UNIVERSIDAD METROPOLITANA
SCHOOL OF ENVIRONMENTAL AFFAIRS
SAN JUAN, PUERTO RICO

# INTERACTION BETWEEN THE DUNE APHID *SCHIZAPHIS RUFULA* AND ITS HOST-PLANT *AMMOPHILA ARENARIA*: A COMPARISON OF INSECT MULTIPLICATION ON DIFFERENT HOST-PLANT POPULATIONS

Partial requisite for the acquirement of the
Degree of Master's in Science in Environmental Management
Specialization in Natural Resource Management and Conservation

By
Jeselyn Calderón-Ayala

December, 4, 2012

**DEDICATION**

*To mom, dad, my 3 sisters and Leinã'ala*

*For their never ending support and for being pillars, not only in my life, but for my career endeavors*

*Thank you; receive all my gratitude, and all my love.*

# ACKNOWLEDGEMENTS

First of all, I want to give thanks to Dr. Eduardo de la Peña, for inviting me to work in his laboratory and trusting me with this amazing opportunity that has change my career, forever. Without him this work would not have been possible, and my love for the coastal ecosystems would have stayed submissive. To Dries Bonte and Luc Lens for letting me work in the Terrestrial Ecology Unit (TEREC) without any doubt or hesitation. To Martijn L. Vandegehuchte for providing indirect guidance with his previous work on *Ammophila arenaria* systems.

To the School of Environmental Affairs, for their permission to let me choose my own research line and be able to work it abroad, at a continental scale. To the Office of Internships and Scholarships of Universidad Metropolitana, especially to its Vice-rector Jacqueline E. Guzmán-Silva and the Annual Fund, for always believing in me. To the José Jaime Pierluisi Foundation and its coordinator Lisa Ramirez and to the Puerto Rico Council on Higher Education (CES-PRIDCO) for providing extra funding in order for me to achieve my desire to do research and have a learning experience overseas. To Leinã'ala S. Hall from University of Hawai'i at Hilo and the ESA/SEEDS Network, not for only helping me *in-situ* with the research work in Belgium, but for proofreading and editing the document in whole. I am tremendously grateful to have worked with a student with such quality; without Leinã'ala this Thesis would not have been translated into English.

Last but not least I want to thank Dr. Juan F. Arratia for exposing me to the initial opportunity that eventually led to the collaboration between my Advisor, Dr. Eduardo de la Peña, and myself. In addition, I want to thank Dr. Arratia for providing the funds to meet my Advisor in Portland, Oregon to summarize the results of this research and also, to be able to present it at the 2012 Ecological Society of America (ESA) Annual Meeting.

## TABLE OF CONTENT

# LIST OF TABLES

## LIST OF FIGURES

## LIST OF APPENDICES

# RESUMEN

Las dunas costeras son grandes acumulaciones de arena que se encuentran a lo largo de la costa, formadas por corrientes marinas, oleaje, viento y vegetación. La vegetación provee la estabilización de la duna, creando a su vez un hábitat idóneo para un sin número de organismos como insectos, crustáceos, reptiles, aves, entre otros. *Ammophila arenaria* es una hierba perene que domina dunas móviles (incluyendo antedunas embriónicas y antedunas) a lo largo de la costa Europea. Por su fácil colección, transplantación, propagación y adaptación a las dunas de arena, esta hierba es comúnmente usada en Europa y mundialmente para propósitos de estabilización de dunas. El afido de duna, *Schizaphis rufula* es una especie de herbívoro comúnmente asociado a esta hierba a lo largo del rango de distribución de la planta huésped. Estudios para ganar conocimiento acerca de las interacciones entre *A. arenaria* y *S. rufula* no son solamente necesarios para entender los rasgos biológicos básicos de estas dos especies, sino que son útiles para la creación de estrategias de manejo óptimas para ecosistemas de duna. La meta de este estudio era estudiar la multiplicación de *S. rufula* en varios genotipos de *A. arenaria* provenientes de diferentes localidades dentro del rango de distribución natural de esta especie. También lo dirigimos hacia evaluar si *S. rufula* prefiere los genotipos locales o alopátricos de esta hierba. Plántulas de Bélgica, Inglaterra y Portugal fueron expuestas a una población de áfido colectado en Bélgica. Luego del proceso de inoculación, se le permitió a los áfidos multiplicarse y alimentarse en los diferentes genotipos de las plantas por 20 días. El número de áfidos fue utilizado para generar una curva de crecimiento poblacional para cada combinación de áfido-planta. La dinámica de crecimiento entre las poblaciones de *A. arenaria* fueron comparadas usando un “one-way” ANOVA. Las cuatro combinaciones planta-herbívoro revelaron diferencias significativas en la multiplicación entre las poblaciones, con una reproducción de áfidos más alta en poblaciones de Bélgica (local) y de Wales, Inglaterra. Por lo tanto, el origen de la población de la planta influencia la multiplicación de áfidos, no obstante, no se descubrió correlación directa con el origen geográfico de la planta huésped en esta prueba. Otras variables como rasgos específicos de la planta y defensas deben ser evaluados con más detenimiento para determinar el mecanismo de selección tras el patrón observado. Nuestro estudio indica que se debe tomar precaución al escoger material vegetativo para propósitos de restauración en sistemas de dunas costeras debido a los impactos potenciales en la fauna asociada.

# ABSTRACT

Coastal dunes are large sand accumulations along the shoreline formed by sea currents, surges, wind or vegetation. The vegetation provides dune stabilization, while creating suitable habitats for a large number of organisms including insects, crustaceans, reptiles and shorebirds. *Ammophila arenaria* is a perennial grass that dominates mobile dunes (including both embryonic foredunes and foredunes) along the European shoreline. Because of its easy collection, transplantation, propagation and adaptation to sand dunes, is commonly used in Europe and elsewhere for dune stabilization purposes. The dune aphid *Schizaphis rufula* is a common species associated with this grass along the distribution range of the host plant. Studies to gain insights into the interactions between *A. arenaria* and *S. rufula* are not only necessary to understand basic biological traits of these two species, but can also be useful to guide optimal management strategies on dune ecosystems. The goal of this study was to study the multiplication of *S. rufula* on various genotypes of *A. arenaria* coming from sites within the natural distribution range of this grass species. We also aimed to evaluate whether *S. rufula* prefers local or allopatric genotypes of this grass. Seedlings from Belgium, United Kingdom and Portugal were exposed to one aphid population collected in Belgium. After inoculation, aphids were allowed to multiply and feed on different genotypes of the plants for 20 days. Aphid numbers were used to generate a population growth curve for each aphid-plant population combination. Growth dynamics were compared between *A. arenaria* populations using a one-way ANOVA. The four plant-herbivore combinations tested revealed significant differences in multiplication among populations, with higher aphid reproduction on local (Belgium) populations and plants from Wales, United Kingdom. Therefore, plant population origin does influence aphid multiplication; nonetheless, no direct correlation with host plant geographic origin was discovered in this assay. Other variables such as specific plant traits and defences must be further assessed to determine the mechanism behind the observed pattern. Our study indicates that caution should be taken when choosing plant material for restoration purposes in coastal dunes due to the potential impacts on the associated fauna.

# CHAPTER I

# INTRODUCTION

## Background of the problem

Dunes are large sand accumulations along the shoreline formed either by sea currents, surges, wind or vegetation (Haslett, 2009). Certain plant species and animals are adapted to dune conditions and colonize them as they form through saltation and aeolian processes. These processes have been defined as large inputs of sand and active wind displacement (Herrmann, Durán, Parteli, & Schatz, 2008). Generally, dunes are found in an active or stabilized state. Dunes in an active state, also referred to as dynamic, have loose sediments undergoing constant displacement, which alters its size, form and position. On the contrary, stabilized or impeded dunes, are covered by vegetation, which contributes to a much lower level of sand displacement, loss and tendency of inland migration (DNER, 2010a; Haslett, 2009).

In Europe, cemented dunes, are the only natural means of shoreline reinforcement, while in the tropics, coral reefs, thalassia beds and mangrove forests provide additional coastal protection against tidal surges, hurricanes and hurricane-generated waves. They also prevent storm waves from flooding inland higher elevation zones along the coast (Miller, Thetford, & Yager, 2001).

Dune recuperation is a very slow process that occurs naturally over time. In the occurrence of severe damage, management becomes imperative in order to accelerate dune reconstruction. During recent years, dune restoration projects have been directed towards the development of new reinforcement techniques, such as gate systems and the identification of appropriate plant species for sand stabilization. The most common approaches methods are sand fencing to enhance sand accumulation and planting of native grasses for natural restoration and promotion of secondary succession (DNER, 2010a; Miller et al., 2001).

A major threat currently faced by dune systems, stems from large scale operations that extract sand for construction purposes. Sand removal by unnatural causes exacerbates the process of erosion and contributes to saline intrusion in fields and aquifers that were once protected from the effects of rough seas (Valeiras, 2007). This phenomenon was documented by both Valeiras (2007) and the Department of Natural and Environmental Resources (DNER, 2010b) in Puerto Rico, most frequently in Isabela, Hatillo, Camuy, Arecibo, Barceloneta and Loíza. Of these locations, the dune formations at Isabela and Loíza contain the largest accumulation of sand on the north shore of the island (DNER, 2010a). In Europe, sand is extracted from the continental shelf (water depth < 60 meters (m)) every year, affecting innumerable coastal processes and marine habitats, including the ocean sea bed, benthic organisms, and waves and currents which alter sediment transport and accelerate erosion, accretion and coast retreat (Krause, Diesing, & Arlt, 2010; Kortekaas, Bagdanaviciute, Gissels, Alonso-Huerta, & Héquette, 2010). The Baltic Sea in particular, which connects to the North Sea, contains various sites for sand extraction that have been exploited for more than a century. The extracted sand is used for industrial purposes, construction and beach nourishment, especially large quantities of sand that are removed and used for coastal defense purposes. This process of sand extraction from the active beach profile, known as draw-drown, causes a net loss of available beach material (Kortekaas et al., 2010).

Rapid coastal development on the shoreline also contributes to the degradation and destruction of coastal dune areas. Reconstruction and restoration of these systems represents a challenging management task after the zone has been dominated by resorts and high-cost residential complexes. New dunes, undergoing any restoration process, will tend to be small in relation to pristine dune mounds that could reach up to 98 feet, documented first in Puerto Rico before overexploitation. Restored dunes will likely never be able to return to the typical linear formation that characterizes them naturally (DNER, 2010b; Antunes do Carmo, Schreck Reis & Freitas, 2010).

Dunes are created and maintained naturally by the vegetation that covers them. Vegetation associated with dune systems is generally located inland, above the upper tidal zone limits (Lomba, Alves, & Honrado, 2008). The vegetation serves as dune stabilization, while creating habitats for a large number of organisms that might include insects, crustaceans, reptiles and shorebirds (Haslett, 2009). Plant colonizers or pioneers, are herbaceous halophytes which can forage and are resistant to sand displacement, water scarcity and high salinity. This type of vegetation is important because it traps and compacts moving sand on the dunes (Acosta, Ercole, Stanisci, Pillar, & Blasi, 2007). Some of these species provide shade, reducing the evaporation of moisture from the soil surface and creating particular conditions for new species establishment. The extensive root systems of plant species that colonize dunes hold sand in its place. Moreover, this vegetation allows the dunes to continue accumulating sand over a long period of time, contributing to its solidification. Vegetation assists trapping the sand particles, while their deep fibrous roots provide stabilization (Lonard & Judd, 2011; Miller et al., 2001).

Sarre (1989) reported that the vegetation helps decrease the speed of wind near the surface, and causes deposition of the sand being transported when it encounters the vegetation. The presence of vegetation therefore decreases the rate of dune deflation (Judd, Summy, Lonard, & Mazariegos, 2008). Among the vegetation that contributes to sand accretion and coastal protection we can find sea oats *(Uniola paniculata)*, bitter panicum grass *(Panicum amarum)* and its variations and the American marram grass or American beachgrass *(Ammophila breviligulata),* native to eastern North America along the Atlantic Ocean and the Great Lakes coasts (Lonard & Judd, 2011; Miller et al., 2001).

Exclusively in Europe, *Ammophila arenaria* is a perennial grass that can be found dominating mobile dunes, including both embryonic foredunes and foredunes (Lomba et al., 2008). It is the most commonly used for this purpose because of its easy collection, transplantation, propagation and adaptation (Antunes do Carmo et al., 2010).

Marram grass *Ammophila arenaria* (L.) Link is a dominant grass species of dynamic coastal dunes and it is naturally distributed along all European coasts south of latitude 63 degrees North (°N) (Huiskes, 1979). The species has been introduced in several areas of the world for dune stabilization (i.e. East and West coasts of USA, South Africa, Australia, New Zealand and Chile) where in some cases has become highly invasive. Hilton, Duncan, and Jul (2005, p. 175 & 184) reported that "*Ammophila* invasion of active dune systems […] is clearly associated with the dune forming processes […] by stabilizing naturally mobile dunes and accelerating vegetation succession." The interactions occurring in the rhizosphere of this dune grass and the aboveground herbivore arthropod community form one of the best documented cases of the role of multitrophic interactions on plant community succession and dynamics (van der Putten, Vet, Harvey, & Wackers, 2001; Zoon, Troelstra, & Maas, 1993).

**Statement of the problem**

Strong prevailing winds, usually coming from high-energy ocean swells, transport massive quantities of particles and sediments that lead to the formation of dune-barriers on the coast (Short, 2010). Specifically, *Ammophila arenaria* play an important role on dune formation by its dune fixation capacity, trapping the sand blown by these winds. Though it provides such an important ecological service, *A. arenaria* has been documented as introduced and invasive across the world and furthermore, capable of the displacement of entire native plant communities and drastically reducing their natural habitat (Vandegehuchte, 2010a). Among the herbivore community associated with this grass, we find the dune aphid *Schizaphis rufula* Walker 1849.

Globally, aphids are major insect pests, causing significant economic loss to markets in the production of crops such as grains, vegetables, fruits, flowers and wood (van Emden & Harrington, 2007). As documented by Vandegehuchte, de la Peña and Bonte (2010b), *S. rufula* has been recently discovered in sand dune areas in Belgium, Europe and is known to feed and live specifically on the leaves of *A. arenaria* grass. Studies in the laboratory, demonstrates how yellowish-brown leaves can serve as an indicator of aphid infestation on the plant, leading it to its death.

Currently, much is still unknown regarding the interaction between *Schizaphis rufula* and *A. arenaria* (de la Peña, personal communication, April 2011). Given the wide geographical range of distribution of *Ammophila arenaria* and the aphid *S. rufula* the occurrence of genotypic variation and performance differ among the different non-overlapping geographical areas (de la Peña, personal communication, April 2011). Studies to gain insight in the interactions among the *A. arenaria* and *S. rufula* are necessary to optimize management strategies on dune ecosystems.

**Justification**

Dune restoration and dune building is a common challenge for littoral areas under natural and anthropogenic pressure. Changes in the system are often evaluated as a whole, sometimes overlooking the importance of the interactions occurring in the vegetation that geographically adapts to them. Plant associations within a (bio) geographical area can serve as a basis for bioindication in monitoring and management of coastal dune systems (Lomba et al., 2008). Acosta et al. (2007) reported how methodical studies of the interactions occurring on plant communities adapted to coastal ecosystems contributes to the understanding of variables such as dune morphology, vegetation zonation, local adaptation, plant invasion and herbivore defences. A gap still exists, however, in the understanding of adaptation to the local environment, herbivory and plant traits in ecological terms (Bischoff & Trémulot, 2011).

Understanding the role of geographic variation and plant genetic differentiation in the interaction between *A. arenaria* and its associated herbivores (i.e. dune aphid: *Schizaphis rufula*) is necessary to understand the functioning of foredunes, improve dune management and understand the invasive character of the species outside its natural range (de la Peña, personal communication, April 2011).

**Research Question**

How is aphid performance on different *Ammophila arenaria* grass populations?

**Goal**

Evaluate the interaction between the dune aphid *Schizaphis rufula* and its host-plant *Ammophila arenaria*: a comparison of insect multiplication on different host-plant populations

**Objective**

Compare multiplication of the dune aphid *Schizaphis rufula* on different Atlantic genotypes of *Ammophila arenaria;* to evaluate if this aphid *S. rufula* multiplies better on sympatric populations than on populations from other geographic areas (allopatric).

# CHAPTER II

# LITERATURE REVIEW

## Historical background

Dunes are large accumulations of sand deposited by wind and tidal surge on the high-tide zone, on which environmental conditions favor the establishment of floristic associations (Valeiras, 2007). Dunes, as permeable structures, generally protect the shorelines from flooding, allowing the groundwater system to be recharged while creating a barrier for saltwater intrusion (Carter, 1991). Among the impacts that affect the dunes as critical habitat for numerous organisms, those that prevail are human impacts such as destruction, sand extraction, excessive touristic and recreational activities, coastal development and coastal erosion (Acosta et al., 2007; Valeiras, 2007).

Since the past 100 years, a new threat is affecting the world's dune coastal ecosystems. Little or no attention is being directed toward sea level rise and global warming issues. Furthermore, studies investigating responses of dune vegetation to these factors remains inconclusive. On its Fourth Assessment (AR4), the Intergovernmental Panel for Climate Change (IPCC) reports how sea level is expected to rise 0.18 meters (m) between the years 1985 and 2030 (IPCC, 2007). According to Carter (1991), sea level rise directly affects the coastal processes that naturally stabilize the shoreline by changing wave patterns, accelerating coast erosion, flooding, avulsion and altering sediment fluxes. Coastal ecosystems worldwide are under threat due to these phenomena. Areas under the most danger are located in low-lying countries like The Netherlands and islands such as Puerto Rico, since their dune areas are on the shore and are therefore, the first systems to be affected by changes offshore (Noest, 1991). Currently, as a proactive measure against sea level rise and climate change, The Netherlands are dredging more than 18 million cubic meters of sand from the bottom of the North Sea and pouring it onto the new coast band, creating a new dune system, broadening the beach and gaining territory. Although the coast is safe under current sea level conditions, this technique is being developed as a management strategy to protect the coast at the levels projected fifty years from now (Rijckaert, 2009).

As mentioned before, natural vegetation on dune ridges is crucial for the conservation of the dune morphology as the vegetation traps blowing-sand, protecting the shoreline from erosion (Acosta, Ercole, Stanisci, Pillar & Blasi, 2007). It is believed that *Ammophila arenaria*, as a $C_3$ photosynthetic plant that succeeds within moderate sunlight intensity and moderate temperatures, will be directly affected by global warming. These plants, already identified as invasive species in some locations, will tend to grow faster as temperatures continue to rise and groundwater and $CO_2$ patterns are altered in their respective cycles (Carter, 1991; Ricklefs, 2008a).

The dune aphid *Shizaphis rufula* is a common species associated with *Ammophila arenaria* grass along the distribution range of the host plant. Studies to gain insight into the interactions between *A. arenaria* and *S. rufula* are necessary not only to understand basic biological traits of these two species but can be useful to guide future management strategies on dune ecosystems. *S. rufula* is a specialized herbivore species known to live and infest the leaves of *A. arenaria*. However, it was neither discovered nor described in Belgium until 2007, after a field survey conducted by Vandegehuchte, de la Peña &

Bonte (2010c). It can be found across Europe, including Britain, Corsica, Denmark, Finland, Germany, Ireland, Poland, Sicily, Sweden, the Netherlands and Ukraine.

Little is still known about *S. rufula*'s dynamic and it is thought that, *A. arenaria*'s displacement may affect the aphid presence on the grass. The aphid seems to prefer young, vital shoots (seedlings), and in addition, it has recently been discovered that belowground interactions also affects its performance (Vandegehuchte et al.; 2010c).

**Conceptual/theoretical framework**

*a) Ammophila arenaria*

*Ammophila arenaria* is known to be the major dune-forming grass. The Gauls, a group from ancient Western Europe (modern North Italy, France, Belgium and South Netherlands) were the first to utilize this species of grass. They adapted dune seeding programs to protect their capital cities from being flooded with sand in 600 B. C. (Green, 1965). Sand fixation was recorded in 1316 in Germany (Withfield & Brown, 1948) where *Ammophila* was used to stabilize main dune complexes, and this practice was reported in the 17$^{th}$ and 18$^{th}$ centuries as well (Vandegehuchte, 2010a).

*Ammophila arenaria* is native to coastal areas and abundant on mobile and fixed dunes where vegetation has developed, creating suitable substrate. Among the most common subspecies we can find the North American spp. *breviligulata*, native from sand dunes along the Atlantic coast of North America, the Great Lakes and also found in Newfoundland. It is believed that spp. *breviligulata* was introduced on the British Isles around 1953 (Huiskes, 1979).

Atlantic populations of *Ammophila arenaria* spp. *arenaria* can be found throughout the coast of the North Sea, the Baltic and the Atlantic to northern Portugal. On the other hand, *Ammophila arenaria* spp. *arundinacea* ranges from central to southern Portugal, the Mediterranean and the Black Sea, being defined as a Mediterranean population (Huiskes, 1979).

*A. arenaria* species are classified as perennial, meaning that they can grow and produce inflorescence from the same root over the course of several years. Perennials can die off-season and grow again bigger and stronger after this cycle (Alderson, 2012). Specifically for *Ammophila*, growth is slower in winter, while the leaves start growing vigorously during spring and summer, dying in autumn (Huiskes, 1979).

Clonal spread, or asexual reproduction developed by interconnected rhizomes, is more successful for *Ammophila*'s reproduction than seedling dispersal. Dispersed seeds can desiccate easily, or the transport can be affected by sand burial and erosion, not allowing the establishment of the population. In some cases, when reproduction is successful, a single genet can age over a hundred years (Huiskes, 1979).

It is common to find *Ammophila arenaria* along the Belgian coast of Europe; specifically in the north-west geographical area defined as the coastal plains. North-west flat coastal plains are characterized by dunes and polders; land areas close to or below sea level that have been reclaimed by water. Strong prevailing winds, coming from high-energy ocean swells specifically occurring in the North Sea, transport massive quantities of particles and sediments that lead to the formation of dune-barriers on the coast (Short, 2010). These winds are responsible for sand accretion and dune formation on site, propitiating the establishment of this grass.

*Ammophila arenaria* can also be found naturally distributed along all European coasts south of latitude 63°N. The two subspecies, *Ammophila arenaria* spp. *arenaria* and *Ammophila arenaria* spp. *arundinacea*, can be found along this range. The former on the north range, specifically on atlantic dune systems and the latter from Portugal southwards along the Mediterranean and Black Sea coasts (Huiskes, 1979; Vandegehuchte, 2010a).

b) Genetic diversity and local adaptation of *Ammophila arenaria*

Populations are constantly changing, and in nature, those changes are generally determined by size, distribution, structure and genetic composition of the population in question. Limiting factors within populations are commonly dictated by the availability of resources, but occasionally, intraspecific interactions can also contribute to changes in the genetic composition of these populations, confining them to different geographical ranges. It is known that even at small geographical scales, plant species have demonstrated notable genetic differentiation (Ricklefs, 2008b).

The adaptation of living organisms to their environment is a function that assures the existence and continuation of the species. Established by the Natural Selection Theory, organisms should possess the genetic capability to adapt to the changing environment, while passing critical genetic information on to their offspring to prevent extinction. Evolution traits can also be referred to as evolutionary adaptations, and in addition to gene flow, these are responsible for the introduction of new genes to populations from the same species (Mader, 1990; Ricklefs, 2008c).

Adaptation of plant species to their local environment is a well-documented phenomenon, while little is known about the selection mechanisms of its associated organisms, (i.e. herbivores to their host-plant). These kinds of interactions are important to management projects because they influence the outcomes of treatments like the introduction of non-local genotypes for restoration or the re-vegetation of areas of concern such as dune systems (Bischoff et al., 2011).

De la Peña, Bonte, and Moens (2009) reports how population differentiation and local adaptation on plants can be triggered by the selection pressure exerted by herbivores. Traits like herbivory defences, as an example, continually change as the plant is subjected to herbivore pressure. Plant defences against herbivory or host-plant resistance (HPR) can provoke the plant to react to functional traits, developing survival strategies such as avoidance of herbivores by changing growth patterns and location. Change of location as a response of HPR, can directly contribute to the evolution of the plant dynamic, such as the developing of different population genotypes. Eventually, HPR can determine the geographic structure of the population and its herbivore community (de la Peña et al., 2009; O'neal & Hodgson, 2008). This geographic structure, in combination with the herbivore selection pressure, is the variable responsible of genetic differentiation and ultimately, local adaptation of different plant populations (de la Peña et al., 2009).

*Ammophila arenaria* dispersal mechanism is primarily clonal growth. The word "clonal" in the term "clonal growth" implies that the individuals will descend from the same "parent", bearing the same genotype (Ricklefs, 2008d). This type of dispersal represents low intraspecific gene flow among populations, "creating large genetic distances between geographically separated populations" concludes de la Peña et al. (2009). Meanwhile, Rodríguez-Echevarría, Freitas, and van der Putten (2008, p. 125+) explains how "the molecular characterization of A. arenaria populations indicate that there are marked genetic differences between populations separated by large geographical distances." Nonetheless, more studies needs to be performed to support these statements.

A more in-depth research study by Rodríguez-Echevarría et al. (2008) indicated that populations from Belgium, England, France and Portugal presented minimal genetic diversity, while the lowest diversity values were found on the Netherlands and Southern Portugal populations. It must be mention that populations from England, Wales and the Netherlands were classified as *Ammophila arenaria arenaria* (Tutin et al., 1980), while populations from Portugal and Mediterranean France were from the southern subspecies *Ammophila arenaria arundinace* (Tutin et al., 1980), which still indicates genetic differences among populations. The study suggest how even when genetic differentiation can still be inconclusive, results are not always correlated with geographical distance (Rodríguez-Echevarría et al., 2008).

c) Aphid *Schizaphis rufula* growth dynamic

When working with dune aphid *Schizaphis rufula*, herbivore of *A. arenaria*, the collection of just a few individuals results enough, since it is a specialist aphid species which mostly multiplies by apomictic parthenogenesis. Parthenogenesis, as defined by Ricklefs, 2008d, (p.162) is "the asexual female reproduction without fertilization by male gametes, usually involving the formation of diploid eggs whose development is initiated spontaneously." It is believed that for Aphidoidea, *S. rufula*'s superfamily, this reproduction system evolved in the Triassic period. Parthenogenetic females used to lay eggs, similar to other modern parthenogenetic species, but they developed the ability of viviparity, or giving birth to live offspring that have developed within the mother's body. Parthenogenesis provides *S. rufula* the capability of rapid dispersal and plant infestation (Blackman & Eastop, 2007).

Aphids can be morphologically recognized by their piercing-sucking mouthparts, which penetrate the phloem tissue of herbaceous plants in order to extract sugars and other nutrients. Mostly, they can be found on the undersides of leaves and stems. Densities can range from low to high, creating persistent colonies in approximately 2 days, multiplying the size of the population up to hundreds of individuals. Aphid infestation affects plant fitness, including height and photosynthesis and it is common to observe them utilizing the cauda or tail to remove the honey-dew produced from their anus, while feeding. This honey-dew, or sugary excretions, makes the plant prone to mold, therefore having a debilitating effect on it (Tilmon, Hodgson, O'neal, & Ragsdale, 2011; Blackman & Eastop, 2007).

Like every individual that compose it, any population will dynamically grow until achieving an equilibrium point. The equilibrium point or the carrying capacity of a population is achieved when densities increase until the maximum number supported by the habitat is reached. It is believed that after reaching its capacity, population-crash follows. This concept was made widely popular by the economist Thomas Robert Malthus in 1798 (Smith, 1966). Aphid's population growth can usually be represented with the SIGMOID method, an S-shaped curve which describes growth of the population over time. It is common to utilize this method under controlled experimental systems where populations are kept and growth under confinement (Colinvaux, 1986).

d) Management strategies and conservation

A clear understanding of the *Ammophila* system is crucial for restoration projects all over the world, especially for coastal conservation and regeneration projects. When introducing a grass for sand compaction processes it is necessary to know exactly where a population originates and the location where the sample seeds where collected. Usually, plant material should be collected locally, but in some cases non-local plants are introduced. There is limited information available related to the introduction of non-local *Ammophila* populations. Local plant material should be preferred over imported material, to provide a bigger genetic pool that could help optimizing transferred grasses and enhance resistance (Rodríguez-Echevarría et al., 2008).

**Case studies**

a) Evidence of population differentiation of *Ammophila arenaria* dune grass and its associated root-feeding nematodes

The entire arrangement of plant populations and communities within the landscape can be determined by soil composition, occasionally resulting in local adaptation of those communities. De la Peña et al. (2009) worked with nematodes, parasitic unsegmented roundworms from different species and geographical locations throughout Europe. Nematodes can affect soil dynamic and consequently, the nutrient cycle and availability. *Pratylenchus brzeskki* and *Pratylenchus dunensis* are two species of endoparasitic nematodes that can be found associated with *A. arenaria* in coastal dune ecosystems throughout Europe. Sixteen plant-herbivore interactions between these nematodes and the *Ammophila arenaria* grasses coming from the same nematode-location were studied. Belowground-herbivore response information, in term of growth and multiplication were collected. In most of the cases, it is expected from the plant-herbivore combination coming from the same location to adapt to its local conditions. However, variation can occur, and it was found that nematodes growth development on sympatric host was adverse, meaning success of local combinations should not be assumed. Nematodes also had a negative effect on plant biomass growth, and overall, the outcome among combinations ended up being case-dependent.

b) Aphid-transmitted viruses: use of barrier plants as a management tool

Aphids are known to function as major virus vectors, specifically of the "aphid-borne non-persistent transmitted viral diseases" (ABNPV) which cannot be controlled by insecticides. A study done by Hooks and Fereres (2006) using barrier plants as management tools, explains that aphid virus transmission accounts for 50% of the 600 known viruses transmitted by invertebrates. Factors such as host-plant selection, visual stimuli and color attraction influence on the aphids preference to inhabit and feed on a plant. It is believed that the transmission of viruses by aphids can be controlled by plant resistance that might have been developed genetically. Power (1991), reported that even when aphids were abundant on different oat habitats, incidence of the transmitted *Barley yellow dwarf virus* (BYDV) was consistently lower on genetically diverse oat plantings, reducing virus transmission. More in-depth studies of the environmental parameters that limit aphid's effects on crops are still needed.

c) Population genetic structure of economically important Tortricidae (Lepidoptera)

In a study by Timm, Geertsema, and Warnich (2010), the population genetics structure of the codling moth *C. pomonella*, the litchi moth *Cryptophlebia peltastica*, the macadamia nut borer *Thaumatotibia batrachopa* and the oriental fruit moth *G. molesta* were studied. The analysis of population genetics can provide insights of the structure and arrangement of these populations. DNA extractions were obtained from the specimens and analyzed using the amplified fragment length polymorphism AFLP. It was found that all of these four economically important pests from the Tortricidae family have the same genetic structure, not only regional but it can even be found on isolated populations along a local geographical setting. It is suggested that this outcome was a result of limited dispersal ability, which in this case, can be a positive response in relation to pest management strategies. Timm et al. (2010) concludes reporting how insecticide use and anthropogenic movement could also be limiting these populations, where in most of the cases they act sedentary and choose to stay near their habitat or orchard.

**Legal frame**

In this study, since our sites and related organisms are directly linked to the European coastal zone, we take into consideration, the legal dictations of dune ecosystem management in Belgium, and more generally Europe. The grass of interest, *Ammophila arenaria*, can be found dominating dynamic coastal dunes, naturally distributed along all European coasts south of latitude 63 degrees North (°N) (Huiskes, 1979). *Schizaphis rufula*, *A. arenaria*'s associated herbivore, has recently been discovered on sand dunes in Belgium, Europe and is known to feed and live specifically on the leaves of this grass (Vandegehuchte, de la Peña, & Bonte, 2010b).

Model Law on Sustainable Management of Coastal Zones and European Code of Conduct for Coastal Zones (Council of Europe, 2000).

A group of Specialists on Coastal Protection, under the order of the Committee of Ministers of the Council of Europe, met for the first time in 1996 where they discussed how a high amount of technical and scientific research had been performed in the field. Still, it implied the need of integrated management and planning of coastal areas, the definition and implementation of these concepts and instruments for sustainable coastal management. Therefore, the Council of Europe, in efforts with the European Union for Coastal Conservation (EUCC) and the United Nations Environment Programme (UNEP) proposed to take action and prepared drafts for the code of conduct and a Model Law on coastal protection.

The code of conduct draft needed to include recommendations, principles and defined rules for local, regional and national authorities, developers, coastal engineers and users. Likewise, the Model Law needed to define the concept of integrated management based on sustainable development, while establishing guidelines and instruments for it appropriate application. As a result, it was expected that the preparation of these documents led to, either amend legislation or the creation of new laws and acts on coastal zones, land use planning, conservation and decision-making.

The Council of Europe Ministers adopted the document on April 1999. It is intended to provide management guidance strategies to the local authorities and also, to the commercial sector. Assessment for the management of direct threats such as habitat destruction and indirect threats such as habitat degradation and health impacts on wildlife and humans are included in these documents (Council of Europe, 2000).

I. Model Law on Sustainable Management of Coastal Zones

a) Definitions

Title 1, Article 1 - First of all, the Law starts defining the two most common terms used throughout the document. The coastal zone is defined as "a geographical area covering both the maritime and the terrestrial part of the shore, including salt-water ponds and wetlands in contact with the sea". The document aspire all territorial waters and public territories bordering seas and oceans to be covered by this law, including estuaries and deltas.

Article 2 - Integrated management is defined as "sustainable development and use of coastal zones which takes into consideration economic and social development linked to the presence of the sea while protecting landscapes and the coastal zone's fragile biological and ecological balances for present and future generations".

Title 2, Article 8 - Among the principles stated in the law concerning directly to threatened ecosystems such as dune ecosystems, it is suggested that fragile areas and threatened ecosystems (covered in Title 12) should be protected, restricting access, in some cases.

b) Dunes and vegetation cover

Title 9, Article 48 – "Vehicular traffic, movement and parking of motor vehicles and mountain bikes on dunes and beaches shall be prohibited, except in areas and trails provided for that purpose".

Title 12, Article 60 - "Dunes shall be classified as sensitive zones, or as nature reserves. Access may be restricted and special soil stabilization measures shall be taken using biological methods and preserving herbaceous or tree cover."

Title 13, Article 65 - “Parts of coastal zones where the soil and coastline are fragile or prone to erosion shall be classified as critical zones. Access may be prohibited and specific stabilization measures shall be taken. Buildings and other structures, recreational facilities, roads and car parks shall be prohibited in these areas”.

Article 66 - Coastal woodlands shall be classified in order to prevent their destruction and protect their plant cover and its soil-stabilizing role. Cutting down or uprooting plants which also help to stabilize soil shall be prohibited. However, under certain circumstances which may benefit the environment and in furtherance of nature conservation objectives, destabilization and uprooting of may be allowed as a form of dynamic management.

Article 67 - Excavation and sand removal should be regulated. “Submarine prospective and excavation for mining, historical or archaeological purposes and the extraction of sand or gravel from the fringe of the coastal zone or from watercourses shall require prior administrative authorization. Such authorization shall be preceded by an Environmental Impact Assessment and by an opinion from the scientific committee mentioned in Article 28”.

c) Management and public participation

The public should be included and also, participate in the creation of any decision-making process or draft of any concerning coastal zones before any plan is finally adopted. Methods include writing or at public hearings, and their opinions should be considerate, while they have the right to appeal in court. National and regional authorities should work together to create and enforce coastal and marine reserves and furthermore, to develop international agreements.

For this purpose, the Pan-European coastal, ecological and research centre networks were identified to locate significant diversity and to help designate protected coastal and marine areas. In addition, it provides a platform for the exchange of scientific data and information to use as an instrument for public awareness, integrated management and sustainable use of coastal zones.

## II. Analogous laws, regulations, and orders for the maritime-terrestrial zone and dune ecosystem management in Puerto Rico

In Puerto Rico, the general policy declares "to avoid the activities that can cause the deterioration or destruction of the natural systems that are critical for the preservation of the environment, such as... dunes" (DNER, 2008, p.30.3).

a) Law number 132 of June 25, 1968, Sand, gravel and stone act (Ley de arena, grava y piedra) (DNER, 2008).

b) Law number 241 of August 15, 1999, New wildlife law (Nueva ley de Vida Silvestre).

c) Regulation number 6916, Regulation for the extraction, excavation, removal and dredging of earth crust components (Reglamento para regir la extracción de materiales de la corteza terrestre) (DNER, 2008).

d) Regulation number 4860, Marine spatial planning guidelines for the submerged lands of Puerto Rico (Reglamento para el aprovechamiento, vigilancia, conservación y administración de las aguas territoriales, terrenos sumergidos y la zona marítimo terrestre.

e) Planning regulation number 13 (amended in 2002), Special flood hazard areas (Reglamento sobre áreas especiales de riesgo a inundación) (DNER, 2008).

f) Planning regulation number 17, Zoning regulation for the coastal zones and the access to beaches and coasts of Puerto Rico (Zonificación de la zona costanera y de accesos a las playas y costas) (DNER, 2008).

g) Administrative order number 2-93 (OA-2-93), To establish the public policy on the conservation of the sand resources in Puerto Rico (Para establecer política pública sobre la conservación de los recursos de arena en Puerto Rico) (DNER, 2008).

# CHAPTER III

# METHODOLOGY

The goal of this research was to evaluate the interaction between the dune aphid *Schizaphis rufula* and its host-plant *Ammophila arenaria* by making a comparison of insect multiplication on different host-plant populations. We established the objective of analyzing the multiplication of dune aphid *Schizaphis rufula* on different atlantic genotypes of *Ammophila arenaria* grass to evaluate if dune aphid *S. rufula* multiplies better on its local *Ammophila arenaria* population than on *Ammophila* populations from other geographic areas.

The study area was located in Ghent, Belgium and for the experimentation, the system was replicated in the Laboratory under controlled conditions. Support for materials and internship was provided by the José Jaime Pierluisi Foundation and the CES-PRIDCO – P uerto Rico Council on Higher Education fellowships, altogether. For Ghent University acceptance letter, please refer to Appendix 2.

## Seeds handling

Experimental design: Acquisition

Three Atlantic and one Mediterranean population were used for this research. The subspecies *Ammophila arenaria* spp. *arenaria* was defined as the "local" variation, with two populations from Belgium and one from the United Kingdom. One population of *Ammophila arenaria* spp. *arundinacea,* from Portugal was cultivated, defined as Mediterranean.

We performed a laboratory replication of the grass-dune aphid system, using samples from both ranges. Therefore, we utilized seedlings from:

a) De Panne, Belgium (Local - Atlantic)

b) Westende, Belgium (Local - Atlantic)

c) Ynyslas, Wales, United Kingdom (Atlantic)

d) Comporta, Portugal (Mediterranean)

De Panne and Westende can be found on the north-west coast of Europe, all within an approximate 30 kilometer radius of one another. Ynyslas can be found west of Belgium on the west coast of the United Kingdom, while the Comporta population is the only population sampled from the Mediterranean, which is south of the U. K., on the Portugal's west coast near Spain.

Sterilization

Surface sterilization of the *A. arenaria* seeds was performed following the Sauer and Burroughs Method (1986). Seeds were submersed in 4% household bleach solution for 5 minutes, rinsed 10 times with water, submersed in 10% ethanol for 5 minutes and then rinsed another 10 times with water. This sterilization method effectively eliminates horizontally transmitted fungi that could otherwise colonise the young seedlings.

Transplanting

Seeds were subsequently germinated at a light regime of 9/15 hours dark/light in plastic trays filled with 190 cubic centimetres ($cm^3$) of sterile dune sand that was autoclaved for 1 hour at 120 degrees Celsius (°C) and 1 atmosphere (atm). The sand was saturated with water. Twenty-four days after the seeds were placed on the sand to germinate, seedlings were selected and transplanted into 20 1-liter (l) plastic pots filled with 550 grams (g) of autoclaved dune sand and placed under a 9/15 hours dark/light regime. Pots were covered with perforated plastic caps to maintain moisture and allow sufficient ventilation. Moisture level was reset to near saturation daily. Pots were randomised on the growth bench. Plants were watered every two days and fertilizer (Hoagland's nutrient solution) was applied once every two weeks. This process was performed until seedlings grew enough for them to be able to resist aphid treatment.

**Aphid handling**

For this research, aphids were allowed to reproduce parthenogenically, multiply and become adults on *Ammophila arenaria* host-plants before being utilized as treatment for the controlled experiment. Aphids were obtained from a laboratory culture that was propagated from one wild individual collected from the Westhoek Nature Reserve in De Panne, Belgium. Plants were inoculated with one adult aphid on day 92 after the seedlings were first planted in the pots. Left to reproduce parthenogenically on the plant, aphids were counted daily to provide a population growth curve for each replicate. Previous experiments with this system have shown that populations usually grow to a peak density after which aphid numbers rapidly decline (adapted from de la Peña & Vandegehuchte, 2010).

**Data analysis**

Values obtained were checked for normality and heteroscedasticity, which determines if variance in the group was the same among the data identified by a normal distribution. Aphid numbers were used to generate a population growth curve for each replicate. *S. rufula* growth dynamics were compared between *A. arenaria* populations using a one-way ANOVA.

# CHAPTER IV

# RESULTS AND DISCUSSION

The principal objective of this research was to evaluate interaction dynamic of dune aphid *Schizaphis rufula* on different Atlantic genotypes of *Ammophila arenaria;* to evaluate if *S. rufula* multiplies better on sympatric populations than on populations from other geographic areas (allopatric). The necessity of the research derives from the lack of knowledge about the genetic diversity of *Ammophila arenaria* grass populations and *S. rufula*'s adaptation to them. The recent discovery of the association of this specific aphid species with the grass has triggered numerous questions regarding selection mechanisms, local adaptation, genetic traits, above-belowground herbivory and plant defense.

For this specific research, aphids were left to reproduce parthenogenically and were counted daily to collect multiplication data (Table 1). After 20 days of aphid infestation on *Ammophila* seedlings from 4 different plant populations, daily figures for individual plants were gathered. Table 1 demonstrates quantification dates, aphid presence and aphid quantity for every plant; standard deviation of aphid numbers is also represented.

As discussed in Chapter II, like every individual that compose it, any population will dynamically grow until achieving an equilibrium point. The equilibrium point or the carrying capacity of a population is achieved when densities increase until the maximum number supported by the habitat is reached. It is believed that after reaching its capacity, population-crash follows (Colinvaux, 1986). It is evident that aphid numbers exponentially grew for every population, achieving the equilibrium point around day fifteen. After increasing to its maximum supported density, populations declined and plants were uprooted. Aphid numbers were used to generate a population growth curve for each replicate (Figure 1). *S. rufula* growth dynamics were compared between *A. arenaria* populations using a one-way ANOVA.

The 4 plant-herbivore combinations tested revealed significant differences among populations, with preliminary results demonstrating more successful aphid reproduction on sympatric (De Panne, Belgium) grass populations. Nonetheless, aphid multiplication on plants from Wales, United Kingdom overlaps with Belgium aphid numbers in a specific time lapse (Figure 1). If we take this into consideration, we can conclude that, overall, *S. rufula* performed better on local or less distant populations, compared to populations from Portugal (Vandegehuchte, de la Peña, Breyne, & Bonte, 2011; Vandegehuchte, 2010a). However, it would have been expected that figures from Westende, Belgium would also overlap with figures from De Panne since it is the nearest site geographically, separated by only 15 kilometers (km). This result implies how plant material origin plays a role for the occurrence of *S. rufula* and also, how the outcomes were strongly case-dependent.

No specific support for local adaptation of *S. rufula* was discovered with this study. Comparatively, on a study by van der Putten, Yeates, Duyts, Schreck, Reis & Karssen (2005), with different populations of *Ammophila arenaria* established outside their native range and introduced in USA, South Africa, Australia and New Zealand, it was found that plants supported a comparable number of root-feeding nematodes. Suggestions have arisen about how some species of *A. arenaria* herbivores (i. e. endoparasitic nematodes) can be considered as generalists rather than specialists, which lead us to question the adaptability of these species to *A. arenaria*, despite geographical location. At the same time, the invasiveness of *Ammophila arenaria* must be taken into consideration, as this study clearly presents the grasses' capacity to withstand above and belowground herbivory outside of its native range, all the while establishing successfully (Vandegehuchte, 2010a).

For this specific research, no direct correlation with host-plant geographic location was discovered (Figure 2). A bigger seed pool from an extended geographical range could have provided the possibility to determine a correlation assay. Rodríguez-Echevarría et al. (2008) discussed how there is a necessity to perform research outside the native range of *Ammophila*, because there are previous studies (i.e. Gray, 1985) that suggest how there is ecological variation among populations, but this remark has to be still tested at a continental scale. Results for this particular study suggested that "European populations of *A. arenaria* are genetically diverse and that these genetic differences are not always correlated with geographic distance" (Rodríguez-Echevarría et al. 2008, p. 125). In further support of this statement, Vandegehuchte, de la Peña, and Bonte (2011a) and Vandegehuchte (2010a) discussed how aphids and nematodes could clearly distinguish between different *Ammophila arenaria* plant populations. Conservation issues also arise from this complex scenario, if we take into consideration that different plant genotypes will support different invertebrate species, therefore, the interactions occurring in the rhizosphere of *Ammophila* can indirectly affect the whole coastal ecosystem and its biological structure.

# CHAPTER V

# CONCLUSIONS AND RECOMENDATIONS

## General conclusions

This study evaluated and compared the multiplication of the dune aphid *Schizaphis rufula* on sympatric and allopatric populations of the European beachgrass *Ammophila arenaria*. *Ammophila arenaria* has shown to be a successful dune compacting grass since 1316 (Withfield & Brown, 1948), with rapid establishment capability and the capacity to function as a multifunctional study system for above-belowground herbivory. Other selection factors such as adaptation mechanisms and herbivore defense can also be tested. Despite its invasive characteristic, *Ammophila arenaria* has demonstrated proper fulfillment of an ecological service by sheltering an entire ecosystem - serving as a living skeleton by providing habitat for a diverse ecological community (i. e. herbivores).

The 2007 discovery of the dune aphid *Schizaphis rufula* on *A. arenaria* grass in Belgium has triggered multiple research questions, the answers of which could potentially end up addressing management issues for dune ecosystems worldwide. After analyzing the results for the multiplication behavior of *S. rufula* on different population genotypes of *A. arenaria*, both entomologists and environmental managers should be concerned about the possibility of *S. rufula* successfully colonizing other geographic areas outside of Europe. Aphids in general, have long been known as pests of cereals and grasses, most of them tolerant to modern pesticide treatments (van Emdem & Harrington, 2007). *S. rufula*'s potential to expand outward from its native range should not be underestimated.

A recent study by Meadows (2011) documented not only how "noxious" invasives like *Ammophila arenaria* are dominating the interiors of California, but describes how the grass is opportunistically utilizing its traits and the changes in climate to expand its range. *Ammophila* takes advantage of its light-capturing efficiency and has emigrated into cooler ecosystems, such as the north coast and mid-elevation mountains; areas richest in native grasses, and previously free from exotics. As if the invasion was not enough of a concern, *A. arenaria* shelters the deer mouse (*Peromyscus maniculatus*), which feeds on the seeds of endangered *Lupinus tidestromii*, the Californian coastal lupine. This is a recent example of the negative effects exotics can exert on the environment, especially, on those ecosystems undergoing dynamic and constant change (Meadows, 2011).

**Limitations**

The methodology of this research was performed under controlled laboratory conditions. Hence, life cycle and growth dynamic of *Schizaphis rufula* was easier to understand and assess. However, performing similar methodology *in-situ*, on the different *A. arenaria*'s populations geographical locations sites could have extended not only analysis and results, but my understanding of the interactions occurring at each site. The action of performing the research under laboratory conditions and not on the European dune ecosystems per se, limited the findings and insight of the results. These limiting factors have to be taken into consideration.

**Recommendations**

In terms of the use of *Ammophila arenaria* as the preferred vegetation material for dune fixation and coastal dune protection, caution should be taken when choosing non-local populations for restoration purposes. Local material should always be the primary option, since even the use of slightly geographically separated populations could have a significant detrimental effect on the local invertebrate community, leading to the non-equilibrium of the ecosystem. Often, dune ecosystems are evaluated from a macroscopic perspective, without assessing minor changes in the microscopic ecosystem functions that could also have impacts on species aside from the "indicator species." Interactions within the rhizosphere of *Ammophila arenaria* are so complex, that they can affect the whole plant, while at the same time, these biotic interactions could involve ecosystem costs (Vandegehuchte , 2010a).

In terms of entomological research, other variables such as specific plant traits and defences must be further assessed to determine the mechanism behind the observed pattern (Figure 1) and investigate if it could have possibly affected the analysis. As an example, we believe that host-plant resistance (HPR) has proven to be successful in deterring aphids due to physical and chemical factors of plant tissue that may interfere during probing (stylet penetration) (Goussain, Prado, & Moraes, 2005). Also, we must take into consideration the abrasive characteristic of grasses from the Poaceae family, which can function as primary defense that deters feeding and reduces foliage digestibility, affecting herbivore performance (Massey & Hartley, 2006).

One of the principal reasons this research was possible, as wells as several other investigations of *Ammophila arenaria* systems in Europe, stems from a rigorous legal frame (Council of Europe, 2000). In Puerto Rico, simple management tasks such as charges against irresponsible citizens and petitioning for stricter protection of coastal ecosystems become an uphill battle. Meanwhile, a very different system, Europe's Model Law on Sustainable Management for Coastal Zones and the Code of Conduct for Coastal Zones, could serve as a model for our legal frame. Europe has a long history of managing coastal erosion, protection and land reclamation (i. e. the Black sea, the Mediterranean sea and The Netherlands land reclamation battles) (Pranzini & Williams, 2012). Protection of Belgium's dune ecosystems date back to the 1980's and has shown to be beneficial, not only environmentally but socio-economically.

The recommendations that could be derived from this research emphasize the use of the legal frames of other continents (in this case Europe) as examples to enforce Puerto Rico's analogous laws, regulations and orders (see Appendix 6 & 7). We are losing coastal and marine ecosystems at a very rapid rate and the anthropogenic pressure they are subjected to accelerate the impacts that could eventually lead to the total destruction of the natural resources that compose them. Taking a step forward to accept that our environmental management system lack enforcement, understanding and support from our community is key to minimize and change the detrimental scenario we are currently facing. Involving the community in hands-on experiences and research findings so they can relate to conservation efforts is one way that could possibly enhance the creation of action-oriented techniques. Providing education and actively engaging our community in projects that promote a sustainable shoreline, could positively impact our Island. I trust that, though this research presents a small element in a more integrated project, it could function as a motor for the visualization of a different management approach for the Puerto Rican coastal dune ecosystems.

In the 1970's and 1980's, Puerto Rico's dune ecosystems were healthy and functioned as primary coastal defense for the north shore (DNER, 2010b). Today, the Isabela dune systems are barely an example of what used to sit in their place. This deprives not only our generation from the opportunity of identifying and assessing the diversity of our dune ecosystems, but our children may also be prevented from understanding the components of healthy coastal ecosystems if degradation persists as it is.

With this research, I intend to provide not only methodology for the replication of this study, but also the inspiration to start a movement that could positively mobilize environmental agencies and environmental managers into gaining insight about the plant-insect interactions occurring on the grasses that compact sand dunes around the island; an initiative that could provide enough relevant scientific information to support the enforcement of legal measures and policies that protect our coastlines.

## LITERATURE CITED


Acosta, A., Ercole, S., Stanisci, A., Pillar, V. D. P., & Blasi, C. (2007). Coastal vegetation zonation and dune morphology in some Mediterranean ecosystems. *Journal of Coastal Research*, *23*(6), 1518-1524.

Alderson, L. (2012). *Growers dictionary guide to plant classification*. Retrieved from http://www.the-herb-guide.com/growers-dictionary.html

Antunes do Carmo, J., Schreck Reis, C., & Freitas, H. (2010). Working with nature by protecting sand dunes: lessons learned. *Journal of Coastal Research*, *26*(6): 1068-1078.

Bischoff, A., & Trémulot, S. (2011). Differentiation and adaptation in *Brassica nigra* populations: interactions with related herbivores. *Oecologia*, *65*: 971–981.

Blackman, R. L., & Eastop, V. F. (2007). Taxonomic issues. *Aphids as crop pests*. Electronic version: Cab International.

Carter, R. W. G. (1991). Near-future sea level impacts on coastal dune landscapes. *Landscape Ecology*, *6*(1/2): 29-39.

Colinvaux, P. (1986). Sigmoid growth in laboratory populations. *Ecology*. Canada: John Wiley and Sons.

Council of Europe. (2000). *Model law on sustainable management of coastal zones and European code of conduct for coastal zones*. (Nature and Environment Series N$^{o}$ 101, ISBN 92-871-4152-5). Council of Europe Publishing.

de la Peña, E., Bonte, D., & Moens, M. (2009). Evidence of population differentiation in the dune grass *Ammophila arenaria* and its associated root-feeding nematodes. *Plant and Soil*, *324*: 307-316.

Department of Natural and Environmental Resources of Puerto Rico [DNER]. (2008). Puerto Rico coastal zone management program (Revision and update, August 2008, Executive Summary). San Juan, Puerto Rico.

Department of Natural and Environmental Resources of Puerto Rico [DNER]. (2010a). *Dunas de Arena*. (Tarea 5.1, Educación Ambiental, Aportación Federal: NA08NOS4190468). San Juan, Puerto Rico.

Department of Natural and Environmental Resources of Puerto Rico [DNER]. (2010b). Plan de manejo para el área de planificación especial de Isabela-Aguadilla. San Juan, Puerto Rico.

Goussain, M. M., Prado, E., & Moraes, J. C. (2005). Effect of silicon applied to wheat plants on the biology and probing behavior of the greenbug *Schizaphis graminum* (Rond.) (Hemiptera: Aphididae). *Neotropical Entomology, 34*(5): 807-813.

Gray, A. J. (1985). Adaptation in perennial coastal plants: with particular reference to heritable variation in *Puccinellia maritima* and *Ammophila arenaria. Vegetatio, 61*: 179-188.

Green, D. L. (1965). Developmental history of European beachgrass (*Ammophila arenaria* (L.) Link) plantings on the Oregon coastal sand dunes. Oregon State University.

Haslett, S. K. (2009). Coastal sand dune systems. *Coastal Systems*. New York: Routledge, Taylor & Francis Group.

Herrmann, H. J., Durán O., Parteli E. J. R., & Schatz V. (2008). Vegetation and induration as sand dunes stabilizators. *Journal of Coastal Research*, *24*(6): 1357-1368.

Hilton, M., Duncan, M., & Jul, A. (2005). Processes of *Ammophila arenaria* (Marram grass) invasion and indigenous species displacement, Stewart Island, New Zealand. *Journal of Coastal Research*, *21*(1): 175-185.

Hooks, C. R. R., & Fereres, A. (2006). Protecting crops from non-persistently aphid-transmitted viruses: A review on the use of barrier plants as a management tool. *Elsevier*. *120*: 1-16.

Huiskes, A. H. L. (1979). Biological flora of the British Isles. *Journal of Ecology*, *67*: 363-382.

Intergovernmental Panel for Climate Change [IPCC]. (2007). *Climate Change 2007: Synthesis Report*. Adopted section by section at IPCC Plenary XXVII. Valencia, Spain.

Judd, F. W., Summy, K. R., Lonard, R. I., & Mazariegos, R. (2008). Dune and vegetation stability at South Padre Island, Texas, United States of America. *Journal of Coastal Research*, *24*(4): 992-998.

Kortekaas, S., Bagdanaviciute, I., Gyssels, P., Alonso-Huerta, J. M., & Héquette, A. (2010). Assessment of the effects of marine aggregate extraction on the coastline: an example from the German Baltic sea coast. *Journal of Coastal Research, 51*: 205-214.

Krause, J. C., Diesing, M., & Arlt, G. (2010). The physical and biological impact of sand extraction: a case study of the Western Baltic Sea. *Journal of Coastal Research, 51*: 215-226.

Lomba A., Alves P., & Honrado J. (2008). Endemic sand dune vegetation of the Northwest Iberian Peninsula: diversity, dynamics, and significance for bioindication and monitoring of coastal landscapes. *Journal of Coastal Research*, *24*(2B): 113-121.

Lonard, R. I., & Judd, F. W. (2011). The biological flora of coastal dunes and wetlands: *Panicum amarum* S. Elliott and *Panicum amarum* S. Elliott var. *amarulum* (A.S. Hitchcock and M.A. Chase) P. Palmer. *Journal of Coastal Research*, *27*(2): 233-242.

Mader, S. S. (1990). Adaptation and gene flow. *Biology 3rd Edition*. Iowa: Wm C. Brown Publishers.

Massey, F. P., & Hartley S. E. (2006). Experimental demonstration of the antiherbivore effects of silica in grasses: impacts on foliage digestibility and vole growth rates. *Proceedings of the Royal Society B, 273*: 2209-2304.

Meadows, R. (2011). Warming climate could boost exotic grasses in California. *The Ecological Society of America Frontiers in Ecology and the Environment, (9)*7: 371.

Miller, D. L., Thetford, M., & Yager, L. (2001). Evaluation of sand fence and vegetation for dune building following overwash by Hurricane Opal on Santa Rosa Island, Florida. *Journal of Coastal Research*, *17*(4): 936-948.

Noest, V. (1991). Simulated impact of sea level rise on phreatic level and vegetation of dune slacks in the Voorne dune area (The Netherlands). *Landscape Ecology*, *6*(1/2): 89-97.

O'neal M. E., & Hodgson, E. W. (2008). Iowa State University. (2008). *Host plant resistance to the soybean aphid*. Ames, Iowa: U. S. Department of Agriculture & Iowa State University.

Power, A.G. (1991). Virus spread and vector dynamics in genetically diverse plant populations. *Ecology 72,* 232–241.

Pranzini, E., & Williams, A. T. (2012). Coastal erosion and protection in Europe. *To be published*.

Ricklefs, R. E. (2008a). $C_3$ photosynthesis. *The economy of nature*. New York: W. H. Freeman and Company.

Ricklefs, R. E. (2008b). Intraspecific competition. *The economy of nature*. New York: W. H. Freeman and Company.

Ricklefs, R. E. (2008c). Adaptation. *The economy of nature*. New York: W. H. Freeman and Company.

Ricklefs, R. E. (2008d). Sexual reproduction mixes the genetic material of two individuals. *The economy of nature*. New York: W. H. Freeman and Company.

Rijckaert, A. (2009). *Dutch construct dunes against rising seas*. United Kingdom, Retrieved from http://www.telegraph.co.uk/expat/expatnews/6613101/Dutch-construct-dunes-against-rising-seas.html

Rodríguez-Echevarría S., Freitas, H., & van der Putten, W. H. (2008). Genetic diversity and differentiation of *Ammophila arenaria* (L.) Link as revealed by ISSR markers. *Journal of Coastal Research*, *24*(1): 122-126.

Rodríguez-Echevarría S., de la Peña E., Moens M., Freitas H., & van der Putten W. H. (2009). Can root-feeders alter the composition of AMF communities? Experimental evidence from the dune grass *Ammophila arenaria*. *Basic and Applied Ecology*, *10*: 131-140.

Sarre, R. D. (1989). Aeolian sand drift from the intertidal zone on a temperate beach: potential and actual rates. *Earth Surface Processes and Landforms, 14*: 247-258.

Sauer, D. B., & Burroughs, R. (1986). Disinfection of seed surfaces with sodium hypo-chlorite. Phytopathology, 76: 745-749.

Short, A. D. (2010). Sediment transport around Australia—Sources, mechanisms, rates, and barrier forms. *Journal of Coastal Research*, *26*(3): 395-402.

Smith, R. L. (1966). Population growth. *Ecology and Field Biology*. New York: Harper and Row Publishers.

Smith, R. L., & Smith, T. M. (2001). Adaptation. *Ecology and Field Biology*. Sixth Edition. USA: Benjaming Cummings.

Tilmon, K. J., Hodgson, E. W., O'neal, M. E., & Rasgdale, D. W. (2011). Biology of the soybean aphid, *Aphis glycines* (Hemiptera: Aphididae) in the United States. *Journal of Integrated Pest Management*. *2*(2): DOI: http://dx.doi.org/10.1603/IPM10016

Timm, A. E., Geertsema, H., & Warnich, L. (2010). Population genetic structure of economically important Tortricidae (Lepidoptera) in South Africa: a comparative analysis. *Bulletin of Entomological Research*, *100*: 421-431.

Tutin, G. T., Heywood, V. H., Burges, Moore, D. M., Valentine, D., Walters, S. M., & Webb, D. A. (1980). *Flora Europaea*. Cambridge: Cambridge University Press.

Valeiras, E. J. (2007). Extracción de Arena en el área de planificación especial de Isabela. *Programa de Manejo de Zona Costanera*, *1*: 8-9.

Vandegehuchte, M. L. (2010a). *Environmental and genetic aspects of aboveground-belowground interactions in the Ammophila arenaria system*. PhD Thesis, Ghent University, Ghent, Belgium.

Vandegehuchte, M. L., de la Peña E., & Bonte D. (2010b). Interactions between root and shoot herbivores of *Ammophila arenaria* in the laboratory do not translate into correlated abundances in the field. *Oikos*, *119*: 1011-1019.

Vandegehuchte, M. L., de la Peña E., & Bonte D. (2010c). Aphids on *Ammophila arenaria* in Belgium: first reports, phenology and host range expansion. *Belgian Journal of Zoology*, *140*: 77-79.

Vandegehuchte, M. L., de la Peña E., Breyne, P., & Bonte D. (2011). Non-local genotypes of a resident grass species reduce invertebrate species richness. *Insect Conservation and Diversity (The Royal Entomological Society)*, DOI: 10.1111/j.1752-4598.2011.00181.x

Vandegehuchte, M. L., de la Peña E., & Bonte D. (2011a). Contrasting covariation of above- and belowground invertebrate species across plant genotypes. *Journal of Animal Ecology, 80*: 148-158.

van der Putten, W. H., Yeates, G. W., Duyts, H., Schreck Reis C., & Karssen G. (2005). Invasive plants and their escape from root herbivory: A worldwide comparison of the root-feeding nematode communities of the dune grass *Ammophila arenaria* in natural and introduced ranges. *Biological Invasions, 7*: 733-746.

van der Putten, W. H., Vet L. E. M., Harvey J. A., & Wackers (2001). Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. *Trends in Ecology and Evolution*, *16*: 547-554.

van Emdem H. F., & Harrington, R. (2007). *Aphids as crop pests*. Electronic version: Cab International.

Whitfield, C. J., & Brown, R. L. (1948). *Grasses that fix sand dunes*. U. S. Department of Agriculture [USDA]. Yearbook, 70-74

Zoon, F. C., Troelstra, S. R., & Maas, P. W. T. (1993). Ecology of the plant-feeding nematode fauna associated with sea buckthorn (*Hippophae rhamnoides* L. ssp. *rhamnoides*) in different stages of dune succession. *Fundamental and Applied Nematology, 16*: 247-258.

# Tables

Table 1.

*Daily populations of aphids on individual plants*

| Dates | Average No. of Aphids | Std Dev of No. Aphids | Count of No. Aphids |
|---|---|---|---|
| **21-07-2011** | **1.4333** | **1.8445** | **60** |
| CP | 1.5833 | 1.8809 | 12 |
| DP | 1.0833 | 1.6765 | 12 |
| ON | 1.6667 | 2.2293 | 12 |
| WB | 1.5000 | 2.0671 | 12 |
| YW | 1.3333 | 1.5570 | 12 |
| **22-07-2011** | **3.4333** | **3.1211** | **60** |
| CP | 4.1667 | 3.6639 | 12 |
| DP | 3.0000 | 3.3845 | 12 |
| ON | 2.5833 | 2.2747 | 12 |
| WB | 3.9167 | 2.5746 | 12 |
| YW | 3.5000 | 3.7050 | 12 |
| **23-07-2011** | **4.5667** | **3.9931** | **60** |
| CP | 4.6667 | 3.2004 | 12 |
| DP | 4.0833 | 4.3788 | 12 |
| ON | 3.2500 | 2.6328 | 12 |
| WB | 5.7500 | 4.0704 | 12 |
| YW | 5.0833 | 5.3506 | 12 |
| **24-07-2011** | **5.9000** | **4.9735** | **60** |
| CP | 5.8333 | 3.7132 | 12 |
| DP | 5.2500 | 5.4793 | 12 |
| ON | 4.8333 | 3.6886 | 12 |
| WB | 6.6667 | 4.7737 | 12 |
| YW | 6.9167 | 6.9995 | 12 |
| **25-07-2011** | **7.1667** | **5.8778** | **60** |
| CP | 7.1667 | 5.0782 | 12 |
| DP | 6.5833 | 6.9079 | 12 |
| ON | 5.8333 | 4.8586 | 12 |
| WB | 8.0833 | 5.4516 | 12 |
| YW | 8.1667 | 7.3588 | 12 |
| **26-07-2011** | **8.2333** | **6.9144** | **60** |
| CP | 8.1667 | 6.5343 | 12 |
| DP | 8.0833 | 8.5754 | 12 |
| ON | 6.2500 | 5.2592 | 12 |
| WB | 9.5000 | 6.2158 | 12 |
| YW | 9.1667 | 8.1779 | 12 |
| **27-07-2011** | **9.3333** | **7.8280** | **60** |
| CP | 9.0000 | 7.0453 | 12 |

| | | | |
|---|---|---|---|
| DP | 9.5833 | 9.6621 | 12 |
| ON | 6.8333 | 5.9212 | 12 |
| WB | 10.6667 | 6.9326 | 12 |
| YW | 10.5833 | 9.5865 | 12 |
| **28-07-2011** | **13.4500** | **10.1103** | **60** |
| CP | 13.3333 | 10.0393 | 12 |
| DP | 15.2500 | 11.6082 | 12 |
| ON | 8.4167 | 5.9001 | 12 |
| WB | 15.8333 | 10.2055 | 12 |
| YW | 14.4167 | 11.6343 | 12 |
| **29-07-2011** | **17.4333** | **14.4085** | **60** |
| CP | 16.5000 | 12.6095 | 12 |
| DP | 22.5000 | 18.7010 | 12 |
| ON | 7.7500 | 5.9563 | 12 |
| WB | 20.5000 | 13.4401 | 12 |
| YW | 19.9167 | 15.5064 | 12 |
| **30-07-2011** | **21.6167** | **20.2167** | **60** |
| CP | 20.1667 | 16.7865 | 12 |
| DP | 27.7500 | 23.5222 | 12 |
| ON | 7.4167 | 6.5845 | 12 |
| WB | 25.8333 | 22.0406 | 12 |
| YW | 26.9167 | 22.6854 | 12 |
| **31-07-2011** | **25.1500** | **25.5667** | **60** |
| CP | 24.5000 | 22.1380 | 12 |
| DP | 31.1667 | 28.8974 | 12 |
| ON | 7.0833 | 6.3455 | 12 |
| WB | 29.5000 | 28.4205 | 12 |
| YW | 33.5000 | 28.9843 | 12 |
| **8/1/2011** | **29.5000** | **31.0541** | **60** |
| CP | 26.5833 | 25.4789 | 12 |
| DP | 37.3333 | 36.6143 | 12 |
| ON | 15.0000 | 24.7533 | 12 |
| WB | 30.8333 | 30.2830 | 12 |
| YW | 37.7500 | 35.6527 | 12 |
| **8/2/2011** | **31.9000** | **31.9293** | **60** |
| CP | 30.2500 | 26.1434 | 12 |
| DP | 42.3333 | 39.6263 | 12 |
| ON | 10.6667 | 8.5102 | 12 |
| WB | 33.6667 | 32.5697 | 12 |
| YW | 42.5833 | 36.6022 | 12 |
| **8/3/2011** | **36.4000** | **34.1369** | **60** |
| CP | 34.2500 | 28.2332 | 12 |

| | | | |
|---|---|---|---|
| DP | 46.6667 | 41.7249 | 12 |
| ON | 15.9167 | 10.6469 | 12 |
| WB | 37.6667 | 35.2067 | 12 |
| YW | 47.5000 | 40.5653 | 12 |
| **8/4/2011** | **42.5000** | **36.3572** | **60** |
| CP | 41.6667 | 30.4581 | 12 |
| DP | 53.0000 | 43.6328 | 12 |
| ON | 21.9167 | 13.6346 | 12 |
| WB | 41.8333 | 37.9876 | 12 |
| YW | 54.0833 | 43.5816 | 12 |
| **8/5/2011** | **35.4333** | **32.3211** | **60** |
| CP | 34.0000 | 25.6267 | 12 |
| DP | 44.8333 | 39.6641 | 12 |
| ON | 16.9167 | 12.0488 | 12 |
| WB | 34.9167 | 32.5505 | 12 |
| YW | 46.5000 | 39.5210 | 12 |
| **8/6/2011** | **29.1667** | **30.4153** | **60** |
| CP | 26.5000 | 24.5672 | 12 |
| DP | 35.5833 | 36.7855 | 12 |
| ON | 13.2500 | 11.2260 | 12 |
| WB | 30.5000 | 29.7581 | 12 |
| YW | 40.0000 | 39.1733 | 12 |
| **8/7/2011** | **19.7333** | **23.5040** | **60** |
| CP | 19.5000 | 21.6900 | 12 |
| DP | 26.9167 | 29.3674 | 12 |
| ON | 6.6667 | 6.3580 | 12 |
| WB | 18.5833 | 20.9044 | 12 |
| YW | 27.0000 | 29.1735 | 12 |
| **8/8/2011** | **11.0000** | **16.1182** | **60** |
| CP | 13.6667 | 18.0722 | 12 |
| DP | 17.5000 | 22.9367 | 12 |
| ON | 2.4167 | 3.6546 | 12 |
| WB | 8.2500 | 9.5644 | 12 |
| YW | 13.1667 | 16.9804 | 12 |
| **8/9/2011** | **1.9000** | **4.4786** | **60** |
| CP | 4.0000 | 7.6277 | 12 |
| DP | 3.7500 | 4.5352 | 12 |
| ON | 0.2500 | 0.8660 | 12 |
| WB | 1.1667 | 3.4597 | 12 |
| YW | 0.3333 | 1.1547 | 12 |
| **Grand Total** | **17.7625** | **24.3873** | **1200** |

*Note*: a) DP: De Panne, Belgium, b) WB: Westende, Belgium, c) YW: Ynyslas, Wales, United Kingdom and d) CP: Comporta, Portugal

# Figures

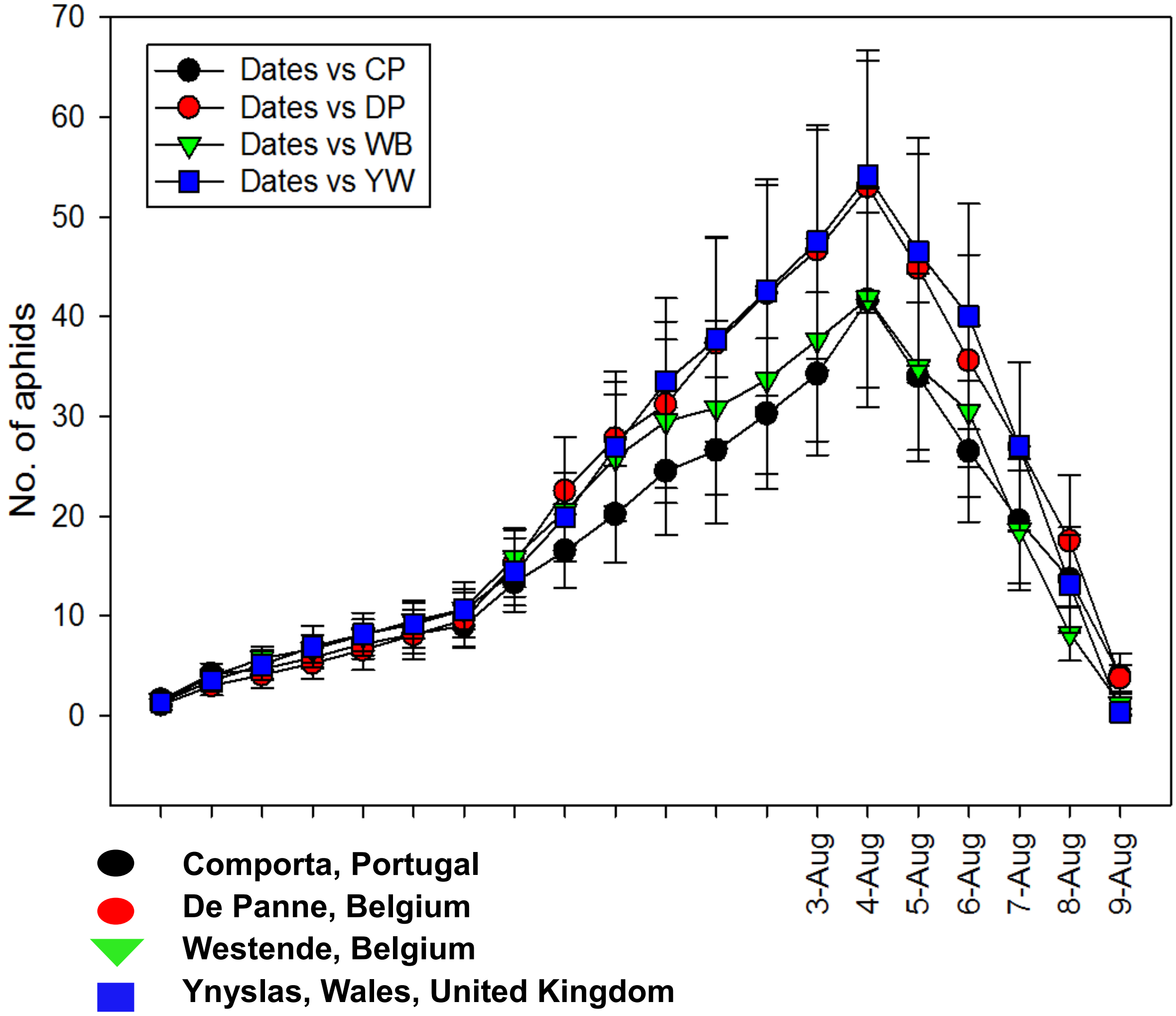


*Figure1*. Aphids vs. time population growth curve. Each curve represents the aphid-plant combination per population locality.


*Figure 2*. Geographical areas demonstrating seed pool collection points:
a) De Panne, Belgium, b) Westende, Belgium, c) Ynyslas, Wales, United Kingdom and d) Comporta, Portugal

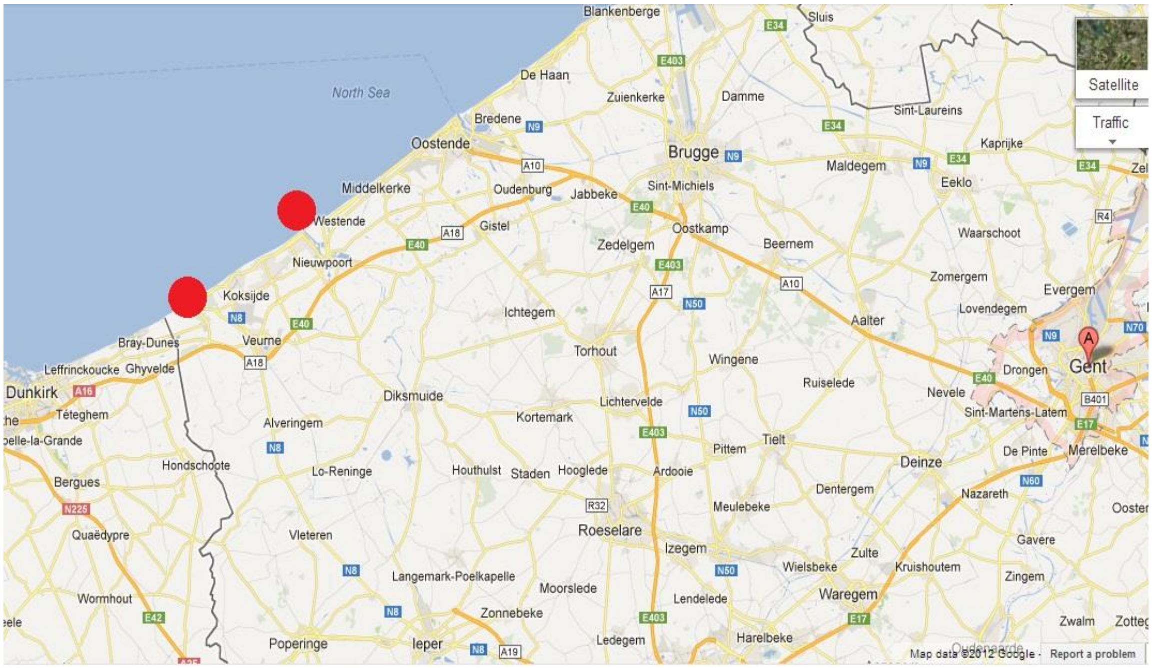


*Figure 3*. Geographical areas demonstrating seed pool collection points Detailed view of the two Belgium localities, Westende and De Panne.

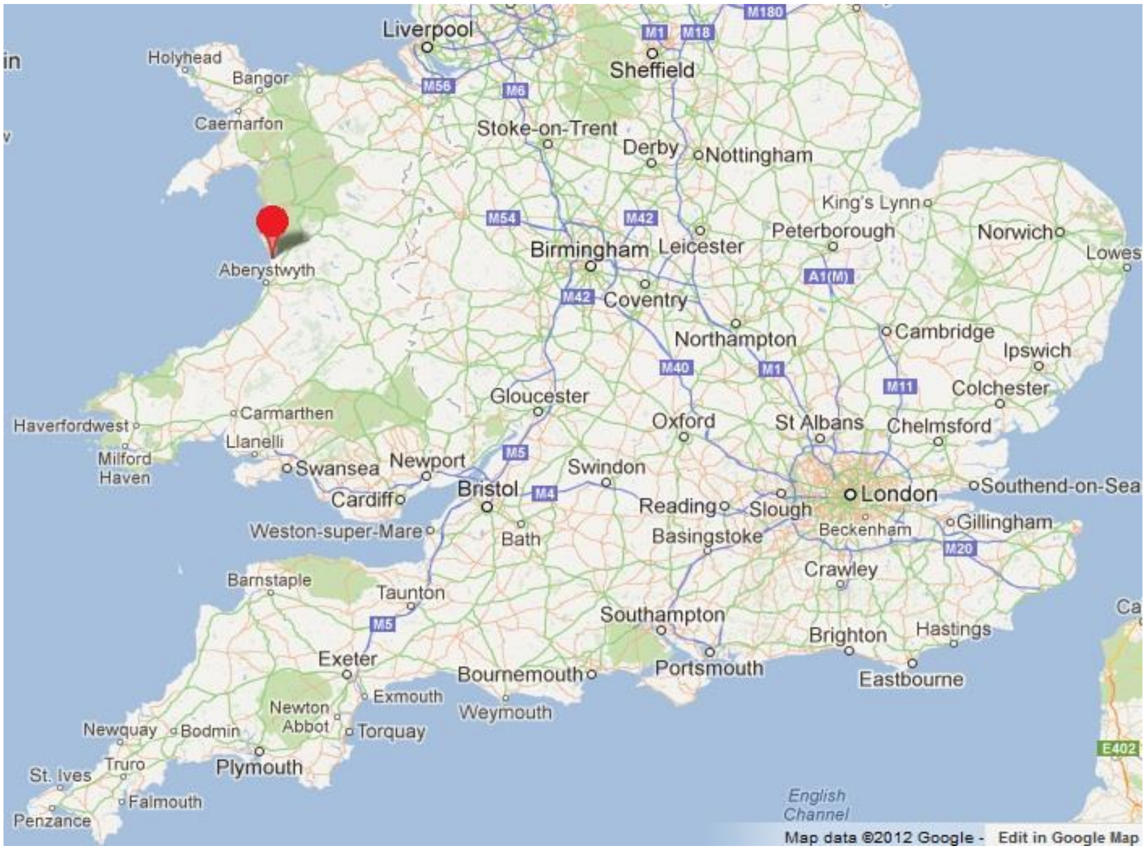

*Figure 4.* Geographical area demonstrating seed pool collection point: Detailed view of Ynyslas, Wales, United Kingdom.

*Figure 5*. Geographical area demonstrating seed pool collection point:
Detailed view of Comporta, Portugal.

# Appendix

Appendix 1. Example of data collection sheet

| | A | B | C | D | E | F | G | H | I | J | K | L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *ILA ARENARIA* : A COMPARISON OF INSECT MULTIPLICATION ON DIFFERENT HOST-PLANT POPULATIONS | | | | | | | | | | | |
| 2 | | | | | | | | | | | | |
| 3 | Observer: | | | | | | | | | | | |
| 4 | Period: | | | | | | | | | | | |
| 5 | | | | | | | | | | | | |
| 6 | Population | Date | | | | | | | | | | |
| 7 | CP | | | | | | | | | | | |
| 8 | DP | | | | | | | | | | | |
| 9 | WB | | | | | | | | | | | |
| 10 | YW | | | | | | | | | | | |
| 11 | | | | | | | | | | | | |
| 12 | | | | | | | | | | | | |
| 13 | | | | | | | | | | | | |
| 14 | | | | | | | | | | | | |
| 15 | | | | | | | | | | | | |
| 16 | | | | | | | | | | | | |
| 17 | | | | | | | | | | | | |
| 18 | | | | | | | | | | | | |

Appendix 2. Ghent University acceptance letter

UNIVERSITEIT GENT

FACULTEIT WETENSCHAPPEN

Vakgroep Biologie
Onderzoeksgroep Terrestrische Ecologie

| uw kenmerk | ons kenmerk | datum |
|---|---|---|
| - | - | 02-10-2012 |
| **contactpersoon** | **e-mail** | **tel. en fax** |
| - | luc.lens@ugent.be | **T** +32 9 264 52 13<br>**F** +32 9 264 87 94 |

Betreft: acceptance letter Jeselyn Calderón-Ayala

To whom it may concern:

This letter is an official acceptance letter from Terrestrial Ecology Group of Ghent University to Jeselyn Calderón-Ayala, and states that she is welcome to visit the Terrestrial Ecology Group during the period April 2011 to September 2011. During her stay she will be based in our research group and we will provide lab and office space during this period.

Yours sincerely,

Dr. Eduardo de la Peña on behalf of:

Prof. dr. Luc Lens
Director of the Terrestrial Ecology Unit, Department of Biology
Ghent University
K.L. Ledeganckstraat 35, 9000 Gent
Belgium

Appendix 3. *Ammophila arenaria* host plants and aphids (*Schizaphis graminum*)



*2) Schizaphis graminum*, greenbug
*3) Ammophila arenaria* host-plants

*Note:* that the aphid in the picture is *Schizaphis graminum* and not *Schizaphis rufula,* nonetheless they look very similar and have the same biology and life cycle. Little or no photographs can be found of *Schizaphis rufula.* Photograph demonstrates use of host plants for parthenogenesis.

Appendix 4. Experimental seedlings planted on the pots

Appendix 5. *Ammophila arenaria* grass *in-situ*: Het Zwin Nature Reserve, Knokke-Heist, Belgium

Appendix 6. Central mobile dune system in Het Zwin Nature Reserve, Knokke-Heist, Belgium

Appendix 7. Dune ecosystem access restriction, De Panne, Belgium