THE BIOGEOGRAPHY OF Ageratina adenophora: A MEXICAN TRANS-GLOBAL INVADER

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THE BIOGEOGRAPHY OF *Ageratina adenophora*: A MEXICAN

TRANS-GLOBAL INVADER

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Abstract

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The Biogeography of *Ageratina adenophora*: A Mexican Trans-Global Invader

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Invasive plants are a global problem often resulting in negative impacts upon populations of native plants and the environments in which they grow. Invasive plants inhibit native species and take over areas where they have been introduced. These invasive species create ecological problems for wildlife foraging, as they often transform the vegetation of native habitats resulting in conditions unsuitable for grazing. In addition, invasive plant species pose problems for humans in the areas of health, economics, and land management. *Ageratina adenophora*, a plant native to Mexico, has become a trans-global invader, particularly in China and India. In an effort to understand how the plant becomes a successful invader, a series of greenhouse experiments at The University of Montana were conducted to test for allelopathy and volatile chemical reactions. This study was designed to determine the effects of *Ageratina adenophora* regarding seed germination, mortality, and plant biomass on plants from the invaded environments of China and India. These tests compared seed germination and mortality from Mexican plants, where *Ageratina adenophora* is native. A second experiment tested the growth rates and biomass of plants in *Ageratina adenophora's* native environment of Mexico, versus those plants from invaded China and India. This was done with the hypothesis that Mexican plants would grow successfully in the presence of *Ageratina adenophora* while Chinese and Indian plant's growth would be inhibited. The findings suggest that indeed, there is a growth rate correlation between Mexican plants surviving in the presence of *Ageratina adenophora* and the Chinese and Indian plant's growth rates were inhibited.

Keywords: *Ageratina adenophora*, invasive species, allelopathy, Mexico, China, India

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Chapter One

Introduction

There is a significant need to understand invasive plants, their importance in introduced ecosystems, and their role in the environments in which they have evolved and developed symbiotic, and other healthy relationships in their native environments. The problems of invasive plants pose threats to ecosystem health, particularly to plants in their native communities and their relationships with interdependent biological life (Cox 2004, 4).

The phenomena of invasive plants and associated threats to diverse plant communities occur without clear, effective, or absolute removal techniques in newly invaded communities where these problems manifest. There are several hypotheses that attempt to understand why this happens, and how it affects the landscape. Invasive plants role on plant communities and how they can transform from small and somewhat insignificant plants at home to substantial plant invaders abroad is one of the most perplexing questions for ecology-based scientists today and accounts for significant research currently (Callaway and Aschehoug 2000, 521; Hierro, Maron and Callaway 2005, 10; Callaway *et al.* 2008, 1043).

This work will examine the problem of negative allelopathic effects of *Ageratina adenophora* in the invaded communities of South and East Asia, and its role in native communities of Mexico. This study includes The Novel Weapons Hypothesis, to develop understanding of growth and inhibition indicated through *A. adenophora*. The Novel Weapons Hypothesis proposes that some invasive plants may succeed by bringing novel chemical interactions to the communities, in which they invade, supporting dominance of

invasive plants over those plants that are native (Callaway and Aschehoug 2000, 521, Inderjit *et al.* 2007, 876). The native plant communities are thought to have evolved within their respective niches, keeping native communities in healthy biological status.

Native plant communities and natural neighbors have become well adapted to one another, and chemicals present in native plant communities typically play a regulatory role, preventing native plants from out-competing with one another to maintain a healthy equilibrium (Hierro 2005, 16). As native plants grow in a naturally evolved community with a peculiar chemical composition, there are instances in which an introduced plant does not become invasive and contribute to the detriment of that plant community. Many introduced plants do become invasive through negative allelopathy and become a problem (Cappuccino and Arnason 2006, 189, Hierro, Maron, and Callaway 2005, 6). Invasive plants may colonize disturbed habitat, are not a members of original plant communities, are locally abundant, and is of little economic value, but costly to control (Ghersa 2007).

This fundamental assumption that release of novel weapons creates strong suppression by invasive plants to a native plant's community. This phenomenon enables invasive plants to attain higher densities upon introduction, becoming dominant enough to create virtual monocultures in their introduced range (Callaway *et al.* 2005, 577). The strength and pervasiveness of population control of plants by their enemies is still a mystery, and commonly, invasive plants may not have been examined within their native communities. Often invasive plants are not significantly abundant in their home ranges, as compared to introduced communities where they flourish and create unnatural monocultures and other community mortality among natives. These problems contribute

to the lack of knowledge to eradicate invasive, as this lack of study exists in not fully understood within their native environments (Hierro, Maron and Callaway 2005, 5; Hierro 2005, 2)

Introduction of invasive plants may be through anthropogenic actions outside of natural biological occurrence. Such introductions may occur as invasive plants attach to shipments of food from abroad; invasive plants may travel as fragments to a new region by inevitable attachment to an airplane or the undercarriage of a motor vehicle and can be carried in lesser cases by faunal migration or weather patterns. It is those plants that may use negative allelopathy, which gives them advantage in the sites where they invade (Inderjit *et al.* 2008, 876).

This chemical-based plant action, allelopathy, has been under study since 372 B.C. by a disciple of Aristotle and following this, 25-220 A.D. a book was reviewed by Chinese researchers, who discovered pesticidal and allelopathic reactions among plants. Much later, into the 20th Century Rice (1984) defined allelopathy as both a stimulant and inhibiting mechanism to plants in the microcosm. Muller discovered its importance of plant interference such as plant dominance, succession and community formation, and thought that plant interactions may be "novel" as expanded by Callaway (2000).

In this work, the study of biogeography and invasive plants investigates a worldwide invader, *Ageratina adenophora*, also known as Sticky Snakeroot, Crofton Weed, and Mexican Devil throughout its regions of invasion. This study focuses upon *A. adenophora*'s role in its native Mexico and in the regions of its invasions where it grows abroad. Although *A. adenophora* has become a transglobal threat and is one of the

world's worst invaders, this study will emphasize invasion and allelopathic growth in India and China, in contrast to *A. adeonphora*'s native Mexico.

This study proposes The Novel Weapons hypothesis that *A. adenophora* possesses chemicals that are novel, in the plant communities where it invades, and gives it an allelopathic advantage to plants in their native communities, that have not evolved to accommodate this introduction. This thesis will contribute to a better understanding of *A. adenophora's invasion* status abroad in relation to its performance in its home range.

Chapter Two: Biogeography, Invasive Plants, and Allelopathy, presents an introduction to the field of biogeography. Within this context attention will turn to case studies of invasive plants and allelopathy in diverse species communities. This will provide a background in biogeography, examples of allelopathy, and how invasive plants may prosper within areas of introduction. Also, an explanation of some key terms will be discussed, and a brief history of when biogeographical sciences began and expanded is included.

Chapter Three: places emphasis upon *A. adenophora*, its physiology and strategies for success. This chapter includes an examination of what the scientific community knows of *A. adenophora*'s relationship to its native region, and how it performs in areas of invasion. Consideration is given to climate, physiology, significance, invasive status, current solutions, and a discussion of *A. adenophora* in theses areas of invasion.

In Chapter Four: Methods, discusses two greenhouse experiments conducted to compare allelopathy within differing populations of plants coming from areas where *A. adenophora* may be found to be invasive in South and East Asia and Native Mexico This

project, conducted on The University of Montana Missoula campus, analyzed *A. adenophora* and allelopathic relationships within these communities. Plants from these regions were grown in a closed environment with leaf litter of *A. adenophora* in close proximity to plant seedlings. These experiments supported understanding of growth rates from all plants included, and to examine the status of allelopathy on plants from the three regions as affected by the leaf litter. Both experiments used *A. adenophora* leaf litter to observe effected growth rates, documented allelopathic effects upon Chinese and Indian plants, and effects on Mexican plants. The outcome of native Mexican and non-native Chinese and Indian were impacted differently is explained in depth.

In Chapter Five: Results and Discussion, the two experiments will be analyzed, and the relevance of the experiments will be discussed. The chapter will expand upon the implications of two experiments. The experiments will be examined independently and comparatively. The analysis will illustrate the relevance of this work and the interconnectiveness of the project.

Chapter Two

Biogeography, Invasive Plants and Allelopathy

Elements of biogeography comprise a combination of several disciplines including geography, biology, ecology, and, in some instances, anthropology. Biogeography encompasses spatial patterns and processes, landscape change, and anthropogenic effects and modifications of biotic communities. Today biogeography looks at existing interdependent systems and how they relate to the world, particularly for this study, the allelopathy and biogeographical status of *Ageratina adenophora*. These scientific practices can be traced back to the early 1800s. The foundations of biogeography were formed prior to theories of natural selection, even preceding those ideas of Charles Darwin and Alfred Wallace (Brown 2004, 2). The basis for the study of allelopathy traces its roots to over 2000 years ago, but is relatively new to mainstream plant research presently.

Foundations of Biogeography and Allelopathy

As mentioned briefly, studies as early as 372 B.C. Theophrastus, a student of Aristotle, observed an effect similar to allelopathy of pigweed on alfalfa (Jelenic 1987 in Chou 2006, 1). Observations and scientific acknowledgments were made relatively shortly after this. In 25 A.D. in China, pesticide control and plant suppression were observed in Shengnong *Ben Tsao Jing* where a description of what is now known as allelopathic activity was recorded. These studies have solid relevance to allelopathy and the potential relationships this could present. In addition, a Chinese pharmacologist wrote a book about herbal supplements where he described the chemical constituents of

toxins and nutrients, specifically to humans and furthered understanding through these examples.

Plants thereafter were often traded or introduced for agricultural purposes in new colonies as early as the 1400s, becoming invasive through anthropogenic action. In historical times, there was neither concern regarding plants threatening a biological system nor were they known to pose a threat to biodiversity and ecosystem health. These actions represented the first widespread and global alteration of native plant communities, and negative effects upon agricultural crops (von Humboldt 2004 [1805], 50).

In the early 1800s, resources around the world became increasingly available and plants were primarily used for food and medicine (von Humboldt 2004 [1805], 50; De Candolle 1832 in Chou 2006, 1). In historical times, there was no concern regarding plants threatening a biological system nor were they known to pose a threat to biodiversity and ecosystem health. These actions represented the first widespread and global alteration of native plant communities, and negative effects upon agricultural crops (von Humboldt 2004 [1805], 50).

At this time in the 1800's there was a vague understanding of plants in a home range and the negative effects of plant introduction into new communities. Farmers noticed that plants within their crops showed inhibition or stimulation among their neighboring plants as the practice of agriculture grew. The biogeography of introduced plants was simpler, in these earlier times as researchers viewed plants for medicine and harvested plants for use in herbal tinctures to cure ailments, aid in the recovery of illness, and to promote well-being. Plants were not considered, by humans, to be used for much else than direct anthropogenic benefits namely agricultural and medicinal uses (von

Humboldt 2004 [1805], 50). There was neither any study of invasive plants in these efforts to cultivate medicinal plants, nor was there a perspective on humans spreading plants into non-native regions (von Humboldt 2004, 51-54).

In the late nineteenth century a study of relationships among species and species dispersal were examined. Today biogeography looks at existing interdependent systems and how they relate to the world (Haeckel 2004 [1876] 180). The strategy of studying invasive plants in their natural communities is not consistent. Perhaps with this scope of biogeographical examination a better understanding of how plants interact at home and abroad (Hierro, Maron, and Callaway 2005, 4), and this could improve understanding of allelopathy and the many effects that come from such a phenomenon may be improved

Certainly, understanding of allelopathic effects has evolved since the initial ideas of how plant species behave and transform landscapes. One of the most elusive questions in ecology presently is why some species succeed much more in their introduced ranges than in their native range, and how allelopathy contributes to the success of introduced plants (Weidenhamer 2006, 85). In efforts to further understanding in biogeography, scientific studies in the early twentieth century developed methods to explain how invasive species travel, how they can hybridize and cross-pollinate and ultimately how invasive plants can have such a powerful, negative impact upon plant community diversity.

Invasive species, no matter the quantity, are observed to create ecological problems for wildlife, human heath, and economics as they often transform the vegetation of native habitats into conditions unsuitable for grazing, for accessing clean water, and

for the consumption of valuable local plants (Holt 2004, 1559; Cox 2004, 65; Sax *et al.* 2005, 65; Radosevich 2007, 545).

Invasive plants may reach such high densities to become dominant within the communities they invade (Aliotta, Cafiero, and Otero 2006, 285). This status in the new ecosystem, as a dominant invader, poses the greatest threat to native ecosystems and invasive plants may create virtual monocultures within these communities (Denslow and Hughes 2004, 1284; Hierro and Callaway 2003, 29; Mack *et al.* 2000, 692).

In the mid-nineteen hundreds pioneering scientists examined the close relationship between ecology and geography and discovering plant commonalities complementing one another, leading to linkages in the exploration of biogeographical and ecological problems. The relationship of ecology and geography relative to environmental processes was a major focus of these earlier works. Discoveries, provided information in their own right, and enlightenment of scientists studying the disciplines together (Wulff 2004 [1943], 514). Island biogeography emerged in the early twentieth century, and remains a foundation for biogeographical scientists both spatially and physically. Island biogeographical studies helped these early, groundbreaking naturalists understand biogeographical isolation among species, and species dispersal (Darwin 2004 [1859], 141; Diamond 2004, 1001).

Biogeography has evolved to become broader in scope, and ecological research of invasive plants has exploded as a subdiscipline, which may be attributed to island biogeography. As we obtain knowledge acquired through increasingly in-depth research, details of ecosystem functions are acquired (Pysek and Richardson 2006, 23). This

science of plants and biological interaction has encouraged the study of a spatial scope of environments where native and invasive plants occur.

<div align="center">Biogeography of Plants</div>

Plant research within biogeography ranges from the study of chemical reactions among plant species both native and invasive, to the studies of loss of plant consumers and natural enemies, and plant competition in a native community (Whittaker 2004, 931). These studies demonstrate relationships that affect plant growth and success (Callaway and Ridneour 2004, 437). Current research has evolved from the earlier efforts to understand plant communities, agriculture, and wilderness by pioneers in biogeography (Lomolino, Sax and Brown 2004, 5).

Plant biogeography currently focuses largely upon invasive plants and efforts of elimination for the purposes of land management, agriculture (Kohli, Batish, and Singh 2006, 467), native plant communities and wildlife habitat (Radosevish, Jodie and Claudio 2007, 8). Not only does analysis of these problems create significant economic burdens, invasive plants are troublesome to recreationists lack access to areas owing to research activities or efforts to eliminate further degradation (Hammitt and Cole 1998, 51). Recreationists may also contribute to species introductions through the unintentional spread from travel on foot, by vehicle undercarriage, or by other means. (Eiswerth 2005, 130).

Plant species act individually and within a community, both of which may enable travel, migration to new communities, without the assistance of humans, all of which may be examples better, understood through island biogeography (Hengeveld, Giller and Riddle 2004, 454). Although island biogeography provides insight into the proposed

questions, it does not cover the full spectrum of invasive plant introduction or success of invasion, as this in part, falls within the broader domain of the biological sciences. The scope of biogeography holds principles from island biogeography, examining plant dispersal, evolution, and species interaction (Diamond 2004, 1005). This early foundation of understanding plant migration and anthropogenic introduction continues to serve as a building block for understanding allelopathy in modern times.

Spatial aspects and community communication, or relationships between evolved communities among coexisting and invasive species are discussed within the biogeography of plants. The studies include plant migration and introduction outside of their natural barriers (Hierro, Maron, and Callaway 2005, 5). This illustrates how species have evolved in their native regions to become successful among their new neighbors. In addition to using this study of plants in their native communities, an understanding may be attained to how a species may be an aggressive invader abroad and if so, the question of how success was established may be clarified.

In the past, anthropogenic actions were thought to be the most prevalent reason for plant dispersal into new areas. Plants were introduced as ornamentals, and through agriculture, inadvertently creating invasive plants and problems that would inevitably follow. In addition to these methods of introduction, plants may travel through waterways chiefly oceans and rivers and may attach to animals that migrate, through airborne travel by birds, wind, and other climatic elements (Mack, *et al.* 2000, 689).

There is not a complete understanding of how invaders may become dominant, and interfere with natural systems that have evolved to become modern communities. There are several theories, however, examining how this phenomenon occurs (Hierro,

Marron Callaway 2005, 7). There is a steady trend in biogeography to discover invasive plants and find solutions to the intrusions of diversity. Biogeography may focus upon invasive species and how they work within their own communities when compared to their success abroad.

Invasive plants may have negative impacts upon introduction to new communities and posses phytotoxic effects that inhibit growth in native plants. Negative allelopathy may cause a loss in biodiversity in healthy plant communities (Cox 2004, 377). Invasive plants result in negative impacts to populations of native plants and the biological communities in which they grow (Kohli, Batish, and Singh 2006, 467). Invasive plants may inhibit native species and create an inbalance in communities that may have evolved together over time (Sinkkonen 2006, 379).

One current hypothesis states that as many plants are transplanted into non-native areas they do not necessarily become invasive out-compete native plants for elemental resources to survive and account for loss of habitat and human resources (Hierro Maron and Callaway 2005, 3). Indeed plants may become invasive, creating large biological problems with a potential to create large changes to the landscape and interdependent life therein.

Recently, there has been significant research on one relevant plant and one tree species that illustrate the issues with the invasion of non-native species into ecosystems. The first example, *Pinus albicaulis,* known commonly as the Whitebark Pine, a tree native to the Rocky Mountains, has a high mortality rate owing to introduction of a non-native, pest-driven disease. The potential extinction of that species has far-reaching impacts across the entire ecosystem (McKinney and Tomback 2007, 1044). The second

case study discusses the introduction and rapid spread of *Centaurea maculosa,* known commonly as Spotted Knapweed into the Rocky Mountain West.

Disruption of Ecosystem Processes Case Study One

In an effort to understand the impacts an invasive species can have on a native system, the following case study illustrates the interconnectedness, and thus biogeographical impacts, of such plant species. Invasive plant-related negative outcomes and the effects of retention and restoration of native communities can be demonstrated by a tree species such as *Pinus albicaulis (*Whitebark Pine), and blister rust a disease causing mortality to the species. This tree is an upper subalpine species, and is native to the North American continent, occurring primarily in the Northern Rocky Mountains (McCaughey and Schmidt 2001; 29).

Pinus albicaulis is both a keystone species (Schrag *et al.* 2007, 10) and a foundation species in high-elevation ecosystems (Resler and Tomback 2008, 161). Many species rely on Whitebark Pine for success, including the Clark's Nutcracker, Red Squirrel, Grizzly Bear, and to a smaller extent the Black Bear (Arno 1986, 92). These species work within an increasingly fragile ecosystem. Global warming and *Cronartium ribicola*, the pathogen that creates Blister Rust in five-needled white pine trees, are the two largest threats to *P. albicaulis*, and the tree is thought to be close to extinction throughout the majority of its native ranges (Schrag *et al.* 2007, 10).

Cronartium ribicola is currently a particularly large threat to *P. albicaulis* and is a disease for which no solution is known (Keane, Gray, and Dickinson 2007, 1). Its effects on these tree stands are widespread and it causes mortality in trees in only a few years (Resler and Tomback 2008, 161). The climatic trend of increasingly warmer

temperatures intensifies the effects of blister rust and these threats to this tree. Mortality

and possibly the end of *P. albicaulis* as a species exist, and this example of invasive

disease is only a part of the seemingly countless ways in which invasive plants and

disease can affect ecosystem processes and native species whether plant, wildlife or

human. In addition, plants can be the foundation for many species survival and success

within their native ranges.

<div align="center">Disruption of Ecosystem Processes Case Study Two</div>

One of the worst invasive species to the North America West, *Centaurea*

maculosa is native to Eurasia. This plant invades throughout the North American

continent in similar climates to it home range (Callaway and Ridenour 2004, 438). It was

probably introduced with alfalfa seeds from Europe, has successfully invaded the

Northern Rocky Mountains, and has become established in more than seven million acres

throughout the United States (Callaway and Ridenour 2004, 438). *C. maculosa* is

thought to have become successful through negative allelopathy and is extremely

destructive. Upon introduction, it may create monocultures where it becomes established

and spreads without many enemies to hinder its success (Callaway *et al.* 2005). Plants such

as *C. maculosa* are biogeographically significant, as these plants travel across regions and

dramatically change the landscapes in which they invade.

Efforts to control *C. maculosa* include biocontrol, or the release of specialist

insects (Callaway and Ridenour 2004, 439) to eliminate the target plant, as the insect

used is a specialist consumer. These efforts have not proven to be completely effective.

Further, efforts have been made to determine the chemical effects of this plant abroad.

Currently, *C. maculosa* is successful in the Northern Rocky Mountains, and there are not solution to control its invasive status (Callaway, and Ridenour 2004; 440).

Biogeographical analysis helps to determine if an invasive plant reacts differently in its invaded range, allowing it to dominate (Hierro, Maron, and Callaway 2005, 9). There are efforts to understand why some invasive plants do well in one location, but are not as successful in another invaded area.

<div align="center">Trends in Invasive Plants Study of Allelopathy</div>

Allelopathy is known to contain plant chemistry that acts as secondary plant products released into the environment. This is a strategy through volatilization, leaching, root exudation and decomposition of plant residues in the soil (Hierro and Callaway 2003, 29). In order to manage the allelopathy of invasive plants that are intruding upon native communities, biogeographical approaches to gain an understanding of the issues on a spatial scale may be implemented. This focus upon understanding a single aspect of a plant's success, chemical behavior at home and abroad is to test negative allelopathic effects of one plant to another in a controlled environment (Callaway and Ridneour 2004, 436). Plant biogeography has much to offer in the analysis of invasive plants. In addition to chemical manipulation, biogeography explores mechanisms that enable successful invasive plants to occur in substantially higher abundance in areas of introduction. Among these areas of research is analysis of allelopathy (Hierro, Maron, and 2005, 9; Inderjit *et al.* 2005, 876; Callaway and Ridenour 2004, 436)

The history of allelopathy started with Hans Molisch, an Austrian professor of botany, who coined the term from the Latin words: *allelo* and *pathy* meaning mutual

harm (Chou 2006, 1). His discoveries of allelopathy started when he observed fruit from different regions that seemed to affect one another in the ripening process, specifically apples that caused other fruits to ripen at a faster rate when in a closed environment. He attributed this to their ethylene gas (Chou 2006, 2). His studies initiated research into the phenomena of plant invasion through chemical integration allowing plants to modify those environments in which they are introduced.

In the 1960s, allelopathy was linked to plant ecology and ecosystem function, with observation of chemical inhibition to native plants by invasive plants (Chou 2006, 2). Later botanist Elroy Rice (1984) studied allelopathy in more depth. His studies furthered understanding of current chemical communication among plants in the areas of stimulation and inhibition. Rice observed that many answers emerged from field studies of allelopathy, as opposed to analysis in a closed environment similar to those of Molisch in 1937 (Rice 1992, 31). The concept of allelopathy contains hypotheses that explain why plants occurring in a new area attributed to anthropogenic causes allow higher significant abundance in their introduced range than in their native range? Native plant communities are often crowded out by invasive plants and out-competed in their native range (Callaway *et al.* 2008, 1043). Several studies have shown the effects of allelopathy and rigorously examined the ecological environments therein. These studies have furthered the science of understanding this phenomena and how it may further future exploration.

Allelopathy can be attributed to how plants react chemically to neighboring plants at home or abroad (Chou 2006, 2). An examination of plant communities suggests that plants may compete with one another for resources such as nutrients, water, light, and

pollinators, better known as resource competition (Weidenhamer 2006, 86). These relationships keep native plants in balance as they are competing for the same resources and have evolved to growing in these specific communities.

Plants are constantly communicating with one another through chemical stimulation and suppression, and provide a necessary environment for native plants that have evolved there (Chou 2006, 5). When plants become invasive, they often escape chemical competition in introduced areas that keep them in control within their native regions, and can use their previously adapted chemical weapons on unsuspecting and unadapted foes in the environment into which they are introduced (Callaway and Ridenour 2004, 436).

Plant communities can encompass healthy forms of competition for some plants, and lack of success for less well-adapted plants. Chemical stimulants released by plants may indirectly affect their neighbors causing inhibition or stimulation. This may modify the soil in some way, and requires further understanding (Callaway and Ridenour 2004, 436). Allelopathy plays a key role in an invasive plant disturbing a diverse plant community (Weidenhamer 2006, 87).

Allelopathy can occur under several conditions. The following case studies attempt to explain how allelopathy occurs within a plant community, and how it can inhibit the naturally occurring flora therein. The case studies found here use existing knowledge with pioneering research by Darwin and Mulisch, whose ideas stimulated scientists and researchers and evolved an understanding of how these biological systems work.

As noted above invasive plants are thought to gain a noticeable advantage in a native plant community. These invasive additions have enemies from abroad that contain novel chemicals permitting them to outcompete specialist enemies that have evolved without highly competitive invasive plants in their native ranges known as The Novel Weapons Hypothesis (Callaway and Ridenour 2004; 437). The fundamental assumption is that chemical release from enemies in a native plant community enables plants to attain higher densities in their introduced range. While native herbivores and pathogens can cause substantial damage (Callaway and Ridenour 2004, 437), an understanding of the strength and pervasiveness of population control of plants by their enemies is incomplete. Explicit comparisons of the effects of enemies in the native and introduced range are crucial for testing allelopathy hypotheses.

The Novel Weapons Hypothesis (Callaway and Ridenour 2004, 437), and the allelopathic properties of *A. adenophora*'s status in its native habitat and abroad (Inderjit *et al.* 2008, 867) suggest that some invasive plants bring novel ways of interaction to natural plant communities (Callaway and Aschehoug 2000, 521). This hypothesis proposes that invasive plants exude allelochemicals that are not necessarily effective in their native ranges, where competitive neighboring plants have evolved as part of a well-adapted community. However, these same species are suggested to be highly inhibitory to those native plant communities where they invade. This biogeographical theory helps to demonstrates that plants are fluid in movement, and allelopathy, positive or negative, is always part of these biogeographical interactions.

As plants move across boundaries, transplanted by man, or through natural dispersal, species demonstrate their approach as spatially based. As biogeography looks

at plants in place-based foundation and the evolution that occurs with plants in their native communities or abroad, it demonstrates a connection of ecology biogeography.

A hypothesis preceding the Novel Weapons Hypothesis is the Evolution of Increased Competitive Ability (Muller-Scharer, Schaffiner, and Steigner 2004, 418; Callaway and Aschehoug 2000, 521). This hypothesis argues that exotics, which have been liberated for a long period from their enemies at home, whose evolutionary chemical makeup is absent in the introduced range, species posses traits that help them outcompete plants where they invade. This argues that invasive plants can use more resources for traits that provide greater competition, such as size or fecundity.

Again, Callaway and Ridenour (2004), suggest that some invasive plants transform from "native weaklings to invasive bullies" by exuding biochemicals that create chemical inhibition. Plants or soil microbes in invaded communities are negatively affected owing to the disruption of balanced competition. There is reason to think that plants do not evolve in order to out-compete plants areas of introduction, but instead evolve to compete in natural communities. The Novel Weapons Hypothesis is relatively new (Callaway *et al.* 2005, 578), and negative allelopathy of invasive plants has been somewhat understudied. It is now believed that biogeographical studies of the allelopathy from invasive plants in natural plant communities may support the role of allelopathy in community theory (Baldwin 2003, 42).

Allelopathy Case Study One

An example of allelopathy was a project conducted by two ecologists to examine allelopathic effects of *Centaurea diffusa* on newly invaded ranges in North America. This compared new ranges with effects in native communities in Eurasia, Callaway and Aschehoug (2000) compared the success of this Eurasian plant on three species of bunch grass that coexist with *C. diffusa (*Hierro and Callaway 2003, 31). These species were all grown with similar species from Eurasia of similar size. Eurasian seeds were harvested from the foothills of the Caucus Mountains in the Republic of Georgia. American seeds were collected from the Rocky Mountains in Montana, USA. Every grass species was common in its respective sites. The ground cover of *C.diffusa* was very low at less than one percent of cover. In the Montana Rocky Mountains, *C. maculosa*, a plant closely related to *C. diffusa*, covered up to ninety percent of a given area.

Each of the seven species were planted alone and in all grass-*Centaurea* combinations, and all combinations were grown in sand or in sand mixed with activated carbon expected to suppress any potential allelopathic effects because of its ability to absorb organic compounds. *C. diffusa* had stronger negative effects upon North American species than upon its native Eurasian species. The Eurasian species showed a reduced *C. diffusa* biomass in the experiment.

Centaurea diffusa had no significant negative effect upon Eurasian grass species, but significantly inhibited all North American species. North American grasses had no competitive effects on the chemical absorption of *C. diffusa* within the experiment. The important implications for the role of allelopathy in exotic plant invasion, activated carbon had exceptionally different effects on the interactions between *C. diffusa* and the

grass species from the different biogeographical regions (Hierro and Callaway 2003; 32).

Allelopathy Case Study Two

A second example of allelopathy is the study of *Alliaria petiolata* is native to Europe and successfully invades forest habitats throughout North America. In its native region, it occurs along forest edges, other shady habitats, and in forest understory (Callaway et al 2008; 1044). Seedlings emerge in early spring and grow into evergreen rosettes during the first year. Flowers are born in late spring on one or more stalks of the second year plants, and mature later into soliloquies. Since its arrival into the northeastern United States, it invaded from Ontario to the Carolinas and as far west in the United States as the Pacific Ocean, it has been regarded as highly invasive in these introduced regions (Callaway et al 2008 1044).

Alliara petiolata was observed to form dense, monospecific stands that take over and eliminate native plant communities within a variety of habitats, including forest edges, floodplains, and forest understory (Callaway et al 2008; 1044). Currently this plant has become highly successful and taken over many plant communities through allelopathy to gaining success.

After rigorous chemical testing, it was determined that *A. petiolata* does indeed have allelopathic affects on plant microbial communities in North America, reducing native soil chemicals to their inhibition. However, *A. petiolata* showed little affect on those species in its native communities in Europe, and thus, they grew without signs of allelopathy and without inhibition.

Allelopathy Case Study Three

Bartholomew (1970) revealed that a shrub and grass community of California Chaparral and costal sage communities, with a characteristic bare zone, is not necessarily empty of vegetation owing to allelopathy. Previous studies suggested that volatiles were the main reason for this bare zone, and this was the accepted hypothesis for several years. Finally, Bartholomew, in his research, suggested that small mammals and birds contributed to the lack of vegetation. Before this the role of animals in the bare zone was believed to be minimal at best. Bartholomew carried out an extensive experiment to determine the actual reason for this area devoid of any vegetation, given it was so close to adjacent shrub and grassland communities.

Bartholomew's observations confirmed his previous idea that the work of herbivores native to the area complemented chemical plant-to-plant inhibition. He argued that California chaparral and sage provided excellent cover for rodents, rabbits and birds. In addition, the neighboring grassland provided an ample food source for this wildlife to graze and consume seeds.

Bartholomew conducted several experiments including wildlife monitoring, using stations around the site of observation. He put out a source of seeds to provide a larger food source. Bartholomew thought that the source of food would determine if these animals were coming to the area for food. In efforts to explain his ideas further, experimental traps were set to hold the small mammals in order to observe which species were accessing the bare zone. In the process of this experiment, seeds were put onto sandpaper to prevent them from falling out of the area. The seeds were accessible from

wildlife that may graze in the areas, using the adjacent shrubs and grasslands for food and cover.

Bartholomew determined that indeed, wildlife feeding in the bare zone was this cause of plant mortality. In addition, he discovered a small amount of volatile toxins; however more lack of plant growth was not due to allelopathy, but instead could be attributed to grazers. These discoveries are important to the understanding of allelopathy and the possibility of other hypotheses. The idea of other sources of conflict within a community and how those may affect native species is important. As scientists succeed in gaining a greater understanding of how invasive plants work within biological systems creates further understanding of allelopathic effects.

There are many different ideas of how plants invade, why they may become successful in their new communities, and how this ties into biogeography. As there are many different approaches, biogeography helps us understand plant allelopathy within a context of place.

As biogeographical scientists strive to gain understanding into the problem threatening biodiversity and the overall well-being of a native community, whether plant, wildlife or anthropogenic contributions are involved, we can proceed with another kernel of knowledge and a step toward healthy ecosystems. The Novel Weapons Hypothesis of, Callaway and Ridenour (2004) and the allelopathic effects to *A. adenophora*, and its status in native Mexico and regions of China and India where it has become a substantial invader, is, in part, the focus of the research within this thesis and will perhaps add to a new collection of studies to further reach a solution.

Chapter Three

Ageratina adenophora

Ageratina adenophora, a plant native to central Mexico, aggressively invades several regions through out the world. This project, however will primarily focus primarily on South and East Asia (Wang and Wang 2006, 397). There are however, regions where *A. adenophora* invades in addition to South and East Asia and a few of these regions will be discussed briefly. *A. adenophora* flourishes in subtropical regions, similar to its native range of Central America (Hong-Bang 2007, 75; Wang and Wang 2006, 406). As it invades, the plant can grow successfully along riparian areas and into disturbed habitat, such as new roadways and other human-related developments (Liu *et al.* 2007, 237; Xiao-yu *et al.* 2004, 319).

Ageratina adenophora chokes out native plant life in regions of invasive establishment, causing harm to natural plant communities and the ecosystem processes therein (Liu *et al.* 2005, 341). Further environmental processes and human-activity are negatively impacted as well, and it is costly for land managers to control (Zhang *et al.* 2008, 561; Liu *et al.* 2005, 341).

Plants such as *A.adenophora* create high monetary costs, loss of biodiversity, and interruption of affected anthropogenic activities. Anthropogenic activities include agriculture, livestock grazing, recreation, harvesting of native plants for cultural purposes, water supply and land development (Eiswerth 2005, 132). *A. adenophora* contributes to these problems as it invades quickly and successfully in most of the plant communities where it is introduced (Lichti and Hoshovsky 2000, 30).

Ageratina adenophora is fatally toxic to horses and most livestock, and is not edible to cattle or goats. The toxic disease caused in horses is known as "blowing disease," and may take several years to become evident. The symptoms of blowing disease manifest as coughing, difficult breathing, and violent blowing after exertion is the result of the acute edema, leading to hemorrhaging (Morris 1989, 282). *A. adenophora* 's introduction into regions abroad started in the early 19th century, spreading from Mexico to Britain as an ornamental plant where it thrives (Cronk and Fuller 1998, 269; Wang and Wang 2006, 397). Later, in the early 1940s, it was introduced into South and East Asia, specifically into Myanmar, where it continues to spread. *A. adenophora* is now devastating areas of China, India and other similar regions of introduction (Xiao-yu *et al.* 2006, 116).
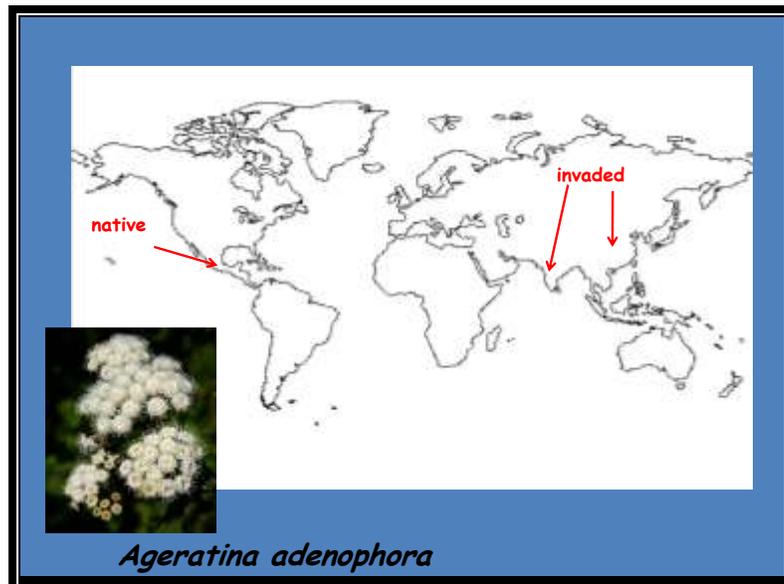

Figure 3.1. Map of *A. adenophora* distribution. [1]

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[1] Figure 3.1. *A. adenophora* distribution in its native and invaded areas, relevant to the project.

Physiology of *Ageratina adenophora*

The physiology of *A. adenophora* makes it a likely candidate for invasion. Its features include large purple stems that grows from 0.5-1.7 meters tall, are very hairy, and roots into the soil upon contact (Muniappan *et al.* 2008, 63). Its leaf blades generally grow five to ten centimeters long and may produce ten to sixty flowers per head (Bossard, Randal and Hoshovsky 2000, 31). The seeds have pappus hairs enabling the plant to withstand diverse environmental conditions that are not present in its native environment. The hairs allow seeds to travel long distances by acting as a parachute, enabling seeds to be carried by wind or water.

Figure 3.2. *A. adenophora.* [2]

A. adenophora buds begin to appear in late winter. They then grow aggressively, flowering in early spring usually in March. Seeds are established without pollination or fertilization, and fifteen to thirty percent of the 7,000 to 10,000 seeds produced by each plant are not viable. However, a high number of seeds, 4000 to 5000, can become

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[2] Figure 3.2. *A. adenophora* growing in the field.

established. Seeds mature and are shed between April and mid-June, and the lower leaves of the plant drop after seed fall. Dense stands can contribute up to 60,000 viable seeds per square meter to the seed bank making it is successful in its introduced ranges (Bossard, Randal and Hoshovsky 2000, 31). In addition, seeds that are buried lose their viability quickly and at a constant rate. This does not mean that seeds are unsuccessful; however, because of high seed production this high mortality has little effect on the plant's potential for spreading.

Germination occurs between June and March, with peak germination for over eighty percent of the viable seeds in August and September (Bossard, Randal and Hoshovsky 2000, 31). Light is necessary for seeds to germinate, so conditions, such as bare soil, are essential for establishment. Once germinated, seedlings can withstand a considerable amount of shading, compensating for reduced light intensity by increasing leaf area. Deep shade, however, will kill seedlings (Xiao-yu 2006, 116).

Seedlings grow rapidly and are fully established and able to regenerate from the crown, if damaged, within eight weeks of germination. In second-year and older plants, new growth begins with the first major summer rains, usually in June. Growth rates of seedlings and mature plants remain high during summer but decline in the cooler winter months (Wang and Wang 2006, 397).

Ageratina adenophora reproduces as noted above, asexually and by dispersal of seeds (Muniappan and Viraktamath 1993). These seeds favor disrupted environmental sites. They also travel as many invasive plants do in mud sticking to animals, machinery, and vehicles, and by adhering to footwear or clothing (Bossard, Randal and Hoshovsky 2000, 31).
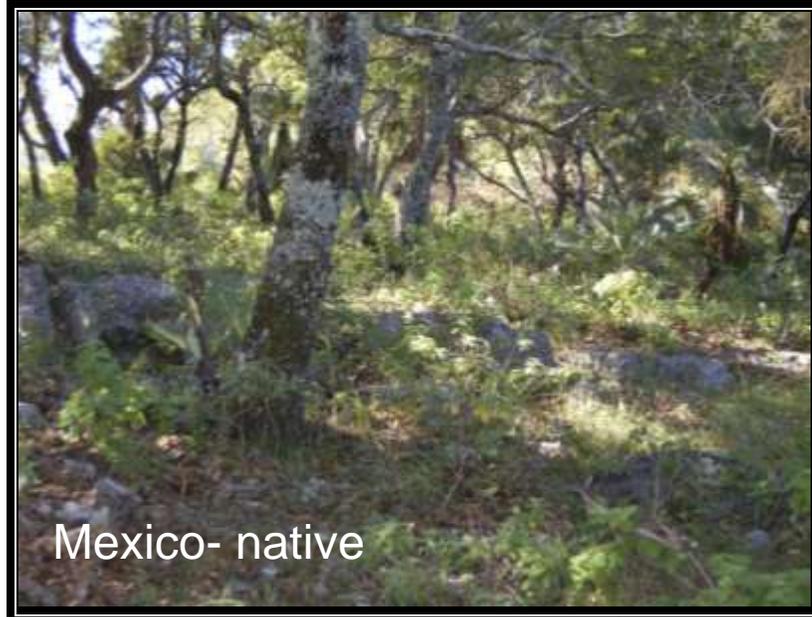
Figure 3.3. *A. adenophora* in Mexico. [3]

Ageratina adenophora grows native in Mexico on a significantly smaller scale than it does in India, and China. This plant is understudied in its native Mexico as it is not an aggressive species in its home range, nor does it grow overly successful at home, as it does abroad (Muniappan, Raman and Reddy 2004, 64

Trans-global Invasion

Ageratina adenophora often takes over native plant communities in areas of invasion, and is such a strong inhibitor it may change soil biota to its own advantage, crowding out native species through allelopathy (Hong-Bang *et al.* 2006, 73).

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[3] Figure 3.3. *A. adenophora* growing in its native Mexico.

Figure 3.4. *A. adenophora* in India. [4]

Invasion in India

In India, *A*. adenophora is a widespread problem. It is hypothesized that

allelopathy plays a strong role in *A. adenophora*'s spread, damage to native plant

communities, and other issues as noted previously. This chemical destruction may be a

key player in the success it attains in India.

Figures 3, 4 and 6 provide visual observation of *A. adenophora* is not a substantial

plant in its native community of Mexico, where it grows in small patches (figure 3). In

India (figure 4), *A .adenophora* grows without inhibition and outcompetes several plants

growing in natural communities, as in the case of China (Xiao-yu, Zhao-hua; Wei-guo

2004, 319, and Dong *et al.* 2007, 283).

Unfortunately, there is little scholarly research into *A. adenophora's* invasion in

India or on its native Mexico that is available to researchers in the United States. Perhaps

Indian research is limited largely to that country's scholarly journals and technical reports

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[4] Figure 3.4. *A. adenophora* invasive in India.

and Mexican research is nonexistent. As the research is inaccessible to researchers abroad, it limits discussion of Indian problems and reaction to *A. adenophora*. Fortunately, owing to the availability of public research, the discussion of China's experience with *A. adenophora* will be slightly more in depth. The discussion will be extended to Australia and the United States. In addition to these mentioned nations, A. *adenophora* has also invaded New Zealand, South and Southwest Africa, and other smaller and less impacted nations of Southeast Asia. In these areas similar to Mexico and India, the available research is limited.

Invasion in China

Concerning native and invasive communities, China is one of the world's hotspots with regard to biodiversity, with some 30,000 native plants species (Ding *et al.* 2008, 319). *A. adenophora* was introduced into China in the 1940s from Myanmar, originally as an ornamental plant. It now spreads into Yunnan, Guangxi and Hainan Provinces (Xie *et al.* 2001, 1337). Geographic predictions for *A.adenophora* in China are illustrated in figure 5. They suggest that this spread may result from several different factors, as the ease with most invasive plants; it may spread with different means depending upon the location of the disturbance and the local environment.

Huge disturbances, such as the Three Gorges Dam and the recently completed rail link to Tibet, could further spread invasive species to disturbed communities in the country (Ding *et al.* 2008, 317) as disturbance is a key component for the introduction of invasive plants and allelopathy.
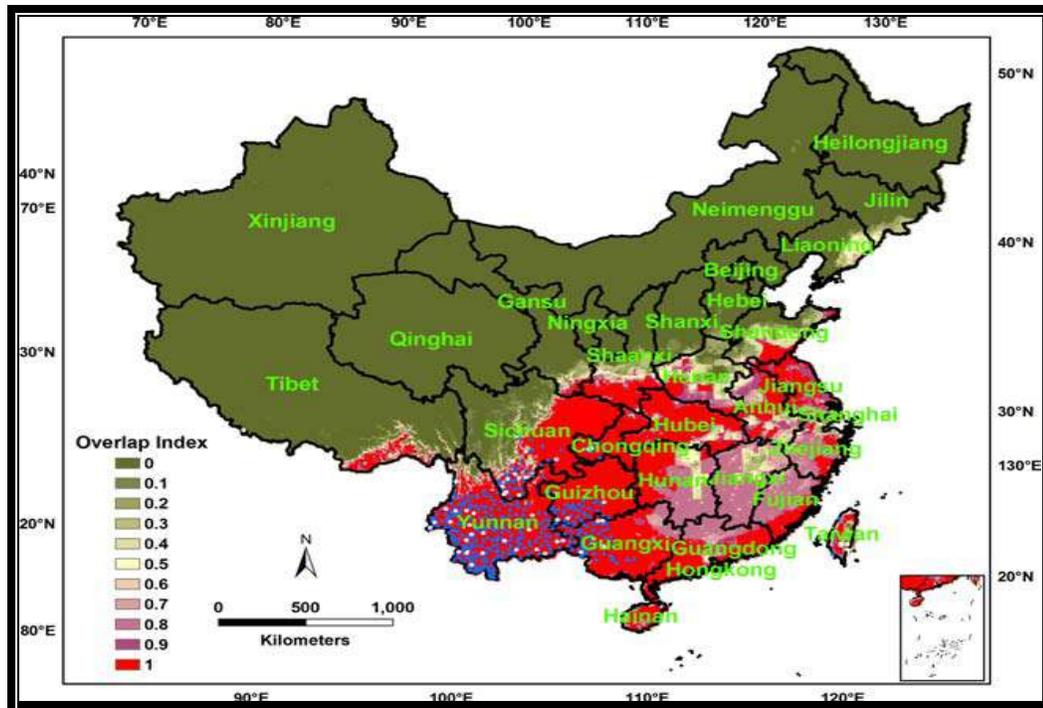
Figure 3.5. *A. adenophora* distributions in China. [5]

Disturbance in China is more frequent and widespread, as the country has undergone an economic boom in the last twenty- five years. As more money is available in the country, there is more trade that is international and a higher risk of invasive plants through shipments from overseas. This enormous increase in trade has resulted in many invasive plants introductions (Ding *et al.* 2008, 317). These introductions allowed an invasion of *A. adenophora* to spread throughout South and East Asia. *A. adenophora* is now currently one of the worst invaders in Chinese plant communities. Figure 6 illustrates how *A.adenophora* can grow in dense monocultures and thickets (Ding et. al. 2008, 317).

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[5] Figure 3.5. Geographic predictions for *A.adenophora* in China. The blue triangles represent predicted models where *A adenophora* is to establish and grow. The white circles represent 51 testing data used to predict the different colors represent regions where A. *adenophora* is present with predicted varying degrees of likelihood and the extent of invasion predictability throughout China.

Figure 3.6. *A. adenophora* in China. [6]

Problems of invasive plants have drawn the attention of the government and the

public in China, bringing focus to the high and still rising price of invasive plants. *A.*

adenophora threatens China's native plant communities, accounting for huge monetary

costs and a sustainable loss of native plants. Invasive plants create problems in the local

economy and loss of native vegetation as well and threats to livestock, and riparian

vegetation, and damaged waterways (Zhu *et al.* 2007, 1143; Zhu *et al.* 2007, 144).

Invasive plants create problems for land managers to consider, and with strategies to rid

the land of the invader, imposing monetary costs along with these severe biological

impacts (Ding *et al.* 2008, 317).

Additional Regional Invasions

Since its introduction into Australia in the early 1900s, *A. adenophora* has

become widespread in Queensland coastal areas and on the New South Wales Northern

Coast and as far south as Wollongong (Trounce and Dayson 2003, 1). Isolated invasions

also occur in the northern and central tablelands. *A.adenophora* has become an invasive

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[6] Figure 3.6. *A. adenophora* in China.

plant in thirty-nine governmentally managed areas of coastal New South Wales (Trounce

and Dayson 2003, 1). Most invasions lye between Gosford and the Queensland border.

In Sydney, *A.adenophora* has been established for more than 30 years and has spread

rapidly from its introduction into this area since the early 1970s (Trounce and Dayson

2003, 1).

Mechanical and chemical controls are implemented in addition to biological control,

and provide no agents to control the plant. These efforts of Australian land managers are

to rid their region of the plant and restore natural systems. (Trounce and Dayson 2003,

643). In figure 7, the map illustrates the areas that have succumbed to *A. adenophora*

invasion in the areas shaded in black. The grey areas represent potential future spread.
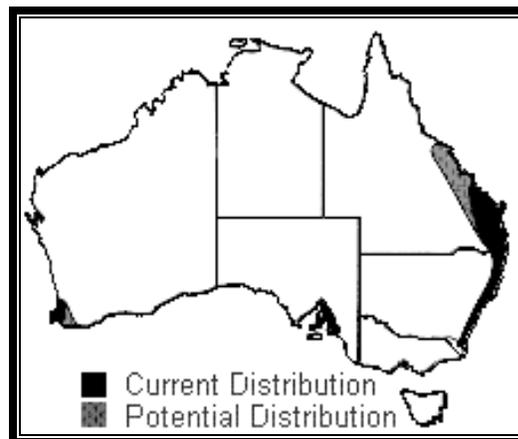
Figure 3.7. *A. adenophora* distributions in Australia. [7]

Ageratina is also found in invaded areas of the United States, where it is a

significant problem. *A. adenophora* can be found in California, and Hawaii. Efforts by

the United States Department of Agriculture focus on gaining control and eliminating the

plant. Currently there are efforts underway to exterminate the species and research is

current throughout the country.

Figure 3.7. Map of Australia 2010. Black shading indicates current *A. adenophora* invasions, grey indicate potential areas of spread. [7]

California State scientists and land managers accounts for *A. adenophora* to be moderately invasive in those areas where it has established. It flourishes in over ten counties in California, from Marin County south to San Bernardino (Bossard, Randal, and Hoshovsky 2000, 30). In California, *A. adenophora* can be especially hard to control, as it prefers to grow on steep slopes and mechanical control here can be ineffective in this type of landscape. *A. adenophora* has been naturalized in California since the early Twentieth Century, from Mexico (Bossard, Randal and Hoshovsky 2000, 31).

Hawaii is a conducive environment for *A. adenophora.* The species successfully invades in the local tropical climate. Here the plant covers five regions among the islands, mostly on the big island, with its mild climate.   *A. adenophora* does not threaten other species in higher elevations; however, it is invasive where it does exist (Motooka 2003, 184).

Figure 3.8. *A. adenophora* in Hawaii. [8]

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[8] Figure 3.8. *Ageratina adenophora* in Maui, Hawaii, February 27, 2009.

As *A. adenophora* remains a problem species throughout the world, there are efforts to resolve the ecosystem-based dilemma. Perhaps, field and laboratory research can piece together answers to solve the questions of invasion related to *A. adenophora* and its many intricacies to regain native plant life and repair the ecosystem problems therein.

Chapter Four

Methods

In order to test for negative allelopathy on selected plant species, two experiments were conducted in a greenhouse, to examine *A. adenophora* on species in regions where it invades, namely China and India in comparison to species from its native habitat in Mexico. The experiments were designed to determine if *A. adenophora* would inhibit plants from China and India, and leave plants from Mexico, to grow freely without chemical suppression, or an absence of allelopathy. This would help to determine if these plants reacted in a controlled environment of a greenhouse, are comparable to plants in the field and give insight into the intricacies of their chemical strategies.

Several species of seedlings were collected from the invaded areas of China and India where *A. adenophora* is present and in Mexico where *A. adenophora* grows naturally. The Mexican plants, for the experiments, will remain labeled simply by the plots where they were harvested in Mexico, rather than by specific Latin names. Scientific and common names are listed in detail in Figures 4.1 and 4.4 for the seedling species from China and India used in Experiments One and Two. The seedlings were collected and provided by three colleagues: Inderjit in India, Yu-Long Feng in China, and Alfonso Valiente-Banuet in Mexico and supervised by Ragan Callaway in the U.S.A.

The hypothesis for Experiment One examined volatile allelopathic effects from *A. adenophora.* This was prepared by using *A. adenophora* leaf litter (treatment) and *Quercus macrocarpa* leaf litter (control) placing it in close proximity, but not in physical contact, with plant seedlings. This strategy would help to determine if there was inhibition of the Chinese and India plants, stunting their growth or preventing growth all

together, while the Mexican plants were hypothesized to react without inhibition when exposed to the treatment group. The hypothesis was that Chinese and Indian plants would grow with inhibition from *A. adenophora*'s allelopathy, while Mexican plants would grow unaffected.

The hypothesis for Experiment Two was that *A. adenophora* leaf litter, placed into the same soil of growing seedlings, would have negative allelopathic effects inhibiting the Chinese and Indian plant species which were the most successful in Experiment One, and the species from Mexico that were most successful from Experiment One would grow without inhibition.

<div align="center">Experiment One</div>

Experiment One was to test the effects of airborne allelopathy, or the chemical volatiles of *A. adenophora* on plants from the three countries. There were several important steps to prepare the experiment and ensure that the plants would receive equal treatment and grow under equal, and thus repeatable, conditions.

Plants from China and India for *Ageratina adenophora* Experiment One		
Country of Origin	Scientific Name	Common Name
China	*Sida szechuensis*	Badusan
China	*Siegesbekia orietalis*	St. Paulswort
China	*Peperomia tetraphylla*	Acorn Peperomia
China	*Hemiphragma heterophyllum*	Unknown
China	*Amranthus tricolor*	Joseph's Coat
China	*Eupotarium Chinense*	Hemp Agrimony
China	*Qxalis comiculata*	Creeping Wood Sorrel
China	*Eupatorium japonicum*	Unknown
China	*Cucubalus baccifer*	Berry-bearing catchfly
India	*Prosopis cineraria*	Ghaf
India	*Cassia fistuca*	Golden Shower
India	*Lawsonia alba*	Henna
India	*Bambosa aroundinacea*	Bamboo
India	*Bombyx ceifa*	Unknown
India	*Dillenia indica*	Elephant Apple
India	*Lagerstroemia indica*	Crepe myrtle
India	*Withania sominifers*	Indian Ginseng

Table 4.1. Plant used in first experiment. [9]

Experiment One Preparation

Experiment One began on January 20, 2008. Several steps were taken to ensure uniformity, precision, and retesting for the future if needed. In the first step of the experiment, 990 rocket pots were washed, sterilized and dried. Each pot was washed with household-quality dish soap, rinsed with tap water, and soaked in a 50% water/ 50% bleach solution for an hour. The same process was applied to the ten racks that held the rocket pots. The second step, after the pots and racks were soaked, was to rinse them with tap water and air-dry them. After the rocket pots were dried, they were prepared for seed planting.

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[9] Table 4.1. Plants used for Experiment One (Mexican plants not included for lack of specie's names).

Figure 4.2. rocket pots. [10]

The third step consisted of using Kimwipes, a cloth-gauze padding, in the bottom of the pots in order to hold the mixture of soil and plant material from filtering out of the bottom of each rocket pot. Each Kimwipe sheet was folded three times, and placed one per pot into the bottom of each rocket pot. This allowed the moisture to remain in the pots for a longer period. The Kimwipes were placed in the 990 rocket pots, ready for further preparation.

In the fourth step, Sand/Soil mixture was blended, using twenty milliliters of sterile sand and thirty milliliters of a sterile innoculum/ soil mix. The thirty milliliters of sand/soil mix consisted of five milliliters of autoclaved soil and twenty-five milliliters of the sterilized sand. (The sand was 20/30 grit, from Lane Mountain, Washington). A total mix of thirty liters of this mixture were put together in order to plant seedlings in 990 rocket pots.

Each of the 990 rocket pots was filled with 30 milliliters of this sand/soil mixture and placed into the racks. These racks, made to hold up to 200 rocket pots, were for this

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[10] Figure 4.2. A rack of rocket pots with *A. adenophora* leaf litter. Colored tags denoting country of origin.

experiment of allelopathy, only filled with 100 pots per rack. This short pot/rack ratio
was intended to cut down on possible allelopathy among plants in the racks, as *A.
adenophora* allelopathy alone is the subject of the experiment. This feature of planting
the racks only half-full and randomizing them in the racks gave plants more room and cut
down on contact between each plant during the experiment.

Before planting in the pots, the seedlings themselves needed preparation. Many
species would not germinate owing to the hard coating of the seeds. In these cases, the
seedlings were scored with a razor blade and others scratched with sandpaper owing to a
lack of environmental factors that help to open the seed naturally.

There were a total of thirty- three plant species, with sixteen species from Mexico,
nine species from China, and eight species from India. These plants were all collected in
areas where *A. adenophora* is present. Each of the thirty-three species was planted into
thirty pots each. In addition to organization, ninety pots split into each of the ten racks
total, with five seeds planted per pot. This is the 990 pots for the total of Experiment
One.

Figure 4.3. Experiment One ready for the greenhouse. [11]

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[11] Figure 4.3. Rocket Pot-filled racks and bins ready for the greenhouse.

The ten racks of ninety-nine pots were each put into separate bins, measuring 115-L (84x40x35cm). Five of these bins had loose, leaf litter of *A. adenophora,* which was shipped to the University of Montana from Inderjit, at the University of Delhi in India. There were also five bins for a control group that were filled with the control litter collected from The University of Montana campus, to act as a control for the experiment. *Quercus macrocarpa* has not been shown to have a negative allelopathic affect upon any of the seedlings planted in this experiment. It is merely present to ensure CO_2 release and other elements of decomposition from decaying litter and acts to make the experiment uniform.

<center>Post Preparation, Monitoring, and Data Collection</center>

Once the full plant flats were filled with planted pots, they were watered and placed into the greenhouse. Following this, the seedlings were watered with a mist of water every two to five days, depending on the moisture in the soil, ensuring that it did not dry up. On February 4, 2008, the *A. adenophora* leaves had been over-watered and were too wet to use for the experiment in this condition. They were then put into a warming oven at sixty-six degrees centigrade until dry. These leaves were then taken out of the warmer and put back into the bins on February 6, 2008. The leaves following the drying phase in the warmer did not have their pungent smell any longer and perhaps this would affect any contribution of inhibition or facilitated growth from allelopathy. On February 11, 2008 a new shipment of *A. adenophora* leaves were received to replace the leaves that were dried in the warmer.

An inventory of the seedling's germination was recorded on February 8, and February 22, 2008. It was then determined how many of the five seeds per pot had

germinated and a worksheet in Microsoft Excel was created, to save into a database. On February 29, 2008, the largest plant in each pot was measured and the remaining smaller plants were plucked out in an effort to look at the most successful plants and their growth rate. The last measure for germination and height was conducted on March 21, 2008, measurements were taken to determine plant mortality.

In Experiment Two, only the most successful plants from Experiment One were examined and tested for allelopathic volatiles. This included eight species from China, eight species from India and six species from Mexico. The seedlings were prepared in the same manner as in Experiment One, with a few exceptions. These plants were treated to examine growth and biomass, but did not have leaf litter of any kind for a control. These were projected to further test for effects of *A. adenophora* and determine its chemical interactions with chosen seedlings.

Experiment Two

The changes in Experiment Two allowed for further investigation, and were conducted to determine how inhibitory *A. adenophora* would be with the most successful plants from Experiment One. The following chart displays the chosen species and helps to provide a visual observation of the plants used.

Plants from China and India for *Ageratina adenophora* Experiment Two		
Country of Origin	Scientific Name	Common Name
China	*Sida szechuensis*	Badusan
China	*Siegesbekia orietalis*	St. Paulswort
China	*Hemiphragma heterophyllum*	Unknown
China	*Amranthus tricolor*	Joseph's Coat
China	*Eupotarium Chinense*	Hemp Agrimony
China	*Qxalis comiculata*	Creeping Wood Sorrel
China	*Eupatorium japonicum*	Unknown
China	*Cucubalus baccifer*	Berry-bearing catchfly
India	*Prosopis cineraria*	Ghaf
India	*Cassia fistuca*	Golden Shower
India	*Lawsonia alba*	Henna
India	*Bambosa aroundinacea*	Bamboo
India	*Bombyx ceifa*	Unknown
India	*Dillenia indica*	Elephant Apple
India	*Lagerstroemia indica*	Crepe myrtle

Figure 4.4. Plants for Experiment Two. [12]

Experiment Two Preparation

Preparation for Experiment Two began on May 23, 2008, and 440 rocket pots and the four racks that hold the rocket pots were washed. The cleaning was done with tap water and standard household-quality dish soap and each pot and rack were washed individually. The pots and racks were then submerged into a 50 % water/ 50% bleach solution for an hour. Then the pots and racks were rinsed with tap water and air dried, getting them ready for potting a soil-sand mixture and seedlings. As in Experiment One, the racks used to hold the rocket pots, which are structured to hold 200 pots at a time, were only filled with 100 pots at a time in order to create space between the pots in an effort to avoid additional chemical reaction between each species.

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[12] Table 4.4. Plants used for the second experiment (Mexican plants not mentioned for lack of specie's names).

The seeds were then sorted into twenty separate pots per species to create 220 pots for a treatment and 220 pots for a control. Hence, there were 440 pots for the entire experiment. In Experiment Two, the *A. adenophora* leaf litter was measured by taking a small and general pinch of litter to put into each pot with an effort to make each pot's content as equal as possible.

The seedlings were prepared for planting with consideration of those seedlings that needed more preparation, such as some tree species and other seedlings that needed to be scored with a razor blade, or scarifying with sandpaper, before planting them into the soil. Hence, seeds were counted, and prepared for germination to ensure a uniform number of seedlings for each pot. Five seedlings from each species went into their own, individual pots for twenty pots per each species. The twenty-two species were then put equally into twenty separate rocket pots, to equal ten pots per species in the *A. adenophora* group and ten pots per species for the control group.

To further the pot preparation for seed planting, several steps were taken to ensure uniformity between the seedling and a repeatable experiment. This entailed folding Kimwipes, and placing one in the bottom of each rocket pot following this the sand-soil mixture with a 5:1 ratio was poured into each pot. The seedlings were then planted, placing five seedlings from one species in each pot with twenty pots per one species. In 220 pots *A. adenophora* leaf litter was incorporated into the sand-soil mixture, and 220 pots with sand-soil mixture only, as the control. This equaled a total of 440 planted pots. The seedlings also received one round of Miracle Grow Fertilizer, or, one to six pellets in each pot.

Figure 4.5. Experiment Two planted pots. [13]

Post Preparation, Monitoring, and Data Collection

The seedlings were then watered every two to three days with a light mist in order to keep the soil moist. Harvests were taken during a three-day period. This included weighing the seedlings that had grown through the duration of the experiment. The plants were then recorded individually by average weight. The first harvest conducted on July 21, 2008, the second July 22, 2008, and the third on July 23, 2008. The plants were then dried in three separate batches, until each plant was weighed and averaged for each species in each rocket pot. The weights and averages demonstrate what level of impact *A. adenophora* has on the seedlings and how successful the seedlings were at their peak in the experiment.

Data Analysis

Statistics were run to determine the mean of the each plant grown, and to measure the species richness and mortality in Experiment One. In Experiment Two, the total mass of the plants per each pot were weighed to obtain an average for growth within the *A.*

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[13] Figure 4.5. Rocket pots for Experiment Two in the greenhouse.

adenophora treatments to determine biomass. Dr. Ragan Callaway also ran statistical

models to quantify the impacts and outcomes of the growing seedlings in the presence of

A. adenophora, and those in a controlled environment where there was not any other

plant litter present.

Chapter Five

Results and Discussion

When this project was started, there was a question of whether *Ageratina adenophora* was releasing volatile chemicals. The question of whether *A. adenophora* would inhibit plants from regions where it invades, and plants from its native Mexico, was the focus of the experiments. These questions are of great importance in understanding invasive plants, and how they negatively impact the world around them. The following experiments were efforts to determine if there were indeed volatiles, and if so, how this contributes to the science of allelopathy and *A. adenophora* today.

Plant Germination, Growth, and Testing for Experiment One

Seedlings for Experiment One were planted and placed into the greenhouse in early January 2008. Here the plants grew steadily through the first few months of the year, and were watered and inventoried every few days to ensure that they had sufficient nutrients, heat and light to allow seedlings to mature; this at least if there was not a presence of allelopathy. This allowed testing of the hypothesis for Experiment One to be carried out.

The hypothesis for Experiment One focused on whether *A. adenophora* would impact said plants, facilitate growth or leave these plants essentially unaffected. In order to determine this, the two experiments conducted were analyzed for the outcome and statistical models were run to determine the percentages of growth, inhibition and overall effects from *A. adenophora* leaf litter.

The plants were thoroughly inventoried four times, and germination and mortality were measured and recorded to determine species richness and mortality to

asses the outcome. Plants growth was recorded a total of four times and placed back into

the greenhouse until the last inventory on March 25, 2008, for a total of fifty-nine days.

These totals were compiled and the germination rates were recorded and considered.

The plants were harvested for the last time and each plant was individually

recorded, thirty plants, planted per each species. The findings were placed into a

Microsoft Excel spreadsheet to determine plant mortality and growth (measured in

millimeters). The tallest plant from each of the 990 rocket pots were plucked and

recorded to represent the respective pots where they were grown. This was taken into

account to determine if allelopathy had affected any of the plants and statistics were ran

to make this discovery.

Statistical Analysis of Experiment One

A statistical analysis using the Statistical Package for the Social Sciences (SPSS)

for a multivariate approach to understanding the project outcomes was conducted by Dr.

Ragan Callaway. The standard error and how each species differed in both the treatment

and control were calculated. This gave closer insight to the project, and allowed for

analysis of the hypothesis and scientific analysis of allelopathy of *A. adenophora*, if there

was any, and if so, to what extent.

The mortality of Indian plants was extremely high in the treatment bins, proving

there were indeed volatiles and *A. adenophora* inhibited the growth of the Indian plants.

The Chinese plants were also affected, but not as statistically significant as Indian plants

and both species from both invaded regions, versus species from Mexico. This showed

that *A. adenophora* was more inhibiting to Chinese and Indian plants than it was to

Mexican plants. Hence, volatile interaction and allelopathic inhibition was greater with

nonnative plants. In addition to mortality, the researcher considered species richness for

Experiment One, and analyzed data using SPSS. The tests showed varied outcomes and

another sign of allelopathy for those plants abroad, more so than in plants from nonnative

regions than in Mexico.

Chinese plants showed higher species richness in exposure to the control, versus

the treatment exposure more so than the other two country's plants. In Experiment One,

these findings were statistically significant. Indian plants also had high species richness

in the control versus the treatment bins and showed to be statistically significant as well.

Mexican plants actually grew successfully and more rapid with the treatment, showing a

positive effect from *A. adenophora*.

Plant Germination, Growth, and Testing for Experiment Two

On March 23, 2008, seedlings for Experiment Two were planted and placed in the

greenhouse to begin their cycle of growth that would later suggest allelopathy. The

plants were also watered and observed for germination to be recorded later. This

experiment had duration of fifty-nine days, allowing enough time for germination, and

for a test to determine allelopathic effects on all plant subjects. The purpose of

Experiment Two was to test those plants that were most successful in Experiment One,

from all three countries, to determine if there was further allelopathy and if there would

be a different outcome with using a control different from Experiment One.

In Experiment One, the control was leaf litter from *Quercus macrocarpa* collected

from The University of Montana campus. This was a plant not known to have

allelopathic effects on any of the plants involved. In Experiment Two, the control was

simply to leave the bins empty of any leaf litter, to ensure that *Q. macrocarpa* did not

have a negative effect on plants in Experiment One, and to conduct another thorough test for allelopathy.

The plants for Experiment Two were watered approximately every three days. They were also checked for germination or mortality and this would be accounted for later during the harvest. The plants were kept in temperatures that had been conducive to their germination and growth weight in their native environments. At 20-22°C during a 16 h light period 72at 18-20°C during the night; relative humidity was $30 - 55$ %. Also, the seedlings were planted with a miniscule amount of fertilizer from a Miracle Grow mixture, to assist in keeping the plants healthy as part of the secondary experiment.

Plants for Experiment Two were harvested after an almost two month growing period and inventoried by weight in grams, and by mortality. They were then entered into a Microsoft Excel spreadsheet and statistically analyzed for biomass. The results were somewhat independent from those of Experiment One and essentially constituted a new experiment in which to test the plants for allelopathy.

<div align="center">Statistical Analysis for Experiment Two</div>

Outcomes of running an SPSS statistical model for Experiment Two gave two results. First was species richness, which was similar to Experiment One in terms of statistical significance. Chinese and Indian plants both grew more successfully in the control than they did with *A. adenophora* and at a high statistically significant rate. The Mexican plants again grew more successfully with *A. adenophora* leaf litter, but this outcome was not statistically significant.

Examining biomass, there was a positive effect of *A. adenophora* on plants from Mexico

and India, but a slightly less prominent effect on Chinese plants. Further, a slightly more

positive effect on Mexican plants than on those of India were found.

Discussion for Experiments One and Two

Perhaps allelopathy provided native plants with an advantage over non-natives

and The Novel Weapons Hypothesis will further become accepted as a truism for

invasive plant strategy, however small this experiment contributes to the study.

Conducting two experiments provided the researcher with an opportunity to gain greater

knowledge of how allelopathy affects plants, both in their native and invasive regions,

and to gain insight into how this may be applicable within plant communities. These

experiments may contribute to a better understanding of volatiles, which can be

inhibitory in communities where an invader is found. The experiments were unique in

scope, preparation, testing and reporting, thus contributions to biogeographical studies of

invasive plants and concepts testing allelopathy. Experiments One and Two both showed

strong allelopathic effects and demonstrated that Mexican plants were not suppressed by

A. adenophora, while non-native plants were distinctly effected by *A. adenophora*

although to a different extent in each experiment.

Outcomes and Significance of Experiment One

As mentioned, Experiment One measured species richness and mortality in order

to quantify *A. adenophora*'s positive or negative effects upon the plants used in the

experiment. The first test in Figure 5.1 measured mortality and is displayed in a graph

following the statistical models run using SPSS. This graph indicates how successful *A.*

adenophora is in India, or at least to Indian plants in the greenhouse, causing a

statistically significant mortality rate. Test of the Chinese plants were statistically

significant as well, but not to the same extent. The Mexican plants did not have a high

rate of morality. This shows a standard error of about ten percent for Indian plants, five

percent for Chinese plants and ten percent for Mexican plants as shown in Figure 5.1
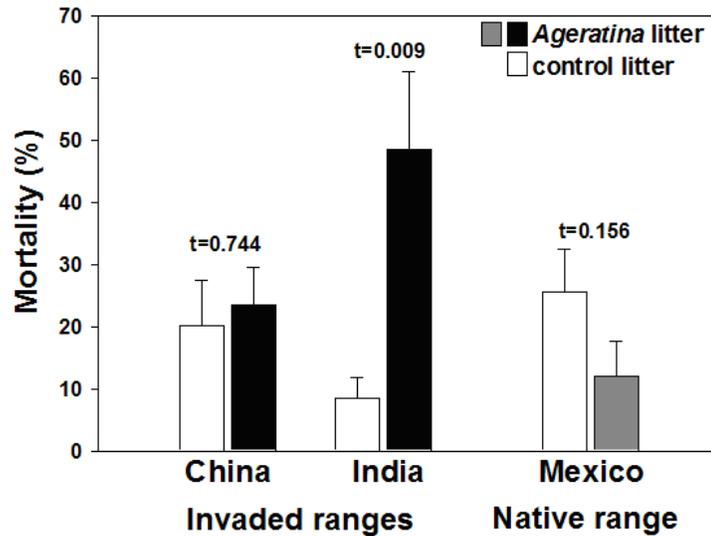
where mortality of the plants is illustrated.

Figure 5.1. Mortality rates for Experiment One. [14]

These findings suggest that allelopathy from *A. adenophora* have negative effects

on the mortality of non-native plants, which have a high mortality rate from *A.*

adenophora's volatiles. Species richness was also great in the non-native plant

assemblages within the control group, and lower in the *A. adenophora* group, suggesting

again the impacts of volatiles. Species richness, as shown in Figure 5.2 indicates that

native plants grew well in the *A. adenophora* group and reveal that there was not a strong

inhibitory effect on those plants. Again, Chinese and Indian plants were affected to a

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[14] Figure 5.1. Measurements of mortality.

degree of statistical significance, and exhibited more growth and success in the control groups than in the *A. adenophora* group. Mexican plants were not negatively impacted by *A. adenophora* and grew more successfully that in the control group.
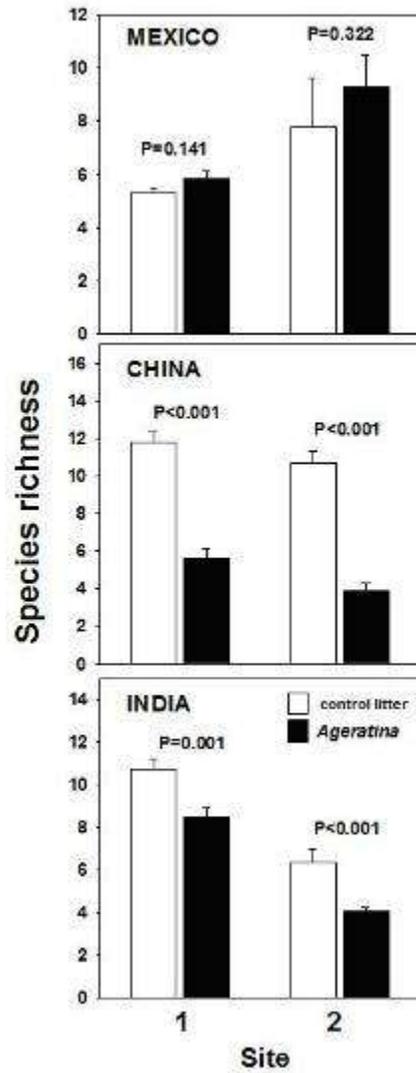

Figure 5.2. Species richness of Experiment One. [15]

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[15] Figure 5.2. Density of species richness in each plant region in the experiment.

Outcomes and Significance of Experiment Two

Experiment Two showed positive signs of allelopathy and statistical significance

as well, *A. adenophora* was consistently inhibitory in both experiments. This

demonstrates that the hypothesis for each plant experiment was positive and gave the
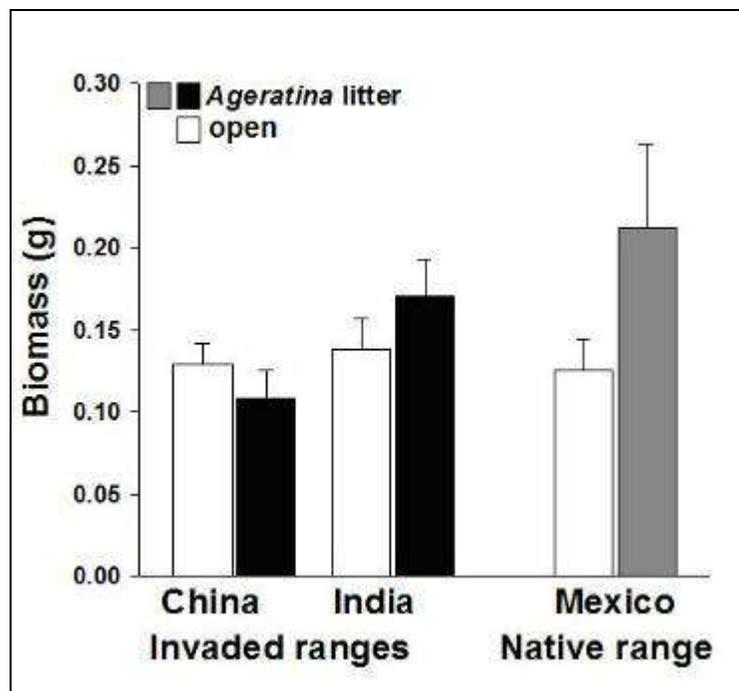
outcomes of positive hypotheses for each test.

Figure 5.3. Biomass of Experiment Two. [16]

Biomass was measured for the control and *A. adenophora* groups illustrated in

Figure 5.3.   There is definitely a higher growth rate for non-native plants with a standard

error of about 0.05. Also, native plants showed a higher growth rate with the treatment

group present and appeared to be unaffected. When a statistical model was run for non-

native plants as one unit, there was an even higher rate of inhibition from *A. adenophora*.

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[16] Figure 5.3 Biomass, or success of plants in the presence of *A. adenophora*.

Hence, biomass was denser, as it was measured by weight of successful seedlings that germinated and survived for the harvest. Dead plants were discarded and not accounted for in this experiment.

Chapter Six

<u>Conclusion</u>

As biogeography furthered the understanding of the natural world and its inhabitants, the foundation was set within geography for the study of science of invasive plants like *Ageratina adenophora*. Through studies of biological life and plants in their natural and invasive environments, we can open the doors to our knowledge of how these plants perform in their respective environmental roles. Field and laboratory experiments are a key to looking at the biogeographical effects and relationships of plants within the Trans-global ecosystems. It is through this understanding that we can preserve native plants in their niches, and have a deeper understanding of the role invasive plants play is their migrations abroad.

It is of great importance to acknowledge the study and insight into allelopathy and how an invasive plant's strategies can affect a plant community with such strength and influence. Allelopathy is at the forefront of explaining how plants invade and become noxious and gives insight into how this phenomenon relates to the rest of the biological world. The possibilities of allelopathy can set the stage for extreme and unrelenting invasion of a plant into a new, and hence, invaded community. In these studies, such as this thesis we may understand how these concepts and knowledge gained present a source of answers to gain control and solve the complex problems of invasive plants.

Plants like *A. adenophora* are a substantial example of the invasion processes and the negative impacts on environment. Invasive plants are costly, degredating to the natural world in new areas, present and create problems to health both for humans and all biological life involved. Studying the foundations of biogeography can grasp the roots of

this science and contribute to the understanding of how this observation of life and place began, and what we can learn from those elements of the past. The tools learned from these pioneering scientists and perhaps insight from this study can perhaps prepare one better for the scientific analysis of *A. adenophora*. Research on *A. adenophora* and the laboratory experiments conducted for this thesis were an attempt to further the examination of the plant and contribute to its still understudied features in the scientific community.

This study established some of *A. adenophora*'s elemental factors, representing its capability of inhibiting plants where it invades with allelopathic volatiles and causes, in some cases, high rates of mortality. This mortality may lead to destruction of a plant community, in India, China, and other parts of the globe where *A. adenophora* can be found in an invasive status. Moreover, *A. adenophora* through negative allelopathy can be degredating to a community's species richness and cause loss of biomass in a plant population, allowing for further disturbance and invasion. The features of *A. adenophora,* which were assumed before these experiments were affirmed, are applicable to the plants in these three nations to resolve the problems of invasion. These findings can better prepare managers for dealing with their plants and furthers our understanding of other invasive plants.

Ageratina adenophora is only one invasive plant, but furthering the study of allelopathic volatile effects can be a lens to understand the proximity of one plant to another, how one invasive plant may react to several species where it is present, and how this directly represents chemical suppression. We can observe how one native plant may react in comparison to their native neighbor, finding that one may be highly inhibited,

while another is completely unharmed and flourishes despite the invasive plant and its effects from one plant to the next, in that community.

Also of importance in this thesis were the studies of whether *A. adenophora* affected each plant similarly or differently in respect to the three measurements of mortality, species richness and biomass. While some of the plants used were tree seedlings, others shrubs and others small to moderate in size. All seedlings for the experiments were selected because they were the plants that grew within close proximity to where *A. adenophora* is found to invade or subside in its areas of introduction and native plant communities. Further study may look at these experiments to perhaps add more in depth to the phenomena for invasion of *A. adenophora* in China and India, an addition to native Mexico.

This thesis research was based largely on Chinese scholarly journals and the few that exist from India and Mexico, which were available for American researchers. Future directions could be to substantiate these outcomes and gain a more worldly study of *A. adenophora* in its native and invasive regions. Perhaps this project conducts further education of the effects of allelopathy, within the tests of these regions and others areas of invasion (Australia, United States, regions in Africa, and Europe). Researchers must observe *A. adenophora* in Mexico, as an addition to invasive plant research as they are often understudied in their native environments (Hierro 2005, 2). It is likely that *A. adenophora* is understudied due to lack of a crucial reason to study it in Mexico, as it may not be as relevant as it is in its invasive regions throughout the world. Further, through my research study of *A. adenophora* it appears to be insignificant in Mexico and does not pose a threat there, nor is it known to be of interest in the study of flora in that

country.   This thesis is one of the first to analyze *A. adenophora* and the aspect of its occurrence in these three regions, and to experiment with its allelopathic effects on these region's plants. *A. adenophora* is not extensively studied, and is relatively new to scholars who have conducted investigations (Dong, *et al.* 2008, Hong-Bang *et al.* 2007, Lichti and Hoshovsky 2000, Munniappan *et al.* 2009, Morris 1989, Zang *et al.* 2008, Tripathi 1981).

It would he of great importance to contribute to the study of this plant and conduct field or laboratory tests to determine the biological role it plays in native Mexico, what contributions, and how significant, or not it is to its community there. This could provide information about chemical content and communication, wildlife habitat, crop management and many other valuable resources that may be affected and may affect *A. adenophora*. Also of importance is to study *A. adenophora* and its effects on each individual plant that may be related to the areas impacted. This may give insight to how to keep *A. adenophora* manageable in croplands, habitat for other, indigenous life and to prevent further spread once established.

This thesis and future studies will contribute to a better understanding of invasive plants, potentially the strategies plants use to become invasive, and their biogeography. This thesis examined how a Mexican native has allelopathic affects on Chinese and Indian plants through leaf litter and what conclusions can be drawn from those results in a laboratory environment. An article, (Inderjit *et al.* 2010 in press) reports on several different mechanisms to compare leaf litter of *A. adenophora* from India on plants from China, India and Mexico. These studies may contribute to the understanding and

strategies to examine the negative allelopathy of *A. adenophora* in these regions around

the world.

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Appendix A

Volatile chemicals from leaf litter are associated with invasiveness of a Neotropical weed in Asia

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Abstract. Some invasive plants successfully outcompete their neighbors in the invaded ranges while happily coexist in diverse communities in their native range. We find that volatile signals may contribute to the success of *Ageratina adenophora,* an aggressive neotropical invasive plant.   In the field in its native Mexico, *A. adenophora* has neutral to positive effects on other species, but in two non-native ranges, China and India, it strongly inhibits other plants. In controlled experiments volatiles from *A. adenophora* litter caused higher mortality of species native to India and China, but not of species native to Mexico. Litter from *A. adenophora* plants from non-native populations produced chemically different VOCs than litter from native populations. Biogeographic differences in the impacts of *A. adenophora* suggest that evolutionary trajectories may affect interactions within communities, and differences in composition of volatiles between ranges suggest that *A. adenophora* may be experiencing strong selection on biochemical composition in its non-native ranges.

Why some plant species are able to attain near monocultural dominance in communities where they are exotic but not where they are native remains poorly understood (Callaway and Maron 2006). But this biogeographic shift in dominance is the essence of exotic "invasion" and in the absence of disturbance strong competitive interactions are likely to play an important role in establishing the dominance of invaders (Levine *et al.* 2003, Víla and Weiner 2004). Release from specialist herbivores or pathogens may allow plants to be more competitive in non-native ranges (Keane and Crawley 2002), or successful invaders may possess competitive advantages because they come from a more competitive species pool or happen to possess inherent traits that give them an advantage relative to their new neighbors. One explicit hypothesis for why some species become more competitively dominant in their new ranges is the Novel Weapons Hypothesis (NWH), which poses that some invaders possess allelopathic, anti-herbivore, or antimicrobial chemicals to which native organisms have not adapted, and that these novel compounds provide invaders with direct or indirect competitive advantages (Callaway and Aschehoug 2000, Callaway and Ridenour 2004). Evidence for the NWH comes from, experiments in which chemicals produced by invaders were applied to species from invaded and native ranges (Callaway *et al.* 2008, He *et al.* 2009), studies comparing the allelopathic effects of invaders and native plants (Ens *et al.* 2009a, b), and literature comparisons of the novelty of chemicals produced by invasive species relative to chemicals identified in the native flora (Cappuccino and Arnason 2006).

Early studies of allelopathy focused on volatile organic compounds (VOCs) (Muller *et al.* 1964, Muller 1965), but interest in VOCs as agents of allelopathy faded after their ecological importance in the field was challenged by experiments showing that herbivores were likely to cause the patterns attributed to VOCs (Bartholomew 1970). But recently, VOCs have gained attention in other ecological roles as factors in communication among plants (Karban 2007, Karban and Shiojiri 2009), signals to the predators of herbivore enemies (Kessler and Baldwin 2002) and in self-nonself recognition (Eoms et al. 2006). And, they have re-emerged as potential factors in allelopathic interactions as well (Eom *et al.* 2006, Barney *et al.* 2005, and Karban 2007).

A plant species that may employ VOCs in allelopathy is *Ageratina adenophora* (Sprengel) R. M. King and H. Robinson [Syn. *Eupatorium adenophorum*, Asteraceae], a species producing a large number of volatiles with broad biocidal properties (Palá-Paúl *et al.* 2002). *Ageratina adenophora* is a shrub native to central Mexico but invasive in subtropical climates throughout the world including Africa, India, the Philippines, Europe, China and Australia. *Ageratina adenophora* can establish virtual monocultures where diverse native communities once flourished (Wang and Wang 2006) and appears to be expanding its range in the areas of our study sites in India and China (Zhu *et al.* 2007). In addition to bioactivity of its VOCs, there is some evidence for *A. adenophora* allelopathy based on tissue extracts and field applications of activated carbon (Song *et al.* 2000, Zhang *et al.* 2008). These results plus the powerful and unusual smell of its litter make *A. adenophora* an interesting candidate for comparing the biological effects of VOCs on competitiors from both native and invasive ranges to gain insight into whether allelopathy might play a role in its invasive abilities.

Effect of Ageratina adenophora *in the field*

In the native range, there were two field sites; one in northeast Mexico in the Sierra Madre Oriental near Balcón de Moctezuma in the state of Tamaulipas, and the other in central Mexico in the state of Querétaro. The Moctezuma site was at 1400 m a.s.l., 23º36'N; 99º13', and is open oak woodland dominated by *Quercus oleoides*. The Querétaro site was at 19º52'N; 100º54'W. In India, we sampled two sites in Mussoorie (30°28.750'N; 78°03.216'E; 1663 m elevation and 30°28.272'N; 78°03.463'E; 1866 m, respectively). At each site we randomly placed 20 1m x1m quadrats in large patches of *A. adenophora* and 20 quadrats in random locations without *A. adenophora* but surrounding the patches. We counted the number of different species in each quadrat. We also sampled two sites in the same way in China, the first located at Xujiaba, 2430 m a.s.l., 24°32'N, 101°1'E; and the second at Taizhong, 1420 m a.s.l., 24°27'N, 100°54'E. Both sites were in Jingdong County, Yunnan Province, and were 15 km from each other. At each site in Mexico and in China we randomly located 20 shrubs and then randomly located 1 x 1 m quadrat under each canopy. In Mexico and China the cover of each species was estimated in each quadrat.

VOC bioassay

We tested the effect of VOCs emitted from *A. adenophora* leaf litter on the germination of *B. biternata* and *B. arundinacea* seedlings at the University of Delhi. Eight seeds of either *B. biternata* or *B. arundinacea* were placed on Whatman filter paper #1 in each of three 7.5-8.0 cm diameter Petri dishes. To maintain uniform moisture, a thin layer of cotton soaked in 10 mL distilled water was placed below the filter paper. Three Petri dishes were then placed in glass chambers (190 mm x 100 mm, i.d. x height) with either 20 g of *A. adenophora* leaf litter, collected as it fell naturally in the field, or without litter. Litter was air-dried and then used for bioassay experiments. For each species and treatment combination there were 5 glass chambers, thus 5 replicates. Glass chambers without *A. adenophora* leaf litter were used as controls. The chambers were incubated at 28-32°C and a 12h:12h light/dark cycle. Seed germination was counted daily and root and shoot lengths were measured after 7 (*B. biternata*) or 8 (*B. arundinacea)* days. Mean root or shoot lengths were compared with t-tests.

Biogeographic comparisons

Seeds were collected in the field and from growing plants growing in communities with *A. adenophora*. We planted seeds from each of the three regions in 200 cm^3 "rocket pots" and then placed these pots in 115-L (84x40x35cm) plastic bins that contained either 1.5 L of *A. adenophora* litter collected from plants from the three regions but grown from seed in a common garden, or a 1.5-L mixture of field-collected litter from *Quercus*

macrocarpa, *Acer platanoides*, and *Populus tremuloides*, collected on The University of Montana campus. We initially used seeds of 16 species from Mexico, 9 species from China, and 8 species from India. For each species we planted 5 seeds in each of 33 rocket pots for an initial total of 990 replicates. Pots contained 5 ml of soil and 25 ml of silica sand. Rocket pots for each species were then divided equally into the two litter treatments. Five bins were used for each of the litter treatments; thus each bin contained 3 replicates of each species. Litter was air-dried for 2 days and then placed in the bottom of the bins; rocket pots were placed in racks which suspended the rocket pots above the litter. Bins were covered with lids leaving 120 cm^2 of each cover open, allowing circulation but ensuring that air within the bins was affected by VOCs; attested to by the exceptionally strong smell within the bins and throughout the greenhouse. These seedlings were grown in a greenhouse from 23 January 2008 to 21 March 2008 with ambient light supplemented by metal halide bulbs, keeping photosynthetically active radiation (PAR) during the day above 1200 µmol/m^2/s with a day length of 13 hours. We watered plants every two to three days. During the experiment germination was counted as plants emerged but new germinants were thinned so that the survival and final mass of the first germinant was measured in each rocket pot. The final total dry (at 60°C) biomass was measured after 59 days and survival of these individuals was measured.

Identification and quantification of volatiles

We grew *A. adenophora* from seeds of nine separate populations collected in each of three different countries, Mexico, China and India. Plants were grown side by side in a

greenhouse at the Max Planck Institute of Chemical Ecology with additional illumination by sodium lamps. Temperature was maintained at 20-22°C during a 16 h light period and at 18-20°C during the night; relative humidity was $30 - 55$ %. Plants were potted in commercial soil (Tonsubstrat, Klasmann, Geeste/Groß-Hesepe, Germany) and watered every day with tap water. Five months after germination, leaves of plants from three populations of the three different origins were collected and dried for 10 d at room temperature (approx. 25°C). Dried leaves were homogenized using metal beads with agitation by a paint shaker two times for 3 minutes each. To collect VOCs from homogenized *A. adenophora* leaves, we applied a closed-loop stripping method. We placed 200 mg of homogenized leaves in a 1L desiccator and VOCs were collected for 2 h by recirculating desiccator air through a charcoal trap with a pump. VOCs were eluted from the trap with 60 µl of ethyl acetate containing 20 ng µl^{-1} nonyl acetate as an internal standard (IS).

Plant material from the same harvest was also used for direct solvent extraction of potential VOCs. Here 200 mg homogenized leaves were extracted with 1ml of ethyl acetate containing 20 ng µl^{-1} IS for 24 h at room temperature under continuous rotation. Solvent extracts were purified and dried over Na_2SO_4. All extractions were performed as four replicates on three populations from each of the three regions. Products of volatile collection and solvent extractions were identified by gas chromatography (Agilent Hewlett-Packard 6890, Agilent Technologies, Santa Clara, CA, USA) coupled to a Network Mass Selective Detector (MS) (Agilent Hewlett-Packard 5973, Agilent Technologies) or a Flame Ionization Detector (FID). For analyses, 2 µl of ethyl acetate extracts were injected at 230°C. Compounds were separated on a DB5-MS column (30

m length, 0.25 mm inner diameter and 0.25 µm film by JandW Scientific; GC-program

40°C for 2 min, first ramp 3°C min^{-1} to 175°C, second ramp 90°C min^{-1} to 250°C, final 3

min hold). GC-MS carrier gas: helium at 1 ml min^{-1}; GC-FID carrier gas: hydrogen at 2

ml min^{-1}. All terpene products were identified by using Agilent Technologies software

with the Wiley 275.L and NIST 98.1 MS libraries, as well as by comparison of mass

spectra and retention times with those of authentic standards where available.

RESULTS

Ageratina adenophora *decreases plant species diversity in its invaded range*

In its native range in Mexico, *A. adenophora* had no significant effect on quadrat-

based species richness at each site tested separately (Fig. 1), but across both sites *A.*

adenophora had a significant stimulating or positive effect (site x treatment ANOVA;

$F_{treatment}$= 5.45; df=1,76; P=0.022). In contrast, species richness under *A. adenophora*

canopies or in *A. adenophora* patches at all sites in the invaded ranges of China and India

was significantly lower than in the open where there was no *A. adenophora*. The total

number of species recorded at Moctezuma in Mexico was 45, with 34 species occurring

under *A. adenophora* and 35 species occurring in the open; a decline in diversity of 3%.

At Querétaro there were 38 species, with 30 found in the open and 26 under *A.*

adenophora; a decline in diversity of 13%. In China there were 111 species at Xujiba,

with 98 of these species found in the open away from *A. adenophora* but only 54 species

beneath *A. adenophora*; a decline in species richness of 54%. There were 91 species in

total at the Taizhong in China and 79 were found in the open; however, only 35 were found under *A. adenophora*; a decline in richness of 56%. In India, at both sites species richness declined significantly in locations with *A. adenophora* compared to the locations where *A. adenophora* has not yet invaded. At Mussoorie 1, there was a decline of 85% (7.5±0.3 to 1.2±0.2 species/m^2; t-test, df=1, 38; t= 20.76; P<0.0001) and at Mussoorie 2 there was a decline of 69% (20.7±0.7 vs. 6.4±0.7 species/ m^2; t-test, df= 1, 38; t= 14.96; P<0.0001; Fig. 1). Biogeographical differences in total cover were even more striking. The total cover of other species associated with *A. adenophora* canopies in Mexico was 71% higher than in the open (44.8 ± 2.6 vs. 76.8 ± 6.2 percent; t-test, df=1,38; t=4.78; P < 0.001), a strong facilitative effect. But in China *A. adenophora* canopies decreased total cover by 75% at the high site (102.6 ± 6.3 vs. 25.5 ± 3.8 percent; t-test, df=1,38; t=10.46; P<0.001), and by 79% at the low site (83.3 ± 6.9 vs. 17.2 ± 3.6 percent; t-test, df=1,38; t=8.44; P < 0.001); indicating strong competitive effects.

Volatiles of A. adenophora *leaf litter collected from invasive plants inhibit the growth of other species*

Bambusa arundinacea seed germination was not inhibited (t=0.180, P=0.858) after 7 d by *A. adenophora* VOCs, but the germination of *B. biternata* seeds was highly suppressed (for final percentages; t=6.767; P<0.0001; (See Fig. S1 in supporting information). The growth of the roots and shoots of both *B. arundinacea* (t_{root}=15.232, P<0.0001; t_{shoot}=8.224, P<0.0001) and *B. biternata* (t_{root}=9.329, P<0.0001; t_{shoot}=14.109,

P<0.0001) was significantly suppressed by VOCs from *A. adenophora* leaf litter (See Fig. S1).

Volatiles of A. adenophora *cause more mortality of species from invasive vs. native*

ranges

This experiment served another purpose, because ecologically realistic concentrations of VOCs are very difficult to determine. Biogeographic differences in a chemical's effects yield strong evidence for whether or not allelopathy actually contributes to invasive success, but also adds to the argument for the ecological relevance of the interaction. The germination or growth of some individual species showed significant responses, either positive or negative, to VOCs emitted from *A. adenophora* litter, but exposure to VOCs had no significant overall effect on the germination or biomass of the combined pool of species from any of the three regions. However, VOCs from *A. adenophora* litter increased the mortality of all species from the combined invaded ranges, but in the same VOC treatments the mortality of species from the native range tended to be lower than in controls (Fig. 2). In a two-way ANOVA with region as a fixed factor (combining species from both invaded regions), bin as a fixed factor, and with the mean for each species as a single replicate, the region x treatment interaction was significant (F=5.201; df=1,37; P=0.029).

Major volatiles of A. adenophora *leaf litter differ between populations from native and*

invasive ranges

Many monoterpenes and sesquiterpenes were identified in the headspace analysis of *A. adenophora* leaf litter produced by plants raised from seeds collected from Mexico, China and India (see Fig. S2; Table S1). The native Mexican populations were significantly different from both the invasive Chinese and Indian populations in terms of emitting six VOCs (Fig. 3; see Table S2). The Chinese and Indian populations, however, showed similar pattern in emitting VOCs. Two monoterpenes, 2-carene and α-phellandrene, were at higher concentrations in the headspace of litter of plants from the invasive ranges; whereas the monoterpene β-pinene, and the three sesquiterpenes (E)-β-caryophyllene, (E)-α- bergamotene and bicyclogermacrene, were at higher levels in the headspace of litter from plants of the native range. Direct extraction of leaf liter with organic solvent gave the same terpenes in the same proportions as present in the headspace (See Fig. S3).

DISCUSSION

Ageratina adenophora, an invader throughout the subtropics, appears to have no negative effects on adjacent plants in its native range in Mexico, and some cases *A. adenophora* has strong facilitative effects. In contrast, in non-native populations in China and India the presence of *A. adenophora* shrubs correlated with substantial decreases in the diversity and cover of native species. Although our limited number of field sites warrants caution in extrapolating to the whole of its invasive range, other studies of *A. adenophora* in Asia have shown effects on native species that are similar to or stronger

than ours. Based on regression relationships at the scale of 25 m^2 plots in southwest

China, Lu and Ma (2005) found that, native species richness averaged >25 species per

plot when *A. adenophora* cover was near zero; however, when *A. adenophora* cover

reached ≈60%, native species richness declined to <5 species per plot. To our

knowledge, no other study has quantitatively estimated the impact of an invasive plant

species in the field in both its native and non-native ranges, but a number of studies have

clearly documented strong negative impacts of many invaders in their non-native ranges

(Hejda *et al.* 2009).

Many factors are likely to contribute to the invasive success of *A. adenophora*,

but our findings point to one factor in particular: the allelopathic effects of VOCs from

the litter of *A. adenophora* are stronger on species from the non-native range than from

the native range; a finding consistent with the Novel Weapons Hypothesis. Our results

add a new dimension to an emerging body of work emphasizing the biological

importance of VOCs emitted from plants as allelopathic agents (Kessler and Baldwin

2002, Karban 2007). Ens *et al.* (2009a) examined VOCs produced by the roots of the

invasive *Chrysanthemoides monilifera* in Australia and detected three volatile

compounds that were found exclusively in *C. monilifera* roots and its rhizosphere.

Higher production of terpenes was observed in *C. monilifera* roots, while *Acacia

longifera,* a native species, produces more alkanes. Extracts from leaves and roots of *C.

monilifera* suppressed more native Australian species than extracts from the dominant

native *Acacia* (Ens *et al.* 2009b). This study, however, lacks biogeographical

comparisons of VOCs in native and invaded ranges.

Production of a novel volatile compound to which plant species growing in invaded regions appear to more susceptible, may contribute to the invasive success of *A. adenophora.* However, this may not fully explain *A. adenophora* invasion and does not rule out the importance of other mechanisms. For example, Niu *et al.* (2007) found that *A. adenophora* and soil biota in the invaded range interacted to establish positive feedbacks on *A. adenophora*, and that *A. adenophora* modifies soil communities in ways that negatively impact other species in the invasive range, suggesting the possibility that escape from soil pathogens may be important for invasion. In China, field surveys found an absence of specialist herbivores on *A. adenophora* (with the exception of a galling insect, *Procecidochares utilis* Stone, which was introduced into China in 1984), and virtually no native generalists attack the plant (Feng *et al.* 2009). *Procecidochares utilis* was introduced as a biological control agent in India in 1963, but has been reported to cause only minor damage (Jayanth 2000). As for any exotic invasion, the relative importance of different mechanisms must be disentangled in order to explain the process.

So far, the NWH has been explored in the context of regional novelty; i.e. the idea that some secondary metabolites might be unusually potent as allelopathic, antibiotic, or defense agents because of their novelty to organisms in non-native ranges. However, it was not necessarily assumed that the invasive species would *introduce* new compounds in its non-native ranges, as we have demonstrated here. Our results raise two potentially different interpretations of biochemical novelty in *A. adenophora* invasion, and do not favor one interpretation over the other. First, we used leaves from India in our biogeographical experiment with VOCs (Fig. 2); and thus the effects in this experiment may be due the adaptive naiveté of species from China and Mexico to chemicals

produced by *A. adenophora*, no matter what the biogeographical source of the invader. But we also found that VOCs from leaf litter from populations of *A. adenophora* from China and India (grown under common conditions) were strikingly different in biochemical composition than VOCs from the leaf litter of *A. adenophora* grown from seed collected from Mexican plants. Although our population-scale sampling was small to exclude founder effects, the finding that all populations from India and China differed from all populations from Mexico raises the possibility that invasive populations of *A. adenophora* have evolved to produce much higher levels of some biologically effective compounds in their non-native ranges. Both of these perspectives on novelty are conceptually consistent with ideas for the importance of biochemical novelty in evolutionary arms races between plants and their herbivore enemies (Ehrlich and Raven 1966). In other words, evolving a new version of a defense compound, or simply possessing a defense compound that is novel in a particular part of the world, are both strategies that may lead to ecological success.

The concentrations of 2-carene in the VOCs of leaf litter produced by *A. adenophora* plants from the invaded range (grown in a common garden) were three orders of magnitude higher than concentrations in VOCs from litter produced by *A. adenophora* plants from the native range (Fig. 3). α-Phellandrene emission was about twice as high for populations from the invaded ranges as for populations from the native range. 2-Carene has been reported to have allelopathic effects (Vokou *et al. 2003*), and Kpoviessi *et al.* (2009) found that α-phellandrene, a constituent of the essential oil of *Justicia anselliana*, had allelopathic effects. If these compounds contribute to the allelopathic effects of *A. adenophora* VOCs, high concentrations may have been selected

for in the non-native ranges (Callaway and Ridenour 2004). However, β-pinene and trans-β-caryophyllene were emitted in far higher amounts from litter from Mexican plants than from the litter of Indian or Chinese plants and these chemicals have also been reported to have important defense properties (Köllner *et al.* 2008). Perhaps these compounds are less active as allelopathic agents than 2-carene and α-phellandrene. Or, perhaps they are important in defense against specialist enemies which should be less abundant in the invaded ranges (Feng *et al.* 2009).

Our spatially and regional explicit results suggest regional evolutionary trajectories (Thompson 2005) which have several interesting implications for community ecology. First, they add to a growing body of literature indicating that there is some degree of species-specificity in plant-plant interactions. Second, our results suggest that assemblages of plants, or communities, may be more tightly knit entities than generally thought. Under these conditions, interactions among plant species may drive natural selection. Finally, regional evolutionary trajectories suggest that novel competitive mechanisms used by invaders may disrupt coevolved interactions among long-associated native species. Mixing species that do not share common evolutionary trajectories may have important consequences.

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Figure Legends

FIG. 1. Quadrat-based plant species richness under *Ageratina adenophora* canopies and in the open (mean + SE) at sites in Mexico, India, and China. Error bars represent 1 SE and P values above paired bars are from independent t-tests.

FIG. 2. Effects of volatiles from leaf litter of *Ageratina adenophora* on the mortality of species native to its invaded (China or India) ranges and species from its native (Mexico) range. Error bars represent 1 SE and ANOVA results are in text.

FIG. 3. Biogeographical differences in the emission of β-pinene, 2-carene, α-phellandrene, (*E*)-β-caryophyllene, (*E*)-α-bergamotene and bicyclogermacrene. Error bars represent 1 SE. P values shown for two-way ANOVA.

FIG. S1. Seedling (root and hypocotyls) length and germination (%) (mean + SE) of *Bidens biternata* and *Bambusa arundinacea* grown in the presence or absence of *Ageratina adenophora* leaf litter.

FIG. S2. Volatiles collected from headspace of leaf material of three different origins.

FIG. S3. A comparison of volatiles collected through solvent extraction or from headspace of leaf material of three different origins.
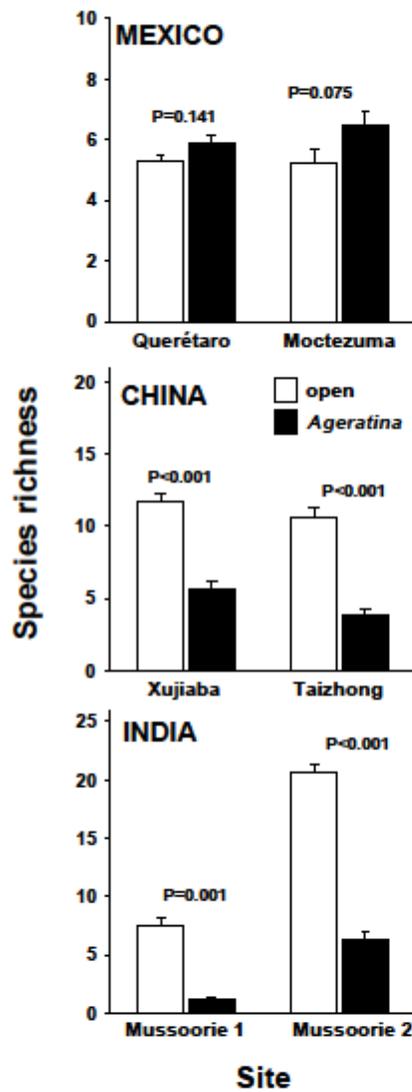
Fig. 1. Quadrat-based plant species richness under *Ageratina adenophora* canopies and in the open (mean + SE) at sites in Mexico, India, and China. Error bars represent 1 SE and P values above paired bars are from independent t-tests.
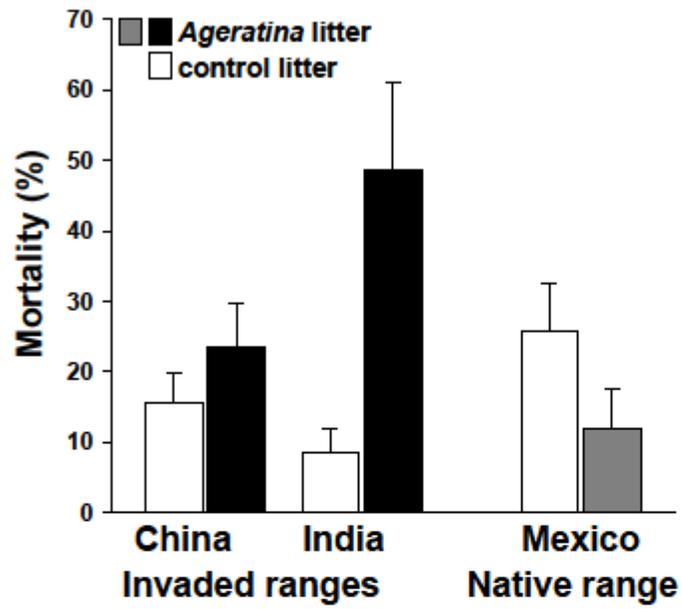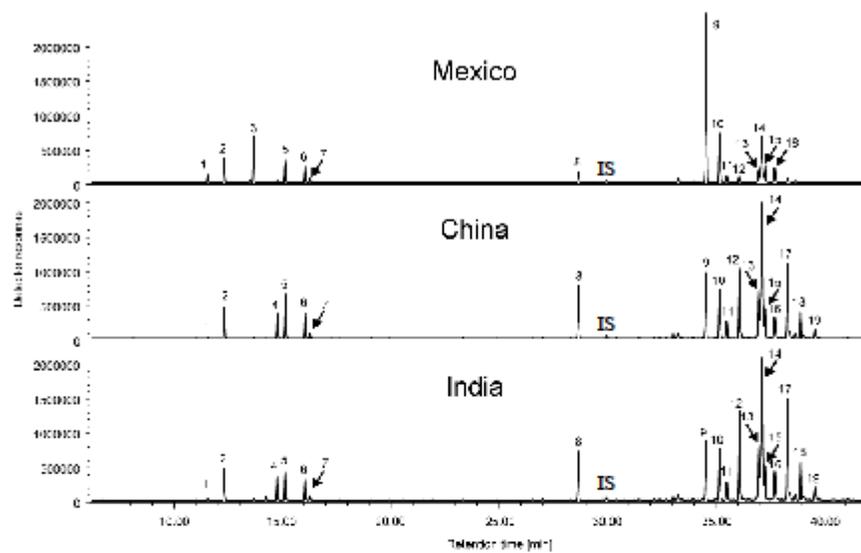
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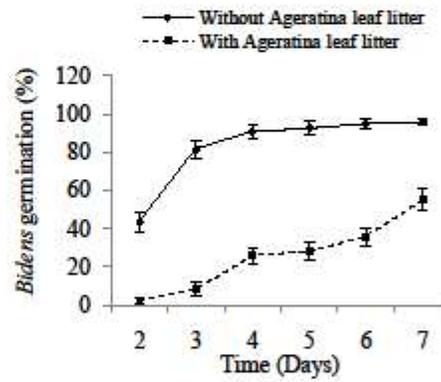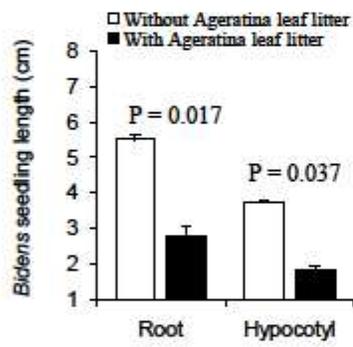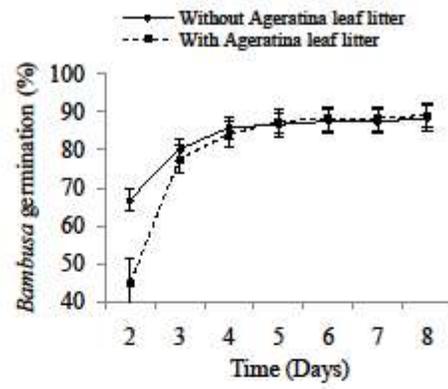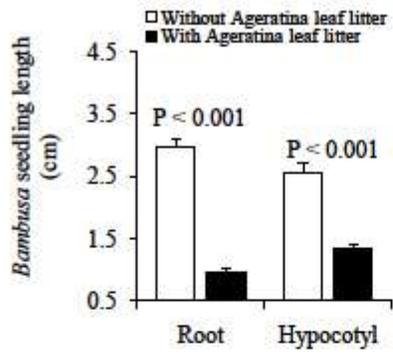
Supplementary Fig. S1

Fig. 2. Effects of volatiles from leaf litter of *Ageratina adenophora* on the mortality of species native to its invaded (China or India) ranges and species from its native (Mexico) range. Error bars represent 1 SE and ANOVA results are in text.
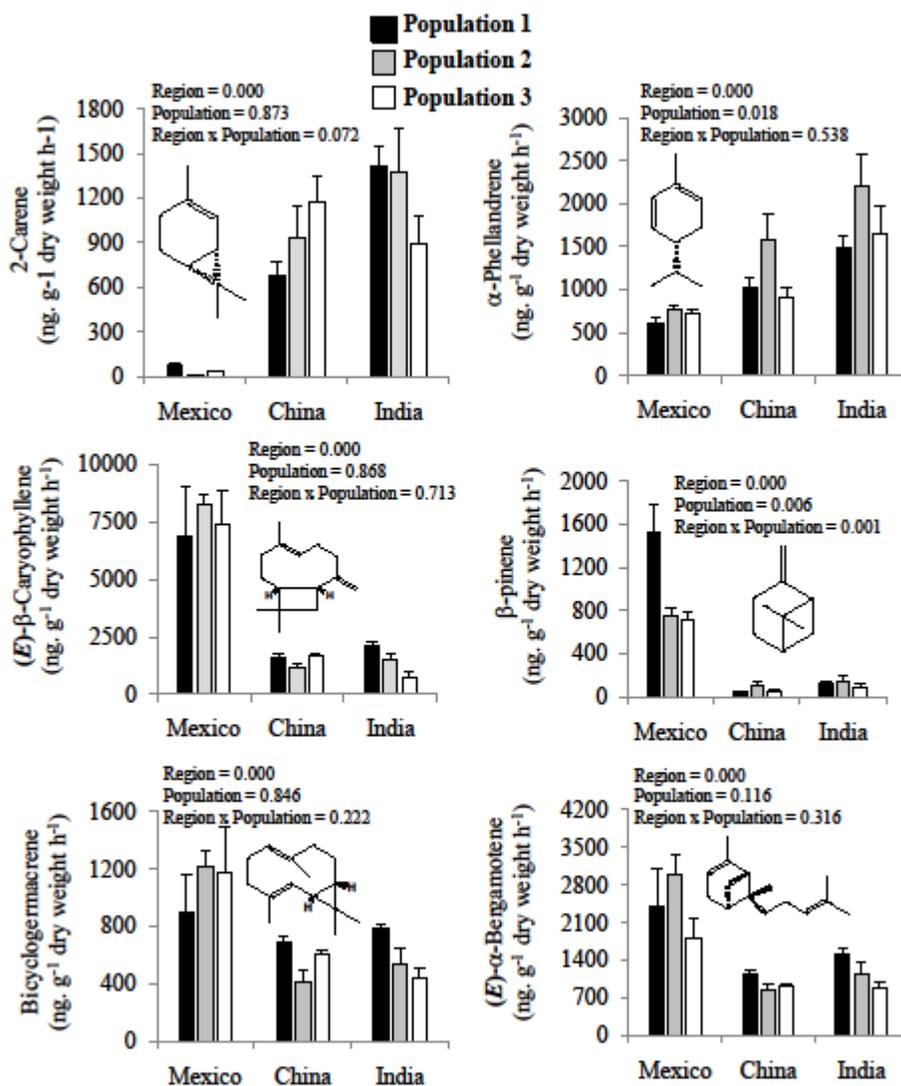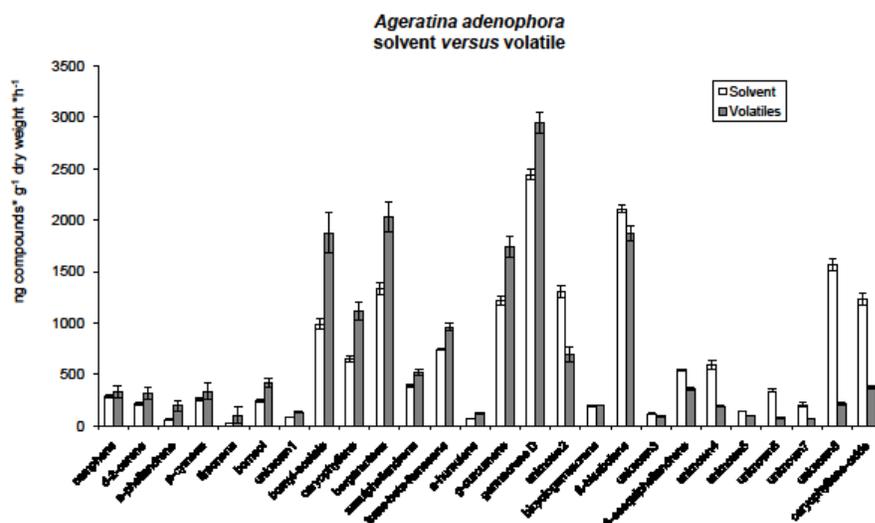
Supplementary Fig. S2

Fig. 3. Biogeographical differences in the emission of β-pinene, 2-carene, α-phellandrene, (E)-β-caryophyllene, (E)-α-bergamotene and bicyclogermacrene. Error bars represent 1SE. P values shown for two-way ANOVA.

Supplementary Fig. S3