THE ASSESSMENT OF POTENTIAL BOTANICAL INSECTICIDES FOR LOCAL USE IN RURAL HIGHLAND ECUADOR

by

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Abstract

In rural highland Ecuador, the potato provides the basic source of calories and nutrients to the local population. The indiscriminate use of synthetic pesticides applied in its production is taking a heavy toll on those people it is meant to sustain. Pesticide poisoning is currently the second-leading cause of death in many of the intensely agricultural provinces of the country, while sub-lethal effects deteriorate the quality of life for up to two-thirds of those exposed to the chemicals.

_Tecia solanivora_ (Povolny), the Guatemalan potato moth, is presently the greatest threat to potato production in the Andes region from Venezuela to Ecuador. This pest devastates production yields both in the field and in stores, with losses frequently exceeding 50%. The damage is caused by larvae burrowing into the tuber, excavating galleries and diminishing its quality. The pest has only recently become established in Ecuador, and there are currently no effective measures developed for its control.

There is a clear need for the development of a non-toxic, efficacious measure of control for this insect pest in Ecuador. The major goal of this project was to identify botanical pesticides that can be produced locally for use against the Guatemalan potato moth as well as for other insect pests.

Crude methanolic extracts of five local botanicals, Marco (_Ambrosia artemisioides_), Matico (_Eupatorium glutinosum_), Mashua (_Tropaeolum tuberosum_), Pumin, (_Hyptis pectinata_), and Santa Maria (_Tanacetum cinerariaefolium_), were screened for activity against the potato tuber moth, _Phthorimaea operculella_, and the cabbage looper, _Trichoplusia ni_. Extracts of Matico and Mashua were the most effective in controlling the potato tuber moth, although each botanical studied warrants further
investigation as a component in an integrated pest management system. The efficacy of the five plants studied was highly variable in controlling the cabbage looper, although Pumin performed best overall.
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Chapter One: Introduction

1.1 General Introduction

The health and environmental impacts of synthetic pesticides are now well known, and are of greatest risk to those living in countries that still allow the most potent toxins to be used. Chronic pesticide exposure is particularly relevant to rural poor populations, where men, women, and children all work and live in close proximity to where chemicals are applied and stored. Although a new generation of reduced-risk pesticides is becoming available to growers in industrialized countries, these are effectively out of reach of the resource-poor subsistence farmers of the south (Ecobichon, 2001). The tragic irony is that these are the farmers most in need of less toxic products, as education on safe application methods, regulations on allowable residues, and access to suitable safety equipment is lacking (Merino and Cole, 2002, Yanggen et al., 2004). It is also in these regions where agriculture is the most human-intensive, pesticides being mixed by hand and applied with backpack sprayers at alarming rates by individuals lacking protective gear (Espinosa et al., 2002, Merino and Cole, 2002, Yanggen et al., 2004). Furthermore, the children of subsistence farmers are often required to take part in the family farming activities, with children as young as ten routinely applying toxic pesticides without supervision.

While botanical insecticides have a long history of use, predating synthetics by hundreds of years, their use has diminished since the discovery of the insecticidal value of DDT at the time of the Second World War (Shaalan et al., 2005). Higher plants
contain a host of secondary metabolites that may effectively control pest problems in crops worldwide. A review by Roark (1947) found approximately 1200 plant species with potential insecticidal value. However, while thousands of new compounds are screened each year, very few are ever developed into commercial pesticides. There are various reasons for this, including the high cost associated with regulatory approval, difficulty in ensuring supply, and grower confidence in botanicals (Isman, 2006). These issues are obstacles to widely varying degrees in different regions of the world. A country such as Ecuador, with a more lenient regulatory environment (Yamagiwa, 1998), an abundance of natural starting materials, and a tradition of botanical use stands to benefit more from this type of research than a country such as Canada.

To increase the likelihood of their adoption as insect control products, routine screening of botanical extracts should identify a targeted geographical region for their adoption based on such criteria. To my knowledge, no studies of this type have been directed at the Andes region of Ecuador, despite the clear need for new products, and the high potential for their adoption.

In the search for novel compounds, plants are usually selected based on phylogeny or potential chemical constituents. Often, a tradition of use is overlooked, although familiarity may play an important role in the plant’s eventual adoption as a pest control product (Rosenthal and Berenbaum, 1991). A better strategy for discovering potential botanical insecticides would be to undertake a collaborative research project in a region where people have a history of association with plants in their environment. Instead of combing through the literature to identify plant species with potential value based on taxonomy, it may be more useful to work with indigenous peoples who may
have greater knowledge than can be drawn from the literature; i.e., knowledge of activity, abundance, distribution, and seasonality of potential anti-insect species. This approach may also help to ensure the chosen plants are relatively non-toxic to humans, especially if they have a history of medicinal use.

This thesis aims to identify plants that may have the ability to control insect pests of potato production in Ecuador, and to test for these properties in the laboratory.

1.2 Potato Production in Ecuador

The potato represents the basic source of calories in the cool Andean highlands of Ecuador, and is an important staple food throughout the country (Yanngen et al., 2004). Potatoes originated in the Andes region of South America and have been cultivated there for more than 8000 years. Current productivity and mechanization rates are low, due in large part to insecurity of land tenure, land shortages, and over-farmed small-scale holdings. Typically only medium and large-scale farmers who produce crops for export (banana, African Palm, sugar cane) can afford the high cost of mechanization. Small farmers of cacao, coffee, rice, corn and potatoes are less technologically advanced (Yamagiwa, 1998). Another problem in the agricultural sector, notably in the highland regions, is the low quality of infrastructure and irrigation systems. This is a problem of great concern to the communities of Totoras, Quindihua, and Marco Pamba, my informant communities (Pers. Comm.).

Potato cultivation and related activities generate employment for more than 100,000 people in Ecuador every year, half of whom are women. Some 90 percent of producers are small and medium-scale and there is only limited export, mainly to
Colombia (Yanngen et al., 2004). While potatoes are the key source of life and livelihood in this region of the world, there is a high cost associated with their production. Fundacion Natura, (an organization in Ecuador which promotes environmental conservation and sustainability), asserts that of all the crops farmed in the highlands, the use of pesticides for potato production poses the highest risk, due to the number of people involved in its production, the generalized consumption of the products in Ecuador, and the use of highly toxic pesticides on the crop. Potato production uses the greatest quantities of pesticides compared to other crops in the region; 90% of pesticides purchased are applied to potatoes (Yanggen et al. 2004).

Within Ecuador, potatoes are susceptible to a variety of pests; among the microbial diseases, bacterial wilt, late blight and numerous viruses are the most damaging. Insect pests of greatest importance include the potato tuber moth complex, the potato leafminer fly, *Liriomyza huidobrensis* (Blanchard) and the Andean Potato weevil, *Premnotrypes vorax* (Hustache) (Crissman et al., 2002).

The potato tuber moth complex consists of three closely related species of moths in the family Gelichiidae; the common potato tuber moth, *Phthorimaea operculella* (Zeller), the Guatemalan potato moth, *Tecia solanivora* (Povolny), and the Andean potato tuber moth, *Symmetrischema plaesiosema* (Turner). The moth complex causes great economic loss, by damaging the appearance of the tubers, which affects sales, as well as damaging the tubers so severely that they cannot be used for seed, or for human or animal consumption (Barragan, 2005).

The Guatemalan potato moth is currently the most serious pest of potato in Ecuador. The pest was first reported in northern Ecuador in 1996, and has since spread to
every potato-producing region of the country. As yet, growers are unable to efficiently control this insect (Pollet et al., 2004). Harvest losses frequently exceed 50%, despite multiple insecticide treatments (Bosa et al., 2005). When no control measures are utilized, a situation faced by many subsistence farmers, damage is dramatic, with losses as great as 100% occurring in as little as three months (Barragan, 2005).

Although the use of pesticides has been the single most popular method of pest control, in recent years, farmers have begun to adopt integrated pest management (IPM) practices. El Instituto Nacional Autonomo de Investigaciones Agropecuarias, (INIAP) has been especially influential in the adoption of alternative measures of pest control (Cole et. al., 2002).

1.3 Pesticide Exposure in Ecuador

In the late 1940s, agrochemicals were introduced to the world; in Ecuador, the use of fertilizers resulted in rapid yield increases and intensified production (Yanngen et al., 2004). The first pesticides introduced were inorganic fungicides and organochlorine insecticides, used to control the Andean potato weevil and a variety of foliage-damaging pests. Today, these products are used indiscriminately in many farming communities. One study (Crissman et al., 2002), found that farmers utilized 28 different commercial insecticides in the production of potatoes, and that potato fields received on average seven pesticide applications per season, each application containing an average of 2.5 different products. One quarter of all potato-production costs among small and medium-sized producers in the highland regions of Ecuador are directly attributed to pesticide product and application costs (Cole et. al., 2002).
Each year, three million people worldwide are hospitalized due to pesticide intoxication, with an estimated 220,000 deaths, and this figure is increasing in spite of the growing awareness of the dangers of these products (Ecobichon, 2001). Eddleston et al. (2002) report that in parts of the developing world, pesticide poisonings cause more deaths than infectious disease. In the potato-producing highland region of Ecuador, pesticide poisonings represent a significant health problem with a prevalence of 4 deaths per 10,000 people, and 4 poisonings per 100 citizens reported each year. In the intensely agricultural province of Carchi, up to two-thirds of potato-producing households show significant neurological impairment attributed to pesticide intoxication (Cole et al., 1998). According to the Ecuadorian Ministry of Public Health, deaths due to pesticide poisonings are second only to motor vehicle accidents, for both men and women (cited in Yanggen et al., 2004).

The extent of this problem is most pronounced in the Indigenous population, as this sector of the population is most involved in agricultural activities, as well as the least literate. It is also this sector of the population that is least able to access medical facilities, as they are largely rural inhabitants. This highlights the fact that a great number of poisoning may go unreported.

Most cases of accidental pesticide poisoning worldwide result from a few basic deficiencies; failure to wear protective clothing, unsafe storage and disposal of products, lack of personal hygiene, insufficient safety training, and weaknesses in occupational health regulations (Ecobichon, 2001). The scale of these deficiencies has remained virtually unchanged in Ecuador for at least the past 20 years. Research from 1989 indicates that backpack sprayers were commonly used; safety equipment was largely
neglected due to discomfort, lack of custom, and/or cost (Grieshop and Winter, 1989). Protective clothing was not worn due to the widely held belief that it is not necessary, although 60% of informants had personally experienced symptoms associated with poisoning (Grieshop and Winter, 1989). Although most believed they could become sick from pesticides, most also believed that they were not in any danger (Grieshop and Winter, 1989). Upon recognition of symptoms of poisoning, most did nothing at all. Only two per cent of those with recognizable symptoms of poisoning reported going to a doctor (Grieshop and Winter, 1989).

The situation today remains strikingly similar; pesticides are often mixed by hand in large barrels without gloves, resulting in considerable dermal exposure (Merino and Cole, 2002). Pesticides are still applied almost exclusively with backpack sprayers in the absence of safety gear (Yanggen et al., 2004). In a study done in Carchi province, it was found that when preparing pesticide mixtures, 86% of farmers did not use gloves, 92% did not use a mask, and 97% did not use eye protection (Yanggen et al., 2004). During fumigation, only 38% used a piece of plastic between their back and the sprayer, 26% wore a protective poncho, and 26% protective pants (Yanggen et al., 2004). In my experience in Bolivar province, where farmers work on much smaller-scale plots for their own subsistence, I saw no evidence of protective gear whatsoever (Fig. 1.1).

In another study, the majority of farmers were found to have made direct contact with pesticides during application, most having wetted their backs and their hands with the products (Espinosa et al., 2002). Considerable dermal contact also occurs on the legs during foliar applications in later stages of potato plant growth (Crissamn et al., 1998). Pesticides are generally stored at home, or close to farmhouses due to fear of robbery
(Merino and Cole, 2002). Household gauze swab tests found pesticide residues on a variety of household surfaces, and pesticide-soaked clothing has been shown to be a heavy source of contamination of the entire household (Merino and Cole, 2002). Excess product is often disposed of improperly; applied to other crops, thrown away with containers in the field, or spread around the farmhouse (Cole et al., 2002).

Figure 1.1 Pesticide applicators in Bolivar, Ecuador, with no evidence of protective gear.

Even today, education on pesticide hazards and safe practices is limited; only a small minority of farmers reported receiving this type of information from vendors (Espinosa et al., 2002). Only one-fifth of farmers had received any training concerning the safe use of pesticides, which came principally from pesticide industry representatives (Yanggen et al., 2004). The cultural belief system relating to an individuals’ ability to build a tolerance to the toxic effects of pesticides persists; the capacity to tolerate the
nausea and other immediate effects of pesticide intoxication are generally associated with strength and manliness (Yanggen et al., 2004).

The most unfortunate part of the improper beliefs and practices is that in Ecuador, farmers use some of the world’s most toxic pest control products. The liquid formulation of the carbamate carbofuran is the single most heavily utilized insecticide (Dale, 2003, Antle et al., 1998). In Carchi, farmers apply on average 1.3kg of carbofuran/ha on their potato crops (Stoorvogel et al., 2004). This product is banned in Europe and on July 25, 2008 the US Environmental Protection Agency (EPA) announced that it intends to ban all forms of it in the US as well. It is classified by the Food and Agriculture Organization (FAO) of the United Nations as highly toxic, due to its ease of absorption and its high acute toxicity. Some carbamates, including the heavily-utilized carbofuran, are extremely toxic to the central nervous system, and are suspected carcinogens and mutagens as well (Kamrin, 1997).

Another highly toxic product, the organophosphate methamidophos, is used most often in the control of chewing and sucking insects. The United States Environmental Protection Agency has classified methamidophos as a Class I compound (most toxic), and the World Health Organization considers it Class 1b (highly hazardous) (Dikshith et al., 2003).

Strategies for reducing the impact of pesticides have focused on eliminating the most toxic compounds, substituting less toxic products with effective alternatives, improving equipment and promoting the use of personal protective equipment. (Crissman et al., 2002). The most likely of these strategies to be effective within Ecuador is to find
effective alternatives to the toxic products in use both currently as well as for the foreseeable future.

1.4 Ethnobotany and Ethics

Ethnobotany has been defined as the study of the interactions between people and the plants found in their environment (Ford, 1978). The term itself was coined in 1895 by the US botanist John William Harshberger, and originally involved merely compiling lists of plants with their relevant uses to humans. In the 20th Century, the field experienced a shift from the raw compilation of data to a truly multidisciplinary field, at the interface of anthropology, botany, phytochemistry, and numerous other fields. The founding father of today’s form of ethnobotany is widely recognized as Richard Evans Schultes.

By the 1990’s, there emerged a general dissatisfaction with the methodological rigor within the field of ethnobotany. The criticism was based on the insufficiency of information given in research papers (e.g., number of informants interviewed, method used for data analysis), and with the qualitative nature of the research process (e.g., absence of criteria to assess relative importance of named species, reliance on single informants). In response to these criticisms, several authors elaborated quantitative ethnobotanical methods. These provided researchers with new criteria for the selection of potential biological activity e.g., Heinrich, (2000); Johns et al., (1990); Leaman et al., (1995); Moerman, (1991), as well as the ability to compare knowledge between geographical areas, cultural groups and communities (Begossi, 1996, Heinrich, 2000), and to identify patterns in ethnobotanical data (Johns et al., 1994).
This field of study has given rise to the concept of ethnopharmacy, the search for novel drugs based on an ethnobotanical approach. Ethnographic studies have long been a successful source for clues to plants with bioactive principles. These plants then become targets of search for bioactive compounds, often with startling success (Macias et al., 2007).

This approach to drug discovery can be extended to the search for novel pesticides. Working with local peoples in the development of a botanical insecticide can help to minimize a few of the problems related to the adoption of such a product. The search for novel compounds can be narrowed drastically by utilizing local knowledge on the flora of the region, especially where a tradition of plant use exists. Second, a community or region is more likely to adopt such a product if they have experience working with plant products, whether for medicinal or other use.

Within the past few decades, public and scientific interest in ethnobotany has skyrocketed. With this increased attention has come increased concern for the protection of intellectual rights, especially of indigenous peoples and their traditional knowledge. This has become a central issue to ethnobotany, in both commercial and academic endeavours.

While recording and publishing ethnobotanical information may be crucial to the conservation of disappearing traditional knowledge, this activity renders the information public, and thus available to individuals and organizations with commercial interests. Faced with such implications, researchers must take care to observe ethical guidelines set out by other researchers in the field. These actions include seeking prior informed
consent, outlining potential risks, and articulating foreseeable implications of the study (Posey and Dutfield, 1996).

1.5 Rationale, Objectives and Hypothesis

The boundaries of agricultural research have lately expanded, with a greater recognition of the cultural environment in which the agricultural system exists. It is imperative that researchers begin to recognize this increasing complexity, and adapt their research methods accordingly. Researchers are now using not only multi-disciplinary methods, but also transdisciplinary approaches. Transdisciplinarity involves not only the integration of diverse scientific disciplines, but also the involvement of community members and policy-makers. In the case of this project, individuals from three distinct communities helped generate knowledge, which, in turn was presented to members of INIAP. Relevance and accountability to my intended beneficiaries were crucial concepts in the design of this research project. To conduct this ethnobotanical research, anthropological, botanical, and toxicological studies were conducted. This research was designed with the intention of not only documenting but also applying research tools to affect change. Instead of simply measuring results, the intention was to create results through usable findings, to be shared with the communities that participated in the research, as well as those in the greater realm of potential impact.

My research has strived to identify botanical pesticides that can easily be formulated in Indigenous communities throughout Ecuador for local use on the most devastating insect pest to potato crops. A viable alternative to such toxic pesticides as methamidophos and carbofuran will help to reduce the negative impacts on human and
environmental health within these communities. In communities without any means of controlling the potato tuber moth, the development of a botanical insecticide will lead to greater yield and a higher quality of harvested tubers. Information has been distributed to local communities regarding sustainable harvest and preparation of the insecticides.

Despite the growing body of knowledge on the potential of botanical extracts as crop protectants, this potential has yet to be realized within Ecuador. More work must be done in collaboration with local farmers in order to promote their traditional knowledge and practices on a larger scale, in addition to finding other alternatives to pesticide use.

The overall objective of this thesis was to identify and evaluate traditional plant use. This was achieved by interviewing community members in order to identify plants used by rural Ecuadorian farmers as insect repellants or insecticides. Quantitative ethnobotanical techniques were used to fully understand the data. Finally, based on this information, the efficacy of selected plants was evaluated against two species of insects using different bioassays.

Specific Objectives

1) to discover new ways of identifying potential insect control products
2) to identify potential anti-insect plants in Bolivar province, Ecuador,
3) to assess the anti-insect potential of selected plants through bioassays with relevant insects
4) to suggest plants for further study

1.6 Personal Significance of Research

Conducting research in the Ecuadorian Andes required me to travel independently and inventively between the multiple communities where my research collaborators
resided. I traveled the paved highways on public buses, and hitched my way into the more remote communities. Always keeping in mind that vehicle accidents were the leading cause of death in this country, I generally sat in the back of any pick-up truck that offered me a ride. I pretended that I enjoyed the fresh air, but really, I felt somehow safer outside of the restrictive cab of the truck. If our truck should plummet from these high and dangerous roads, I felt sure that I could leap to safety. One evening, heading home from a day in the field, I waved down a truck heading in my direction, and climbed into the back. Most people traveled this way, so I quickly greeted the three others who had already taken their place. A little further down the road, a young boy, perhaps just into his teenage years, climbed in to join us. He was wearing a backpack sprayer of some pesticide unknown to me, and I instinctually shrank away from him. I didn’t want to risk my own skin absorbing the chemicals that were seeping out of his equipment, soaking into his clothing and skin. In the next second I realized the incredible tragedy of this moment. This boy was likely doing what he did most days of his life. Waking up at dawn, putting on the unwashed, pesticide-soaked clothing worn the day before, filling his backpack sprayer with chemicals mixed by his father or himself, without gloves or other safety equipment, then lifting the heavy equipment onto his back. He would have spent the day wandering the potato fields, dousing the foliage with the pesticide, breathing in the overspray, and soaking his clothing in the process. I shrank away from one minor possible contact, but this boy’s life was immersed in the product.
Chapter Two: Knowledge of Insecticidal Plants in Bolivar Province, Ecuador

2.1 Introduction

While insect pests represent a significant threat to agricultural crops, plants within their natural environment are generally well protected from herbivores. Protection can be either structural, in the form of thorns, spines, or waxes; or chemical, through toxins that deter the herbivore or reduce the plant’s digestibility (Buchanan et al., 2002).

For millennia humans have capitalized on the chemical defenses of plants, utilizing secondary plant products as agents of pest control. This practice is known to have occurred as far back as two thousand years in ancient Egypt and India, while in Europe and North America, there is documented evidence of such use as long as 150 years ago (Isman, 2006a). In the first century AD, Greek philosopher Pliny the Elder recorded pest control methods using plant materials in his Natural History, and the Chinese have recorded the use of powdered chrysanthemum as an insecticide from the same time (Shaalan et al., 2005).

In Ecuador, as in many developing countries, there exists a strong tradition of plant use as medicine, and to a lesser extent, insect repellants and crop protectants. Both Indigenous and Mestizo (mixed Spanish and Indigenous ancestry) peoples continue to have a close association to the plants in their environment, with virtually everyone capable of naming a number of plants with direct human uses as medicines or insecticides.

Though a long history of plant use exists within the country, there is little information on botanical pest control in Ecuador to be found within the scholarly
literature. However, plant knowledge has been documented in other types of resources, such as pamphlets and books available within the country.

In order to compile a list of plants with potential as insecticides, I traveled to 3 rural communities within Bolivar province, Ecuador, to interview research collaborators. As a second source of information on insecticidal species, I evaluated literature printed and circulated within Ecuador regarding pest control with plant materials. Finally, I searched the scientific literature for species named in my first two sources, with the assumption that plants named most commonly within Ecuador, but absent in the scientific literature are perhaps the most promising source of novel compounds. I suggest that a collaborative approach with local Indigenous peoples is a more efficient and effective method of discovering botanical insecticides. Potential insecticidal plants selected from this phase of the research are subjected to laboratory bioassays in the following two chapters.
2.2 Methodology

2.2.1 Study Site

Figure 2.1 Map of Ecuador
(http://www.cfhi.org/web/index.php/program/showcountry/id/6).

Ecuador is located in South America, sharing political borders with Colombia and Peru. The country is small, less than 300,000 sq km, but the landscape is incredibly diverse owing to the two ranges of Andes Mountains running north and south throughout the country. The mountain ranges effectively create 3 distinct zones; the hot, humid lowlands of the coast; the temperate highlands between the ranges, and the rainy lowlands to the east. Due to its location on the equator, the country experiences consistent day length throughout the year (Stoorvogel et al., 2004). The country sustains a wide
variety of distinct ecosystems and accordingly, over 16,000 species of vascular plants have been recorded, over one-quarter of them endemic (Jorgensen and Ulloa, 1994).

The population is 65% Mestizo, 25% Indigenous Amerindian, and 10% other (World Almanac, 2005), though other sources claim that Indigenous peoples represent as high as 40% of the population (Wahbe et al., 2008). There are 13 Indigenous nationalities in Ecuador; the Shuar, Achuar, Siona, Secoya, Cofan, Huaorani, Zaparo, Chachi, Tsachila, Awa, Epera, Manta, Wancavilca, and Quichua. Within Bolivar province, where this research took place, the most prominent is Quichuan (Wahbe et al., 2008). The Quichua are the largest Indigenous group in Ecuador, numbering over 2 million in a population of 14 million. Despite significant challenges, the Indigenous culture has remained strong within the country and Indigenous languages, while threatened, are still in use (Wahbe et al., 2008).

On my first visit to Ecuador in May 2005, I formed a relationship with two Masters of Agriculture students, David Silva and Maria Arguello, from the Universidad Estatal de Bolivar (UEB), in Guaranda, the capital city of Bolivar province. With their help, I began to visit rural farming communities within the province to discuss potential research issues. This region was chosen as the project site due to several factors; first, there existed a prior relationship between the Institute for Aboriginal Health (IAH) at the University of British Columbia and the local Indigenous community in Guaranda. This partnership has existed since 2003, when representatives from IAH visited Guaranda to discuss potential collaborations with Indigenous communities in the region. In addition, the community expressed an interest in working on a project to help alleviate issues faced
by farmers. Finally, the highly Indigenous population was likely knowledgeable in the use of plants.

Through a series of discussions with community members, it was decided that the dependence on the toxic pesticides used in potato production was the most pressing issue. On my return to Ecuador the following year, I visited a total of three Indigenous communities; Santa Rosa de Totoras (Totoras), Marco Pamba, and Qindihua, in order to conduct surveys to determine which plants are known to have anti-insect properties, with the intention of finding a botanical product to replace the chemical pesticides currently in use. These communities exist at between 2700 and 4500m above sea level, and consist of individuals largely of Quichua origin. The first language is Quichua, though most members, especially the youngest, speak Spanish as well. These communities maintain a strictly agriculture-based economy with very limited access to such services as education or health care. Most members of these communities depend on subsistence farming of potatoes for survival. The potatoes grown in this region of Ecuador are not sold across national boundaries, and thus are not subject to any outside regulatory restrictions. The benefit of this is that the growers need only satisfy the demands and meet the requirements of their own domestic markets. In most instances, regulatory approval is the greatest challenge in the development of new botanical insecticides (Isman, 2006a); it seems that a region such as Bolivar province in Ecuador can best benefit from this type of research, as rural subsistence farmers can utilize the abundance of plant materials at their disposal without the requirement of regulatory approval.
2.2.2 Ethical Considerations

A set of Good Practices has been developed by the Canadian Institutes of Health Research (CIHR) for researchers and research ethics boards involved with Aboriginal Peoples (http://www.cihr-irsc.gc.ca/e/documents/ethics_aboriginal_guidelines_e.pdf). All aspects of my research were conducted in full awareness of these practices, striving to respect the knowledge, culture and traditions of the communities I worked with. This was achieved by conducting the research as a partnership with the communities, and involving community members in as many phases of research as possible. It was of great importance in this research to ensure that its emphasis respected the myriad of viewpoints on this issue by different community members, as well as myself and the other researchers. The use of pesticides in the region is an issue with many sides. While the products are clearly poisoning the land and its people, these people must also be fed. It was very important to respect the long-standing tradition of pesticide use in potato production as an integral part of the community fabric. It is easy for external researchers to believe that traditional plant use is more defining of Indigenous culture, and therefore, what is best and right, but in fact, pesticides have been widely used for over half a century, the lifetime of most community members. It is a romantic notion to envision walking into a community and reawakening their traditions, but in reality, what they now know best is chemical products like carbofuran and methamidophos.

In addition to the CIHR Good Practices, the set of ethical guidelines proposed by Scott and Receveur (1995) were followed. Accordingly, this project was discussed with Vicente Emilio Pilco Llagcha, Totoras Indigenous Community President, as well as with faculty at UEB, which maintains a research station in Totoras, and has a strong working
relationship with the community. Verbal informed consent was gained from each participant, and participation in the research project was explained as entirely voluntary. Each participant was informed that the results of the study would be shared with all community members at the completion of the project. The final results were shared with the original collaborators, the Instituto Nacional de Investigaciones Agropecuarias (INIAP), The Centro Internacional de Papas (CIP), as well as with faculty and students at UEB.

2.2.3 Quantification in Ethnobotanical Studies

Only recently have methodological issues been explicitly addressed in ethnobotanical studies. If it is not clear how the data are compiled, it is difficult to evaluate their quality. While quantitative methods may not always be possible or even necessary with this type of data, it must be a goal of the researcher to make the data as reproducible as possible. Quantitative models facilitate the examination of patterns in ethnobotanical data and the testing of hypotheses relating to the use of plants (Johns et al., 1994). If a plant is used in a consistent fashion it is reasonable to assume that there are biological and/or cultural bases for the phenomenon. Testing with bioassays, the comparison of traditional uses of a plant with those in scientific publications can further rationalize their use (Johns et al., 1990).

2.2.4 Interviews

Community members participated on a voluntary basis and interviews were taken with the help of an interpreter, UEB Masters student Maria Arguello. Interviews took place at agricultural fairs, or at community meetings where agricultural issues were being discussed. Participants were selected based on willingness and availability. The aims of
the project were explained to each participant, and verbal consent was sought for recording and using any information gained through the process.

Surveys comprised 5 basic, open-ended questions, the interpreter asking the questions and recording responses. When mixtures of plants were suggested, a record was made for each component and added to the total number of insecticidal plants reported.

A total of 50 informants were interviewed, 31 men and 19 women.

2.2.4 Ecuador-Specific Literature

Literature produced within Ecuador relating to botanical insecticides (Fig. 2.2) was reviewed. Plants named as insecticidal or insect-deterrent or repellant were recorded. Literature was obtained through the UEB and local experts.

Figure 2.2 Literature produced and circulated in Ecuador
2.2.5 Scientific Literature

The top ten plants named in interviews, along with any of the plants listed at least twice in the Ecuador-specific literature, were researched using Science Direct from the University of British Columbia Library. The genus of the plant and the word insect were entered into the search engine, and the results were reviewed for relevant information. Reports of each genus used in the control of insects were recorded.

2.3 Results

In total, 36 plants were named by local informants as having insecticidal value. Almost half of the participants listed Marco (*Ambrosia artemisioides* Meyen and Walp.) among plants they knew to be insecticidal. Matico (*Eupatorium glutinosum* Lam), Ajenjo (*Artemisia absinthum* L.), and Mashua (*Tropaeolum tuberosum* Ruiz and Pavon), were each named by nearly a quarter of participants. A significant portion of the respondents reported mixtures of plants (76%) and/or fermentation (22%) as a production method. The ten most commonly named species are listed in Table 2.1.
Table 2.1 Ten most commonly named plant species in interviews

<table>
<thead>
<tr>
<th>Local Name</th>
<th>Scientific Name</th>
<th>Plant Family</th>
<th>Times Reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marco</td>
<td><em>Ambrosia artemesioides</em></td>
<td>Asteraceae</td>
<td>28 (56%)</td>
</tr>
<tr>
<td>Matico</td>
<td><em>Eupatorium glutinosum</em></td>
<td>Asteraceae</td>
<td>12 (24%)</td>
</tr>
<tr>
<td>Mashua</td>
<td><em>Tropaeolum tuberosum</em></td>
<td>Tropaeolaceae</td>
<td>12 (24%)</td>
</tr>
<tr>
<td>Ajenjo</td>
<td><em>Artemisia absinthum</em></td>
<td>Asteraceae</td>
<td>12 (24%)</td>
</tr>
<tr>
<td>Ruda</td>
<td><em>Ruta graveolens</em></td>
<td>Rutaceae</td>
<td>10 (20%)</td>
</tr>
<tr>
<td>Ajo</td>
<td><em>Allium sativum</em></td>
<td>Liliaceae</td>
<td>10 (20%)</td>
</tr>
<tr>
<td>Ortiga</td>
<td><em>Urtica sp.</em></td>
<td>Urticaceae</td>
<td>9 (18%)</td>
</tr>
<tr>
<td>Pumin</td>
<td><em>Hyptis pectinata</em></td>
<td>Lamiaceae</td>
<td>7 (14%)</td>
</tr>
<tr>
<td>Verbena</td>
<td><em>Verbena litoralis</em></td>
<td>Verbenaceae</td>
<td>7 (14%)</td>
</tr>
<tr>
<td>Santa Maria</td>
<td><em>Tanacetum cinerariaefolium</em></td>
<td>Asteraceae</td>
<td>6 (12%)</td>
</tr>
</tbody>
</table>

Within the five sources of Ecuador-specific literature, Marco was again the most frequently mentioned species. Of the 10 species mentioned most commonly in interviews, only four were mentioned within two or more sources of Ecuador-specific literature. Species named at least twice in the Ecuador-specific literature are listed in Table 2.2.
Within the scientific literature, Santa Maria, a known insecticidal plant was cited 15 times. There is a strong association between plants listed in Ecuador-specific literature and that found in the scientific literature, while there is almost a negative association between plants named in interviews and found within the scientific literature. Species reported in the scientific literature are listed in Table 2.3.

<table>
<thead>
<tr>
<th>Local Name</th>
<th>Scientific Name</th>
<th>Plant Family</th>
<th>Times Cited</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marco</td>
<td><em>Ambrosia artemesioides</em></td>
<td>Asteraceae</td>
<td>4 (80%)</td>
</tr>
<tr>
<td>Ruda</td>
<td><em>Ruta graveolens</em></td>
<td>Rutaceae</td>
<td>4 (80%)</td>
</tr>
<tr>
<td>Aji</td>
<td><em>Capsicum sp.</em></td>
<td>Solanaceae</td>
<td>3 (60%)</td>
</tr>
<tr>
<td>Ajo</td>
<td><em>Allium sativum</em></td>
<td>Liliaceae</td>
<td>3 (60%)</td>
</tr>
<tr>
<td>Eucalipto</td>
<td><em>Eucalyptus globulus</em></td>
<td>Myrtaceae</td>
<td>3 (60%)</td>
</tr>
<tr>
<td>Cebolla</td>
<td><em>Allium aflatunense</em></td>
<td>Liliaceae</td>
<td>2 (40%)</td>
</tr>
<tr>
<td>Ortiga</td>
<td><em>Urtica sp.</em></td>
<td>Urticaceae</td>
<td>2 (40%)</td>
</tr>
<tr>
<td>Tabaco</td>
<td><em>Nicotiana tabacum</em></td>
<td>Solanaceae</td>
<td>2 (40%)</td>
</tr>
</tbody>
</table>
Table 2.3 Reports in scientific literature of plants named in Tables 2.1 and 2.2

<table>
<thead>
<tr>
<th>Local Name</th>
<th>Scientific Name</th>
<th>Family</th>
<th>Times Cited</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ecualipto</td>
<td><em>Eucalyptus globulus</em></td>
<td>Myrtaceae</td>
<td>22</td>
</tr>
<tr>
<td>Santa Maria</td>
<td><em>Tanacetum cinerariaefolium</em></td>
<td>Asteraceae</td>
<td>15</td>
</tr>
<tr>
<td>Ajenjo</td>
<td><em>Artemisia absinthum</em></td>
<td>Asteraceae</td>
<td>13</td>
</tr>
<tr>
<td>Pumin</td>
<td><em>Hyptis pectinata</em></td>
<td>Lamiaceae</td>
<td>7</td>
</tr>
<tr>
<td>Ajo</td>
<td><em>Allium sativum</em></td>
<td>Liliaceae</td>
<td>6</td>
</tr>
<tr>
<td>Tabaco</td>
<td><em>Nicotiana tabacum</em></td>
<td>Solanaceae</td>
<td>4</td>
</tr>
<tr>
<td>Cebolla</td>
<td><em>Allium aflatunense</em></td>
<td>Liliaceae</td>
<td>3</td>
</tr>
<tr>
<td>Ruda</td>
<td><em>Ruta graveolens</em></td>
<td>Rutaceae</td>
<td>3</td>
</tr>
<tr>
<td>Ortiga</td>
<td><em>Urtica sp.</em></td>
<td>Urticaceae</td>
<td>2</td>
</tr>
<tr>
<td>Aji</td>
<td><em>Capsicum sp.</em></td>
<td>Solanaceae</td>
<td>2</td>
</tr>
<tr>
<td>Marco</td>
<td><em>Ambrosia artemesioides</em></td>
<td>Asteraceae</td>
<td>1</td>
</tr>
<tr>
<td>Matico</td>
<td><em>Eupatorium glutinosum</em></td>
<td>Asteraceae</td>
<td>1</td>
</tr>
<tr>
<td>Cola de Caballo</td>
<td><em>Equisetum sp.</em></td>
<td>Equisteraceae</td>
<td>0</td>
</tr>
<tr>
<td>Verbena</td>
<td><em>Verbena litoralis</em></td>
<td>Verbenaceae</td>
<td>0</td>
</tr>
<tr>
<td>Mashua</td>
<td><em>Tropaeolum tuberosum</em></td>
<td>Tropaeolaceae</td>
<td>0</td>
</tr>
</tbody>
</table>

2.4 Discussion

The data on insecticidal plant use obtained from rural farmers in Bolivar province is remarkably consistent. Merely 4 of the reports were encountered only once. This can
be accounted for by the homogeneity of the study site both geographically and socially. The study area was small, and the population interacts over the area, often collaborating on agricultural projects and sharing skills and information. There are no agricultural specialists in the region – virtually all men, women, and even older children (though no children were selected as interviewees) participate in agricultural activities, and all possess the basic repertoire of information related to farming activities. A child of only 10 years, Martha Coles Quilatoa, though not an official informant in this research project, led me around her community and pointed out many plants she knew to be repellent to insects. Each of her observations was corroborated in the official interviews undertaken with adults. It is assumed that consistency of use is directly related to efficacy from either a biological or cultural perspective (Johns et al., 1990). Certain plant organs such as leaves are mentioned more frequently than other, such as roots. This may be indicative of knowledge of sustainability, and is another important factor in the consideration of cultural efficacy.

I assumed Trotter and Logan’s model of effectiveness based on informant agreement, or consensus, which was put forward in the classic paper in 1986. They argue that a consistent pattern of usage of a plant within and between cultural groups is more likely to be found for plants with scientifically demonstrable biological effectiveness than for those without such effects (Trotter and Logan, 1986). The model assumes that the more agreement there is concerning its use, the more biologically effective the plant will be. This suggests that the use of ethnopharmacological and ethnobotanical studies in the identification of bioactive species is an effective technique for identifying bioactive
species. In fact, such studies have proven more successful than traditional approaches such as chemotaxonomy or random searches (Sousa Araujo et al., 2008).

Another important, but seemingly contradictory concept in the search for botanical insecticides is that of “cultural constructions of efficacy”. The criteria utilized by Indigenous peoples in selecting a plant for use as medicine or insecticide may differ from the criteria a scientific researcher may use. While a researcher may consider biological activity to be the paramount attribute by which to select a plant, colour, size, potency, availability, toxicity, susceptibility to disease, storage properties, pharmacological activity, tradition of use, and other characteristics are variables of equal, if not greater weight to Indigenous peoples (van der Geest and Whyte, 1988). Though ideally there will be an overlap between Indigenous and biological constructs of efficacy, it is important that the two are not conflated. Establishing a balance between biological activity and these other features is paramount for any botanical insecticide program to succeed in a place such as Ecuador.

I give credence to both of these concepts in my research by rationalizing the two in the following manner; Indigenous people’s selection and use of plants ultimately depends on their cultural constructions of efficacy. While species with greater biological activity may exist, the likelihood of their adoption is minimized if the plant is not considered culturally efficacious. Of the plants named insecticidal by my informants, I hypothesize that those mentioned most frequently will show the greatest activity. Alternately, there may be plants in a region that have greater activity, but are not utilized as a result of cultural rationale that may not be explained by science. Indeed, many Solanaceous plants in the region are highly toxic to insects, but are not considered useful
as insecticides due to their human toxicity or the reverence imparted to them as shamanic plants.

This implies that a researcher may have to accept lower efficacy than desired, as the most biologically efficacious plant may not be culturally efficacious. The greatest likelihood we can have in introducing a botanical insecticide to a region is to select the most effective plant from a list of plants with accepted cultural efficacy. There are often complex cultural undercurrents regarding plant use that are beneath the scrutiny of a researcher. One plant mentioned frequently in my surveys, the edible tuber called Mashua (*Tropaeolum tuberosum* Ruiz and Pavon), was openly spoken of as an insecticide, but when I asked farmers about it when I saw it growing in their fields, most were reluctant to talk about it. I discovered that the reason for their disinclination to speak of it was that it was perceived as “Indian food”, inferior to the white potatoes eaten throughout Ecuador. They were embarrassed that an outsider had seen that they use it as food. The most interesting fact is that Mashua is far superior to potatoes in terms of nutritional content, as well as resistance to pests and adverse growing conditions. Its yield is high, around 30 tons per hectare per year (Yamagiwa, 1998), though is currently cultivated on only a very small scale for consumption.

Another interesting finding is that of the 10 plants most commonly named in interviews, only 4 were found in the Ecuador-specific literature. This suggests that the two sources of information may draw from different wells of knowledge. This may also be evidence that there is little flow of knowledge in either direction, from farmer to academics, or vice versa. Perhaps even within Ecuador, the academic and institutional centres of learning look past traditional knowledge as sources of reliable information on
plant products. Alternately, it may suggest that plants with demonstrable activity, mentioned in the Ecuador-specific literature are simply not considered culturally efficacious to the Indigenous farmers of Bolivar.

Plants named most commonly in interviews, and mentioned at least twice in Ecuador-specific literature, but which are not substantiated by reports in the primary literature are potentially the most novel and interesting species warranting further study. Using this criteria for plant selection, the most interesting plants for study include Mashua and Verbena, (mentioned 0 times in scientific literature), and Matico and Marco (mentioned once each in the scientific literature). Santa Maria, a known insecticide, was selected for use as a positive control (mentioned 15 times in scientific literature).

Overlaying these choices with availability of botanical material altered the selection of plants only slightly. Pumin, mentioned frequently in all 3 cases, is a very abundant weedy plant, and was chosen for study, whereas Verbena was not studied, as it was less accessible.
Chapter Three: Screening of Plant Extracts for Insecticidal Potential against the Potato Tuber Moth, *Phthorimaea operculella* (Zeller)

### 3.1 Introduction

The potato tuber moth complex is the most damaging pest to potatoes in Ecuador. The principal species are the common potato tuber moth (PTM), *Phthorimaea operculella* (Zeller), the Guatemalan potato moth (GPM), *Tecia solanivora* (Povolny), and the South American potato tuber moth, *Symmetrischema plaesiosema* (Turner). The moth complex causes significant economic loss, by damaging the appearance of the tubers, which affects sales, as well as damaging the tubers so severely that they cannot be used for seed, or for human or animal consumption (Barragan *et al.*, 2004).

The Guatemalan potato moth was first reported in northern Ecuador in 1996, and has since spread to every potato-producing region of the country (Barragan *et al.*, 2000). As yet, growers are unable to efficiently control this insect (Barragan *et al.*, 2004, prs. comm). Harvest losses frequently exceed 50%, despite multiple insecticide treatments. When no control measures are utilized, a situation faced by many subsistence farmers, damage is dramatic, with losses as great as 100% occurring in as little as three months (Bosa *et al.*, 2005). An example of the damage done to untreated potatoes in stores can be seen in Figure 3.1.
In Ecuador, the control of GPM has proven very difficult for numerous reasons; the income generated from the sale of the tubers does not allow for adequate phytosanitary control (Barragan et al., 2004), there are poor storage techniques, an absence of biological control measures, and lack of knowledge of integrated pest management (Pollet et al., 2004).

A specific granulovirus, (PoGV, Baculoviridae) which infects PTM has been identified in various parts of the world and is currently the most promising candidate for the biological control of the tuber moth complex in stores and fields. Although the host range of granuloviruses is generally quite limited, PoGV has been found to be highly effective against both PTM and the closely related species GPM. A powder product formulated by grinding virus-infected larvae and mixing with talc at a rate of 5kg per ton...
of potatoes significantly reduces GPM damage in stores. However, the current \textit{in vivo} propagation and formulation techniques are limiting its potential use. Furthermore, its low field stability due to UV degradation is considered a limiting factor for its use in fields (Sporleder and Kroschel, 2006).

Potential exists for the development of a botanical insecticide to control the GPM in Ecuador. An aqueous extract of \textit{Picconia excelsa} (Aiton) has been found to deter adult GPM when applied to tubers, and is being used successfully in the Canary Islands of Spain (Cabrera et al., 2004). In a survey of literature published from 1915 to 1993, preparations from 35 plant species were found to be effective against PTM either in storage or in the laboratory (Das, 1995). As GPM and PTM are very closely related species, it is assumed that plant extracts effective against one will be similarly effective against the other.

Botanical insecticides generally degrade rapidly under normal environmental conditions; sunlight, humidity, and rainfall break down most botanicals ensuring lesser persistence and therefore reduced impact on beneficial and non-target organisms (Isman, 2006). Most botanicals have low mammalian toxicity based on oral LD50 (Isman 2006). Each of the plants used in this study is also used medicinally, suggesting they are relatively non-toxic to humans if used in similar concentrations.

Drawbacks to botanicals do exist, however. There are often greater interspecific differences in bioactivity, meaning that a single botanical cannot likely be used for a range of insect pests (Isman, 1993). Rapid degradation, while a plus for the environment and non-target species, means more frequent applications may be necessary if protection is required over a prolonged period. This equals greater cost in human labour and/or
products. Though generally considered less toxic than synthetics, botanicals are sometimes toxic to fish, birds, reptiles, and amphibians (Shaalan et al., 2005).

While the best-known examples of botanical insecticides, such as neem, pyrethrum and rotenone are more refined products; there is evidence that crude extracts can act as effective insecticides or deterrents as well. Simple crude extracts from plants have been used as insecticides in many countries for centuries (Crosby, 1971). There are advantages to using plant extracts as pest control agents over single-component products. Extracts generally contain several different constituents that may act synergistically; the extract showing greater activity than would be expected by looking at the components in isolation (Berenbaum, 1985). Furthermore, insecticide resistance and desensitization is much less likely to develop as different constituents may have different modes of action (Feng and Isman, 1995). Finally, crude extracts may be cheaper to prepare if plant materials are available locally, which is the case in the present study. In a study undertaken in Indonesia, a cost analysis found that crude aqueous extracts of locally sourced seed extracts of *Annona squamosa* were more economical than synthetic insecticides (Leatemia, 2003).

Based on the findings of the surveys discussed in the previous chapter, five plants were selected for further investigation in the laboratory: Marco (*Ambrosia artemisioides*), Figure 3.2; Matico (*Eupatorium glutinosum*), Figure 3.3; Mashua (*Tropaeolum tuberosum*), Figure 3.4; Pumin, (*Hyptis pectinata*), Figure 3.5; and Santa Maria (*Tanacetum cinerariaefolium*), Figure 3.6.
Marco (*Ambrosia artemisioides*) was the most commonly named species in interviews (Table 2.1). More than half of the informants named this species as having insecticidal value.

The genus *Ambrosia* contains more than 40 species, and belongs to the family Asteraceae. The genus is well studied, and many members contain sesquiterpene lactones (Jakupovic *et al.*, 1988), compounds known to be toxic to insects (Picman, 1986). *Ambrosia artemisioides* has been found to contain a number of alantolactone derivatives, epi-eudesmanes, oplopanone derivatives, scopoletin, kaempferol and kaempferol 6-methyl ether, although these constituents appear to vary between geographic regions (Silva *et al.*, 1992, Jakupovic *et al.*, 1988).
Marco is widely known as a medicinal plant throughout its range, which extends from Venezuela to Northern Chile at altitudes of 2200m to 3200m above sea level (Jørgensen and Ulloa Ulloa, 1994). It is considered to be a magical plant, and is used to clean “bad air” (Lombeyda, 1998). An infusion of the leaves is used to treat menstrual irregularities and dysentery, an infusion of the flowers is used as a vermifuge; lightly roasted fresh leaves are used to alleviate the symptoms of rheumatism. In Colombia, the leaves are used as an infusion to cleanse the body (Sarria, 2003). Marco is a weedy plant, widely distributed throughout the province of Bolivar, abundant in disturbed soils, along roadsides, rivers and fields (Jørgensen and Ulloa Ulloa, 1994). The plant grows year round, allowing for continual, sustainable harvest of this fast-growing weed. This plant, if efficacious, is ideal as a botanical insecticide.
The second species, locally known as Matico, *Eupatorium glutinosum* has well-characterized medicinal properties (El-Seedi *et al.*, 2002a). The genus *Eupatorium* comprises close to 600 species distributed mainly within the tropical regions of the Americas (Maia *et al.*, 2002). This particular species can be found in Ecuador and Peru, growing in a fairly limited range at an altitude of around 3000m (El-Seedi *et al.*, 2002a). Numerous species have been used in traditional medical systems throughout the world, many of which have been found to have insecticidal properties as well (Jacobson, 1990). Matico has been used in Ecuador and Peru as an astringent, antirheumatic, antimicrobial, and to cure stomach ulcer, headache and diarrhea (El-Seedi *et al.*, 2002b, and Sarria,
2003), and in Colombia, an infusion of the leaves is used as a bath to cleanse the body (Sarria, 2003). The genus *Eupatorium* is known to have feeding-deterrent properties (Jacobson, 1990), although there is tremendous variation in the chemistry of this genus (El-Seedi et al., 2002).

Figure 3.4 Mashua, *Tropaeolum tuberosum*

Mashua, *Tropaeolum tuberosum*, is an indigenous Andean edible tuber that has been cultivated for centuries in northwestern South America. The tubers are harvested both for daily dietary consumption as well as for medicinal purposes. Compared with other root and tuber crops, Mashua has relatively high levels of vitamins. It also has the highest fat content of all the Andean root and tuber crops (Guimaraes, 2001). Mashua is
used to treat kidney and liver problems, skin eczema, as well as prostate disorders (Grau et al., 2003). These properties may be related to the presence of antioxidant phenolic compounds (Chirinos et al., 2008). Mashua is currently grown in the highlands to a much lesser extent than the potato, but thrives in the same soils (Grau et al., 2003).

Figure 3.5 Pumin, *Hyptis pectinata*

Pumin, *Hyptis pectinata*, is a pantropical member of the mint family, and is used extensively in ethnomedicine. Its essential oil has been found effective against larvae of *Aedes aegypti* (Silva et al., 2008). In Brazil, it is commonly used in the treatment of inflammations, bacterial infections, and body aches (Arrigoni-Blank et al., 2008). The
essential oil has been found to contain sesquiterpenes, beta-caryophyllene and caryophyllene oxide accounting for 79% of its composition (Silva et al., 2008).

Figure 3.6 Santa Maria, *Tanacetum cinerariaefolium*

*Tanacetum cinerariaefolium* is an introduced plant, and is widely known as the source of pyrethrum. Pyrethrum is an ancient insecticide, with documented use as early as the 1800’s in Europe, and centuries earlier in China (Shaalan et al., 2005). The insecticide is produced by grinding the dried flowers and extracting the active principles with a non-polar solvent such as hexane (Isman, 2006). It is the first natural product to be used against adult mosquitoes (Silva et al., 2008). Pyrethrum is produced on a
commercial scale within Ecuador, but the plant is grown on a limited scale in the province of Bolivar.

3.2 Materials and Methods

3.2.1 Plant Acquisition

The collection and identification of plant species (except Mashua) was undertaken with the aid of the translator, as well as select participants (Vicente and Maria Pilco, Maria Arguello). Plants were identified by Maria Arguello and Juan Gaibor, and their scientific name, as well as their local name was recorded for each. Each species was photographed in its natural environment, and the exact location of plant collection was recorded using a GPS. Plant materials were pressed and air-dried in the shade. A voucher specimen of each species was deposited at the Centro Internacional de la Papa in Quito, Ecuador. Dried, diced Mashua tuber was obtained in Salinas, Ecuador. Dried materials were then brought to UBC in Vancouver, Canada for testing.

3.2.2 Plant Extracts

At UBC, the dried plant materials were ground using a mortar and pestle, and 100g amounts were extracted with 95% methanol (3 X 200ml) over 3 days. The extracts were vacuum filtered (Whatman No. 1) and evaporated in vacuo using a rotovapor. Dried extracts were re-suspended in a small volume of 95% methanol and transferred to pre-weighed vials. After evaporation of the methanol the vials were re-weighed to determine the extract weight. Ethanol was used as the solvent in bioassays.
3.2.3 Insects

Adult potato tuber moths have a wingspan of approximately 15mm, and a length of approximately 8.5mm. They are grey in colour, with three pairs of black spots on the wings which appear as an X from a distance. The eggs are 0.5 mm in diameter, smooth, and white, turning yellow over time. The larvae have 4 instars, the first measuring about 1mm in length, and remaining white until the 3rd instar. The fourth and final instar reaches a length of 10mm, becoming greenish with tones of pink. The pupa is 6mm in length, starting green in colour and becoming brown. The pupae are sexually dimorphic, with females larger than the males (Onore, G. et al., 2005). In the Andes, GPM can produce 3 to 4 generations per year (Notz, 2005). The life stages of the tuber moth are shown in Figure 3.8.

PTM were obtained from laboratory cultures reared by Dr. Lerry Lacey at the USDA station in Wapato, WA, USA. The insects were maintained at 25° C and a photoperiod of 16L:8D. Pupae received from Dr. Lacey were placed in oviposition cups, which were covered with mesh fabric. Filter paper was placed on top of the mesh fabric, and weighed down with a Petri dish. Eggs were collected on filter paper, and placed on clean, dry tubers for larval development. Development occurs within the tubers, 4th instar larvae emerging to pupate at approximately 17 days. Pupae were collected and put into oviposition cups for egg collection.
3.3 Screening Plant Extracts Against *Phthorimaea operculella*

3.3.1 Oviposition Deterrence

Extracts were screened for oviposition deterrent effects on adult *P. operculella*. One ml aliquots of extracts in ethanol were applied to filter paper at concentrations of 0.5%, 1% and 5% for Marco; 0.25%, 0.5%, and 2.0% for Matico; and 10% for Mashua, Pumin and Santa Maria. Twenty-five pupae of mixed sex were introduced into oviposition cups, enclosed with mesh fabric. Treated and control (ethanol only) filter paper was placed on top of the fabric, and weighed down with a small Petri dish to ensure
contact with the fabric. Filter paper was collected and changed daily, and eggs laid were counted.

3.3.2 Contact Toxicity (Eggs)

The toxic effects of Marco and Matico were tested on the eggs of *P. operculella*. Filter papers with newly deposited eggs were collected from oviposition cups, and eggs were counted to ensure equal numbers per paper. Filter papers (42.5mm) containing a specific number of eggs (200) were dipped in 1 ml of 1.0% and 5.0% extract in ethanol or in ethanol alone. Filter papers were allowed to dry and placed individually on tubers in separate containers. After 17 days, the number of pupae that had emerged from the tubers was recorded.

3.3.3 Contact Toxicity (Larvae)

The toxicity of the plant extracts was also evaluated using 4th instar larvae of *P. operculella*. Fourth instar larvae were collected after they had exited tubers. Fourth instar larvae are non-feeding, so it can be assumed that any mortality seen will have resulted from contact toxicity. Twenty larvae were placed individually in Petri dishes (9cm diameter), containing a cabbage leaf disc (1.5 cm diameter) with 150ul of 5% or 10% extract in ethanol. Extracts tested were Marco, Matico, Mashua, Santa Maria, and Pumin. The cabbage leaf was used as the substrate because it maintained turgidity after the solution was applied. Controls were treated with ethanol alone. Petri dishes were placed in plastic boxes lined with moist paper towels to maintain humidity, and were stored in a growth chamber at 26°C to allow for pupation. After 4 days, the numbers of pupae were counted. The procedure was repeated in triplicate.
3.3.4 Contact Toxicity (Pupae)

The toxic effects of the plant extracts were tested on pupae of *P. operculella*. Newly emerged pupae were collected, and placed in groups of 25 in separate cups. Five hundred ul of extract at concentrations of 5% and 10% of Marco, Matico, Mashua, Santa Maria and Pumin was applied topically to each group of 25 pupae; controls were treated with 500 ul of ethanol alone. Pupae were allowed to dry, and then oviposition cups were closed with mesh fabric to allow for adequate ventilation. Emergence was recorded after 4 days. Procedure was repeated in triplicate.

3.4 Results

3.4.1 Oviposition Deterrence

Both Santa Maria and Pumin exhibited complete oviposition deterrence at 10%, suggesting it may be effective at lower concentrations. Unfortunately, due to material limitations, no further testing was possible. Matico was the most active oviposition deterrent at low concentrations, exhibiting an oviposition deterrent index (ODI) of 72.3% at a concentration of 0.25%. It is ~3 times more active than Marco at 0.5%. Mashua has an ODI of 60% at 10%, suggesting it is not an effective oviposition deterrent (Table 3.1).
Table 3.1. Oviposition deterrence of the plant extracts at different concentrations against PTM.

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>Marco</th>
<th>Matico</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentrations tested</td>
<td>5%</td>
<td>1%</td>
</tr>
<tr>
<td># of eggs laid on treated</td>
<td>81</td>
<td>29</td>
</tr>
<tr>
<td># of eggs laid on control</td>
<td>338</td>
<td>118</td>
</tr>
<tr>
<td>ODI*= {((C-T/C+T)*100)}</td>
<td>61.34</td>
<td>60.54</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>Matico</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentrations used</td>
<td>2%</td>
</tr>
<tr>
<td># of eggs laid on treated</td>
<td>13</td>
</tr>
<tr>
<td># of eggs laid on control</td>
<td>272</td>
</tr>
<tr>
<td>ODI*= {((C-T/C+T)*100)}</td>
<td>90.88</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>Santa Maria</th>
<th>Mashua</th>
<th>Pumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentrations used</td>
<td></td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td># of eggs laid on treated</td>
<td></td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td># of eggs laid on control</td>
<td></td>
<td>48</td>
<td>162</td>
</tr>
<tr>
<td>ODI*= {((C-T/C+T)*100)}</td>
<td>100</td>
<td>60.0</td>
<td>100</td>
</tr>
</tbody>
</table>

3.4.2 Toxicity (Eggs)

A one-way ANOVA on the number of pupae of potato tuber moth produced from eggs sprayed with Marco or Matico extracts produced a significant F value \(F_{3, 11} = 84.6, p = 0.01\). My results indicate that Marco was significantly more active than Matico at 1% (Tukeys test, p<0.05). However, the extracts were equally effective at 5% (Figure 3.8). Treatment of eggs with 5% Marco and Matico extracts resulted in about 30% and 24% of pupae respectively compared with the control.
Figure 3.8 Effects of the plant extracts (1% and 5%) on the emergence of pupae following the treatment of PTM eggs (n = 200 eggs). Means(± SE) followed by the same letters are not significantly different (Tukey’s test, p<0.05).

3.4.3 Toxicity (Larvae)

A one-way ANOVA on the number of pupae following the spraying of larvae with the plant extracts (5%) produced a significant F value (F_{4, 14} = 6.6, p = 0.01) (Fig. 3.9).

My results indicate that Marco and Mashua were the most active (Tukey’s test, p<0.05) followed by Matico and Santa Maria. Pumin was the least active. Treatment of larvae with 10% Mashua and Marco resulted in a complete disruption of the life cycle of the tuber moth, with no pupae being produced. Mashua was equally effective at 5%, suggesting it would continue to be an effective product at lower concentrations.

A one-way ANOVA on the number of pupae following the spraying of larvae with the plant extracts (10%) produced a significant F value (F_{4, 9} = 20.8, p = 0.01) (Fig. 3.9).
All extracts were significantly more active than Pumin (Tukeys test, p<0.05).

![Bar chart](image)

Figure 3.9 Effects of the plants extracts (5% and 10%) on the number of pupae (% control) following treatment of PTM larvae (n= 5 larvae).

Means (± SE) followed by the same letters are not significantly different (Tukey’s test, p<0.05). Capital letters indicate 5% extracts, lowercase letters refer to 10% extracts.

### 3.4.3 Toxicity (Pupae)

A one-way ANOVA on the emergence of potato tuber moth following spraying the pupae with the plant extracts (5%) produced a significant F value (F$_{4, 14}$ = 71.9, p = 0.01). The results indicate that Marco and Mashua were significantly the most active (Tukey’s test, p<0.05) followed by Matico and Santa Maria (Figure 3.10). Pumin was the least active.

A similar trend was also observed with 10% extracts (one-way ANOVA; F$_{4, 14}$ = 6.6, p = 0.01). Again Marco and Mashua were significantly the most active (Tukeys test, p<0.05) followed by Santa Maria and Matico. Pumin was the least active (Figure 3.10).
Figure 3.10 Effects of the plants extracts (5% and 10%) on mean emergence (% control) of PTM adult following treatment of pupae

Means (± SE) followed by the same letters are not significantly different (Tukeys test, p<0.05). Capital letters refer to 5% extract and lowercase letters refer to 10% extract.

3.5 Discussion

Some of my extracts were very active oviposition deterrents as well as toxic against different stages of the potato tuber moth. Matico showed the most promise as an oviposition deterrent, with an ODI of 72.3% at a concentration of 0.25%. Since Marco showed ~60% deterrence at 1%, it could still be used in the stores to deter female moths from laying eggs on potatoes. Santa Maria and Pumin could be active at low concentrations as they exhibited 100% oviposition deterrence at 10% but were not tested at lower concentrations. Mashua was the least active exhibiting 60% ODI at 10%. This is a very interesting finding in terms of controlling potato tuber moth as it is very hard to control this insect because of its behaviour. Since the larval stages develop inside the
potatoes, it is hard to control them with insecticides. If we can manipulate the oviposition behaviour of this pest through these plant extracts, we will be able to control the damage caused by this pest to the crop.

My data shows promising results for some of the extracts as contact toxins against different life stages of the moth. Both Marco and Matico were toxic to the eggs at 5% and had a significant effect on the number of subsequent pupae (~30 and 24% following treatment of eggs with 5% of Marco and Matico respectively). Since only Marco and Matico were evaluated in this experiment, it would be interesting to test the effects of other extracts as ovicides.

There was a consistent trend in the toxicity of the extracts against both larvae and pupae of the PTM. In each case, Mashua and Marco were the most toxic, followed by Matico, Santa Maria, with Pumin as the least toxic extract. It is striking that the two most effective extracts were also the first and second most commonly named plants in community interviews. Surprisingly, they are also both the least cited in the scientific literature.

A treatment of 10% Marco or Mashua, applied directly to tubers in storage, might effectively prevent further infestation by reducing adult development from both the larval and pupal stages. While damage has already been done to infected tubers at this point in the moth’s life cycle, there is still benefit that can be realized from treating this stage, as preventing adult development will ensure that infestation can be contained. As Matico proved to be an effective oviposition deterrent, it can be considered a third option for treatment of the stored tubers against infestation by the GPM.
Perhaps the greatest potential for the introduction of botanicals into the potato production system is to integrate their use with other tools of integrated pest management (IPM). IPM relies on the concerted use of various controls, including biological control, the use of resistant cultivars, cultural control, as well as limited chemical control. Botanical insecticides are often slow-acting, or with non-toxic modes of action, and may not be highly effective on their own; however their use as part of an IPM system can prove highly effective.

Many farmers in Ecuador currently store seed potatoes in open structures, piled on the ground (Fig. 3.11). This leaves them very susceptible to infestation by the GPM. 

Figure 3.11 Typical storage of seed potatoes, piled on the ground in open stores.

The plants tested in this chapter show promise in reducing or eliminating infestation of potatoes in stores used in conjunction with other phytosanitary controls.
Recommended IPM strategies to defend against infestation in stores include cleaning and disinfecting the store before storing potatoes, storing the potatoes off the ground, on wooden shelves or platforms, inspecting the sacs for any life stage of the GPM. Cultural controls to prevent infestation in the field include soil preparation and management, irrigation, location of plantings, seed source and variety selection (Horne, 2006).
Chapter Four: Screening of Plant Extracts for Insecticidal Potential Against Cabbage Looper, *Trichoplusia ni*

4.1 Introduction

The cabbage looper, *Trichoplusia ni* Hubner, (Noctuidae) is one of the most damaging pests of crucifers in the New World as well as an important greenhouse pest. Annual losses and the cost of control measures make the cabbage looper an economically important pest (Mota-Sanchez et al., 2002). The species also attacks other vegetable crops including lettuce, beet, peas, tomato, as well as many weedy plants. Additional hosts include flower crops such as chrysanthemum, hollyhock and snapdragon, and field crops such as cotton and tobacco. The larval stage damages crops by feeding on foliage and fruiting structures. Larvae are light green with white or pale yellow stripes, and three pairs of prolegs. The adult moth has dark brown mottled fore wings, each having a small silvery spot near its centre; the hind wings are light brown. The wingspan of the moths is over 4 cm (Davidson and Lyon, 1979). Cabbage loopers overwinter in the pupal stage, the pupae enclosed within silken cocoons which are attached to the host plant or to nearby objects. Moths emerge in the spring and deposit pale green, dome-shaped eggs singly on the host plant. Once hatched, the destructive larvae reaches full development in 2 to 4 weeks, depending on temperature. New adults emerge approximately 10 days after pupation. (Davidson and Lyon, 1979). Life stages of cabbage looper are shown in Fig. 4.1.
Because the cabbage looper has evolved resistance against many synthetic insecticides (Mota-Sanchez et al., 2002) and the microbial insecticide *Bacillus thuringiensis* (Janmaat and Myers 2005), there is a need to develop new methods that could protect crops in integrated pest management systems. Furthermore, as the feeding stage of the PTM exists within the tuber, bioassays to determine the efficacy of the
selected plant extracts as antifeedants were impractical. The cabbage looper is an ideal candidate for such bioassays. For these reasons, the selected plant extracts were tested against the cabbage looper in the lab.

4.2 Materials and Methods

4.2.1 Plant Acquisition

The collection and identification of plant species (except Mashua) was undertaken with the aid of the translator, as well as select participants (Vicente and Maria Pilco, Maria Arguello). Botanical identification was performed by Maria Arguello and Juan Gaibor, and their scientific name, as well as their local name was recorded for each plant. Each species was photographed in its natural environment, and the exact location of plant collection was recorded using a Garman Global Positioning System. Collected plant materials were air-dried in the shade. A voucher specimen of each species was deposited at the Centro Internacional de la Papa in Quito, Ecuador. Dried, diced Mashua tuber was obtained from a local plant-processing cooperative in Salinas, Ecuador. Plant materials are dried in ovens at 150 degrees for 4 to 5 hours. Dried plant materials were then brought to UBC in Vancouver, Canada for laboratory testing.

4.2.2 Plant Extracts

At UBC, the dried plant material was ground using a mortar and pestle, and 100g amounts of each plant were extracted with 95% methanol (3 X 200ml) over 3 days. The extracts were vacuum filtered (Whatman No. 1) and taken to dryness using a rotovapor. Dried extracts were re-suspended in a small volume (2ml) of 95% methanol and were transferred to pre-weighed vials. Methanol was evaporated over night in a fume hood,
then vials were re-weighed to determine the extract weight. Dry extracts were stored under refrigeration until use. Methanol was used as a carrier solvent in all bioassays.

4.2.3 Plants Used in Bioassays

Cabbage plants (*Brassica oleracea* var. Stonehead) used in the bioassays were routinely grown in plastic pots with a mixture of sandy loam soil and peat moss (4:1) in a greenhouse at the University of British Columbia, Vancouver, BC, Canada. Leaves used in the bioassays were collected from 5-6 weeks old cabbage plants.

4.2.4 Insects

Cabbage loopers, *Trichoplusia ni*, were obtained from Dr. Murray Isman’s laboratory-reared colony which has been maintained at UBC for over 50 generations. These insects are reared on an artificial diet, Velvetbean Caterpillar Diet [No. F9796, Bio-Serv Inc. (Frenchtown, NJ.)] and maintained in the insectary of the University of British Columbia (UBC). The diet was supplemented with finely ground alfalfa, to improve acceptability, and vitamins [No. 8045, Bio-Serv Inc. (Frenchtown, NJ.)]. The insects were maintained at 25° C and a photoperiod of 16L:8D.

4.3 Screening of Plant Extracts against *Trichoplusia ni*

4.3.1 Larval Growth Inhibition

In order to assess the effects of the plant extracts on larva, methanolic extracts were incorporated into artificial diet at 1000ppm, by the method of Isman and Rodriguez (1983). Control diets were treated with methanol alone. Two newly hatched neonate larvae were placed in an individual cell in a plastic assay tray [(No. 9067, BioServe Inc., (Frenchtown NJ.))] with approximately 1g of treated or control diet. Larvae were
maintained in a growth chamber at 26 C and photoperiod 16L:8D. After 24 hours, one of the two larvae was removed, leaving one per cell (n=20 for each treatment). This was to ensure there was one healthy larva per cell. Larval weights were determined after 10 days, and the mean larval weight for each treatment was expressed as a percentage of the control. A dose response was performed on extracts that showed bioactivity; the procedure was repeated as above, at four –five concentrations (500, 1250, 2500, and 5000 ppm fresh weight) and EC$_{50}$ values were calculated for each plant extract with rosemary as the positive control. EC$_{50}$ values are defined as effective concentrations causing 50% growth inhibition compared to the negative control.

**4.3.2 Antifeedant Activity**

Extracts were screened for antifeedant effects on third instar *T. ni* larvae via a leaf disc choice bioassay (Akhtar et al., 2007) as shown in Fig. 4.2. Methanolic extracts at concentrations ranging from 6.25 ug/cm$^2$ to 50ug/cm$^2$ were applied to clean, dried cabbage leaf disks (*Brassica oleracea* var. Stonehead, No 8 cork borer); 10ul of solution was applied to each side of the leaf disc. One treated and one control disc (after being dried) were placed in each compartment [(4.2cm X 3.0cm (length X width)] of a plastic assay tray (No. 9067 BioServe., Frenchtown NJ) with a small piece of moistened cotton to prevent desiccation. The distance between the two discs was ~ 0.7 cm. After 3h of starvation, one larva was introduced gently into the center of each compartment using forceps and allowed to feed.
Figure 4.2 Leaf disc choice bioassay

(Akhtar, 2003)

The trays were covered with plastic lids. The plastic trays with larvae and test discs were put into a clear plastic box [(39 x 27 x 14 cm (length x width x height)] lined with moistened paper towel and the box was placed at room temperature (23 °C). Bioassays were terminated when ~50% of the control disc had been eaten (normally 3-5 hours). Feeding deterrence was calculated using the formula:

\[ \frac{(C-T)}{(C+T)} \times 100 \]

where C and T are areas consumed of the control and treated leaf discs respectively (Akhtar et al., 2007). The number of larvae was 25 per treatment. A dose response was performed on extracts that showed bioactivity at an initial screening concentration (50 µg/cm²) at four–five concentrations (50, 100, 200 and 400 µg/cm²) and DC₅₀ values were calculated with rosemary as the positive control. DC₅₀ values are defined as concentrations causing 50% feeding deterrence compared with the negative control.
4.3.3 Oviposition Deterrence

Oviposition response of *T. ni* moths was measured according to oviposition choice bioassay (Akhtar and Isman, 2003) as shown in Fig. 4.3. *Trichoplusia ni* larvae were reared on normal diet from neonates (< 24 h) until pupation.

Figure 4.3 Oviposition cage (top) and the control and the treated leaves with sugar solution (bottom) for moths to feed on.
Pupae were sexed and put in separate plastic containers until emergence. After eclosion, pairs of moths (one male and one female) were introduced into each cage with a control and a treated cabbage leaf. Pairs of moths (n = 25), were used per treatment. Each leaf (approximately 100-110 cm²) was sprayed with 0.5 ml of MeOH or a methanolic solution of the test chemical on each side. Eggs were counted on each cabbage leaf after 48 h. ODI (oviposition deterrence index) was calculated using the formula: \[ ODI = \frac{(C-T)}{(C+T)} \times 100 \] where C and T are the numbers of eggs laid on the control and treated leaf discs respectively (Akhtar and Isman, 2003).

4.3.4 Contact Toxicity (through spraying)

Mortality was determined 24 hours after spraying larvae directly with test solutions (Akhtar et al., 2007). Third instar *T. ni* larvae were sprayed in 90 mm x 15 mm Petri dishes (Falcon®) lined with Fisher Scientific filter paper (90 mm diameter). Small plastic hand spraying bottles (50 ml capacity) were used. Larvae were then transferred to Petri dishes (90 mm x 15 mm) with a small piece of artificial diet. Each Petri dish contained 10 larvae. Three replicates, each consisting of 10 larvae, were used per treatment.
4.4 Results

4.4.1 Larval Growth Inhibition

A one-way ANOVA of the mean growth of *T. ni* larvae feeding on the plant extracts at 1000 PPM fwt produced a significant F value ($F_{4, 14} = 489, p = 0.01$) (Fig. 4). Results indicate that Pumin and Matico were the most active growth inhibitors of *T. ni* larvae (Tukey’s test, $p < 0.05$) followed by SM. Marco and Mashua were the least active.

![Figure 4.4 Chronic growth inhibition of *T. ni* larvae](image)

4.4.2 Antifeedant Activity

Pumin, Santa Maria and Matico were subjected to dose response. Rosemary oil was used as a positive control. While a typical dose response would test at four or five different concentrations, only two or three different concentrations of extracts were tested due to material limitations. My results indicate that some of the extracts were more active than the positive control at the highest concentration. All three extracts, Santa Maria, Pumin and Matico resulted decreased growth (30-40% larval weight relative to the
control) at 2500 PPM, whereas rosemary produced 30% larval weight at 5000 PPM. (Fig. 4.5).

Figure 4.5. Chronic growth dose response of Pumin, Santa Maria and Matico. Rosemary oil was used as a positive control.

Dose response relationships (concentration versus feeding deterrence) of plant extracts against third instar *T. ni* larvae in leaf disc choice bioassays are shown in Figure 4.6. Based on the DC\textsubscript{50} values (Table 4.1), Pumin was the most active feeding deterrent (DC\textsubscript{50} = 64.8 µg/cm\textsuperscript{2}) followed by Santa Maria and Marco. All three were more active than the positive control, rosemary (DC\textsubscript{50} = 64.8 µg/cm\textsuperscript{2}). Matico and Mashua were less active than the positive control. High R\textsuperscript{2} values (Table 4.1) indicate a strong
dose response relationships (concentration versus feeding deterrence) of plant extracts.

Figure 4.6 Dose response relationships (concentration versus feeding deterrence) of plant extracts against third instar Trichoplusia ni larvae in leaf disc choice bioassays
Regression lines were calculated from 4-5 points, n = 20-25 for each point (each point represents mean feeding deterrence [%] of larvae for a particular concentration of the test substance when given a choice between control and treated leaf discs).

Table 4.1 Antifeedant effects of plant extracts on third instar *T. ni* larvae.

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>DC$_{50}$ values* ($\mu$g/cm$^2$)</th>
<th>R$^2$**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Santa Maria (SM)</td>
<td>121.2</td>
<td>0.96</td>
</tr>
<tr>
<td>Marco</td>
<td>135.1</td>
<td>0.92</td>
</tr>
<tr>
<td>Matico</td>
<td>230.4</td>
<td>0.97</td>
</tr>
<tr>
<td>Mashua</td>
<td>369.3</td>
<td>0.98</td>
</tr>
<tr>
<td>Pumin</td>
<td>64.8</td>
<td>0.88</td>
</tr>
<tr>
<td>Rosemary</td>
<td>205.3</td>
<td>0.94</td>
</tr>
</tbody>
</table>

* DC$_{50}$ = concentration of the test substance to deter feeding by 50% in leaf disc choice bioassay calculated from Figure 4.5.

** R$^2$ coefficient of determination

### 4.4.3 Oviposition Deterrence

A one-way ANOVA on the oviposition deterrence of *T. ni* female moths by plant extracts at 1% produced a significant F value ($F_{4,167} = 7.6$, $p = 0.01$) (Fig.4.7). The results indicate that Santa Maria demonstrated the strongest oviposition deterrent effect (78.9%) followed by Matico (62.5%), Marco (40.0%) and Pumin (33.0%). Mashua was the least active oviposition deterrent (17.0%) (Tukey’s test, $p < 0.05$) (Fig. 4.7).
Means (+/-SE) followed by the same letter are not significantly different

4.4.4 Contact Toxicity

Based on a contact toxicity bioassay, Matico was the most toxic (48.2% toxicity) followed by Marco (36.7% toxicity), Mashua (22.2% toxicity), Rosemary (6.9% toxicity) and Santa Maria (2% toxicity) when tested at 10%. Pumin showed no toxicity against third instar larvae (Table 4.2).

Mashua (~3 times) Marco (~ 5 times) and Matico (~ 7 times) were more active than the positive control, Rosemary oil.
Table 4.2. Toxicity of plant extracts (10%) against third instar *T. ni* larvae in 24h contact toxicity bioassay through spraying.

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>Mortality (%)</th>
<th>95% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Santa Maria</td>
<td>2</td>
<td>0.4-4.5</td>
</tr>
<tr>
<td>Marco</td>
<td>36.7</td>
<td>34.2-39.2</td>
</tr>
<tr>
<td>Matico</td>
<td>48.2</td>
<td>45.7-50.7</td>
</tr>
<tr>
<td>Mashua</td>
<td>22.2</td>
<td>19.7-24.7</td>
</tr>
<tr>
<td>Pumin</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rosemary</td>
<td>6.9</td>
<td>4.4-9.4</td>
</tr>
</tbody>
</table>

N= 3*10

* Confidence interval

**4.5 Discussion**

There was considerable variability with respect to the efficacy of the different extracts in the bioassays. No single extract was consistently good or poor in controlling the cabbage looper. Pumin proved to be the most effective botanical in controlling *T. ni*, with good results as a growth inhibitor and an antifeedant. It was ineffective as a contact toxin, however. Santa Maria was the most effective oviposition deterrent, and was also effective as an antifeedant. Matico was the most effective contact toxin, and was the second most effective oviposition deterrent and growth inhibitor. Mashua was the least effective extract. Marco did not perform well in any of the bioassays. As it appears that Santa Maria, Pumin, and Matico are all have different modes of action, and thus affect the test organism in different ways, it would be most beneficial to growers to utilize a
combination of the plants. It would have been interesting to look at synergistic effects of the three plants used together.

Advantages of antifeedants over acutely toxic compounds include the reduced effects on natural enemies, pollinators and other non-target organisms, as well as a decreased likelihood of the development of resistance (Isman, 2002).
Chapter Five: Summary and Discussion

5.1 General Conclusions

The overall objective of this thesis was to identify plants that have the ability to control insect pests of potato production in Ecuador, and to test for these properties in the lab.

The collaborative approach with Indigenous communities proved to be an effective means of identifying potential insecticidal plants. The theory of consensus was supported by my findings, as the most commonly named species were found to be the most effective in controlling the PTM. My research also supported the hypothesis that the plants named most frequently by research collaborators, and mentioned least frequently in the scientific literature are the best source for novel insecticides. Both Mashua, *Tropaeolum tuberosum*, and Marco, *Ambrosia artemidioides*, were under-represented in the scientific literature, and were found to be the most promising plants for the control of the PTM.

There currently exists no effective means of controlling the Guatemalan potato tuber moth, *Tecia solanivora*, in fields or in stores. Without access to effective control measures, farmers are faced with the potential loss of their entire store of potatoes. The greatest likelihood of controlling the pest may result from the use of integrated pest management (IPM) measures including the use of botanicals. Many farmers in Ecuador currently store their seed potatoes in open locations, without any phytosanitary precautions. The great majority of infestation occurs in these situations, where adult moths have easy access to tubers on which they lay their eggs. Mashua and Marco
proved to be very effective at disrupting the lifecycle of the PTM when extracts were applied to the larvae and pupae at concentrations of 5%. In addition to contact toxicity, some of these plant extracts also possess mild oviposition deterrent effects against female tuber moths. Both Santa Maria and Pumin exhibited an oviposition deterrent index (ODI) of 100% at 10%, though they were not tested at lower concentrations. Matico was the most active oviposition deterrent at low concentrations, exhibiting an ODI of 72.3% at a concentration of 0.25%.

Other measures can be taken to further ensure that adult moths have limited access to tubers. The tuber moth negatively impacts farmers in three ways; by increasing the cost of production by necessitating the purchase of chemicals; by harming health and the environment through the application of such toxic chemicals, and through crop loss from ineffective or prohibitively expensive chemicals. The use of botanicals in an IPM system can effectively address each of these issues. The botanicals studied in this project are all abundantly available in the region, so they can be collected and prepared free of direct cost. We have to assume that the extracts studied are safe to the environment and to humans as prepared and at the rates required for efficacy. Finally, these products, while less efficacious than some conventional pest control products, are certainly more effective than no product at all.

My findings have been shared with INIAP, as well as with Birgit Schuab, a PhD student working at CIP in Quito on ecological control of *Tecia solanivora*. 
5.2 Limitations

Perhaps the greatest limitation to this study was the time available to conduct research in Ecuador. I had originally hoped to complete a significant portion of the laboratory work within the province of Bolivar, using *Tecia solanivora* as my test insect, and where I could have extended the work to the field. Unfortunately, I discovered that the pace of research feasible in that location would not allow for even preliminary bioassays. While I was able to obtain a permit to import the closely related *P. operculella* for research at UBC, the potential specificity of botanicals to particular species stands as a limitation. The inexperience I had with rearing insects was also a limitation, as my colony collapsed two separate times. Bringing plant material into Canada from Ecuador necessitated that it be dried; this imposed the further limitation that investigating the fermentation products of selected plant materials was not possible. In consideration of how these extracts could be produced locally, I used a polar solvent for the extraction of all plant materials, including Santa Maria, however, the insecticidal properties in Santa Maria are extracted using a non-polar solvent such as hexane. The choice of extraction methods can have an effect on the efficacy of active plant constituents. Of primary consideration is the type of solvent used since polar solvents will extract polar molecules, and non-polar solvents will extract non-polar molecules. The purpose of a general screen for bioactivity is to extract as many potentially active constituents as possible. This is achieved by using solvents ranging from water (the most polar solvent), to hexane (non-polar). If incomplete screening of botanical material is attempted, the solvents should be carefully selected as different solvent types can significantly affect the potency of extracted plant compounds.
5.3 Future Research

The collaboration with indigenous peoples was a successful approach to discovery of botanical insecticides; further research in different communities should be carried out.

The results of the research warrant further investigation in a field setting. The use of fermentation in the production of botanical insecticides is a very interesting topic for further research. It was mentioned by greater than 20% of informants, which suggests there is a great likelihood that it is a useful and perhaps crucial step in the preparation of insecticides.

Field trials, effects on non-target species, development of resistance, economic feasibility are all issues that should be investigated further.
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