Understanding the interactions between the dune aphid *Schizaphis rufula* and parasitoid wasps:

Top-down control, aphid resistance and parasitoid recognition ability

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Introduction

In nature, no organism exists in absolute isolation. The interactions of an organism with its environment are fundamental to the survival of that individual and the functioning of the ecosystem as a whole (Wikipedia contributors, 2016a). The loss of one keystone species and its interactions can disrupt the functioning of the ecosystem dramatically (Sole and Montoya, 2001). In recent decades, nature has experienced a tremendous loss of biodiversity due to the high level of fragmentation and disappearance (Shah, 2014). These phenomena already demonstrated their negative impact on a lot of ecosystems (Hooper et al., 2012). They become less resilient with decreasing biodiversity and increasing fragmentation (Sole and Montoya, 2001, Marcantonio et al., 2014). Humanity is the main reason for the loss of biodiversity, fragmentation and with it the loss of interactions between species (Malavasi et al., 2014, Provoost et al., 2011). Because of the great importance of interactions for an ecosystem, understanding these is essential for the conservation of the remaining ecosystems.

An interspecific interaction between organisms can be subdivided into predation, competition and symbiosis. The latter is defined as a close and often long-term interaction between two different biological species. Symbiosis can be subdivided by its effect on both organisms. A symbiotic interaction can be beneficial (mutualism), beneficial and neutral (commensalism) or beneficial and harmful (parasitism) for the species (Wikipedia contributors, 2016d, Wikipedia contributors, 2016a). Furthermore, the effect of symbiosis may encompass more than two species via top-down and bottom-up forces in a community (Dyer and Letourneau, 1999).

Yellow dune system

The ecosystem that this thesis will focus on is the yellow dune system. Bottom-up control has already been studied in this ecosystem (De La Peña et al., 2006, Van der Stoel et al., 2002). De la Peña et al. did a study to explore the interactions between the pioneer dune grass Ammophila arenaria, arbuscular mycorrhizal fungi (AMF) and the root-feeding nematode Pratylenchus penetrans. Their objectives were to determine whether AMF can suppress nematode infection and reproduction and to explore the mechanisms of nematode control by AMF (De La Peña et al., 2006). The role of bottom-up control in yellow dune habitats has already been well described, but the role of top-down control remains a question.

People have drastically altered coastal dune landscapes through centuries of exploitation and more recently modification of the coast. This constant and substantial pressure leads to the disappearance and fragmentation of our dune landscape (Stancheva et al., 2011, Malavasi et al., 2014, Provoost et al., 2011). Dune landscapes can be classified into more dune habitats/types according to the vegetation, succession or fixation rate of the sand (Bertels et al., 2005). The main abiotic factor that distinguishes it from most other habitats is the great importance of wind dynamic for the appearance and functioning of the habitat. It determines whether or not there is soil development. This development is largely responsible for the way in which plant succession in a dune landscape occurs. This creates a self-regenerating landscape, which contains a mosaic of several plant successional stages, each successional stage representing a so-called dune habitat (Bonte and Provoost, 2005). The habitat this study will mainly focus on is yellow dune habitat, also called mobile, shifting or dynamic dune habitat, defined as large masses of sand that can still be relocated by wind and are not fixated entirely by Ammophila arenaria (L.) link) (Marram grass) or Leymus arenarius (Romão, 1996). Due to the relocation of the sand, soil development is absent. Because of that, only a few plant species can occur at low densities in shifting yellow dunes. Leymus arenarius is more dominant in
regions north of latitude 63°N, where A. arenaria is less dominant. L. arenarius can also establish in embryonic dunes, which are mainly dominated by Elymus farctus (Heukels et al., 2005). Embryonic dunes are situated on coastlines and are the pioneers in dune formation.

Another serious problem for dynamic yellow dunes today is the tendency of increasing fixation and succession on coastal dunes throughout Northwest Europe (Provoost et al., 2011). Due to the fixation of sand and formation of more nutrient-rich soil, other grass species like Carex arenaria and Festuca rubra can ascend and a more species-rich habitat arise. The habitat is called dune scrub (Romão, 1996). Also a more diverse soil biota can occur in fixed dunes. The decreasing densities of A. arenaria from dynamic to fixated dunes are influenced by the increasing harmful soil biota, like root-feeding herbivores (De La Peña et al., 2006, Van der Stoel et al., 2002). Due to the species-rich conditions, more organic matter accumulates and more competitors, like Calamagrostis epigejos, establish (Bertels et al., 2005).

Because of the endangered status of this habitat, institutions like Natuur en Bos (ANB) and Natuurpunt are committed to protecting and restoring residual relicts (Agentschap Natuur en Bos, 2016). In Belgium the coast possesses only a few noteworthy relicts (Westhoek, Ter Yde, het Zwin). More about the habitat status can be told by understanding the species interactions in this ecosystem. It can be the key to preserving and restoring this vulnerable habitat successfully. Yellow dune organisms can be used as indicators for conservation (Espejel et al., 2008). Ecological indicators can be used to assess the condition of the environment, to provide an early warning signal of changes in the environment or to diagnose the cause of an environmental problem (Dale and Beyeler, 2001).

**Aphids in dune systems**

Although shifting yellow dunes show low plant diversity, they harbour a broad range of insect species (Bonte et al., 2001). They contain also a few aphid species. The species Schizaphis rubula (Walker 1849), Laingia psammatae (Theobald 1922) and Metopolophium sabiae (Prior 1976) have already been reported on A. arenaria (Vandegehuchte et al., 2010). Schizaphis rubula is mostly present on the leaves, while Laingia psammatae can be found living between the flowers or fruits. This study will focus on the aphid species S. rubula.
The life cycle of aphids (figure 2A) is most of the year asexual (summer cycle). Sometimes sexual reproduction occurs, with the production of overwintering eggs (winter cycle). Aphids represent a very important group of plant pests with high reproductive potential through parthenogenesis and viviparity (Agarwala and Das, 2012). They are causing direct (sucking) and indirect (transmission of viruses) damage on cultivated and wild-growing plants (Dedryver et al., 2010). In an experimental study of Van Moorleghem C., the grazing effects of S. rufula on A. arenaria were tested. In the presence of S. rufula, there was a significant decrease in dry weight of A. arenaria compared with the control experiments. In field observations, these results had not been seen yet. Several additional factors, which control the harmful densities of S. rufula in the field, were not taken into account in the experimental study (Van Moorleghem, 2014). Examples of additional factors are predation rate, physical stress by windblown sand, the impact of underground fauna on aboveground herbivory and parasitism.

**Parasitism in dune systems**

The role of bottom-up control in yellow dune habitats has already been described well, but the role of top-down control remains a question. That is why this thesis will focus on the interaction between an aphid species and parasitoid wasps in a yellow dune system, a typical example of top-down control.

In other systems there are a lot of predators or parasites that influence the top-down control of aphids. (i) ground-dwelling generalist predators; mostly spiders, carabid and staphylinid beetles; (ii) flying predators, like coccinellid beetles and syrphid flies and (iii) parasitoid wasps. According to Schmidt et al., parasitoid wasps are the most important group in controlling aphid densities (Schmidt et al., 2003). In dune systems, there have not been many studies addressing top-down control. There is very scarce information on the groups controlling aphids in coastal dunes. Because this thesis will only tackle one of the groups and since Smith et al. reported that parasitoids are important in other systems, the study will focus on parasitoid wasps.

Parasitoid wasps live a part of their lives at the expense of other organisms and nearly always kill their host. This is in contrast with true parasites, which keep their host alive (Wikipedia contributors, 2016b). Parasitoid wasps can be subdivided into two groups according to their lifestyles, namely koinobionts and idiobionts (Wikipedia contributors, 2016b). The eggs are transported with an ovipositor inside the aphid. The larvae do not affect the development of their host, until they reach an adult stage. The wasp larva then attaches the body of the aphid to the leaf and spins a cocoon within the aphid, in which it pupates. The dead aphid takes on a golden brown, papery, swollen appearance, often called a mummy (Williams et al., 2011). When the larvae have developed, they emerge. (Figure 2B)

Previous studies on the ecology of parasitoid wasps of cereal aphids on winter wheat approved that parasitoid wasps were likely to provide the most important contribution towards top-down control of cereal aphid densities in the treatments in which they occurred at natural densities (Schmidt et al., 2003). The role of parasitoid wasps in the top-down control of S. rufula in dynamic dunes is an unexplored field and opens perspectives to perform top-down experiments.

Due to the highly fragmented situation of the current yellow dune habitats, the question arises if there are differences in resistance against parasitoids in different aphid populations along the coast. The large distances between suitable areas can limit the gene flow between populations. Because of
that, evolution can increase the differences in resistance against parasitoid wasps between the aphid populations. For example, de la Peña et al. found a lot of variation in endosymbiotic communities in *S. rufula* according to species and location (de la Peña et al., 2014). The high variation in endosymbiotic communities might point out the low level of gene flow between the aphid populations. By knowing the status of resistance of aphid populations, more about the well-being of the population can be told and new management measures can be implemented.

The interaction between *S. rufula* and his parasitoid wasps has been partly disentangled, but the process between hatching and actually parasitizing *S. rufula* is an unexplored field. Especially the recognition ability of the parasitoid wasps to find the *S. rufula* populations is a main issue in this thesis. Is the encounter between an aphid and a parasitoid wasp pure chance or a reward for active searching?

A lot of research on this topic has been done in agricultural systems. Volatiles play an important role in the recognition. VOC (volatile organic compounds) is one of the indirect pathways, which plants use to attract parasitoids or predators to eliminate the herbivory. In tomato plants and other dicot crops, the pathways and the role of volatiles in attracting parasitoid wasps have already been defined (Kessler and Baldwin, 2001, Thaler, 1999). The most important volatile in attracting parasitoids are jasmonates (jasmonate acid + biological active derivatives), one of the final products in the octadecanoid pathway (Devoto and Turner, 2005). In monocots, especially maize and rice, the role of volatile terpenes has been studied in detail (Degenhardt, 2009). In natural systems, certainly yellow dune habitats, the ability of parasitoids to actively find aphid populations has not been studied yet.

![Figure 2: A: general life cycle aphids; B: general life cycle parasitoid wasps](image)

Figure 2: A: general life cycle aphids; B: general life cycle parasitoid wasps
Objectives
The aim of this master thesis is to understand the ecological interactions between the aphid S. rufula, his parasitoid wasps and other potential influencing links. By knowing the interactions, the goal of understanding the whole ecosystem of the shifting yellow dune habitat, comes closer. New insights in the ecosystem help to manage the few relics in a proper manner. Of course this extensive subject needs to be tackled in some specific and purposeful objectives.

The first objective of this master thesis is to study the top-down control of S. rufula populations by parasitoid wasps. Parasitoid wasps influence aphid populations by parasitizing individuals. Still, the question remains whether the interaction is strong enough to have a significant impact on aphid populations and to indirectly decrease the damage to the A. arenaria plants. By performing a top-down experiment, we want to answer this question.

The second objective tackles the differences in resistance of S. rufula populations against parasitoid wasps. The high fragmentation of the study areas triggered our attention on the fact that differences in resistance between populations can occur. This assumption has not been tested yet. By doing a resistance-experiment (top-down experiment with different populations) the study answers the question. Studying the underlying reasons, causing the differences in resistance, fall beyond this thesis.

The last focus of this thesis is the recognition ability of parasitoid wasps to detect S. rufula populations on yellow dune grasses. By performing simple choice experiments, the presence of recognition abilities can be observed. In total five choice experiments were done to get an answer as full as possible. In the first choice experiment, the parasitoid wasps had to choose between plants with and without aphids. During the experiments another more specific question arose. Are parasitoid wasps able to recognise different densities? The second choice experiment answered this question. We know that S. rufula lives on different host grasses in nature, but are there specific grass species whose recognition by parasitoids is limited? By opposing two different (aphid-present) grass species, an evolutionary advantage can be shown to live on a particular grass species. The possible effect of recognition ability can be caused by the plants, the aphids or a combination. First a pure aphid choice experiment was performed without plants. The parasitoids had to choose between aphids or no aphids. The second choice experiment was between treated and untreated plants. There were no aphids present on the plants.
Materials & methods

Study sites
In the past centuries, shifting yellow dunes explored a tremendous decline and fragmentation. Therefore, finding suitable yellow dune relicts is not a matter-of-course. Eventually, five suitable study sites were selected where sand is still sufficiently mobile to maintain the specific character of the dune landscape. The Belgian study sites are De Westhoek and Ter Yde, both situated closely to the French border. De Westhoek in De Panne, an area of 340 hectares, contains the largest remaining yellow dunes of Belgium and is the oldest nature reserve in Flanders (Provoost et al., 2011). The other nature reserve that still contains mobile sand dunes is Ter Yde in Oostduinkerke (Bonte and Provoost, 2005). Both nature reserves are managed by Natuur en Bos (ANB) (Natuur en Bos, 2016a, Natuur en Bos, 2016c). In the Netherlands, we selected three study sites. Retranchement, a part of the nature reserve Het Zwin, is situated against the Belgian border. Het Zwin is also managed by ANB (Natuur en Bos, 2016b). The penultimate site is De Verdrongen Zwarte polder in Nieuwvliet, managed by Het Zeeuwse Landschap (Wikipedia contributors, 2014, Landschap, 2016a, Landschap, 2016b). This area is called a tidal creek, a habitat situated after a trench in the dunes and overflowed with springtide (Wikipedia contributors, 2016c). The last study site is situated closely to the coastline, namely the beach of Breskens. The wide beach of Breskens starts to form some immature dunes where A. arenaria and L. arenarius dominate and wind dynamics play an important role.

Study species

1. Schizaphis rufula (Walter, 1849) (figure 4A)
The aphid species that is used in the study was already mentioned above, S. rufula. In 2007, S. rufula (Walker, 1849), was discovered for the first time in Belgium (Vandegehuchte et al., 2010). In Northwest Europe, Schizaphis rufula is an aphid species, which is highly abundant in coastal sand dune landscapes (Nieto Nafria, 2013). In these habitats it is usually found on young tussocks of sand stabilizing dune grasses like Ammophila arenaria and Leymus arenarius (Vandegehuchte et al., 2010). S. rufula is relatively abundant in coastal dunes and the distribution range is very wide in Northwest Europe.

Taxonomic classification (Norenburg, 2016a)
Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Tribe: Hemiptera
Superfamily: Aphidoidea
Family: Aphididae
Genus: Schizaphis
Species: Schizaphis rufula

2. Aphidius ervi (Haliday, 1834) (figure 4B)
In the study of Van Moorleghem et al. mummies of S. rufula formed by parasitoids were collected and identified. They found two parasitoids of the genus Aphidius, namely A. rhopalosi phi and A. avenae (Van Moorleghem, 2014). The aim of this study was to collect mummies and to try to cultivate the parasitoid wasps. Due to the low densities of aphids in spring and the absence of mummies, cultivation was not possible. At the end of August and September the densities of aphids
in the field were much higher, but mummies were still scarce. Low numbers of mummies and the lateness of the density peak of the aphids have made it necessary to turn to A. ervi. This parasitoid wasp species is available on the web and is used as a biocontrol agent in a lot of crop systems. The high attractiveness to green aphids, the cosmopolitan nature of the aphid and its belonging to the same genus, makes from A. ervi an appropriate surrogate (Biological control news, 2016). Basic tests in the lab proved that A. ervi parasitizes on S. rufula. Also mummies were formed.

**Taxonomic classification** (Norenburg, 2016)

*Kingdom:* Animalia  
*Phylum:* Arthropoda  
*Class:* Insecta  
*Tribe:* Hymenoptera  
*Superfamily:* Ichneumonoidea  
*Family:* Braconidae  
*Genus:* Aphidius  
*Species:* *Aphidius ervi*

**Experiments**

Six dune grasses were selected. *A. arenaria, E. farctus* and *L. arenarius* are known host grasses of *S. rufula* (Vandegehuchte et al., 2010) and *C. arenaria, F. arenaria* and *C. epigejos* were collected as backup. In October, when the ear of the dune grasses is mature, the seeds were collected. The seeds of *A. arenaria, E. farctus, C. arenaria, F. arenaria* and *C. epigejos* were collected in De Westhoek and *L. arenarius* was collected in Het Zwin. By spreading the ears between newspapers, the seeds were dry after one week. Seeds were surface sterilized. By first washing the seeds in 30 % EtOH for two minutes, then in a 10 % bleach solution for five minutes and finally with a large amount of sterile dH2O. After this treatment, seeds were kept overnight in a sufficient amount of distilled water to soak off any remaining bleach residues before being plated onto petri dishes containing a 1% agar growth medium. These petri dishes were then put under lights, subjecting them to an 8h night/16h day rhythm. When seeds germinated, seedlings were planted in polypropylene cups containing sand collected from nature reserve Westhoek, De Panne (Van Moorleghem, 2014)(figure 4C, D & E). The plants were kept under 8h night/16h day light rhythm and 24 degrees Celsius. The germination rate of *A. arenaria* was low. Therefore seeds of 2003 were also used to ensure enough germination. After one month the plants were big enough to maintain aphid populations and perform experiments. A. arenaria was used to maintain the aphid populations.

In September, we collected *S. rufula* populations in the five study sites. Zip bags were filled with sand and young tussocks of *A. arenaria* on site to create an in sito habitat. Leaves with aphids were cut off and placed in the bags. The aphids moved from the severed leaves to the young healthy tussocks. The bags were kept under ambient temperature and prevailing night/day rhythm in the month of October. Every week new young tussocks from *A. arenaria* were added to the bags to support the aphid populations. The young tussocks were checked on being clean of aphids to avoid contamination.

In the month of November the cultivated plants were suitable to maintain aphid populations. The bags were replaced by growthtubes (figure 4F). The populations were subjected to an 8h
night/16h day light rhythm. The A. arenaria plants were refreshed weekly. To avoid contamination, the tubes were taped off.

**Choice experiments**
In total, five choice experiments are performed. The setup of the experiment is built up by two transparent boxes connected with a transparent tube. The boxes are 21cm wide and 25cm high. The tube between the two boxes is 8cm long. The upper side of the box contains a removable cover. The middle of the tube is provided with an opening to deposit the parasitoids. To eliminate variation in light, the boxes are provided with two lamps and cardboard boxes shielding incoming sunlight (figure 4G).

The mummies of the parasitoids, delivered in a bottle of 100ml that contains 250 mummies grown on buckwheat, were ordered two weeks in advance by Biobest. In a plastic box mummies were spread out to start the hatching process and kept under ambient temperature. After two days most of the mummies were hatched. Within the plastic box a mixture of sugar and water kept the parasitoids longer alive.

1) aphid/no aphid choice experiment
In total twenty A. arenaria plants were selected to use in this experiment. The length of each plant was measured. Half of the plants were treated by placing one adult aphid from the Westhoek population on each plant. After two days the treated plants contained aphid densities between ten to thirty individuals.

2) High/low density choice experiment
In total twenty A. arenaria plants were selected for use in this experiment. The length of each plant was measured. All plants were treated by placing one adult of the Westhoek population on each plant. After two days some plants contained high (+20) and other low densities (+5).

3) Grass species 1/grass species 2 choice experiment
In total four grass species were selected: A. arenaria, L. arenarius, C. epigejos and E. farctus. Ten plants of each species were treated by placing one adult aphid of the Westhoek on each plant. After two days A. arenaria and C. epigejos contained a workable number of aphids. The other species turned out to be no suitable host plant.

4) Pure high/low aphid density choice experiment
In this experiment no plants were used. The aphids were placed on a piece of plastic, floating on a wet medium in petri dishes (figure 4H). The aphids can freely move on the plastic, but take no risks to cross the water. Before the experiment aphids of the Breskens population were placed on the plastic. The high density treatment contains ten aphids and the low density treatment three aphids.

5) Pure treated/no treated plant choice experiment
In total twenty A. arenaria plants were selected. The length of each plant was measured. Because the choice experiment took more days, the plants were treated by five aphids the evening before to eliminate time variation. Before the experiment, the aphids were taken away from the plant to eliminate the aphid interaction and to purely look at the role of the plant.

To prevent aphids from escaping, plastic long cocktail glasses that were punctured at the bottom were placed upside down over each plant individually before each experiment. During the experiment the glasses were removed.
The methodology for the different choice experiments is the same. The first two plants or petri dishes are placed in the boxes and a parasitoid wasp is placed in the tube. The transportation of the parasitoids from the plastic box to the tube took place with another plastic tube (20 cm long, 0.5 cm diameter). By making an opening in the cover of the plastic box parasitoids could be picked up and dropped in the tube of the setup. We used only female parasitoids for the experiment. By looking at the ovipositor, the females were easily recognizable. One parasitoid was used for three choices. After that, a new female parasitoid replaced the old one. When five parasitoid wasps made three different choices (total of fifteen choices), the plants or petri dishes got replaced by new ones. At the end of the experiment, we used four combinations of plants or petri dishes, which make sixty choices in total. During the experiment, the plants were randomly changed from box to avoid biased results. After the choice experiment every plant was cut off to measure the dry weight.

Resistance/top-down experiment
Forty suitable A. arenaria plant individuals were selected and measured to perform the resistance experiment. The culturing of population of the Westhoek had failed by this time, so four populations were left. The forty plants were divided over four populations, which means that each population contained ten plants. The plants were treated by one adult aphid from the corresponding populations. On top of the sand, a tailor-made white paper was attached to optimize the counts of the death aphids (figure 4I). To prevent aphids from escaping, plastic long cocktail glasses that were punctured at the bottom were placed upside down over each plant individually. After two days the aphids were counted and the experiment could start. Five treated plants of each population, in total twenty plants, were selected to undergo the top-down experiment (test group), while the other twenty were used for control (control group). The twenty polypropylene cups from the test group were placed in a box. To optimise the translocation between the plants, sand was used to fill up the gaps between the cups (figure 4I). Also twenty female parasitoids were selected, because only the females are able to parasitize.

The experiment took place in a breeding chamber (40*40*50 cm). The box with the treated plants was placed in the breeding chamber. The temperature was twenty-four degrees Celsius and lights were present. The cocktail glasses were removed and the parasitoids were released for three hours. After three hours the parasitoids were removed and the cocktail glasses were added on top of the plants to avoid aphid escaping and contamination. The next ten days the living individuals, the death individuals and the mummies were counted and noted. After ten days the plants were cut off to measure the dry weight.

Statistics
All statistical analyses were conducted using the statistical package SAS® version 9.4 (SAS Institute Inc, 2016c). To avoid putting covariant independent variables in the models a correlation matrix was made, which showed the Pearson correlation coefficients between length and biomass of the plants.

Choice experiments:
The data of the choice experiments are Bernouilli distributed, a special case of the binomial distribution for which the two possible outcomes need to be 0 (fail) and 1 (success). To evaluate the data, the proc freq function in SAS was used (SAS Institute Inc, 2016a). In this test we reject the null hypothesis when there is a significant deviation from a 0.5 distribution.
The variation in length or biomass from the plant could be the trigger to choose that plant instead of the tested factors. A correlation matrix between the discrete dependent data and the continuous independent data (length or biomass) will perhaps clarify matters. Therefore the SAS function Proc probit is used (SAS institute inc, 2016b).

**Top-down/Resistance experiment:**

First a covariant test between the length and the count data will be done to test whether correction for length is needed. The data of the top-down/resistance experiment are typical count data, which are Poisson distributed. By using the glimmix procedure, the least square means will be measured and compared with a post hoc tukey test. For the top-down control we want to test the significant difference in aphid numbers between the treatments over the days. The first two days no parasitoid wasps were added. The count of the second day is just before the treatment. From day three there is a possibility to observe an effect of top-down control. For the resistance experiment comparisons between the populations need to be done. The exactly same tests as performed in the top-down control will be used. The only change is that we will compare the treatments over the days, but within each population.

We are also interested in the difference between day three, four and five with day two as control group. Each count of aphids will be subtracted with the count of aphids of day two in the same plant. In doing so, we can visualise the differences in aphid growth between treatments and also between different populations. When a negative value is obtained, there is a decrease in aphid density. Like this, we try to visualise the top-down control and the resistance of the population in another way. Indirectly there can also be an effect on the biomass of the plants. Therefore we will compare the least square means of the biomass between the test and control plants in a tukey test.
Figure 3: A: *Schizaphis rudula*; B: *Aphidius ervi*; C & D: seed germination; E: polypropylene cups with seedlings from different plants; F: Growthtubes with aphid populations; G: setup choice experiments; H: Aphids placed on a piece of plastic, floating on a wet medium in petri dish; I: Experimental setup resistance experiment. Pay attention to the gaps filled up with sand and the tailor-made white papers.
Results
The results of the correlation test between the length and biomass show that there is indeed a correlation. For the choice experiments the Pearson correlation coefficient is 0.7928 (p-value = 4.9x10^{-8}). The Pearson correlation coefficient for the resistance-experiment is lower, but still significant (0.3703, p-value = 0.0187) (table 1, appendix). Without conducting this test, covariant independent variables would be used in further statistics. Be using the length, the biomass is indirectly incorporated in the statistics. Tables with the p-values are added in the appendix.

Choice experiments

1) Aphid/no aphid choice experiment
Thirty-five against twenty-four parasitoid wasps choose for aphids. After statistics the result is not significant (p-value = 0.1925). Although there is no significance, the difference cannot be neglected. From a biological perspective, there is a difference in choice between the presence and absence of aphids. Therefore, a Wald chi-square test for the correlation between the length and the choices shows a significant result (p-value = 0.0161). This means that when the parasitoid chooses for a plant, they choose significantly for plants with a higher length. This test suggests that the results obtained in the first procedure can be biased due to the length of the plants. Parasitoids could be triggered by the length instead of the presence or absence of aphids.

2) High/low density choice experiment
Thirty-five against twenty parasitoid wasps chose for plants with the low density of aphids. The p-value is 0.0581, which is close to significance. Also here, from a biological perspective, there is a clear difference in choice pattern between low and high density. The Wald chi-square test for the correlation between the length and the choices shows no significant result. We can assume that some factors, other than length and biomass of the plant, trigger the parasitoid wasps to choose for plants with low density of aphids.

3) Grass species one/grass species two choice experiment
There was clearly no difference of choices between two different plants. A. arenaria was visited thirty-one times, where C. epigejos was visited twenty-nine times. The Wald chi-square test for the correlation between the length and the choices show a highly significant result (p-value = 0.0043). A. arenaria show a significant higher length than C. epigejos. This result shows that also the factor length plays no important role in the choice patterns of parasitoid wasps.

4) Pure high /low aphid density choice experiment
Here, too, no significant result is obtained (p-value = 0.6989). Based on this result and the result of the high/low density choice experiment, we can assume that parasitoid wasps recognise aphid densities only when plants are involved. Without the plants the parasitoid wasps are lost.

5) Pure treated / untreated plant choice experiment
The last experiment gives no significant result either (p-value = 1). The Walt chi-square test shows no correlation between the length of the plants and the choices (p-value = 0.3050). Straight out this result the direct interaction between the plant and the aphids causes recognition abilities of the parasitoid wasps. Only aphids or plants have no effect on the recognition abilities.
Resistance/top-down experiment

Both the covariance analysis and the glimmix procedure with length as dependent variable, show a non-significant difference of length between treatments and treatments within each population (table 2, appendix). Therefore, correction for the length is not necessary. During the resistance/top-down experiment, we noticed that the plants were drying out fast. Because this experiment only wanted to test the top-down control, it is important to minimise the bottom-up control of the plants. Therefore, the statistical analysis will only focus on the first five days.

Top-down control

Figure 4 shows that there are some clear patterns of top-down control. Because the parasitoid wasps are added to the test group after 2 days, it is important that the first two days are not significant. When there is already a difference between test and control group in the first two days biased results could be obtained. After the treatment there is clearly some decrease in aphid density in the test group. In the tukey comparison test, day three and five show a significant decrease in aphid density. There is no significance observed on day four, but there is clearly some decrease (table 3, appendix).

It seems interesting to check whether there are significant differences between the days before and after the treatment (figure 5). The aphid numbers from day three, four and five will be distracted with the aphid numbers of day two within the same plant. Between the test and control group the pure growth of the number of aphids is visualized compared with the number of aphid just before treatment (figure 5). Between day two and day three the control still increases, while the test group decreases in aphid density. In the tukey test this effect is significant (table 4, appendix). Day four and day five compared with day two produce no significant results in the tukey test, but the pattern that the numbers of aphids are increasing more slowly in the test group is visible (table 4, appendix). In other words, the parasitoids have some effect on the aphid densities, certainly the first day after parasitism.

There is also an indirect effect on the biomass of the plants. When we compare the least square means of the biomass between the test and control plants in a tukey test, a significant result is obtained (table 5, appendix). The biomass of the control plants (mean = 0.002945g) seems to be significantly smaller in comparison with test plants (mean = 0.005070).
Figure 4: Top-down experiment: chart of the least square means of the number of aphids between the treatments over five days. We clearly see some patterns of top-down control after the treatment. The test group (group that experienced the treatment with parasitoid wasps) is each time lower than the control group. After a Tukey comparison test significant results for day three and five are obtained.

Figure 5: Top-down control: graph in which we make the comparison between day three, four and five and day two as control. Between the test and control group the pure growth of the number of aphids is visualized compared with the number of aphid just before treatment 1: difference between day one and day two; 2: difference between day two and day three; 3: difference between day two and day four; 4: difference between day two and day five. Significant result is obtained for the first day after treatment. In general, the growth of aphids of the control group is higher than the test group. The first day after treatment (2) there is a small decrease of aphids in the test group.
Resistance experiment

Figure 6 shows some differences between the populations in average number of aphids. For Breskens top-down control is not really present. The population decrease in test as control after the treatment with parasitoids, although the decrease in the test group is higher. In Ter Yde, top-down control is present. The number of aphids in the control group gradually increases in time, while the test group stagnates. Zwarte Polder starts with more aphids in the control group than in the test group. There is no clear top-down control visible. Both the test and control group gradually increase in aphid numbers. Het Zwin shows some top-down control on day three, but afterwards the test population seems to recover. When we look at the table, obtained by performing a tukey comparison test, there is no trend visible. Only two comparisons are significant (Zwarte Polder day three: p-value =0.0327; Ter Yde day five: p-value = 0.0009) (table 6, appendix).

In figure 7, only in Ter Yde there is a stagnation of the test group, while the control group increase. The glimmix procedure in SAS confirm that Ter Yde show significant differences, which means that there is top-down control in Ter Yde and no resistance of the aphids against the parasitoid wasps (table 6, appendix). The other populations show no significant difference between test and control group over the different days and compared with day two (table 7, appendix). In Het Zwin there seems to be some top-down control on day three, but the population recovers. The population of Breskens show a negative trend in the control and the test group. But there is a larger decrease of aphids in the test group compared with the control group. In Zwarte Polder there seems to be a lower increase in aphids from the test group, but not significantly.

Also mummies were formed in the ten days resistance/top-down experiment. In total nine mummies had been developed at the end of the experiment. In the following graph you see that the average number of aphids is already very small, when mummies arise. There was only mummy formation in Ter Yde and Zwarte polder.

Figure 6: The line indicates the number of aphids during the days. The bars show the number of mummies. After day six, when the aphid density is already very low, the mummies are formed.
Figure 7: Resistance experiment: the average number of aphids is visible over the five days within each population. B: Breskens: no significant top-down control is visible in the population. The difference with the other populations is the decrease in the number of aphids after the treatment. Y: Ter Yde: There is a significant effect of top-down control. Test group stagnates, while the control group continues to increase. ZP: Zwarte Polder: there is also no significant effect of top-down control. Both test and control group continues to increase. ZW: Het Zwin: In the first day after treatment there seems to be some effect of top-down control, but then the population recovers. In general, variation between populations is visible.

Figure 8: Resistance experiment: graph in which we make the comparison between day three, four and five and day two as control. Between the test and control group the pure growth of the number of aphids is visualized compared with the number of aphid just before treatment within each population. B: Breskens: the decrease of the numbers of aphids is visible. Here we can observe that the decrease is higher for the test than the control group. Y: Ter Yde: there is a rapid increase in the control group and a stagnation of the test group. Significant result obtained; p-value = 0.0229 (table 6: appendix). Both ZP (Zwarte Polder) as ZW (Het Zwin) show no effect of top-down control. In general, variation in resistance is visible.
Discussion

Most of the hypotheses stated in the introduction are proven in our results. First there are clearly some recognition abilities of parasitoid wasps to detect aphid populations. In this part, the underlying mechanisms will be discussed. Also the hypothesis that aphid populations are controlled by parasitoid wasps is demonstrated. This result has important influences on the way we look at yellow dune ecosystems. They can have an important role in the conservation of the dynamic dunes. The high variation in resistance against parasitoid wasps between populations is proven in our results. High fragmentation of yellow dunes supports evolution to act differently on the remote populations. This statement will be supported by an example from previous studies in yellow dunes.

Recognition ability of the parasitoid wasps: underlying mechanisms.

From the first choice experiment, the parasitoid wasps seem to have some recognition abilities. The presence of aphids trigger their attention to choose A. arenaria plants with aphids present. Although there is no significance, there is some trend visible (thirty-five against twenty-four). An unexpected outcome (thirty-five against twenty) is the biologically significant choice for low densities instead of high densities. You would expect that they choose for high densities because more aphids are present to parasitize. The underlying reasons why the wasps choose for a particular side are not studied in this thesis, but are very interesting for further research. Therefore, an enumeration of the possible underlying reasons could be of benefit for future research.

Parasitoid wasps are able to recognise colours. The visual cues seem to be important to select the suitable host at short distances, while the role at long distance is negligible and granted to the olfactory cues (Powell et al., 1998). There are two main olfactory cues that could play a role in the recognition ability of the parasitoid wasps, semiochemicals and volatiles. Although many herbivorous insects have evolved to remain as inconspicuous as possible, they often produce semiochemicals for long-range, intraspecific communication. Sex pheromones and aggregation pheromones are being well-known examples. Such pheromones are obviously potential host location cues and a number of natural enemies are known to have exploited this opportunity. Although the emission of sex pheromones in aphids is limited to the sexual stage, the value of it is multiple times demonstrated (Powell et al., 1998). More recently, researchers identified another semiochemical: bacteria-produced honeydew volatiles (Verheggen, 2016). Another important cue, already mentioned in the introduction, is volatiles. Aphid-induced plant volatiles seem to have a more important function than the semiochemicals mentioned above. The plants call the parasitoids for help, which is in contrast to the insects, that want to remain as inconspicuous as possible (Powell et al., 1998). There are a lot of cues involved in the parasitoid-aphid recognition. The question remains whether a combination of cues or just one decisive cue causes the reaction of parasitoid wasps. This question can be answered by the two last performed experiments. None of them seems to have an effect on the recognition ability of the parasitoid wasps. In the first experiment, the volatiles of the plants were excluded. Only the semiochemicals and visual cues could play a role in the choice of the parasitoid wasps. In the last experiment, the semiochemicals and the visual cues were excluded. Only the volatiles could play a role in the choice of the parasitoid wasps. From this result, we could conclude that a combination of the semiochemicals and the volatiles trigger the parasitoid wasps.

Of course these results should be regarded with some caution. Our results have not the power to prove the role of olfactory and visual cues, but open perspectives to perform studies on the volatiles, semiochemicals and visual cues in this ecosystem, because some trends are visible. Some proposed
research questions are, which volatiles are secreted by \textit{A. arenaria}, which semiochemicals are secreted by \textit{S. rufula}, are the olfactory cues able to trigger the choice of the parasitoid wasps and is the production of volatiles density-dependent? After these studies also the trends in our investigation could be clarified. It could be that low aphid densities trigger the plant to emit more volatiles than higher densities. This could clarify the result obtained in our study (high/low density choice experiment). Another research question of societal importance is the role of air pollution (production of VOC by humans (EPA, 2014) in the story of recognition abilities of parasitoid wasps. In other studies the impact of air pollutants on BVOC (biogenic volatiles organic compounds) has already been studied and shows significant negative results (Calfapietra et al., 2013). In the future, the recognition ability could be changed due to the change in air pollution and emission of human VOC’s.

From the findings of the thesis of Van Moorleghem, aphids prefer to settle on \textit{A. arenaria} in the very harsh and unpredictable yellow dunes instead of more fixated dunes. More complex and diverse vegetation types harbour a greater community of natural enemies. Therefore aphids would prefer the yellow dune habitat. Another hypothesis is the resource concentration effect. \textit{S. rufula} could have problems with locating \textit{A. arenaria} host plants within fixated dunes where \textit{A. arenaria} individuals are less aggregated (Otway et al., 2005, Van Moorleghem, 2014). Another hypothesis is the “escape hypothesis”, which states that in dynamic sand dunes the continuing supply of fresh sand triggers vertical growth of \textit{A. arenaria} and allows it to escape harmful soil biota (De La Peña et al., 2006). Aphids could benefit greatly from this escape through better food quality (Van Moorleghem, 2014). A new hypothesis could be added when the role of volatiles has been clarified. The volatiles are directly diffused in the air. The problem in a yellow dunes habitat is the harsh wind condition. The parasitoid wasps try to orientate and find suitable aphid populations based on the volatiles. Wind dynamics can dilute the volatile concentrations and disarrange the navigation system of the parasitoid wasps. Therefore, life in yellow dune habitats decreases the probability of parasitoid wasp encounters and increases the fitness of the aphid populations.

The broader picture of top-down control in a yellow dune system

In the thesis of Van Moorleghem, they proved there was a significant effect of the aphids on the biomass of the \textit{A. arenaria} plants. In field observations \textit{A. arenaria} plants seems to have no effect from the presence of the aphids. There were some contradictions between the field and experimental studies. Our study proves that there is top-down control of \textit{S. rufula} by parasitoid wasps (figure 4 and 5). This can explain the contradiction. In yellow dune systems, the harmful \textit{S. rufula} densities are partly controlled by parasitoid wasps. Of course, a lot of other insect species can play a role in the top-down control. Spiders; carabid, coccinellid and staphylinid beetles; syrphid flies are insects also reported in yellow dune systems (Schmidt et al., 2003). Their role in the top-down control has not been tested, but can only reinforce the significant effect found in this master thesis. Also between the biomasses of the \textit{A. arenaria} plants from the test and control group is there a significant difference. Parasitoid wasps have a direct negative impact on the aphids and have a significant positive effect on the biomass of the plants (table 5, appendix).

In the study of Van Moorleghem C., also hyperparasitoid wasps from the genus \textit{Apooanagyrus sp.}, \textit{Dendrocerus sp.} and \textit{Pachyneuron sp.} (Hymenoptera) were found (Van Moorleghem, 2014). Hyperparasites are parasites which parasitize other parasitoid wasps within their host (Wikipedia contributors, 2015). Because the hyperparasitoid wasps parasitize the larvae of the parasitoid wasps
the impact of top-down control on the aphid densities is already caused. The hyperparasitoids will have an influence on the parasitoid densities, which can have an indirect impact on the top-down control of the aphids. Because we tried to imitate the natural densities of the parasitoid wasps in nature (one parasitoid wasp / plant) the influence of the hyperparasitoid wasps should be minimized. Yet, previous studies about the influences of hyperparasitoid wasps are contradictory. The high levels of hyperparasitism that Schmidt et al. (Schmidt et al., 2003) found, did not appear to prevent an effective control of cereal aphids by their parasitoid wasps. Still, hyperparasitism has been shown previously to reduce the effectiveness of primary parasitoid wasps (Rosenheim, 1998).

During the experiment aphid responses were visible against parasitoid wasp attacks. In general, two main escape responses are observed in a broad range of aphid species and also during our experiment, so-called dropping and kicking. Other behaviour responses are walking away and body rotation (Wyckhuys et al., 2008) (Powell et al., 1998). Parasitism has a direct effect on the top-down control of aphids, but also indirect effects provoked by parasitism lead to expiring or surviving of aphids. The first behaviour is called kicking, defined as an aphid body raising and contacting the parasitoid with one of its legs. In 2008, Wyckhuys et al. (Wyckhuys et al., 2008) observed that kicking was the most frequent defence behaviour and mostly used by fourth and alatoid (=nymph bearing wing pads destined to become an aphid with wings (USDA, 2014)) instar aphids. The second behaviour is dropping of the feeding place. Chau et al. (Chau and Mackauer, 2000) studied the dropping behaviour in relation with age. There was a clear positive correlation with increasing age. First instar aphids have low energy reserves and therefore should not favour dropping as escape tactic. The risk to find no suitable plants in the surroundings or being predated is much higher for first instar aphids than for fourth instar aphids, which have a higher mobility and energy reserve. In yellow dune habitats, another factor hinders the aphids to recapture the young tussocks again. Due to the harsh wind conditions, the aphids are just blown away when they end up in the sand. Because in the experiment the wind conditions and predation are not taken into account, most of the aphids survive when they drop. Therefore, under natural conditions the top-down effect would be even more extreme.

**Aphid resistance: facultative endosymbionts and mummy formation**

In the resistance experiment, large differences between the populations are visible. The aphid density of the population of Breskens decreases after treatment in both the test and the control group (figure 7 and 8). The aphids were collected from the host plant L. arenarius, the other population from the host plant A. arenaria. This can explain the differences between Breskens and the other populations. The aphids of Breskens are probably less adapted to A. arenaria. Ter Yde shows no resistance against parasitoid wasps. The control group keeps increasing, while the test group stabilises. Het Zwin and Zwarte polder show relatively low resistance after the first day, but the population densities recover after day five (figure 7 and 8). The high differences between populations could have to do with the increasing fragmentation of the coastline. There is no more gene flow and evolution strikes different between these populations. An example is the role of facultative bacterial symbionts. Although these symbionts are not required for the survival of the aphid, they may transfer beneficial features to their hosts such as increased resistance against natural enemies and pathogens, protection from heat shocks, and more importantly influence survival and fitness on specific host plants (Leonardo and Mondor, 2006, Oliver et al., 2010). The study of de la Pena et al. showed variation in the endosymbiont community according to species and location. All populations sampled along the North Sea coast were associated with the facultative
endosymbiont *Serratia symbiotica*. *Hamiltonella defensa* was only detected in some specimens coming from the population in Het Zwin. *H. defensa* is an endosymbiont already reported in aphids and other sap-feeding insects as protecting its hosts from parasitoid wasps (de la Peña et al., 2014). There is a possible role of *H. defensa* in the defence against parasitoid wasps. The results give a high defence mechanism for Het Zwin, maybe due to the presence of *H. defensa*. It would be interesting to look at the dispersal of facultative endosymbionts over aphid populations. Important here is that facultative symbionts are also found extracellularly in the aphids' haemolymph, which facilitates horizontal transmission. In situ, it is possible to transfer them between lineages of the same or even different species by microinjection of symbiont-containing haemolymph (Chen and Purcell, 1997) or by feeding aphids with an artificial diet containing symbiotic bacteria (Douglas, 1998) (Gehrer and Vorburger, 2012). In aphids, the only natural mechanism of horizontal transfer known so far is by sex. During mating, infected males may transmit facultative symbionts to uninfected females through their ejaculate (Moran and Dunbar, 2006). Recently, researchers demonstrated that parasitoid wasps can transfer endosymbionts of aphids between clones by sequentially stabbing infected and uninfected aphids. This is an undescribed route of horizontal transmission. To have a successful transmission, the aphids need to survive the attack of the parasitoid. Now, transmission is possible over greater distances and is not limited to the period of sexual reproduction. In the case of *H. defensa*, the wasps are spreading a symbiont that is detrimental to their own fitness. They may thus be under selection to minimize such transfers (Gehrer and Vorburger, 2012). Casting our mind back to the current major problems of the Belgium coast, particularly the fragmentation, transmission of endosymbionts across the population boarders seems non-existent. More research about long distance migration and flight capacity of *S. rufula* needs to be accomplished to underpin previous statement.

The development of the mummies takes a long time (figure 6). After six days the first mummies were formed. Due to the rapid dry-out of the *A. arenaria* plants, we cannot draw strong conclusions around the mummy formation. In Het Zwin, there was no mummy formation. This can also point at the presence of facultative endosymbionts that decrease the impact of the parasitoid wasps (Oliver et al., 2003). Also in Breskens there was no mummy formation. In the introduction we already stated that the aphids have to stay alive for successful mummy formation. Due to the rapid dry-out of the plants in Breskens a handful of aphids were present after day six.

**Implications for conservation and management**

The fact that we already find significant effects on top-down control without including the predation rate on the ground, wind dynamics and other aphid controlling insects, obtaining harmful densities of *S. rufula* in natural yellow dune system seems to be unrealistic. With the increasing fragmentation and overbuilding of the coast, suitable yellow dune habitat decreases together with the young *A. arenaria* tussocks and the aphid populations. The population will become small and less resilient. When the top-down effect still remains the same, aphid populations will not survive the persistent pressure. This little interaction can play a major role as an indicator for conservation in yellow dune systems. When the young tussocks in yellow dunes contain aphids and are not harmful for the *A. arenaria* population, the ecosystem reaches equilibrium. Deviations from that equilibrium can indicate serious problems. The aphid populations can become harmful for the *A. arenaria* individuals. This indicates a problem with the top-down control by parasitoids and predators in the yellow dune system. The task for nature conservationists is to research the underlying reason for the decrease of top-down control and come forth with some useful management measures to increase the predator
and parasitoid populations again. Another possible deviation is the abrupt loss of the *S. rufula* population. This can indicate that the nature reserve is not big enough to prevent a healthy population and has to be extended. Other possibilities are the persistent pressure of the humans on the ecosystem or the loss of connectivity with other populations. In that case, management measurements have to be introduced to prevent the disappearance of the yellow dune ecosystem.

Nature conservationists have to keep in mind the high variation between populations. Het Zwin is the biggest nature reserve in the top-down and resistance experiment. Their higher resilience against aphids is visible. Due to the fragmentation and loss of some aphid populations, beneficial endosymbionts can be lost along with the resistance against the parasitoid wasps. Because of this, the small populations will suffer even more. The high variation can also be an indicator for the high fragmentation between the populations along the coast. To reduce the variation more connectivity between the yellow dune habitats is needed.

**Conclusion**

Basic research has been done to understand the interaction between *S. rufula* and his parasitoid wasps, but proposed additional research will allow us to understand the interaction in full. Also the role of other predators and parasitoids in the top-down control needs to be tested.

The role of bottom-up control was already clarified in yellow dunes, but the top-down control was a missing piece in the puzzle of this ecosystem. My thesis partly fills the gap that we need to fully understand the yellow dune ecosystem. Also for conservationists, it is easier to suggest management measures based on documented evidence. Thus, this thesis could play an important role in the conservation and protection of yellow dune systems in Northwest Europe.
Summary

An organism’s interactions with its environment are fundamental to the survival of that organism and the functioning of the ecosystem as a whole. The loss of one keystone species and his interactions can disrupt the functioning of the ecosystem dramatically. Because of the great importance of interactions for an ecosystem, understanding these is essential for the conservation of the remaining ecosystems. The habitat this study will mainly focus on is yellow dune habitat. The role of bottom-up control in yellow dune habitats has already been described well, but the role of top-down control remains a question. People have drastically altered coastal dune landscapes throughout centuries of exploitation and more recently modification of the coast. This constant and substantial pressure leads to the disappearance and fragmentation of our dune landscape. Another serious problem is the tendency to increase fixation and succession on coastal dunes throughout Northwest Europe. This creates possibilities for strong competitors to dominate the dune system. A dune system can be subdivided into more dune habitats, each with their specific plant species. Yellow dunes are mainly characterized by a grass species *Ammophila arenaria*, which contains an important aphid species *Schizaphis rufula*. In previous studies, *S. rufula* was shown to cause significant damage to *A. arenaria* individuals. In field observations, these results have not yet been seen. Several additional factors, which control the harmful densities of *S. rufula* in the field, were not taken into account in the experimental study. Our study will scrutinize one of the additional factors, namely parasitism by parasitoid wasps.

The aim of this master thesis is to understand the ecological interactions between the aphid *S. rufula*, his parasitoid wasps and other potential influencing links. Of course this extensive subject needs to be tackled in some specific and purposeful objectives. The first objective of this master thesis is study the top-down control of *S. rufula* populations by parasitoid wasps. The second objective tackles the differences in resistance of *S. rufula* populations against parasitoid wasps. The last one checks the recognition ability of parasitoid wasps to detect *S. rufula* populations on yellow dune grasses.

Five study sites were selected where sand is still relocated by wind dynamics. In De Westhoek, Ter Yde, Het Zwin, Zwarte Polder and Breskens *S. rufula* populations were collected. Also six typical dune grass species were collected in these areas. After a surface sterilization treatment, the seeds germinated until they were ready for use in the experiments. Aphid populations were kept in an artificial habitat in situ. Because cultivation of the natural parasitoid wasps was impossible, *A. ervi* was elected as appropriate surrogate. They are available on the web. The setup of the choice experiment is built up by two transparent boxes connected with a transparent tube. The upper side of the box contains a removable cover. The middle of the tube is provided with an opening to deposit the parasitoids. Five choice experiments are completed. The first one is an aphid/no aphid choice experiment. Because the densities are also important, the second experiment is a high/low density choice experiment. *S. rufula* feeds on different grass species. Therefore an experiment is performed were two grass species are placed across each other. The last two experiments are pure high / low aphid density choice experiments, where plants are eliminated, and pure treated /untreated plant choice experiment where aphids are not present. For the resistance/top-down experiment, we selected forty suitable *A. arenaria* plants. Twenty plants will undergo the treatment and the others are used as control. The treatment consists of twenty parasitoid wasps who parasite the aphids on the *A. arenaria* individuals for three hours. After the treatment the aphids from the test and control group are counted for the next ten days. All statistical analyses were conducted using the statistical package SAS® version 9.4.
The results of the choice experiment are interesting. Clear patterns are visible in the aphid/no aphid experiment and the high/low density experiment. In the latter low densities are chosen above high densities. The underlying mechanisms that are responsible for the choice of the parasitoid wasps are not studied in this thesis, but can be interesting for further experiments. First the visual cues can play a role in the recognition. The visual cues seem to be important to select the suitable host at short distances, while the role at long distance is negligible and granted to the olfactory cues. There are two main olfactory cues that could play a role in the recognition ability of the parasitoid wasps, semiochemicals and volatiles. The latter will play a more important role, because semiochemicals are produced by the aphids themselves, who want to be less conspicuous as possible and volatiles by the suffering plants. Our basic experiments open perspectives for new research questions.

The results of the top-down experiment clearly show some significant patterns of top-down control. There were some contradictions between the field and experimental studies. Our study proves that there is top-down control of S. rufula by parasitoid wasps. This can explain the contradiction. Also hyperparasitoid wasps can play a role in the top-down control of aphids. Hyperparasites are parasites that parasitize other parasitoid wasps within their host. Because the hyperparasitoid wasps parasitize the larvae of the parasitoid wasps the impact of top-down control on the aphid densities is already caused. Because we tried to imitate the natural densities of the parasitoid wasps in nature (one parasitoid wasp/plant) the influence of the hyperparasitoid wasps should be minimized. Also the response of the aphids against the parasitoid wasp attacks can play an important role in the top-down control. Dropping of the feeding place was one of the responses visible in our experiments. Because of the harsh wind conditions and the presence of predators, ending on the sand could be deadly for the S. rufula individuals. These factors reinforce the significant result of top-down control found in this thesis. In the resistance experiment, large differences between populations are visible. The high differences between populations could have to do with the increasing fragmentation of the coastline. There is no more gene flow and evolution strikes differently between these populations. An example is the role of facultative bacterial symbionts. Although these symbionts are not required for the survival of the aphid, they may transfer beneficial features to their hosts, such as increased resistance against natural enemies and pathogens. In previous studies the endosymbiont H. defensa was detected in some aphid individuals of Het Zwin. H. defensa can play an important role in the resistance against parasitoid wasps. The resistance found in Het Zwin and Zwarte Polder can be caused by the presence of the endosymbiont bacteria H defensa. Horizontal transmission of the endosymbiont over populations seems non-existent in the fragmented coastline of Belgium and The Netherlands. More research about long distance migration and flight capacity of S. rufula needs to be accomplished to underpin previous statement.

This interaction can play an important role as an indicator for the health condition of yellow dune ecosystems. Nature conservationists have to keep an eye on the S. rufula densities and the condition of the A. arenaria individuals in the field. When a deviation of equilibrium is visible, management measurements have to be introduced to prevent the disappearance of the yellow dune ecosystem. Also nature conservationists have to keep in mind the high variation between populations. This can be an indicator for the level of fragmentation of the yellow dune habitats.

The role of bottom-up control was already clarified in yellow dunes, but the top-down control was a missing piece in the puzzle of this ecosystem. My thesis partly fills the gap that we need to fully understand the yellow dune ecosystem. Also for conservationists, it is easier to suggest management
measures based on documented evidence. Thus, this thesis could play an important role in the conservation and protection of yellow dune systems in Northwest Europe.

Samenvatting

Interacties tussen organismen zijn essentieel voor de overleven van dat organisme en het functioneren van een ecosysteem. Het verlies van een belangrijke soort en zijn interactie in een voedselweb kan het functioneren van een ecosysteem serieus in de war brengen. Daarom is het zo belangrijk dat we de interacties in een ecosysteem begrijpen om goeie beheersmaatregelen te nemen. De habitat waarop deze thesis zich richt, zijn dynamische kustduinen. De rol van bottom-up controle in dynamische duinen is al onderzocht, maar de rol van top-down controle is nog niet bekend. De mensheid heeft doorheen de jaren het duinlandschap aangetast door de kust voor economische en toeristische gelegenheden uit te buiten. Die constante druk heeft tot het verdwijnen en de fragmentatie van de kust in Noordwest-Europa geleid. Een ander probleem is de tendens van toenemende fixatie en successie van de Europese dynamische duinen. Daardoor kunnen competitieve soorten zich vestigen en domineren in de duinen. Een kustduin kan ingedeeld worden in verschillende duinhabitats, elk met hun specifieke duingrassen. Dynamische kustduinen zijn vooral gekarakteriseerd door A. arenaria, die een belangrijke soort bladluis bezit, namelijk S. rufula. In voorgaand onderzoek bleek dat de bladluis S. rufula significante schade veroorzaakte aan A. arenaria individuen. Veldobservaties ondersteunen die experimentele resultaten niet. Verschillende andere factoren die de schadelijke densiteiten van S. rufula onderdrukken, werden niet in rekening gebracht in het voorgaande experimenteel onderzoek. Onze studie zal één van de andere factoren, namelijk parasitisme door sluipwespen, onder de loep nemen.

Het doel van deze masterthesis is om de ecologische interacties tussen S. rufula en de sluipwespen beter te begrijpen. Drie specifieke en doelgerichte objectieven worden opgesteld om de algemene vraag zo goed mogelijk te beantwoorden. De eerste bekijkt de invloed van de top-down controle door sluipwespen op de bladluis. De tweede zal kijken of er verschillen zijn tussen populaties in de resistentie tegen sluipwespen. De laatste onderzoekt het herkenningsvermogen van sluipwespen om bladluizen te detecteren.


De resultaten van de top-down experimenten tonen duidelijke significante patronen van top-down controle. Uit vorig onderzoek bleken tegenstrijdigheden tussen veldobservaties en experimenten. Ons onderzoek toont aan dat er top-down controle van S. rufula individuen is. Dat kan de tegenstrijdigheid verklaren. Ook hyperparasieten kunnen een belangrijke rol spelen in de top-down controle. Hyperparasieten zijn parasieten die andere primaire sluipwespen parasiteren. Omdat de hyperparasieten de larven van de primaire sluipwespen parasiteren, is de schade van de sluipwespen op de bladluizen al aangericht. Daardoor zullen hyperparasieten geen directe invloed hebben op de top-down controle van bladluizen, maar op de dichten van de sluipwespen. Omdat we in onze experimenten de natuurlijke dichten van sluipwespen hebben proberen te benaderen, zou de invloed van hyperparasieten geminimaliseerd moeten zijn. De respons van de bladluizen op de aanval van een sluipwespaan kan een belangrijke invloed hebben op de top-down controle. Zich laten vallen op de grond was één van de responses zichtbaar in het experiment. Door de harde windcondities en de predatoren op de grond, zou eindigen op de grond wel eens het doodvonnis kunnen betekenen voor een bladluis. Deze factoren versterken alleen maar de significante resultaten die wij bekomen in ons experiment. In het resistentieexperiment zijn er grote verschillen zichtbaar tussen populaties. De grote verschillen tussen de populaties zouden wel eens te maken kunnen hebben met de toenemende fragmentatie van de kust. Er is geen of verminderde gene flow en evolutie slaat verschillend toe in de populaties. Een voorbeeld is de rol van facultative endosymbionten. Die endosymbionten zijn niet nodig voor de overleving van de bladluizen, maar toch kunnen ze zorgen voor een verhoogde resistentie tegen sluipwespen. In vorig onderzoek is de endosymbiont H. defensa gedetecteerd in verschillende individen in Het Zwin. H. defensa kan een belangrijke rol spelen in de verhoogde resistentie tegen sluipwespen. De hoge resistentie gevonden in Het Zwin kan dus een resultaat zijn van de aanwezigheid van gunstige endosymbionten. Horizontale transmissie van endosymbionten tussen populaties lijkt niet bestaande in een
gefragmenteerde kust als die van België. Onderzoek over de verspreiding en lange afstand migratie van bladluizen moet de vorige uitspraak onderbouwen.

De bestudeerde interactie kan een belangrijke rol spelen als indicator over de gezondheidstoestand van dynamische duinen. Natuurbepaarders moeten de S. rufula densiteiten en de toestand van de A. arenaria in de gaten houden. Wanneer ze een afwijking van het evenwicht zien, moeten er beheersmaatregelen getroffen worden om de verdwijning van dynamische duinen tegen te gaan. Ook moet de hoge variatie in resistentie tussen populaties in de gaten gehouden worden. Dat kan een indicator zijn van de fragmentatietoestand van de dynamische kustduinen.

De rol van bottom-up controle was al bestudeerd in dynamische duinen, maar de rol van top-down controle was nog een ontbrekend puzzelstuk. Mijn thesis vervolledigt de puzzel met als resultaat dat we terug een stap dichter zijn bij ons doel om het systeem volledig te begrijpen. Als conservator van een dynamische duin is het makkelijker om beheersmaatregelen voor te stellen gebaseerd op goed gedocumenteerde literatuur. Deze thesis kan dus een grote rol spelen in de bescherming en conservatie van de dynamische kustduinen in Noordwest Europa.

Acknowledgement
I want to sincerely thank Eduardo de la Peña for helping me to find my own way of accomplishing this master thesis with thorough guidance and clear counselling. I also want to thank the people from the terrestrial ecology unit in Ghent, who supported me to achieve this result. I also want to thank my family and friends for believing in me and supporting me where possible. Thanks a lot to my former teacher Raymond Reynaert for reading and correcting my master thesis. And last but not least, my girlfriend, who continued to support me all the way.
References


Appendix

Correlation test between length and biomass in choice and resistance/top-down experiment

![Scatter Plot](image1)

![Scatter Plot](image2)

Table 1: left Scatter plot: the correlation between length and biomass for the choice experiments (corr = 0.7928, p-value = 49x10^-8). Right scatter plot: correlation between length and biomass in the resistance/top-down experiment (corr = 0.3703, p-value = 0.0187)

Resistance/top-down control: glimmix procedure with length as dependent variable

<table>
<thead>
<tr>
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</thead>
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Table 2: left: least squares means of the length in control and test; and the tukey comparisons test. No significance is obtained. Right: results of the tukey test after slicing for the population. For each population no significance is obtained.

Top-down experiment: tables of the glimmix procedure

<table>
<thead>
<tr>
<th>Tests of Effect Slices for day*treat Sliced By day</th>
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<tbody>
<tr>
<td>day</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
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Table 3: Results of the tukey comparison test between the treatments over 5 days. The first two days, we see no significance. Day 3 and 5 show high significance and day 4 none.

<table>
<thead>
<tr>
<th>Tests of Effect Slices for day*treat Sliced By day</th>
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<tbody>
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<td>day</td>
</tr>
<tr>
<td>1</td>
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<td>2</td>
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<td>3</td>
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<td>4</td>
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</tbody>
</table>

Table 4: tukey test after distracted day 3, 4 and 5 with day 2 as control. 1: difference between day 1 and day 2; 2: difference between day 2 and day 3;
Top-down experiment: glimmix procedure with biomass as dependent variable

<table>
<thead>
<tr>
<th>treat</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>DF</th>
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Table 5: least square means of the biomass in the control and test group + tukey comparison test. Highly significant result is obtained.

Resistance experiment: tables of the glimmix procedure

<table>
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<tr>
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<tr>
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<td>ZW</td>
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Table 6: tukey comparison test sliced by day*pop. Low significant levels obtained. Only day three in Zwarte polder and day five in Ter Yde show significant top-down effects.

Table 7: Tukey comparison test for the differences between day three, four and five and day 2 as control. Only Het Zwin shows a significant result (p-value = 0.0229).