodcock, 1998; Bruce, Wadhams and Woodcock, 2005). The perception of these signals is mediated by olfactory receptor neurons (ORNs) present on the insect antenna which converts chemical signals into an electrical signal which is sent to the CNS (Hansson, 2002; Bruce, Wadhams and Woodcock, 2005). Elevated temperatures may influence the emissions of volatile compounds from plants by increasing biosynthesis levels and emissions rates (Niinemets, Loreto and Reichstein, 2004; Holopainen and Gershenzon, 2010; Jamieson *et al.*, 2012). However, the quality and quantity of these produced and emitted VOCs depend on many variables such as plant - climate change interactions, plant traits and herbivore damages.

### ❖ *Temperature*

Elevated temperatures also affect wheat and insect relationship. Dong *et al.*, 2013 have studied the elevated temperature influences on the wheat growth phenology. Under warming conditions, the developmental period of winter wheat is shorter as the time between re-greening and maturity is reduced by six days. Aerial biomass also shows improvement at maturity with higher temperatures (Hou *et al.*, 2012). This improvement is mainly explained by a rise in tillering numbers. Nevertheless, the winter wheat yield does not seem to be affected by this warming as the plant performs a photosynthesis which compensates the advance in the reproductive period. Due to the modification in winter wheat phenology, plants may reach maturity earlier during the growing period and affect aphids by reducing the food source. Aphids can react to this situation by adopting other behaviours such as earlier migration in spring hosts (Dong *et al.*, 2013).

According to Bauerfeind and Fischer, 2013, an increase in temperature may also affect plant quality by reducing the carbohydrates content, phenols and terpenoids (Kuokkanen *et al.*, 2001; Veteli *et al.*, 2002; Bohinc and Trdan, 2012; Bauerfeind and Fischer, 2013) which impact the aphid population and reduce their infestation. Other factors are correlated with temperature and can impact aphid infestation, such as earlier ripeness, higher predator attack and low fecundity (Triltsch, Freier and Rossberg, 1998).

As warming accelerates the emergence of wheat ears, *S. avenae* may appear earlier (Dong *et al.*, 2013). Between 10 and 20°C, the development rate of the English grain aphid increases more on ears than on the flag leaf. After 25°C, individuals developmental rate scale down both on ears and the flag leaf (Dixon and Acreman, 1989).

Concerning the size of aphids, it seems to decrease with increasing temperature. Aphids are larger on the flag leaf at 10°C while they are larger on ears at 15°C. In a range from 25 to 30°C, apterous *S. avenae* were the same size on ears and the flag leaf. At 15°C, aphids were smaller on the flag leaf than on ears. Thus, if the host plant quality increases, aphids will be larger only if the growth rate is more important than the developmental rate. The fecundity does not seem to be affected by the temperature and the feeding site before 25°C, while it decreases and becomes minimal at 30°C. The reproduction reaches its maximum on ears at 15°C while the maximum is reached at 10°C on the flag leaf (Dixon and Acreman, 1989).

### ❖ *Drought and precipitations*

Apart from elevated temperature and CO<sub>2</sub>, variation in precipitation events also has a significant effect on the interaction between aphids and wheat. According to Knapp *et al.*, 2008, extreme precipitations may destabilise terrestrial ecosystems due to changes in plant development and chemical composition as well as disruption in plant-herbivore interactions. Increased drought frequency can impact plant damages during herbivore attacks by changing plant water status and physiology (McDowell *et al.*, 2011).

A potential consequence of extreme precipitations is the asynchrony between the development, behaviour and life cycle between plants and insects (Weltzin *et al.*, 2003; Trotter, Cobb and Whitham, 2008; Wade *et al.*, 2015). Plants under water stress can react by modifying their morphology, physiology and chemical composition to be more adapted or tolerant to these situations such as changing their resource allocation (Blum, 1996; Chaves, Maroco and Pereira, 2003; Wade *et al.*, 2015) and osmotic adjustments to protect themselves and minimize antioxidants systems (Chaves, Maroco and Pereira, 2003; Barnabás, Jäger and Fehér, 2008). Some plants may also change silicon uptake to immobilise higher antioxidants proportions and avoid cellular damage to be more resistant against attacks. However, these chemical changes also influence food quality for herbivory and in turn affect herbivory performances (Huberty and Denno, 2004; Chown, Sørensen and Terblanche, 2011). Thus, altered precipitations could impact on multitrophic interactions by promoting the presence of herbivore populations in systems where phenological periods become asynchrony for each element (Jamieson *et al.*, 2012).

Those three climatic parameters will play an important role on plant-pest interactions, especially in aphids. Few studies are conducted on the combined effect of elevated temperature and elevated carbon dioxide concentration on plant – aphid interactions.

## 2. Objectives

In this study, we aim at evaluating the impact of climate change on plant-aphid interactions.

To do so, we identified two different plant-aphid models: (1) *Sitobion avenae*, the English grain aphid and (2) *Aphis fabae*, the black bean aphid.

The English grain aphid is a specialist cereal pest infesting winter wheat. It especially occurs before prebooting and feeds on wheat ears which leads to yield losses. The black bean is a generalist species. Both species have different feeding preferences and behavior.

This study specially aims at

(1) comparing the population dynamics of *S. avenae* on winter wheat under contrasted climatic conditions.

(2) comparing life history traits (including development and reproduction) of *S. avenae* under contrasted climatic conditions.

(3) comparing host preferences of *S. avenae* facing wheat plants grown under contrasted climatic conditions.

(4) comparing the volatile organic compounds (VOCs) emitted from winter wheats grown under contrasted climatic conditions.

Taking into account the existing literature, we raise several hypotheses for each specific objective.

(1) The aphid population will increase under an elevation in temperature as higher temperatures will accelerate their development. An increase in carbon dioxide will favour more local infestations and a better aphid development.

(2) Aphids will produce fewer nymphs, live shorter lives and be heavier under higher temperature. On the contrary, an elevation in CO<sub>2</sub> concentration will increase the growth rate and the fecundity of aphids.

(3) Wheat plant quality will be improved under elevated CO<sub>2</sub> as the content of carbohydrates and other nutrients will be richer which will result in a higher attraction of aphids.

(4) The emission of wheat VOCs will be more important under elevated temperature and CO<sub>2</sub> concentration because the vapour pressure will be greater and the carbon production in the plant in excess. Thus, aphids will be more attracted by plants grown under elevated carbon dioxide concentration and elevated temperature.



### 3. Materials and methods

#### 3.1 Aphid rearing

Populations of *Sitobion avenae* were reared on winter wheat in a conditioned chamber. The species is originated from a Shanxi Taiyuan strain (China). This non-virotic strain was selected to prevent the transmission of viruses to wheat. The chamber was maintained at  $23 \pm 1^\circ\text{C}$  and  $60 \pm 10\%$  of relative humidity (RH), with a 16:8 light: dark photoperiod under cool white light-emitting diode (LED) lights ( $77 \text{ Imol/sqm/s}$ ). Winter wheat from the Julius variety (Gembloux, Belgium) used for the rearing of aphids was sown in plastic trays containing organic soil from La plaine CHASSART (Wagnelée, Belgium). About forty seeds were sown in one plastic tray (30 x 19 cm). After one week, the cereals were inserted in insect cages for feeding. The cereals used for the experimentations were of the SAHARA variety (Anvers, Belgium). This variety was sown in small plastic pots with the same soil than the one used for aphid rearing. Once sown, winter wheats were placed in climatic chambers and watered initially with 100 ml of water. Then, every two days, 50 ml of water were poured to the plants. Two different varieties of winter wheat were used because the SAHARA variety was not resistant enough for the aphid rearing.

*Aphis fabae* were reared on swamp beans (variety Grosse Ordinaire, Huy, Belgium). The sowing of swamp beans was made in small plastic pots. The substrate used for the sowing was composed by  $\frac{3}{4}$  of organic soil from La plaine CHASSART (Wagnelée, Belgium) and  $\frac{1}{4}$  of perlite Xmineral P40 19 060 10L. Once the swamp beans sown, the different plastic pots were disposed in a bigger plastic container (40 x 40 cm). Initially, the water was poured in this container in order to reach the second marking line which represents about 500 mL of water. After that, the plants were watered every two days. *Aphis fabae* were reared in two climate chambers under two temperature regimes: the first conditioned chamber was maintained at a temperature reaching  $20 \pm 1^\circ\text{C}$ . The other one was at  $23 \pm 1^\circ\text{C}$ . In each chamber, aphids were kept in PLEXIGLAS® cage (60 x 50 x 50 cm) made of transparent windowpane (PLEXIGLAS® GS, clear 0F00 GT, 8 mm thick; Evonik Industries, Essen, Germany). Half of the PLEXIGLAS chambers were receiving ambient air containing  $450 \pm 50$  ppm  $\text{CO}_2$  (termed a $\text{CO}_2$ ) while the other half were receiving enriched air containing  $800 \pm 50$  ppm  $\text{CO}_2$  (termed e $\text{CO}_2$ ) thanks to a  $\text{CO}_2$  gas tank (> 99% purity, Airliquide, Paris, France). The relative humidity was always set at  $60 \pm 10\%$  with a 16:8 light: dark photoperiod under cool white light-emitting diode (LED) lights ( $77 \text{ Imol/sqm/s}$ ). Carbon dioxide concentrations, temperature, and RH were continuously monitored in each PLEXIGLAS cages with MCH-383 SD data loggers (Lutron, Taipei, Taiwan).

### 3.2 Weekly evaluation of *Sitobion avenae* populations under contrasted climates

To study climate change influence on the interaction between winter wheat and aphids, climatic chambers, called Ecotrons, were set up. More information about the dimensions of the Ecotrons is in Appendix 1. Those climatic chambers were developed to forecast the impact of climate change on agricultural systems, biodiversity, insect-plant interactions and yield. Six chambers were available. Three chambers were set at to simulate the climate recorded in 2014-2015 in Gembloux, and three other chambers were set at a simulated 2093-2094 climate.

For the 2014-2015 Ecotrons, the climate information and modelling come from data collected by CRA-w<sup>6</sup> after some trials on the field, at Ernage (Belgium) in 2015 with winter wheat. Climatic parameters of the Ecotron 2093-2094 were modeled by using information given by the Royal Meteorological Institute of Belgium (RMI). The climatic forecasting were based on the RCP scenario 8.5 defined by the IPCC. Here are the forecasting in terms of mean temperature and anthropogenic CO<sub>2</sub> emissions for the RCP scenario 8.5 (Figure 15 and 16).

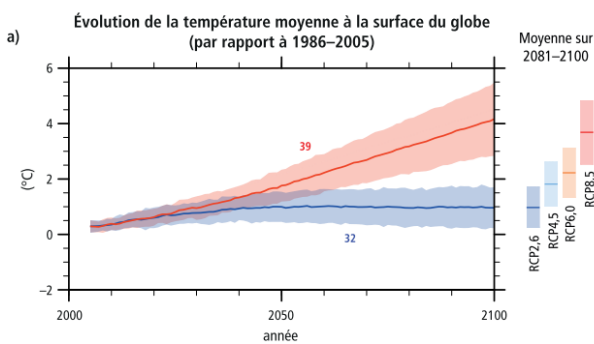


Figure 15 - Evolution of mean temperature in surface for the 2081-2100 period compared to 1986-2005 (IPCC, 2013)

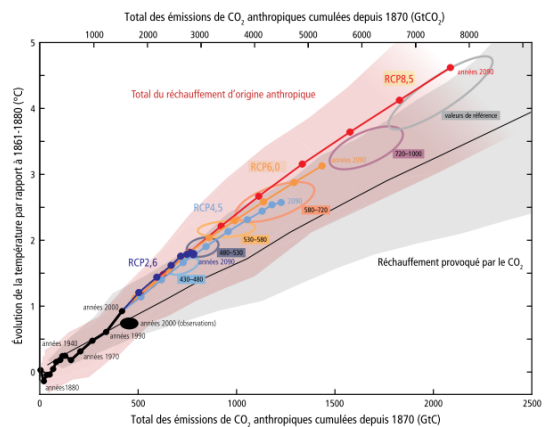
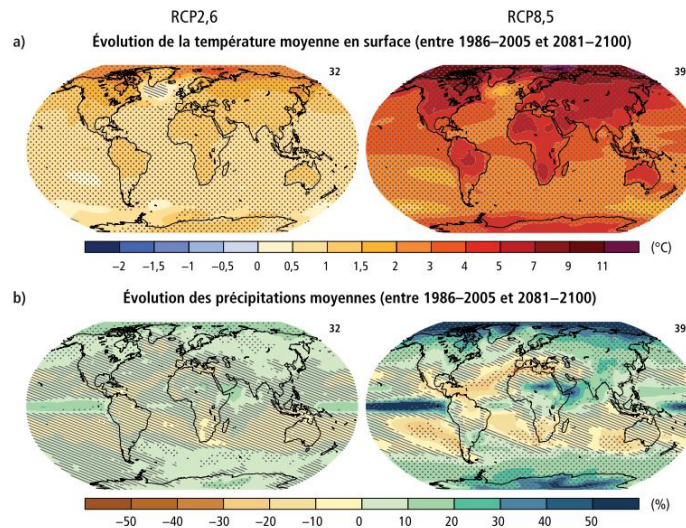


Figure 16 - Anthropogenic CO<sub>2</sub> emissions cumulated since 1870 (GtCO<sub>2</sub>) (GIEC, 2014)

This scenario represents the 2081-2100 period. The 2093-2094 year was chosen because it represents the typical year of this scenario as it characterises by extreme events such as droughts and floods, as illustrated in Figure 17.

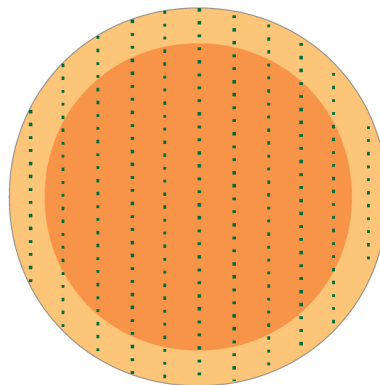
<sup>6</sup> CRA-w is a scientific unit of the government of Wallon Region which conducts researches on precision farming and breeding, risk management as well as chemical products analyses.



**Figure 17 - (a) Evolution of mean temperature in surface for the 1986-2005 period and the 2081-2100 period (b) Evolution of mean precipitation for the 1986-2005 period and the 2081-2100 period (GIEC, 2014)**

Climatic data from Ernage and the RMI were converted with an adapted program called ALAROO. This program calibrates the data and gives the standard deviation and the mean of each climatic parameter such as temperature, precipitation and photoperiod. Once calibrated, the data were formulated to be read by the computer. Thus, each climatic replicate was modelled and programmed with ALAROO (Appendix 2 and 3).

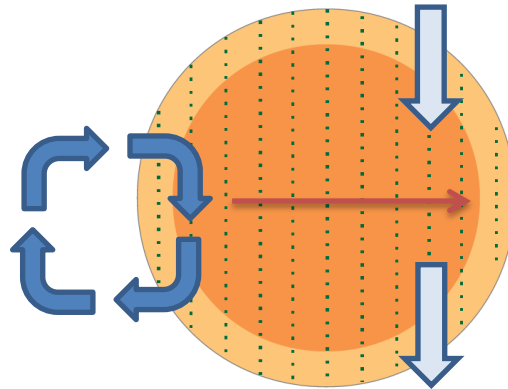
About 500 seeds of winter wheat were sown in each Ecotron chamber in a lysimeter characterised by a diameter of 1.63 m and a height of about 1.5 m (Figure 18). Each seed was planted at a distance of 147 mm from each other. The variety of winter wheat sown was SAHARA (Anvers, Belgium) because this variety is resistant to diseases and has the same yield than those present on the field after a trial with SAHARA wheat.



**Figure 18 - Schematic representation of the planting**

During the growth of seedlings, different climatic parameters were monitored and controlled. The first parameter was the CO<sub>2</sub> concentration. For the 2014-2015 condition, this concentration is set at 380 ppm. While the 2093-2094 condition is characterised by a concentration of 775 ppm. Based on a program, the amount and frequency of rain were simulated. In addition, plants were lighted with different lamps as halogen, plasma and LED

to correspond to the spectral range of solar radiation of plants (PAR). Moreover, the crop was ventilated thanks to two systems (Figure 19). The first one was characterized by the circulation of air in a closed system (dark blue arrow). The second one was represented by the injection of air in the chamber and the removal of it (light blue arrow). The wind speed inside the chamber was constant and at 0.5 m/s (bold red arrow).

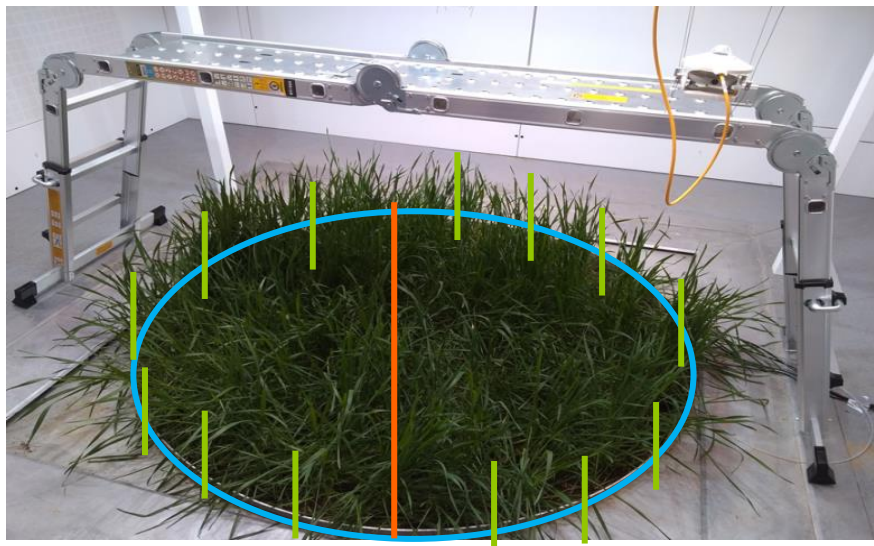


[Figure 19 - Ventilation system in the Ecotron](#)

To evaluate *S. avenae* population dynamics, 50 parthenogenic females originated from the rearing were introduced on winter wheat plants, with the help of a paintbrush 2 Marabu UNIVERSAL (Gembloux, Belgium), in each of the six climatic chambers (Ecotrons). Each parthenogenic female was placed randomly on a plant at the developmental stage 37-39 of winter wheat which corresponds to prebooting (the flag leaf just visible and the flag leaf ligule), according to Zadoks code.

Once the aphids introduced, an evaluation of the population size was performed on a weekly base. The day of counting, a number is generated randomly. This number is present on the blue rope and gives the position of a transect delimited with a small rope crossing both sides of the Ecotron (orange rope). The transect helps to determine which plant is observed to perform aphid counting (Figure 20). Twenty plants selected randomly on the transect are examined and the number of aphids is recorded by counting the number of winged and wingless individuals, the position of the insects on the plant (ear, stem or leaf) as well as the growth stage of the cereal. The position of the plant inside the Ecotron is also recorded. This position is calculated from the extremity to the center of the Ecotron. This extremity is considered as 0 value.

The counting is performed in one day for the three replicates of the same climate year.



**Figure 20 - Methodology of aphid counting**

Another experimentation, at the plant scale, was performed on the same day of the insect inventory. In each chamber, four plants were selected randomly to assess the aphid growth and position every week. To be able to identify the plant evaluated, a red wool yard Mouliné Spécial DMC 321 (Gembloux, Belgium) was tied at the winter wheat stem (Figure 21). The measures taken for this experimentation were the aphid number, the developmental stage of the plant and the aphid position. At the end of the experimentation, the aphid size, the tibia size as well as the weight of ten aphids were recorded.



**Figure 21 - Selected plant for the experimentation at the plant scale**

### **3.3 Fitness evaluation**

Each week, ten random individuals were removed from each replicate of the same climate year. When the insects are removed from the plant, a red wool yard is placed next to it to replace insects on the same plant. The insects are weighted by ten with the help of a semi-micro analytical balance Kern ABT 120-SDM (Balingen, Germany). Once the weight is obtained, each insect is observed with binocular lens composed by an ocular micrometer (PL 10x/20) (Euronex, Holland) to measure the size of their body as well as the tibia length of their posterior left leg (Figure 22).



[Figure 22 - Measured tibia of the aphid](#)

### 3.4 Aphid feeding preferences

#### 3.4.1 *Sitobion avenae*

The preference choice of *S. avenae* when facing winter wheat grown under different climatic conditions was assessed. The four different climatic modalities obtained from the rearing chambers included: **(Modality 1)** ambient Temperature – ambient CO<sub>2</sub>, **(Modality 2)** ambient Temperature – elevated CO<sub>2</sub>, **(Modality 3)** elevated Temperature – ambient CO<sub>2</sub> and **(Modality 4)** elevated Temperature – elevated CO<sub>2</sub>. Each modality was then tested in dual choice, according to the six combinations listed in Table 1.

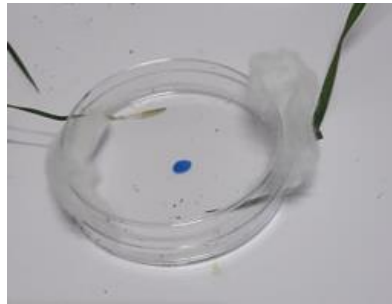
[Table 1 - Combinations and modalities tested during the experimentation](#)

Combination	Modality	VS	Modality
<b>A</b>	1		2
<b>B</b>	1		3
<b>C</b>	1		4
<b>D</b>	2		3
<b>E</b>	2		4
<b>F</b>	3		4

To evaluate the choice of this aphid species, plastic Petri dishes were used (Ø75 mm). These Petri dishes were previously cut from each side to introduce leaves of wheat without cutting or hurting them. Then, a circle filter paper Whatman™ 55mm Cat No 1001-055 (Darmstadt, Germany) was introduced in its lid to avoid the impact of electrostatic electricity of the plastic on aphids. In addition to reduce this impact on the insect, the bottom of the Petri dish was rinsed off with distilled water.

Plants aged from two weeks were selected from all the different modalities. Once selected, the seedling was removed from the pot with the seed. Then, the seedling was covered with a watered paper which itself was wrapped with aluminium.

Selection of the tested combination was made by using a random draw. When the combination was defined, one plant from each modality was selected and introduced in the Petri dish by the slot shown in Figure 23. The aphid was, then, introduced in the center of the Petri dish (blue dot on Figure 23). Once the aphid introduced, the lid was put on the top. Both slots were recovered with cotton wool to reduce the odour from other elements surrounding the Petri dish. When the plants were prepared, the host plant selection started with the introduction of a single aphid. After observing six females, all Petri dishes were cleaned with ethanol 99% during five minutes.



[Figure 23 - Representation of the experimental model \(\*Sitobion avenae\*\)](#)

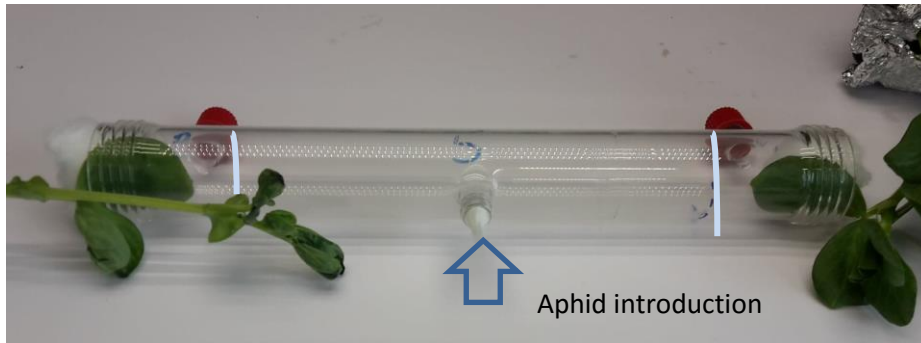
During the experimentation, two data were recorded: the leaf selected by the aphid and the time needed to select the leaf. The choice was recorded when the aphid was on the leaf. In addition, the plant position was changed randomly. Six repetitions of one aphid were done at once to avoid the starving of aphids. If the aphid did not move after fifteen minutes, it was considered as no choice.

### 3.4.2 *Aphis fabae*

The host plant selection of *Aphis fabae* when facing swamp beans grown under different climatic conditions was assessed. The different climatic modalities were the same as the one used for the English grain aphid. Each modality was tested in between, which represents six combinations (Table 1).

To evaluate the aphid choice, cylinder olfactometers were used (Figure 24). The olfactometers consisted in tubes of 32 cm long, with an internal diameter glass tube of 3.6 cm (Verre Labo Mula, Vaulx en Velin, France). The tubes were previously cleaned with Extran MA 3. The next day, all the material was rinsed off with tap water and distilled water. Then, the olfactometers were dried in an oven during one hour.

Swamp beans aged from two weeks were taken from different climatic conditions. Once selected, each plant was covered with aluminium paper to avoid VOCs emissions from the soil. One plant from one condition was settled at each extremity of the olfactometer. Then, the large extremities were covered with cotton wool to avoid the entrance of other VOCs. Once the plants settled, the aphid was introduced by the main entrance of the olfactometer with a paintbrush. The two little entrances were closed with a plug to avert the escape of the aphid as the picture below shows (Figure 24).



[Figure 24 - Representation of the experimental model \(\*Aphis fabae\*\)](#)

The choice made by the aphid was taken into account when it crossed the line previously drawn at two-third of the main entrance. If the aphid did not move after ten minutes, it was considered as no choice.

Every three tests, the olfactometer was cleaned with pentane. The solvent was left to evaporate for five minutes. The data collected for this experimentation included the time needed to make a decision as well as the choice made by the insect.

Both choice experimentations were done in a ventilated room with panes on the windows to avoid the penetration of light in the room. This room ensures the presence of uniform lighting which reduces the light bias.

### 3.5 VOCs sampling

To identify the volatile organic compounds (VOCs) attracting *S. avenae* to winter wheat, VOCs from winter wheat grown under different climatic conditions were sampled. As a reminder, the different climatic conditions are ambient temperature – ambient CO<sub>2</sub> (1), ambient temperature – elevated CO<sub>2</sub> (2), elevated temperature – ambient CO<sub>2</sub> (3) and elevated temperature - elevated CO<sub>2</sub> (4).

To sample VOCs from winter wheat of SAHARA variety, one pot of seedling from each modality was taken. The plants used were aged from two weeks. A blank was done and consisted in the same apparatus except that there was no plant. The experimentation started by the pot transfer from the plastic container to a 600 mL beaker. The step is realised one day before the sampling. The same day, four guillotines, four glass bells, eight glasses as well as plastic plugs are laid out in a bath of EXTRAN MA3 during one night. Then, the next day all the material is rinsed out with tap water and distilled water. After the cleaning, the material is let in a steam room until dry.

Once all the material is cleaned, the experiment can start. The plant is placed in a beaker (Figure 25). The guillotine is placed on the beaker and is stabilised with two additional glasses. The guillotine is tight enough to hold on all the seedlings of winter wheat. To avoid the entrance of other VOCs such as soil, roots and so on, the guillotine hole is covered with cotton and aluminium. When all the holes are filled, a glass chamber is deposited above the plants. The glass chamber is connected with a push-pull air system.





[Figure 25 - Sampling of winter wheat VOCs](#)

Air is pulled from the hole located above the glass chamber at 400 mL/ min. In the same time, air is pushed at 500 mL/min inside the chamber through a hole located at the bottom right of the glass chamber.

The odour sampling is made with a tenax - carbograph cartridge inserted in the hole above the glass chamber. The cartridge consists in 8,9 cm long and 0,64 cm external diameter stainless steel thermal desorption sampling tube packed with two sorbent beds: Tenax TA (mesh: 35/60) and Graphitised Carbon black (mesh: 40/60) (Markes International, UK). The combination of Tenax TA and Carbopack X is interesting to use as both sorbents are hydrophobic which ensures a quantitative retention and a release of compounds in volatility from 1,3-butadiene to the highest boiling components (Kännaste, Copolovici and Niinemets, 2014). Before using the cartridges, they were conditioned at 310°C for 12 hours with a nitrogen flow at 50 mL/min (model TC-20, Markes International, UK).

The sampling is done on four replicates and realised during eight hours. Once the sampling is finished, the volatiles are thermodesorbed on gas chromatograph coupled with a mass spectrometer (GC-MS) (model QP2020 NX, Shimadzu, Kyoto, Japan). In this system, the trap was cooling at -30°C and then desorbed at 280°C for 5 minutes. The cartridge was thermally desorbed (model TD30R, Shimadzu, Kyoto, Japan) at 280°C for eight minutes prior to the injection, at 60 mL/min. In each sample, four microliters of butylbenzen (CAS number: 104-51-8.99%, Sigma Aldrich) concentrated at 8.5 ng/μl was injecting as an internal standard.

The entire sample was auto injected thanks to an auto injector (model AOC-20i Plus, Shimadzu, Kyoto, Japan). The injection mode was 'split' by a ratio of 5. The carrier gas used for the injection was helium (column flow: 0.94 mL/min and total flow: 6.6 mL/min) and its pressure was at 45.1 kPa. The temperature program started at 180°C for 5 minutes and then at 300°C for 20 minutes, at 200 C°/min. Once the cartridge is thermally desorbed, the detected peaks were identified with the software 'Shimadzu Postrun'. The identification of volatile organic compounds was done based on their mass spectrum by using spectral libraries FFNSC and NIST17 (The mass spectrometer spectra match factor was minimum 80%).

## 3.6 Statistical analyses

To respond to the different goals, several statistical analyses were realised on the software RStudio 'version 3.6.0'.

### 3.6.1 Population dynamics in the Ecotron (2014-2015 and 2093-2094)

To determine the modelling and the evolution of both population curves, a general linear model (GLM) test was done by comparing two models: mean aphid per plant related to the observation dates (model 1) and mean aphid per plant related to the observation dates and the two climates year (model 2). To see if the two models were different between them, an ANOVA test was performed.

### 3.6.2 Aphid migration on winter wheat

To evaluate the quantity of aphids on leaves, stems and ears, an independence chi-squared test was realized for each observation date between both climates.

### 3.6.3 Aphid fitness

To determine if aphids have the same mean tibia size, body size and body weight between each observation dates of the 2014-2015 and 2093-2094 climate year, a Shapiro and Bartley test were performed to see if the application conditions are respected; population normality and homoscedasticity, i.e. variances equality of the population. If both conditions were respected, an ANOVA test was completed. Otherwise, a Kruskal-Wallis test was performed. When a significant difference was obtained with the Kruskal-Wallis test, a post hoc test, Wilcoxon test, was done to see where the variabilities were observed. To notice if there was a difference for the same variables between both climate years, a t-test was performed. To notice if there was a difference in the evolution of body weight at each observation date in 2015 and 2094, a linear model was used.

### 3.6.4 Aphid host plant attraction

To identify the host plant preferences for *S. avenae* and *A. fabae*, a GLM test was primarily realised. This test determines the modality mainly select by both aphid species. To see if there was a selection in plants, an ANOVA test was performed. To visualise the variabilities between each choice, some box plots were generated and a One-Way ANOVA test was executed. Then, another GLM test was performed to determine if the time needed to make a decision is similar for each modality. The results were also obtained by an ANOVA test. Some box plots were also generated and the variabilities were evaluated with a One-Way ANOVA test.

### 3.6.5 VOCs sampling from climatic enclosures

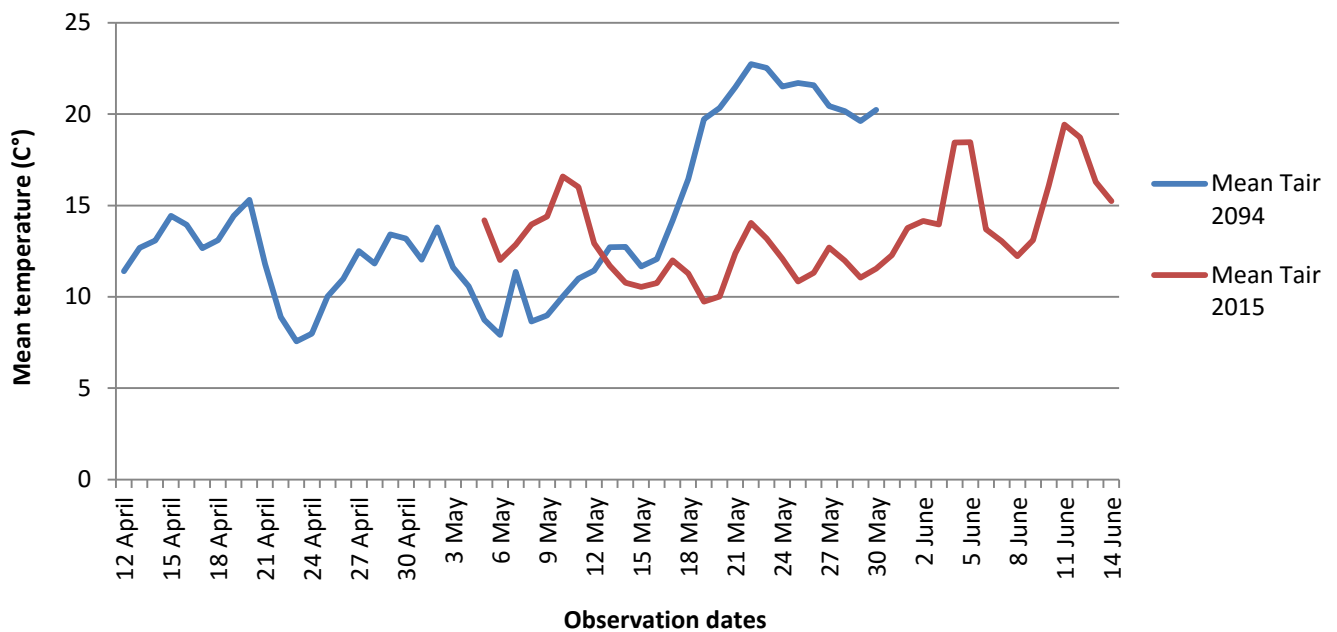
Statistical analyses were performed at two levels: area and concentration. First, an ANOVA test was realised for the total areas to identify the impact of one factor (temperature or CO<sub>2</sub> or both) on the total area of each sample. To identify which chemical compound is responsible for the differences observed, a PERMANOVA test was performed on the relative areas and the concentrations. Then, Principal component analyses (PCA) were performed for the two levels.

## 4. Results

### 4.1 Assessment of population dynamics in the Ecotron

As the aphid development and performances are affected by temperature and CO<sub>2</sub> concentration, we focus on the mean air temperatures observed in the Ecotrons from the beginning until the end of the experiment.

Figure 26 illustrates the evolution of daily mean temperatures in the climatic chambers, Ecotrons, representing the 2015 and 2094 climate.



[Figure 26 - Evolution of daily mean temperatures in 2015 and 2094, for each observation date](#)

As a reminder, aphids from the 2094 chambers are introduced on 29<sup>th</sup> April 2019 (Ecotron date = 12<sup>nd</sup> April 2094) while aphids from the 2015 chambers are introduced on 22<sup>nd</sup> May 2019 (Ecotron date = 5<sup>th</sup> May 2015). In 2094, mean air temperatures drop more frequently below 10°C and during a longer period of time. While, in 2015, mean air temperatures drop below 10°C around 19<sup>th</sup> May 2015. Around 17<sup>th</sup> May, an elevation of temperature is observed in 2094 (22°C) while mean air temperatures stay around 15°C, in 2015. The CO<sub>2</sub> concentration is set at 380 ppm in 2015 and 775 ppm in 2094.

As the population dynamics of aphids is assessed weekly, it is interesting to represent the evolution of weekly temperatures throughout the experiment (Table 2). This table will be used later to discuss the impact of temperature on aphid performances.

Table 2 - Weekly evolution of mean air temperature in 2015 and 2094, for each observation date

Observation dates	Mean airT 2015 (°C)	Mean airT 2094 (C°)
J+3	16.6	14.4
J+7	12.0	8.9
J+14	12.1	13.4
J+21	12.3	7.9
J+28	13.1	12.7
J+35	15.2	20.3
J+42	/	20.4

#### 4.1.1 Population dynamics of aphids

To assess the population dynamics of aphids, seven countings were realised in the Ecotrons representing the 2093-2094 climate year and six in the Ecotrons representing the 2014-2015 climate year. About six weeks after the insect introduction, the population was killed with a bio-insecticide, Bio-Pyretrex Garden (Deinze, Belgium) to reduce its impact on the yield crop.

To investigate population dynamics in 2015 and 2094, the data are expressed in mean number of observed aphids per plant, at each observation date after the introduction (Figure 27).

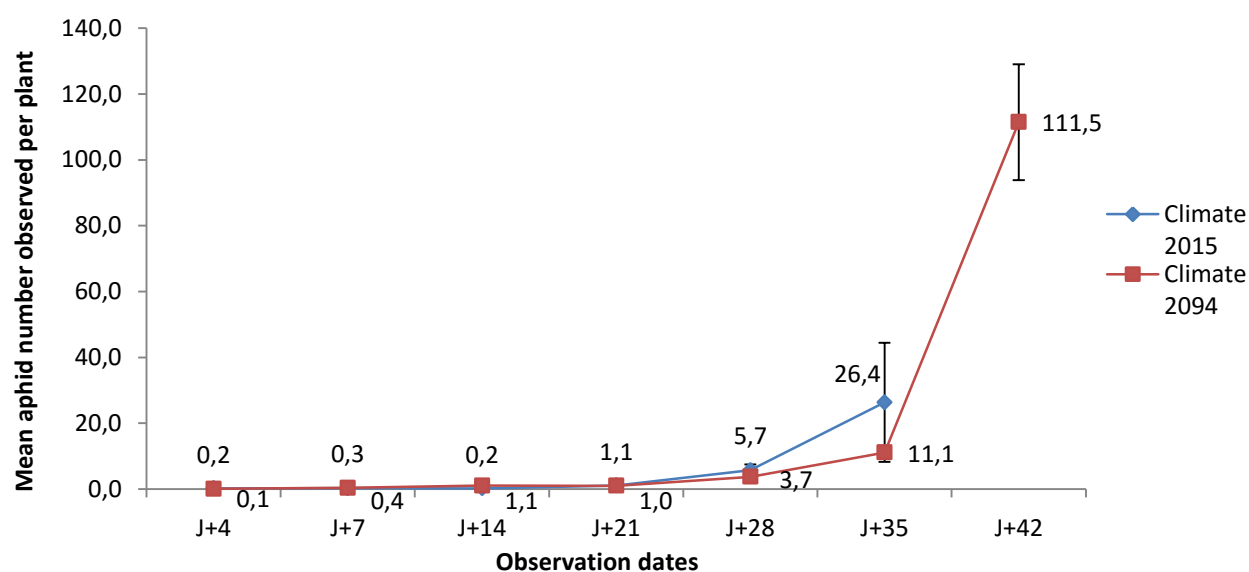


Figure 27 - Evolution of aphid population, per plant, in 2015 and 2094 ('J', in 2015, represents the aphid introduction day: 22<sup>nd</sup> May 2019 while 'J' in 2094, represents the aphid introduction day: 29<sup>th</sup> April 2019)

In 2015, the aphid population stays stable at the beginning of the experiment and starts to increase 21 days after the introduction (Figure 27). Indeed, the mean number of aphids per plant is around  $0.2 \pm 0.3$ ,  $0.3 \pm 0.3$  between J+4 and J+14 and goes up to  $26.4 \pm 18.1$  at J+35.

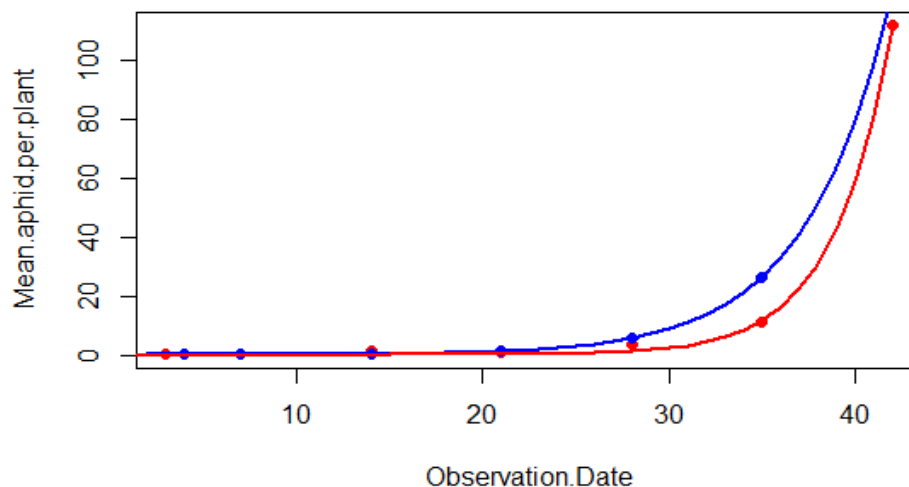
In 2094, the aphid population fluctuates gently at the beginning of the experiment and then starts to rise 28 days after the introduction (Figure 27). The mean aphid number per plant

oscillates between  $0.1 \pm 0.1$  and  $1.1 \pm 0.7$  between J+4 and J+21. Then, the mean aphid population per plant climbs up to  $111.5 \pm 17.6$  at J+42.

When comparing the two climates, we can observe that the aphid population stays relatively stable between the aphid introduction and J+21. From J+28, both aphid populations rise. However, the mean aphid number per plant increases more rapidly in 2015 than in 2094 from this observation date. In 2015,  $5.7 \pm 1.8$  and  $26.4 \pm 18.1$  aphids are observed, on average, per plant at J+28 and J+35 while, only  $3.7 \pm 1.5$  and  $11.1 \pm 1.5$  aphids, on average, are observed at the same observation dates in 2094.

As more aphids are present per plant at J+35 in 2015, a modelling is realised to predict the potential evolution of aphid population for J+42 and demonstrate if the two population dynamics follow the same trend for the 2014-2015 and 2093-2094 climate year (Figure 28).

The figure, below, illustrates the evolution of aphid population dynamics in 2015 and 2094, on winter wheat. The curve representing the 2015 climate is expressed by the equation  $y = 0.012 * e^{0.022x}$ . The curve representing the 2094 climate is expressed by the equation  $y = 0.000145 * e^{0.3225x}$ . The coefficient of determination ( $R^2$ ) equals 0.999 which means that the modelling shape at 99.9% the evolution of aphid population dynamics in 2015 and 2094.



**Figure 28 - Evolution of aphid population dynamics in 2015 and 2094 (Blue line= 2014-2015 and Red line = 2093-2094)**

As the modelling shows, both curves follow the same shape until J+21. Then, the aphid population dynamics in 2015 increases more rapidly than in 2094 creating a gap with the 2094 curve, particularly pronounced at J+35 ( $F = 9.046$ ,  $df = 9$ ,  $p = 0.0148$ ). At J+42, both lines converge even if the predicted mean of observed aphids per plant in 2015 seems to be slightly higher than the one in 2094. Thus, aphids are more numerous per plant in 2015 compared to 2094.

#### 4.1.2 Aphid migration on winter wheat

To assess the aphid migration on wheat, the aphid position on winter wheat is assessed weekly in 2015 and 2094. The position of aphids is recorded on three parts of the plant: the leave, the stem and the ears. Figure 29 illustrates the aphid position on winter wheat in 2015, for each observation date.

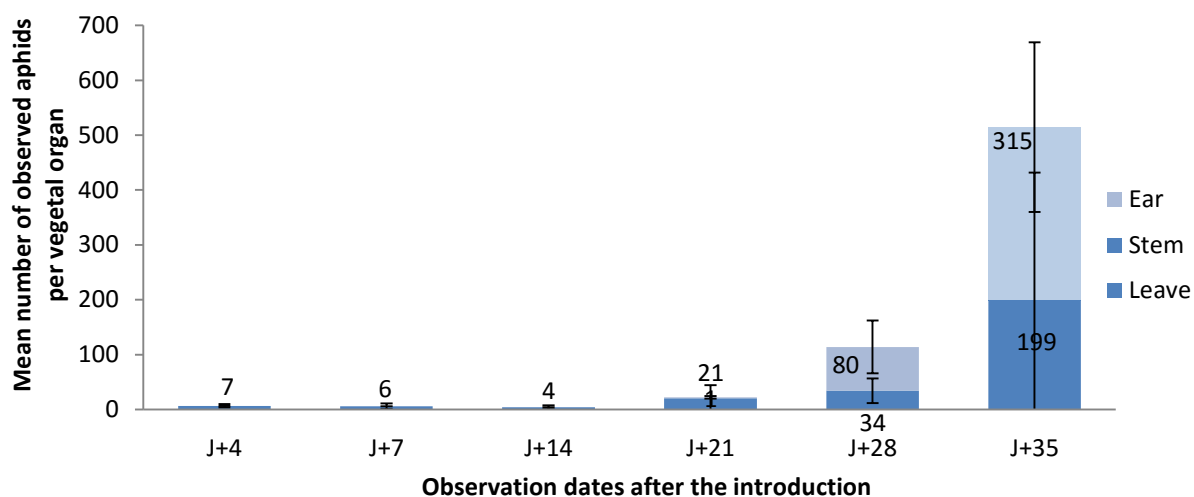
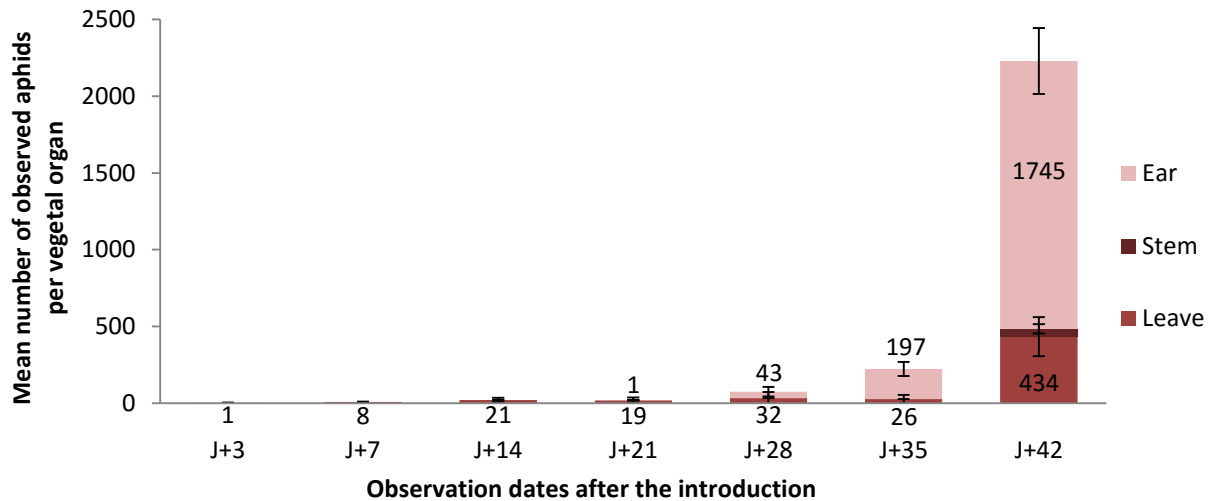


Figure 29 - Mean aphid migration on winter wheat, in 2015

During the first three weeks, aphids are only present on wheat leaves, in 2015. Then, they gently migrate on ears at J+21 and become more important at J+28 and J+35 with  $80 \pm 47.96$  and  $315 \pm 154.5$  aphids observed, respectively. On average, wheat plants at J+28 are at BBCH65<sup>7</sup> which represents the phenological stage ‘full flowering’, according to Zadoks code.

The mean migration of aphids is also investigated under the 2093-2094 climate year (Figure 30). Aphids are only present on wheat leaves between J+3 and J+14. At J+21, few aphids are observed on ears ( $1 \pm 0.58$ ), and from J+28 to J+42, aphid migrate to ears with  $43 \pm 31.26$  individuals observed at J+28 and  $1745 \pm 215.53$  at J+42. Aphids are noticed on stems only at J+42.

<sup>7</sup> BBCH code is a scale which helps to identify the phenological growth stages of a cultivated plant



[Figure 30 - Mean aphid migration on winter wheat, in 2094](#)

In 2094, aphids appear on ears when wheat plants reach the growth stage BBCH 59 which represents the stage ‘End of heading’.

To conclude, aphids appear at an earlier growth stage of winter wheat in 2094. Indeed, individuals are noticed at the stage ‘Ending of heading’ for the climate year 2094. In the beginning, the same number of aphids are present on ears in 2015 ( $1 \pm 2.31$ ) and 2094 ( $1 \pm 0.58$ ) (chi-squared =  $9.88e-32$ ,  $df = 1$ ,  $p = 1$ ). Then, aphids are more abundant on ears in 2015 relative to 2094 (chi-squared =  $11.13$ ,  $df = 1$ ,  $p = 8.45e-04$ ). Indeed,  $43 \pm 31.26$  aphids, on average, are observed on ears in 2094 while  $80 \pm 47.96$  aphids are observed in 2015. The same analyses are done for J+35, as  $315 \pm 154.5$  aphids, on average, were observed on ears in 2015 compared to  $197 \pm 46.07$  in 2094 (chi-squared =  $88.516$ ,  $df = 1$ ,  $p = < 2.2e-16$ ).

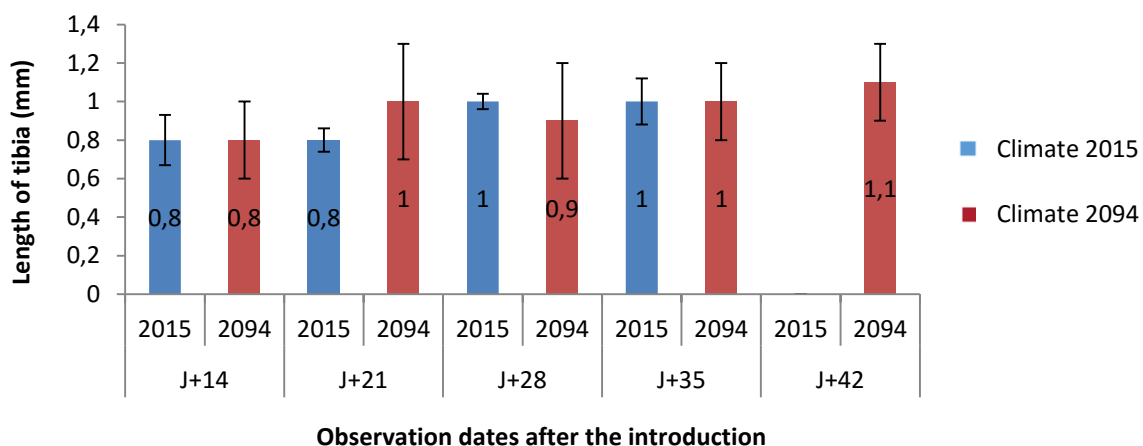
## 4.2 Aphid fitness assessment

### 4.2.1 Aphid mean fitness

To compare the aphid fitness under contrasted climates, different variables are recorded: the tibia size of aphids, their body size as well as their mean weight.

#### ❖ *Assessment at the Ecotron scale*

To assess the fecundity of aphids, the left tibia length of aphids was measured each week (Figure 31).

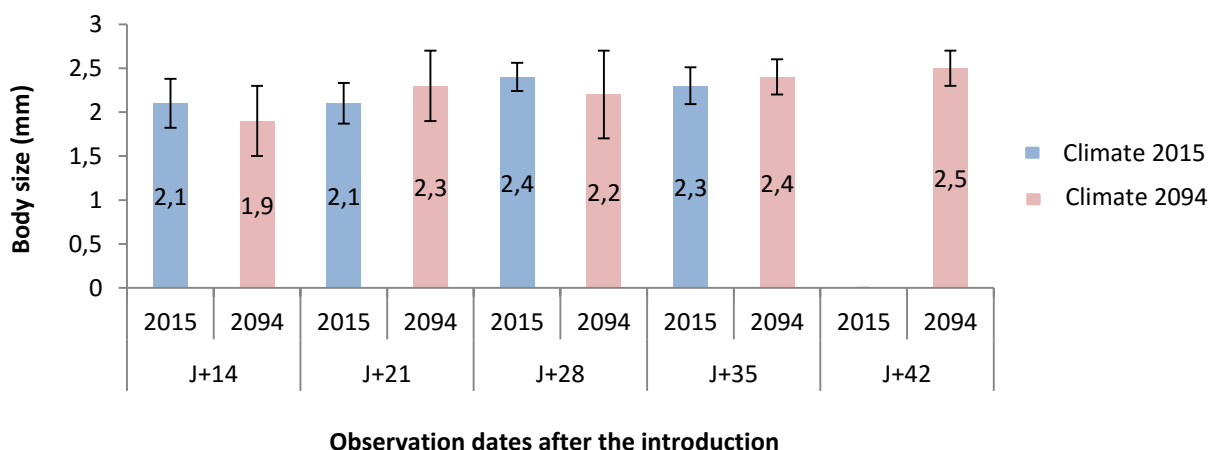


**Figure 31 - Evolution of aphid tibia length in 2015 and 2094, for each observation date**

Under the 2015 climate, aphids fecundity increases significantly throughout the experiment ( $H = 13.683$ ,  $df = 3$ ,  $p = 0.003$ ). Indeed, aphids have a longer tibia at J+35 than at J+14 (pairwise.wilcox.test,  $p = 0.007$ ) and J+21 (pairwise.wilcox.test,  $p = 0.046$ ).

Under the 2094 climate, aphids are more fecund at the end of the experiment ( $H = 30.467$ ,  $df = 4$ ,  $p = 3.932e-06$ ). Aphids have a longer tibia at J+35 (pairwise.wilcox.test,  $p = 0.001$ ) and J+42 (pairwise.wilcox.test,  $p = 1.3e-06$ ) than at J+14. In addition, the tibia length is higher at J+35 (pairwise.wilcox.test,  $p = 0.022$ ) and J+42 (pairwise.wilcox.test,  $p = 0.035$ ) than at J+28.

To assess the aphid growth, the aphid body was measured weekly (Figure 32).



**Figure 32 - Evolution of aphid body size in 2015 and 2094, for each observation date**

In 2015, aphids are taller at the end of the experiment (J+35) than at the beginning (J+14) ( $H = 8.2935$ ,  $df = 3$ ,  $p = 0.0403$ ) (Figure 32).

In 2094, aphids are taller at the end of the experiment ( $H = 35.974$ ,  $df = 4$ ,  $p = 2.929e-07$ ). Indeed, aphids are taller at J+21 (pairwise.wilcox.test,  $p = 2.0e-07$ ), J+35 (pairwise.wilcox.test,  $p = 1.2e-05$ ) and J+42 (pairwise.wilcox.test,  $p = 1.3e-06$ ) than at J+14.



To assess the aphid body weight, the same ten individuals used for the assessment of fecundity and size are weighted. Figure 33 illustrates the adult body weight in 2015 and 2094.

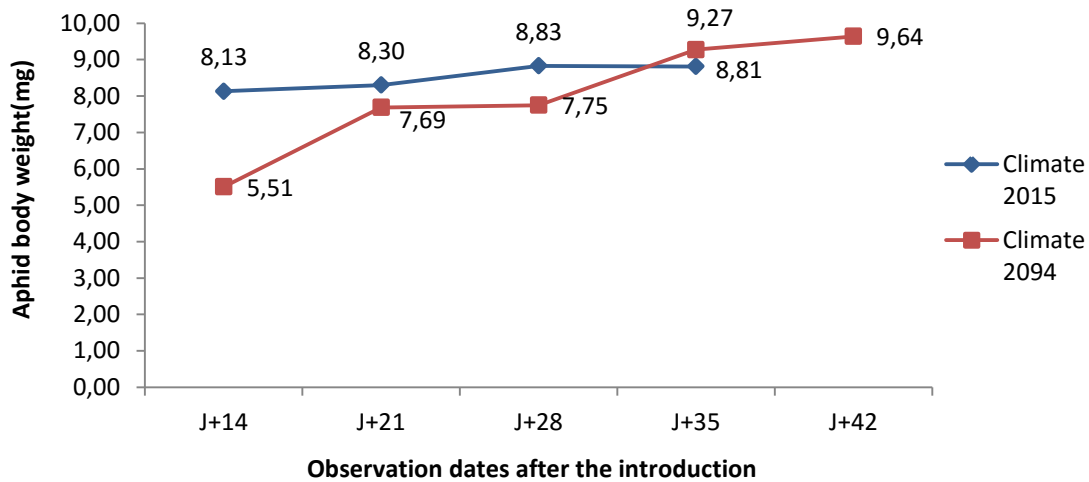


Figure 33 - Evolution of aphid body weight in 2015 and 2094, for each observation date

Aphids have the same body weight from the beginning until the end of the experiment in 2015 ( $F = 0.051$ ,  $df = 1.10$ ,  $p = 0.825$ ) and in 2094 ( $F = 2.166$ ,  $df = 1.13$ ,  $p = 0.165$ ).

When comparing the two climates, the evolution of tibia length is similar in 2015 and 2094 ( $F = 0.883$ ,  $df = 1.266$ ,  $p = 0.348$ ). Aphid body size evolves in the same way in 2015 and 2094 ( $F = 0.152$ ,  $df = 1.266$ ,  $p = 0.697$ ). In addition, the evolution of aphid body weight is similar under both climatic conditions ( $F = 1.5$ ,  $df = 1.13$ ,  $p = 0.233$ ) resuming a high variability between each weighting.

#### ❖ Assessment at the plant scale

As a reminder, four plants selected randomly are followed throughout the experiment. The results below illustrate the mean aphid fitness in both climate years, assessed at the end of the experimentation before the insecticide application (Figure 34). The black rope represents the body weight of aphids.

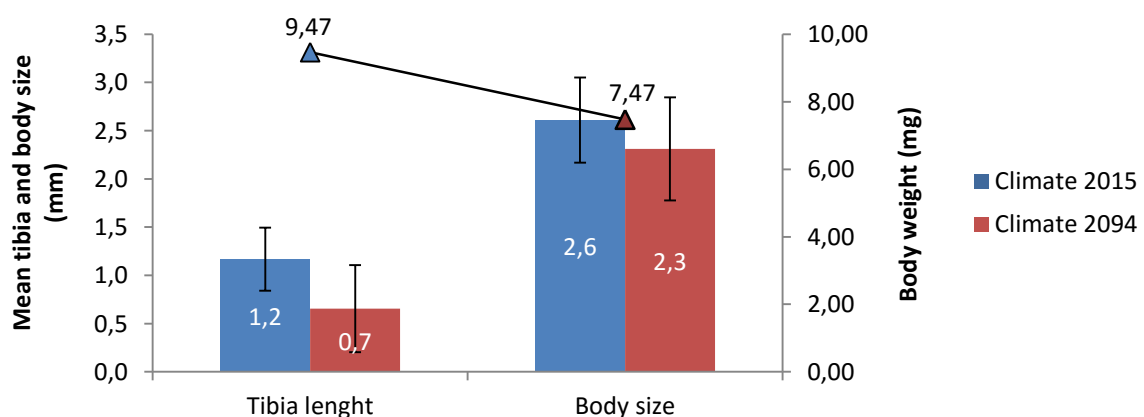


Figure 34 - Evolution of mean fitness of aphids in 2015 and 2094, for the four followed plants

In 2015, aphids have a longer tibia (t.test = 18.862, df = 191.99, p < 2.2e-16), are taller (t.test = 27.664, df = 177.97, p < 2.2e-16) and heavier (t.test = 2.404, df = 19.893, p = 0.026) compared to 2094.

### 4.3 Host plant attraction

#### 4.3.1 Winter wheat attraction for the English grain aphid

To evaluate the aphid host plant attraction, aphids are submitted to plants grown under several climatic conditions. No difference is observed in the host plant selection when aphids are facing plants grown under **(Condition 1)** ambient Temperature – ambient CO<sub>2</sub> and elevated Temperature - ambient CO<sub>2</sub> (glm.test, p = 0,528), **(Condition 2)** ambient Temperature – ambient CO<sub>2</sub> and ambient Temperature – elevated CO<sub>2</sub> (glm.test, p = 0,209), **(Condition 3)** ambient Temperature – ambient CO<sub>2</sub> and elevated Temperature – elevated CO<sub>2</sub> (glm.test, p = 0,209), **(Condition 4)** elevated Temperature - ambient CO<sub>2</sub> and ambient Temperature – elevated CO<sub>2</sub> (glm.test, p = 0,209), **(Condition 5)** elevated Temperature - ambient CO<sub>2</sub> and elevated Temperature – elevated CO<sub>2</sub> (glm.test, p = 0,528) and **(Condition 6)** ambient Temperature – elevated CO<sub>2</sub> and elevated Temperature – elevated CO<sub>2</sub> (glm.test, p = 1). Aphids do not select preferentially plants grown under elevated temperature and/or higher CO<sub>2</sub> concentration as the following figures from 35 to 40 represent.

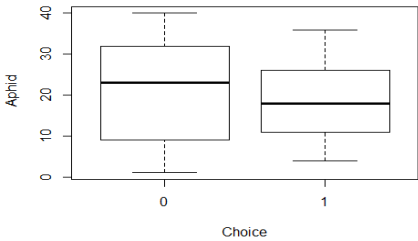


Figure 35 - Choice made by an aphid where modality 0 = 'eTaCO<sub>2</sub>' and 1 = 'aTaCO<sub>2</sub>'

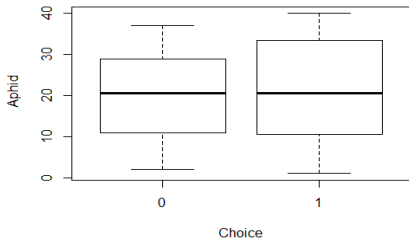


Figure 36 - Choice made by an aphid where modality 0 = 'aTeCO<sub>2</sub>' and 1 = 'aTaCO<sub>2</sub>'

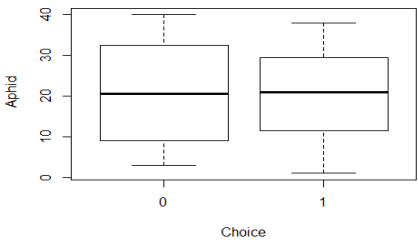


Figure 37 - Choice made by an aphid where modality 0 = 'eTeCO<sub>2</sub>' and 1 = 'aTaCO<sub>2</sub>'

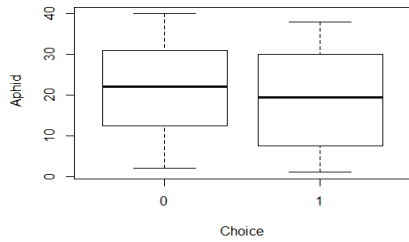


Figure 38 - Choice made by an aphid where modality 0 = 'aTeCO<sub>2</sub>' and 1 = 'eTaCO<sub>2</sub>'

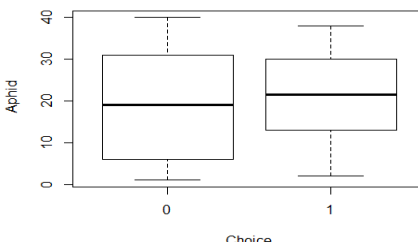


Figure 39 - Choice made by an aphid where modality 0 = 'eTeCO<sub>2</sub>' and 1 = 'eTaCO<sub>2</sub>'

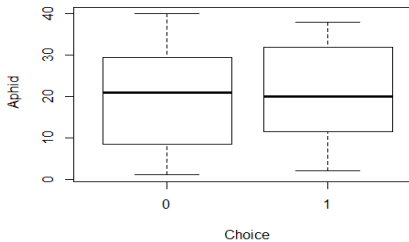


Figure 40 - Choice made by an aphid where modality 0 = 'eTeCO<sub>2</sub>' and 1 = 'aTeCO<sub>2</sub>'

To assess if aphids take the same amount of time to select one modality from another one, an ANOVA test is realised. The results obtained from the test are summed up in Table 3.

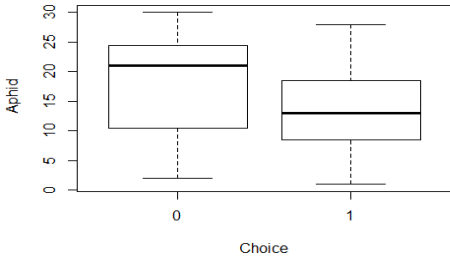
**Table 3 - Time needed for the English grain aphid to make a decision when facing different feeding conditions**

	Condition1		Condition2		Condition3		Condition4		Condition5		Condition6	
	Fvalue	Pr(>F)	Fvalue	Pr(>F)	Fvalue	Pr(>F)	Fvalue	Pr(>F)	Fvalue	Pr(>F)	Fvalue	Pr(>F)
<b>Choice</b>	0.738	0.397	0.836	0.3674	4.180	<b>0.0492*</b>	0.205	0.654	0.205	0.654	0.145	0.706
<b>Blocks</b>	1.722	0.148	3.069	<b>0.0173*</b>	0.871	0.5268	0.576	0.746	0.576	0.746	1.018	0.413

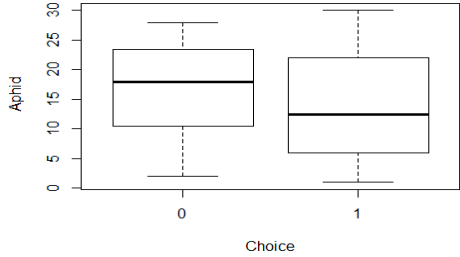
Aphids take more time to select wheat plants cultivated under elevated temperature – elevated CO<sub>2</sub> than ambient temperature - ambient CO<sub>2</sub> (F = 4.180, df = 1.32, p = 0.0492). However, no difference in time is observed for the other conditions. Some box plots representing the time needed to make a decision are in Appendix 4.

**4.3.2 Fava bean attraction for the black bean aphid**

The same conclusions are observed for the black bean aphid when it is exposed to fava beans grown under the same climatic conditions as the ones used for winter wheat. No difference in terms of host plant attraction is observed when plants are grown under: **(Condition 1)** ambient Temperature – ambient CO<sub>2</sub> and elevated Temperature - ambient CO<sub>2</sub> (glm.test, p = 0.149), **(Condition 2)** ambient Temperature – ambient CO<sub>2</sub> and ambient Temperature – elevated CO<sub>2</sub> (glm.test, p = 0.715), **(Condition 3)** ambient Temperature – ambient CO<sub>2</sub> and elevated Temperature – elevated CO<sub>2</sub> (glm.test, p = 0.467), **(Condition 4)** elevated Temperature - ambient CO<sub>2</sub> and ambient Temperature – elevated CO<sub>2</sub> (glm.test, p = 0.074), **(Condition 5)** elevated Temperature - ambient CO<sub>2</sub> and elevated Temperature – elevated CO<sub>2</sub> (glm.test, p = 0.277) and **(Condition 6)** ambient Temperature – elevated CO<sub>2</sub> and elevated Temperature – elevated CO<sub>2</sub> (glm.test, p = 0.467). Thus, aphids do not prefer plants grown under elevated temperature and/or higher CO<sub>2</sub> concentration as the figures from 41 to 46 below show.



**Figure 41 - Choice made by an aphid where modality 0 = 'eTaCO<sub>2</sub>' and 1 = 'aTaCO<sub>2</sub>'**



**Figure 42 - Choice made by an aphid where modality 0 = 'aTaCO<sub>2</sub>' and 1 = 'aTeCO<sub>2</sub>'**

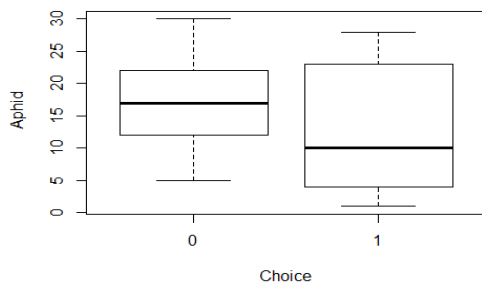


Figure 43 - Choice made by an aphid where modality 0 = 'aTaCO<sub>2</sub>' and 1 = 'eTeCO<sub>2</sub>'

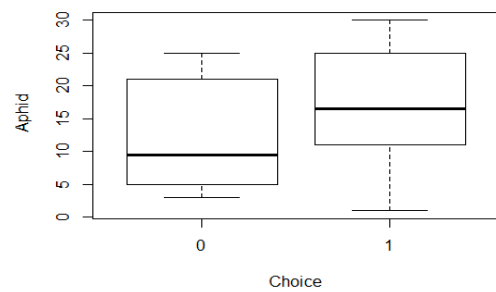


Figure 44 - Choice made by an aphid where modality 0 = 'eTaCO<sub>2</sub>' and 1 = 'aTeCO<sub>2</sub>'

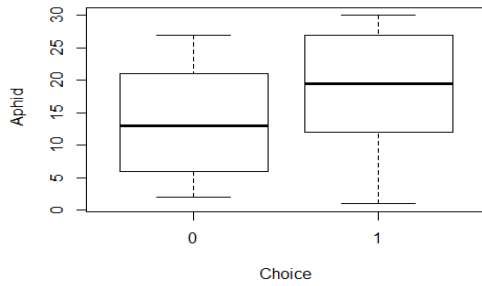


Figure 45 - Choice made by an aphid where modality 0 = 'eTaCO<sub>2</sub>' and 1 = 'eTeCO<sub>2</sub>'

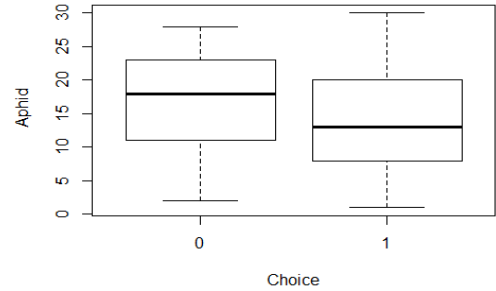


Figure 46 - Choice made by an aphid where modality 0 = 'eTeCO<sub>2</sub>' and 1 = 'aTeCO<sub>2</sub>'

To visualise if aphids take the same time to select wheat plants grown under those conditions, an ANOVA was realised. Table 4 sums up the different results obtained.

Table 4 - Time needed for the black bean aphid to make a decision when facing different feeding conditions

	Condition1		Condition2		Condition3		Condition4		Condition5		Condition6	
	Fvalue	Pr(>F)	Fvalue	Pr(>F)	Fvalue	Pr(>F)	Fvalue	Pr(>F)	Fvalue	Pr(>F)	Fvalue	Pr(>F)
<b>Choice</b>	0,828	0.372	1.263	0.272	0.17	0.683	0.879	0.358	1.166	0.291	0.241	0.628
<b>Blocks</b>	2.715	0.053	0.531	0.714	1.27	0.309	1.436	0.253	0.405	0.803	2.507	0.069

Aphids take the same amount of time to select the modalities of a same condition. The box plots representing the time taken by an aphid to make a decision are in Appendix 5.

Before the assessment of host plant attraction, the experimental apparatus is tested to validate the set-up of both insect models and limit the set-up bias. For the study conducted on *S. avenae*, forty repetitions are realised by submitting aphids to wheat, on one side, and cotton, on the other side of the Petri dish. The experimental set-up is validated as aphids select more preferentially wheat (chi-squared = 12.902, df = 1.24, p = 3.28e-04).

The experimental set-up used for *A. fabae* is also tested. Forty repetitions are done by submitting aphids to two fava beans grown under the same condition which is represented by ambient Temperature and ambient CO<sub>2</sub>. Aphids do not make a difference between the same plants (chi-squared = 1.286, df = 1, p > 0.05) which allow us to validate the experimental apparatus.

#### 4.4 Sampling of winter wheat volatile organic compounds (VOCs)

No significant difference was observed in host plant selection for both aphid species. However, Chen, Wu and Ge, 2004 have observed that alate *S. avenae* select preferentially winter wheat grown under elevated CO<sub>2</sub>. To see if the absence of responses from apterous *S. avenae* is linked to VOCs, a study is conducted on wheat VOCs emission.

To determine if there is a significant impact of elevated CO<sub>2</sub> concentration and elevated temperature on the volatile profile of winter wheat, an evaluation on the interaction between 'elevation in temperature' and 'elevation in CO<sub>2</sub> concentration' is assessed. The evaluation is divided into two steps. The first one aims to determine if the total emissions of chemical compounds differ between each sample. The second evaluation aims to identify which VOC differs in terms of relative area and concentration in each sample. A tentative of identification of the chemical compounds emitted by winter wheat is realised in Appendix 6.

##### ❖ Total areas

A significant difference is obtained when plants are grown under elevated CO<sub>2</sub> and elevated temperature ( $F = 3.488$ ,  $df = 3.12$ ,  $p = 0.050$ ). More precisely, the total emissions in VOCS are different between aTaCO<sub>2</sub> and aTeCO<sub>2</sub> (pairwise.t.test,  $p = 0.015$ ).

To assess more in detail which chemical compound varies between in each climatic condition, a PERMANOVA test is realised by considering the relative area of all the chemical compounds detected in one sample. In addition to it, a PCA is performed to illustrate the results obtained from the PERMANOVA. The PCA is done between dimension 1 and 2 as the percentage of explained variances is higher.

##### ❖ Relative areas

A significant difference is observed when the parameters 'CO<sub>2</sub>' and 'temperature' are combined ( $F = 3.450$ ,  $df = 3.12$ ,  $p = 0.004$ ). The main variation is noticed for the combination 'aTeCO<sub>2</sub>' and 'eTaCO<sub>2</sub>' (pairwise.t.test,  $p = 0.028$ ) and the combination 'aTaCO<sub>2</sub>' and 'eTaCO<sub>2</sub>' (pairwise.t.test,  $p = 0.026$ ) (Figure 47).

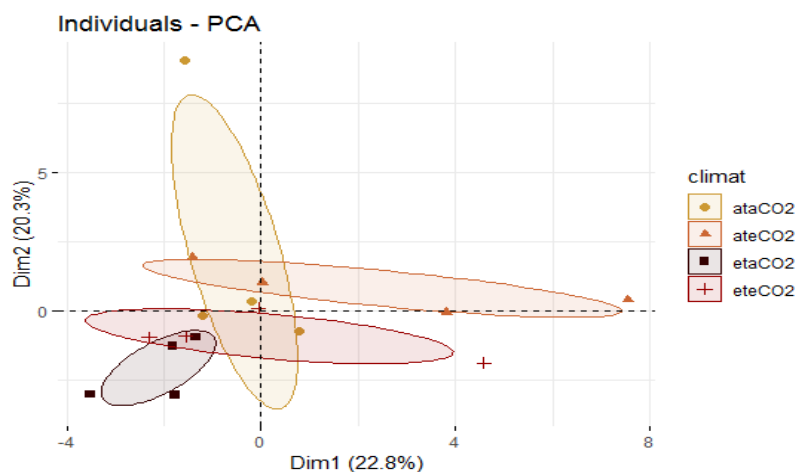


Figure 47 - Principal component analysis for the different climatic conditions: temperature ('aT and eT') and CO<sub>2</sub> ('aCO<sub>2</sub> and eCO<sub>2</sub>'), expressed by dimension 1 and 2

To identify which relative area of chemical compounds induces a difference between both climatic conditions, the contribution of volatiles to dimension 1 and 2 is analysed.

As Figure 48 below shows, compounds mainly from the alkane family contribute to dimension 1. There are other contributions from the ketone, acid and alcohol family.

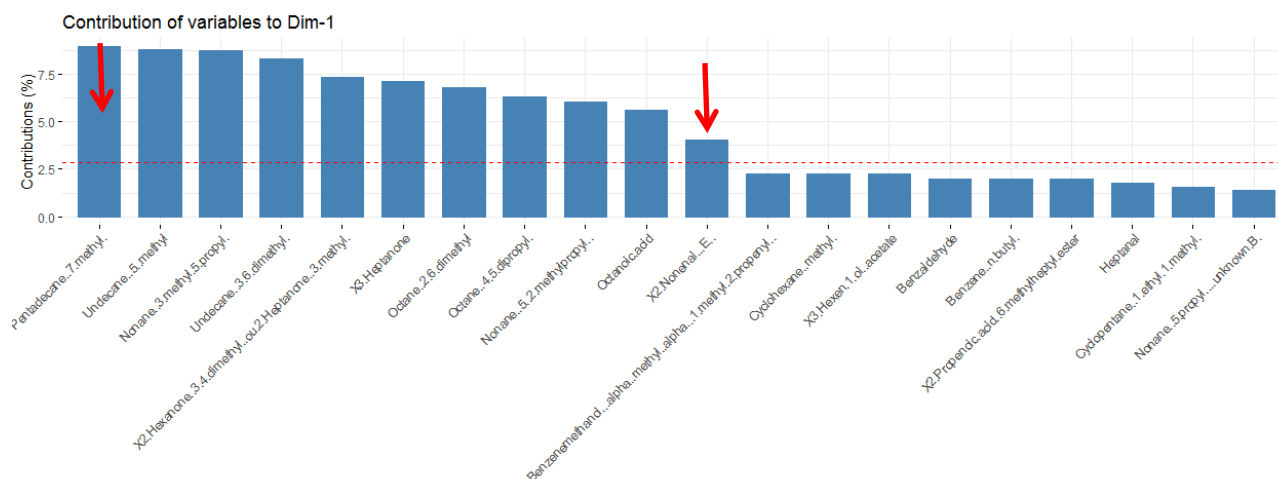


Figure 48 - Contribution of chemical compounds to dimension 1

The contribution of chemical compounds to dimension 2 is different from dimension 1. Other compounds are observed in Figure 49 such as 4-Nonenal, 3-methylundecane and benzaldehyde.

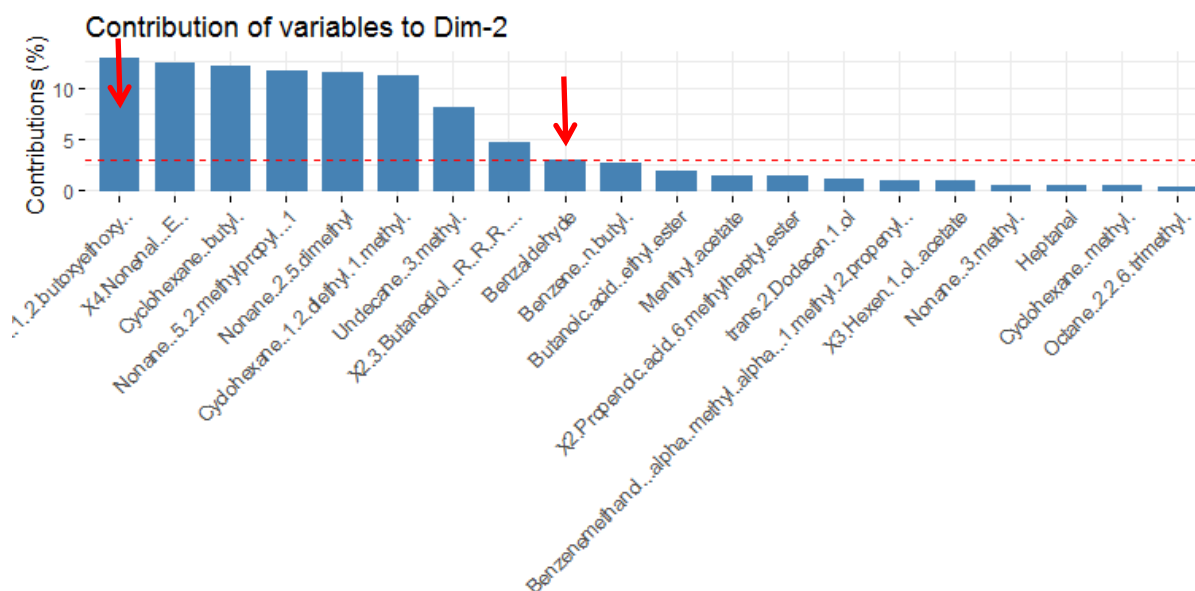
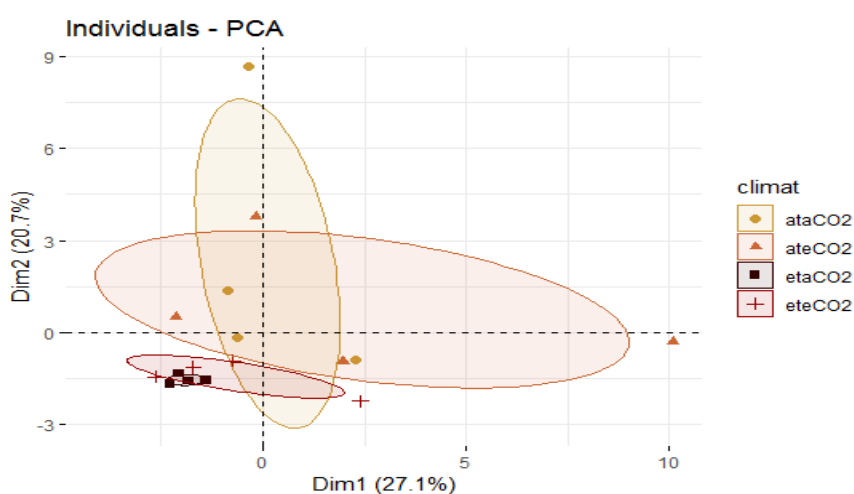


Figure 49 - Contribution of chemical compounds to dimension 2

### ❖ Concentrations

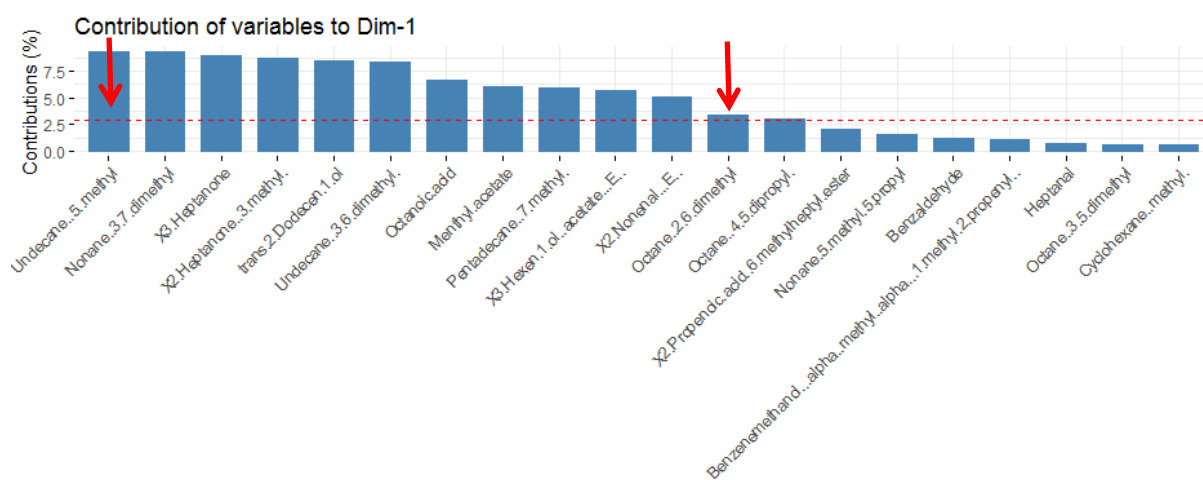
The same analyses are realised in terms of concentration. The goal of it is to determine if the chemical compounds vary in concentration for each climatic variable. The same trends are observed.

A significant difference is observed between the combination 'temperature' and 'CO<sub>2</sub>' (F = 3.518, df = 3.12, p = 0.002). VOCs concentrations vary between 'aTaCO<sub>2</sub>' and 'eTaCO<sub>2</sub>' (pairwise.t.test, p = 0.028), 'aTaCO<sub>2</sub>' and 'eTeCO<sub>2</sub>' (pairwise.t.test, p = 0.030), 'aTeCO<sub>2</sub>' and 'eTaCO<sub>2</sub>' (pairwise.t.test, p = 0.035) as well as 'aTeCO<sub>2</sub>' and 'eTeCO<sub>2</sub>' (pairwise.t.test, p = 0.028) (Figure 49). Again, a PCA is realised between dimension 1 and 2 (Figure 50).



**Figure 50 - Principal component analysis for the different climatic conditions: temperature ('aT and eT') and CO<sub>2</sub> ('aCO<sub>2</sub> and eCO<sub>2</sub>'), expressed by dimension 1 and 2**

As figure 51 illustrates, chemical compounds from the alkane family mainly contribute to dimension 1. Other chemical families such as acid, alcohol and ketone also contribute to this dimension.



**Figure 51 - Contribution of chemical compounds to dimension 1**

The contribution of chemical compounds to dimension 2 is more varied. Chemical compounds belonging to the alkane and alcohol family as well as cyclic compounds are noticed (Figure 52).

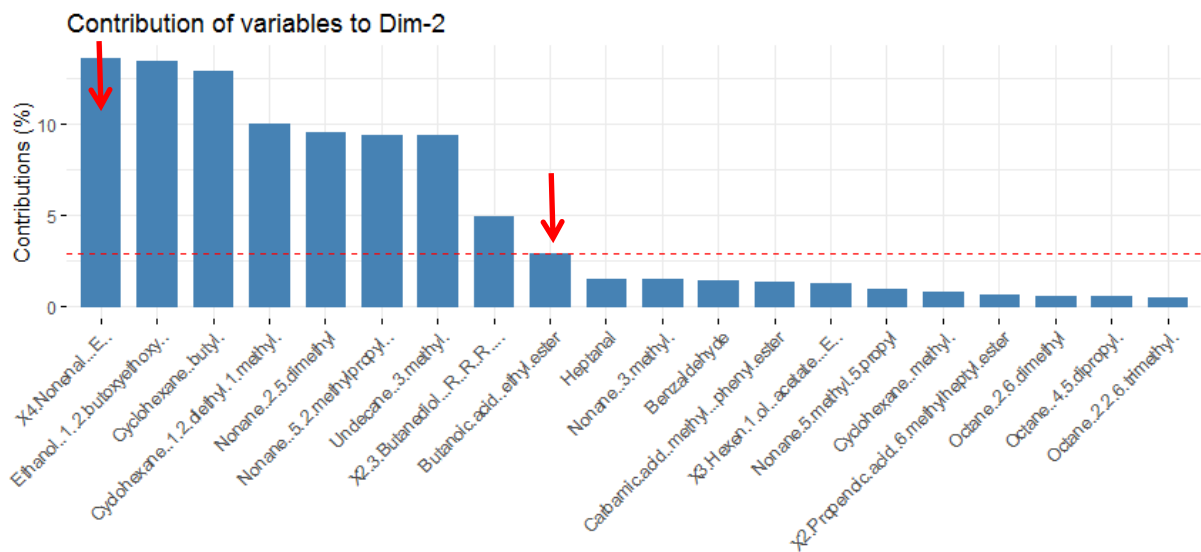


Figure 52 - Contribution of chemical compounds to dimension 2

## 5. Discussion

The global goal of this study is to evaluate the impact of contrasted climatic conditions on aphid fitness and host plants selection.

Specially, this study aims to (1) evaluate the population dynamics of *S. avenae* on winter wheat, (2) compare life history traits of *S. avenae*, (3) identify the host plant attraction of *S. avenae* when facing wheat plants grown under contrasted climatic conditions, (4) analyse the VOCs emitted by winter wheat under contrasted climatic conditions.

The major conclusions we can draw from this work include: The population of *S. avenae* is more important under a 2015 climate than under a 2094 climate. Furthermore, aphids tend to be less fecund and smaller in 2094 than in 2015. Regarding host plant selection, aphids make no difference between plants grown under any tested climatic conditions. However, the VOCs emitted by winter wheat appear to be different according to the climatic condition.

I will discuss in more detail these different elements.



## 5.1 Assessment of population dynamics in the Ecotron

### 5.1.1 Population dynamics of aphids

As a reminder, a modelling of both population dynamics has shown a significant difference between the 2014-2015 and 2093-2094 climate year. The aphid population increases more rapidly in 2015 as more aphids per plant were observed.

This difference observed in population dynamics under the two climates is explained by the physiological responses of aphids facing different temperatures during their development. In the chambers set at the conditions of the 2094 climate year, aphids faced longer and colder episodes. Indeed, the mean air temperature was below 10°C between the 22<sup>nd</sup> and 24<sup>th</sup> of April 2094 and between the 5<sup>th</sup> and 9<sup>th</sup> of May 2094. These cold periods increase the development time of each instar resulting in a slower growth rate which in turn reduces the aphid abundance (Dean, 1974; Jeffs and Leather, 2014). Moreover, below 10°C, the mortality of the fourth instars is higher (Lykouressis, 1985) leading to a lower adult emergence. In addition, aphids from the 2094 climate are submitted to more fluctuations in temperature. The first week, the mean air temperature drops from 14 to 9°C while in the third week, those values decrease from 13 to 8°C. The important temperature variations have reduced aphid development, longevity and reproduction (Dean, 1974) leading the production of fewer nymphs (Kuo, Chiu and Perng, 2006).

Aphid population is also facing an increase in temperature in 2094. Indeed, during the sixth week of the experiment, the mean air temperature climbed from 13 to 20°C and remains constant the next week (Table 2). Temperatures around 20°C may have favoured a better fecundity and a lower development time leading to an increase in aphid population in 2094 (Dean, 1974; Duffy, Fealy and Fealy, 2017). In 2015, air temperatures were quite similar which may help aphids to be more adapted and take advantage in terms of reproduction and development. However, aphids develop faster and produce more nymphs at 20°C than at 15°C (Dean, 1974; Kuo, Chiu and Perng, 2006). As more aphids were observed in 2015 than in 2094, despite the better temperatures in 2094, the combination between elevated CO<sub>2</sub> concentration and elevated temperature may have decreased the aphid abundance in 2094 (Newman *et al.*, 1999).

At an individual scale, the aphid population has been multiplied by ten between the last two observation dates (J+35 and J+42). In addition, most of the aphids are present on ears and stems. The abundance is so important that there is a crowding of aphids on ears. The reasons of this increase may be linked to constant temperatures (20°C) as well as the absence of rain.

### 5.1.2 Aphids migration on winter wheat

For both climate years, *S. avenae* migrates from leaves to ears as this species especially feeds on ears (Weppeler, 2009; Honek *et al.*, 2017).

As a reminder, aphid appearance on ears is earlier in 2094, as aphids are observed on wheat plants characterised by the growth stage 'End of heading' (BBCH59). Climate change accelerates significantly the phenological stage of winter wheat. Wheat plants from the 2094 chambers are facing higher daily minimum temperature relative to 2015 from J+21 to J+35. An increase of 1°C in minimum temperature is known to shorten the period from tillering (BBCH 20) to stem elongation (BBCH30) while it prolongs the period from stem elongation (BBCH 30) to booting (BBCH 40) as well as the period from anthesis (BBCH 60) to milk (BBCH 70) (Wang *et al.*, 2008). In addition, as the observed nighttime temperature is about 21°C in the 2094 chambers, a reduction in the duration of anthesis and physiological maturity may perhaps appear. Prasad *et al.*, 2008 have found that wheat plants exposed to 20°C nighttime temperature instead of 14°C have a reduction in the duration of anthesis stage by one day and by four days for the physiological maturity. Semenov, 2009 has also found that the maturity of wheat is earlier in warmer climates.

Besides the influence of daily minimum and maximum temperatures, aphid appearance at an earlier growth stage of winter wheat is explained by the earlier flag leaf senescence. Thanks to a parallel study conducted on climate change impact on winter wheat phenology at TERRA, in the Ecotrons, some results have shown that the daily temperatures in 2094 are optimal for the photosynthesis. In addition, higher CO<sub>2</sub> concentration favours an earlier senescence of the wheat flag leaf if ears have emerged and have a photosynthetic activity (Zhu *et al.*, 2009). As ears have emerged earlier in 2094, earlier flag leaf senescence has appeared favouring aphid migration from leaves to ears. Aphid appearance at an earlier growth stage of winter wheat may provoke earlier damages on cereal productivity and yield.

During the assessment of aphid migration, more individuals are observed on ears in 2015. The higher aphid number per ear is explained by the reproduction site. On ears, aphids produce more nymphs than on mature leaves leading to more young individuals. In addition, the fecundity and survival are improved on ears, in 2015, as the food is richer on ears around flowering for aphids than on mature and senescence leaves (Watt, 1979).

## 5.2 Aphid fitness assessment

### 5.2.1 Aphid mean fitness

By assessing the life history traits of aphids from four followed plants, we found that the fecundity, the growth rate as well as the weight are higher in 2015 relative to 2094.

As explained before, aphids are facing better temperature conditions in 2015, at the beginning of the experiment, leading to a better fecundity and survival (Barlow, 2008). The important fluctuations in temperature in 2094 have reduced considerably the fecundity and longevity of aphids. Kuo, Chiu and Perng, 2006 have observed a drop in fecundity and longevity when temperatures decrease from 15°C to 10°C and from 10 to 6°C. However, the increase in temperature from 13 to 20°C observed the last weeks of the experiment, in 2094, has increased the aphid fecundity (Kuo, Chiu and Perng, 2006). Thus, it seems that the

combined effect of temperature and CO<sub>2</sub> concentration has reduced the aphid fecundity in the 2094 chambers.

It has been observed that aphids are taller in the 2015 chambers compared to the 2094 chambers. Aphids seem to grow better with increasing temperatures. At the beginning, temperatures in 2015 are beneficial for aphid growth as Dixon and Acreman, 1989 have shown that apterous *S. avenae* are larger on ears and flag leaf at 15 than 10°C. In addition, cooler temperatures in the 2094 chambers have slowed the growth rate of aphids at the beginning.

The body weight of aphids is also more important in 2015 as aphids are living under optimal temperatures. Temperatures around 15°C, in 2015, contribute to a better body gain of *S. avenae*. While, temperatures dropping below 10°C or upper 15°C reduce its weight on ears and flag leaf (Dixon and Acreman, 1989).

The lower body weight in 2094 could have also been linked to the drought. At first, the drought observed in both climate years was considered as a factor on the differences observed in aphid performances. Indeed, wheat plants from 2094 were characterised by small leaves flag. In addition, wheat plants from 2015 were characterised by a rolling of the leaves, induced by an absence of water soil. In both climate years, the plants were characterised by a higher stem elongation rather than a mass growth. When rain episodes appeared, as plants were relatively dense, the amount of water percolating from the top of the plant to roots was limited, reducing the water soil content. As both climate years were marked by drought stress, the difference in aphid performances in each climate may be explained by another factor, the CO<sub>2</sub> concentration.

By assessing the life history traits of aphids throughout the experiment, similarities are observed between 2015 and 2094 in fecundity, body size and body weight. Those similarities may be explained by the experimental set-up. After measuring the tibia length, body size and body weight, the repositioning of individuals on leaves was not easy in 2094. It was then difficult to take weekly the same individuals at a similar development stage which lead to variabilities between data.

As the fecundity and the body size of aphids are similar in 2015 and 2094, it seems that the positive effects of temperature are mitigated by the elevation in CO<sub>2</sub>.

The lower body weight of aphids in 2094 compared to 2015, at the beginning of the experiment, could be explained by the fluctuations in temperature observed in 2094. Nonetheless, as the temperatures continue to fluctuate and the body weight to increase, it suggests that elevated CO<sub>2</sub> play a role in this variable.

Higher carbon dioxide concentration enhances the photosynthesis activity of C3 plants, such as wheat, and promotes a higher production of carbohydrates in the phloem sap. Large quantities of honeydew were observed on wheat leaves in 2094 as aphids excreted the

excess in carbohydrates content in honeydew (Ryan *et al.*, 2015). Aphid performances are also influenced by nitrogen levels in their host plants (Aqueel and Leather, 2011). Throughout the experiment, wheat plants have grown rapidly the first weeks of the experiment, in the 2094 chambers (unpublished results, Antoine, M., 2019). This rapid growth may have reduced the content of soil nitrogen. As less nitrogen is available in the soil, wheat plants have reallocated the nitrogen resources to the upper leaves, which may have promoted a variation in the nutritional intake of aphids and a higher body weight.

However, no studies have demonstrated the impact of elevated CO<sub>2</sub> on *S. avenae* weight. It can be interesting, in the future, to conduct some studies on this topic. Indeed, most of the studies are carried out on the impact of elevated CO<sub>2</sub> on aphid population fecundity or growth rate. It may also be interesting to carry out experiments on the impact of elevated CO<sub>2</sub> on host plant quality (winter wheat) and *S. avenae* performances. This topic could be discussed by taking samples of winter wheat either every week or either at the end of this experiment. Each plant content, such as carbohydrates and amino acids, could be analysed to determine if the amino acid content decreases. In addition, several aphid adults could be weighted to assess the influence of this variable. Thereafter, it may allow linking aphid sap intake, sap content and aphid weight.

### 5.3 Host plant attraction

#### 5.3.1 Winter wheat attraction for the English grain aphid

During the experiments, *S. avenae* and *A. fabae* did not select preferentially plants grown under elevated CO<sub>2</sub> concentration and/or temperature.

Initially, the absence of host plant selection was explained by the chosen experimental apparatus. During pretest experiments, the experimental set-up has been questioned for both species. The validation of the apparatus has been questioned for the English grain aphid, because aphids seemed to be influenced by the electrostatic electricity of the plastic in Petri dishes. Indeed, the small size of this species seemed to be more susceptible to electrostatic electricity. The pressure applied can generate stress for the insect and decrease its capacity to select a plant. However, in our study, the experimental apparatus is validated, generating no further stress on aphids and allowing normal behavioural responses.

In the case of *A. fabae*, in the beginning, the experiment was performed near a window. We found that the choice made by the insect was influenced by the light as aphids were going to the plant closest the window. Following these observations, the experiment was realised in a room where the lightening is uniform. As both experimental set-ups are validated, the influence of it was neglected.

The absence of choice is also explained by the capacity of apterous *S. avenae* to detect differences in volatile organic compounds (VOCs) from plants cultivated under contrasted climatic conditions. The volatile profile of chemical compounds in terms of concentration mainly differs between the modality 'temperature' and 'CO<sub>2</sub>'. The major chemical

compounds responsible for it belong to the alkane family. Other chemical compounds belong to the alcohol, ketone and aldehyde family.

Olfactometry and electroantennography trials have shown that apterous and alate *S. avenae* respond mainly to C6 and C7 alcohols, green leaf volatiles, which are represented by alcohols with 6 carbons, aldehydes and acetates (J. H. Visser and Fu-shun, 1995; Bruce, Wadhams and Woodcock, 2005) and benzaldehyde (Quiroz and Niemeyer, 1998). Visser and Fu-shun, 1995 have observed an antennary response of alate individuals to the compound (Z)-3-Hexen-1-ol and some terpenes such as  $\gamma$ -terpinene and  $\alpha$ -terpineol (Quiroz and Niemeyer, 1998). However, no preferential choice is observed for *S. avenae*. Aphids may need a particular ratio of host-plant volatile (kairomones) to induce an attraction to the plant. Bruce, Wadhams and Woodcock, 2005 had found that when the blend of kairomones is modified or incorrect, *S. mosellana*, a cereal specialist, is no longer attracted to wheat. In the case of *S. avenae*, the ratio of host-plant volatiles may not be correct to attract the insect.

Furthermore, the tested plants were two weeks (BBCH 12) (Zadoks, Chang and Konzak, 1974). But, *S. avenae* seems to become more present at the booting stage (BBCH 40) (Wang *et al.*, 2018). Thus, the variabilities in chemical concentrations are perhaps not sufficient to induce a choice in the plant under contrasted climatic conditions.

In addition, apterous *S. avenae* are not sensitive to the same volatiles as alate morphs. A study conducted by Quiroz and Niemeyer, 1998 on the olfactometry responses of alate *S. avenae* has shown that aphids react to benzaldehyde and (Z)-3-Hexen-1-ol with a concentration of 0.64 and 0.32 ng from 1 g of fresh wheat respectively. As the detected concentrations, in our experiment, are quite similar, 0.18 ng/g for benzaldehyde and 0.57 ng/g for (Z)-3-Hexen-1-ol, it seems that the absence of response to those volatiles depends on other parameters.

The aphid morph plays a role in the capacity of host plant selection. Apterous females mainly ensure the population development and reproduction by exploiting host plants and defending their colony (Newton *et al.*, 1987). Alate females favour the species dispersion and are responsible for the population maintenance by finding other host plants to migrate and establish a new population (Powell, Tosh and Hardie, 2006). Thus, apterous females may detect plant odours by the primary rhinaria. However, those rhinaria play mainly a role in the detection of alarm pheromone. On the contrary, alate adult females possess secondary rhinaria on their antennae which allow alate females to select host plants among an array of volatile (Pettersson, 1970; Niemeyer, 1990).

The same conclusions can be drawn for the other species *A. fabae*, in terms of morphology. No host plant preference was noticed when apterous individuals were submitted to elevated temperature and/or elevated CO<sub>2</sub>. However, a study conducted by Blanchard *et al.*, 2017 have found that alate *A. fabae* select preferentially plants grown under elevated CO<sub>2</sub>

concentration as the plants produce more nectar and flowers. Again, alate females are able to detect the volatiles necessary for their migration and the colony dispersion.

The assessment of aphid population dynamics and performances in the 2094 Ecotrons show the impact of climate change on plant-aphid interactions. In a climate change context, aphid development and performances will not be more important compared to 2015. However, further studies need to be carried out over a longer period of time such as one year. Indeed, as longer and colder episodes affect negatively the aphid development and performances, it is interesting to assess the impact of heat episodes on aphids. Climate change will favour the appearance of aphids on ears at an earlier phenological stage of winter wheat. It will lead to earlier attacks on plants and will contribute to earlier damages. Crop yield and productivity may be reduced which may impact negatively farmers and to a larger extent, humans.

## **6. Perspectives and recommendations**

### **6.1 Perspectives**

Other topics could be covered about climate change impact on aphids and plants. Climate change will not only influence the aphid performances and migration on wheat. Other studies including aphid morphology, aphid interspecific competitions, predators and parasites and honeydew production may be interesting to investigate.

#### **6.1.1 Interspecific competition**

Sun and Ge, 2011 have found that elevated CO<sub>2</sub> conditions may affect the spatial distribution of cereal aphids on cereal crops especially wheat. In addition, this modification in spatial distribution may modify interspecific competition between cereal aphid species. Several cereal aphids tend to be reduced in presence of others because other aphid species decrease the effect of elevated CO<sub>2</sub> (Sun and Chen, 2009). Thus, interspecific competition may reduce the presence of some species which are perhaps less harmful to crops and promote the presence of detrimental aphid species.

#### **6.1.2 Alarm pheromone**

Several studies were carried out by Kunert *et al.*, 2005, Sun, Su and Ge, 2010 and Sun and Ge, 2011 on the elevated CO<sub>2</sub> impact on the alarm pheromone production. According to those studies, elevated CO<sub>2</sub> concentration may reduce alarm pheromone production by *S. avenae*. This reduction in production may be explained by the modification of plant answers to elevated CO<sub>2</sub>. Under elevated CO<sub>2</sub>, plants have a better growth which can limit the contact and the perception of alarm pheromone by other aphids during attacks. In the future, a decrease in alarm pheromone may perhaps help predators to attack more individuals before the insect retreat.

#### **6.1.3 Pest predators and parasitoids**

It seems that under elevated CO<sub>2</sub> concentration, the parasitoid populations will have the same efficiency than under ambient CO<sub>2</sub> concentration to parasitize aphids (Stacey and

Fellowes, 2002). However, drought episodes may reduce the attacks of parasitoids and natural predators on aphids (Aslam, Johnson and Karley, 2012). As few studies are carried out on the combined effect of elevated temperature and CO<sub>2</sub> concentration, it is interesting to assess the performances of predators and parasitoids to attack or parasitize aphids. Nowadays, parasitoids and predators are integrated in agriculture to fight against several pests as for example aphids. Those technics are more environmentally friendly but take more time to obtain a similar efficiency to chemical products as insects need time to be adapted to the environment. If the adaptation of these predators is more rapid in a climate change context, it may convince growers to use more pest predators.

#### **6.1.4 Honeydew production**

Under elevated CO<sub>2</sub>, the production of honeydew by aphids may increase as C3 plants favour photosynthesis activity instead of photorespiration. Better photosynthesis activity enhances the carbohydrates content (Sun, Jing and Ge, 2009) which may lead to a higher amino acid content. A higher honeydew production may contribute to a higher development of sooty mold which in turns may weaken plants.

#### **6.1.5 Aphid morph colour**

The influence of climate change on the morph colour of *S. avenae* may also be fascinating. In the course of the experiment, the morph colour of aphids changed. Aphids turned from dark green into pink (Appendix 7). Alkhedir, Karlovsky and Vidal, 2010 have found that the colour modification may occur under light intensity variation. However, pink individuals were also observed in the rearing where the light conditions were different from the ones observed in the Ecotrons. Other biotic and/or abiotic factors may modify and influence the colour morphs. To go further, an assessment of the morph performances may be interesting to carry out to determine if the reproduction, longevity, development and weight is similar between morph colour and under several climatic conditions. By defining the factors linked to the colour modification, perhaps, in greenhouses, several abiotic parameters could be monitored to favour one particular colour such as the one which has low performances.

#### **6.1.6 Aphid feeding abilities**

Huberty and Denno, 2004 have observed that drought leads to a loss in water content and turgor cell pressure (Archer *et al.*, 1995). However, aphids require a positive turgor pressure to extract nutrients from plants such as leaf nitrogen. The reduction of water in leaves also favour the production of a more viscous phloem sap reducing the sampling of leaf nitrogen which may affect negatively aphid performances.

#### **6.1.7 Plant defences**

The modification of plant defences may also vary in a climate change context. It is interesting to know if plants would be more able to defence against herbivory attacks particularly aphid attacks. As the study has shown, *S. avenae* will not be more important when facing climate change. Nonetheless, plants may produce more or less repulsive volatiles compounds which in turn may lead to an absence or perpetuation of feeding.

### 6.1.8 Plant nutritional aspects

An elevation in CO<sub>2</sub> concentration seems suitable to C3 plants as it enhances the photosynthesis activity and produces more carbohydrates. According to Oehme *et al.*, 2013, elevated CO<sub>2</sub> may modify the content of carbohydrates and the availability of essential amino acids in the phloem sap for aphids. They found that for another cereal aphid, *R. padi*, elevated CO<sub>2</sub> increases the content of amino acids in wheat which leads to a higher relative growth rate for the aphid. The same studies could be performed to determine how the amino acid composition evolve and if it is profitable for *S. avenae*.

## 6.2 Recommendations

As the Ecotron experiment was conducted for the first time this year, several phenomena were difficult to explain. In some rooms, the absence of rain was noticed. In addition, the wall temperatures were too high compared to the ones observed in real conditions which are representing by the sky temperature. Those differences had enhanced a quicker development of wheat leading to high stem elongation and higher leaves numbers. It is difficult to conduct a first experiment in a new site where all the parameters are not controlled.

If another study has to be carried out in the Ecotron about the impact of climate change on plant-aphid interactions, it may be interesting to increase the number of sampling inside each crop. In this study, twenty plants were observed weekly. By observing forty plants weekly, it may help the experimenter to have more data and less variability. At the beginning of the counting, some weeks, no individuals were observed. In addition, some concentrations of individuals were observed at some particular locations such as near the air circulation.

A study more specific between aphids present on ears and leaves could also be carried out in terms of performances. Dixon and Acreman, 1989 have noticed some variability in adult weight and fecundity between the flag leaf and ears for the same temperatures. In addition, a collection of pink aphids could occur to assess the difference in performances between two morphs. As the colour modification was unexpected, no study on it was carried out.

At the laboratory scale, an assessment of host plant selection on both winged and wingless individuals could also be conducted to see what is the real impact of the wing formation on the VOCs detection. In this case, the experimental apparatus has to be redefined. To a larger extent, this assessment may be conducted on plants at BBCH 12 and BBCH 40. In this study, an evaluation of host plant preferences on plants reaching the stage BBCH 40 was difficult to settle up.



## 7. Conclusion

This study has shown that in a climate change context, the population dynamics of *S. avenae* will not be more important on wheat in 2094 as the aphid population is affected by the variations in temperature and the higher CO<sub>2</sub> concentration. Elevated temperature will also accelerate the plant growth by reducing some phenological stages and accelerating others which will lead to an appearance of aphids on ears at an earlier growth stage. Life history traits of aphids are negatively affected by climate change as aphids are less fecund, have a lower increase rate and body weight in 2094. Elevated CO<sub>2</sub> may have also modified the nutritional quality of wheat plants leading to a change in aphid performances. However, further researches are required to understand the impact combined of elevated temperature and elevated CO<sub>2</sub> concentration on life history traits. In terms of host plant selection, no preferential feeding is observed by apterous females as this morph exploits the host plant in situ and enhances the development of aphid population. However, it is noticed that the emission of volatile compounds changes under higher CO<sub>2</sub> concentration and elevated temperature which may attract more alate females which are responsible for the migration and the establishment of new colonies.

In the future, plant-aphid interactions may not differ from the interactions observed in the 2015 climatic conditions. However, the earlier appearance of *S. avenae* on cereal ears may impact farmers. Therefore, it is necessary to deepen our understandings about climate change impact on aphid migration to promote crop protection and sustainable food production.

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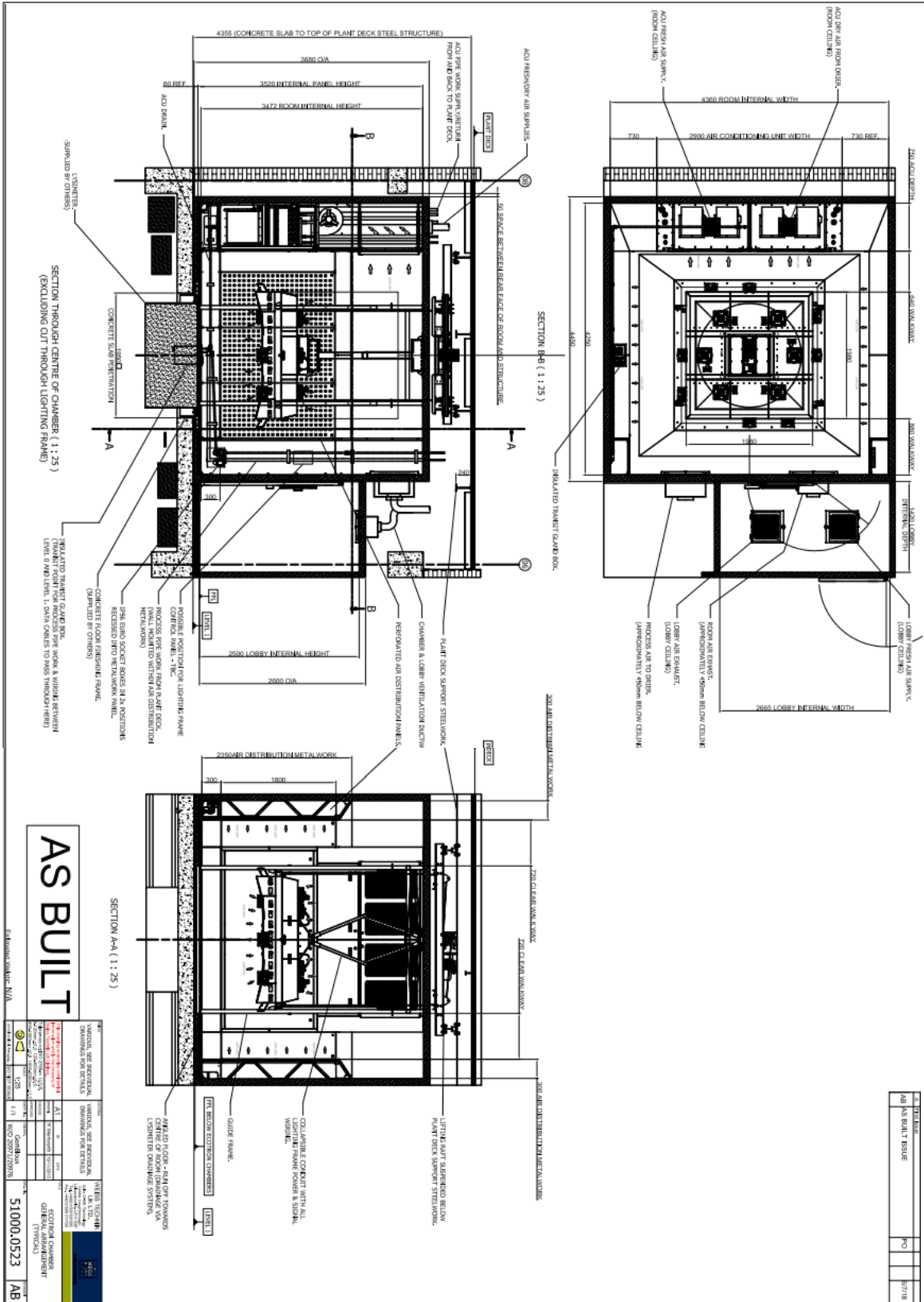
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# Appendices

## Appendix 1 - Dimensions of the climatic chamber (Ecotrons)



# Appendix 2 - Modelling of the 2014-2015 climate year for the air temperature, the rain and the sunlight, in the climatic chambers (Ecotron)



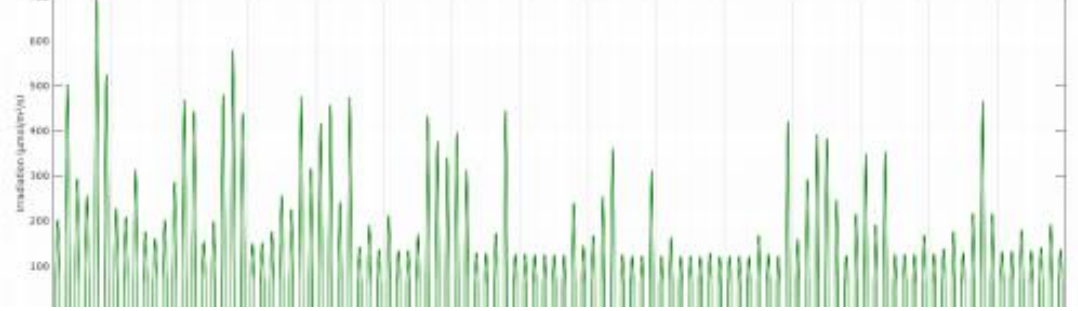
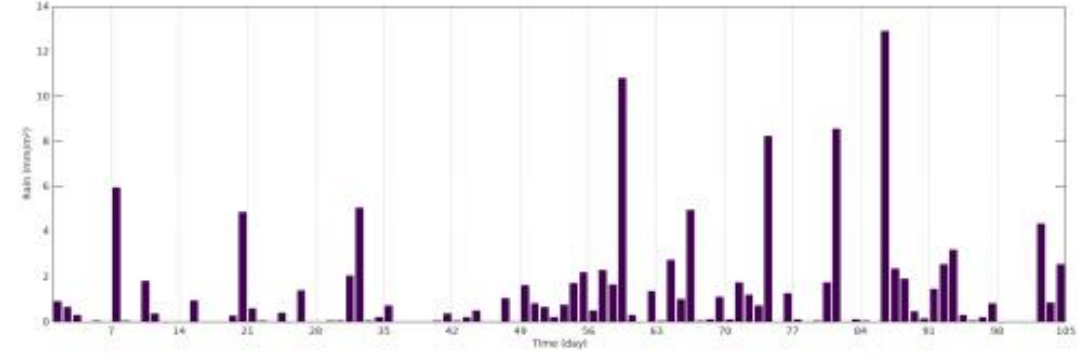
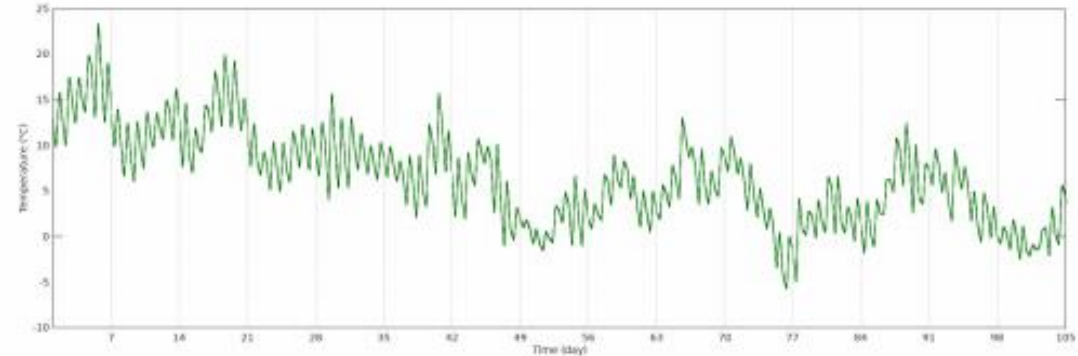
SEE  
 Meteorological condition  
 Crop season 2014-2015



### Shooting period

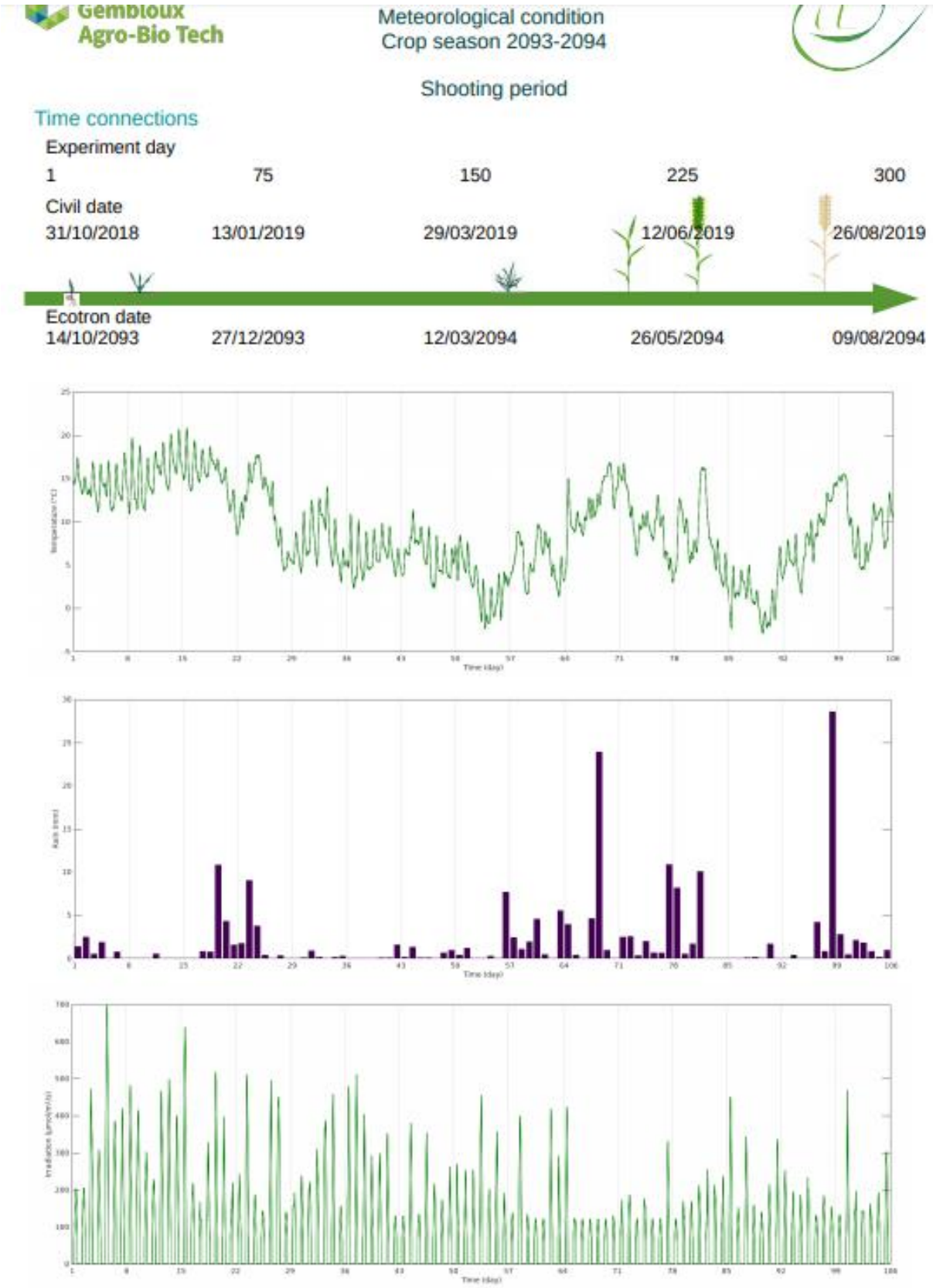
#### Time connections

Experiment day	75	150	225	300
Civil date	13/01/2019	29/03/2019	12/06/2019	26/08/2019
Ecotron date	27/12/2014	12/03/2015	26/05/2015	09/08/2015

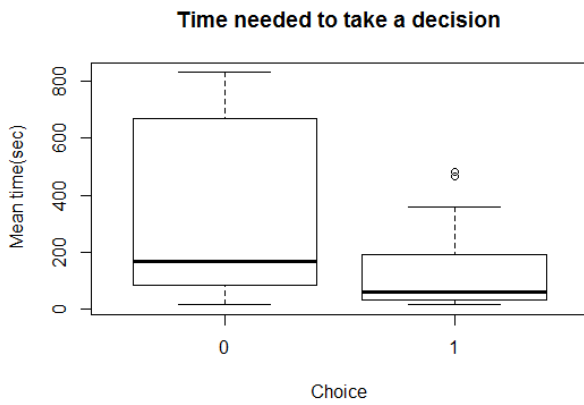




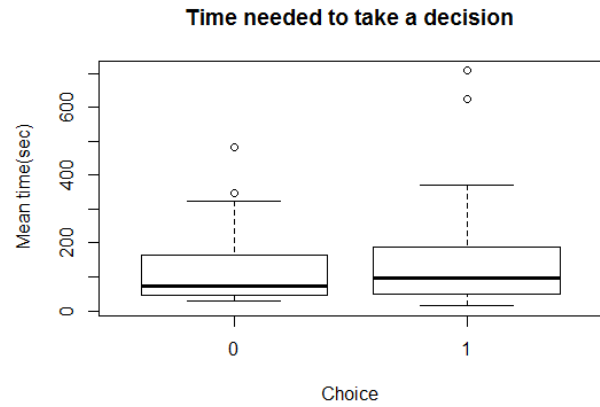
# Appendix 3 - Modelling of the 2093-2094 climate year for the air temperature, the rain and the sunlight, in the climatic chambers (Ecotron)



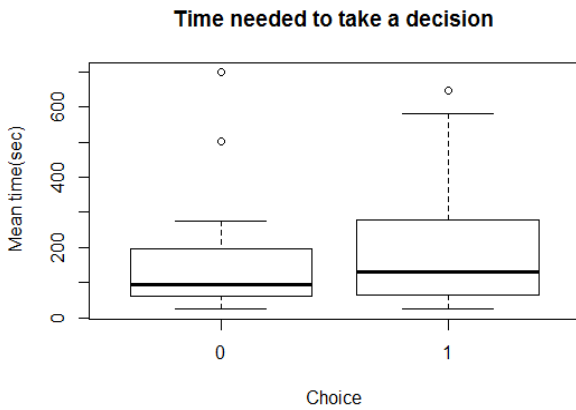
## Appendix 4 - Time needed to take a decision according to several climatic conditions, for winter wheat



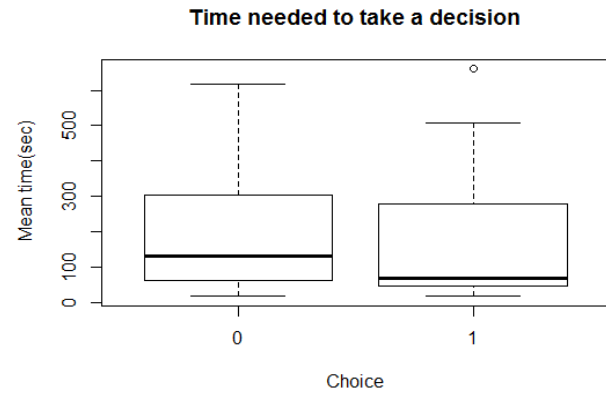
**Figure A - Time needed to choose the modality 0 (eTaCO2) and 1 (aTaCO2)**



**Figure B - Time needed to choose the modality 0 (aTeCO2) and 1 (aTaCO2)**



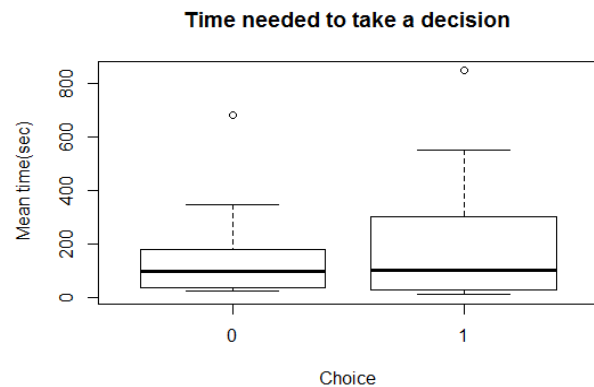
**Figure C - Time needed to choose the modality 0 (eTeCO2) and 1 (aTaCO2)**



**Figure D - Time needed to choose the modality 0 (eTaCO2) and 1 (aTeCO2)**

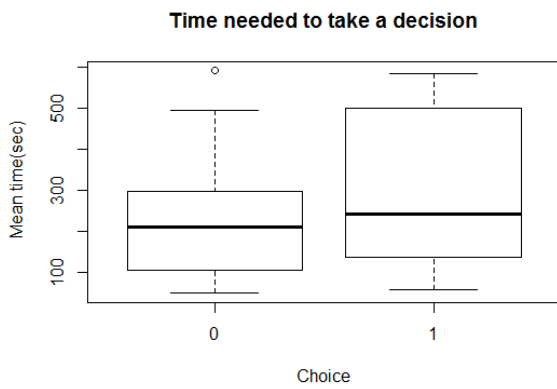


**Figure E - Time needed to choose the modality 0 (eTeCO2) and 1 (eTaCO2)**

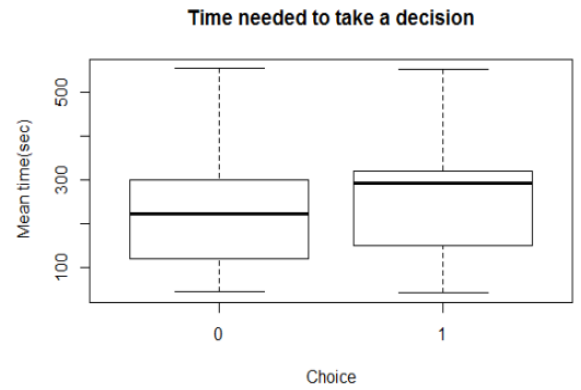


**Figure F - Time needed to choose the modality 0 (eTeCO2) and 1 (aTeCO2)**

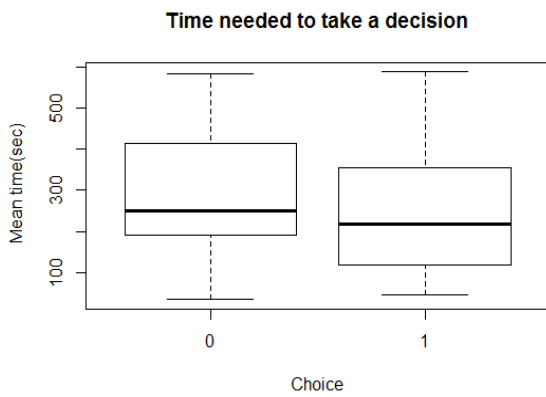
## Appendix 5 - Time needed to take a decision according to several climatic conditions, for fava beans



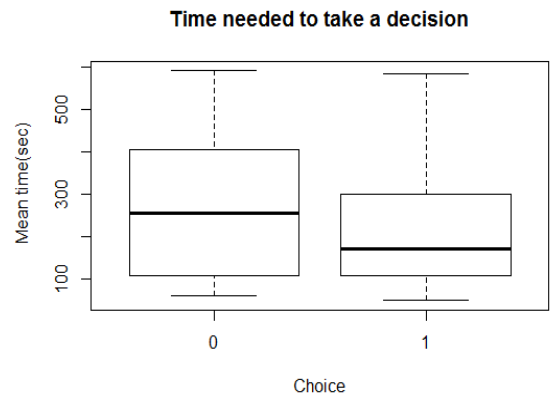
**Figure G - Time needed to choose the modality 0 (aTaCO2) and 1 (eTaCO2)**



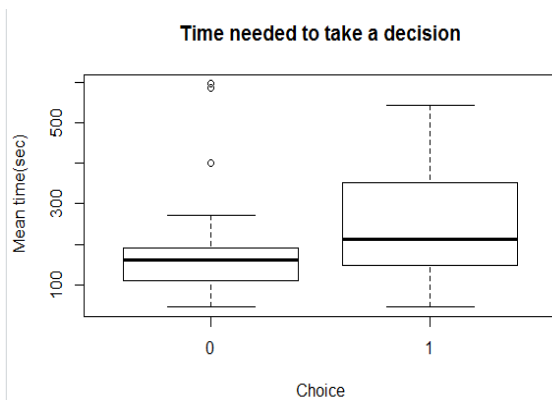
**Figure H - Time needed to choose the modality 0 (aTaCO2) and 1 (aTeCO2)**



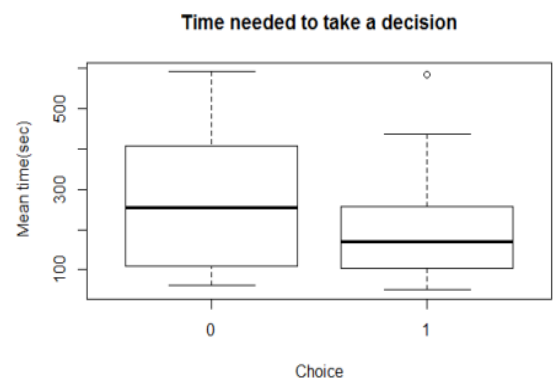
**Figure I - Time needed to choose the modality 0 (aTaCO2) and 1 (eTeCO2)**



**Figure J - Time needed to choose the modality 0 (eTaCO2) and 1 (aTeCO2)**



**Figure K - Time needed to choose the modality 0 (eTaCO2) and 1 (eTeCO2)**



**Figure L - Time needed to choose the modality 0 (eTeCO2) and 1 (aTeCO2)**

**Appendix 6 - A tentative identification of chemical compounds emitted by winter wheat under several climatic conditions, expressed in ng/h per g of fresh plant**

Chemical compounds	aTaCO <sub>2</sub>	aTeCO <sub>2</sub>	eTaCO <sub>2</sub>	eTeCO <sub>2</sub>	References
<b>Fatty acid</b>					
Octanoic acid	0,79 ± 1,86	0,45 ± 0,53	0,00 ± 0,00	0,06 ± 0,11	(Buško <i>et al.</i> , 2010)
<b>Ester</b>					
Butanoic acid, ethyl ester	0,00 ± 0,00	0,00 ± 0,00	1,25 ± 1,51	1,66 ± 1,67	(Aragüez and Valpuesta Fernández, 2013)
Carbamic acid, methyl-, phenyl ester	0,48 ± 1,54	0,56 ± 1,81	0,00 ± 0,00	0,00 ± 0,00	(Schlagbauer and Schlagbauer, 1972)
2-Propenoic acid, 6-methylheptyl ester	0,02 ± 0,07	0,02 ± 0,05	0,00 ± 0,28	0,00 ± 0,00	
<b>Alkane</b>					
Cyclohexane, 1,2-diethyl-1-methyl-	0,36 ± 1,10	0,11 ± 0,22	0,00 ± 0,00	0,03 ± 0,10	
Cyclohexane, butyl-	0,76 ± 1,65	0,48 ± 0,54	0,00 ± 0,00	0,00 ± 0,00	(Seitz, 1995)
Cyclohexane, methyl-	0,03 ± 0,08	0,00 ± 0,00	0,00 ± 0,00	0,15 ± 0,49	(Fowler <i>et al.</i> , 2012)
Cyclopentane, 1-ethyl-1-methyl-	0,00 ± 0,00	0,10 ± 0,33	0,00 ± 0,00	0,00 ± 0,00	(Rios-Hernandez, 2003)
Decane, 2,2,6-trimethyl,	0,02 ± 0,07	0,08 ± 0,27	0,00 ± 0,00	0,09 ± 0,28	
Nonane, 2-5-dimethyl	0,93 ± 2,67	0,50 ± 0,93	0,02 ± 0,06	0,17 ± 0,33	(Seitz, 1995)
Nonane, 3,7-dimethyl	0,02 ± 0,06	0,06 ± 0,21	0,00 ± 0,00	0,03 ± 0,11	(Moura <i>et al.</i> , 2016)
Nonane, 3-methyl-	0,10 ± 0,22	0,10 ± 0,33	0,01 ± 0,02	0,06 ± 0,18	
Nonane, 5-(2-methylpropyl)-	0,03 ± 0,10	0,35 ± 0,39	0,00 ± 0,00	0,05 ± 0,16	
Nonane, 5-methyl-5-propyl	0,03 ± 0,10	0,35 ± 0,39	0,00 ± 0,00	0,05 ± 0,16	
Octane, 2,6-dimethyl	0,02 ± 0,05	0,24 ± 0,51	0,01 ± 0,05	0,09 ± 0,19	(Müller <i>et al.</i> , 2013)
Octane, 2,2,6-trimethyl-	0,00 ± 0,00	0,00 ± 0,00	0,15 ± 0,49	0,23 ± 0,46	
Octane, 4,5-dipropyl-	0,04 ± 0,08	0,19 ± 0,44	0,00 ± 0,00	0,06 ± 0,19	(Müller <i>et al.</i> , 2013)
Undecane, 3,6-dimethyl-	0,00 ± 0,00	0,22 ± 0,71	0,00 ± 0,00	0,12 ± 0,39	
Undecane, 3-methyl-	0,00 ± 0,00	0,13 ± 0,42	0,04 ± 0,12	0,18 ± 0,56	(Siddiquee <i>et al.</i> , 2015)
Undecane, 5- methyl	0,03 ± 0,08	0,09 ± 0,30	0,00 ± 0,00	0,05 ± 0,15	(Siddiquee <i>et al.</i> , 2015)
<b>Alcohol</b>					
2,3-Butanediol, [R-(R*,R*)]-	164,16 ± 60,21	188,34 ± 94,54	29,29 ± 54,25	63,19 ± 35,99	(Chen <i>et al.</i> , 2019)
Ethanol, 1-(2-butoxyethoxy)-	0,03 ± 0,10	0,14 ± 0,44	0,08 ± 0,24	0,10 ± 0,32	(Leff and

					Fierer, 2008)
3-Hexen-1-ol, acetate, (Z)-	1,19 ± 2,83	3,71 ± 7,98	0,55 ± 0,79	2,00 ± 3,46	(Hamilton-kemp and Andersen, 1985)
3-Methyl-2-phenyl-4-penten-2-ol	0,00 ± 0,00	0,13 ± 0,42	0,04 ± 0,12	0,18 ± 0,56	(Cramer <i>et al.</i> , 2005)
<b>Aldehyde</b>					
Benzaldehyde	0,00 ± 0,00	0,00 ± 0,00	0,73 ± 0,29	0,00 ± 0,00	(Quiroz and Niemeyer, 1998)
Heptanal	0,00 ± 0,00	1,25 ± 0,63	0,00 ± 0,00	0,00 ± 0,00	(Chen <i>et al.</i> , 2019)
2-Nonenal, (E)-	0,10 ± 0,33	0,17 ± 0,55	0,02 ± 0,07	0,03 ± 0,08	(Cramer <i>et al.</i> , 2005)
4-Nonenal, (E)-	0,02 ± 0,07	0,15 ± 0,49	0,01 ± 0,02	0,02 ± 0,07	
<b>Ketone</b>					
2-Heptanone, 3-methyl-	0,64 ± 2,05	1,21 ± 3,87	0,00 ± 0,00	0,13 ± 0,42	(Seitz, 1995)
3-Heptanone	0,31 ± 0,99	1,02 ± 3,25	0,00 ± 0,00	0,07 ± 0,23	(Drakulic <i>et al.</i> , 2016)
<b>Mono-terpene</b>					
Menthyl acetate	0,00 ± 0,00	0,24 ± 0,45	0,00 ± 0,00	0,05 ± 0,17	(Seitz, 1995)

Appendix 7 - Morph colour



## Appendix 8 - R codes

### ❖ Population dynamics in the Ecotron (2014-2015 and 2093-2094)

```
>library(lme4)
>setwd("D:/florence/Master2 Agro/TFE/Ecotron")
>Evolution <- read.csv2("PositionEcoAll1.csv")
>View(Evolution)
>fit2 <- glm(Mean.aphid.per.plant ~ Observation.Date, data=Evolution, family=gaussian(link="log"))
>fit2 <- glm(Mean.aphid.per.plant ~ Climat+Observation.Date+Climat:Observation.Date, data=Evolution,
family=gaussian(link="log"))
>anova(fit2, test="F")
>plot(Mean.aphid.per.plant~Observation.Date, data=Evolution, col=c('blue', 'red')[as.numeric(Climat)], pch=16)
>points(1:42, predict(fit2, type="response", newdata=data.frame(Observation.Date=1:42, Climat="C15")),
type="l", col='blue', lwd=2)
>points(1:42, predict(fit2, type="response", newdata=data.frame(Observation.Date=1:42, Climat="C94")),
type="l", col='red', lwd=2)
```

### ❖ Aphid migration on winter wheat

```
### 2015###
>Dist1 = c(21,34,199)
>Dist2 = c(0,0,1)
>Dist3 = c(1,80,315)
># Création d'une matrice comparative :
>tableau = matrix(c(Dist1, Dist2, Dist3),3,3,byrow=T) # (2 : nombre de lignes et 4 nombres de colonnes)
>### Réalisation du test khi-deux - les résultats sont sauvegardés dans "khi_test"
>khi_test = chisq.test(tableau)
>khi_test # affiche le résultat du test
>khi_test$observed
>khi_test$expected
>khi_test$residual
>str(khi_test)
>khi_test$stdres

### 2094###
>Dist1 = c(19,32,26)
>Dist2 = c(0,0,1)
>Dist3 = c(1,43,197)
># Création d'une matrice comparative :
>tableau = matrix(c(Dist1, Dist2, Dist3),3,3,byrow=T) # (2 : nombre de lignes et 4 nombres de colonnes)
>### Réalisation du test khi-deux - les résultats sont sauvegardés dans "khi_test"
>khi_test = chisq.test(tableau)
>khi_test # affiche le résultat du test
>khi_test$observed
>khi_test$expected
>khi_test$residual
>str(khi_test)
>khi_test$stdres
```

## ❖ Aphid fitness

```
####2015####Tibia
>setwd("D:/florence/Master2 Agro/TFE/Ecotron")
>f15 <- read.csv2("Fit15.csv")
>View(f15)
>is.numeric(f15$Date)
>f15$Date<-as.numeric(f15$Date)
>is.vector(f15$Enceinte)
>f15$Enceinte<-as.vector(f15$Enceinte)
>is.numeric(f15$tibia)
>f15$tibia<-as.numeric(f15$tibia)
####implémentation de la matrice de résultat en format csv
>Aov_data<-f15
>Aov_data ## Visualisation du tableau de donnée
#### Normalité
>shapiro.test(residuals(lm(Aov_data$Date~ Aov_data$tibia)))
#### Homoscédasticité
## si un seul facteur AV1
>bartlett.test(Aov_data$tibia,Aov_data$Date)
#### Test ANOVA si shapiro et barlett p-value>0.05
>fit <- aov(tibia~Date, data= Aov_data) # y est la variable numérique. A indique les groupes de facteurs
>summary(fit)
#### Test Kruskal-Wallis
kruskal.test(tibia~Date, data = f15)
pairwise.wilcox.test(f15$tibia, f15$Date, p.adjust="bonferroni")

####2015####Taille
>setwd("D:/florence/Master2 Agro/TFE/Ecotron")
>f15 <- read.csv2("Fit15.csv")
>View(f15)
>is.numeric(f15$Date)
>f15$Date<-as.numeric(f15$Date)
>is.vector(f15$Enceinte)
>f15$Enceinte<-as.vector(f15$Enceinte)
>is.numeric(f15$taille)
>f15$tibia<-as.numeric(f15$taille)
####implémentation de la matrice de résultat en format csv
>Aov_data<-f15
>Aov_data ## Visualisation du tableau de donnée
#### Normalité
>shapiro.test(residuals(lm(Aov_data$Date~ Aov_data$taille)))
#### Homoscédasticité
## si un seul facteur AV1
>bartlett.test(Aov_data$taille,Aov_data$Date)
#### Test ANOVA si shapiro et barlett p-value>0.05
>fit <- aov(taille~Date, data= Aov_data) # y est la variable numérique. A indique les groupes de facteurs
>summary(fit)
#### Test Kruskal-Wallis
kruskal.test(taille~Date, data = f15)
pairwise.wilcox.test(f15$taille, f15$Date, p.adjust="bonferroni")
```



```

####2015####Taille
>setwd("D:/florence/Master2 Agro/TFE/Ecotron")
>w15 <- read.csv2("w15.csv")
>View(w15)
>is.numeric(w15$Date)
>w15$Date<-as.numeric(w15$Date)
>is.vector(w15$Enceinte)
>w15$Enceinte<-as.vector(w15$Enceinte)
>is.numeric(w15$poids)
>w15$tibia<-as.numeric(w15$ poids)
####implémentation de la matrice de résultat en format csv
>Aov_data<-w15
>Aov_data ## Visualisation du tableau de donnée
#### Normalité
>shapiro.test(residuals(lm(Aov_data$Date~ Aov_data$ poids)))
#### Homoscédasticité
## si un seul facteur AV1
>bartlett.test(Aov_data$poids,Aov_data$Date)
#### Test ANOVA si shapiro et barlett p-value>0.05
>fit <- aov(poids~Date, data= Aov_data) # y est la variable numérique. A indique les groupes de facteurs
>summary(fit)
#### Test Kruskal-Wallis
kruskal.test(poids~Date, data = w15)
pairwise.wilcox.test(w15$poids, f15$Date, p.adjust="bonferroni")

####2094####Tibia
>setwd("D:/florence/Master2 Agro/TFE/Ecotron")
>f94 <- read.csv2("f94.csv")
>View(f94)
>is.numeric(f94$Date)
> f94$Date<-as.numeric(f94$Date)
>is.vector(f94$Enceinte)
> f94$Enceinte<-as.vector(f94$Enceinte)
>is.numeric(f94$tibia)
> f94$tibia<-as.numeric(f94$tibia)
####implémentation de la matrice de résultat en format csv
>Aov_data<- f94
>Aov_data ## Visualisation du tableau de donnée
#### Normalité
>shapiro.test(residuals(lm(Aov_data$Date~ Aov_data$tibia)))
#### Homoscédasticité
## si un seul facteur AV1
>bartlett.test(Aov_data$tibia,Aov_data$Date)
#### Test ANOVA si shapiro et barlett p-value>0.05
>fit <- aov(tibia~Date, data= Aov_data) # y est la variable numérique. A indique les groupes de facteurs
>summary(fit)
#### Test Kruskal-Wallis
kruskal.test(tibia~Date, data = f94)
pairwise.wilcox.test(f94$tibia f94$Date, p.adjust="bonferroni")

```

```

####2094####Taille
>setwd("D:/florence/Master2 Agro/TFE/Ecotron")
> f94 <- read.csv2("f945.csv")
>View(f94)
>is.numeric(f94$Date)
> f94$Date<-as.numeric(f94$Date)
>is.vector(f94$Enceinte)
>f15$Enceinte<-as.vector(f15$Enceinte)
>is.numeric(f94$taille)
> f94$tibia<-as.numeric(f94$ taille)
####implémentation de la matrice de résultat en format csv
>Aov_data<- f94
>Aov_data ## Visualisation du tableau de donnée
#### Normalité
>shapiro.test(residuals(lm(Aov_data$Date~ Aov_data$ taille)))
#### Homoscédasticité
## si un seul facteur AV1
>bartlett.test(Aov_data$ taille,Aov_data$Date)
#### Test ANOVA si shapiro et barlett p-value>0.05
>fit <- aov(taille~Date, data= Aov_data) # y est la variable numérique. A indique les groupes de facteurs
>summary(fit)
#### Test Kruskal-Wallis
kruskal.test(taille~Date, data= f94)
pairwise.wilcox.test(f94$taille, f94$Date, p.adjust="bonferroni")

####2015####Poids
>setwd("D:/florence/Master2 Agro/TFE/Ecotron")
>w94 <- read.csv2("w94.csv")
>View(w94)
>is.numeric(w94$Date)
>w15$Date<-as.numeric(w94$Date)
>is.vector(w94$Enceinte)
>w94$Enceinte<-as.vector(w94$Enceinte)
>is.numeric(w94$poids)
>w94$tibia<-as.numeric(w94$ poids)
####implémentation de la matrice de résultat en format csv
>Aov_data<-w94
>Aov_data ## Visualisation du tableau de donnée
#### Normalité
>shapiro.test(residuals(lm(Aov_data$Date~ Aov_data$ poids)))
#### Homoscédasticité
## si un seul facteur AV1
>bartlett.test(Aov_data$poids,Aov_data$Date)
#### Test ANOVA si shapiro et barlett p-value>0.05
>fit <- aov(poids~Date, data= Aov_data) # y est la variable numérique. A indique les groupes de facteurs
>summary(fit)
#### Test Kruskal-Wallis
kruskal.test(poids~Date, data = w94)
pairwise.wilcox.test(w94$poids, f15$Date, p.adjust="bonferroni")

```

```

####2015AND2094####Total observation dates#### Tibia
>setwd("D:/florence/Master2 Agro/TFE/Ecotron")
>getwd()
>rm(list = ls())
>d <- read.csv("totti1594.csv", sep=";")
>summary(d)
>is.factor(d$année)
>d$année <- as.factor(d$année)
>is.numeric(d$tibia)
>d$tibia <- as.numeric(d$tibia)
>t.test(tibia ~ année, data = d)

```

```

####2015AND2094####Total observation dates#### Taille
>setwd("D:/florence/Master2 Agro/TFE/Ecotron")
>getwd()
>rm(list = ls())
>d <- read.csv("totta1594.csv", sep=";")
>summary(d)
>is.factor(d$année)
>d$année <- as.factor(d$année)
>is.numeric(d$taille)
>d$taille <- as.numeric(d$taille)
>t.test(taille ~ année, data = d)

```

```

####2015AND2094####Total observation dates#### Poids
>setwd("D:/florence/Master2 Agro/TFE/Ecotron")
>getwd()
>rm(list = ls())
>d <- read.csv("poids1594.csv", sep=";")
>summary(d)
>is.factor(d$année)
>d$année <- as.factor(d$année)
>is.numeric(d$poids)
>d$poids <- as.numeric(d$poids)
>t.test(poids ~ année, data = d)

```

```

####2015AND2094####Four followed plants#### Tibia
>setwd("D:/florence/Master2 Agro/TFE/Ecotron")
>getwd()
>rm(list = ls())
>d <- read.csv("cumulti495.csv", sep=";")
>summary(d)
>is.factor(d$année)
>d$année <- as.factor(d$année)
>is.numeric(d$tibia)
>d$tibia <- as.numeric(d$tibia)
>t.test(tibia ~ année, data = d)

```

```

####2015AND2094####Four followed plants#### Taille
>setwd("D:/florence/Master2 Agro/TFE/Ecotron")
>getwd()

```

```

>rm(list = ls())
>d <- read.csv("cumulta1494.csv", sep=";")
>summary(d)
>is.factor(d$année)
>d$année <- as.factor(d$année)
>is.numeric(d$taille)
>d$taille <- as.numeric(d$taille)
>t.test(taille ~ année, data = d)

###2015AND2094###Four followed plants### Poids
>setwd("D:/florence/Master2 Agro/TFE/Ecotron")
>getwd()
>rm(list = ls())
>d <- read.csv("cumulpoids1494.csv", sep=";")
>summary(d)
>is.factor(d$année)
>d$année <- as.factor(d$année)
>is.numeric(d$poids)
>d$poids <- as.numeric(d$poids)
>t.test(poids ~ année, data = d)

```

### ❖ Aphid host plant attraction

```

###S.avenae###AB
>library(lme4)
>setwd("D:/florence/Master2 Agro/TFE/Expériences ")
>resultAB <- read.csv("Resultats1--2.csv", dec = ".", sep = ";")
>View(resultAB)
###GLMCHOICE
mod0 <- glm(Choix ~ 1, data= resultAB, family = binomial)
## Choix Test : Chisq si Df = Resid. Dev si non Fisher
>anova(mod0)
>anova(mod0, test="Chisq")
>summary(mod0)
###BOXPLOT CHOICE
>boxplot(Individu~Choix, data =resultAB, xlab="Choice",ylab=" Individual")
>res.aov <- aov(Individu~Choix, data = resultAB)
>summary(res.aov)
### GLM CHOICE| BLOCK
>mod1 <- glmer(Choix ~ 1 + (1|Bloc), data= resultAB, family = binomial)
>mod2 <- glm(Choix ~ 1 + Bloc, data= resultAB, family = binomial)
>anova(mod1)
>anova(mod1, test="Chisq")
>summary(mod1)
##Visulation graphique
>plot(mod1)
>plot(mod2)
## Visulation temps/Choix
>boxplot(Tmoysec~Choix, data=resultAB, main="Time needed to take a decision", xlab ="Choice",ylab=" Mean
time(sec)")
>res.aov <- aov(log(Tmoysec)~Choix + Bloc, data = resultAB)

```

```

# Summary of the analysis
>summary(res.aov)

##S.avenae##AC
>library(lme4)
>setwd("D:/florence/Master2 Agro/TFE/Expériences")
>resultAC <- read.csv("Resultats1--3.csv", dec = ".", sep = ";")
>View(result AC)
###GLMCHOICE
mod0 <- glm(Choix ~ 1, data= result AC, family = binomial)
## Choix Test : Chisq si Df = Resid. Dev si non Fisher
>anova(mod0)
>anova(mod0, test="Chisq")
>summary(mod0)
###BOXPLOT CHOICE
>boxplot(Individu~Choix, data =result AC, xlab="Choice",ylab=" Individual")
>res.aov <- aov(Individu~Choix, data = resultAB)
>summary(res.aov)
### GLM CHOICE| BLOCK
>mod1 <- glmer(Choix ~ 1 + (1|Bloc), data= result AC, family = binomial)
>mod2 <- glm(Choix ~ 1 + Bloc, data= result AC, family = binomial)
>anova(mod1)
>anova(mod1, test="Chisq")
>summary(mod1)
##Visulation graphique
>plot(mod1)
>plot(mod2)
## Visulation temps/Choix
>boxplot(Tmoysec~Choix, data=result AC, main="Time needed to take a decision", xlab ="Choice",ylab=" Mean
time(sec)")
>res.aov <- aov(log(Tmoysec)~Choix + Bloc, data = result AC)
# Summary of the analysis
>summary(res.aov)

##S.avenae##AD
>library(lme4)
>setwd("D:/florence/Master2 Agro/TFE/Expériences ")
>resultAD <- read.csv("Resultats1--4.csv", dec = ".", sep = ";")
>View(resultAD)
###GLMCHOICE
mod0 <- glm(Choix ~ 1, data= resultAD, family = binomial)
## Choix Test : Chisq si Df = Resid. Dev si non Fisher
>anova(mod0)
>anova(mod0, test="Chisq")
>summary(mod0)
###BOXPLOT CHOICE
>boxplot(Individu~Choix, data =resultAD, xlab="Choice",ylab=" Individual")
>res.aov <- aov(Individu~Choix, data = resultAD)
>summary(res.aov)
### GLM CHOICE| BLOCK

```

```

>mod1 <- glmer(Choix ~ 1 + (1|Bloc), data= resultAD, family = binomial)
>mod2 <- glm(Choix ~ 1 + Bloc, data= resultAD, family = binomial)
>anova(mod1)
>anova(mod1, test="Chisq")
>summary(mod1)
##Visulation graphique
>plot(mod1)
>plot(mod2)
## Visulation temps/Choix
>boxplot(Tmoysec~Choix, data=resultCD, main="Time needed to take a decision", xlab ="Choice",ylab=" Mean
time(sec)")
>res.aov <- aov(log(Tmoysec)~Choix + Bloc, data = resultCD)
# Summary of the analysis
>summary(res.aov)

##S.avenae##BC
>library(lme4)
>setwd("D:/florence/Master2 Agro/TFE/Expériences ")
>resultBC <- read.csv("Resultat2--3.csv", dec = ".", sep = ";")
>View(result BC)
###GLMCHOICE
mod0 <- glm(Choix ~ 1, data= result BC, family = binomial)
## Choix Test : Chisq si Df = Resid. Dev si non Fisher
>anova(mod0)
>anova(mod0, test="Chisq")
>summary(mod0)
###BOXPLOT CHOICE
>boxplot(Individu~Choix, data =result BC, xlab="Choice",ylab=" Individual")
>res.aov <- aov(Individu~Choix, data = result BC)
>summary(res.aov)
### GLM CHOICE| BLOCK
>mod1 <- glmer(Choix ~ 1 + (1|Bloc), data= result BC, family = binomial)
>mod2 <- glm(Choix ~ 1 + Bloc, data= result BC, family = binomial)
>anova(mod1)
>anova(mod1, test="Chisq")
>summary(mod1)
##Visulation graphique
>plot(mod1)
>plot(mod2)
## Visulation temps/Choix
>boxplot(Tmoysec~Choix, data=result BC, main="Time needed to take a decision", xlab ="Choice",ylab=" Mean
time(sec)")
>res.aov <- aov(log(Tmoysec)~Choix + Bloc, data = result BC)
# Summary of the analysis
>summary(res.aov)

##S.avenae##BD
>library(lme4)
>setwd("D:/florence/Master2 Agro/TFE/Expériences ")
>resultBD <- read.csv("Resultat2--4.csv", dec = ".", sep = ";")

```

```

>View(resultBD)
###GLMCHOICE
mod0 <- glm(Choix ~ 1, data= resultBD, family = binomial)
## Choix Test : Chisq si Df = Resid. Dev si non Fisher
>anova(mod0)
>anova(mod0, test="Chisq")
>summary(mod0)
###BOXPLOT CHOICE
>boxplot(Individu~Choix, data =resultBD, xlab="Choice",ylab=" Individual")
>res.aov <- aov(Individu~Choix, data = resultBD)
>summary(res.aov)
### GLM CHOICE| BLOCK
>mod1 <- glmer(Choix ~ 1 + (1|Bloc), data= resultBD, family = binomial)
>mod2 <- glm(Choix ~ 1 + Bloc, data= resultBD, family = binomial)
>anova(mod1)
>anova(mod1, test="Chisq")
>summary(mod1)
##Visulation graphique
>plot(mod1)
>plot(mod2)
## Visulation temps/Choix
>boxplot(Tmoysec~Choix, data=resultBD, main="Time needed to take a decision", xlab ="Choice",ylab=" Mean
time(sec)")
>res.aov <- aov(log(Tmoysec)~Choix + Bloc, data = resultBD)
# Summary of the analysis
>summary(res.aov)

###S.avenae##CD
>library(lme4)
>setwd("D:/florence/Master2 Agro/TFE/Expériences ")
>resultCD<- read.csv("Resultat3--4.csv", dec = ".", sep = ";")
>View(resultCD)
###GLMCHOICE
mod0 <- glm(Choix ~ 1, data= resultCD, family = binomial)
## Choix Test : Chisq si Df = Resid. Dev si non Fisher
>anova(mod0)
>anova(mod0, test="Chisq")
>summary(mod0)
###BOXPLOT CHOICE
>boxplot(Individu~Choix, data =resultCD, xlab="Choice",ylab=" Individual")
>res.aov <- aov(Individu~Choix, data = resultCD)
>summary(res.aov)
### GLM CHOICE| BLOCK
>mod1 <- glmer(Choix ~ 1 + (1|Bloc), data= resultCD, family = binomial)
>mod2 <- glm(Choix ~ 1 + Bloc, data= resultCD, family = binomial)
>anova(mod1)
>anova(mod1, test="Chisq")
>summary(mod1)
##Visulation graphique
>plot(mod1)

```

```

>plot(mod2)
## Visulation temps/Choix
>boxplot(Tmoysec~Choix, data=resultCD, main="Time needed to take a decision", xlab ="Choice",ylab=" Mean
time(sec)")
>res.aov <- aov(log(Tmoysec)~Choix + Bloc, data = resultCD)
# Summary of the analysis
>summary(res.aov)

##A.fabae##AB
>library(lme4)
>setwd("D:/florence/Master2 Agro/TFE/Expériences/Aphis/AphisCsv ")
>resultAB <- read.csv("Result12.csv", dec = ".", sep = ";")
>View(resultAB)
###GLMCHOICE
mod0 <- glm(Choix ~ 1, data= resultAB, family = binomial)
## Choix Test : Chisq si Df = Resid. Dev si non Fisher
>anova(mod0)
>anova(mod0, test="Chisq")
>summary(mod0)
###BOXPLOT CHOICE
>boxplot(Individu~Choix, data =resultAB, xlab="Choice",ylab=" Individual")
>res.aov <- aov(Individu~Choix, data = resultAB)
>summary(res.aov)
### GLM CHOICE | BLOCK
>mod1 <- glmer(Choix ~ 1 + (1 | Bloc), data= resultAB, family = binomial)
>mod2 <- glm(Choix ~ 1 + Bloc, data= resultAB, family = binomial)
>anova(mod1)
>anova(mod1, test="Chisq")
>summary(mod1)
##Visulation graphique
>plot(mod1)
>plot(mod2)
## Visulation temps/Choix
>boxplot(Tmoysec~Choix, data=resultAB, main="Time needed to take a decision", xlab ="Choice",ylab=" Mean
time(sec)")
>res.aov <- aov(log(Tmoysec)~Choix + Bloc, data = resultAB)
# Summary of the analysis
>summary(res.aov)

##A.fabae##AC
>library(lme4)
>setwd("D:/florence/Master2 Agro/TFE/Expériences/Aphis/AphisCsv")
>resultAC <- read.csv("Resultat13.csv", dec = ".", sep = ";")
>View(result AC)
###GLMCHOICE
mod0 <- glm(Choix ~ 1, data= result AC, family = binomial)
## Choix Test : Chisq si Df = Resid. Dev si non Fisher
>anova(mod0)
>anova(mod0, test="Chisq")
>summary(mod0)

```



```

####BOXPLOT CHOICE
>boxplot(Individu~Choix, data =result AC, xlab="Choice",ylab=" Individual")
>res.aov <- aov(Individu~Choix, data = resultAB)
>summary(res.aov)
### GLM CHOICE| BLOCK
>mod1 <- glmer(Choix ~ 1 + (1|Bloc), data= result AC, family = binomial)
>mod2 <- glm(Choix ~ 1 + Bloc, data= result AC, family = binomial)
>anova(mod1)
>anova(mod1, test="Chisq")
>summary(mod1)
##Visulation graphique
>plot(mod1)
>plot(mod2)
## Visulation temps/Choix
>boxplot(Tmoysec~Choix, data=result AC, main="Time needed to take a decision", xlab ="Choice",ylab=" Mean
time(sec)")
>res.aov <- aov(log(Tmoysec)~Choix + Bloc, data = result AC)
# Summary of the analysis
>summary(res.aov)

###A.fabae##AD
>library(lme4)
>setwd("D:/florence/Master2 Agro/TFE/Expériences/Aphis/AphisCsv ")
>resultAD <- read.csv("Result14.csv", dec = ".", sep = ";")
>View(resultAD)
###GLMCHOICE
mod0 <- glm(Choix ~ 1, data= resultAD, family = binomial)
## Choix Test : Chisq si Df = Resid. Dev si non Fisher
>anova(mod0)
>anova(mod0, test="Chisq")
>summary(mod0)
####BOXPLOT CHOICE
>boxplot(Individu~Choix, data =resultAD, xlab="Choice",ylab=" Individual")
>res.aov <- aov(Individu~Choix, data = resultAD)
>summary(res.aov)
### GLM CHOICE| BLOCK
>mod1 <- glmer(Choix ~ 1 + (1|Bloc), data= resultAD, family = binomial)
>mod2 <- glm(Choix ~ 1 + Bloc, data= resultAD, family = binomial)
>anova(mod1)
>anova(mod1, test="Chisq")
>summary(mod1)
##Visulation graphique
>plot(mod1)
>plot(mod2)
## Visulation temps/Choix
>boxplot(Tmoysec~Choix, data=resultCD, main="Time needed to take a decision", xlab ="Choice",ylab=" Mean
time(sec)")
>res.aov <- aov(log(Tmoysec)~Choix + Bloc, data = resultCD)
# Summary of the analysis
>summary(res.aov)

```

```

##A.fabae##BC
>library(lme4)
>setwd("D:/florence/Master2 Agro/TFE/Expériences/Aphis/AphisCsv ")
>resultBC <- read.csv("Result23.csv", dec = ".", sep = ";")
>View(result BC)
###GLMCHOICE
mod0 <- glm(Choix ~ 1, data= result BC, family = binomial)
## Choix Test : Chisq si Df = Resid. Dev si non Fisher
>anova(mod0)
>anova(mod0, test="Chisq")
>summary(mod0)
###BOXPLOT CHOICE
>boxplot(Individu~Choix, data =result BC, xlab="Choice",ylab=" Individual")
>res.aov <- aov(Individu~Choix, data = result BC)
>summary(res.aov)
### GLM CHOICE | BLOCK
>mod1 <- glmer(Choix ~ 1 + (1 | Bloc), data= result BC, family = binomial)
>mod2 <- glm(Choix ~ 1 + Bloc, data= result BC, family = binomial)
>anova(mod1)
>anova(mod1, test="Chisq")
>summary(mod1)
##Visulation graphique
>plot(mod1)
>plot(mod2)
## Visulation temps/Choix
>boxplot(Tmoysec~Choix, data=result BC, main="Time needed to take a decision", xlab ="Choice",ylab=" Mean
time(sec)")
>res.aov <- aov(log(Tmoysec)~Choix + Bloc, data = result BC)
# Summary of the analysis
>summary(res.aov)

##A.fabae##BD
>library(lme4)
>setwd("D:/florence/Master2 Agro/TFE/Expériences/Aphis/AphisCsv ")
>resultBD <- read.csv("Result24.csv", dec = ".", sep = ";")
>View(resultBD)
###GLMCHOICE
mod0 <- glm(Choix ~ 1, data= resultBD, family = binomial)
## Choix Test : Chisq si Df = Resid. Dev si non Fisher
>anova(mod0)
>anova(mod0, test="Chisq")
>summary(mod0)
###BOXPLOT CHOICE
>boxplot(Individu~Choix, data =resultBD, xlab="Choice",ylab=" Individual")
>res.aov <- aov(Individu~Choix, data = resultBD)
>summary(res.aov)
### GLM CHOICE | BLOCK
>mod1 <- glmer(Choix ~ 1 + (1 | Bloc), data= resultBD, family = binomial)
>mod2 <- glm(Choix ~ 1 + Bloc, data= resultBD, family = binomial)
>anova(mod1)

```

```

>anova(mod1, test="Chisq")
>summary(mod1)
##Visulation graphique
>plot(mod1)
>plot(mod2)
## Visulation temps/Choix
>boxplot(Tmoysec~Choix, data=resultBD, main="Time needed to take a decision", xlab ="Choice",ylab=" Mean
time(sec)")
>res.aov <- aov(log(Tmoysec)~Choix + Bloc, data = resultBD)
# Summary of the analysis
>summary(res.aov)

##A.fabae##CD
>library(lme4)
>setwd("D:/florence/Master2 Agro/TFE/Expériences/Aphis/AphisCsv ")
>resultCD<- read.csv("Result34.csv", dec = ".", sep = ";")
>View(resultCD)
###GLMCHOICE
mod0 <- glm(Choix ~ 1, data= resultCD, family = binomial)
## Choix Test : Chisq si Df = Resid. Dev si non Fisher
>anova(mod0)
>anova(mod0, test="Chisq")
>summary(mod0)
###BOXPLOT CHOICE
>boxplot(Individu~Choix, data=resultCD, xlab="Choice",ylab=" Individual")
>res.aov <- aov(Individu~Choix, data = resultCD)
>summary(res.aov)
### GLM CHOICE| BLOCK
>mod1 <- glmer(Choix ~ 1 + (1|Bloc), data= resultCD, family = binomial)
>mod2 <- glm(Choix ~ 1 + Bloc, data= resultCD, family = binomial)
>anova(mod1)
>anova(mod1, test="Chisq")
>summary(mod1)
##Visulation graphique
>plot(mod1)
>plot(mod2)
## Visulation temps/Choix
>boxplot(Tmoysec~Choix, data=resultCD, main="Time needed to take a decision", xlab ="Choice",ylab=" Mean
time(sec)")
>res.aov <- aov(log(Tmoysec)~Choix + Bloc, data = resultCD)
# Summary of the analysis
>summary(res.aov)

```

### ❖ VOCs sampling from climatic enclosures

```

#####ANOVA#####
##data implementation

Aov_data <- read.csv2(file.choose())
###tout###
###Normalité
shapiro.test(residuals(lm(Aov_data$anova~ Aov_data$tout)))

```

```

##Homoscédasticité
bartlett.test(Aov_data$anova, Aov_data$tout)
##Test ANOVA
fit <- aov(anova ~ tout, data= Aov_data) # y est la variable numérique. A indique les groupes de facteurs
summary(fit)
pairwise.t.test(Aov_data$anova,Aov_data$tout,p.adj="none")
####temperature###
##Normalité
shapiro.test(residuals(lm(Aov_data$anova~ Aov_data$temperature)))
##Homoscédasticité
bartlett.test(Aov_data$anova, Aov_data$temperature)
###Test ANOVA
fit <- aov(anova ~ temperature, data= Aov_data) # y est la variable numérique. A indique les groupes de
facteurs
summary(fit)
pairwise.t.test(Aov_data$anova,Aov_data$temperature,p.adj="none")
###CO2###
##Normalité
shapiro.test(residuals(lm(Aov_data$anova~ Aov_data$CO)))
##Homoscédasticité
bartlett.test(Aov_data$anova, Aov_data$CO)
##Test ANOVA
fit <- aov(anova ~ CO, data= Aov_data) # y est la variable numérique. A indique les groupes de facteurs
summary(fit)
pairwise.t.test(Aov_data$anova,Aov_data$CO,p.adj="none")
#####PERMANOVA PORPORTION#####
####load packages
library('vegan')
##load the matrix
sterol <- read.table(file.choose(), header=TRUE, sep="\t", na.strings="NA", dec=".", strip.white=TRUE)
View(sterol)
##remove columns of factor
comp <- sterol[,-c(1:3)]
View(comp)
##Define factors
sp <-factor(sterol[,1])
sp2 <- factor(sterol[,2])
sp3 <- factor(sterol[,3])
###Equal matrices variiances-covariances
sterol.bray<-vegdist(comp, method="bray")
sterol.bray.MHV <- betadisper(sterol.bray, sp)
anova(sterol.bray.MHV)
permutest(sterol.bray.MHV)
###permutation multivariate analysis of variance (PerMANOVA) non-parametric gold MANOVA (npMANOVA)
adonis(comp~sp, permutations=999)
###Equal matrices variiances-covariances
sterol.bray<-vegdist(comp, method="bray")
sterol.bray.MHV <- betadisper(sterol.bray, sp2)
anova(sterol.bray.MHV)
permutest(sterol.bray.MHV)
### permutation multivariate analysis of variance (PerMANOVA) non-parametric gold MANOVA (npMANOVA)
adonis(comp~sp2, permutations=999)
### Equal matrices variiances-covariances
sterol.bray<-vegdist(comp, method="bray")
sterol.bray.MHV <- betadisper(sterol.bray, sp3)
anova(sterol.bray.MHV)
permutest(sterol.bray.MHV)

```

```

### permutation multivariate analysis of variance (PerMANOVA) non-parametric gold MANOVA (npMANOVA)
adonis(comp~sp3, permutations=999)
###pairwise
pairwise.adonis <- function (x, factors, sim.function = "vegdist", sim.method = "bray",
p.adjust.m = "bonferroni", reduce = NULL, perm = 999)

{
co <- combn(unique(as.character(factors)), 2)
pairs <- c()
Df <- c()
SumsOfSqs <- c()
F.Model <- c()
R2 <- c()

p.value <- c()
for (elem in 1:ncol(co)) {
  if (inherits(x, "dist")) {
    x1 = as.matrix(x)[factors %in% c(as.character(co[1,
      elem]), as.character(co[2, elem])), factors %in%
      c(as.character(co[1, elem]), as.character(co[2,
        elem]))]
  }
  else (if (sim.function == "daisy") {
    x1 = daisy(x[factors %in% c(co[1, elem], co[2, elem]),
      ], metric = sim.method)
  }
  else {
    x1 = vegdist(x[factors %in% c(co[1, elem], co[2,
      elem]), ], method = sim.method)
  })
  ad <- adonis(x1 ~ factors[factors %in% c(co[1, elem],
    co[2, elem])], permutations = perm)
  pairs <- c(pairs, paste(co[1, elem], "vs", co[2, elem]))
  Df <- c(Df, ad$aov.tab[1, 1])
  SumsOfSqs <- c(SumsOfSqs, ad$aov.tab[1, 2])
  F.Model <- c(F.Model, ad$aov.tab[1, 4])
  R2 <- c(R2, ad$aov.tab[1, 5])
  p.value <- c(p.value, ad$aov.tab[1, 6])
}
p.adjusted <- p.adjust(p.value, method = p.adjust.m)
sig = c(rep("", length(p.adjusted)))
sig[p.adjusted <= 0.05] <- "."
sig[p.adjusted <= 0.01] <- "*"
sig[p.adjusted <= 0.001] <- "***"
sig[p.adjusted <= 1e-04] <- "****"
pairw.res <- data.frame(pairs, Df, SumsOfSqs, F.Model, R2,
  p.value, p.adjusted, sig)
if (!is.null(reduce)) {
  pairw.res <- subset(pairw.res, grepl(reduce, pairs))
  pairw.res$p.adjusted <- p.adjust(pairw.res$p.value, method = p.adjust.m)
  sig = c(rep("", length(pairw.res$p.adjusted)))
  sig[pairw.res$p.adjusted <= 0.1] <- "."
  sig[pairw.res$p.adjusted <= 0.05] <- "*"
  sig[pairw.res$p.adjusted <= 0.01] <- "***"
  sig[pairw.res$p.adjusted <= 0.001] <- "****"
  pairw.res <- data.frame(pairw.res[, 1:7], sig)
}

```

```

class(pairw.res) <- c("pwadonis", "data.frame")
return(pairw.res)
}
pairwise.adonis(comp,sterol$out)

#####ACP proportion#####
###load matrix
sol.data <- read.csv2(file.choose())
sol.data
####load packages
library(FactoMineR)
library(factoextra)
library(wesanderson)
library(multcomp)
###PCA
sol.pca <- PCA(sol.data[-(1:3)],graph = FALSE)
##eigenvalues
plot(sol.pca$eig[,1], type="h", lwd=4, ylab="Eigenvalues")
abline(h=1, lty="dashed")
#Other figure
fviz_eig(sol.pca, addlabels = TRUE, ylim = c(0, 100))
###Individual
#temperature 1,2
fviz_pca_ind(sol.pca, geom.ind = "point",axes=c(1,2), col.ind = (sol.data$temperature),
  addEllipses = TRUE, ellipse.type = "confidence", # Ellipses de concentration
  mean.point = FALSE, # retire le centre de gravité des groupes
  legend.title = "temperature",
  palette = c("#CC9933", "#CC6633", "#330000", "#990000", "#3399FF"),
  pointsize= 2)
#temperature 1,3
fviz_pca_ind(sol.pca, geom.ind = "point",axes=c(1,3), col.ind = (sol.data$temperature),
  addEllipses = TRUE, ellipse.type = "confidence", # Ellipses de concentration
  mean.point = FALSE, # retire le centre de gravité des groupes
  legend.title = "temperature",
  palette = c("#CC9933", "#CC6633", "#330000", "#990000", "#3399FF"),
  pointsize= 2)
#temperature 2,3
fviz_pca_ind(sol.pca, geom.ind = "point",axes=c(2,3), col.ind = (sol.data$temperature),
  addEllipses = TRUE, ellipse.type = "confidence", # Ellipses de concentration
  mean.point = FALSE, # retire le centre de gravité des groupes
  legend.title = "temperature",
  palette = c("#CC9933", "#CC6633", "#330000", "#990000", "#3399FF"),
  pointsize= 2)
#CO2 1,2
fviz_pca_ind(sol.pca, geom.ind = "point",axes=c(1,2), col.ind = (sol.data$CO),
  addEllipses = TRUE, ellipse.type = "confidence", # Ellipses de concentration
  mean.point = FALSE, # retire le centre de gravité des groupes
  legend.title = "CO2",
  palette = c("#CC9933", "#CC6633", "#330000", "#990000", "#3399FF"),
  pointsize= 2)
#Co2 1,3
fviz_pca_ind(sol.pca, geom.ind = "point",axes=c(1,3), col.ind = (sol.data$CO),
  addEllipses = TRUE, ellipse.type = "confidence", # Ellipses de concentration
  mean.point = FALSE, # retire le centre de gravité des groupes
  legend.title = "CO2",
  palette = c("#CC9933", "#CC6633", "#330000", "#990000", "#3399FF"),
  pointsize= 2)

```

```

#CO2 2,3
fviz_pca_ind(sol.pca, geom.ind = "point", axes=c(2,3), col.ind = (sol.data$CO),
  addEllipses = TRUE, ellipse.type = "confidence", # Ellipses de concentration
  mean.point = FALSE, # retire le centre de gravité des groupes
  legend.title = "CO2e",
  palette = c("#CC9933", "#CC6633", "#330000", "#990000", "#3399FF"),
  pointsize= 2)
#climat 1,2
fviz_pca_ind(sol.pca, geom.ind = "point", axes=c(1,2), col.ind = (sol.data$stout),
  addEllipses = TRUE, ellipse.type = "confidence", # Ellipses de concentration
  mean.point = FALSE, # retire le centre de gravité des groupes
  legend.title = "climat ",
  palette = c("#CC9933", "#CC6633", "#330000", "#990000", "#3399FF"),
  pointsize= 2)
#climat 1,3
fviz_pca_ind(sol.pca, geom.ind = "point", axes=c(1,3), col.ind = (sol.data$stout),
  addEllipses = TRUE, ellipse.type = "confidence", # Ellipses de concentration
  mean.point = FALSE, # retire le centre de gravité des groupes
  legend.title = "climat ",
  palette = c("#CC9933", "#CC6633", "#330000", "#990000", "#3399FF"),
  pointsize= 2)
#climat 2,3
fviz_pca_ind(sol.pca, geom.ind = "point", axes=c(2,3), col.ind = (sol.data$stout),
  addEllipses = TRUE, ellipse.type = "confidence", # Ellipses de concentration
  mean.point = FALSE, # retire le centre de gravité des groupes
  legend.title = "CO2e",
  palette = c("#CC9933", "#CC6633", "#330000", "#990000", "#3399FF"),
  pointsize= 2)

##Variable
# Color according to group !
group <- as.data.frame(t(sol.data[1,]))
colnames(group) <- "col"
group1 <- group$col
group1 <- group1[-c(1:3)]
fviz_pca_var(sol.pca, axes=c(1,2), , repel = TRUE)
fviz_pca_var(sol.pca, axes=c(2,3), repel = TRUE)
fviz_pca_var(sol.pca, axes=c(1,3), , repel = TRUE)
##contribution to variable
# Contributions des variables à PC1
fviz_contrib(sol.pca, choice = "var", axes = 1, top = 20)
# Contributions des variables à PC2
fviz_contrib(sol.pca, choice = "var", axes = 2, top = 20)
# Contributions des variables à PC3
fviz_contrib(sol.pca, choice = "var", axes = 3, top = 20)

####PERMANOVA CONCENTRATION ####
###load package
library('vegan')
###load the matrix
sterol <- read.table(file.choose(), header=TRUE, sep="\t", na.strings="NA", dec=".", strip.white=TRUE)
View(sterol)
###remove columns of factor
comp <- sterol[-c(1:3)]
View(comp)

```

```

####Define factors
sp <-factor(sterol[,1])
sp2 <- factor(sterol[,2])
sp3 <- factor(sterol[,3])
####Equal matrices variiances-covariances
sterol.bray<-vegdist(comp, method="bray")
sterol.bray.MHV <- betadisper(sterol.bray, sp)
anova(sterol.bray.MHV)
permutest(sterol.bray.MHV)
####permutation multivariate analysis of variance (PerMANOVA) non-parametric gold MANOVA (npMANOVA)
adonis(comp~sp, permutations=999)
#### Equal matrices variiances-covariances
sterol.bray<-vegdist(comp, method="bray")
sterol.bray.MHV <- betadisper(sterol.bray, sp2)
anova(sterol.bray.MHV)
permutest(sterol.bray.MHV)
####permutation multivariate analysis of variance (PerMANOVA) non-parametric gold MANOVA (npMANOVA)
adonis(comp~sp2, permutations=999)
####Equal matrices variiances-covariances
sterol.bray<-vegdist(comp, method="bray")
sterol.bray.MHV <- betadisper(sterol.bray, sp3)
anova(sterol.bray.MHV)
permutest(sterol.bray.MHV)
#### permutation multivariate analysis of variance (PerMANOVA) non-parametric gold MANOVA (npMANOVA)
adonis(comp~sp3, permutations=999)
##pairwise
## Installer les packages
## Créer la fonction de comparaisons multiples
pairwise.adonis <- fonction (x, factors, sim.function = "vegdist", sim.method = "bray",
                           p.adjust.m = "bonferroni", reduce = NULL, perm = 999)
{
  co <- combn(unique(as.character(factors)), 2)
  pairs <- c()
  Df <- c()
  SumsOfSqs <- c()
  F.Model <- c()
  R2 <- c()
  p.value <- c()
  for (elem in 1:ncol(co)) {
    if (inherits(x, "dist")) {
      x1 = as.matrix(x)[factors %in% c(as.character(co[1,
                                                elem]), as.character(co[2, elem])), factors %in%
        c(as.character(co[1, elem]), as.character(co[2,
                                                elem]))]
    }
    else (if (sim.function == "daisy") {
      x1 = daisy(x[factors %in% c(co[1, elem], co[2, elem]),
        ], metric = sim.method)
    }
    else {
      x1 = vegdist(x[factors %in% c(co[1, elem], co[2,
        elem]), ], method = sim.method)
    })
    ad <- adonis(x1 ~ factors[factors %in% c(co[1, elem],
        co[2, elem])], permutations = perm)
    pairs <- c(pairs, paste(co[1, elem], "vs", co[2, elem]))
    Df <- c(Df, ad$aov.tab[1, 1])
  }
}

```



```

SumsOfSqqs <- c(SumsOfSqqs, ad$aov.tab[1, 2])
F.Model <- c(F.Model, ad$aov.tab[1, 4])
R2 <- c(R2, ad$aov.tab[1, 5])
p.value <- c(p.value, ad$aov.tab[1, 6])
}
p.adjusted <- p.adjust(p.value, method = p.adjust.m)
sig = c(rep("", length(p.adjusted)))
sig[p.adjusted <= 0.05] <- "."
sig[p.adjusted <= 0.01] <- "*"
sig[p.adjusted <= 0.001] <- "***"
sig[p.adjusted <= 1e-04] <- "****"
pairw.res <- data.frame(pairs, Df, SumsOfSqqs, F.Model, R2,
  p.value, p.adjusted, sig)
if (!is.null(reduce)) {
  pairw.res <- subset(pairw.res, grepl(reduce, pairs))
  pairw.res$p.adjusted <- p.adjust(pairw.res$p.value, method = p.adjust.m)
  sig = c(rep("", length(pairw.res$p.adjusted)))
  sig[pairw.res$p.adjusted <= 0.1] <- "."
  sig[pairw.res$p.adjusted <= 0.05] <- "*"
  sig[pairw.res$p.adjusted <= 0.01] <- "***"
  sig[pairw.res$p.adjusted <= 0.001] <- "****"
  pairw.res <- data.frame(pairw.res[, 1:7], sig)
}
class(pairw.res) <- c("pwadonis", "data.frame")
return(pairw.res)
}
pairwise.adonis(comp,sterol$out)

#####ACP CONCENTRATION#####
sol.data <- sterol
# load packages
library(FactoMineR)
library(factoextra)
library(wesanderson)
library(multcomp)
##### PCA #####
sol.pca <- PCA(sol.data[-(1:3)],graph = FALSE)
# eigenvalues
#####
plot(sol.pca$eig[,1], type="h", lwd=4, ylab="Eigenvalues")
abline(h=1, lty="dashed")
#Other figure
fviz_eig(sol.pca, addlabels = TRUE, ylim = c(0, 100))
#####Individual#####
#temeprature 1,2
fviz_pca_ind(sol.pca, geom.ind = "point", axes=c(1,2), col.ind = (sol.data$temperature),
  addEllipses = TRUE, ellipse.type = "confidence", # Ellipses de concentration
  mean.point = FALSE, # retire le centre de gravit  des groupes
  legend.title = "temperature",
  palette = c("#CC9933", "#CC6633", "#330000", "#990000", "#3399FF"),
  pointsize= 2)
#temeprature 1,3
fviz_pca_ind(sol.pca, geom.ind = "point", axes=c(1,3), col.ind = (sol.data$temperature),
  addEllipses = TRUE, ellipse.type = "confidence", # Ellipses de concentration
  mean.point = FALSE, # retire le centre de gravit  des groupes
  legend.title = "temperature",
  palette = c("#CC9933", "#CC6633", "#330000", "#990000", "#3399FF"),

```

```

    pointsize= 2)
#temeprature 2,3
fviz_pca_ind(sol.pca, geom.ind = "point", axes=c(2,3), col.ind = (sol.data$temperature),
  addEllipses = TRUE, ellipse.type = "confidence", # Ellipses de concentration
  mean.point = FALSE, # retire le centre de gravit  des groupes
  legend.title = "temperature",
  palette = c("#CC9933", "#CC6633", "#330000", "#990000", "#3399FF"),
  pointsize= 2)
#CO2 1,2
fviz_pca_ind(sol.pca, geom.ind = "point", axes=c(1,2), col.ind = (sol.data$CO),
  addEllipses = TRUE, ellipse.type = "confidence", # Ellipses de concentration
  mean.point = FALSE, # retire le centre de gravit  des groupes
  legend.title = "CO2",
  palette = c("#CC9933", "#CC6633", "#330000", "#990000", "#3399FF"),
  pointsize= 2)
#Co2 1,3
fviz_pca_ind(sol.pca, geom.ind = "point", axes=c(1,3), col.ind = (sol.data$CO),
  addEllipses = TRUE, ellipse.type = "confidence", # Ellipses de concentration
  mean.point = FALSE, # retire le centre de gravit  des groupes
  legend.title = "CO2",
  palette = c("#CC9933", "#CC6633", "#330000", "#990000", "#3399FF"),
  pointsize= 2)
#CO2 2,3
fviz_pca_ind(sol.pca, geom.ind = "point", axes=c(2,3), col.ind = (sol.data$CO),
  addEllipses = TRUE, ellipse.type = "confidence", # Ellipses de concentration
  mean.point = FALSE, # retire le centre de gravit  des groupes
  legend.title = "CO2e",
  palette = c("#CC9933", "#CC6633", "#330000", "#990000", "#3399FF"),
  pointsize= 2)
#climat 1,2
fviz_pca_ind(sol.pca, geom.ind = "point", axes=c(1,2), col.ind = (sol.data$tout),
  addEllipses = TRUE, ellipse.type = "confidence", # Ellipses de concentration
  mean.point = FALSE, # retire le centre de gravit  des groupes
  legend.title = "climat ",
  palette = c("#CC9933", "#CC6633", "#330000", "#990000", "#3399FF"),
  pointsize= 2)
#climat 1,3
fviz_pca_ind(sol.pca, geom.ind = "point", axes=c(1,3), col.ind = (sol.data$tout),
  addEllipses = TRUE, ellipse.type = "confidence", # Ellipses de concentration
  mean.point = FALSE, # retire le centre de gravit  des groupes
  legend.title = "climat ",
  palette = c("#CC9933", "#CC6633", "#330000", "#990000", "#3399FF"),
  pointsize= 2)
#climat 2,3
fviz_pca_ind(sol.pca, geom.ind = "point", axes=c(2,3), col.ind = (sol.data$tout),
  addEllipses = TRUE, ellipse.type = "confidence", # Ellipses de concentration
  mean.point = FALSE, # retire le centre de gravit  des groupes
  legend.title = "CO2e",
  palette = c("#CC9933", "#CC6633", "#330000", "#990000", "#3399FF"),
  pointsize= 2)

#####Variable#####
# Color according to group !
group <- as.data.frame(t(sol.data[1,]))
colnames(group) <- "col"
group1 <- group$col

```

```
group1<-group1[-c(1:3)]
fviz_pca_var(sol.pca,axes=c(1,2), ,repel = TRUE)
fviz_pca_var(sol.pca,axes=c(2,3),repel = TRUE)
fviz_pca_var(sol.pca,axes=c(1,3), ,repel = TRUE)
#contribution to variable
# Contributions des variables à PC1
fviz_contrib(sol.pca, choice = "var", axes = 1, top = 20)
# Contributions des variables à PC2
fviz_contrib(sol.pca, choice = "var", axes = 2, top = 20)
# Contributions des variables à PC2
fviz_contrib(sol.pca, choice = "var", axes = 3, top = 20)
```