INFLUENCE OF DROUGHT ON INTERACTIONS AMONG APHIDS, APHID PREDATORS AND *FUSARIUM* INFECTION ON BARLEY CROP IN SWEDEN

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DECLARATION

This thesis is my original work and has not been presented elsewhere for a degree or any other award.

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DEDICATION

I wish to dedicate this work to the Almighty God for the gift of life, guidance and strength during my studies. I would also like to dedicate this work to my Dad and Mum, my loving family, to my brother Nicholas and my sisters Eunice and Gladys for their moral, emotional, spiritual and financial support.

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LIST OF ABBREVIATIONS AND ACRONYMNS

BYDV	Barley Yellow Dwarf Virus
EU	European Union
GLMM’s	Generalized Linear Mixed Models
Kg/ha	Kilograms per hectare
SLU	Sveriges Lantbruksuniversitet
VPE	Väaxtproduktionsekologi (Swedish name for Department of Crop Production Ecology)
ANOVA	Analysis of Variance
FHB	*Fusarium* Head Blight
RNAi	Ribonucleic Acid interference
HIGS	Host Induced Gene Silencing
WFA	Wet *Fusarium* Aphids treatments
DFA	Dry *Fusarium* treatments
WF	Wet *Fusarium* treatments
DF	Dry *Fusarium* treatment
WA	Wet Aphid treatments
DA	Dry Aphids treaments
VOCs	Volatile Organic Chemicals

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ABSTRACT

Climate-change leads to extreme droughts, but it is difficult to predict how crop pests are affected. Drought may alter behavior of crop pests such as aphids that facilitate transmission of pathogens like *Fusarium graminearum,* causing *Fusarium* foot and root rot in barley crops by feeding close to the susceptible base of plants. Ground dwelling generalist predators like Carabid beetles, and wolf spiders are important aphid enemies during the aphid colonization phase in barley crops in Sweden. This study sought to determine the influence of drought on interactions among aphids, aphid predators, and *Fusarium* infections on spring sown barley (*Hordeum vulgare* L.) in Sweden. To evaluate the influence of drought on predation of aphids by Carabid beetles and Wolf spiders, the experimental design used was factorial with two factors, i) drought and no drought, ii) predators present and predators absent. Similarly, the experimental design used to assess the influence of drought on interactions between aphids and *Fusarium* infections on barley sown during spring was factorial with three factors, i) drought and no drought, ii) aphids and with no aphids, iii) *Fusarium* and with no *Fusarium*. To evaluate the influence of drought on predation of aphids by Carabid beetles and Wolf spiders, the total number of aphids per cage and the proportion of aphids present on different parts of each barley plant sampled (below ground, 0-1 cm above ground, >1cm above ground on stem, and on the leaves) was recorded. Data was subjected to normality test to determine its distribution and analyzed using generalized linear mixed effects models with the lme function in the nlme package in R 3.4.2. To assess the influence of drought on interactions between aphids and *Fusarium* infections, the total number of aphids present at 0-1 cm above ground, >1cm above ground on stem, and on the leaves per plant in each pot was recorded. The data was subjected to normality test and analyzed using generalized linear mixed effects models with the lme function in the nlme package in R 3.4.2. Disease scores on the barley inoculated with *Fusarium* were recorded and the data analyzed using one-way ANOVA test. There was a significant effect of *Fusarium* inoculation on barley plants. In addition, *Fusarium* significantly reduced the number of aphids on the whole plant and 0 to 1 cm above the ground. The study concluded that drought increased proportion of aphids (*Rhopalosiphum padi* L.) below ground. In addition, a strong effect of predation by Carabid beetles and Wolf spiders reduced the number and proportions of these aphids below ground only in dry conditions. This study recommends the need for further studies to establish how drought intensity is likely to affect interactions among pest populations, their natural enemies and plant pathogens.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Europe is among the world's largest and most productive supplier of food and fiber (Olesen *et al*., 2011). In 2008, the region accounted for 19% of global meat production and 20% of global cereal production (Olesen *et al*., 2011). About 80% of the European meat and 63% of the cereals is produced in the EU 27 countries (Olesen *et al*., 2011). Recent studies have indicated that in the instances where global temperature increases, population growth and metabolic rates of insect pests' increases hence leading to crop losses increasing by 10-25 %, especially in temperate areas where cereals such as barley, oats and wheat are grown (Deutsch *et al*., 2018).

The bird cherry-oat aphid (*Rhopalosiphum padi* L.) is one of the most important pests of temperate cereal crops such as barley, causing damage as a virus vector and through direct feeding (Peng *et al*., 2020). The insect pest is a vector for barley yellow dwarf virus (BYDV) which is an economically important disease in many parts of the world where barley is grown. In Sweden, the bird cherry-oat aphid (*R. padi*) invades spring sown barley crops during late May and early June, although this may be determined by weather and location (Jonsson *et al*., 2014). There is a brief cycle of rapid population growth of wingless aphids observed until the time when crop forms head during late June or early July (Jonsson *et al*., 2014). Afterwards, aphid populations diminish rapidly on the crop as a result of declining quality of plant material and also migration of winged aphids to grasslands (Jonsson *et al*., 2014). It is therefore important to employ biological methods of aphid control as part of an integrated pest management in barley crops to reduce the yield losses attributed to the pest invasion.

Aphids' natural enemies are composed of a complex community of arthropods which includes generalist ground-dwelling predators, more specialized leaf dwelling predators that consumes aphids and other soft-bodied prey and parasitoids (Jonsson *et al*., 2014). The most paramount generalist predators include wolf spiders (Araneae: Lycosidae), sheet-web spiders (Araneae: Linyphiidae), ground beetles (Coleoptera: Carabidae) and rove beetles (Coleoptera: Staphylinidae) (Thies *et al*., 2014). The generalist predators are very necessary especially during the aphid colonization stage when aphids are located at the lower parts of the young barley plants, although they also play a role later in the season during the aphid population growth period (Jonsson *et al*., 2014). The important specialists include lady beetles (Coleoptera: Coccinellidae), green lacewings (Neuroptera: Chrysopidae), hoverflies (Diptera: Syrphidae) and parasitoids (Hymenoptera: Braconidae Aphidiinae). These are mostly involved in consuming aphids on the crop as they multiply during late population growth phase (Jonsson *et al*., 2014).

In many ecosystems, aphids sustain several higher trophic groups, including their primary consumers, such as parasitoid wasps, spiders, ladybirds, and carabid beetles (Staudacher *et al*., 2016); the higher-level consumers of these aphid-natural enemies, such as hyper parasitoids (Traugott *et al*., 2008; Lefort *et al*., 2017), small mammals, and birds; and many entomological pathogens and parasites. Examining how climate change, including drought, might influence aphid performance is a major area of current research, specifically with regard to establishing how this might affect the productivity and functioning of agricultural, horticultural, and natural vegetation systems across the globe (Romo & Tylianakis, 2013; Teixeira *et al*., 2020). Experimental studies of aphids indicate that this negative effect of drought is observed across many aphid–plant systems (Aslam *et al*., 2013; Grettenberger & Tooker, 2016; Foote *et al*., 2017), although this has not been assessed quantitatively. Further, there has been no comprehensive analysis of the causes of decreased aphid performance under drought, although several studies suggest that it is mediated through reduced plant fitness (BanfieldZanin & Leather, 2015; Dai *et al*., 2015).

Although many studies have reported reduced aphid fitness when exposed to drought-stressed hosts (Dai *et al*., 2015; Foote *et al*., 2017; Kansman *et al.,* 2020), other studies have reported null (Mewis *et al*., 2012) and positive effects (Oswald and Brewer, 1997). Multiple factors could explain these contrasting observations, including differences in aphid or plant biology. Indeed, in the study by Oswald and Brewer, (2017) a positive effect of drought on aphid performance was detected in the Russian wheat aphid, *Diuraphis noxia* (Mordvilko), and a negative effect was reported for the corn leaf aphid, *Rhopalosiphum maidis* (Fitch). Although both species are cereal-feeding aphids, *D. noxia* and *R. maidis* belong to two distinct aphid tribes, the *Macrosiphini* and the *Aphidini*, respectively (Choi *et al*., 2018), raising the possibility that differences in aphid biology and/or life history could underlie the contrasting responses. Additionally, the specific aphid–plant combination could further influence the effects of drought on aphid performance.

The *R. padi* life cycle is completed on various hosts like the bird cherry tree (*Prunus padus* L.) which is a primary host and cereal crops such as barley, oats, wheat considered to be secondary hosts (Finlay and Luck, 2011). The parthenogenetic populations of the aphid are developed on the secondary hosts during late spring and summer (Rastegari *et al*., 2010). They produce sexual morphs in autumn, that mate and lay overwintering eggs (Finlay and Luck, 2011). The obligate parthenogenetic generation overwinter as mobile parthenogenetic individuals reproduce under mild winter condition (Finlay and Luck, 2011).

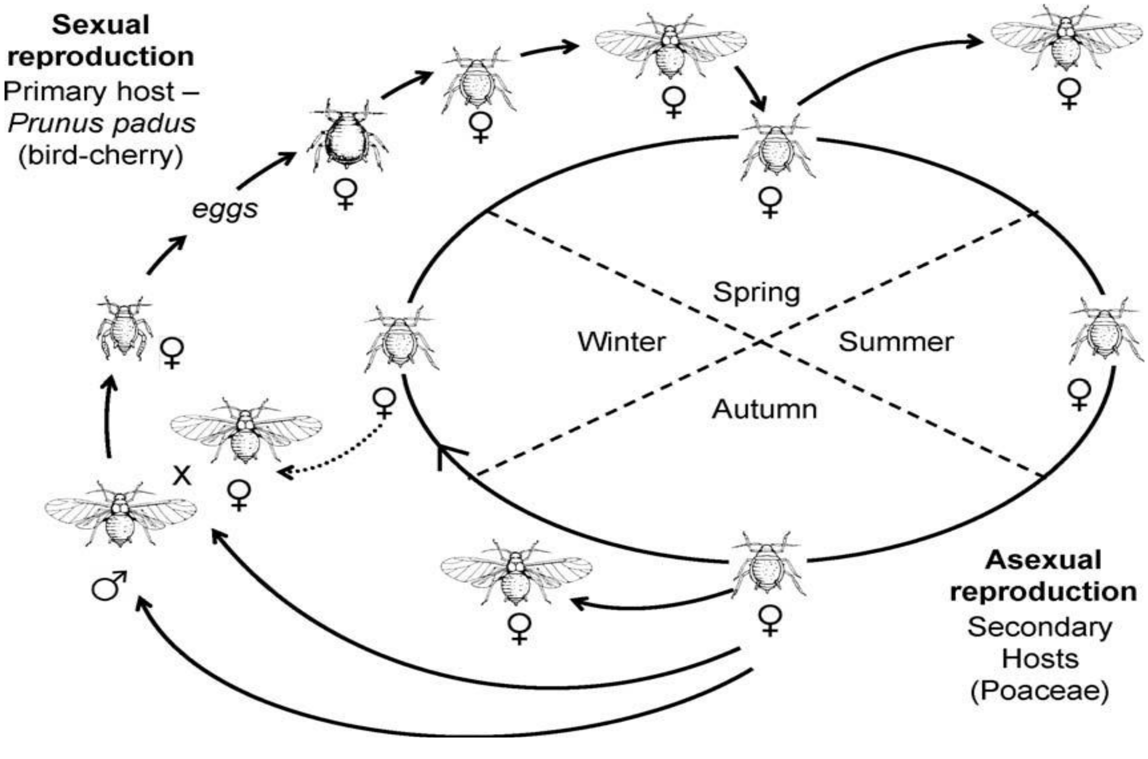

Figure 1. 1 The life cycle of Rhopalosiphum padi ((Finlay and Luck, 2011)

In most temperate areas, both types of aphid life cycle occur but in central Sweden, sexual part of the lifecycle is often skipped and the local balance between them has been found to correspond and have an evolutionary stable strategy (Dedryver and Ralec, 2010). Specialized predators such as Coccinellid beetle (*Cheilomenes* spp.), spiders (Arachnidea) and lacewings (*Chrysopa* spp.), are found at very low density from tillering up to the crop heading stage (Thomson, *et al*., 2010). Studies involving interactions between fungi and insects conducted by several researchers show several layers of complexity whose interactions are either direct, indirect or both (De Zutter *et al*., 2017).

Direct interactions involve insects acting as disease transmitting agents or feeding on fungal particles, or fungi penetrating into the plant through herbivore injured parts (De Zutter *et al*., 2017). Cereal crops including barley are attacked by *Fusarium* disease on the root and stem base. The causal agent of this disease is *Fusarium graminearum* considered as a worldwide fungal pest impacting cereal production (Leplat *et al*., 2015). The losses due to *Fusarium* head blight in Northern and Central America from 1998 to 2002 has been approximated to be $2.7 billion (Leplat *et al*., 2015). Moreover, *F. graminearum* produces mycotoxins which affect human and animal health (Leplat *et al*., 2015). The pathogen survives for several years saprophytically in the soil, on dead organic matter especially crop residues (Leplat *et al*., 2015). *Fusarium graminearum* adapts to a wide range of environmental conditions, and produces extracellular enzymes allowing feeding on different crop residues (De Zutter *et al*., 2017). In addition, *F. graminearum* also competes with other decomposers such as other *Fusarium spp.* belonging to the same complex of species (Leplat *et al*., 2015).

1.2 Statement of the problem

In many parts of the world, it is predicted that climate change will result in more extreme droughts thus negatively affect crop production. Understanding how drought affects invertebrate pests and plant diseases is a key challenge for developing climate resilient agriculture. However, as a result of complex species interactions it can be difficult to predict how different pest problems will be affected by drought. Key pests such as aphids are persistent and are able to complete more generations within a season due to warmer temperatures and cause more damage and losses to barley sown during spring season in Sweden. The aphid (*Rhopalosiphum padi* L.) colonizes barley in late May to Early June in Sweden. In the field, bird cherry oat aphids (*R. padi*) have been mainly observed on lower parts of the plants and sometimes below the soil during dry conditions. This increases niche overlap between *R. padi* and their ground dwelling predators, reducing number of aphids during dry conditions. However, this hypothesis has not been validated.

Ground-dwelling generalist predators such as carabid beetles and wolf spiders are the main natural enemies during the aphid colonization phase and their impact on aphid populations can be substantial. The *Fusarium* pathogen infecting barley cause stem base and root rot disease resulting in death of the affected plants, and it has been hypothesized that aphids can predispose plants to *Fusarium* infection. Estimation of the crop damage by *R. padi* shows that barley yield losses up to 600 kg/ha or about a 15% decrease in yields can occur in Sweden. In the event of drought, complex interactions between crop pest species and their predators are usually anticipated and this could negatively influence barley production. Information on the influence of drought on interactions among aphids, aphid predators and *Fusarium* infections on barley sown in spring has not been documented.

1.3 Justification of the study

World's human population has shown a constant increase up from approximately 2.5 billion to 7.0 billion since 1950 to 2010 (Shaykheeva *et al*., 2016). This population is estimated to increase up again to 11 billion by 2050 (Shaykheeva *et al*., 2016), thus need for global food security. In order to realize increased barley production, there is a need to develop effective drought and aphid management approaches. To make this possible, there is need to understand the influence of drought on aphid, aphid predators and *Fusarium* disease. This will be paramount in mitigating challenges posed by the changing behavior, diversity and abundances of aphids due to current and future climate variation (Finlay and Luck, 2011). Aphids (*Rhopalosiphum padi* L.) that attacks barley crops grown during spring season are consumed by ground dwelling natural predators which includes carabid beetles and wolf spiders (Kuusk *et al*., 2008).

Increased quantity of grain yield in cereals such as wheat due to use of aphid natural enemies have been reported (Ali *et al*., 2018). Barley grain yield losses prevented by aphids' natural enemies are substantial especially for barley organic farmers. This will result in high income to the barley producers hence preventing them from switching to other crops such as corn. The reason is because it is more economical to grow barley which is used in making livestock feeds and beer production (Kerr *et al*., 2019). Production of pest free barley will result to increased yield, more income to producers and hence more job opportunities due to increased supply of raw material for the barley processing industries. It is expected that the findings of this study will contribute to the existing knowledge gap on the influence of drought on interactions among aphids, aphid predators and *Fusarium* infections on barley sown during spring in Sweden. This will enhance generation of an effective integrated strategy for aphid and *Fusarium* management for optimal barley yield production in other climatic regions with similar production challenges.

1.4 Hypotheses

1. There is no influence of drought on the interactions between aphids and their predators on barley sown during the spring season in Sweden.

2. There is no influence of drought on the interactions between aphids and *Fusarium* infections on barley sown during the spring season in Sweden.

1.5 Objectives

1.5.1 General objective

To determine the influence of drought on the interactions among aphids, aphid predators and *Fusarium* infection on barley in Sweden.

1.5.2 Specific objectives

1. To evaluate the influence of drought on interactions between aphids and their predators on barley.

2. To assess the influence of drought on interactions between aphids and *Fusarium* infection on barley.

1.6 Research questions

1. What is the influence of drought on the interactions between aphids and their predators on barley in Sweden?

2. What is the influence of drought on the interactions between aphids and *Fusarium* infection on barley in Sweden?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Biology of aphids

Aphids (Aphididae) are a part of the Hemiptera, a species-rich group of the hemimetabolous insects (Panfilio *et al*., 2019). Reflected in their alternative name, "Rhynchota" all members of *Hemiptera* have mouthparts transformed to a "sucking beak" (Panfilio *et al*., 2019). Mandibles and maxillae are used as stechborsten, the labium forms a sheath around them, maxillary and labial palps are reduced and this innovation initially evolved to optimize the exploitation of plant saps for feeding (Vilcinskas, 2016). The sister group of Hemiptera is most probably the *Thysanoptera* (thrips), which also suck plant saps, but their mouthparts are derived in a different way (Vilcinskas, 2016). Among the hemipteran subtaxon Heteroptera, several branches secondarily switched to predatory behavior retaining sucking mouthparts and feed on invertebrate hemolymph or vertebrate blood (Panfilio *et al*., 2019).

The currently described about 100,000 species of *Hemiptera* were traditionally split into Heteroptera ("typical bugs") and "Homoptera", but the latter group appears to be a paraphylum in most molecular analyses (Song *et al*. 2012, Cui *et al*. 2013). "Homoptera" is now usually split into the clades *Sternorrhyncha* (plant lice, including the aphids), *Cicadomorpha* (cicadas, leaf hoppers, tree hoppers), *Fulgoromorpha* (plant hoppers) and *Coleorrhyncha* (moss bugs or beetle bugs) (Song *et al*. 2012). The old name "Auchenorrhyncha" for the combination of Cicadomorpha and Fulgoromorpha is no longer supported as it seems to be a paraphyletic group united by shared plesiomorphic characters (Vilcinskas, 2016). Nevertheless, molecular analyses differ in the exact placement of these taxa to each other for example, early studies, based on 18s rRNA data, placed Cicadomorpha as the sister group to a combined clade of Fulgoromorpha and the sister groups Coleorrhyncha and Heteroptera (Sorensen *et al*. 2015), another one with the same marker set found only support for the clade combining all those four taxa (Ouvrard *et al*., 2019). More recent studies with mitochondrial genome sequences confused the overall picture by combining Cicadomorpha, Fulgoromorpha and Sternorrhyncha, with the latter two as sister groups (Song *et al*.

2012), or supporting a sister group relation between Cicadomorpha and Heteroptera (Cui *et al*., 2013). In contrast, a recent phylogenomic study, making use of 1,500 orthologous genes and covering all insect orders, placed Coleorrhyncha as sistergroup to a clade combining Fulgoromorpha and Cicadomorpha, while Heteroptera is sister group to these three taxa (Misof *et al*., 2014).

Almost all studies favours monophyly of the clade Sternorrhyncha, which includes the aphids (Aphididae), along with scale insects (Coccoidea), white flies (Aleyrodoidea), and jumping plant lice (Psylloidea) (Sanaei, 2021). All Sternorrhyncha feed on plant saps by tapping the phloem and many species have economic impact by damaging plants directly or acting as vectors of plant pathogens (Bragard *et al*., 2021). In phylogenetic analyses of molecular datasets aphids (Aphididae) and scale insects (Coccoidea) usually appear as sister groups. Most of the 7,700 species of scale insects feed on plant saps, a few exploit fungal food sources (Misof *et al*., 2014). In most species the adult females are wingless and stay permanently attached to their food plant and the bodies of many of these sessile females are covered by wax secretions as a defense against predators (Vilcinskas, 2016). The adult males have wings, but are short-lived and take up no food (Sanaei, 2021). The relationship of the sister groups (Aphididae) and (Coccoidea) to the remaining two clades of Sternorrhyncha varies between different analyses (Panfilio *et al*., 2019).

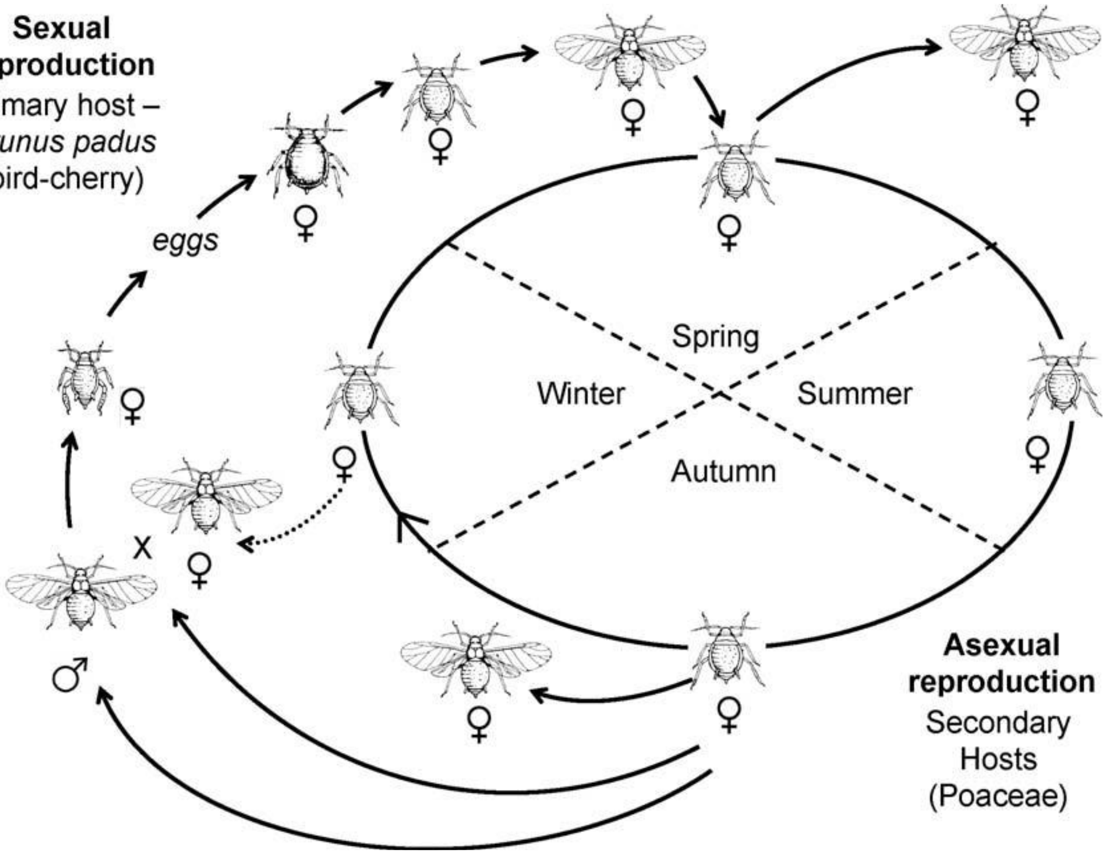

Figure 2. 1 The life cycle of *Rhopalosiphum padi* ((Finlay and Luck, 2011)

2.2 General features of aphids

Typical morphological characteristics include a head that is immovably joined to the thorax, which itself is broadly conjoined with the abdomen, thus the body has a compact oval shape (Madenjian, 2020). As in other Sternorrhyncha the base of the "sucking beak" is located between the coxae of the first walking legs (Madenjian, 2020). Species of the largest family Aphididae have a noticeable pair of siphons on the back (Sarwar, 2020). While morphology is rather uniform throughout the aphids, their life cycles vary strongly, often involving alternating generations, e.g., of winged (*alate*) and wingless (*apterous*), sexual and parthenogenetic forms, with oviparous or viviparous females (Sarwar, 2020).

Several bacterial endosymbionts are described from aphids (Grigorescu *et al*., 2018). In the largest family Aphididae there is a long known mutual relationship (obligatory, “primary” endosymbionts) with Buchnera bacteria, which supplement their hosts’ poor diet of phloem sap with essential aminoacids (Ramsey *et al*. 2010, Wilson *et al*. 2010). In addition, some species also harbour facultative (secondary) symbionts. Aphids from Adelgidae and Phylloxeridae families have no association with Buchnera strains, but other kinds of endosymbionts (Michalik *et al*. 2013, Toenshoff *et al*., 2014).

Aphid evolution is strongly shaped by their dependency on their host plants. For example, about 99% of the species are specialists, associated with one or just a few closely related plant species (Yamamoto *et al*., 2020). About 10% of the species regularly switch between two host species (primary and secondary host) during the seasons (Pureswaran *et al*., 2018).

The primary host is usually a woody plant, which is inhabited in autumn, winter and spring, while the secondary host may be herbaceous plant, exploited during summer season (Pureswaran *et al*., 2018). Life cycles with host alternation evolved several times independently and are found in species of Adelgidae, Phylloxeridae, Hormaphidinae, Eriosomatinae, Anoeciinae and Aphididae (Blackman and Eastop 2006).

Figure 2. 2 (a) Photomicrograph of a winged male adult *Rhopalosiphum padi*

The majority of all aphid species are found in temperate regions of the northern hemisphere and for unknown reasons, tropical trees are not often inhabited by aphids (Aoki and Kurosu, 2021). The complex life cycles of aphids with alternation between flightless parthenogenetic generations and winged sexual morphs seem to be adaptations to the seasonal changes of plant food supply in temperate regions (Peccoud *et al*. 2010).

Figure 2. 3 (b) Photomicrograph of a wingless female adult viviparous *Rhopalosiphum padi*

Probably aphid life cycle evolution has to overcome some hurdles to adapt to the tropical absence of seasons (Aoki and Kurosu, 2021). Besides the classical mode of allopatric speciation, sympatric ecological speciation was proposed for specialized phytophagous insects, e.g., by reduced interactions between "ecotypes" exploiting different host plants (Saunders and Rader, 2021). Aphid research provided several examples demonstrating that speciation processes coincide with host plant switches, for instance in genus Aphis (Peccoud *et al*. 2010). Nevertheless, in many cases the allopatric mode of speciation contributes substantially to biodiversity (Jousselin *et al*. 2013). Here as in many other biological fields, general conclusions of trends and features do not well fit to the complicated ecological interactions and historical processes occurring in real populations.

2.3 Impact of drought on aphid populations

Drought can be defined as a prolonged period of shortage of water in relation to normal conditions (Lloyd-Hughes, 2014). There are three major categories of drought namely agricultural, meteorological, and social-economic (Panu and Sharma, 2009). Droughts are recognized as an environmental disaster and have attracted the attention of environmentalists, ecologists, hydrologists, meteorologists, geologists, and agricultural scientists (Lloyd-Hughes, 2014). Temperatures, high winds, low relative humidity, timing and characteristics of rains including distribution of rainy days during crop growing seasons, intensity, and duration of rain, and onset and termination, play a significant role in the occurrence of droughts (Panu and Sharma, 2009). In contrast to aridity, which is a permanent feature of climate and is restricted to low rainfall areas, a drought is a temporary aberration (Saunders and Rader, 2021). Often, there is confusion between a heat wave and a drought, and the distinction is emphasized between heat wave and drought, noting that a typical time scale associated with a heat wave is on the order of a week, while a drought may persist for months or even years (Lloyd-Hughes, 2014).

Drought risk is a product of a region's exposure to the natural hazard and its vulnerability to extended periods of water shortage (Belal and Ramady, 2014). This study focuses on agricultural drought experienced when soil moisture available to crops declines, leading to negative effects on grain yield as well as agricultural production in a given region (Panu and Sharma, 2009).

In Sweden, barley (*Hordeum vulgaris* L.) is among the most important cereal crops after wheat grown in southern and eastern regions (Tidåker *et al*., 2016). Barley is mainly used as a source of livestock feed and also to some extent for manufacture of beverages such as beer (Tidåker *et al*., 2016). Barley crops in Sweden are colonized by aphids particularly (*R. padi*) during spring season (Jonsson *et al*., 2014). Plant insect herbivores respond differently to drought intensity either severe or moderate thus influencing rate of survival and fecundity hence affecting their abundance (Sconiers and Eubanks, 2017).

The spring season occurs in the months of April and May where winter snow normally begins to melt due to gradual rise in air temperature (Larsen *et al*., 2007). Actually for the last 50 years, Northern Sweden has experienced an increase in the air temperature by 2.8^{o} C and 1.5^{o} C in winter and spring respectively (Larsen *et al*., 2007). Although daylight hours and temperatures begin to increase in March, snow is still possible (Græsli *et al*., 2020). Ski season starts wrapping up in April, snow and cold are possible well in May while midnight Sun typically starts in late May and lasts through August (Græsli *et al*., 2020). The major focus of this study was to unravel the influence of drought conditions on the interactions between these aphids and their ground dwelling natural enemies in order to understand how future drought is likely to impact on interactions between these aphids and their predators.

In Sweden, bird cherry-oat aphid (*Rhopalosiphum padi* L.) is an important insect pest of cereal crops like oats, wheat and barley (Rastegari *et.al*., 2010). This aphid (*R. padi*) colonizes bird cherry trees (*Prunus padus* L.) (primary host) and cereal crops as secondary hosts to complete its life cycle (Rastegari *et.al*., 2010). In Sweden, eggs of *R. padi* found on the buds of the primary host (*P. padus*) during winter hatch into female (fundatrices) that start to feed on the breaking buds (Pettersson and Sandstr, 2000). During early summer, no aphids are left on *P. padus* and the emigrants formed colonize the cereal crops producing alates (winged males) (Finlay and Luck, 2011) due to gradual decline in plant food quality until mid-summer when the maximum populations are realized (Ninkovic *et al*., 2003). At this stage also, the crop is exponentially maturing and drying out hence inhibiting aphid survival (Ninkovic *et al*., 2003). In early September, presence of female migrants (gynoparae) and males are produced where they fly to colonize the primary host (*P. padus*).

Figure 2. 4 Photograph of adult *Rhopalosiphum padi*

Aphids are migratory hence well adapted to exploit new habitats by moving into areas which become more favorable due to climatic change (Czerniewicz *et al*., 2011). Climate and weather changes are likely to affect the population dynamics and status of crop insect pests' temperatures (Finlay and Luck, 2011). This will be due to direct effects on distribution and abundance of pest populations but also effects on the pest's host plants, competitors and natural enemies (Jeffs and Leather, 2014). Temperature affects the survival, development, reproduction and migration of individual insects (Czerniewicz *et al*., 2011). This in turn influence potential distribution and abundance of a particular pest species. Genetic and environmental changes, can cause a change from sexual to asexual populations in aphids (Christoph *et al*., 2011).

The Bird cherry-oat aphid (*R. padi*) is one of the most important pests in cereal fields and its abundance varies greatly between years, especially in Northern Europe (Jeffs and Leather, 2014). The *R. padi* populations abundance in both sexual and asexual generation is determined by climate, with asexual populations prevalent where winter is mild (Gilabert *et al*., 2009). Temperature directly affects the growth and development rate of insects (Jeffs and Leather, 2014). Temperature is a reliable indicator for future aphid population growth rates and aphid performance (Jeffs and Leather, 2014). Higher temperatures expected almost throughout under climate change, are predicted to cause an increase in reproduction rates and lead to shorter lifecycles for insect species in general (Bale *et al*., 2002). For most aphid species there is a strong positive linear relationship between temperature and development from approximately 7ºC to 25ºC, followed by a corresponding decrease at temperatures beyond 30ºC (Jeffs and Leather, 2014). In addition, *R. padi* infests cereals grown in mid to high latitudes region where pest insects are expected to become more abundant as the conditions become warmer (Finlay and Luck, 2011).

2.5 Transmission mechanism of *Fusarium* pathogen in cereal crops

Fusarium species that infect cereals such as barley are initially found in the soil occurring as saprophytic mycelium or thick-walled resting spores depending on the species (Landschoot *et al*., 2011). Planting cereals into *Fusarium* infested soil leads to development of seedling blight and foot rot disease on the plant and airborne inoculum in form of ascospores or conidia infects the ears (Khosht, 2010). Consequently, there is production of *Fusarium* infested grain that act as source of inoculum for developing seedling blight (Khosht, 2010). Apart from wind and rain drops splash, spores of different *Fusarium* species are also dispersed by certain arthropod vectors such as mites, barley thrips, houseflies, picnic beetles among others (Khosht, 2010).

The amount of temperature and moisture determine incidence and severity of *Fusarium* disease on cereals. For example, when ears of wheat were inoculated with *F. graminearum* at 25ºC and exposed to 36 hours and 48 hours of continuous wetness, 18% and 77% of ears became infected respectively (Pfordt *et al*. 2020). The bird cherry oat aphid (*R. padi*) act as an insect vector that transmits barley yellow dwarf virus (BYDV) on barley crops (Wang *et al*, 2015). It is also hypothesized to predispose the barley crop to *Fusarium* disease. *Fusarium spp*. are filamentous, necrotrophic fungi with several species causing severe plant diseases across the world. Some *Fusarium spp*. also contribute to yield losses in cereals, and contamination by producing mycotoxins like deoxynivalenol, zearaleone, fusarin (Wagacha and Muthomi, 2007). *Fusarium spp*. cause *Fusarium* head blight, *Fusarium* foot rot and *Fusarium* seed blight on small grain cereals (Wagacha and Muthomi, 2007). Three important species of *Fusarium spp*. that causes foot rot on cereals like barley are *F. graminearum*, *F. culmorum* and *F. pseudograminearum* but they differ in their pathogenicity (Hutzenlaub, 2010).

Fusarium graminearum was originally identified to be associated with warm and humid areas, whereas *F. culmorum* was found in cooler areas of North and West Europe but currently these borders seem to disappear (Munkvold *et al*., 2019). *Fusarium graminearum* is also present in high amounts frequently in England, Wales, and other parts of Europe including Sweden (Bateman *et al*., 2007). *Fusarium pseudograminearum* thrive well in regions that are characterized by dry conditions and high temperatures (Singh *et al*., 2009). The symptoms of stem base and root rot disease appear as necrotic, brown, elongated spots without distinctive center or as watery-brown to dark brown discolorations of tissue along the stem base and root (Beccari *et al*., 2011). The first visible symptom is usually the browning of the coleoptiles and the stem base (Hutzenlaub, 2010). Plants showing a severe infection at the lower node easily breaks and the tissues at the internodes often becomes softened (Hutzenlaub, 2010).

Fusarium graminearum is a major fungal pathogen of cereals worldwide, causing seedling, stem base and floral diseases, including *Fusarium* head blight (FHB) (Bateman *et al*., 2007). In addition to yield and quality losses, FHB contaminates cereal grain with mycotoxins, including deoxynivalenol, which are harmful to human, animal and ecosystem health (Machado *et al*., 2018). Currently, FHB control is only partially effective due to several intractable problems. RNA interference (RNAi) is a natural mechanism that regulates gene expression (Machado *et al*., 2018). RNAi has been exploited in the development of new genomic tools that allow the targeted silencing of genes of interest in many eukaryotes (Machado *et al*., 2018). Host-induced gene silencing (HIGS) is a transgenic technology used to silence fungal genes *in planta* during attempted infection and thereby reduces disease levels. HIGS relies on the host plant's ability to produce mobile small interfering RNA molecules, generated from long double-stranded RNA, which are complementary to targeted fungal genes. These molecules are transferred from the plant to invading fungi via an uncharacterized mechanism, to cause gene silencing (Machado *et al*., 2018).

Plant pests cause entry of secondary fungal infection by wounding a plant or they may change host susceptibility by inducing alterations in plant defense pathways (Drakulic *et al*., 2017). Upon plant infection, *Fusarium* pathogen undergoes through bio trophy in their host plants before switching to necrotrophic on tissues and crop residues (Hutzenlaub, 2010). It is at this stage where infected material becomes a potential source of inoculum for the next crop in rotation (Kistler *et al*., 2004). *Fusarium* infected heads produce infected seed which causes seedling blight, and conidia developing from seedling blight can give rise to stem base and root rot (Bateman *et al*., 2007). The conidia at the stem bases can be dispersed to the ears and canopy layers through rain splash initiating *Fusarium* head blight in wheat (Zhang *et al*., 2005). It is important to develop measures that can mitigate problems associated with decline in barley yields such as *Fusarium* disease.

An effective strategy for combating plant diseases requires a thorough knowledge of the pathogens, including their biology, ecology and their variability (Elad *et al*., 2007). Knowing the life cycle of a pathogen sheds light on its survival mechanism, interaction with host plants, spread over time and space, and capability of evolving into new forms (pathotypes). *Fusarium oxysporum* f.sp. lentis (Fol), lacks a teleomorphic state, and thus genotypic changes result from anamorphic phenomena rather than sexual reproduction that results in teleomorphic stage. The first step in the sexual-like cycle or process is the formation of a heterokaryon, which is important for the wilt fungus to adapt to changing circumstances (Glass and Kuldua, 1992). At this stage, due to the exchange of genetic material among various forms, the pathogenicity partner changes.

Vascular wilt disease caused by fungi is usually highly destructive whether they occur in cultivated crops or in indigenous wild species. Wilts occur as a result of the presence and activities of the pathogen in the xylem vessels of the plant (Agrios, 2005). Thus, the vascular wounds enhance vascular colonization. The most common sites of direct penetration are located at or near the root tip of both tap root and lateral roots (Hutzenlaub, 2010). Following infection of host roots, the fungus crosses the cortex and enters the xylem tissues. It then spreads rapidly up through the vascular system, becoming systemic in the host tissues, and may directly infect the seed. Entry is either direct through wounds at the point of formation of lateral roots (Ahari *et al*., 2011). Direct introduction of the vascular wilt pathogen to the stem does not allow the activation of resistance mechanism present in the roots for soil borne pathogen getting roots as the main point of entry (Cirulli *et al*., 2008). The mycelium takes an intercellular path through the cortex, and enters xylem vessels through the pits (Hutzenlaub, 2010). Infection occurs ready where the xylem was exposed by wounding of the stem or the root (Pouralibaba *et al*., 2016)

Fusarium wilt occurs in fields in patches and originates either at early (seedling) crop stage or at reproductive (adult plant) stage (Chavdarov, 2006). Seedling wilt is characterized by sudden drooping, followed by drying of leaves and seedling death. The roots appear healthy, with reduced proliferation and nodulation and usually no external discoloration of the vascular system (Hutzenlaub, 2010). Adult wilt symptoms appear from flowering to late pod-filling stage and are characterized by sudden drooping of top leaflets of the affected plant, leaflet closure without premature shedding, dull green foliage followed by wilting of the whole plant or individual branches (Ahari *et al*., 2011). Wilt symptoms in the field include wilting of older leaves, stunting of plants, shrinking, and curling of leaves from the lower part of the plants that progressively move up to the stems of the infected plant making the plant become yellow and die (Pouralibaba *et al*., 2016).

2.6 *Fusarium* wilt disease management

The management of wilt complex can be done through cultural practices, use of resistant varieties, biological control and chemical protection (Degani and Dor, 2021). In the absence of resistant/tolerant varieties, it would be too difficult to manage the disease caused by soil-borne pathogens because of complex soil physico-chemical properties, environmental conditions and biological origin (Degani and Dor, 2021). The following sub-sections deal with possible management options of *Fusarium* wilt.

2.6.1 Cultural practices

The cultural practices include deep plowing and leaving the soil fallow. These practices helped in reducing the pathogen population in the soil but do not eliminate it completely (Silvia *et al*., 2016). Also, management practices to reduce the effects of waterlogged soil include genotype choice and the proper design of field drainage systems to discharge excess water. Selecting cultivars that mature early and adjusting the planting date, if possible, can reduce disease incidence by escaping a portion of lentil growth period from weather conditions favorable to the disease (Rathore *et al*., 2010). Use of clean seed for sowing and/or the use of fungicidal seed treatments can eliminate or reduce contaminating inoculum sources. Lentil crops grown on raised beds produced significantly superior agronomic characteristic; yield attributes trials, seed and straw yield as compared to the flat bed sown crop (Rathore *et al*., 2010). Merkuz and Getachew (2012) reported that growing resistant and moderately resistant varieties on raised seedbed that drain excess water with recommended seeding rate could reduce plant mortality caused by chickpea wilt.

Changes of micro-environment are complex and often interrelated because they affect both host and root pathogens (Ghatak *et al*., 2015). Some factors may affect the lentil plant negatively and the fungus positively, leading to an apparent increase in lentil wilt. Under the traditional management systems, lentil yield from vertisols is far below the potential yield. Feng *et al*. (2010) also demonstrated significant effects of the ridge-furrow system on Siberian wild rye (*Elymus sibiricus*).

2.6.2. Host plant resistance

Breeding for host resistance is the most effective, economical, efficient and environment-friendly disease management method. The search for sources of resistance to diseases is a primary and most eminent research for most of the work carried out in the past and also is continuing presently (Gohel *et al*., 2007).). Successful screening for disease resistance is based on the availability of large and diverse germplasm collections and of precise and accurate screening techniques. To date, host plant resistance screening was being conducted at Debre Zeit International *Fusarium* wilt by screening of germplasm from abroad and indigenous materials. However, the challenges of *Fusarium* wilt have increased since the available host plant resistance source was not obtained as a core factor regardless of the breeding efforts made so far.

2.6.3. Biological control

Biological control relies largely upon an interruption of host parasite relationship through biological means whereby the approach is used to control disease by use of living microorganisms under their natural or artificial circumstance. Biocontrol is the best and effective substitute, environment friendly, especially against soil-borne pathogens, such as *Fusarium* species (Gohel *et al*., 2007). Among several antagonists used for biological management, *Trichoderma* species are used extensively as biocontrol agents against soil- and seed-borne diseases, such as *Fusarium* wilt. These antagonists are saprophytic filamentous fungi, easily growing and produce conidia having long survival period in large quantities (Kumar *et al*., 2013). *T. harzianum* was highly efficient in controlling wilt disease and reducing disease severity to 8.9% when applied as a soil drench (Ref). Kumar *et al*. (2013) observed significant reduction in incidence and maximum grain yield in field trials against *Fusarium* wilt through *T. harizanum* and *Pseudomonas fluorescens* as bioagents.

2.6.4. Integrated disease management

Integrated disease management is a holistic approach that combines available disease management technologies in an economically and ecologically-sound manner. Landa *et al*. (2004) studied the effect of sowing date, resistant genotypes and seed and soil treatments chemically or biologically against *Fusarium* wilt and found it effective against wilt incidence. Blanca *et al*. (2004) reported that the change in date of sowing, host plant resistance and seed/soil treatment with biocontrol agents reduces disease intensity and increases lentil seedling emergence. Thus, an integrated disease management approach is essential to combat lentil *Fusarium* wilt for increased and sustainable yields.

CHAPTER THREE

3.0 MATERIALS AND METHODS

2.4 Role of natural enemies in control of aphids

Cereal aphids (Hemiptera; aphididae) are specialist herbivores and major pest insects in cereal crops in northern Europe (Sigsgaard, 2002). Cereal aphids are serious pests of grain crops causing economic damage directly by feeding on the plants and indirectly by transmitting cereal and barley yellow dwarf viruses (Chapin *et al*., 2001). Both chemical and biological methods have been used to manage aphids and reduce the spread of viruses in cereals (Sigsgaard, 2002). While effective in aphid control, intensive use of insecticides can lead to increased production costs, development of insecticide resistance, increased aphid movement from plant to plant increasing virus spread and negative effects on human health and the environment (Chapin *et al*., 2001).

Pest suppression by natural enemy communities is an important ecosystem service which helps to reduce their spread. Naturally occurring enemies that prey on aphids can prevent populations from multiplying beyond economic thresholds and prevent yield loss thereby reducing the need for insecticide use (Safarzoda *et al*., 2014).

The entomophagous arthropods that attack aphids can be divided broadly into specialists and generalists. Specialists include potentially important control agents of aphids such as parasitoids (Hymenoptera: Braconidae, Aphidiinae), and aphidophagous predators: coccinellids (Coleoptera, Coccinellidae), lacewings (Neuroptera, Chrysopidae), and hoverflies (Diptera, Syrphidae (Müller and Godfray, 2019). Generalists include euryphagous predators such as ground beetles (Coleoptera, Carabidae) and spiders (Lang, 2003).

Generalist aphid predators like carabid beetles and wolf spiders may be important in controlling aphid numbers since they are able to survive on other prey types when aphid densities are low (Tscharntke *et al*., 2004). Aphid predators including representatives from Coccinellidae, Syrphidae, Neuroptera, Cecidiomyidae, Anthocoridae, Miridae are more important later in the spring season by reducing aphid numbers (Finlay and Luck, 2011). In Europe, generalist predators such as Carabidae, Staphylinidae, and Araneae have been shown to reduce *R. padi* numbers particularly during the colonization periods when aphid populations are establishing (Finlay and Luck, 2011).

The degree to which ground-dwelling natural enemies of the bird cherry-oat aphid (*R. padi*) reduce aphid abundance, thereby influencing barley yields on commercial farms in central Sweden has been reported (Östman *et al*., 2003). On average, ground-dwelling natural enemies of pests increased barley grain yields by 303 kg/ha in organic farms compared to conventional farms (Östman *et al*., 2003). This corresponded to a potential 52% reduction in grain yield loss from *R. padi* compared with when natural enemies were excluded (Östman *et al*., 2003). Ground-living natural enemies of aphids could increase grain yield by 23% (Östman *et al*., 2003).

Conventional farms use insecticides to reduce aphid populations making the natural enemies less effective in those fields while in organic farms, aphid population increases which leads to increase in natural enemies (Östman *et al*., 2003). Ground-living natural enemies can reduce the abundance of *R. padi* in cereal fields (Östman *et al*., 2001). Both generalist and specialist aphid natural enemies contribute to pest suppression in disturbed habitats such as crop fields although their relative importance varies (Öberg and Ekbom, 2006). Many generalist predators migrate between crop fields and permanent habitats hence have cyclic colonization patterns in accordance with annual crops (Jonsson *et al*., 2014). Generalist predators can also be sustained by detritivores when herbivore prey is absent (Öberg and Ekbom, 2006). Predators can establish in the field at low pest densities hence have potential to suppress low pest populations in the field (Öberg and Ekbom, 2006). The difference in effects of temperature on aphids and their natural enemies has demonstrated how temperature to greater extent can affect the level of control of these pests (Jonsson *et al*., 2014). For example, below 11°C, the pea aphid (*Acyrthosiphon pisum*) can build up populations at a faster rate than can be regulated by the coccinellid, (*Coccinella septempunctata* L.) but it is opposite when the temperatures are beyond 11°C (Harrington *et al*., 2001).

3.1 Study site description

The experiments were conducted at the Sveriges Lantbruksuniversitet (SLU) Centre for Ecology in Uppsala (Sweden) as shown in Figure 3.1. The site is located at 59.8º N and 17.6º E and is characterized by different temperature ranges, and rainfall patterns leading to autumn, winter, spring and summer seasons (Christidis *et al*., 2012). Each season lasts for three months whereby spring season comes after winter but before summer season (Bergh *et al*. 2003). During spring season, days are longer and it gets warmer and crops grow and flower during this season (Christidis *et al*., 2012).

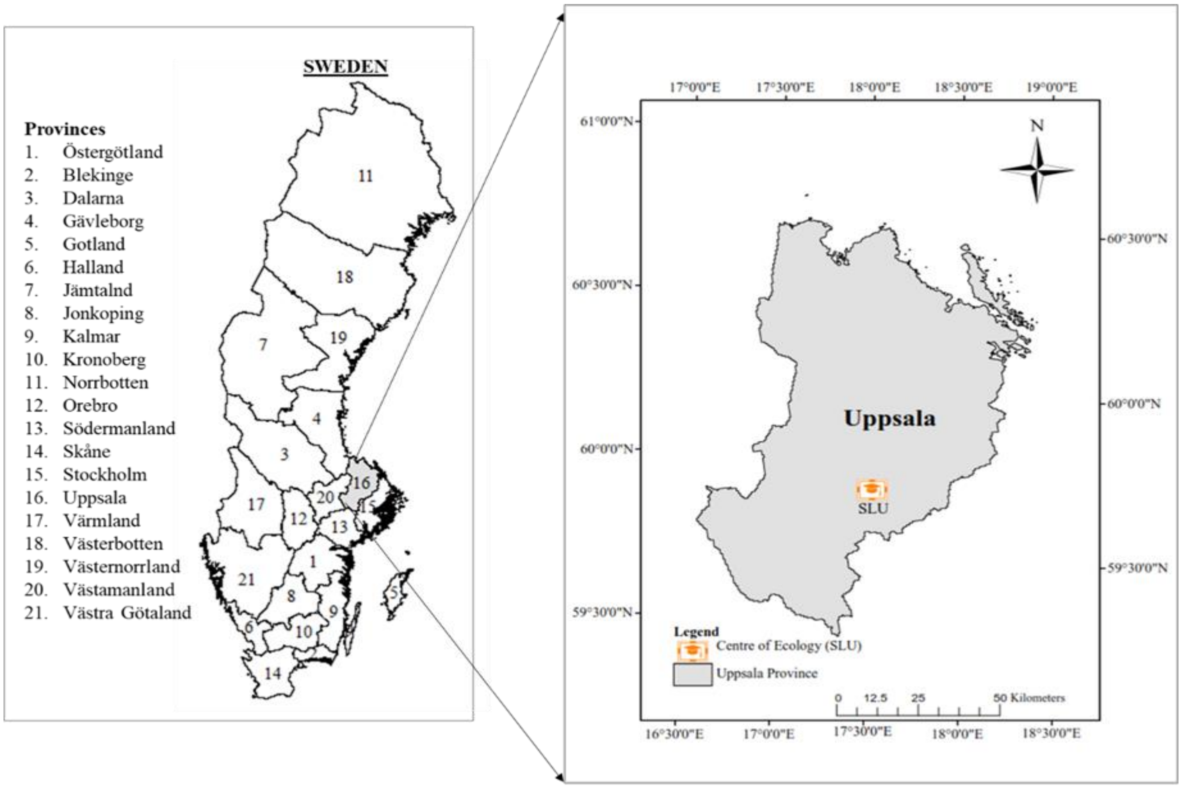

Figure 3.1 Map of Sweden showing the study site at SLU in Uppsala

SOURCE: Esri, HERE, Garmin, Intermap, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordance Survey, Esri Sweden and the GIS User Community

3.2. Experimental material description

The study involved setting greenhouse mesocosm experiments using plastic crates measuring 60 cm x 40 cm x 20 cm. A cage mesh 60 cm high with one side resalable cage and a mesh size of 0.5 mm x 0.5 mm was fixed on top of each crate to prevent insects from entering or exiting the cage. The plastic containers were filled with Hasselfors garden soil up to a depth of 15 cm for aphid predation experiment. Collection of carabid beetles and wolf spiders was done using medium sized pitfall traps in the fields with cultivated barley and oat crops during summer. Seeds for Kara barley variety susceptible to *R. padi* infestation and *Fusarium* disease infecting barley was obtained from Sveriges Lantbruksuniversitet (SLU). The barley seeds were sown in small plastic pots measuring 8.3 cm long x 8.3 cm wide x 7.4 cm deep filled with sand. Potting soil sometimes does not make plants to get infected, thus the reason why sand was used in the experiment. A small (standardized size) agar plugs for cutting inoculum was used during sowing experiment for *Fusarium spp.* inoculation. The strain of *Fusarium spp.* used in this experiment was pathogenic isolate of *Fusarium graminearum* VPE 105. The fungal isolate was obtained from wheat kernels in Sweden, Uppland region. Pathogenicity of the *Fusarium spp*. isolates used in this study was tested by placing sterilized wheat seeds on moist filter paper in a parafilm sealed Petri dish (9 cm) of diameter Hutzenlaub (2010). When the wheat seeds germinated, an agar plug of (5mm) diameter with the fungus was placed adjacent the germinating seed Hutzenlaub (2010). The Petri dish was covered with parafilm and incubated at room temperature (approx.22^{o} C) for 2 weeks. Thereafter, disease symptoms were assessed under a Stereomicroscope (10x) Hutzenlaub (2010). The fungal pure cultures of the inoculum were sourced from Department of Forest Mycology and Plant Pathology at the SLU Centre for Ecology in Uppsala (Sweden) for the purpose of study.

3.3 Culturing of aphids

Culturing of aphid (*Rhopalosiphum padi* L.) was done in the greenhouse set up under controlled conditions of temperature ranging between 19^{o}C to 28^{o}C, and relative humidity of between 32 % to 76 %. Small plastic pots measuring 8.3 cm long x 8.3 cm wide x 7.4 cm deep were filled with Hasselfors garden soil. Kara barley variety seeds were sown randomly in the soil. Ten small plastic pots were then placed in a crate measuring 40 cm long x 30 cm wide x 10 cm deep filled with sand. A cage mesh 60 cm high with a mesh size 0.5 mm x 0.5 mm was fixed on top of each crate to prevent insects from entering or escaping from the cage. The aphids used in both experiments were sourced from a previously prepared culture at the Sveriges Lantbruksuniversitet (SLU), Department of Ecology.

3.4 Raising of barley seedlings

To evaluate the influence of drought on interactions between aphids and their predators on barley sown during the spring season, twenty plastic crates measuring 60 cm long x 40 cm wide and 20 cm deep were used for sowing Kara barley variety seeds at a density of 450 seeds/M^2. Four rows 10 cm apart were made on each crate and thirty barley seeds sown to a depth of 4 cm to make a total number of 120 barley seedlings per cage using potting soil. A cage mesh 60 cm high with one side resalable and a mesh size of 0.5 mm x 0.5 mm was fixed on top of each cage to prevent insects from entering or exiting the cage. A total of twenty cages were used to obtain a substantial sample population for adequate data. To assess the influence of drought on interactions between aphids and *Fusarium* infection on barley sown during the spring season, sixty-four small plastic pots measuring 8.3 cm long x 8.3 cm wide x 7.4 cm deep were filled with sand for sowing barley seed. Three rows 1.5 cm apart were made on each pot and in each row, four Kara barley seeds were sown.

3.5 Experimental design

3.5.1 Evaluation of the influence of drought on interactions between aphids and their predators on barley

For this part of the study, a mesocosm experiment was conducted in a greenhouse to evaluate the influence of drought on interactions between aphids and their predators on barley. A factorial design with two factors namely, i) drought and no drought, ii) predator present and predator absent was used in this experiment. Introduction of adult aphids (*Rhopalosiphum. padi* L.) using eight Petri dishes each with ten male and female adult aphids was done on 8th day after sowing of Kara barley seeds. Two days after aphids (*R. padi*) introduction, two species of (*P. melanarius* and *H. rufipes*) and wolf spider (*Pardosa spp.*) predators were introduced directly by hand in one of these two crates selected in a random manner. Introduction of predators was done on the 11th day in cages with and without predators selected in a randomized manner. A set of two crates with barley seedlings were used in treatment one where there was no drought simulation. This set of experiment was replicated five times to obtain a total of ten crates. In the wet treatment, Kara barley seedlings were supplied with water after every two days after determining the soil field capacity. Monitoring was done from 8th day after aphid introduction and the experiment terminated on 21st day.

For the dry treatment, a set of two crates with barley seedlings were inoculated with adult aphids. Eight petri dishes were used in the experiment where each petri dish contained ten aphids. Two adult species of beetles (*P. melanarius* and *H. rufipes*) and wolf spider (*Pardosa spp*.) were inoculated in one of these two crates selected in a random manner. This set of the experiment was replicated five times to make a total of ten crates. Drought was simulated by watering only during sowing for the purpose of seed germination. Introduction of aphids to colonize host plant was done one-week from the day of barley seed sowing. Four small Petri-dishes each containing ten adult aphids (*R. padi*), were placed at equal distances on the space between the first and the last two rows of barley seedlings. This made a population of eighty aphids in each cage. After 24 hours, dead aphids were replaced to ensure that the original aphid population was maintained.

The aphid predators namely carabid beetles and wolf spiders were collected using pitfall traps method from the fields with cultivated cereal crops mainly barley or oat. The predators were introduced to the aphid colonized barley seedlings in randomized five cages for treatment one and two days after aphid introduction. In each of randomized five cages from both treatments, two species of beetles (*P. melanarius* and *H. rufipes*) and wolf spider (*Pardosa spp*.) were introduced directly by hand. Monitoring of the experiment was done from day four after the introduction of the predators and experiment terminated on the 21st day. Data on the number of aphids located at different parts of the plant (below ground, 0 cm to 1 cm above ground, ≥1cm above ground on stem, and on the leaves) was collected and recorded on 14th, 18th and 21st day. The number of aphid predators was monitored at noon every day for 11 days.

3.5.2 Assessment of the influence of drought on interactions between aphids and *Fusarium* infection

For this part of the study, a factorial design with three factors namely, i) drought and no drought, ii) Aphids and with no aphids, iii) *Fusarium* and with no *Fusarium* was used.

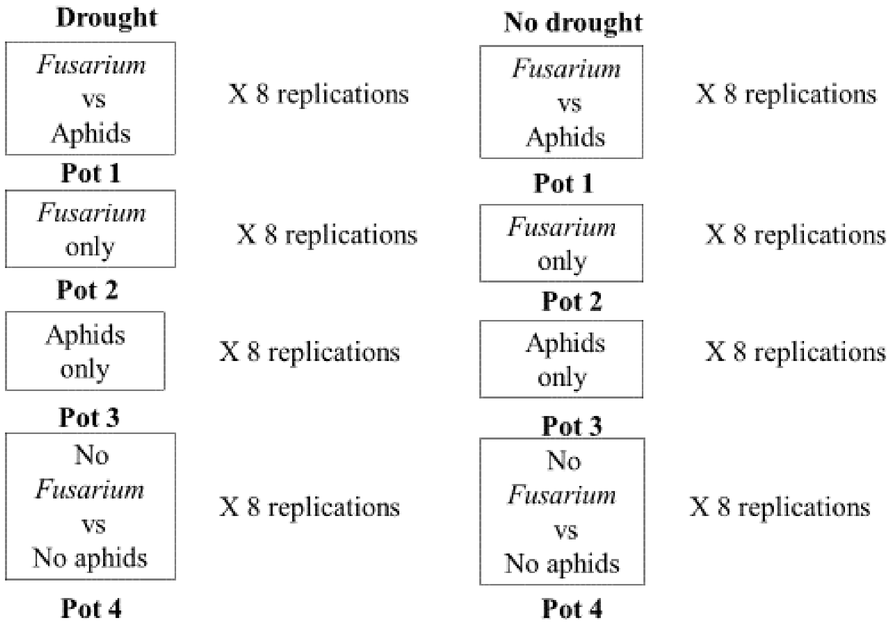

Figure 3.2 Experimental design for the assessment of aphid predisposed *Fusarium* infection on barley

Assessment of *Fusarium* infection on spring sown barley predisposed by bird cherry oat aphid (*Ropalosiphum padi* L.) was performed by setting greenhouse mesocosm experiments. The already prepared fresh culture of *Fusarium graminearum* isolate VPE 105 which was isolated from wheat kernels in Sweden, Uppland region were used in this study. *F. graminearum* is known to cause root and stem base rot disease in wheat and barley according to Hutzenlaub (2010). During *Fusarium* inoculation, an agar plug of (5mm) diameter was used to cut the fungus which was then placed between two barley seeds during sowing and covered with sand to a depth 1 cm. The experiment set up comprised of two treatments namely wet and dry treatments each containing thirty-two pots to make a total of sixty-four pots.

The barley seedlings in both treatments were watered after determination of field capacity of sand. Two adult aphids *(R. padi*) were inoculated directly onto each barley seedling using camel’s hair brush one week after sowing so as to assess the role of the aphids in facilitating *Fusarium* infections on barley. A net of mesh size 0.5 mm x 0.5 mm was fixed on top of each pot inoculated with aphids to prevent them from escaping the pots. The control set comprised of eight pots in both dry and wet treatments with neither *Fusarium* nor aphid inoculations. Data on number of aphids located at different plant parts (stem 0 cm to 1cm, above 1cm and leaves), was collected on fourth and eighth day after aphid inoculation. The set-up of each pot was replicated eight times for both treatments. On the eighth day, aphids were eliminated using camel's hair brush to avoid severe infestation on the host plant. The symptoms and severity of *Fusarium* disease was monitored four days after emergence of barley seedlings to the end of third week, after which disease scoring was done to collect the data on the *Fusarium* disease levels of the barley seedlings.

3.6 Data collection and analysis

3.6.1 Evaluation of the influence of drought on interactions between aphids and their predators on barley

To evaluate the influence of drought on interactions between aphids (*R. padi)* and their predators on barley, aphids were counted 14, 18 and 21 day**s** after introduction of aphid predators. On each count, sixteen barley seedlings per cage were uprooted carefully one at a time and placed on a tray. Aphids were counted below ground, 0 cm to 1 cm above ground, ≥1 cm above ground on the stem, and on the leaves. General linear mixed effects models were used to analyze effects of drought, presence of predators and interaction between drought and predators on the total number of aphids on plants, and the proportion of aphids on different plant parts. Block**s** nested within sampling day were included as random factors. Aphid numbers were log transformed and proportions on different plant parts arcsine-square root transformed prior to analyses. Statistical analyses were conducted using the lme function in the nlme package in R 3.4.2

3.6.2 Assessment of the influence of drought on interactions between aphids and *Fusarium* infection on barley

To assess the influence of drought on interactions between aphids and *Fusarium* infections on barley sown during the spring season, data on number of aphids located at different plant parts namely stem 0 cm to 1cm, above 1cm and leaves, was collected on fourth and eighth day after aphid inoculation. Data on disease scores on the barley inoculated with *Fusarium* was collected during termination of the experiment on 23rd day. Number of aphids were log transformed and proportions on different plant parts were arcsine-square root transformed prior to analyses. The reason for log transformation was to subject data to normality test to determine its distribution and analyze it using generalized linear mixed effects models with the lme function in the nlme package in R software (version 3.4.2). Data on *Fusarium* disease scores were analyzed using one-way ANOVA test.

CHAPTER FOUR

RESULTS

4.1 Influence of drought on predation aphids by ground beetles and spiders on barley

The study involved setting of mesocosm experiments to test the influence of drought on predation of aphids (*R. padi*) by ground dwelling carabid beetles and wolf spiders. Figure 4.1 below shows average number (±SE) per day of *R. padi* in the different mesocosm treatments where aphid numbers increased in dry conditions in absence of predators unlike when they were present. The effect of drought on the number of aphids depended on performance of predator present (t = -2.887, P = 0.06; Table 4.1) but the overall number of aphids on barley plant in dry conditions did not reduce in predator presence. Aphid numbers in fact increased in dry conditions overall. Thus, it seems the more stressed plants in dry conditions the more beneficial for aphids and that the predators couldn't compensate for this even if they were also more effective in dry conditions. Thus, the aphid populations were more driven by bottom up than top down forces.

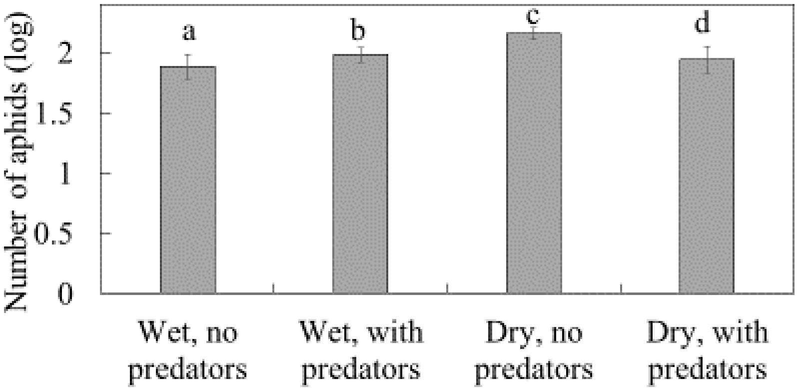

Figure 4.1 The total number of aphids on barley plant averaged across days in the different mesocosm treatments

Figure 4.2 below shows average proportion (±SE) per day of *R. padi* found below ground in the different mesocosm treatments. The results further revealed that effect of drought on proportions of aphids especially below ground was also dependent on the presence of predators (t = -4.327, P = 0.0001; Table 4.2). On the other hand, drought increased proportion of aphids below ground in absence of predators, but strongly reduced proportions of these aphids when predators were present. The reduction of proportions of aphids here was attributed by strong effect of drought on performance of aphid's ground dwelling beetles and wolf spiders.

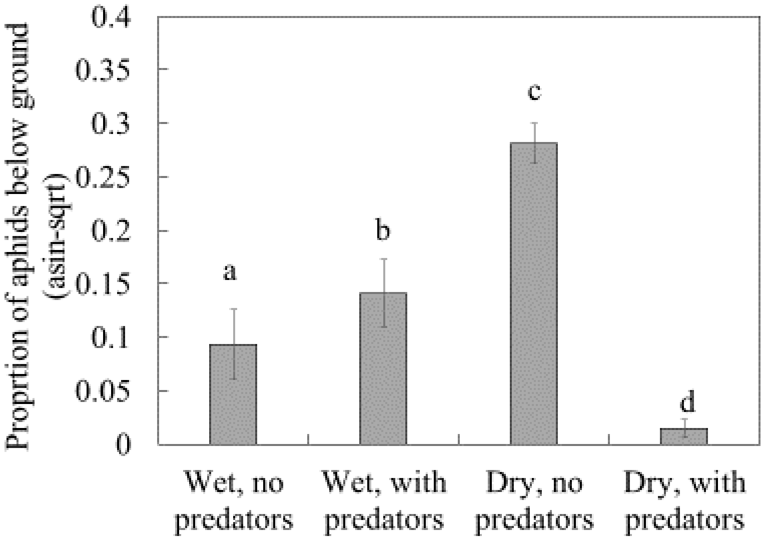

Figure 4. 2 The proportion of aphids on barley stem below ground averaged across days in the different mesocosm treatments

Figure 4.3 below shows average proportion (±SE) per day of *R. padi* found on the barley stems 0 cm to 1cm above the ground in the different mesocosm treatments. The results indicate that drought had no effect on proportion of aphids 0 to 1 cm above the ground (t = -1.2285, P = 0.206; Table 4.3) but predators significantly reduced proportion of aphids present there (t = -2.560, P = 0.0141; Table 4.3).

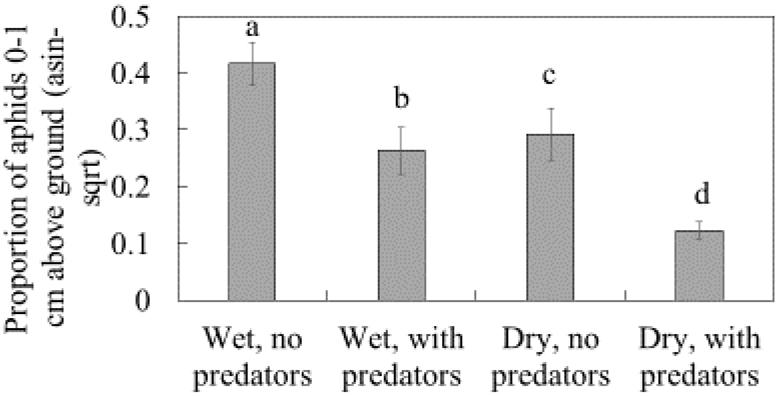

Figure 4. 3 The proportions of aphids on barley stem 0 to 1cm above ground averaged across days in the different mesocosm treatments

Figure 4.4 below shows average proportion (±SE) per day of *R. padi* found on the barley stems ≥ 1cm above the ground in the different mesocosm treatments. In this case, there was no effects of drought (t = -0.983, P = 0.331; Table 4.4) and predator presence (t = -1.05, P = 0.301; table 4.4) on the proportion aphids located on barley stems ≥ 1cm above the ground.

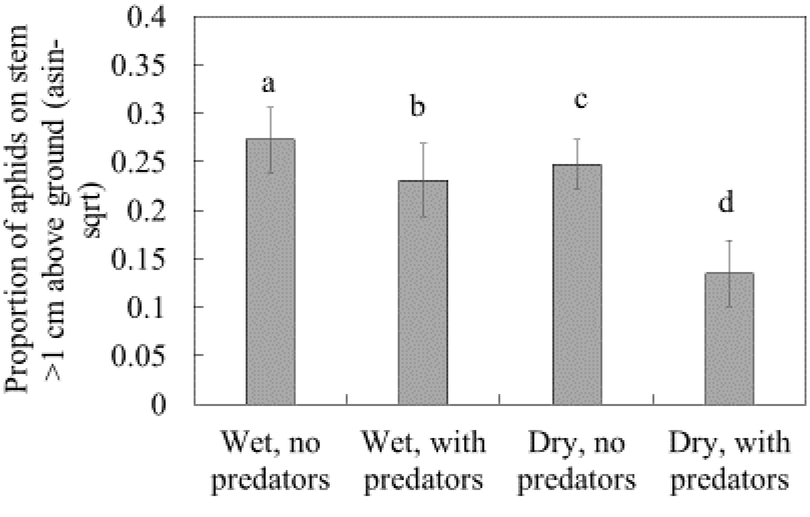

Figure 4. 4 The proportions of aphids on barley stem above 1cm averaged across days in the different mesocosm treatments

Figure 4.5 below shows average proportion (±SE) per day of *R. padi* found on the barley leaves in the different mesocosm treatments. On the barley leaves, it was observed that effect of drought depended on predator presence, since proportion of aphids increased in dry conditions with presence of predators (t = 2.459, P = 0.018; Table 4.5).

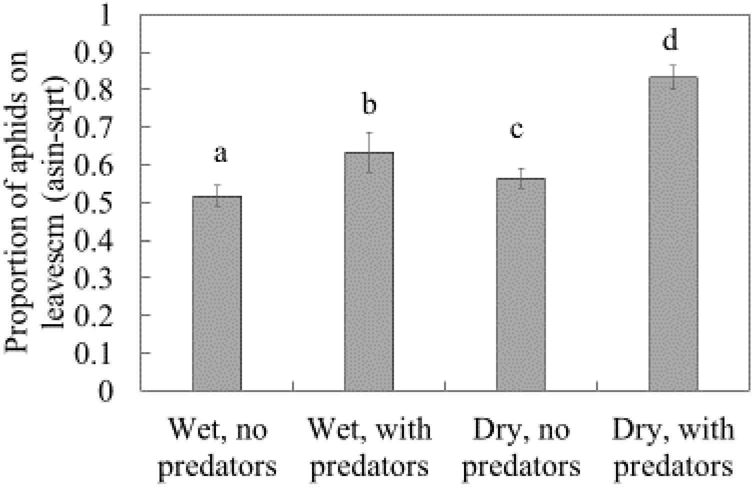

Figure 4. 5 The proportions of aphids on the leaves averaged across days in the different mesocosm treatments

Tables 4.1, 4.2, 4.3, 4.4 and 4.5 below show results from the aphid numbers which were log transformed and their proportions on different plant parts arcsine-square root transformed prior to analyses. The tables contain statistical criterion that indicate various components of analyzed data. These components include coefficient, standard error, degree of freedom, t values and p values. The t values indicate effects of drought as independent variable, predators and number of aphids as dependent variables while p values show the level of significance on the effects of drought on interactions between predators and aphids on different levels of barley plants.

Table 4.1 below shows the Log of total number of aphids averaged across days. The results indicate the effects of drought, presence of predators and interactions between drought and predators on the total number of aphids on barley plants. The results revealed that effect of drought on aphid numbers was determined by the presence of predators since there was a negative t value (-2.887) indicating a decrease in number of aphids on barley plants in dry conditions at 1 % level of significance. In addition, drought increased the number of aphids in absence of predators with a positive t value (3.444) and at 1 % level of significance.

Table 4.1 Log of total number of aphids averaged across days

Variables	**Coefficient**	**Standard error**	**Degree of freedom**	**t-value**	**p-value**
Intercept	1.8918	0.1729	42	10.9385	0.0000
Predator (Yes)	0.0875	0.0661	42	1.3237	0.1928
Drought (Yes)	0.2252	0.0654	42	3.4438	0.0013
Predator and drought (Yes)	-0.2625	0.09095	42	-2.8867	0.006

Table 4.2 below show the square root proportions of aphids below ground averaged across days. The results revealed that the effect of drought on proportion of aphids below ground depended on presence of predators. The p values indicate the level of significance of drought on interaction between predators and the proportion of aphids below ground. The results also revealed that drought significantly increased proportion of aphids below ground as indicated by a positive t value (3.11) at 1% level of significance. The increased proportion of aphids below ground were reduced by predators as indicated by a negative t value (-4.327) at 1% level of significance.

Table 4.2 The proportions of aphids below ground averaged across days

Variables	**Coefficient**	**Standard error**	**Degree of freedom**	**t-value**	**p-value**
Intercept	0.1117	0.0448	42	2.4928	0.0167
Predator (Yes)	0.0294	0.0438	42	0.6706	0.5062
Drought (Yes)	0.1330	0.0426	42	3.1195	0.0033
Predator and drought (Yes)	-0.2595	0.0599	42	-4.3268	0.0001

Table 4.3 below show the square root proportions of aphids on barley stem 0 cm to 1cm above ground averaged across days. The results show effect of drought on proportion of aphids on barley stem 0 cm to 1 cm above ground. The results revealed that there was no significant effect of drought on proportion of aphids 0 cm to 1 cm as indicate by p value of 0.206 but predators significantly reduced proportions of aphids present as indicated by a negative t value (0.141) at 1% level of significance.

Table 4.3 The proportions of aphids on barley stem 0 to 1cm above ground averaged across days

Variables	**Coefficient**	**Standard error**	**Degree of freedom**	**t-value**	**p-value**
Intercept	0.3891	0.0491	42	7.9245	0.0000
Predator (Yes)	-0.1258	0.0491	42	-2.5601	0.0141
Drought (Yes)	-0.0619	0.0482	42	-1.2285	0.2057
Predator and drought (Yes)	-0.0787	0.0674	42	-1.1671	0.2498

Table 4.4 below show the square root proportions of aphids on barley stem 1cm above ground averaged across days. The results show the effect of drought on proportion of aphids on barley stems 1 cm above ground. The results revealed that there was no significant effect of drought and presence of predators on proportion of aphids on barley stems 1 cm above ground as indicated by p values of 0.331 and 0.301 respectively.

Table 4.4 The proportions of aphids on barley stem 1cm above ground averaged across days

Variables	**Coefficient**	**Standard error**	**Degree of freedom**	**t-value**	**p-value**
Intercept	0.5514	0.0557	42	9.8954	0.0000
Predator (Yes)	-0.0763	0.0728	42	-1.0471	0.3011
Drought (Yes)	-0.0698	0.0709	42	-0.9837	0.3309
Predator and drought (Yes)	-0.0821	0.0998	42	-0.8232	0.4151

Table 4.5 below show the square root proportion of aphids on the barley leaves averaged across days. The results show the effect of drought on the proportion of aphids found on the barley leaves depended on presence of predators. The results indicate that drought significantly (P = 0.018) increased proportion of aphids on the barley leaves in dry conditions when predators were present as indicated by a positive t value (2.459) at 1% level of significance.

Table 4.5 The proportions of aphids on the barley leaves averaged across days

Variables	**Coefficient**	**Standard error**	**Degree of freedom**	**t-value**	**p-value**
Intercept	0.8053	0.0553	42	14.5727	0.0000
Predator (Yes)	0.1243	0.0622	42	1.9974	0.0523
Drought (Yes)	0.0407	0.0606	42	0.6715	0.5056
Predator and drought (Yes)	0.2095	0.0852	42	2.4597	0.0181

4.2 Influence of drought on interactions between aphids and *Fusarium* infection on barley

Figure 4.6 below show average proportion (±SE) per day of *R. padi* found on the whole barley plant in the different mesocosm treatments. *Fusarium* significantly (t = 3.6521, P = 0.0006; Table. 4.7); (t = 2.3349, P = 0.023; Table 4.8) reduced number of aphids both on the whole plant and 0 cm to 1 cm on the barley stems above the ground respectively.

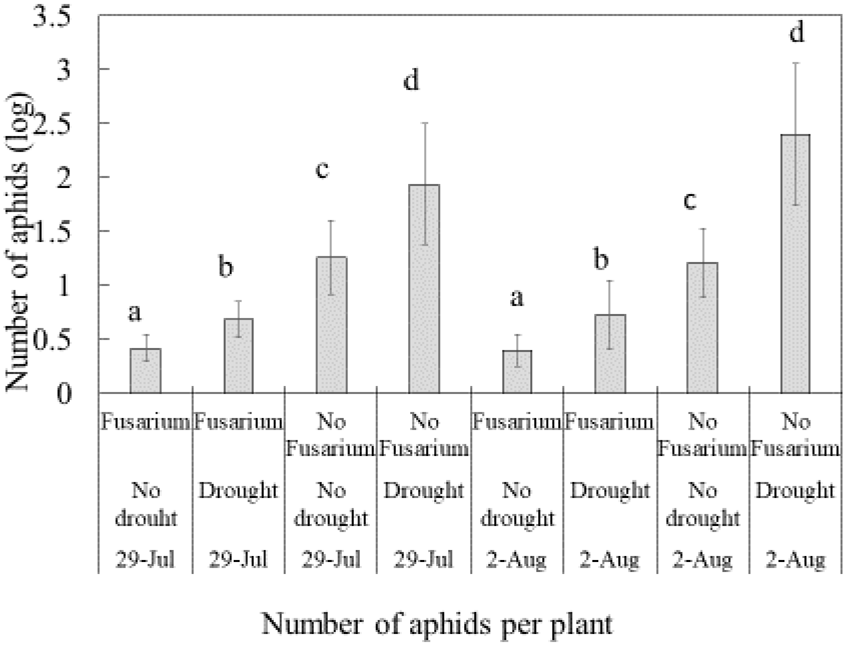

Figure 4. 6 Log total number of aphids per plant averaged across days

Figure 4.7 below shows mean disease index for various combination treatment. As seen in this figure, there was an effect of *Fusarium* inoculation (P = <0.0001; Table 4.6), but not any effect of drought or aphids on *Fusarium*. There was no significant effect of drought on disease, or interaction between drought and *Fusarium* (P = 0.579; Table. 4.6).

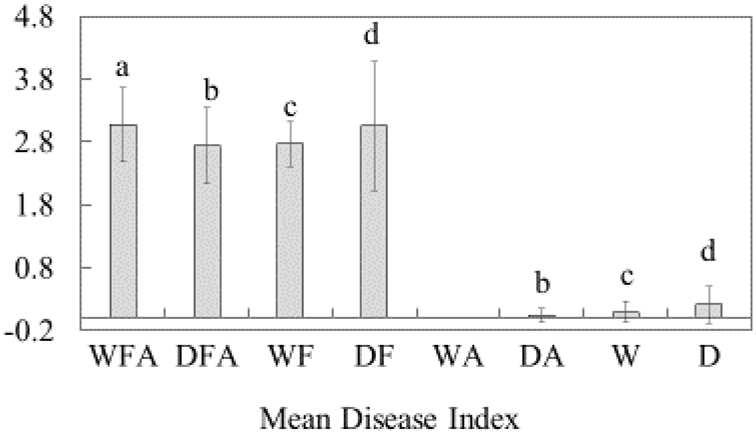

Figure 4. 7 Mean disease Index on X-axis for various combination treatments

Table 4.6 below show the analysis of variance on different treatments used to assess the influence of drought on interactions between aphids and *Fusarium* infections on barley plants. The results show that there was significant (P = 0.0001) effect of *Fusarium* inoculation on barley plants as indicated at 1% level of significance. However, there was no significant (P = 0.57) effect of aphids or drought on the *Fusarium* disease.

Table 4.6 ANOVA table showing different treatments

Variables	**Df**	**Sum Sq.**	**Mean Sq.**	**F value**	**P value**
Drought	1	0.012	0.012	0.415	0.522
Fusarium	1	15.118	15.118	531.740	<0.0001
Aphids	1	0.037	0.037	1.311	0.257
Drought: *Fusarium*	1	0.009	0.009	0.311	0.57

Table 4.7 below show the log of total number of aphids on the barley plants indicating effect of drought on interaction between aphid and *Fusarium* disease. The results indicate that *Fusarium* significantly (t = 3.6521; P = 0.0006) reduced number of aphids on the whole plant. The results further revealed that drought tend to increase number of aphids but this effect is not significant (t = -0.5892; P = 0.558).

Table 4. 7 Log of total number of aphids averaged across days

Variables	Coefficient	Standard error	Degree of freedom	t-value	p-value
Intercept	0.2040	0.0452	59	4.5156	0.0000
Drought No drought	-0.0689	0.0639	59	-1.0790	-1.0789
Fusarium No *Fusarium*	0.2333	0.0639	59	3.6521	0.0006
Fusarium No *Fusarium*: Drought No drought	0.0532	0.0901	59	-0.5892	0.5580

Table 4.8 below show the log of total number of aphids on the barley stems 0 cm to 1 cm above the ground indicating effect of drought on interaction between aphid and *Fusarium* disease. The results revealed that *Fusarium* significantly (t = 2.3349; P = 0.023) reduced number of aphids on the barley stems 0 cm to 1 cm above ground. The results further indicate that there was no significant interaction between drought and *Fusarium* (t = 0.2599; P = 0.7958).

Table 4. 8 Log of total number of aphids on the barley stems 0 to 1cm above the ground averaged across days

Variables	**Coefficient**	**Standard error**	**Degree of freedom**	**t-value**	**p-value**
Intercept	0.0061	0.0128	59	0.4777	0.6346
Fusarium Fusarium	0.0419	0.0179	59	2.3349	0.0230
Drought No drought	0.0005	0.0179	59	-0.0278	0.9779
Fusarium No *Fusarium*:					
Drought No drought	0.0066	0.0254	59	0.2599	0.7958

Table 4.9 below show the square root proportions of aphids on the barley stems 0 cm to 1cm above the ground. The results in this table revealed that there was no significant (t = 1.0155; P = 0.3144) effect of *Fusarium* on the proportion of aphids located 0 cm to 1cm on barley stems. The results also indicate that there was no significant (t = 0.972; P = 0.3354) effect on the interaction between *Fusarium* and drought on proportion of aphids here.

Table 4. 9 The proportions of aphids on the barley stems 0 to 1cm above the ground

Variables	Coefficient	Standard error	Degree of freedom	t-value	p-value
Intercept	0.0403	0.0699	54	0.5762	0.5669
*Fusarium*No *Fusarium*	0.0972	0.0957	54	1.0155	0.3144
Drought No drought	0.0261	0.1007	54	0.2594	0.7963
*Fusarium*No *Fusarium*:DroughtNo drought	0.1329	0.1368	54	0.9720	0.3354

Table 4.10 below show the square root proportions of aphids on the barley stems ≥1 cm above ground. The results indicate that there was no significant (t = -0.4653; P = 0.1498) effect of *Fusarium* on the proportion of aphids located on barley stems ≥ 1 cm above ground. In addition, interaction between *Fusarium* and drought there was no significant (t = 0.3932; P = 0.6957) effect on the proportion of aphids here.

Table 4. 10 The proportions of aphids on the barley stems ≥1 cm above ground

Variables	Coefficient	Standard error	Degree of freedom	t-value	p-value
Intercept	0.0307	0.0513	54	0.5999	0.5511
Fusarium No *Fusarium*	0.0869	0.0595	54	-0.4653	0.1498
Drought No drought	0.0291	0.0625	54	-0.4653	0.6436
Fusarium No *Fusarium*:DroughtNo drought	0.0333	0.0849	54	0.3932	0.6957

Table 4.11 below show the square root proportions of aphids on the leaves. The results in this table indicate that *Fusarium* significantly (t = -1.8214; P = 0.0741) reduced of proportion of aphids on barley leaves. There was no significant (t = -1.1307; P = 0.2632) effect of interaction between *Fusarium* and drought on proportion of aphids found on the leaves.

Table 4. 11 The proportions of aphids on the barley leaves

Variables	**Coefficient**	**Standard error**	**Degree of freedom**	**t-value**	**p-value**
Intercept	1.5198	0.0720	54	21.0857	0.0000
Fusarium No *Fusarium*	-0.1798	0.0987	54	-1.8214	0.0741
Drought No drought	-0.0154	0.1039	54	-0.1486	0.8824
Fusarium No *Fusarium*:DroughtNo drought	-0.1594	0.1410	54	-1.1307	0.2632

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

5.1.1 Evaluation of drought influence on predation of aphids by carabid beetles and wolf spiders on barley

Plant insect herbivores respond differently to drought intensity, whether severe or moderate, thus influencing rate of survival and fecundity hence affecting their abundance (Sconiers and Eubanks, 2017). In Sweden, barley is among the most important cereal crop after wheat grown in southern and eastern regions (Tidåker *et al*., 2016). This crop is mainly used as a source of livestock feed and also to some extent for manufacture of beverages (Tidåker *et al*., 2016). Barley crops in Sweden are colonized by aphids particularly (*R. padi*) during spring season (Jonsson *et al*., 2014). The major focus of this study was to unravel the influence of drought conditions on the interactions between these aphids and their ground dwelling natural enemies in order to understand how future drought is likely to impact on interactions between these aphids and their predators.

Results from the analysis of data on number and proportion of aphids located on different parts of the plant, revealed support for the hypothesis that drought increases top-down regulation of *R. padi* by ground dwelling predators. The results found that the treatment subjected to dry condition and with presence of predators had less number of aphids compared to the control (wet conditions and no predators). This hypothesis was supported by the fact that during dry conditions, i) aphids moved down below ground, ii) presence of predators reduced aphid numbers, and iii) predators also strongly reduced the proportion of aphids below ground. The increased predation effect that resulted during dry conditions was compensated for by an equivalent increase in number of aphids on the plants during dry conditions (as indicated in treatments without predators) and therefore the net effect of drought on aphid abundance ended up being neutral. Although predation performance rates were stronger in dry conditions, the ground dwelling predators also seem to perform better below than above ground under wet conditions. This probably is because predators encounter these aphids more below than above ground when they burrow into the soil.

A greater proportion of aphids on plant parts below ground was observed in the drought treatment of the present study. The possible credible explanation is that during drought seasons the seedlings are more nutritious and also they could be searching for protection. This observations concurs with a study that established that aphids tend to reside close to or below the ground surface during dry conditions and that drought can increase the movement of *R. padi* (Njue *et al*., 2021). Further, Wade *et al*., (2017) established that during drought conditions, the water stressed barley seedlings are more nutritious and have higher turgor pressure on their lower parts.

Presence of predators significantly reduced aphid numbers only during dry conditions, and the proportion of aphids found below the ground was reduced. These observations supported the hypothesis of the present study that predation rates on aphids below ground surface by ground dwelling predators was enhanced during dry conditions. The possible explanation for this is that, the effect seemed to have been largely driven by greater predation rates below but not on the ground surface during dry conditions. This could either be because predators were looking for protection against the drought, or because they were actively tracking down for aphids that had moved beyond the ground surface (Williams *et al*., 2014).

The above observation concurs with a study by Yihdego, (2017) on drought and pest management which established that drought conditions favors survival of pest attackers and thus significant reduction of the number of aphids in dry treatments. Further, other studies revealed that, drought, as predicted under climate change, is likely to alter the prey consumption in Sitka spruce plantations and that severe levels of drought stress, both continuous and intermittent, resulted in an increase in the consumption of prey (Banfield-Zanin, J. A., and Leather, S. R. (2016).

The current study revealed that even though there was increased predation rates in dry conditions, an overall decline in aphid numbers under dry conditions was not realized. This was probably because aphids survived better and/or increased their fecundity during dry conditions (aphid numbers increased in the drought treatment without predators). This concurs with a study by Ahmad *et al*. (2016) which have shown that fecundity of *R. padi* can increase in the event of reduced humidity, perhaps due to increase in plant transpiration rates. Further, recent studies have shown that the effects of water stress on phloem feeders depend on the strength of the drought event. If the plants are exposed to strong water stress, then aphid populations may be negatively affected, but if the level of water stress is moderate their performance may increase (Gutbrodt *et al*., 2011; Banfield-Zanin and Leather, 2015). In some cases, however, aphids may decline also during moderate water stress (Simpson *et al*., 2012).

The results of the current study revealed higher number of aphids in the simulated wet treatment. The possible credible explanation is that aphids in the wet treatment were disturbed during the watering process and hence this could have contributed to the lower aphid numbers in the wet treatment and also since watering was carried out carefully by hand, this is unlikely to have substantially affected the results. Another possibility is that, the drought simulated in the experiment only caused moderate water stress for the barley seedlings. Similarly, Simpson *et al*. (2012) found that aphids may also decline during moderate water stress. In addition, aphids are small, soft bodied insects that feed on plant phloem sap with their piercing and sucking mouthparts (Miller and Foottit, 2009). They pierce the plants using structures known as stylets in order to penetrate plant tissue suck the phloem sap containing nutrients such as sugars and amino acids (Moreno *et al*., 2011).

Apart from the generalist ground dwelling natural enemies of (*R. padi*) used in this experiment, they are also specialized leaf dwelling predators such as Coccinellid beetle (*Cheilomenes spp*.), spiders (Arachnidea) and lacewings (*Chrysopa spp*.) found at low densities and they consume these aphids from tillering to crop heading stage (Thomson *et al*., 2010; Boetzl *et al*., 2020). The use of these specialized aphid natural enemies in this study could probably present different results. The above arguments agree with the observation of the present study that the number of aphids increased in treatments where dry conditions were simulated. The spring season is experienced in the months of April and May where winter snow normally begins to melt due to gradual rise in air temperature (Larsen *et al*., 2007). Actually for the last 50 years, Northern Sweden has experienced an increase in the air temperature by 2.8^{o} C and 1.5^{o} C in winter and spring respectively (Larsen *et al*., 2007).

The results of the current study also revealed that there were significantly higher number of aphids in the presence than in absence of predators under wet conditions. The possible explanation to this scenario could be due to decline in temperatures due to wetness which might have affected the predatory behavior on these aphids. The predator community used in this study for example, *Pterostichus melanarius* is nocturnal while *Harplus rufipes* active during the day but burrow deep into the soil especially when it is cold. The remaining predator *Pardosa spp*. is likely to have predated on less significant number of aphids. Combination of these factors may have their influenced predatory behavior of these predators on aphids in wet conditions. Predators also seemed to perform better above ground than below ground under wet conditions. This could be probably because predators like *Pardosa spp*. (Wolf spiders) does on rare occasion climb on the plant although less significant number of aphids will be consumed subject to the number inoculated. There was an increase in the number of aphids on the leaves in the presence of predators which could be due to bottom up mechanism of these aphids.

5.1.2 Assessment of drought influence on interaction between aphids and *Fusarium* infection on barley

Results from the analysis of data on number and proportion of aphids located on different parts of the plant, and disease scores on *Fusarium* inoculated barley plants did not support the hypothesis of the present study that drought reduce aphid populations but enhance *Fusarium* infections. However, they revealed that *Fusarium* significantly reduced number of aphids on the whole plant and 0 cm to 1 cm above the ground. The possible explanation of this is that, the presence of *Fusarium* pathogen is likely to have induced changes to the barley plants that made them unpalatable to the aphids, thus affecting their survival or reproduction hence decline in population. Also, the barley plants infected with *Fusarium* might have produced chemical substances that influenced the feeding behavior of aphids. Further, infected barley seedlings are likely to have emitted volatile organic chemicals that affected survival and fecundity of the aphids as phloem feeders.

Similarly, Previous research has shown that *Sitobion avenae* aphid survival and fecundity reduce on wheat ears infected with *Fusarium graminearum* where both interacts (Drakulic *et al*., 2015). Plants infected with *F. graminearum* may emit different volatile organic chemicals (VOCs) compared to healthy ones which may alter the behavior of the arthropods either by attractive , repellant or neutral effects (Selitskaya *et al*., 2014; Drakulic *et al*., 2017). *Fusarium* pathogens also produce mycotoxins that are harmful to their consumers and therefore this might have led to decline in aphid population due to increased mortality rate. Mycotoxins like trichothecenes inhibit protein synthesis process by ribosomes and cause direct damage to intestines when ingested (Drakulic *et al*., 2017).

The volatile organic chemicals (VOCs) emitted by the infected barley seedlings might have made the plant environment inhospitable to aphids hence their migration to the floor of planting media (sand). Plant changes due to the disease and secondary metabolites from the pathogen can exert an influence on the aphids, leading to altered preferences and performances such as reproduction, population and survival rates (Zutter *et al*., 2017). The pathogen attacks the roots which may interfere with the uptake of water and nutrients by the xylem and phloem tissues respectively. This in turn might have affected the quality and quantity of phloem tissue contents and accumulation of the mycotoxins like deoxynivalenol in plants, which can influence both reproductive and mortality rates of the aphids for the phloem feeders such like aphids leading to their decline in population. A study by Drakulic *et al*. (2015) shows a greater mortality rate and lower reproductive rate of *S. avenae* aphids on the wheat ears infected with *F. graminearum* due to high concentrations of deoxynivalenol mycotoxin.

Recent studies have shown that presence of disease causing organisms in plant growing in medium like soil can impact the aboveground insect herbivores indirectly through plant- mediated mechanisms or directly through pathogenic or mutualistic interactions (Pineda *et al*., 2017). An increase in the population of the specialist foliar-feeding aphid (*Aphis jacobaea*) was observed due to the interactions between microbial communities which includes plant pathogens inhabiting the soil used by its host plant ragwort (*Senecio jacobaea*) (Pineda *et al*., 2017). Previous studies have shown that a decline in quality of plants nutrients can provide defense mechanism against herbivorous pests, reduce their fecundity and increase developmental time (Rastegari *et.al*., 2010).

The study also found an effect of *Fusarium* inoculation on barley plants, but not any effects of aphids or drought on the disease. However, this did not support the hypothesis that drought will facilitate *Fusarium* infections since aphids will feed on the plant parts close to the susceptible plant base. This might have been due to low aphid numbers inoculated per plant hence failure to detect any potential behavioral differences in their effects on the disease. It is also possible that susceptibility of the barley variety (Kara) used for *Fusarium* infections played a role to this observation. These finding concurs with a study by Bedawy *et al*. (2018) which established that Fusarium being a devastated fungus causes significant losses yield and quality losses in cereals.

The aphid mode of feeding may create some injury to the plant tissue that can act as an entry route for the *F. graminearum* fungus. Aphids also transmit other pathogens like viruses in cereal crops like barley through feeding on infected plant tissues and transferring these pathogens to uninfected plant tissues as they feed on them (Wang *et al*., 2015). Recent studies have shown aphids (*R. padi*) are responsible for transmission of barley yellow dwarf virus in barley crops through complex interactions between proteins that are in the virus and certain compounds associated with these aphids (Wang *et al*., 2015). However, the level of plant tissue injury may also depend on the level of aphid abundance. Studies have found that forage legumes inoculated with *Fusarium roseum* and subjected to a relatively high abundance of pea aphid (*Acyrthosiphon pisum*) feeding had a severe root rot disease possibly due to wounds created by aphids (Valenzuela and Hoffmann, 2015). Analysis of proportion of aphids on different plant parts did not reveal any potential behavioral difference on their role to facilitate transmission of *F. graminearum* probably due to very low aphid numbers and mostly located on the leaves. There are other foliar fungal infections such as leaf rust, powdery mildew and Septoria leaf blotch that infects cereal crops like wheat (EL Jarroudi *et al*., 2015).

Climatic factors such as temperature, rainfall and to some extent air, influences survival, dispersal, penetration, development and reproduction rate of these pathogens (Katelaris, 2016). Increased moisture and temperature encourages and triggers disease development together with germination and multiplication of fungal spores of different foliar fungal infections (Katelaris, 2016). The results of the present study indicate an increased disease development in wet treatment inoculated with *Fusarium* than in the dry treatments suggesting that presence of moisture might have enhanced development of this pathogen in planting media (sand) hence increased disease level. Also, *fusarium grameanarium*, which is prevalent in South Europe has been found to spread towards cooler parts of Central Europe: Finland and Sweden (Ferrigo *et al*., 2016).

Similarly, a study by Parikka, and Tiilikkala, (2012) found that rainfall, warm and moist conditions favours production of *Fusarium* species production hence disease development on the cereal crops. Barley (*Hordeum vulgare*) is among the cereal crops that are infected with *Fusarium* foot and root rot *F. graminearum* although the level of susceptibility to the disease infection may differ with variety.

5.2 CONCLUSIONS

5.2.1 Evaluation of drought influence on predation of aphids by carabid beetles and wolf spiders on barley

From the results of this study, it can be concluded that with increased frequency of drought conditions being experienced in future due to climate change, crop pests and interactions with their natural enemies are likely to present differently. This is evidenced by the fact that the results of this part of the study revealed that drought induces behavioral changes in aphids thus increasing their niche overlap with ground–dwelling predators on barley plants. It also indicates that as plants experience water stress due to drought conditions crop pests such as aphids tends to move down the plant and even beyond ground surface, although there is no reason to support this occurrence. It is likely that under these circumstances aphids tends to seek for more sheltered locations against drought. As intensity of drought conditions vary with time, there is need to develop mechanisms of mitigating the problems brought about by plant herbivores such as insects particularly aphids whose populations seems to present differently on their host plants.

The current study reveals that the simulated dry conditions increased aphid populations on barley plants hence depending on the extent of water stress plants will be exposed to these insects such as aphids are likely to behave differently. It is therefore important to develop crop management practices that can enhance proper growth, increased quantity and quality improvement of crop products. However, this will require knowledge and understanding how pest problems manifest due variations in the intensity of drought as it is likely to keep on being experienced due effects of climate change.

The plant water stress due to moderate drought simulated in this experiment could be an indication of how future drought conditions will lead to complex interactions between crop pest and their predators. However, complex effects of drought on pests may not only depend on the characteristics of the pest and host plant, intensity of water stress, and but also on interactions between pests and natural enemies. This experiment therefore illustrated how complex effects of drought on pests can be since it depends on the characteristics of the pest and host plant, strength of water stress, and on interactions between pests and natural enemies. This complexity needs to be put into account in order to establish more realistic predictions of how increased frequency of drought will affect pest dynamics in the future.

5.2.2 Assessment of drought influence on interaction between aphids and *Fusarium* infection on barley

Interactions between aphids and *Fusarium* pathogen on barley crops revealed significant results where number of aphids on the whole plant and 0 cm to 1 cm above the ground declined. In conclusion, plant nutrient quantity and quality are important for the development, survival and reproduction of plant phloem feeders. It can further be concluded that the aphid development, survival and fecundity were affected as barley plants was infected by *Fusarium* pathogen. The findings of the study further revealed an effect of *Fusarium* inoculation on the barley plants both dry and wet treatments The current study revealed that drought does not significantly influence *Fusarium* infection on barley hence this disease can manifest itself both in moist and dry soils. The determining factor on the disease infection therefore is whether the inoculum is present or not in the growing medium particularly the soil as a result of crop residues from the previous crop which had been infected. Another source of the inoculum for the *Fusarium* disease is an infected planting material which in this case is the seed. The management of this disease therefore would be to ensure that both the planting material and the growing medium are free from the inoculum. Aphids as phloem feeders can cause injuries as they feed on various plant parts. Pathogens like *Fusarium spp*. that cause plant diseases such as *Fusarium* foot and root rot can enter into the plant tissues through these injuries. The level of disease spread to the plants may probably depend on the abundance of these aphids on the plant body. The findings of this study revealed that there was no significant effect between aphids and *Fusarium*. However, there could be low aphid abundances hence no potential behavioral difference was detected in relation to the role of aphids in facilitating transmission of *Fusarium* pathogen as they feed on plant base. Increasing the aphid abundance may thus give more realistic predictions on whether aphids play a role in transmission of *Fusarium* pathogen as they feed on the lower parts of barley plants.

5.3 RECOMMENDATIONS

The study recommends a need to consider complexity of drought on interactions among pests, host plants and natural enemies in order to predict how increased frequency of drought will affect pest dynamics in future.

Based on the findings from this study the following is recommended for future studies;

- Investigation of how increased frequency of drought will affect aphids’ dynamics in barley.
- Determination of the impact of drought intensity on more specialized aphid predators and parasitoids that arrive when the aphid populations continue to develop.
- A field experiment could be conducted to determine effect of predators on *R. padi* since different species of predator community used in this study are present.

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