UNDERSTANDING AND IMPROVING THE RISK ANALYSIS PROCESS FOR APPROVING THE IMPORTATION AND RELEASE OF ENTOMOPHAGOUS BIOLOGICAL CONTROL AGENTS INTO THE UNITED STATES—EVALUATION OF CURRENT METHODOLOGIES AND LESSONS LEARNED FROM BIOLOGICAL CONTROL RESEARCH

By

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To my family, especially my mother Alhaja Mulikat Paraiso

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LIST OF ABBREVIATIONS

APHIS	Animal and Plant Health Inspection Service
ARS	Agricultural Research Service
AQIS	Australian Quarantine and Inspection Service
BA	Biosecurity Australia
BCA(s)	Biological Control Agent(s)
BCRC	Biological Control Review Committee
CFIA	Canadian Food and Inspection Agency
CSIRO	Commonwealth Scientific and Industrial Research Organization
DAFF	Department of Agriculture, Fisheries and Forestry
DEH	Department of Environmental and Heritage
EA	Environmental Assessment
EBCA(s)	Entomophagous Biological Control Agents
EIS	Environmental Impact Statement
EPBC act	Environment Protection and Biodiversity Conservation act
FAO	(United Nations) Food and Agriculture Organization
FONSI	Finding of No Significant Impact
IOBC	International Organization for Biological Control
IPPC	International Plant Protection Convention
ISPM	International Standard for Phytosanitary Measures
NBCI	National Biological Control Institute
NAPPO	North American Plant Protection Organization
NEPA	National Environmental Policy Act
NPPO	National Plant Protection Organization
OPL	Ontario Plant Laboratory

PHD	Plant Health Division
PPA	Plant Protection Act
PPQ	Plant Protection and Quarantine
PPQS	Directorate of Plant Protection, Quarantine and Storage
PRA	Pest Risk Analysis
QEL	Quarantine Entomology Laboratory
RA	Risk Assessment
RC	Risk Communication
RM	Risk Management
RSPM	Regional Standard for Phytosanitary Measures
SPS	Sanitary and Phytosanitary Agreement
TAG	Technical Advisory Group
USDA	United States Department of Agriculture
WTO	World Trade Organization

Abstract of Dissertation Presented to the Graduate School
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UNDERSTANDING AND IMPROVING THE RISK ANALYSIS PROCESS FOR APPROVING THE IMPORTATION AND RELEASE OF ENTOMOPHAGOUS BIOLOGICAL CONTROL AGENTS INTO THE UNITED STATES—EVALUATION OF CURRENT METHODOLOGIES AND LESSONS LEARNED FROM BIOLOGICAL CONTROL RESEARCH

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Classical biological control is a strategy to manage invasive pests. Despite many success stories involving the use of entomophagous biological control agents (BCAs), concerns have been raised about potential negative environmental impacts. The current risk analysis process for entomophagous BCAs in the U.S. is considered by some to be subjective and often arbitrary. The research undertaken in this dissertation developed a modified risk analysis framework to improve the importation and release of entomophagous BCAs. The aim of this revised procedure was to increase involvement and trust by stakeholders. In an effort to apply risk analysis concepts into research, a model example of a potential entomophagous BCA was examined. A case study was explored using the naturally occurring egg parasitoid, *Trichogramma fuentesi* Torre (Hymenoptera: Trichogrammatidae), attacking *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae), a serious non-native pest of *Opuntia* spp. in North Florida. In order to implement a safe and effective biological control program, biological and ecological characteristics of *T. fuentesi* were assessed. Our research showed that one

to two-day-old mated *T. fuentesi* females should be used to increase percent parasitism in the field. However, the correlation between the number of egg parasitized and host egg densities was weak suggesting that *T. fuentesi* would not provide a significant level of control of this pest in the field. Comparison of percent parasitism of *C. cactorum eggs* with preferred hosts showed a relatively low level of parasitism when the wasp attacked the invasive moth (11%) than when native Lepidoptera were attacked (26–75%). In addition, results suggested that inundative releases of *T. fuentesi* could potentially impact native cactus moth and butterfly eggs. The findings from this study demonstrated that *T. fuentesi* is not a good candidate for augmentative biological control against *C. cactorum*. Results from our research study provided a better understanding of the information necessary to improve the pest risk analysis for the importation and release of entomophagous BCAs in the U.S. Inclusion of recommendations developed from the conceptual analysis and experimental studies into decision-making processes will improve implementation and safety of biological control programs in the U.S.

CHAPTER 1
INTRODUCTION

Classical biological control is an important component of an integrated pest management strategy against invasive species. Classical biological control refers to the use of introduced natural enemies against non-native pests (Eilenberg et al. 2001). Despite several examples of successful pest control using entomophagous biological control agents (BCAs) (van Lenteren et al. 2006), there are increasing concerns from the scientific community on the possible negative environmental and economic impacts associated with their importation and release (Howarth 1983; Simberloff & Stiling 1996; Louda et al. 2003). Many consider the current risk assessment process for entomophagous BCAs in the U.S. to be subjective and often arbitrary. In 2007, Interviews were conducted to characterize how phytosanitary decisions were made during the permitting process for entomophagous BCAs by the U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ)—the National Plant Protection Organization (NPPO) for the U.S., and to identify issues related to the risk assessment process that could be improved. Concerns about the potentially irreversible non-target impacts that might result from the importation and release of entomophagous BCAs have resulted in increasingly stringent regulatory requirements by the U.S. NPPO.

Despite numerous scientific publications on the potential risks associated with importation and release of BCAs, there still are divergent opinions among regulators, researchers, environmentalists, and the general public on ways to appropriately manage these potential risks. One approach that could be used to reduce this divergence might be through the implementation of a comprehensive and effective risk communication process. This study examined communication habits of stakeholders involved in biological control and characterized how phytosanitary decisions were communicated to them by decision-makers working for the U.S. NPPO. We used the "mental models" approach to examine how stakeholders perceived risks and understood the associated scientific literature, and we explored possible ways of providing a better understanding of factors that affected the permitting process for entomophagous BCAs.

The second part of the dissertation focused on the evaluation of potential risks associated with entomophagous BCAs under laboratory conditions. The assessment of potential impacts is a crucial step during the risk assessment process. *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) is a serious pest of *Opuntia* spp. (prickly pear cactus) in North America. Presence of *C. cactorum* in the U.S. is of great concern because of its potential adverse impacts to ecological systems and to native and endangered *Opuntia* spp. particularly in the southwestern U.S. Geographical expansion of *C. cactorum* is also a threat to *Opuntia* spp. biodiversity and agricultural production in Mexico. Interest in the natural enemies of *C. cactorum* has increased since the moth was found in Florida in 1989. Previous surveys of natural enemies in Argentina identified egg parasitoids in the family Trichogrammatidae as potential BCAs for *C. cactorum*. *Trichogramma* wasps are widely distributed egg parasitoids used against major lepidopteran pests in greenhouse and field settings. Consequently, a study was conducted in North Florida to identify and to assess occurrence of egg parasitoids attacking this invasive moth in its new homeland. In addition, to further understand the interactions of the parasitoids and the pest, this research examined the following biological parameters in the laboratory: parasitoid functional and numerical response, behavioral characteristics, and host specificity of *Trichogramma fuentesi* Torre (Hymenoptera: Trichogrammatidae). The last part of the dissertation uses the findings from the conceptual analysis and the laboratory experiments to discuss the potential for inundative releases of *T. fuentesi* against *C. cactorum* in the U.S.

CHAPTER 2
LITERATURE REVIEW

Negative Impacts of Invasive Species

The intentional introduction of several non-native species of plants and animals to the U.S. such as corn, wheat, soybeans, and cattle has greatly benefited American agriculture, whereas unintentional introductions of many species have been the source of significant economic and environmental damage [e.g. Brazilian pepper tree (*Schinus terebinthifolius* Raddi, Anacardiaceae) and Zebra mussels (*Dreissena polymorpha* Pallas, Dreissenidae)] (Andersen et al. 2004). One of the main concerns of the U.S. National Plant Protection Organization (NPPO) is the increasing rate of unintentional introductions of non-native species that in many instances become invasive (Waage 2001). The introduction of these non-native species, without their natural enemies, indirectly contributes to their invasiveness (Waage 2001).

Concerns over the impact of invasive species on the U.S. economy have resulted in the development of new policies and action groups. For instance, in 1999, President Bill Clinton issued an Executive Order for the allocation of US $28 million for creation of an Interagency Invasive Species Council to address the threat of invasive species (Pimentel et al. 2005). The Council's principal mission was to develop a strategy to fight the spread of non-native species (Pimentel et al. 2005). In 2005, it was estimated that the annual economic cost of non-native invasive species in the U.S. was almost US $120 billion per year (Pimentel et al. 2005). Arthropod pests alone cause a 13% overall reduction in crop yields. In economic terms, this reduction represents about US $33 billion in lost crop production annually (USBC 2001). Just 40% of these introduced pests accounted for an annual total economic loss of US $13 billion (Pimentel et al.

1999). About US $500 million are spent each year applying pesticides to control crop pests (Pimentel et al. 1999) and another US $1.5 billion are spent each year to manage entomophagous pests of lawns, gardens, and golf courses in the U.S. (Pimentel et al. 2005).

In addition to their negative economic effects, invasive species have been recognized as a threat to the conservation of biodiversity (Convention on Biological Diversity, article 8h). Invasive species may cause permanent changes to ecological communities by shifting the arrangement and abundance of native species (Andersen et al. 2004). Almost half of the current species listed as threatened or endangered in the U.S. are considered to be at risk because of the potential predatory or competitive behaviors of non-native species (Wilcove et al. 1998). Moreover, the presence of invasive species has been documented as a major constraint to reforestation, water management, and recovery of degraded lands in developing countries (Waage 2001).

Invasive pest species are a threat to sustainable development (Abate et al. 2000; Waage 2001; Kairo 2005). A sustainable approach is based on the management of natural resources to meet current human needs while maintaining the earth's capacity to meet the needs of future generations. At the social level, there has been an increasing demand for "green" alternatives to chemical control of invasive species (Charudattan 2001; Pimentel et al. 2005).

The Importance of Biological Control in Invasive Species Management

Biological control can be a key component of an integrated pest management strategy against invasive species. Classical biological control is defined as the intentional introduction of an exotic natural enemy (e.g. insects, mites, nematodes, pathogens) that results in establishment of the enemy and, as consequence, effects permanent control of the target pest (Eilenberg et al. 2001). There is an increased interest in the use of biological control by the U.S. Department of Agriculture (USDA) because of the imminent withdrawal of several major pesticides and insecticides from the market (Charudattan 2001). Furthermore, development and registration of new pesticides involves high costs. Meanwhile, governmental institutions have been mandating a reduction of chemical pesticide usage in food production (Charudattan 2001). For instance, the extensive use of herbicides for weed management resulted in the emergence of herbicide-resistant weed biotypes. The economic and environmental impacts of herbicide-resistant crops and naturally resistant weeds generated a need for pest management alternatives to unilateral chemical controls (Charudattan 2001). In addition, there has been opposition from the general public toward excessive use of insecticides or development of genetically altered food crops (e.g. herbicide-tolerant transgenic crops) to control invasive species (Charudattan 2001; Pimentel et al. 2005). Successful biological control programs directly benefit consumers because they usually have minimal impacts on the environment. Pest control using biological control agents (BCAs) presents a safer alternative to pesticide use in food production systems (Charudattan 2001). The use of biological control minimizes pesticide impacts on people and the environment (Hoddle 2004). At the social level, biological control programs are a sustainable solution for developing countries and resource-poor farmers because of biological control ‘s intrinsic characteristics (e.g., safe to humans, environmentally friendly, and self-sustaining) (Abate et al. 2000; Waage 2001; Kairo 2005).

Examples of Successful Biological Control Programs

There are many examples of successful biological control programs against weed and arthropod pests (Julien 1992; Julien & Griffiths1998). Infestations of purple loosestrife (*Lythrum salicaria* L., Lythraceae), a rhizomatous perennial introduced to North America from Eurasia and Africa, was managed by the release of *Galerucella pusilla* Duftschmidt, *G. calmariensis* Duftschmidt (Coleoptera: Chrysomelidae) and *Hylobius transversovittatus* Goeze (Coleoptera: Curculionidae) (Hight et al. 1995; Blossey et al. 1994; Landis et al. 2003). Management of the expansion of the Australian tree *Melaleuca quinquenervia* (Cav.) S.T. Blake (Myrtaceae) in Florida by three BCAs was another successful example of weed biological control (Serbesoff-King 2003). Melaleuca had become one of Florida's most invasive weeds since its introduction in the 1880s (Fairchild 1947; Morton 1966; Balciunas 1990; Laroche & Ferriter 1993).

Various scientific publications report the success of biological control programs against arthropod pests (van Lenteren et al. 2006). For instance, in Africa, the cassava mealybug [*Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae)] was effectively controlled by a parasitic wasp, *Apoanagyrus lopezi* (DeSantis) (Hymenoptera: Encyrtidae), imported from South America (Herren & Neuenschwander 1991; Hammond et al. 1992). Cassava (*Manihot esculenta* Crantz, Euphorbiaceae), native to South America, and the preferred host of the cassava mealybug, is a staple food for millions of Africans (CGIAR 2008). An economic study showed that this biological control program generated a benefit of US $149.00 for every dollar invested (Norgaard 2006).

In North America, the positive impacts of entomophagous BCAs have been thoroughly documented. In 1888, the ladybird beetle, *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae), was introduced into California to control the cottony cushion scale, *Icerya purchasi* Maskell (Hemiptera: Margarodidae) (DeBach 1973). The cottony cushion scale was severely infesting California citrus groves causing orchard yield to decrease dramatically. The biological control project, which cost only about US $1500, saved the California citrus industry millions of dollars (Caltagirone & Doutt 1989). Control of the alfalfa weevil, *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae) (Bryan et al. 1993) is another good example of successful biological control. The alfalfa weevil, native to Europe, was originally detected in the U.S. in 1904. By 1970, the weevil had spread to all 48 contiguous states and become a serious pest of alfalfa (*Medicago sativa* L., Fabaceae). In 1957, the USDA-Agricultural Research Service (USDA-ARS) released parasitoid species belonging to four families (Eulophidae, Ichneumonidae, Mymaridae, and Braconidae) to reduce weevil populations to manageable levels in the eastern U.S. (Day 1981).

The Risk Analysis Process for Entomophagous Biological Control Agents

Risks Associated with the Importation and Release of Entomophagous Biological Control Agents

The largest risk associated with the introduction and release of BCAs is their potential detrimental environmental effects on non-target organisms (Louda et al. 2003). Unfortunately, once BCAs are successfully established, they are impossible to eradicate and their adverse impacts on non-target organisms may persist indefinitely. For instance, the majority (83%) of parasitoids found attacking native caterpillars in Hawaii are BCAs intentionally released to control pest populations (Henneman & Memmot

2001). Therefore, the main challenge for future biological control programs is to identify specific natural enemies of the targeted pests that are effective in their actions without having detrimental ecological consequences (Howarth 1983; Simberloff & Stiling 1996; Louda et al. 2003). Testing and verifying the existence and magnitude of non-target impacts for entomophagous BCAs has become a significant concern of many biological control practitioners (Boyd & Hoddle 2007). Decisions to approve the importation and release of any new BCA must be considered carefully by appropriate authorities in the importing country, taking into account the risks of doing nothing or the risks associated with the BCA introduction, and the consequences of possible non-target effects (Jayanth et al. 2003) including the likelihood that the agent will spread beyond national borders (van Lenteren et al. 2006).

Several countries have developed new legislation and/or have revised existing regulations to facilitate the introduction of new biological control organisms while minimizing environmental risks (COSAVE 1996; AQIS 1997; ERMA 1997a; b). These regulations have mostly been based on International Standards for Phytosanitary Measures (ISPM # 2, 11) developed by the International Plant Protection Convention (IPPC) (De Nardo & Hopper 2004).

Pest Risk Analysis

The level of risk associated with the unintentional introduction of invasive species has been an ongoing concern that has been elevated to the international trade and environmental policy agendas (Andersen et al. 2004). Consequently, in 1995, the World Trade Organization (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) was ratified by most of the WTO country members (Ebbels 2003). Under the SPS Agreement, member countries agreed to base their phytosanitary decisions on the results of a science-based assessment of potential risks (Andersen et al. 2004). In the area of plant health, this process was called pest risk analysis (PRA).

In the U.S., the task of protecting American agriculture and natural resources against the risks associated with the entry, establishment, or spread of plant pests is the responsibility of the U.S. NPPO—the Plant Protection and Quarantine (PPQ) group within the Animal and Plant Health Inspection Service (APHIS), an agency within the USDA (APHIS 2007). The Plant Protection Act (PPA) of 2000 authorized the Secretary of Agriculture to delegate his/her plant protection authority to employees of USDA-APHIS-PPQ. This federal entity has broad authority to develop and enforce phytosanitary measures that will prevent or delay the introduction and spread of plant pests (PPA 2000). In the last 10 years, new and revised legislation and regulations have been implemented by the U.S. NPPO in response to the identification of new pathways for potential pest introductions resulting from trade globalization, diversification in transportation and increases in tourism (De Nardo & Hopper 2004).

Risk can be defined as the combination of the likelihood of an adverse event and the magnitude of the consequences (Delfosse 2005). Pest risk analysis has three components. Several publications recognize different steps but essentially the key elements are: risk assessment, which is the estimation of the likelihood and consequences of an adverse outcome; risk management, which identifies management options that will reduce or manage the adverse event; and risk communication, which is a two-way exchange of information about the undesirable event (Fisher et al. 1994). A PRA provides the technical justification for the application of phytosanitary measures.

In the area of biological control, a PRA examines the potential adverse effects that the introduction of BCAs can have and these include detrimental economic and/or environmental impacts (Delfosse 2005). It provides the scientific evidence to either allow or deny the importation and subsequent release of BCAs (Barratt & Moeed 2005).

The PPA makes a specific distinction between plant pests and BCAs used in pest management programs. Under the PPA, the U.S. NPPO has authority to regulate the importation and release of BCAs (PPA 2000). In addition, the National Environmental Policy Act (NEPA) of 1973 requires the development and submission of an Environmental Assessment (EA) and Environmental Impact Statement (EIS) before approval to introduce any organism with potential negative environmental impacts. An accepted and preferred alternative during the permitting process of entomophagous BCAs is the preparation and submission of an EA which provides information on the organism and its potential environmental significance in a succinct format (Kubasek & Silverman 2005). The EA document provides basic information on the positive and negative environmental impacts of the proposed action, including a management program in case of adverse consequences, and also suggests alternatives to the plan under consideration. The EA is compulsory under three circumstances: when the activity is federal; when the federal activity is major; and when the proposed activity will have a significant impact on the human environment (Kubasek & Silverman 2005). The EA is reviewed by an external group of experts selected by the agency required to file the EA. The group of experts provides an analysis of potential environmental adverse effects of the proposed action. The revised document is then sent to the Council on Environmental Quality (CEQ) for further comments. Finally, in accordance with the

Administrative Procedure Act's rules on informal rule making, a draft is published in the Federal Register and public comments are submitted for a period of 90 days. When an EA is completed and determined that there are no significant impacts, no EIS needs to be filed (Kubasek & Silverman 2005).

In the U.S., consensus among regulators, decision-makers, and biological control stakeholders on an acceptable and standardized risk assessment framework for entomophagous BCAs has not yet been reached (De Nardo & Hopper 2004; Boyd & Hoddle 2007). In the 1990s, scientists and governments worldwide identified a need for a harmonized regulatory framework during the importation of BCAs in order to ensure a more effective plant and animal protection system (Ebbels 2003). In Europe, an expert panel from the European and Mediterranean Plant Protection Organization (EPPO) published two guidelines on the safe use of biological control and developed a safe list for commercially used BCAs (Bigler et al. 2005). Independently, the European Union (EU) funded a research project entitled "Evaluating Environmental Risks of Biological Control Introductions into Europe" (ERBIC) and developed a document for the environmental risk assessment of exotic natural enemies in inundative biological control (van Lenteren et al. 2003). In 1999, the Organization for Economic Co-operation and Development (OECD) developed guidance documents on appropriate regulations for the use of BCAs (Bigler et al. 2005). Recently, an EU-policy support action document compared regulation procedures used by the EU and U.S. to develop a more efficient process for the EU (REBECA 2011). One of the priorities of the U.S. NPPO has been to improve their understanding and communication of the risks of the importation of

BCAs through the development of a more transparent risk assessment process (Fisher et al. 1994).

The SPS Agreement recognizes the IPPC as the standard setting body for phytosanitary measures to protect plant life and plant health. In response to an increasing demand for the use of BCAs across the globe, the IPPC developed the “Code of Conduct for the Import and Release of Exotic Biological Control Agents” as the third International Standard for Phytosanitary Measures (ISPM # 3) (IPPC 1997). In 1995, the standard was accepted by Food and Agriculture Organization (FAO) member countries. This standard provided a framework for safe importation and release of BCAs in the context of plant protection (Greathead 1997). In 2005, the scope of ISPM # 3 was expanded to include other beneficial organisms (e.g. sterile insects) and the title was changed to “Guidelines for the Export, Shipment, Import and Release of Biological Control Agents and other Beneficial Organisms” (IPPC 2005). To conform to the SPS Agreement, the revised ISPM # 3 required that a NPPO be identified and made responsible for the implementation of phytosanitary measures. The revised ISPM # 3 refers to other phytosanitary standards that provide guidance in the development of PRAs—ISPM # 2 (“Framework for Pest Risk Analysis”) and ISPM # 11 (“Pest Risk Analysis for Quarantine Pests Including Analysis of Environmental Risks and Living Modified Organisms”) (IPPC 2004; IPPC 2007). The revised ISPM # 3 recommends that a dossier be developed for each agent and beneficial organism that includes information on the organism, pests targeted, potential human and animal health safety issues, and potential economic impacts of both the agent or beneficial organism and the pest. In the U.S., the evaluation of this dossier is a central component of the decision making process to allow or deny the importation of BCAs or beneficial organisms.

Permitting Process

The enabling legislation that governs the importation and release of entomophagous BCAs in the U.S. is the PPA of 2000. To obtain approval for (1) importation of BCAs into containment facilities; (2) domestic movement of imported BCAs to other containment facilities; or (3) release of a BCA into the environment, applicants must complete a USDA-APHIS-PPQ federal permit application form (PPQ 526). Furthermore, if the entomophagous BCA has not been previously released in the U.S., the application must be accompanied by supplemental documentation which describes the justification for the proposed action, provides information on the biology/ecology of the pest and the entomophagous BCA, the economic impacts, and any potential detrimental environmental impacts as well as possible mitigation options (Hunt et al. 2008). The dossier format is loosely based on the "Guidelines for Petition for First Release of Non-indigenous Entomophagous Biological Control Agents", Regional Standard for Phytosanitary Measures # 12 published by the NAPPO. The U.S. NPPO makes the final determination to approve or deny a petition based on the information provided by the applicant during the permitting process. Applicants who are employees of a federal agency, have received any federal funds, and/or have employed any federal workers during the project must also write an EA as required by NEPA (NEPA 1970).

Currently, the U.S. has no standardized or published regulatory framework for importing and releasing entomophagous BCAs (Messing 2005). However, the regulatory framework for importation and release of non-native phytophagous BCAs is relatively well-defined (Scoles et al. 2008). As regulations and requirements for importation and release of entomophagous BCAs are being developed, there has been an attempt by the U.S. NPPO to develop more transparent processes for entomophagous agents.

Risk Communication Framework during the Importation of Entomophagous Biological Control Agents

Risk Communication during Pest Risk Analysis

International Standard for Phytosanitary Measures # 11 describes PRA as a process requiring three stages. Stage 1 is an initiation stage that involves identification of what triggers the PRA process. There are generally two initiation points for PRA—the identification of a pathway or the identification of a pest that may qualify as a quarantine pest. In stage 2, pest risk assessment—a risk assessment is conducted to evaluate the probability of introduction and potential economic and environmental consequences. Stage 3, risk management, requires the identification of mitigation options to control/manage identified risks and some assessment of their effectiveness (IPPC 2005). However, according to Fisher et al. (1994), a PRA should include: risk assessment—that estimates the likelihood of occurrence of a hazard and magnitude of the consequences; risk management—which addresses what can be done to mitigate the consequences of the adverse event; and risk communication—which involves two-way exchange of information concerning the likelihood and magnitude of the hazard and the risk management measures to deal with the hazard.

Risk communication is often neglected in PRA. One of the reasons might be that risk communication is a non-technical and subjective concept that is difficult to grasp for many agencies. Risk communication also carries a stigma as it is often viewed as a means to make stakeholders "more rational" (Jasanoff 1989; Goldman 1994). International Standard for Phytosanitary Measures # 11 contains only one sentence explaining the importance of communication of risks in the PRA process. Risk communication is an interactive exchange of information about a potential risk between individuals (Fischnoff 1990). Risk communication can be described as a consensus structure that joins the interests and needs of both senders (applicants and researchers) and recipients (governmental entities, regulators, and decision-makers). The main purpose of risk communication is to provide individuals with enough information to enable them to make an informed decision about a potential risk (Gibson 1985; Fischnoff 1990; Gow & Otway 1990). In addition, risk communication helps identify and explain the benefits gained by accepting a particular risk (Morgan et al. 2002). Mature risk communication is defined as more than providing adequate information. It is a conscious effort to develop a partnership between the senders and the receivers of information (Fischnoff 1995).

Improving Risk Communication in Governmental Agencies

Governmental agencies have long been concerned with communication methods used to more appropriately convey the risks associated with environmental issues (Chess et al. 1995). The U.S. NPPO has a long history of evaluating stakeholder satisfaction with its risk communication efforts (Fisher & Chen 1996). It was one of the first agencies within the USDA to conduct a baseline survey of a wide range of stakeholders to examine how well they meet their customer needs during their risk communication activities (Fisher & Chen 1996).

In 1990, the now defunct National Biological Control Institute (NBCI) was created within USDA-APHIS-PPQ with the objective of promoting, facilitating, and providing leadership in biological control and integrated pest management (APHIS 1996). From 1991 to 1995, NBCI gathered opinions on biological control regulations and related guidelines from a plethora of stakeholders (researchers from universities, integrated pest management working groups, professional society members, industry representatives, environmental groups, federal and state agricultural department officials). The Institute published its final report in 1996 highlighting areas in the current biological control regulatory system that needed improvement (APHIS 1996). One of those areas was customer service and communication activities during the regulatory processes associated with importation and release of phytophagous and entomophagous BCAs (APHIS 1996). In 2006, an internal evaluation of USDA-APHIS-PPQ permitting process for entomophagous BCAs considered that customer service was still an activity that needed attention in order to improve BCA permitting activities in the U.S. (APHIS 2006).

Approaches to Communicating Risk

The one-way model of communication includes a source that generates a message sent via a channel to a receiver (Shannon 1948). Technical communication is the communication of scientific information. Risk communication is a subset of technical communication and is described as the communication of potential risks (Lundgren & McMakin 2004). In the process of risk communication one must consider how the messages are sent and received, how conflicts and misunderstandings are managed, and how decisions are made (Lundgren & McMakin 2004). A few examples of methodologies used in risk communication include the “convergence communication” approach, the “mental noise” approach, and the “mental models” approach. Each method examines risk communication processes based on how the audience perceives risk (Lundgren & McMakin 2004). For instance, during the convergence communication approach, the values (culture, experience, background) of the audience must be taken into account as they may affect the risk communication procedure. A group within the U.S. Department of Defense uses the “mental noise” approach to communicate with their stakeholders. This approach takes into consideration that people’s ability to process information might be altered by their feeling of being at risk (Blakeney 2002). The U.S. Environmental Protection Agency used the “mental models” approach during the communication of radon informational programs to their stakeholders. This approach allowed the integration of people’s’ understanding and views on their current risk situation in the development of the risk message.

Mental Models Approach within Organizations

The importance of understanding mental models within an organization was first demonstrated in large corporations, such as Royal Dutch/Shell. In the 1970s, the world oil business became more multicultural and a need for building consensus across the different management styles became important for oil companies. A management consensus was only made possible by understanding the shared mental models of each cultural group (Sengue 1992). In order to build effective communication frameworks across multicultural groups, the knowledge gaps, general understanding, and misconceptions of each group first needed to be identified (Jungermann et al. 1988; Lave & Lave 1991; Bostrom et al. 1992; Maharik & Fischnoff 1992).

Mental models are representations of the stakeholders assumptions based on personal perceptions. The assumptions can be simple generalizations or complex theories. Mental models help to anticipate and predict the outcome of an unknown event and usually impact the decision-making process (Sengue 1992). The mental models approach attempts to solve communication problems faced by risk specialists by requiring both a consideration of how the stakeholders intuitively think about the risks and which aspects of the scientific literature actually matter to stakeholders (Morgan et al. 2002). The approach is based on a systematic analysis of the beliefs of decision-makers and stakeholders and what specific information each group needs in order to make an informed decision (Morgan et al. 2002).

Conditions for Using the Mental Models Approach

Past studies (Jungermann et al. 1988; Lundgren & McMakin 2004) have demonstrated that the mental models approach is best used when the main purpose of risk communication is to modify behaviors. Additionally, the risk must be associated with conflicting opinions about how to manage the risk and the decision-making process should not be under time constraints.

In this dissertation, the mental models approach was used to propose an improved risk communication framework for the permitting process of entomophagous BCAs as implemented in 2007 in the U.S. The analysis is discussed in Chapters # 3 (p. 49), # 4 (p.82), and # 5 (p. 104). The discussion below focuses on the other risk criteria used during risk assessment for importation and environmental release of entomophagous BCAs.

Practical Application of Pest Risk Analysis into Biological Control Research

Selecting a Successful Entomophagous Biological Control Agent

Selection of an effective entomophagous BCA is a crucial step in the implementation of a biological control program. Various factors are used to determine the effectiveness of the BCA before its release into the environment. Biological parameters are used as technical justification for approval or denial of the introduction of an entomophagous BCA. Host range, functional response, climate suitability, dispersal ability, high reproduction rate, and short life cycle, are just a few examples of critical biological parameters that can be used to examine or determine the future effectiveness of a particular BCA. Much of this same information is used to assess the risks associated with the importation of the agent.

Biological characteristics

In order to select a successful agent, the natural enemy should display certain desirable attributes that relate to field performance. Previous studies demonstrated that abiotic factors, such as the presence of a source of food for energy have a positive impact on the establishment of introduced natural enemies (Boivin et al. 2006). The potential BCA should be host specific. In other words, the BCA should only feed on or parasitize the target pest species or closely related species that are also pests. This characteristic is theorized to reduce unintended non-target effects. The natural enemy should also exhibit several key traits, especially a high reproductive capacity, an effective attack rate, and good capacity for dispersal and searching (van Lenteren et al. 2006).

Functional response and numerical response

Biotic factors such as the temporal or spatial availability of hosts are important in the establishment of the BCA. Functional response describes the behavioral response of an individual biological control agent to increasing host density (Holling 1959). Results from these studies provide information on searching ability and handling time for BCAs. The concept is associated with the numerical response, which corresponds to the number of progeny produced as a function of host density (Holling 1959).

Without a host density dependent numerical response, parasitoids are less likely to be able to reduce host populations (Holling 1959).

Host suitability and preferences

One of the key parameters used to determine potential non-target effects by BCAs is the range of species that the BCA is able to attack. Therefore, host specificity testing has become the central issue in analyzing the risk of a potential BCA (DeNardo & Hopper 2004). While methods to determine host specificity of BCAs used to control weeds have been significantly improved (McEvoy 1996), this is not the case for BCAs used for the control of insect pests (Barratt et al. 2000; Kuhlmann et al. 2006). Even though a focus on non-target effects of non-native enemies is considered the key to safety associated with biological control programs (Haye et al. 2005), an effective and standardized host range testing strategy for entomophagous BCAs has not yet been developed (Duan & Messing 2000; Messing et al. 2006). The well accepted centrifugal-phylogenetic method (Table 1-1) developed by Wapshere (1974, 1989) is used to select host range test species of phytophagous BCAs. The method is based on phylogenetic taxonomic affinities of related plant taxa. Retrospective studies demonstrated of established phytophagous BCAs have revealed that the host range of these insects is limited to phylogenetically related host plant taxa (Bernays 2000; Pemberton 2000; Kuhlmann et al. 2006). There is little or no evidence in the existing literature that this approach has failed (McFayden 1998). This method is complicated for entomophagous BCAs because of unreliable host lists (due to possibly greater number of host taxa attacked), difficulty of assessing behaviors influenced by habitat under laboratory conditions, difficulty in mass-rearing non-target test species, and possible host shifts by BCAs (van Lenteren et al. 2006; Boyd & Hoddle 2007). In addition, until the mid 1980s, parasitoids and predators of plant pests were not subjected to laboratory host range tests unless they were thought to be capable of attacking beneficial organisms and/or other important non-target species (Ertle 1993).

Pre-release studies to determine the susceptibility of non-target plants has been part of weed biological control for over 60 years (Barratt et al. 2000). The pre-release host specificity testing for entomophagous BCAs is still one of the most difficult tasks that an applicant faces during the permitting process for entomophagous BCAs (Barratt et al. 1998). The extremely varied nature of host-parasitoid relationships and the large number of insect taxa precludes the establishment of a single prescriptive set of protocols for parasitoid-host specificity testing (Barratt et al. 1998). However, a process similar to weed biological control using phylogenetic and ecological parameters for possible hosts and non-target species might be applied. In addition, information on parasitoid host range from the country of origin can be indicative of the possible extent of the range of species which might be at risk in the area proposed for release (Barratt et al. 2000).

A review of published methods used to establish host lists for entomophagous BCAs showed that phylogenetic criterion was an important component. Biological, ecological, and socio-economic information were also important considerations during the process. Several studies (Duan & Messing 1996; Duan et al. 1997; Barratt et al. 1997, 1998, 2000) used these criteria during the development of host test lists, and subsequent post release monitoring of inundative parasitoid releases showed few non-target impacts on hosts from different feeding niches (Duan & Messing 1996; Duan et al. 1997). The results demonstrated that host morphological features were as important as phylogenetic relationships for host selection to determine oviposition behaviors of parasitoids.

Example of Non-Target Impacts: *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae), a Spreading Pest in the U.S.

The Argentine cactus moth, *Cactoblastis cactorum*, often is referred to as an example for successful biological control of weeds (Moran & Zimmermann 1984). Prickly pear cactus, *Opuntia* sp. were introduced into Australia in 1788 as ornamental plants and again in 1830 as hosts of the cochineal dye producing scale insect, *Dactylopius coccus* Costa (Hemiptera: Dactylopiidae). Unfortunately, the *Opuntia* cacti became invasive, resulting in the loss of millions of hectares of productive rangeland. In 1925, *C. cactorum* was introduced into Australia from its native Argentina to control the prickly pear. The cactus moth diet is restricted to the genus *Opuntia* but has a wide number of hosts in this genus in its native geographical range (Moran & Zimmermann 1984). Gregarious *C. cactorum* larvae destroy *Opuntia* cacti by feeding internally in the pads (or cladodes) (Dodd 1940). Within a few years, the introduction of *C. cactorum* into Australia restored US $6 million worth of rangeland to agriculture at that time (Dodd 1940). Based on these encouraging results, *C. cactorum* was transferred from Australia into South Africa, Mauritius, and Hawaii to manage other non-native *Opuntia* species that had become weeds in those countries (Moran & Zimmermann 1984). In 1957, *C. cactorum* was introduced into several Caribbean islands (Nevis, Montserrat and Antigua) to control native and non-native *Opuntia* (Simmonds & Bennett 1966). The *C. cactorum* stock introduced into Australia and subsequently into other parts of the world was collected from *Opuntia delaetiana* (Weber) (now known as *O. paraguayensis* Schumann) and from an *Opuntia* species of the "*monacantha*" group (McFayden 1985).

All introduced populations originated from this one introduction to Australia in 1925, which comprised about 3,000 eggs. Unfortunately, little thought was given to the potentially injurious environmental impacts if *C. cactorum* were to arrive in the U.S. (Stiling et al. 2004).

The first record of *C. cactorum* in the U.S. was from Bahia Honda Key, Florida, in October 1989 (Dickel 1991). The pathway by which the moth first arrived in Florida is not known. The moth either spread naturally or was unintentionally transported from the Caribbean with imported *Opuntia* nursery stock (Pemberton 1995; Stiling 2002). In Florida, there are six native *Opuntia* spp. (Benson 1982). Three species are widespread: *Opuntia stricta* (Haw.), *O. humifusa* (Raf.) and *O. pusilla* (Haw.). Three additional species are considered rare in the Florida Keys: *O. corallicola* (Small), *O. cubensis* Britton & Rose and *O. triacantha* (Willd.). In addition, four naturalized species also are common in Florida: *O. ficus-indica* (L.), *O. monacantha* (Mills.), *O. leucotricha* DC., and *O. cochenillifera* (L.) (Johnson & Stilling 1996, 1998). *Cactoblastis cactorum* spread rapidly and all Florida *Opuntia* spp. were attacked (Johnson & Stilling 1998). As many as three *Opuntia* spp. in the U.S. are used by people as food, animal fodder, and for dye production (Zimmermann et al. 2000). Concerns about environmental non-target effects were initially greatest for cacti endemic to the Florida Keys, especially *O. corallicola* (Johnson & Stilling 1996, 1998), one of the rarest plants in North America (Austin et al. 1998). At that time, the total world's population of *O. corallicola* consisted of 12 plants located on a property protected by the Nature Conservancy at Little Torch Key (Gordon & Kubisiak 1998; Stiling et al. 2004). Additional isolated populations of *O. corallicola* were subsequently found in South Florida (Hight et al. 2002).

Inundative Biological Control as a Pest Management Strategy for *Cactoblastis cactorum*

Pemberton and Cordo (2001a, b) reviewed available natural enemies and pathogens to control *C. cactorum*. Several larval and pupal parasitoids were reported from South America including species from the families Braconidae, Chalcidae, Ichneumonidae and Tachinidae. In general, these natural enemies were found to parasitize the pest with a moderate success rate (<30%). For instance, the braconid wasp, *Apanteles alexanderi* Brethes, parasitized approximately 30% of the larvae under field conditions (Parker & Pinnell 1973). Trichogrammatid egg parasitoids also were recorded attacking *C. cactorum* in Argentina (Logarzo et al. 2009). Parasitized eggsticks have also been reported from Florida (Bennett & Habeck 1995). Egg parasitoids of the genus *Trichogramma* have been used successfully as inundative BCAs against a range of agricultural pests such as corn borers, sugarcane borers and cotton bollworm [*Helicoverpa zea* (Boddie)] (Li 1994; van Lenteren 2000). *Trichogramma* spp. parasitize more than 200 insect species belonging to 70 families in 8 insect orders, especially Lepidoptera (Parra et al. 1987). For example, *Trichogramma* spp. have been successful at controlling internal feeding lepidopterans such as the European pine shoot borer , *Rhyacionia buoliana* Denis & Schiffermüller (Kogan et al. 1999), and the European corn borer, *Ostrinia nubilalis* Hübner (Dahlsten & Mills 1999). Although *Trichogramma* wasps are the most widely used entomophagous group in biological control programs (Li 1994), Bennett and Habeck (1992) were the first to suggest the possibility of their use to manage *C. cactorum*.

Assessing the Risk of Releasing a Non-Specific Egg Parasitoid to Control *Cactoblastis cactorum* in North America

Trichogramma species are facultative gregarious egg parasitoids (Rabinovich 1970), attacking eggs of a wide range of lepidopteran and other insect orders (Clausen 1940; Pinto & Stouthamer 1994; Thomson & Stinner 1989). As such, inundative releases of species of *Trichogramma* to control *C. cactorum* might result in adverse impacts on rare butterfly species such as those present in the Florida Keys (Bennett & Habeck 1992). Furthermore, *Trichogramma* wasps also may negatively impact native cactophagous moths that also produce eggsticks, such as *Melitara prodenialis* Walker (Lepidoptera: Pyralidae). Previous studies demonstrated that the efficacy of parasitoids as biological control agents was influenced by several factors including host acceptance, host age, and contact time between host and parasitoid. Therefore, evaluation of host range and other biological parameters that influences host selection constitutes the first step for understanding the potential risks associated with parasitoid introductions. Because of current stringent requirements governing the importation of non-native entomophagous BCAs into the U.S., it was worthwhile to explore the potential use of egg parasitoids already present in Florida and known to attack cactus moth in the field (Pemberton & Cordo 2001a). In Australia, *T minutum* Riley was reported to parasitize up to 32% of the cactus moth eggs in the field (Dodd 1940). Indeed, inundative releases of *T. minutum* were used against many pest insects (Li 1994), including the sugarcane borer *Diatraea saccharalis* F. (Lepidoptera: Pyralidae) in Florida (Wilson 1941). Another potential egg parasitoid species was *T. pretiosum* (Riley), a parasitoid observed to parasitize *C. cactorum* eggs in the Florida Keys (Bennett & Habeck 1995).

***Trichogramma* (Hymenoptera: Chalcidoidea: Trichogrammatidae)**

Presently, the superfamily Chalcidoidea has about 22,000 named species, belonging to 19 families (Noyes & Valentine 1989; Noyes 1998). Most species are less than 3mm in length, averaging 1.5mm, with the smallest about 0.11mm (*Dicopomorpha echmepterygis* Mockford Male, Mymaridae) (Noyes & Valentine 1989; Noyes 1998). Individuals from the family Trichogrammatidae are 1.8 mm in length including the ovipositor. The family currently includes 83 genera and 839 species. Trichogrammatids are primary solitary or gregarious endoparasitoids of Lepidoptera, Hemiptera, Coleoptera, Thysanoptera, Hymenoptera, Diptera, and Neuroptera eggs (Strand & Vinson 1984).

***Trichogramma pretiosum* (Riley)**

Trichogramma pretiosum, a North American species, is thought to be the most common *Trichogramma* species found in the Western Hemisphere (Pinto et al. 1986; Olkowski & Zhang 1990; Zucchi et al. 2010). This species has been documented parasitizing the cotton leafworm [*Alabama argillace* (Hübner), Noctuidae], the velvetbean caterpillar [*Anticarsia gemmatalis* (Hübner), Noctuidae], sugarcane borers (*Diatraea* spp., Pyralidae), *Heliothis armigera* (Hübner, Noctuidae), the cabbage looper [*Trichoplusia* ni (Hübner), Noctuidae], the tomato pinworm [*Keiferia lycopersicella* (Walshingham), Gelechiidae], the Indian mealmoth [*Plodia interpunctella* (Hübner), Pyralidae] and others (Li 1994). Hassan (1993) documented that *T. pretiosum* was released commercially in the U.S. to control pests into cotton, corn and soybean. Wührer and Hassan (1993) showed that *T. pretiosum* had a high egg-laying capacity (53.7 eggs/female). Monje et al. (1999) showed that female wasps preferred to parasitize the smaller *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) eggs over the larger *Diatraea rufescens* Box (Lepidoptera: Pyralidae), and *Diatraea saccharalis* (F.) eggs.

***Trichogramma fuentesi* Torre**

Trichogramma fuentesi has been recorded in several countries in South America (Argentina, Columbia, Mexico, Peru, and Venezuela) and seven states in the U.S. (Alabama, California, Florida, Louisiana, New Jersey, South Carolina and Texas) (Fry 1989; Pinto 1999). The primary hosts of *T. fuentesi* are species belonging to the families Noctuidae, such as *Helicoverpa zea* (Boddie) and *Heliothis virescens* (F.), and Pyralidae, such as *Diatrea saccharalis* (F.), *Ephestia kuehniella* Zeller and *Ostrinia nubilalis* (Hübner) (Fry 1989; Wilson & Durant 1991; Pintureau et al. 1999; Querino & Zucchi 2003). *Trichogramma fuentesi* is widely used for biological control of orchard pests.

Table 2-1. Selection criteria of the centrifugal phylogenetic method for choosing test plants to determine host range of weed biological control agents (after Wapshere 1974, 1989).

Plant Group	Host Range
1	Host plants with similar genetic types (ecotypes/biotypes)
2	Plant species from same genus
3	Host plants from same tribe
4	Host plants pertaining to same subfamily
5	Host plants from same family
6	Host plants belonging to same order

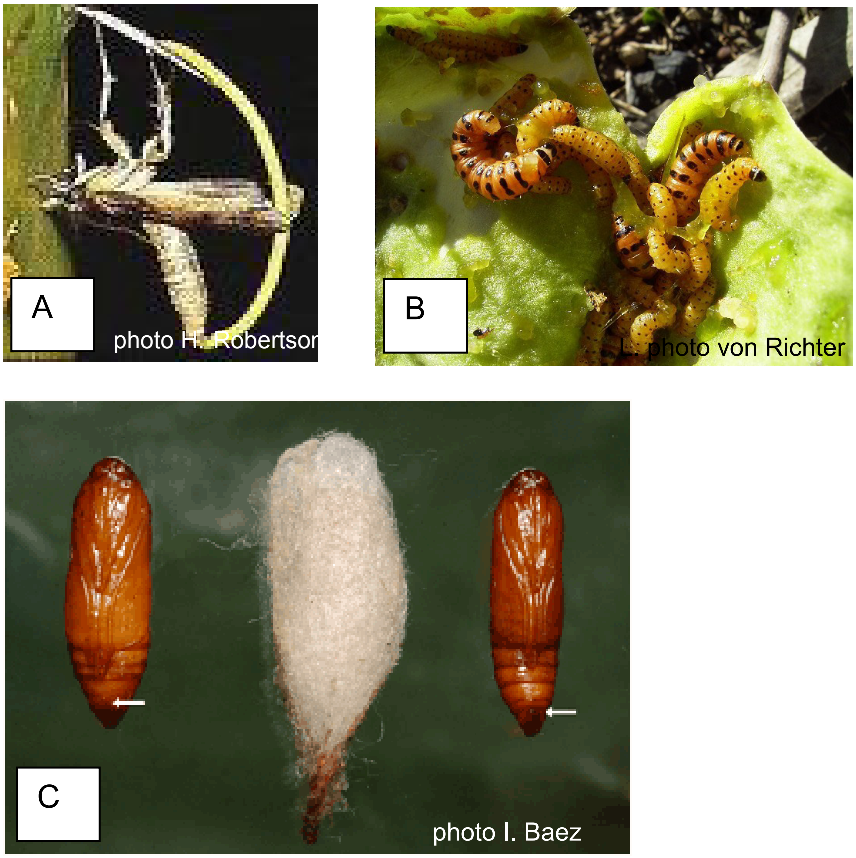


Figure 2-1. Life stages of *Cactoblastis cactorum*: **A**-Female laying eggs on a cactus spine, **B**-Larvae feeding inside *Opuntia* cactus pad, **C**-Male pupa (right) and female pupa (left), and cocoon (center).

CHAPTER 3
COMPARISON OF REGULATORY PROCEDURES FOR THE IMPORTATION AND RELEASE OF ENTOMOPHAGOUS BIOLOGICAL CONTROL AGENTS IN EIGHT COUNTRIES—HOW IS IT DONE?

Concerns in recent years about potentially undesirable environmental impacts of entomophagous biological control agents (BCAs) have generated a need for development of a harmonized risk analysis process for importation and release of these agents. Consequently, there has been interest in developing harmonized technical and regulatory frameworks for plant protection specifically in the area of biological control at the national, regional, and international levels. While a risk-based process has been an integral part of the importation process for weed BCAs in the U.S., this approach has been a relatively new consideration for entomophagous BCAs. Risk can be defined as a combination of the probability of an adverse event and the magnitude of the consequences (Delfosse 2005). In 2007, the U.S. had no standardized regulatory framework for the importation and release of non-native entomophagous BCAs (Messing & Wright 2006; Hunt et al. 2008).

The level of risk associated with the unintentional introduction of invasive species has been elevated onto the international trade and environmental policy agendas (Andersen et al. 2004). In 1995, the World Trade Organization (WTO) Agreement on the application of Sanitary and Phytosanitary Measures (SPS Agreement) was ratified by most of the WTO country members (Ebbels 2003). Under the SPS Agreement, the member countries agree to base their phytosanitary decisions on the results of a science-based assessment of potential environmental and economic risks (IPPC 2005). The International Plant Protection Convention (IPPC) is recognized as the standard setting body for the application of SPS principles to plant health matters. In 1995, as a result of the growing concerns from the scientific community and the general public about the potential negative impacts of BCAs, the “Code of Conduct for the Import and Release of Exotic Biological Control Agents” (International Standard for Phytosanitary Measures/ISPM # 3) was endorsed by the IPPC. In 2005, the document was revised to include other beneficial organisms (IPPC 2005). Other relevant pest risk analysis (PRA) standards include International Standard for Phytosanitary Measures (ISPM) # 2 “Framework for Pest Risk Analysis” (IPPC 2007) and ISPM # 11 “Pest Risk Analysis for Quarantine Pests including Analysis of Environmental Risks and Living Modified Organisms” (IPPC 2004). In addition, at the regional level, the North American Plant Protection Organization (NAPPO) developed the “Guidelines for Petition for First Release of Exotic Entomophagous Biological Control Agents” (Regional Standard for Phytosanitary Measures/RSPM # 12) (NAPPO 2008).

Several regional blocks and/or countries have developed new legislation or have revised existing regulations to facilitate the introduction of new biological control organisms while minimizing their potential environmental risks (COSAVE 1996; AQIS 1997; ERMA 1997a, b). However, there have been few comparative studies of regulatory systems in different countries and much of the information is scattered in less accessible conference proceedings or National Plant Protection Organization (NPPO) websites. A comprehensive and timely comparative analysis will provide critical information that will guide further development of appropriate policies and legislation and development of practical and effective regulatory processes for entomophagous BCAs. This study used ISPM # 3 to identify key criteria that should be used during the decision-making process for entomophagous BCAs. The objective was to develop a summary of regulatory approaches used by different countries as these frameworks relate to the criteria outlined in ISPM # 3. It is anticipated that the findings of this study might allow countries to compare different approaches to risk analysis and facilitate development of national processes that support informed decisions regarding the screening and approval of entomophagous BCAs.

Methodology

Selection of Countries

An initial list of 15 countries was selected for the comparative analysis based on their implementation of an operational regulatory system for the importation and release of entomophagous BCAs. However, because of limited available information and difficulty in language translation, several countries (China, France, Indonesia, Pakistan, Philippines, Russia, and South Africa) were eliminated from consideration. A subset of eight countries (U.S., Australia, Canada, United Kingdom, India, New Zealand, Mexico, and Switzerland) was selected for detailed analysis based on 4 criteria: the ample amount of available information, widespread geographic representation, level of gross domestic product (GDP), and implementation of ISPM # 3 “Guidelines for the Export, Shipment, Import and Release of Biological Control Agents and Other Beneficial Organisms” (IPPC 2005) (Table 3-1). The GDP-Agricultural Sector gave the contribution to agriculture relative to the total GDP. The GDP and GDP-Agricultural Sector were used as an index to select developed countries. Ratification of the SPS Agreement was not a selection criterion, although most countries in the final list have ratified the protocol.

Collation of Information

Internet search engines (e.g. Google®, Google Scholar® or Google Books®) as well as bibliographical databases (e.g. Agricola, CAB abstracts) were used to gather relevant information on the NPPO structure, legislation/regulations and current risk analysis process implemented by the eight countries during their permitting process of entomophagous BCAs. The key words “risk”, “risk assessment”, “risk analysis”, “plant protection organization”, “biological control”, “importation of biological control agents”, and “release of biological control agents” were used to identify relevant information. Selection of the key words was subjective. Reviews of NPPO configuration, PRA process, and permitting system for importation and release for entomophagous BCAs in the Asian-Pacific, North American, and European Union countries were also used as sources of data (Fasham & Trumper 2001; Mason et al. 2005; REBECA 2006; FAO 2007; Loomans 2007; Hunt et al. 2008).

Comparative Process

The status of regulatory biological control program frameworks, as implemented in 2007, were evaluated for each of the eight countries. Comparisons were made between the NPPOs structures, PRA framework, permitting process for entomophagous BCAs, and information required from the petitioner during the importation and release of BCAs. Selected decision-making criteria used by each NPPO during the regulatory process for entomophagous BCA introduction were summarized. The ISPM # 3 was used to identify crucial themes that are of importance to the decision-making process and these were: designation of responsible authority, laws and regulations, general principles (general acceptance of precaution), communication and reporting, documentary responsibilities, reviewing process and consultation, and responsibilities of NPPO before, during and after release.

Results

Australia (AU)

Designation of authority, laws and regulatory requirements

In Australia, the Department of Agriculture, Fisheries and Forestry (DAFF) is responsible for the Australian Government's animal and plant biosecurity policy and the establishment of risk management measures (Figure 3-1). The Secretary of the Department of Agriculture is also the Director of Animal and Plant Quarantine under the Quarantine Act of 1908. The Australian NPPO consists of AQIS (Australian Quarantine and Inspection Service), Biosecurity Australia (BA) and the Office of the Chief Plant Protection Officer which are all within DAFF (FAO 2007). The Biosecurity group within the Department takes the lead in biosecurity and quarantine policy development and the establishment and implementation of risk management measures across the biosecurity continuum (DAFF 2007; Hunt et al. 2008). The importation and release of entomophagous BCAs is also regulated by the Department of the Environment and Heritage (DEH) under the Environment Protection and Biodiversity Conservation Act of 1999 (DEH 2007; Hunt et al. 2008).

General acceptance of precaution

Australia expresses its Appropriate Level of Protection (ALOP) in qualitative terms. The objective of the ALOP is to provide a high level of phytosanitary protection while reducing risk to a low level (FAO 2007). This approach conforms to the WTO's Agreement on the application of SPS Agreement (FAO 2007).

Documentary responsibility of importer

An initial application to import entomophagous BCAs into quarantine includes information on biology, ecology, and potential economic and environmental impacts of the agent and the target pest. In addition, a risk-benefit analysis is included. A host species specificity test list and the methodology used for testing also need to be provided to DAFF for approval (DAFF 2007; Hunt et al. 2008). After completing the host specificity tests and before releasing an entomophagous BCA, a “release package” is submitted by the permit petitioner to AQIS (DAFF 2007) and separately to DEH (DEH 2007) (Figure 3-1). The dossier provides information on the origin, biology, and native range of the agent as well as related species to the agent. Methods used and results from laboratory evaluation of the agent are discussed by the petitioner, including a list of potential non-target organisms, results from host specificity testing, an evaluation of risk of non-target impacts, as well as the appropriate testing methods and statistical analysis (IRA 2007; Hunt et al. 2008). The current economic and environmental status of the target species is also assessed by the petitioner (IRA 2007; Hunt et al. 2008). The document indicates whether and when the agent was approved for biological control in other locations, host records from foreign countries, and results from previous risk assessments or environmental assessments of the BCA in other countries. The permit petitioner needs to indicate any possible conflicts of interest with existing biological control programs (IRA 2007; Hunt et al. 2008).

Australian regulatory process for importation and release of entomophagous BCAs (Figure 3-1)

The importation and release of an entomophagous BCA must be approved by DAFF and DEH. In addition, the host specificity test list must be approved by the co-operators which include the AQUIS within DAFF (DAFF-AQIS), the DEH, the Commonwealth Scientific and Industrial Research Organization (CSIRO), and relevant state/territory government departments or research organizations. Co-operators have the power to veto any decision if their recommendations are not accepted by the petitioner.

Reviewing process/consultation

Public participation is sought at different stages of the approval process; for example, stakeholders are consulted prior to the importation and the release of entomophagous BCAs (Figure 3-1).

Responsibility of NPPO during and following release of BCA

Release of entomophagous BCA is supervised by a federal quarantine entomologist working for DAFF. Post-release evaluations are recommended but not enforced by either DAFF or DEH (Hunt et al. 2008).

Canada (CAN)

Designation of authority, laws and regulatory requirements

The Canadian Food Inspection Agency's (CFIA's) Plant Health Division (PHD) fulfills the obligations as Canada's NPPO. CFIA-PHD is responsible for pest risk assessment and management in Canada. Regulation of entomophagous BCAs was initially implemented in the late 1990's by the CFIA through the authority of the Canadian Plant Protection Act (PPA) of 1990 (DJC 2005). Pest risk assessments are developed and evaluated by the CFIA's Plant Health Risk Assessment Unit, within the Science Division.

General acceptance of precaution

Pest risk assessment is a science based procedure based on information provided and results from host specificity testing. An ALOP approach is used during risk assessment (Mason et al. 2005).

Documentary responsibility of importer

Petitions for import for research purposes include information on the BCA (scientific name, common name, origin, etc…), the proposed action, and a management plan to prevent the spread of target pest. To obtain release approval, the permit petitioner needs to submit additional information on both the BCA and target pest biology, and results from evaluations of host specificity testing. In addition, the possible environmental and economic impacts of the proposed action should be covered in the petition. The information should conform to the NAPPO standard requirements for entomophagous BCAs as described in RSPM # 12 (NAPPO 2008). In order to obtain a permit for release, the permit petitioner needs to provide a detailed description of the agent, information on the release locations, means of transportation used, and reason for release.

Canadian regulatory process for importation and release of entomophagous BCAs (Figure 3-2)

Information submitted by permit petitioner is reviewed by CFIA-PHD for approval for importation and movement of the BCA to a federal quarantine facility for research purposes (CFIA 2006; Hunt et al. 2008). For release approval, the permit petitioner must take into account recommendations from the Ontario Plant Laboratory-Quarantine Entomology Laboratory (OPL-QEL). Once the requirements from the OPL-QEL are met, the petition is sent to the Biological Control Review Committee (BCRC) for review.

Based on recommendations from the BCRC after evaluating the application, the OPL-QEL forwards their recommendation whether or not to approve the release of the proposed entomophagous BCA to the Director of the CFIA-PHD. The Director of the CFIA-PHD will either approve or deny the release of the BCA or request that more research be conducted (CFIA 2006; Hunt et al. 2008).

Reviewing process/consultation

Public opinions are not solicited in Canada during the permitting process of entomophagous BCAs (Hunt et al. 2008). Prior to issuance of a permit for release purposes, CFIA-PHD forwards petitions to the OPL-QEL. The OPL-QEL verifies that the information provided by the permit applicant follows NAPPO standards. Completed petitions are also sent to the BCRC for external review. The BCRC is composed of taxonomists, ecologists, entomologists, botanists, federal and provincial scientists, extension specialists, university researchers, and Environment Canada and Pest Management Regulatory Agency officials. Petitions are also sent to the Mexican NPPO and the U.S. NPPO for comments (Figure 3-2) (CFIA 2006; Hunt et al. 2008).

Responsibility of NPPO during and following release of BCA

Although post-release evaluations are not enforced by the Canadian NPPO; the applicant must develop a plan of action to assess economic and environmental impacts.

European and Mediterranean Region

The European and Mediterranean Plant Protection Organization (EPPO) is an intergovernmental organization responsible for European cooperation in plant protection in the European and Mediterranean region. The organization currently has 50 member countries including Switzerland and the United Kingdom (EPPO 2006). The EPPO is administered by its Executive Committee (seven Governments elected on a rotational basis, meeting twice a year), under the control of its Council (representatives of all member governments, meeting once a year) headed by a Chairman and a Vice-Chairman, which are elected individuals. The technical work for the organization is done by Panels of Experts, under the supervision of Working Parties. Experts are nominated by their respective NPPOs (EPPO 2006). Most of the European countries have ratified the Convention on Biological Diversity (CBD) which stipulates that the introduction of non-native species should be under regulatory control (REBECA 2006). In addition, applicants might need a permit for environmental release as stipulated by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). However, both of these directives are directed to preserve natural habitats and native flora and fauna (Loomans 2007). The organization has regulations and laws to protect natural habitats and indigenous flora and fauna, but the organization does not have specific laws for the regulation of entomophagous BCAs (EPPO 2006; REBECA 2006). Each European country is responsible for developing the appropriate regulations and methodologies for PRA (REBECA 2006).

In 1997, in an effort to provide guidance for safe use of BCAs between the European Union (EU) countries, EPPO and CABI Bioscience organized a workshop on safety and efficacy of BCAs (Bigler et al. 2005). As a result, guidance documents for the safe use of BCAs for research purposes were developed: “First Importation of Exotic BCAs for Research under Contained Conditions”-PM 6/1(1) (EPPO 1999) and “Import and Release of Non-Indigenous BCAs”-PM 6/2(1) (EPPO 2001a), along with a guidance document focusing mainly on the PRA process (EPPO 2001b). In parallel, the EPPO funded the “Evaluating Environmental Risks of Biological Control Introductions into

Europe" (ERBIC) project. The outcome was the development of a document with detailed criteria for risk assessment of BCAs to assist regulators in assessing environmental risks associated with their use (van Lenteren et al. 2003).

In 1999, the Organization for Economic Co-Operation and Development (OECD) was established. The OECD member countries worked to develop a harmonized approach for regulation of BCAs. The initiative resulted in the development of a guidance document stipulating the information requirements during permit submission of BCAs (OECD 2004). However, the EU felt that information requirements proposed by the OECD guidance document would hinder the existing regulatory system for BCAs. Consequently, a commission of the International Organization for Biological Control/Plants West Paleartic Regional Section (IOBC/WPRS) produced a document that provided guidance for a streamline regulatory process for BCAs (Bigler et al. 2005). Recently, an international initiative was created to harmonize the regulatory systems for BCAs between U.S. and EU countries; the REBECA (Regulations for Biological Control Agents) project. One of the objectives of REBECA was to review regulations of BCAs and provide recommendations for an implementation for a more harmonized and efficient regulatory process for BCAs (REBECA 2011). The analysis for each risk assessment criteria as outlined in ISPM # 3 will only be addressed for Switzerland and the United Kingdom.

Switzerland (SW)

Designation of authority, laws and regulatory requirements

Switzerland has developed an operational regulatory system for importation and release of BCAs (REBECA 2006). The Federal Law for the Protection of the Environment regulates the use of entomophagous BCAs under the Ordinance on Plant

Protection implemented by the Federal Office for the Environment. In addition, specific ordinances are used to assist in containment procedures of entomophagous BCAs: “Ordinance on the Contained use of Organisms” (1999), and for the environmental release “Ordinance on the Release of Organisms into the Environment” (1999) (Loomans 2007).

General acceptance of precaution

There is no standardized PRA process for the importation and/or release of entomophagous BCAs (REBECA 2006). The application process is structured according to procedures already in place for plant protection (REBECA 2006).

Documentary responsibility of importer

The applicant must provide information as recommended by the guidance document developed by OECD (OECD 2004) on information requirements for regulation of invertebrates as biological agents (Loomans 2007). The documents require information on the identity, origin, source, distribution, biology, native host range, natural enemies, and potential economic of the BCA. In addition, an evaluation of the human and animal health impacts of releasing the BCAs should be included. Specific environmental criteria, such as information on the establishment in the wild, host specificity data and information on non-target effects are requested (Loomans 2007).

Swiss regulatory process for importation and release of entomophagous BCAs

The applicant must first submit an application to register the entomophagous BCA to the Federal Office of Environment. After registration, the applicant needs to apply for a permit for importation and to submit a dossier containing information as recommended by the OECD guidance document (OECD 2004). Upon approval for importation, decisions to grant approval for environmental release will be based on the quality and quantity of information provided by the applicant (Loomans 2007).

Reviewing process/consultation

No public hearings or consultations are solicited during the decision-making process. The Federal Office of Environment reviews information provided by the permit petitioner and makes final decision.

Responsibility of NPPO during and following release of BCA

A post release evaluation plan is not required by the SW NPPO.

United Kingdom (UK)

Designation of authority, laws and regulatory requirements

To some extent, the UK has an operational regulatory process for BCAs (REBECA 2006). The Ministry of Agriculture Plant Health Division under the Great Britain Plant Health Act (1967) is responsible for all plant health matters. There is no specific legislation regarding the import and release of non-native invertebrate BCAs for the purpose of biological control (Loomans 2007).

General acceptance of precaution

In 1998 with the assistance of the Cabinet Office, the Prime Minister introduced a Regulatory Impact Assessment (RIA) procedure. All regulatory proposals must contain a RIA which is an organized document that offers a cost-benefit analysis, risk assessment, and compilation of potential stakeholders that might be impacted by the proposed action. In addition, a review of alternative non-regulatory options needs to be included in the RIA. Risk communication is an important component of the UK RIA.

Documentary responsibility of importer

The application process is structured according to procedures already in place for nature conservation. The UK does not have a specific form for the approval of importation or release of entomophagous BCAs. However, it is recommended that applications follow the format as recommended in the Department for Environment, Food and Rural Affairs (DEFRA) website (DEFRA 2000). An extensive amount of information is given on the DEFRA website on data requirements to support applications for permits to release entomophagous BCAs. Information on the origin, biology, source, and native host range of the BCA is required. The key requirement is information about the establishment potential of the BCA in the UK. Environmental assessments including evaluation of previous use and host specificity testing should be discussed (DEFRA 2000). For a non-native species, DEFRA requires data to be generated, when not already available, in order to properly assess the survival in the environment (Loomans 2007).

United Kingdom regulatory process for importation and release of entomophagous BCAs

A permit must be obtained before importation or release of entomophagous BCAs. The application is reviewed by the competent authority and decisions are made based on the information provided by the applicant and the recommendations developed by the National Advisory Committee.

Reviewing process/consultation

The National Advisory Committee is comprised of experts from various disciplines. The Committee is consulted during the decision-making process (Loomans 2007). No public hearings or consultations are solicited during the decision-making process.

Responsibility of NPPO during and following release of BCA

A post release evaluation plan is not required by the UK NPPO (DEFRA 2000).

India (IN)

Designation of authority, laws and regulatory requirements

The Directorates of Plant Protection Quarantine & Storage (PPQS) established under the Indian Department of Agriculture & Cooperation of Ministry of Agriculture have been given the responsibility of implementing the regulations relating to the importation of BCAs in India. Their importation is regulated by the Plant Quarantine Order (2003) issued under the Destructive Insects and Pests Act (1914) and amendments issued there under (PPQS 2006). Importation of entomophagous BCAs requires a permit issued by the Plant Protection Adviser.

General acceptance of precaution

The PPQS follows pest risk assessment approach as stated in ISPM # 11 (IPPC 2007) in relation to environmental risks as covering environmental aspects related to the use of entomophagous BCAs (PPQS 2006).

Documentary responsibility of importer

Prior to containment in a federal quarantine facility, the absence of contamination of the entomophagous BCA needs to be confirmed by a certificate issued by the NPPO of the country of origin (PPQS 2006). Prior to the first importation into India, information on the origin, distribution, biology, host specificity, and potential non-target impacts of the entomophagous BCA should be submitted (PPQS 2006). In addition, the following information on the target pests should be provided to the Plant Protection Adviser: origin, biology, ecology, economic and environmental impacts, possible benefits and conflicting interests surrounding its use, and known natural enemies, antagonists and competitors already present or used (PPQS 2006).

Indian regulatory process for importation and release of entomophagous BCAs

For importation approval, the permit petitioner must submit PQ 12 form to the Plant Protection Adviser at least two months prior to proposed action. The application is reviewed by a Technical Committee established under the chairmanship of the Department of Agriculture and Cooperation (PPQS 2006). Upon approval for importation, the PPQS might grant permission for carrying out experimental studies under confinement in isolated fields. After receiving results from field evaluation and after consultation with Technical Committee, PPQS will approve or deny environmental release of the BCA (PPQS 2006).

Reviewing process/consultation

A Technical Committee composed of representatives from the Directorates of Plant Protection Quarantine & Storage, Indian Council of Agriculture Research, Project Directorate of Biological Control, National Centre of Integrated Pest Management, Forest Research Institute, National Bureau of Plant Genetic Resources, Division of Entomology, Nematology, and Plant Pathology from the Indian Agriculture Research Institute review applications and develop recommendations prior to approval for importation (PPQS 2006). There is no public notification or solicitation during the decision-making process.

Responsibility of NPPO during and following release of BCA

The PPQS ensures that monitoring of release of entomophagous BCAs takes place (PPQS 2006).

Mexico (MX)

Designation of authority, laws and regulatory requirements

Under the Secretaria de Agricultura, Ganadelia, Desarollo Rural, Pesca y Alimentacion (SAGARPA) (Ministry of Agriculture, Livestock, Rural Development, Fisheries and Food), the Direccion General de “Sanidad Vegetal”, is responsible for permitting the importation and release of non-native entomophagous BCAs in Mexico. The importation and/or release of entomophagous agents is regulated through the Plant Health Act, articles 101 and 102 (1980) (Mason et al. 2005).

General acceptance of precaution

Risk assessment is based on concepts described in ISPM # 2 and ISPM # 11 (IPPC 2004, 2007).

Documentary responsibility of importer

The permit petitioner must provide information according to RSPM # 12 on the origin, distribution, biology and ecology, host specificity, natural enemies of the BCA, and results from previous use of the agent (Mason et al. 2005). In addition, evaluation of the economic and environmental impacts and general information (origin, distribution, biology, and ecology) of the target pests should be provided. Potential benefits and any conflict of interest associated with its use should be included in the document (NAPPO 2008).

Mexican regulatory process for importation and release for entomophagous BCAs (Figure 3-3)

The Direccion General de “Sanidad Vegetal” in collaboration with the “Centro National de Referencia de Control Biologico” (National Center of Biological Control

Reference) reviews import applications and recommendations are given to the Plant Health Director of the Department of Agriculture.

Reviewing process/consultation

If needed, additional consultations with the “Consejo Nacional Consultivo Fitosanitario” (National Consultative Phytosanitary Advisory Group) are made prior to environmental release. The National Consultative Advisory Group is composed of professionals from academics, research, and government. There is no public notification or solicitation during the decision-making process.

Responsibility of NPPO during and following release of BCA

Conforming to RSPM # 12, a pre-release plan must be developed for monitoring of non-target impacts (NAPPO 2008). However, the plan is not enforced by the MX NPPO.

New Zealand (NZ)

Designation of authority, laws and regulatory requirements

In New Zealand, the Environmental Risk Management Authority (ERMA) is responsible for reaching decisions concerning necessary phytosanitary issues. The introduction of entomophagous BCAs falls under the Hazardous Substances and New Organism (HSNO) Act of 1998 (ERMA 2006). The ERMA is comprised of three entities: the decision-making “Authority”, the “Agency”, and Ngā Kaihautū tikanga taiao (Ngā Kaihautū)—a Māori advisory committee. The Authority is composed of experts appointed by the Minister of Environment. The Authority functions like a quasi-judicial entity with court-like procedures. The Agency provides guidance to the applicant and provides recommendations to the Authority after evaluation of petitions. Ngā Kaihautū advices the ERMA on matters relevant to native people (Māori) (Hunt et al. 2008).

Biological control agents are also regulated under the Biosecurity Act of 1993 by the Ministry of Agriculture and Forestry (MAF) which is in charge of New Zealand Import Health Standards (IHS) that prevent unwanted introduction of organisms (Hunt et al. 2008).

General acceptance of precaution

The New Zealand approach to assessing the risks of introduction of a BCA is based on a full ecological risk, cost and benefit analysis (ERMA 2006; Hunt et al. 2008). The HSNO Act requires the need for caution where there is scientific and technical uncertainty (Sheppard et al. 2003).

Documentary responsibility of importer

For importation approval, the applicant must provide information on the proposed action, identity, biology, and ecology of the BCA. Containment specifications (physical and operational) must also be included. An assessment of the risks, costs and benefits of importing the BCA must be developed (Hunt et al. 2008). For approval of full or conditional release of the BCA, comprehensive environmental risk, cost, and benefit assessments must be developed. Guidelines on processes to conduct analysis are based on the Information factsheet "Estimating the Beneficial Effects of Biocontrol Agents" and "A Technical Guide to Identifying Assessing and Evaluating Risks, Costs and Benefits" available on the ERMA website (ERMA 2006). One important element of the risk evaluation is the assessment of host range (Hunt et al. 2008).

New Zealand regulatory process for importation and release of entomophagous BCAs (Figure 3-4)

For importation approval of an entomophagous BCA, the applicant must submit a permit application to ERMA for evaluation. At this stage, the Authority can decide whether notification of the public of the proposed action is necessary. The application must also satisfy requirements from the MAF's Import Health Standards. For release of the BCA, the applicant must first get in contact with the ERMA Agency staff for proper guidance during the process. The ERMA Agency staff evaluates the application which is then accessible for comments by the Minister, and other interested parties. Based on the issues raised during the consultation period including those from Ngā Kaihautū, the ERMA Agency staff develops a report for the decision-making Authority. The Authority makes the decision to deny the BCA for release or approve full or conditional release. Under conditional release the BCA can only be released under specific conditions.

Reviewing process/consultation

An important aspect of HSNO Act is the "Public's Right to Know" section which provides the provision for public notifications and hearings of the proposed action. Public notifications include summary statements in daily newspapers, in government newsletters, or on the ERMA website. The public has an opportunity to comment on proposed action prior to importation and release of BCAs. In addition, the petitioner has an opportunity to set up a public hearing to provide any additional relevant information before a final decision is made (Hunt et al. 2008).

Responsibility of NPPO during and following release of BCA

Although post-release evaluations are not enforced; the applicant must develop a plan of action to assess economic and environmental impacts.

United States (U.S.)

Designation of authority and law/regulatory requirements

In the U.S., there is no well defined regulatory process for importing and releasing entomophagous BCAs (Messing 2005). The Plant Protection Act (PPA 2000) gives broad jurisdiction to the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA-APHIS-PPQ) to regulate the importation, interstate movement and release of entomophagous BCAs. Permit conditions are based on regulations as stated in 7 CFR section 330 Parts 200-212 (CFR 2001). Many of the regulations used today are the ones developed under the previous Federal Plant Pest Act of 1957 (Hunt et al. 2008).

General acceptance of precaution

Permits are issued only when sufficient safeguards are in place and BCAs do not represent or have an unacceptable level of risk (PPA 2000). Regulations for entomophagous BCAs became more stringent since the terrorist attacks of 2001. A "Guilty until proven innocent" approach is thought to be used during the decision-making process for importation and release of entomophagous BCAs (Ruesink et al. 1995; Simberloff 1996, 2005).

Documentary responsibility of importer

For importation approval, along with the permit form (PPQ 526), general information (scientific name, host range, and geographic origin) on the entomophagous BCA must be provided. Containment specifications of an imported BCA into an USDA-APHIS-PPQ quarantine facility are also included. For field release approval, the applicant must submit a dossier with information that follows the format provided by RSPM # 12 (NAPPO 2008) (Figure 3-5).

U.S. regulatory process for importation and release of entomophagous BCAs (Figure 3-5)

For the importation and release of entomophagous BCAs, a permit (PPQ 526), administered by USDA-APHIS-PPQ, must be completed and submitted seeking approval to bring the BCA into a federal quarantine facility. If the applicant wanted to move the BCA to another quarantine facility, a separate PPQ 526 form must be submitted and approved by USDA-APHIS-PPQ. An additional PPQ 526 application for field release approval is required from the applicant. At this stage, a dossier conforming to RSPM # 12 “Guidelines for Petition for First Release of Exotic Entomophagous Biological Control Agents” (NAPPO 2008) must accompany the PPQ 526 application. Federal employees who have received any federal funds for the project must submit an Environmental Assessment under the National Environmental Policy Act of 1972. If USDA-APHIS-PPQ considers that the risk poses by the proposed action is high, an Environmental Impact Statement (EIS) is required. After consultation with Canadian and Mexican counterparts from NAPPO, USDA-APHIS-PPQ decides whether the proposed action has possible non-target impacts on endangered and threatened species. If potential for negative impacts is verified, USDA-APHIS-PPQ must consult with the U.S. Fish and Wildlife Service (FWS). Under the Endangered Species Act (ESA) of 1973, submission of a BA will be required from the applicant. If the USDA-APHIS-PPQ considers that the release of the entomophagous BCA will cause no deleterious impact, a determination of no jurisdiction is made and the BCA can be field released according to the state laws.

Reviewing process/consultation

During the decision-making process, representatives from FWS and NAPPO are consulted. In 2007, there was no public notification or solicitation of stakeholder input during the decision-making process.

Responsibility of NPPO during and following release of BCA

As indicated in RSPM # 12, a pre-release plan must be developed for monitoring of non-target impacts (NAPPO 2008).

Discussion

All eight countries outlined above were found to have regulations for the importation and release of entomophagous BCAs implemented to a certain degree. However, the analysis showed that regulatory procedures were not harmonized across countries. Two countries (SW, UK) did not have a specific risk assessment approach or regulations for the approval of importation and release of entomophagous BCAs (Table 3-2). During the decision-making process, for all eight countries, the applicants were required to submit a dossier with information on the identity, biology, ecology, native host range, and distribution of the proposed entomophagous BCAs. Different guidance documents and formats were used by countries during the process. However, all the guidance documents required an evaluation of potential environmental impacts (Table 3-3). For all the countries, evaluation of possible negative environmental impacts required an analysis of the host range and results from host specificity tests (Table 3-3). Comparative analysis of regulatory procedures of the eight countries demonstrated that six of them (AU, CAN, IN, MX, NZ, UK) used a form of participatory/collaborative-based risk analysis process during the decision-making process (Table 3-4). Within the PRA process, six countries (AU, CAN, IN, MX, NZ, UK) had integrated subject matter expert consultations (Table 3-5). These experts have different backgrounds and include government employees to environmental groups. In addition, two countries (AU, NZ) solicited public comments and one country (NZ) considered public hearings necessary prior to the importation and/or release of entomophagous BCAs (Table 3-5). Most of the NPPOs (AU, CAN, IN, MX, NZ, US) required a post-release monitoring plan but only IN enforced the completion of the plan (Table 3-6). This comparative analysis provided information on how to improve and implement a workable participatory/collaborative–based procedure within the existing permitting process for entomophagous BCAs in the U.S. Specific recommendations to improve the U.S. process include the use of a group of experts to provide recommendations to USDA-APHIS-PPQ during the decision-making process. In addition, the public should have the opportunity to comment on the proposed action prior to environmental release of the entomophagous BCA.

Nevertheless, difficulties in implementing these suggestions in the U.S. include the "Guilty until proven innocent" approach used during risk assessment and the consequent stringent regulations applied to the importation and release of entomophagous BCAs. In addition, the fear of possible litigations might prevent the implementation of a more transparent regulatory process. Chapters # 4 and # 5 in this dissertation describe how the regulatory system for permitting entomophagous BCAs, as used in 2007, could be modified to improve the decision-making process.

Table 3-1. Criteria used in countries examined in the pest risk analysis comparative study (CIA 2011).

Countries	IPPC Membership	WTO-SPS Agreement Implementation	Conformed to ISPM # 3	PRA	GDP (million $US)	GDP Agricultural Sector (%)
World					61,963,429	6.0
Australia	X	1995	X	X	1,219,722	4.1
Canada	X	1995	X	X	1,563,664	2.3
European Union	X	1995			16,106,896	1.9
India	X	1995	X	X	1,430,020	17.0
Mexico	X	1995	X	X	1,004,042	4.3
New Zealand	X	1995	X	X	138,003	4.6
Switzerland	X	1995			522,435	8.4
UK	X	1995			2,258,565	1.2
US	X	1995	X	X	14,624,184	1.2

Table 3-2. Comparison of general acceptance of precaution during pest risk analysis and decision-making during permitting for entomophagous biological control agents in 8 countries (Fasham & Trumper 2001; Mason et al. 2005; REBECA 2006; FAO 2007; Loomans 2007; Hunt et al. 2008).

General Principles	AU	CAN	SW	UK	IN	MX	NZ	US
General Acceptance of Precaution								
“Appropriate Level of Protection” approach reduces risk to a very low but not to zero	X	X				X		
Full ecological risk, cost and benefit analysis					X		X	
“Guilty until Proven Innocent” approach			X	X				X
Specific Time Frame	X	X	X			X	X	
Process in Place for Decision Reversal	X	X					X	X

Table 3-3. Comparison of documentary responsibilities of importer, prior to import, for pest risk analysis and decision-making during permitting for entomophagous biological control agents in 8 countries (Fasham & Trumper 2001; Mason et al. 2005; REBECA 2006; FAO 2007; Loomans 2007; Hunt et al. 2008).

Documentation Requirements	AU	CAN	SW	UK	IN	MX	NZ	US
Documentary Requirements Related to the Target Organism								
Accurate identification of the target organism(s) generally at the species level	X	X	X	X	X	X	X	X
Its known biology and ecology	X	X	X	X	X	X	X	X
Its economic importance and environmental impact	X	X	X	X	X	X	X	X
Possible benefits and any conflicting interests surrounding its use		X			X	X		X
Known natural enemies, antagonists and other BCAs or competitors of the target pest		X	X		X	X		X
Documentary Requirements Related to the BCA								
Sufficient characterization of the BCA for accurate identification to the species level at minimum	X	X	X	X	X	X	X	X
Voucher specimens deposited in recognized specimens	X	X						X
Key published		X						
Summary of all available information on its origin, world, distribution, biology, natural enemies, hyperparasites and impact on its area of distribution	X	X	X		X	X	X	X
Information on host specificity and any potential hazards posed to non-target	X	X	X	X	X	X	X	X
Approval of host specificity list required by Federal Agency	X							
Approval of host specificity not required but recommended		X	X					

Table 3-4. Comparison of communication and reporting processes during pest risk analysis and decision-making during importation and release of entomophagous biological control agents in 8 countries (Fasham & Trumper 2001; Mason et al. 2005; REBECA 2006; FAO 2007; Loomans 2007; Hunt et al. 2008).

Communication and Reporting	AU	CAN	SW	UK	IN	MX	NZ	US
Clear Risk Assessment Guidelines and Policies								
Required information for applications is available on website	X	X				X	X	X
Risk assessment criteria publicly available online		X					X	
Public Notifications								
Notification of proposed release	X						X	
Applications available online before evaluation								
Risk assessment posted on public website								
Risk assessment summary posted on government website	X						X	
Risk assessment summary published in daily newspaper							X	
Risk assessment summary published in government newsletter	X						X	
Release of risk assessment published in Federal Register								X
Public notifications of approved release								
Community informed about issues relating to safety	X				X			

Table 3-5. Comparison of reviewing and consultation processes for pest risk analysis and decision-making during permitting process for entomophagous biological control agents in 8 countries (Fasham & Trumper 2001; Mason et al. 2005; REBECA 2006; FAO 2007; Loomans 2007; Hunt et al. 2008).

Reviews and Consultation	AU	CAN	SW	UK	IN	MX	NZ	US
Public Participation								
Solicit public comments in risk decision process prior to importation	X							
Solicit public comments in risk decision process prior to release	X						X	
Formal procedures in place for hearings during decision process							X	
Approval process includes public comment periods	X						X	
Use of Secondary Sources								
Use of risk assessments from foreign countries	X							
Use data or results from previously submitted risk assessments	X							
Use of Experts								
Consultation with scientific experts	X	X		X	X	X	X	
Consultation with members of regulatory body	X	X			X	X	X	X

Table 3-6. Comparison of responsibilities of the National Plant Protection Organization (NPPO) before, during and following release of biological control agent in 8 countries (Fasham & Trumper 2001; Mason et al. 2005; REBECA 2006; FAO 2007; Loomans 2007; Hunt et al. 2008).

Responsibility	AU	CAN	SW	UK	IN	MX	NZ	US
Release								
Release supervised by quarantine entomologist	X							
Documentation								
Documentation on measures undertaken to ensure levels of contamination acceptable to the importing NPPO		X				X		X
Monitoring and Evaluation								
Post-release monitoring enforced					X			
Post-release monitoring required but not enforced	X	X				X	X	X

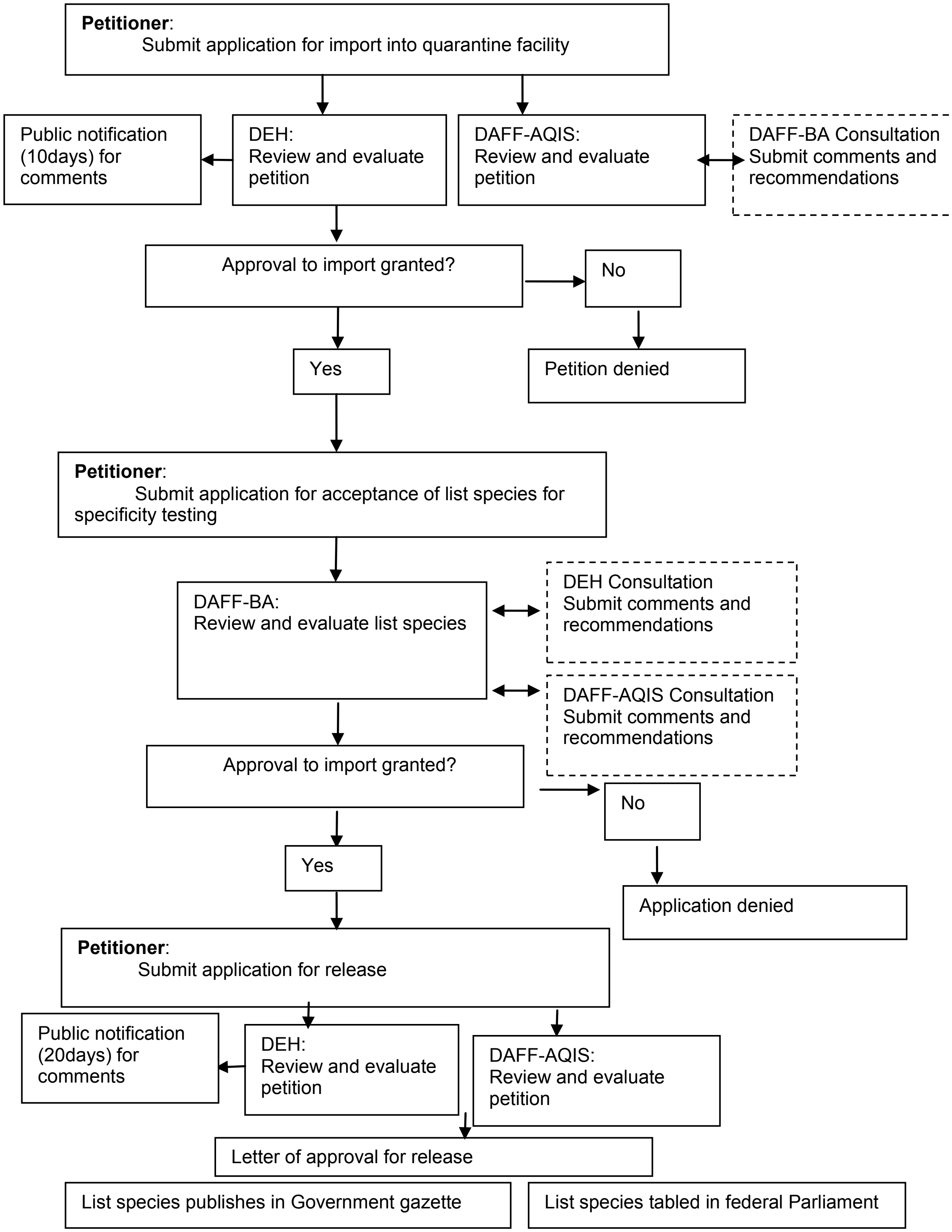


Figure 3-1. Australian permitting process for entomophagous biological control agents (Based on Hunt et al. 2008).

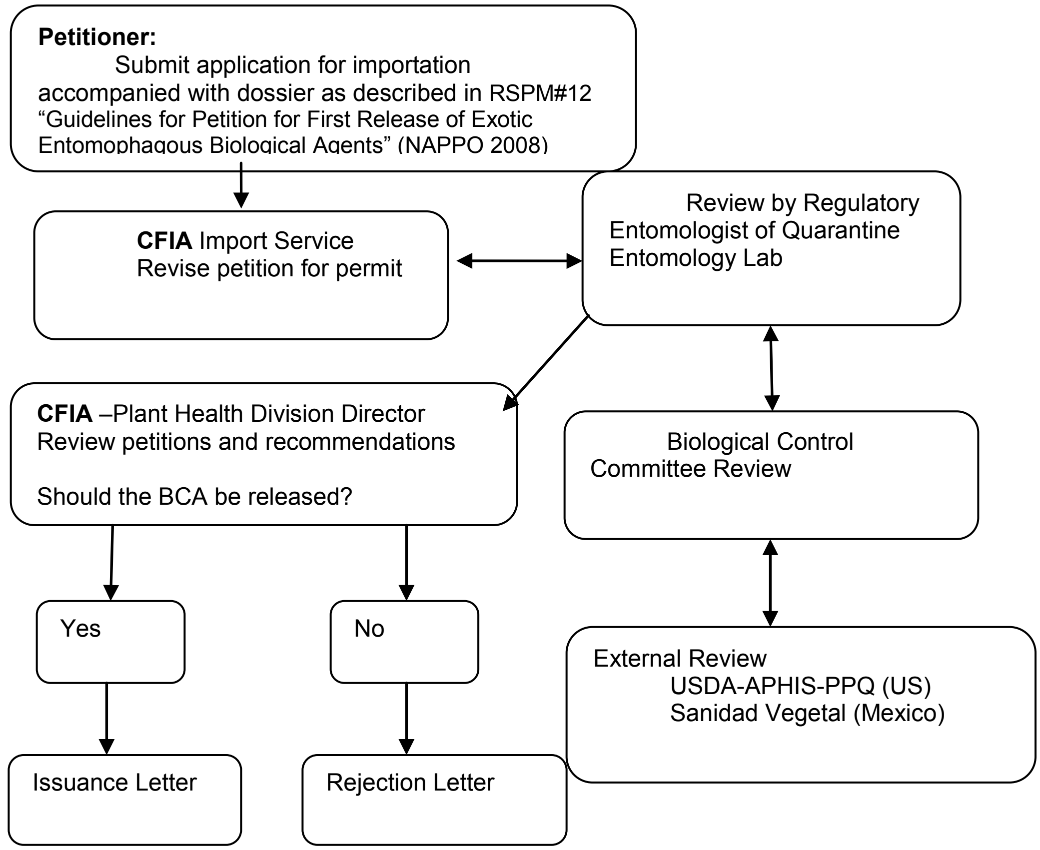


Figure 3-2. Canadian permitting process for entomophagous biological control agents (Based on Hunt et al. 2008).

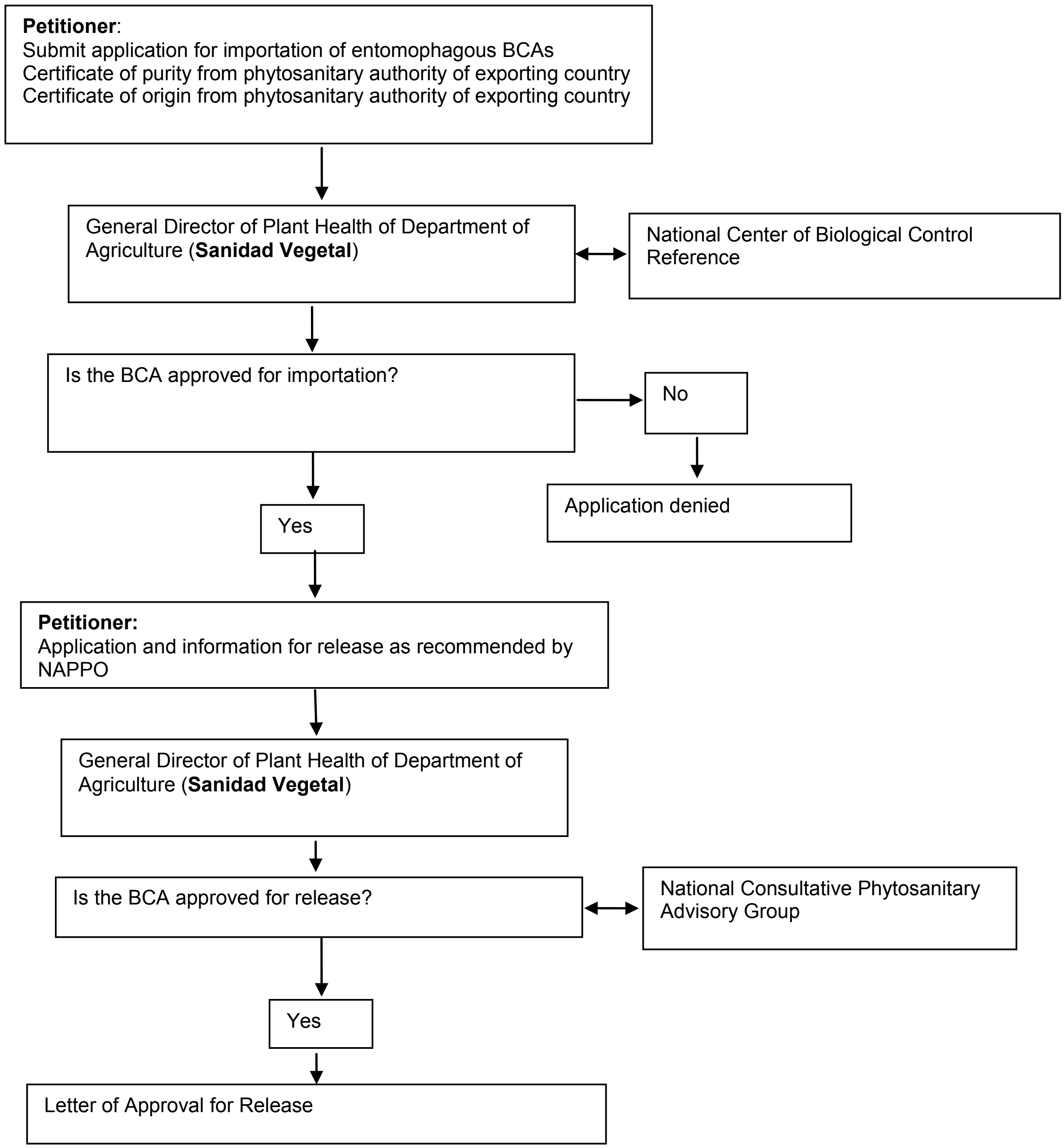


Figure 3-3. Mexican permitting process for entomophagous biological control agents (Based on Mason et al. 2005).

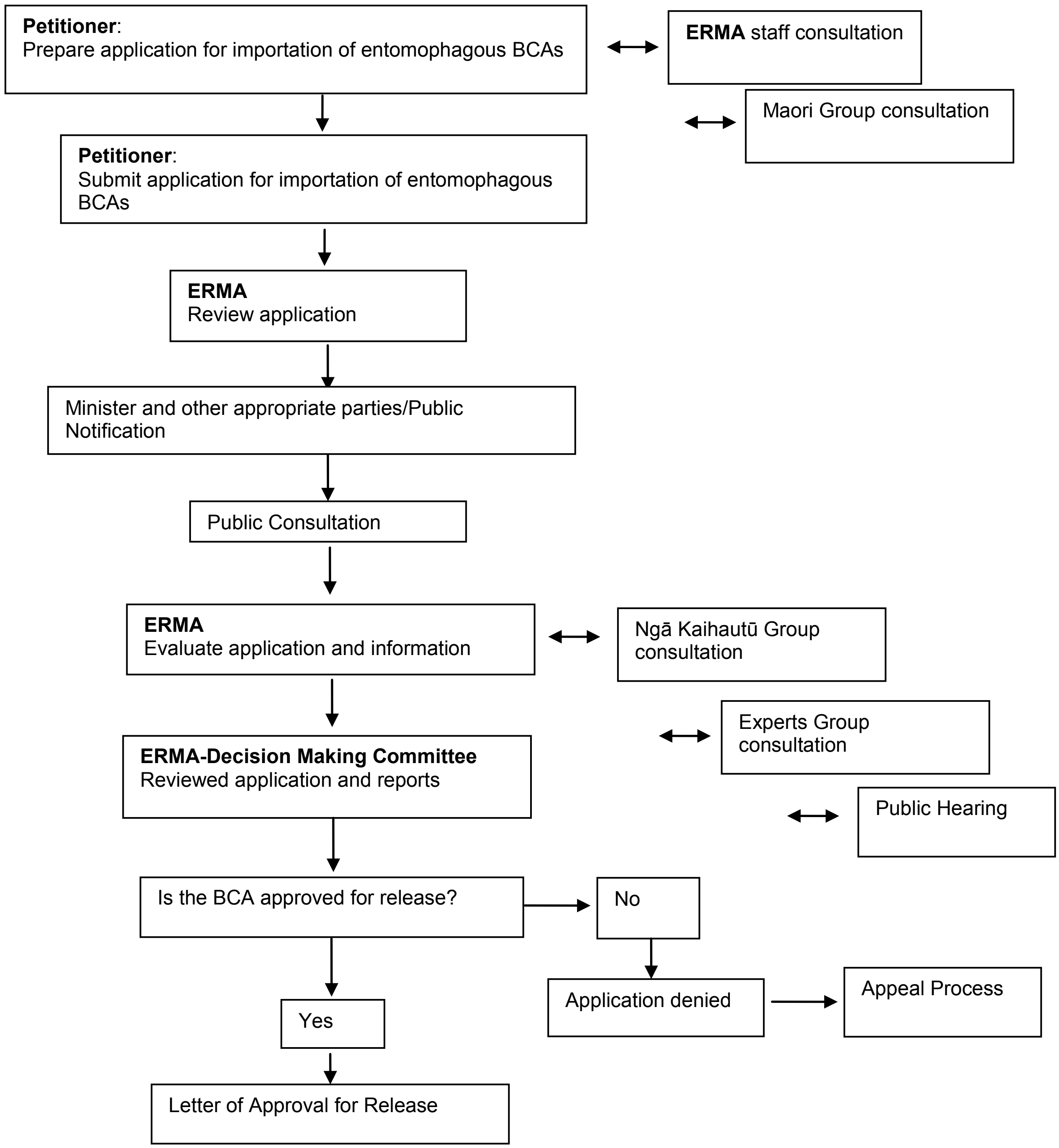


Figure 3-4. New Zealand permitting process for entomophagous biological control agents (Based on Hunt et al. 2008).

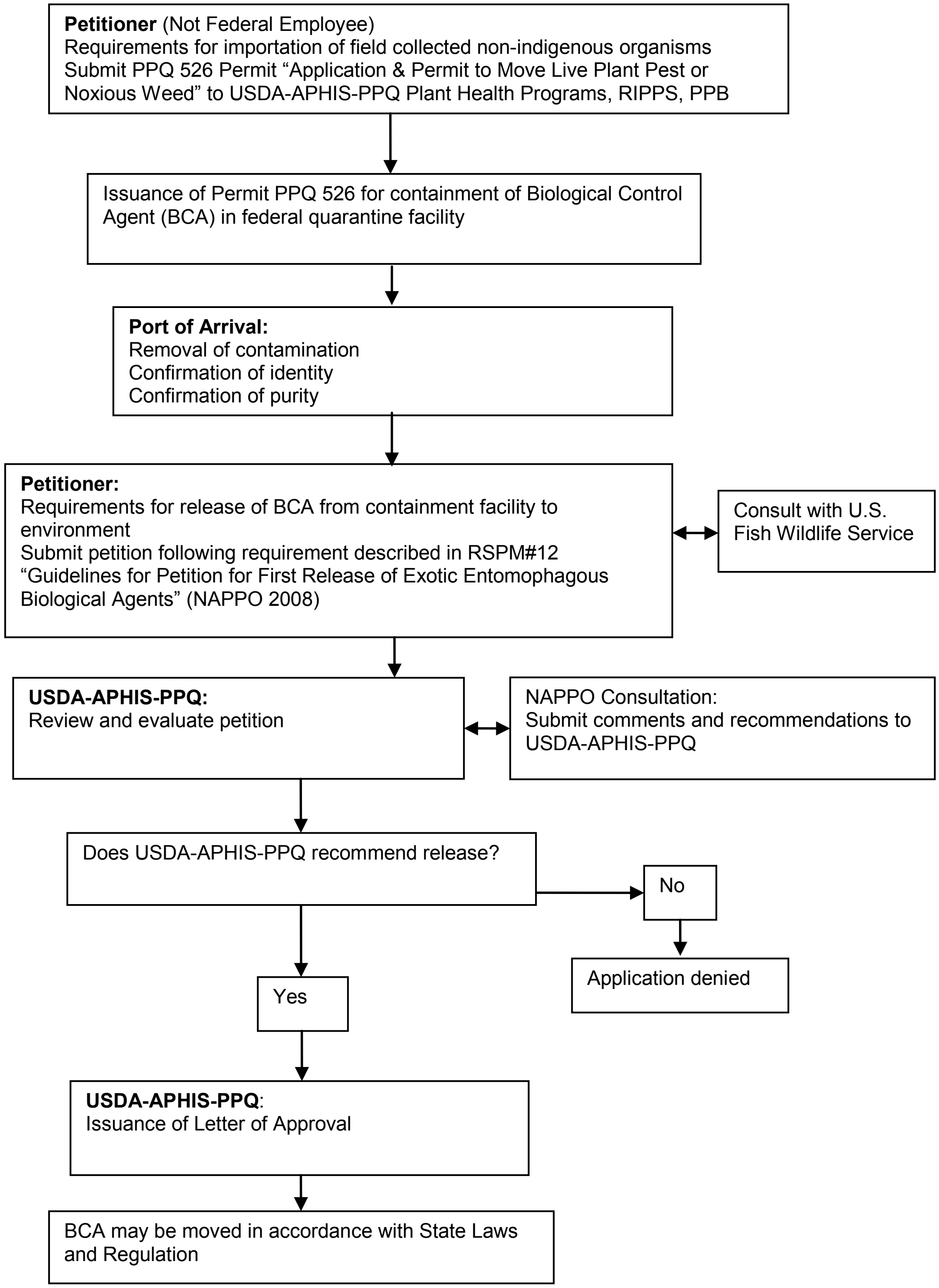


Figure 3-5. U.S. permitting process for entomophagous biological control agents as implemented in 2007 (Based on Hunt et al. 2008).

CHAPTER 4
RISK COMMUNICATION DURING THE IMPORTATION AND RELEASE OF ENTOMOPHAGOUS BIOLOGICAL CONTROL AGENTS IN THE U.S.—IS THERE ROOM FOR IMPROVEMENT?

Classical biological control can be a key component in invasive species management programs. During the last century, many programs successfully used biological control agents (BCAs) against arthropod pests (van Lenteren et al. 2006). However, when successful, the establishment of these agents is irreversible and, once in a new area, they are very difficult if not impossible to eradicate (Simberloff & Stiling 1996; van Lenteren et al. 2006). Therefore, decisions to approve the importation and release of any new BCA must be considered carefully by appropriate authorities, particularly taking into account the likelihood of occurrences and consequences of any non-target effects. This should be done by conducting a pest risk analysis (PRA) (IPPC 2004; IPPC 2005).

A PRA summarizes the available scientific evidence in order for a decision to be made on the importation and release of entomophagous BCAs (Barratt & Moeed 2005). According to Fisher et al. (1994), a PRA is comprised of risk assessment, which estimates the likelihood of occurrence of a hazard and the magnitude of the consequences; risk management, which identifies options to mitigate the consequences of adverse events; and risk communication, which involves two way exchange of information between decision-makers and stakeholders. At the international level, the International Standard for Phytosanitary Measures (ISPM) #2 “Framework for Pest Risk Analysis” describes PRA as having three stages: risk initiation (which involves the identification of pests or pathways of concern), risk assessment, and risk management (IPPC 2007).

Regulations for import and release of entomophagous BCAs in the U.S. require applicants to obtain a Federal permit (PPQ 526) for: (1) Importation of BCAs into containment facilities; (2) domestic movement of imported BCAs to other containment facilities; and (3) BCA release into the environment (Figure 4-1). Furthermore, if the BCA has not been previously released in the U.S., the application must be accompanied by a dossier which describes the justification for the proposed action. The dossier must also provide information on the biology and ecology of the pest and the BCA, the economic and any potential detrimental environmental impacts of the BCA as well as possible mitigation options (Hunt et al. 2008; Mason et al. 2005). The format of the dossier can be based on the Regional Standard for Phytosanitary Measures # 12, “Guidelines for Petition for Release of Entomophagous Agents for the Biological Control of Pests”, developed by the North American Plant Protection Organization (NAPPO 2008). The International Standard for Phytosanitary Measures # 3, “Guidelines for the Export, Shipment, Import and Release of Biological Control Agents and Other Beneficial Organisms” developed by the International Plant Protection Organization also provides guidance on the importation and release process for biological control practitioners in the U.S. (IPPC 2005). In addition, ISPM # 2, “Framework for Pest Risk Analysis” and ISPM # 11, “Pest Risk Analysis for Quarantine Pests including Analysis of Environmental Risks and Living Organisms” give information on the risk analysis process (IPPC 2007; IPPC 2004).

In several countries such as Australia and New Zealand, a risk communication framework is defined and integrated within the PRA process during the permitting process of entomophagous BCAs (Hunt et al. 2008). For instance, in Australia, public comments are solicited during the importation approval process and during the environmental release of the BCAs (Hunt et al. 2008). In New Zealand, petitioners have the opportunity to defend their application in a "court-like" setting which might consist of relevant governmental decision-makers, experts, stakeholders, and members of the general public (Hunt et al. 2008). In the U.S., prior to 2007, environmental assessments were only required for federal employees and the information needed to be published in Federal Registry for public comments. However, the importance and role of risk communication is still a poorly defined concept for many federal and state agencies in the U.S. (Walls et al. 2004). Risk communication can be described as a consensus structure that joins the interests and needs of both senders and recipients (Fischnoff 1990). Within a PRA framework, "senders" include researchers and other permit applicants who may work for Universities, Federal or State governments, or the private sector. Senders are the ones that provide information on the potential risks associated with the importation and release of entomophagous BCAs. "Recipients" are governmental entities that regulate the permitting process. The National Research Council (1996) defines risk communication as "an interactive process of exchange of information and opinions among individuals, groups, and institutions". Therefore, the main purpose of risk communication is to provide individuals with enough information to enable them to make an informed decision about a potential risk (Gibson 1985; Fischnoff 1990; Gow & Otway 1990).

Governmental agencies have long been concerned with communication methods used to convey the risks associated with environmental issues (Chess et al. 1995). The USDA-APHIS has a long history of evaluating stakeholder satisfaction with its risk communication efforts (Fisher & Chen 1996). It was one of the first agencies within the USDA to conduct a baseline survey of a wide range of stakeholders to examine how well they were meeting their stakeholders' needs during risk communication activities (Fisher & Chen 1996). In 1995, the now defunct USDA-APHIS-PPQ, National Biological Control Institute (NBCI) identified areas in the current biological control regulatory system that could be improved. One of those areas was customer service and communication during the permitting process of BCAs (APHIS 1996). Unfortunately, with the demise of NBCI, recommendations made to improve risk communication by USDA-APHIS-PPQ were not implemented. In 2006, a follow-up internal evaluation of the USDA-APHIS-PPQ entomophagous BCAs permitting process revealed that customer service (stakeholder communication) remained an activity that should be improved (APHIS 2006).

In recent years, there has been an increased interest in risk communication among government agencies in the U.S. (Chess et al. 1995; Fisher & Chen 1996; APHIS 2009). However, there has been a lag in implementation of risk communication practices during the permitting process for the importation and release of BCAs (Messing 2005; Hunt et al. 2008). In this study, areas of risk communication practice are identified that could be improved in order to enhance the current permitting process in the U.S. Also, how USDA-APHIS-PPQ stakeholders receive information on risks pertaining to the permitting process of entomophagous BCAs and how they viewed the agency's risk information activities and performance are described. Based on the results, recommendations for improving risk communication practices during the BCA permitting process in the U.S. are suggested.

Materials and Methods

The data described below were collected by means of a web-based questionnaire. A modification of the "mental models" approach was used to develop the questionnaire. The "mental models" approach developed by Morgan et al. (2002) identifies gaps and misconceptions on critical problems from both the target audience and the experts, by gathering information from both groups. In our study, the approach involved a series of five steps. First, an expert model was created (Figure 4-1) (based on a survey of the literature) to determine the nature and magnitude of the risk communication problem during the permitting process. Subsequently, the expert model was used to conduct interviews with a small group of knowledgeable stakeholders in order to elicit their perceptions of the risk communication deficiencies and problems. Then, a confirmatory questionnaire was developed (Appendix F) and a quantitative survey was conducted and administered to an expanded group of stakeholders in order to estimate the prevalence of the identified beliefs. We identified areas in the current risk communication framework (during permitting of BCAs) that needed improvement. The last step in the mental models process was not covered in the present study. This step involves the evaluation of the improved risk communication framework to assess its efficiency and practicality with permit petitioners.

A committee comprised of 30 experts from various agencies and backgrounds, including risk analysts, academic researchers, and members of the private sector, was assembled. Individual members were selected based on their experience and knowledge about the USDA-APHIS-PPQ permitting process and risk communication procedures. The Dillman method (Dillman 2000) was used to develop and administer a survey of 15 open-ended questions (Appendix C). This method attempts to maximize response rates by minimizing the cost of responding, while establishing trust with the respondents. Open-ended questions were designed to generate perspectives from committee members on the risk communication practices of USDA-APHIS-PPQ and the critical points that should be targeted during the risk communication process. In accordance with the Dillman method, a personalized letter of notice was sent to the selected committee members explaining the goals of the study, the reason for their inclusion in the expert committee, and the reason for sending them the questionnaire (Appendix A). Approximately one week later, each participant received the questionnaire with a cover letter (Appendices B and C). A follow-up notice was sent a week later thanking those participants that had already responded and requesting a response from those who had not yet responded. Two weeks later a reminder was sent to non-respondents (Appendix E). Based on the results of the survey, topics and priorities were identified and addressed in a confirmatory questionnaire. A second web-based questionnaire comprised of 18 close-ended questions was developed (Table 4-1; Appendix F). Each question was reviewed and pre-tested (by graduate committee members) to ascertain its clarity. This study used 18 questions of which 16 were subjected to statistical analysis, and 2 questions, requesting general information (Q1, 2), were not included. The first question requested background information (name, company affiliation, and contact information) about the respondent. In addition, respondents were asked about the following: their involvement in biological control (Q 3), their frequency of risk communication in context of their profession (Q 4), the importance of risk communication (Q 5), the sources and frequency of risk communication (Q 6, 7), their satisfaction with risk communication from and interactions with USDA-APHIS-PPQ (Q 8, 9), channels for risk communication (Q 10), their ranking goals for risk communication (Q 11), effectiveness of USDA-APHIS-PPQ in fulfilling communication goals (Q 12), their familiarity with guidance documents (Q 15), the adequacy of USDA-APHIS-PPQ website (Q 16), and their knowledge on who to contact (Q 17). The last question (Q 18) solicited additional comments from the respondent.

A combination of several databases and directories (e.g. Government agency staff, university faculty, and professional societies) were used to compile a list of 500 decision-makers and stakeholders. Different words used during the search for potential respondents included “biological control”, “entomology”, “regulatory entomology” and “quarantine”. A modification of the Dillman method (Dillman 2000) was used to administer the web-based questionnaire. An introductory message was sent along with the web-based questionnaire. In addition, a note thanking respondents for their participation was automatically sent with the web-questionnaire. An electronic message reminding those who had not responded was sent 2 weeks later with the electronic link to the web-survey.

The respondents were grouped into five categories based on their affiliation as follows: federal, state, university, non-governmental agency, and private sector. The Kruskal-Wallis test (H value) was used to assess pairwise comparisons of groups of stakeholders that had an independent distribution with ordinal and rating responses as used in the survey (Sokal & Rohlf 1981). This non-parametric test also was used to determine whether the distributions of the responses were statistically different across the different groups of stakeholders.

Results

Response Rate and Respondent Characterization

Out of the 500 web-based questionnaires sent, 105 participants responded, 29 were undeliverable due to incorrect email addresses, and 5 opted-out from participating. An adjusted response rate of 23% was determined. Responses to the web-survey mostly came from participants involved in research (62%)—92% from the university group, 49% from the federal group, and 57% from the state category (Table 4-1). A smaller percentage of the respondents (19%) were involved in regulatory aspects during the implementation of biological control programs (30% from the federal group and 36% from the state group). Less than 4% of the respondents were involved in commercial production of BCAs (Figure 4-2).

Importance of Risk Communication

The majority of respondents across the three major types of affiliations (university researchers, federal, and state employees) considered risk communication to be an important component during the permitting process of entomophagous BCAs (Table 4-1). Participants from the private sector were evenly divided on the importance of risk communication (Table 4-1).

Risk Communication Framework

Four diagrams showing the relationship between risk analysis, risk management, and risk communication were presented in the questionnaire (Figure 4-3). In various literature sources (CFIA 2000; APHIS 2007; IPPC 2007), risk communication is integrated within risk analysis, and illustrated as an independent processes interconnected to the risk assessment and risk management elements (Model A, Figure 4-3). When respondents were asked which of the four diagrams best described existing risk communication practices, 34% of the respondents considered Model A to be the best representation. The federal and state group chose Model A (36%, 31% respectively) or Model D (27%, 38% respectively) whereas the university group selected Model A (33%) or Model B (37%). Within the private sector category, Models B and D received the same level of significance (50%).

Frequency and Sources of Risk Communication

Nearly 80% of respondents indicated that they communicated about risks in the context of their professions monthly or more frequently (Table 4-1). Specifically, more than 80% of researchers across the different types of affiliations communicated at least monthly about risk in the context of their profession (Figure 4-4). Nearly 70% of federal and state regulators indicated they had weekly communications about risk (Figure 4-4).

To accomplish these risk communication activities, respondents relied on a combination of traditional communication channels such as face-to-face meetings, telephone exchanges, televised programs, pamphlets, and scientific publications, and electronic communication channels, such as e-mails, list servers, Federal Registry site, and blogs (Table 4-1). Most of the risk communication information received by stakeholders was conveyed by USDA-APHIS-PPQ (29.9%), university researchers (28.9%), and state and local plant protection agencies (21.6%); with less information from environmental groups (15.5%) and the Cooperative Extension Service (10.3%) (Figure 4-5). The majority of stakeholders received information from USDA-APHIS-PPQ once a year or less frequently (72.6%). Environmental groups followed by Cooperative Extension Service personnel were the least involved in the transfer of risk information (17.4% and 15.4% respectively) (Figure 4-5).

Goals of Risk Communication

Overall, the respondents believed that explaining the risks associated with the importation and release of entomophagous BCAs should be the most important goal of risk communication activities (mean score 3.64, Figure 4-6). On the other hand, they also believed that one of the main objectives of these interactions should be to explain the decisions made during the importation of entomophagous BCAs (mean score 3.29). In decreasing importance, they considered that the process should encourage good practices among biological control practitioners (mean score 3.06), respond to external peer review recommendations (mean score 2.88), and explain the different petition requirements needed during the importation of process for BCAs (mean score 2.13). Based on the analysis with Kruskal-Wallis, there was a statistical difference in the way the various groups of stakeholders ranked the key goals of risk communication ($H = 12.5$; 4 d.f.; $P = 0.01$).

Respondent Satisfaction with Risk Information and Interactions

The respondents were somewhat familiar with which entities to contact during the permitting process (Table 4-1). About one third of respondents across the different groups were satisfied with quality of the content of the risk message (30.0%, Figure 4-7) and with the risk communication exchanges and interactions (26%, Figure 4-8) they received from USDA-APHIS-PPQ. When risk communication interactions occurred between USDA-APHIS-PPQ and their stakeholders, analysis with Kruskal-Wallis demonstrated that federal, state and the university groups ranked the Agency's effectiveness in fulfilling risk communication goals the same way ($H = 5.1$; 4 d.f.; $P = 0.3$) (Figure 4-9). In contrast, professionals from the private sector (60%) believed the

Agency to be ineffective in explaining the risks pertaining to the importation of entomophagous BCAs (Figure 4-9).

Need for more Guidance Documents

The federal and state respondents were somewhat familiar with international and regional standards for phytosanitary measures related to pest risk analysis or specific to importation and release of BCAs. Private sector respondents were unfamiliar with the various ISPM and RSPM guidance documents (Table 4-1). Although the USDA-APHIS-PPQ website provides some information, the respondents recognized the need for more information from USDA-APHIS-PPQ focusing on the risks pertaining to the importation and release of entomophagous BCAs (Table 4-1).

Public Involvement

Respondents from state and university groups felt that biological control stakeholders were not appropriately included during the decision-making process of the permitting of arthropod BCAs (Table 4-1).

Discussion

To date, there has been relatively little attention given to understanding risk communication activities during the permitting process for the importation and release of entomophagous BCAs in the U.S. Therefore, although risk communication is an important component of the PRA process, it is still an ambiguous concept for many Agency professionals and their stakeholders (Walls et al. 2004). There is a need, therefore, to identify ways in which risk communication can be improved and thus lead to the development of an improved framework that will satisfy the needs of stakeholders. This new framework would address some of the key concerns expressed by biological control practitioners, environmental groups, and the general public (Thomas & Willis 1998; Simberloff 2005).

One area that should be clarified is how risk communication is currently integrated within the PRA framework. Although Models A or B (Figure 4-2) were more frequently chosen by respondents (68.2%), various scientific publications and many respondents (Table 4-1) indicated that Model D (Figure 4-3) best described how risk communication should be integrated. In Model D, risk communication is an integral element of risk assessment and management components within the PRA. The difference in opinions between what is the current practice and what should be targeted demonstrated a flaw in the current risk communication framework. Although Model D may seem unobtainable for a novel risk communication framework, it should form a basis for a more participatory based PRA model. Consultations between the general public or experts and USDA-APHIS-PPQ during the permitting process for BCAs should provide an additional source of knowledge to validate the identification of risk factors and management options.

Although one of the current concerns of USDA-APHIS-PPQ is to increase public involvement in decision-making process (APHIS 2009), there is lack of information on risk communication activities by the Agency. Previous studies showed that stakeholders had little knowledge of the risk analysis framework pertaining to the importation and release of entomophagous BCAs, consequently limiting their participation (APHIS 2006; APHIS 2009). This study showed that stakeholder perception and understanding of the process, the communication channels used, and the efficiency of the risk message should be improved in order to increase participation by stakeholders. In addition, the survey showed that stakeholders received information from only a few sources and that the information was received very infrequently. Only 1% of respondents thought that the USDA-APHIS-PPQ website was efficient at providing guidance. It seems that USDA-APHIS-PPQ is aware of these issues. Indeed, in November 2009, the Agency conducted a survey of their registered stakeholders to obtain feedback on how they could improve the overall delivery of information on their website (APHIS 2009). The development of website links relating to critical issues relating to PRA will provide improved information and guidance to stakeholders. Our study showed that new channels of communication should be investigated to increase stakeholder access to risk related information. This might include the use of television, national public radio, or newspaper during the communication of risk.

Even when stakeholders received information from USDA-APHIS-PPQ, this message did not always meet their needs. For instance, university and private sectors respondents said that USDA-APHIS-PPQ was ineffective in communicating risk pertaining to the importation and release of entomophagous BCAs (Figure 4-9). The difficulty faced by USDA-APHIS-PPQ in fulfilling stakeholder needs may come from the fact that different groups of stakeholders view risk communication goals differently (Figure 4-6). Therefore, there is a need to identify the main goals of the risk communication efforts, specific to the different types of stakeholders and respond accordingly.

A majority of the respondents was not satisfied with the quality of risk the communication messages or the risk message exchanges and interactions from USDA-APHIS-PPQ (Figure 4-7). For instance, 60% of respondents from the private sector were either dissatisfied or very dissatisfied with the risk communication messages and interaction with USDA-APHIS-PPQ (Figure 4-8). This result seemed to confirm the negative perception of USDA-APHIS-PPQ's customer service record from their stakeholders as illustrated by Warner & Getz (2008). A greater level of involvement in the decision-making process by stakeholders and expert peer review groups may increase the stakeholders' trust in the decisions and improve the stakeholders' perception of the quality of the risk communication message. In October 2009, a proposed rule was submitted by USDA-APHIS-PPQ for the mandatory development of an Environmental Assessment (EA) before the importation of entomophagous BCAs (APHIS 2009). Under the National Environmental Policy Act (NEPA), the development and submission of an EA is required when a proposed action such as the introduction of any organism has potentially significant environmental impacts (Kubasek & Silverman 2005). An external group of experts selected by the governmental agency reviews the EA. The group of experts then provides an analysis of potential adverse environmental effects of the proposed action. In accordance with the Administrative Procedure Act's rules on informal rule making, a draft is published in the Federal Register and public comments are accepted by the stakeholders for a period of 60 days. One of the major advantages of this process is that it requires public participation in the decision-making process. In addition, the development of a standardized risk communication framework with clear and identified risk communication activities will increase the quality of the interactions between the agency and its stakeholders.

The results from this survey provide baseline data to evaluate USDA-APHIS-PPQ's risk communication performance during the importation and release of entomophagous BCAs. Based on the findings of this study, the following are being suggested to enhance the risk communication framework:

- increase of transfer of guidance documents and information pertaining to PRA process of entomophagous BCAs through the use of additional media;
- greater involvement of cooperative extension faculty in stakeholders' education about PRA;
- identification and development of risk communication messages specific to different types of stakeholders;
- development of a PRA framework with a detailed time frame which will increase stakeholder involvement in the decision-making process.

Table 4-1. Questionnaire.

	Questions
1.	About Yourself
2.	In which group will you categorize yourself?
3.	How would you categorize your involvement in biological control?
4.	How often do you communicate risk in the context of your profession?
5.	Do you view risk communication as an important component during the importation process of entomophagous BCAs?
6.	From which entity(ies) do you receive information pertaining to risks associated with importation of BCAs and what is relative importance of each source?
7.	How often do you receive information about risks associated with the importation of entomophagous BCAs from USDA-APHIS-PPQ
8.	How would you rate your level of satisfaction with the risk communication information that you perceive from USDA-APHIS-PPQ pertaining to the importation of entomophagous BCAs?
9.	How would you rate your level of satisfaction of the Risk Communication interactions with USDA-APHIS-PPQ concerning the importation of entomophagous BCAs?
10.	What percentage best describes the communication channel(s) through which you receive the information on risks pertaining to the importation of entomophagous BCAs?

Table 4-1. Continued

	Questions
11.	Rank the following key goals of the risk communication process during the importation of entomophagous BCAs in order of importance
12.	How effective is USDA-APHIS-PPQ in fulfilling each risk communication goal during the importation of entomophagous BCAs?
13.	What is your degree familiarity with the different guidance documents pertaining to the importation of entomophagous BCAs?
14.	Which of these models best represent your perception of risk communication as it is currently incorporated during the importation process of entomophagous BCAs
15.	Do you think there is a need for more guidance documents from USDA-APHIS-PPQ concerning the importation of entomophagous BCAs?
16.	Does your USDA- APHIS-PPQ website provide you with enough explanations and guidance about importation of entomophagous BCAs?
17.	Do you have the information (phone numbers, emails, fax number, address) of points of contact that you can reach if you have any questions during the importation of entomophagous BCAs process?
18.	In your opinion, is the public adequately involved in the importation of entomophagous BCAs process?

Table 4-2. Summary of questions and responses obtained from 5 categories of biological control stakeholders (Federal, State, University, Private, and Non-Governmental).

Question	Stakeholder Categories[a]			
	Federal (40%)	**State (18%)**	**University (34%)**	**Private (6%)**
3. Biological control area of involvement	Research (57%) Regulation (24%)	Regulation (53%) Research (42%)	Research (92%)	Commercial production (67% Conservation (17%)
5. Is RC[b] important?	Yes (92.5%)	Yes (90%)	Yes (100%)	Yes (33%) No (33%)
7. RC Frequency from PPQ[c]	At least yearly	At least yearly	Yearly to never (91%)	Never (60%)
10. Main RC channels	Mailed letters Scientific publications Scientific conferences emails	Scientific publications Scientific conferences	Scientific publications Scientific conferences emails	Meetings (lunch, social, or board)
13. Familiarity with guidance documents	Somewhat to very familiar	Somewhat familiar to familiar	Unfamiliar to somewhat familiar	Unfamiliar
15. Need for more guidance documents	Yes, mostly to definitively (60%)	Yes, somewhat to mostly (65%)	Yes, mostly to definitively (56%)	Yes, definitively (100%)
16. Is PPQ website provide enough guidance	Yes, somewhat to mostly (54%)	Not at all to yes, somewhat (65%)	Yes, somewhat (68%)	Yes, somewhat (100%)
17. Knowledge of point of contacts	Yes, somewhat to mostly (57%)	Yes, somewhat to mostly (59%)	Yes, somewhat to mostly (85%)	Yes, somewhat (100%)
18. Is public involvement adequate?	Yes, somewhat to mostly (53%)	Not at all to undecided (52%)	Yes, somewhat to not at all (74%)	Yes, somewhat to undecided (100%)

[a]Non-Governmental Organization – less that 2%

[b]Risk Communication

[c]Plant Protection and Quarantine Organization

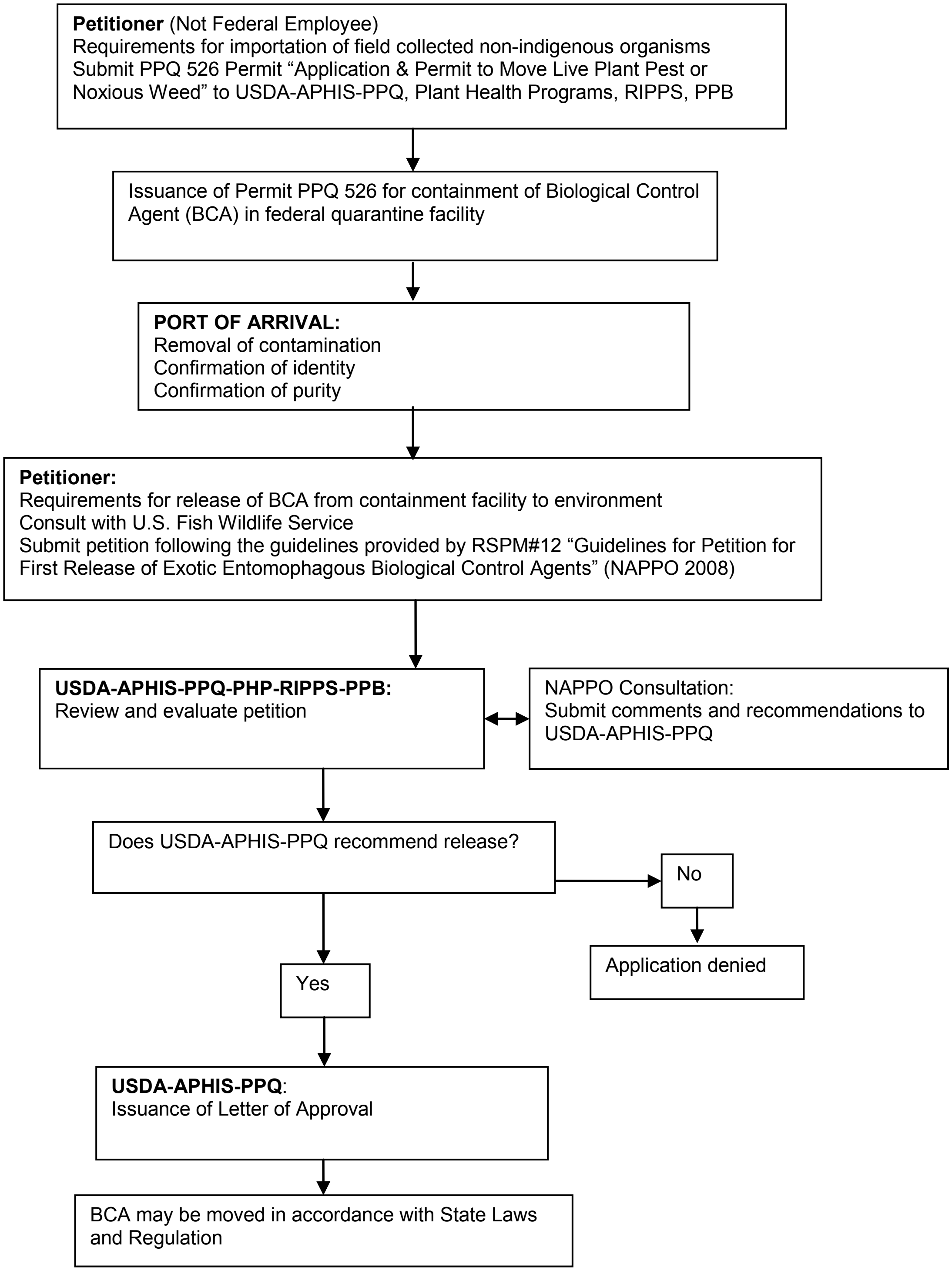


Figure 4-1. Expert model of the permitting process used by USDA-APHIS-PPQ in 2007 (Hunt et al. 2008).

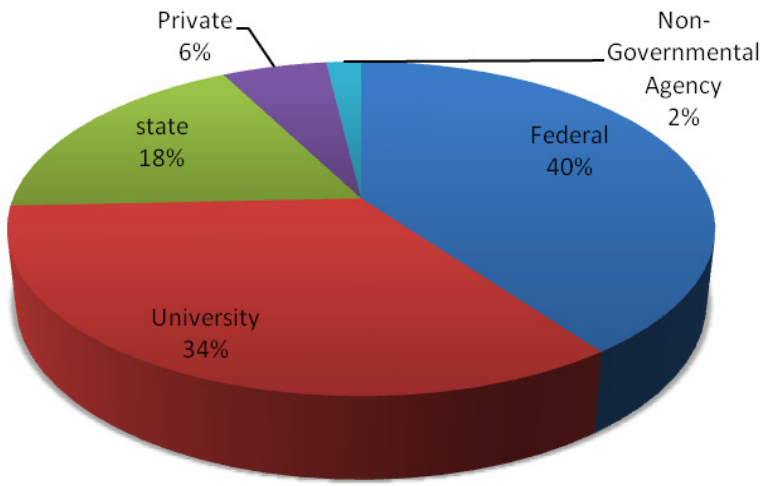


Figure 4-2. Distribution of respondents to Question 1: "In which group will you categorize yourself?"

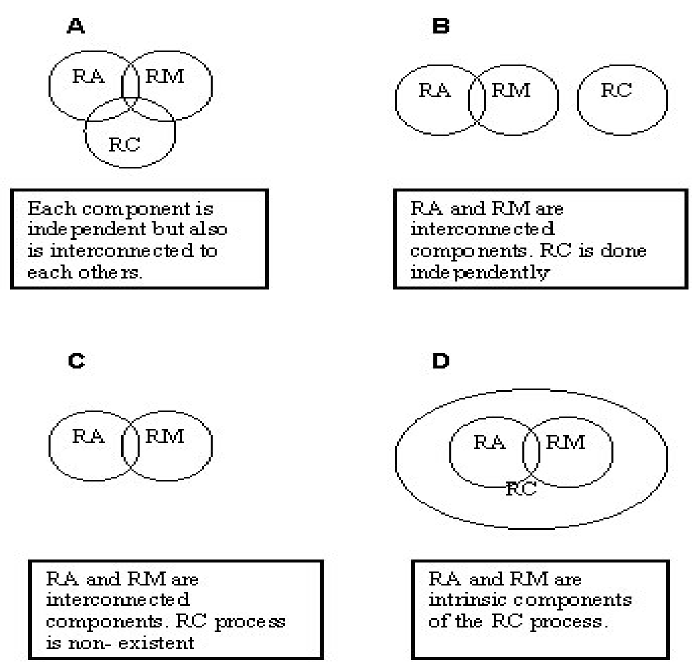


Figure 4-3. Different model choices of pest risk analysis structure presented in questionnaire. RA = risk analysis, RM = risk management, RC = risk communication.

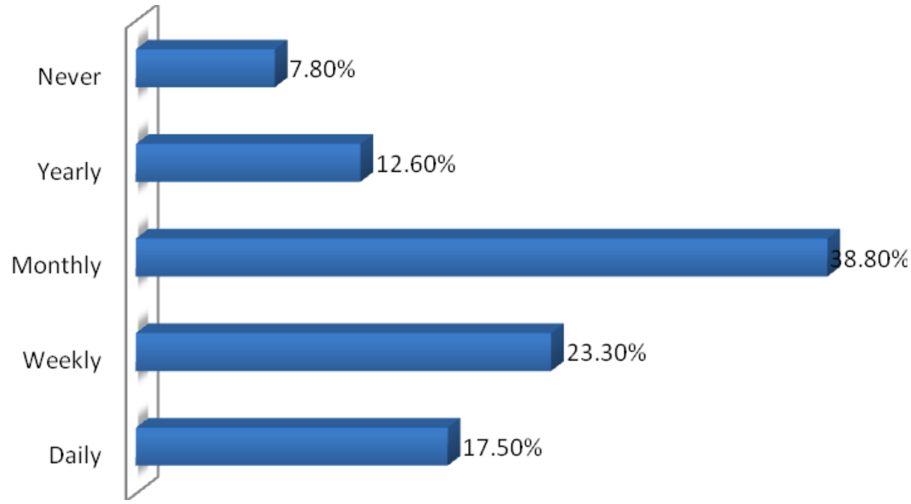


Figure 4-4. Distribution of respondents to Question 4: “How often do you communicate risk in the context of your profession?”

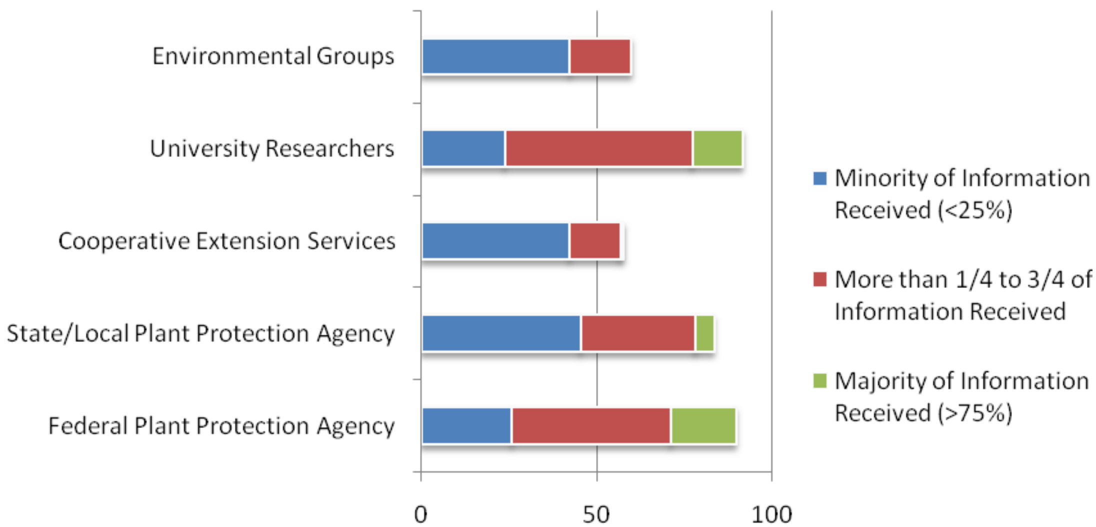
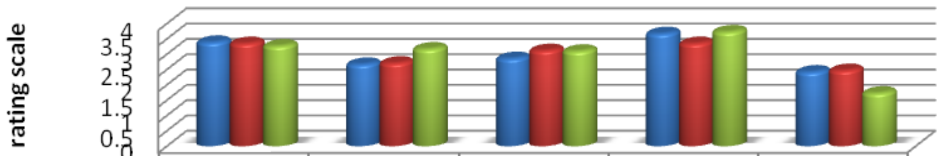


Figure 4-5. Distribution of respondents to Question 6: “From which entity(ies) do you receive information pertaining to risks associated with importation of BCAs and what is the relative importance of each source (percentage)?”



	Explain decisions	Respond to external peer review recommendations	Encourage good practices among biological control practitioners	Explain risks	Explain petition requirements
Federal	3.39	2.67	2.86	3.67	2.42
State	3.35	2.71	3.12	3.35	2.47
University	3.27	3.17	3.1	3.73	1.73

Figure 4-6. Distribution of respondents to Question 11: “Rank the following key goals of the risk communication process during the importation of entomophagous BCAs in order of importance (5-very important to 1-least important).”

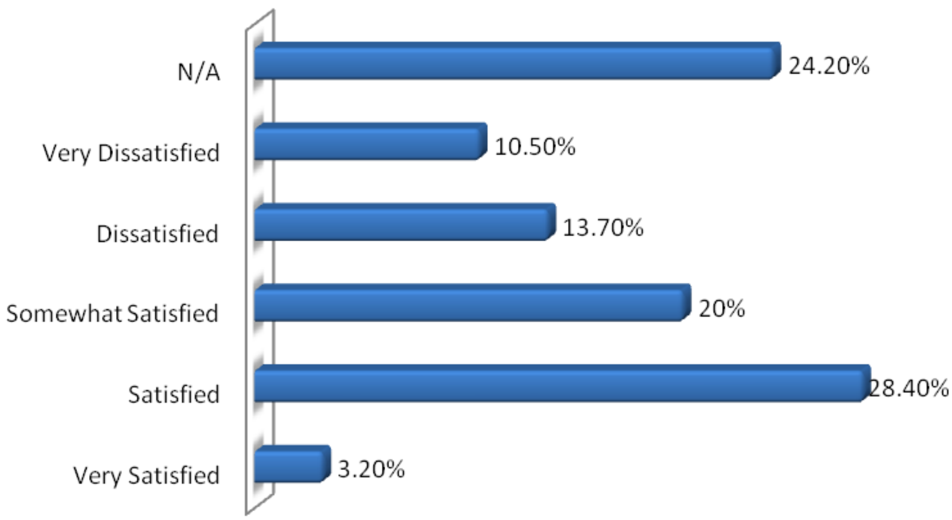


Figure 4-7. Distribution of respondents to Question 8: “How would you rate your level of satisfaction with the risk communication message/ information that you receive from USDA-APHIS-PPQ pertaining to the importation of entomophagous BCAs?”

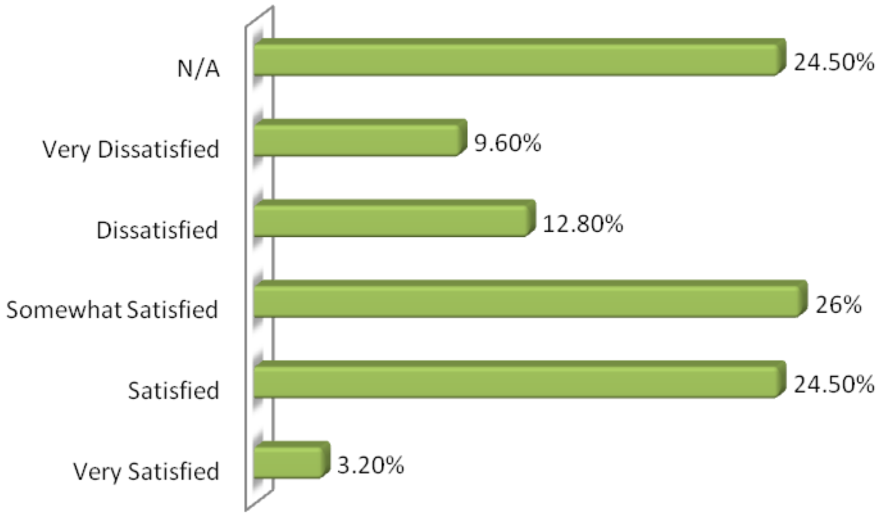
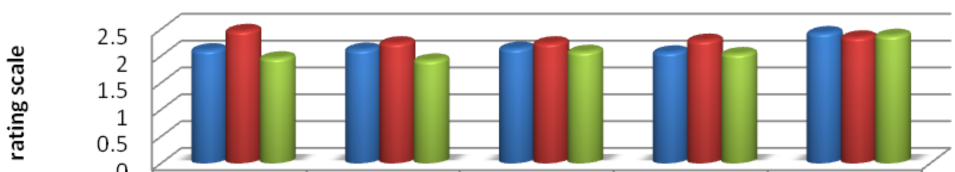


Figure 4-8. Distribution of respondents to Question 9: “How would you rate your level of satisfaction with the risk communication exchanges/ interactions that you receive from USDA-APHIS-PPQ pertaining to the importation of entomophagous BCAs?”



	Explain decisions	Respond to external peer review recommendations	Encourage good practices among biological control practitioners	Explain risks	Explain petition requirements
Federal	2.11	2.12	2.14	2.07	2.43
State	2.47	2.24	2.24	2.29	2.35
University	1.96	1.92	2.08	2.04	2.38

Figure 4-9. Distribution of respondents to Question 12: “How effective is USDA-APHIS-PPQ in fulfilling each risk communication goal during the importation of entomophagous BCAs?”

CHAPTER 5
"COLLABORATIVE" RISK ASSESSMENT DURING THE PERMITTING PROCESS OF ENTOMOPHAGOUS BIOLOGICAL CONTROL AGENTS—A BETTER PROCESS?

The task of protecting American agriculture and natural resources against the risks associated with the entry, establishment, and spread of plant pests in the U. S. is the responsibility of the U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ) (APHIS 2011), the U.S. National Plant Protection Organization (NPPO). The Plant Protection Act (PPA 2000) authorizes the Secretary of Agriculture to delegate authority for plant protection authority to USDA-APHIS-PPQ. This federal entity has broad jurisdiction to develop and enforce phytosanitary measures that will prevent and or delay the introduction and spread of plant pests (PPA 2000). In the 1990s, scientists and governments across the globe identified a need for harmonization of regulatory and phytosanitary procedures during the permitting process for entomophagous biological control agents (BCAs) in order to ensure more effective plant and animal protection (Ebbels 2003). However, regulations and legislation vary considerably between countries (Chapter # 3). In an effort to harmonized PRA processes and also provide procedures to assess risks associated with the importation and release of BCAs, the International Plant Protection Convention (IPPC) developed the International Standard for Phytosanitary Measures (ISPM) # 2 "Framework for Pest Risk Analysis" (IPPC 2007). New and revised legislation and regulations were implemented by the U.S. NPPO in response to a need for a comprehensive, harmonized regulatory process for the importation and release of BCAs (De Nardo & Hopper 2004). A major addition of the Plant Protection Act of 2000 was USDA-APHIS-PPQ's statutory authority to regulate organisms intended to control pant pests. Prior to this Act, a legal definition of a biological control agent was not present in the statutes of the U.S., and the U.S. NPPO regulated them under statutes and regulations pertaining to plant pests. In 2001, following an agreement with Canada and Mexico, the U.S. NPPO, under the North American Plant Protection Organization (NAPPO), requires a permit for environmental release of an entomophagous BCA (NAPPO 2008).

One of the priorities of USDA-APHIS-PPQ has been to improve their understanding of the potential risks associated with the importation and release of entomophagous BCAs. Decisions for approving or denying the importation of BCAs are made by conducting a pest risk analysis (PRA) (Hunt et al. 2008; Mason et al. 2005). Risk-based analysis provides a structured framework for making rational decisions when outcomes are uncertain (Simberloff 2005). One of the main objectives of a PRA is to confirm absence of contamination and identification of BCAs or associated organisms whose introduction could lead to detrimental agricultural/environmental impacts as well as to identify those BCAs that pose little risk (Page 1978). Quality control of BCAs during consignment is an important step because it establishes presence of harmful contaminants associated with the imported BCAs. The permit petitioner is responsible for providing information on the BCA following the format recommended by the Regional Standard for Phytosanitary Measure # 12 "Guidelines for Petition for First Release of Exotic Entomophagous Biological Control Agents" (NAPPO 2008). Information about the proposed action, biology and ecology about the target pest and BCA, potential economic and environmental impacts are required. The science-based PRA reduces the scientific uncertainty associated with potential risks by examining information helpful in evaluating management options (DeKay et al. 2002)

Uncertainty is inherent to all risk analyses (Koch et al. 2009). Uncertainty can be categorized into epistemic, linguistic, and stochastic (natural variability) (Burgman 2005). Epistemic uncertainty is generally described as a shortfall in knowledge which may lead to undesired outcomes (DeKay et al. 2002). Pest risk analysis is mostly affected by epistemic uncertainties (Rafoss 2003; Koch et al. 2009). The precautionary principle is sometimes used during decision-making when there is uncertainty (Graham 2001), and was accepted as a valid approach by the Rio Convention on Biodiversity in 1992 (Simberloff 2005). The principle states that precautionary measures are authorized for environmental decisions even when a causal relationship has not been fully established (Simberloff 2005). The principle is often invoked in complex environmental situations where detrimental impacts are often irreversible and losses difficult to recover. Therefore, the precautionary approach is more likely to be used in cases where traditional regulatory approaches are inadequate (Dekay et al. 2002), and there is no consensus about the likelihood of occurrence of potential detrimental impacts (Simberloff 2005). The precautionary approach ensures that the absence of scientific certainty is not used as a reason for postponing actions that are intended to protect people and the environment (Arrow & Fisher 1974; Dixit & Pindyck 1994; Farrow & Morel 2001). For intentional introduced non-native species such as entomophagous BCAs, rigorous quarantine laws adopting a “guilty until proven innocent” approach are believed to be used in the U.S. (Ruesink et al. 1995, Simberloff 1996, 2005). This approach is justified by the fact that in the event of unintended environmental impacts from an authorized release of an entomophagous BCA, the U.S. NPPO will be the responsible party not the researcher. Several scientists argue that such approach is used as justification for arbitrary denial of entomophagous BCAs permits. However, there has been recognition that a level of zero risk in the case of BCA is unobtainable and that a certain level of risk is acceptable considering the benefits.

The lack of transparency in the process of assessing data during the permitting process for entomophagous and phytophagous BCAs by USDA-APHIS-PPQ creates additional distrust among stakeholders in the current risk assessment process (Mason et al. 2005; Warner & Getz 2008). In this study, the hypothesis tested is that credibility in the current risk analysis process can be improved through the development of a collaborative approach between decision-makers and stakeholders. Collaboration involves the process of joint decision-making among key stakeholders, experts and decision-makers (Daniels & Walker 2001). Decisions resulting from a collaborative-based risk analysis model are more widely accepted by participants because they provide legitimacy to environmental decisions (Fischnoff 2005a,b). It builds trust among participants and allows a balanced review of information during the risk assessment process (Fischnoff 2005a,b). The mental models approach (Morgan et al. 2002) was used to identify gaps, misconceptions and critical problems in participant’s comprehension of the risk analysis process by gathering information from both decision-makers and stakeholders. The approach is based on a systematic analysis of participant’s beliefs and the identification of knowledge gaps during the decision-making process (Morgan et al. 2002). Based on the findings, a modified collaborative-based risk analysis process was developed.

Materials and Methods

Committee of Experts

A committee of 30 experts with various backgrounds covering five professional affiliations (federal, state, university, environmental and the private sector) was chosen (Table 5-1). A combination of several databases and directories (e.g. government agency staff, university faculty and professional society) was used to compile the committee of experts. The members of the expert committee were selected based on their experience and knowledge concerning the permitting process for BCAs and risk assessment procedures used to evaluate entomophagous and phytophagous BCAs for importation and release. All of the experts had submitted at least one petition for importation and/or release of a potential entomophagous BCA or been actively involved in a classical biological control project that involved importation and release of natural enemies.

Development of an Expert Conceptual Model and Interview Protocol

The mental models approach (Morgan et al. 2002) was used to compare and analyze the different beliefs held by the group of experts. The mental models approach consists of a series of 5 steps described as follows: 1-development of an expert conceptual model based on scientific data to assess issues associated with the risk analysis process; 2-administration of interviews to elicit stakeholders' beliefs; 3-creation of a confirmatory questionnaire to evaluate the proportion of stakeholders which share the same beliefs; 4-correction of misconceptions; and 5-development of an improved process based on findings.

A conceptual diagram was developed based on information from scientific publications and the USDA-APHIS-PPQ's website (Figure 4-1, Chapter 4). The diagram captured the different steps involved in the permitting process for entomophagous BCAs when submission was made by a non-federal employee. The conceptual diagram was used to assess the group's understanding of relationships and interaction of key factors during the risk analysis process of entomophagous BCAs and to identify specific issues affecting the efficiency of the permitting system. Open-ended questions directed participants to specific topics in the expert conceptual model. A modification of the Dillman method (Dillman 2000) was used to develop a semi-structured interview with 15 open-ended questions (Appendix C). The questionnaire can be found in Table 4-1. The open-ended questions were designed to characterize settings or events that might affect the operation and efficiency of the risk analysis framework. The questionnaire was reviewed and pre-tested to ascertain the clarity of each question.

Comparison of Mental Models for Decision-Makers and Stakeholders

Results from the interviews allowed for the characterization of themes and patterns of issues related to the risk assessment process. They were used to compare the beliefs of decision-makers and stakeholders. Findings were summarized and used to identify the commonalities and divergences among participants. Recommendations were used to develop a suggested collaborative risk analysis process to be used during the importation and release of entomophagous BCAs.

Results and Discussion

Conceptual Expert Model

Not surprisingly, the interview results generated different mental models for decision-makers and stakeholders on issues impacting the permitting process for entomophagous BCAs (Appendix D). The mental models for each group were compared and categorized into eight categories, which corresponded to crucial components of the risk analysis process (Table 5-2). These components were described as follows: risk assessment, risk communication, submission of a petition for importation of entomophagous BCA, inspection at the port of entry, submission process for petition to release a BCA, external review process, decision-making process, and issuance of letter of approval for release. Problem areas pertaining to the risk analysis process during permitting were characterized into five categories (risk assessment process, stakeholder participation, risk communication, decision-making, and selection of expert group members during decision-making process) (Table 5-2).

Comparison of Mental Models for Decision-Makers and Stakeholders by Categories

Risk analysis process

Stakeholders (permit petitioners) believed that a “guilty until proven innocent” approach was used during the risk analysis process and that it was often used by USDA-APHIS-PPQ decision-makers as a justification to prevent the importation or release of candidate entomophagous BCAs. Decision-makers argued that due to high uncertainty related to complex environmental scenarios for entomophagous BCAs, an “innocent until proven guilty” risk analysis approach could not be used. They pointed out that there was often a lack of transparency in the information provided by the permit petitioner. In addition, they indicated that there was no accepted standardized process to quantify uncertainty during their risk analysis process. The precautionary principle postpones taking actions that may result in irreversible outcomes when there is a prospect for obtaining improved information (Simberloff 2005). Uncertainty is often described as a gap of knowledge or as lack of, incomplete, erroneous, dated, or inaccurate knowledge. However, Wynne (1992) defined scientific uncertainty as a subjective combination of complex social and cultural factors. Therefore, uncertainties can never be fully resolved before taking action (Graham 2001). In addition, the precautionary approach is always appropriate for environmental decisions because risks are often not demonstrated until later when damages have already occurred and are irreversible (Graham 2001). The modified risk analysis process (Figure 5-1) proposed in this study is based on a collaborative model. A collaborative-based process is a two-way interaction model that considers concerns and information from both key stakeholders and decision-makers. It also assumes shared responsibility for subsequent outcomes from those actions (Selin & Chavez 1995) unlike in the current risk approach used for permitting entomophagous BCAs in the U.S.

Stakeholder participation

Stakeholders often felt that the risk analysis process during entomophagous BCA permitting was subjective and arbitrary. They believed that the general public should be more involved in the decision-making process in order to increase transparency in the process. Participation is defined as a process where individuals, groups, and organizations choose to take an active role in making decisions that affect them (Wanderman 1981; Wilcox 2003; Rowe et al. 2004). The complex and dynamic nature of environmental problems requires flexible and transparent decision-making procedures that embrace a diversity of expertise (Stringer & Reed 2007). To achieve this, stakeholder and/or expert involvement needs to be increasingly embedded into environmental decision-making process (Stringer & Reed 2007). The modified collaborative-based risk analysis process (Figure 5-1) requires the development of an Environmental Assessment (EA). The EA document provides basic information on the positive and negative environmental impacts of the proposed action, including a management program in case of adverse consequences, and also suggests alternatives to the implementation plan under consideration (NEPA 1970). A similar approach is recommended by the Regional Standard for Phytosanitary Measures # 12 ("Guidelines for Petition for First Release of Exotic Entomophagous Biological Control Agents") developed by the North American Plant Protection Organization (NAPPO) and the International Standard for Phytosanitary Measures # 3 ("Guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms") published by the International Plant Protection Convention (NAPPO 2002; IPPC 2005). However, in accordance with the rules on informal rule making of the U.S. Government Administrative Procedure Act, during the development of an EA, the proposed action must solicit public comments for a period of 60 days (Kubasek & Silverman 2005).

Risk communication

Results from these interviews revealed that a well-orchestrated risk communication process is needed in order to reduce information gaps between decision-makers and stakeholders. Participants considered that risk communication was an important component of the risk analysis process. Results showed that both decision-makers and stakeholders believed that risk communication should be integrated within this process (Table 5-2). The role of risk communication in environmental decision-making has significantly increased within USDA-APHIS over the last decade and remains an important social issue (Santos & Chess 2003). It provides a means to determine what matters most to USDA-APHIS-PPQ decision-makers (Wardman 2008). However, the majority of the stakeholders interviewed were dissatisfied with the risk communication interactions and messages received from decision-makers (Table 5-2). The critical issues identified were: unacceptable communication channels used during risk communication activities, problems with the main source of information, and poor quality of risk communication interactions between decision-makers and stakeholders. The areas of risk communication that needed improvement are further discussed in detail in Chapter 4 of this dissertation.

External review process and selection of expert group members

This study showed that stakeholders felt that there was a lack of external peer consultations during the decision process of issuing permits for entomophagous BCAs. Generally, decisions made after the development of a risk assessment are based on expert judgment (Daniels & Walker 2001). The stakeholders believed that an independent assessment of the facts by an expert group would provide a more transparent decision than USDA-APHIS-PPQ's seemingly arbitrary decisions. An expert can be described as someone with an appropriate degree of knowledge about an issue and that has efficient communication skills (Meyer & Booker 1990). An expert group must be carefully selected because its composition affects the outcome of the risk assessment (Daniels & Walker 2001). For instance, the Food and Agriculture Organization used a stratification approach for the selection of experts for issues of food safety based on their affiliation, technical expertise, professional recognition, publications, and ability to draft clear reports (Meyer & Booker 1990). The selection of experts for external review could be done in a way similar to the Technical Advisory Group (TAG), an expert group that advises USDA-APHIS-PPQ on risk assessment of importation and release of phytophagous BCAs (Scoles et al. 2008). The TAG is composed of representatives of federal agencies that might be affected by decisions, such as the USDA-Agricultural Research Service, USDA-Forest Service, USDA-Natural Resources Conservation Service, U.S. Environmental Protection Agency, etc (Scoles et al. 2008). In addition, state employees may also be incorporated in the TAG. In the proposed modified risk analysis process for entomophagous BCAs, a representative from the State Plant Regulatory Office (SPRO) could make the connection between the state and federal decision-makers.

Decision-Making Process

Approval for import to federal quarantine facility

In the modified risk analysis process (Figure 5-1); the permit petitioner must initially contact the USDA-APHIS-PPQ service staff office to discuss the scientific soundness of the proposed action. A consultation with the U.S. Fish and Wildlife Service (FWS) also is suggested at this time rather than later in the process. This first step ensures that possible environmental and economic issues are identified early before the release is made. Based on the initial contact and submitted documentation, risk analysts from USDA-APHIS-PPQ will provide recommendations whether or not to approve the initial importation of the BCA into a quarantine facility. No public hearing is required at this initial stage.

Approval for environmental release

In the modified risk analysis process (Figure 5-1), all applicants (federal and non-federal employees) are required to automatically prepare an EA for the environmental release of an entomophagous BCA. Under NEPA, submission of the EA calls for public comments during the decision-making process. The EA and the host species list for the assessment of the BCA host range would be reviewed by a newly proposed group of outside experts and recommendations will be sent to risk analysts within USDA-APHIS-

PPQ. After evaluation of the EA, USDA-APHIS-PPQ would reach a decision based on the recommendations from the expert group and comments from the general public. In addition, recommendations from representatives from NAPPO (Canada and Mexico) also woulb be taken into account during the decision-making process.

Final decision

If USDA-APHIS-PPQ denies the stakeholders' permit to release the BCA, a written response is prepared by USDA-APHIS-PPQ outlining their concerns, justifying their denial, and identifying potential steps to rectify the problems with the EA. The applicant has the option of resubmitting the application after conducting further research which addresses USDA-APHIS-PPQ concerns. After the stakeholder submits supplemental information, USDA-APHIS-PPQ re-evaluates the application, and, in case of continued refusal to permit release of the entomophagous BCA, communicates their reasons to the applicant. If approval for release is granted, USDA-APHIS-PPQ staff notifies the stakeholder and sends the information to the SPRO in the state where the entomophagous BCA will be released. After communicating with the SPRO, the stakeholder can release the entomophagous BCA following state laws.

Summary

This study explored how decisions were made and who was involved in making them during the process of approving the importation and release of entomophagous BCAs. Based on the findings, the implementation of a collaborative-based risk analysis process during the permitting process of entomophagous BCAs was investigated. The modified risk analysis process incorporated expert and public participation during the decision-making process. In addition, the permit petitioner had the opportunity to resubmit the application if approval for release was denied. Following approval from the federal authority, a direct liaison with a state official was integrated into the process.

Table 5-1. Type of professional affiliation and number of expert committee members.

Professional affiliation	n
University	
Professor (biological control)	4
Professor (integrated pest management)	1
Professor (plant pathology)	1
Lecturer (regulatory sciences)	1
Researcher (biological control)	2
Researcher (environmental sciences)	1
State	
Researcher (biological control)	2
Researcher (environmental sciences)	1
Extension specialist	2
Federal	
U.S. Department of Agriculture-Agricultural Research Service (entomologists)	3
U.S. Department of Agriculture-Animal and Plant Health Inspection Service-Plant Protection and Quarantine (risk analysts)	6
Private sector	3
Environmental groups	2

Table 5-2. Characterizing the mental models of decision-makers and stakeholders during the permitting process of entomophagous biological control agents (BCAs) in the U.S.

	Mental models			
Components of the permitting process	Stakeholders (permit petitioners)	Decision-makers	Issue themes	Recommendations
Risk Analysis (RA) process	•Stringent risk analysis process due to misuse of precautionary approach •Lack of public involvement during decision-making process •Unclear decision criteria •Absence of clear timeframe for each step of RA process •Insufficient guidance document on RA process	•Due to uncertainty in potential irreversible environmental impacts BCAs (Biological Control Agents) follow a precautionary approach •Difficulty in balancing appropriate level of environmental protection •No clear method to evaluate uncertainty	•RA process is inadequate •Stakeholder participation needs to be increased	•Development/adoption of a standardized RA process •Experts/Public involvement during decision making process •Incorporate an appellate system in case of permit refusal
Risk Communication (RC) process	•The most important goal of RC is to explain risks associated with importation of BCAs •Dissatisfaction with National Plant Protection Organization effectiveness in explaining risks to permit petitioners •Dissatisfaction with information and interactions with regulators/decision-makers •No clear direct point of contacts	•The most important goal of RC is to explain risks associated with importation and release of BCAs to biological control practitioners •Problems of transparency and consistency from permit petitioners	•Need to characterize RC goals and identify importance •Need to improve efficiency of RC activities	•Creation of additional media outlets to accomplish RC activities •Improve customer service •Development of International Standard for Phytosanitary Measures (ISPM) on RC or addendum to ISPMs related to PRA

Table 5-2. Continued

Components of the permitting process	Stakeholders (permit petitioners)	Decision-makers	Issue themes	Recommendations
Submission of petition (PPQ 526) for movement of BCA into Federal Quarantine Facility	•Insufficient guidance documents explaining the permitting process •Unnecessary procedural steps at initial stage •No clear point of contacts identified for additional inquiry •Reluctance of carriers to ship BCAs due to liability issues	•USDA-APHIS-PPQ website and other published articles provide enough guidance information for permit petitioners[a] •The online submission (e-permit) create a quicker process •A toll free number and email contacts for additional information on organism permits are available on USDA-APHIS website page	•All the steps of the RA process should be clearly defined	•Improve website by the creation of online links on topics related to entomophagous BCAs •Provide additional guidance documents on relevant information on legislation/ regulations, permitting unit organization, permitting process with corresponding time frame
Inspection at the port of entry	•Strict quarantine requirements difficult to fulfill •Refusal of BCAs at port of entry (hand carrying) because of lack of instructions and training from Customs and Border Protection (CBP) inspectors	•Problems of transparency from petitioners •Lack of education/ knowledge from permit petitioners	•Improve RC between regulators and permit petitioners	•Improve CBP inspectors training on issues pertaining to entomophagous BCAs •Identification of point of contacts at port of entry •Standardization of documentation and notification requirements for hand carrying
Submission of petition (PPQ 526) for movement of BCA into Federal Quarantine Facility	•Insufficient guidance documents explaining the permitting process •Unnecessary procedural steps at initial stage •No clear point of contacts identified for additional inquiry •Reluctance of carriers to ship BCAs due to liability issues	•USDA-APHIS-PPQ website and other published articles provide enough guidance information for permit petitioners[a] •The online submission (e-permit) create a quicker process •A toll free number and email contacts for additional information on organism permits are available online	•All the steps of the RA process should be clearly defined	•Improve website by the creation of online links on topics related to entomophagous BCAs •Provide additional guidance documents on relevant information on legislation/ regulations, permitting unit organization, permitting process with corresponding time frame

Table 5-2. Continued

Components of the permitting process	Stakeholders (permit petitioners)	Decision-makers	Issue themes	Recommendations
Submission of petition (PPQ 526) for release of BCA into environment	•Frequent loss petitions/documents •Inability to track updated petition submission status •Lack of information about the data requirements	•Submission status can be tracked online •Permit petitioners can communicate with PPQ staff through emails •Data requirement format might followed Regional Standard for Phytosanitary Measures # 12 as recommended in USDA-APHIS-PPQ website	•Lack of transparency and reliability of risk assessment process	•Designated risk analyst assigned to petitions and in direct contact with petitioner •Online access to previously approved petitions for guidance •Improve quality of RC interactions between permit petitioners and decision-makers
External Review	•Lack of standardized peer review process •Lack of external consultation	•Option of external consultation with North American Plant Protection and Fish and Wildlife Service representatives during decision-making process	•Decision-making process is arbitrary and subjective •Integrate involvement of an Expert Group during decision-making process	•Creation of an Expert Group representing stakeholders interests •Development of a standardized guidelines for evaluation
Decision-Making Process	•Difficulty in balancing appropriate level of environmental safety •Undefined decision criteria •Blend of two different expertise (scientific and bureaucratic) •No appellate system	•Difficulty in balancing appropriate level of environmental safety	•Risk assessment process is inefficient •Decision-making process is inadequate	•Standardized approach to testing non-target impacts •Possibility to resubmit petition after conducting more research upon recommendations
Submission of petition (PPQ 526) for release of BCA into environment	•Frequent loss petitions/documents •Inability to track updated petition submission status •Lack of information about the data requirements	•Submission status can be tracked online •Permit petitioners can communicate with PPQ staff through emails •Data requirement format might followed Regional Standard for Phytosanitary Measures # 12 as	•Lack of transparency and reliability of risk assessment process	•Designated risk analyst assigned to petitions and in direct contact with petitioner •Online access to previously approved petitions for guidance •Improve quality of RC interactions between permit petitioners and decision-

Table 5-2. Continued

Components of the permitting process	Stakeholders (permit petitioners)	Decision-makers	Issue themes	Recommendations
		recommended in USDA-APHIS-PPQ website		makers
Issuance of Letter of No Further Regulation/ Accordance with State laws and regulations	•Lack of authority from USDA-APHIS-PPQ to regulate entomophagous BCAs •Inconsistency of coordination between the State and Federal level •Confusion between Federal permits versus State approvals	•The Plant Protection Act (2000) gives a broaden authority to USDA-APHIS-PPQ to regulate entomophagous BCAs •	•Authority and jurisdiction during decision making process is unclear	•Create direct link between USDA-APHIS-PPQ and State Plant Regulatory Officials

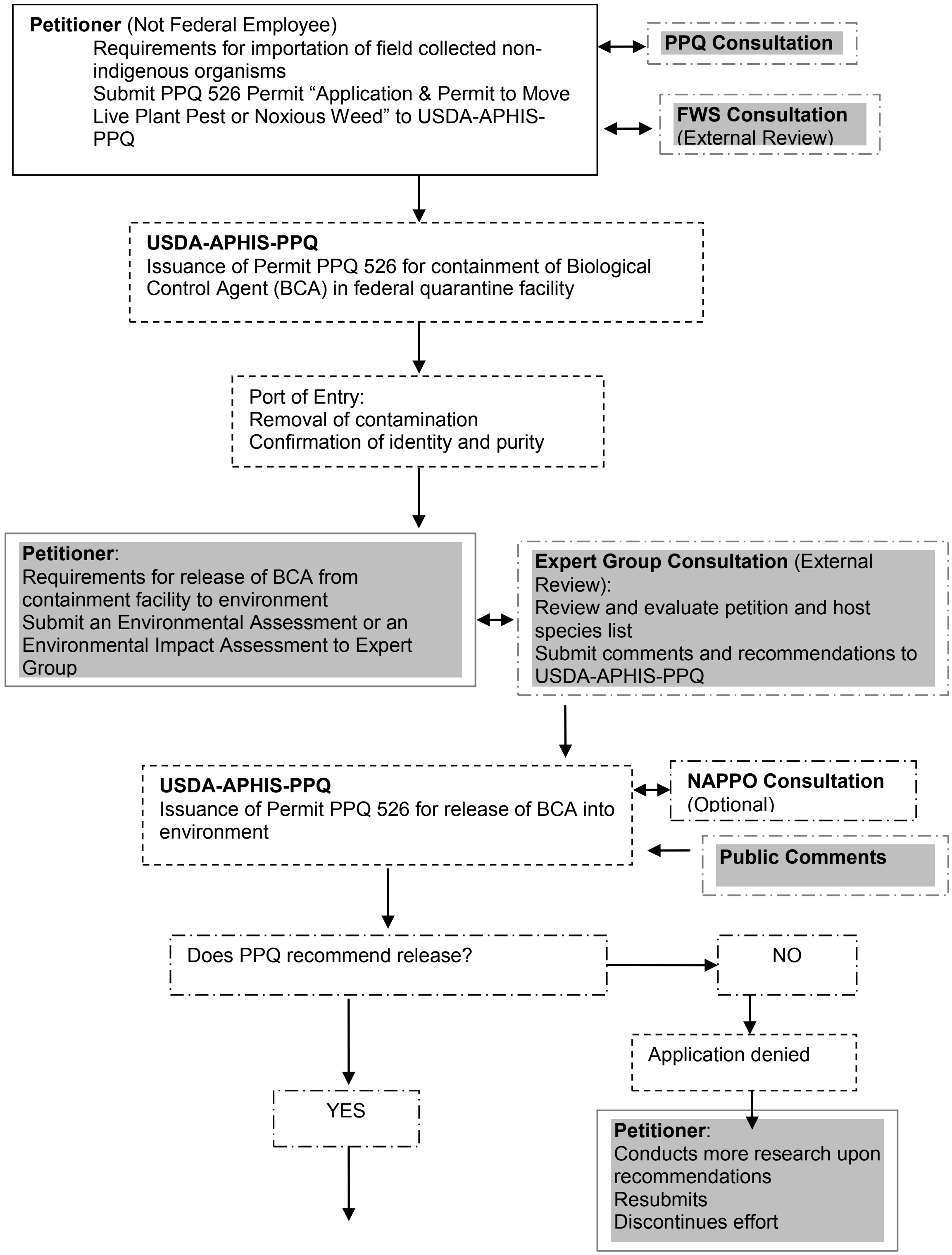


Figure 5-1. Novel risk assessment process for the permitting process for importation and release of entomophagous BCAs. Steps in gray boxes are additional to existing pest risk analysis process.

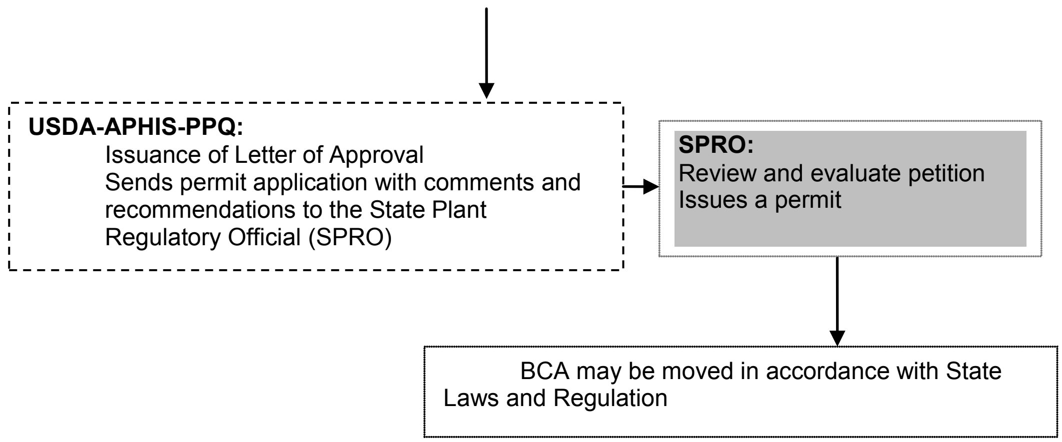


Figure 5-1. Continued

CHAPTER 6
EGG PARASITOIDS ATTACKING *CACTOBLASTIS CACTORUM* (LEPIDOPTERA: PYRALIDAE) IN NORTH FLORIDA

The cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae), is often cited as the perfect example of a successful weed biological control agent (Moran & Zimmermann 1984). In 1925, the cactus moth was introduced from its native Argentina into Australia to control prickly pear cactus, *Opuntia* spp., which had originally been brought into Australia from Mexico for commercial purposes (Dodd 1940; Mann 1970). The cactus became invasive and made large tracts of rangeland unfit for grazing cattle. Within a few years after the introduction of *C. cactorum* into Australia, US $6 million worth of rangeland was restored, equivalent to more than US $60 million in today's dollars (Dodd 1940; Williamson 2009). Based on these promising results, *C. cactorum* was imported from Australia to South Africa, Mauritius, and Hawaii to manage other non-native and invasive *Opuntia* spp. (Moran & Zimmermann 1984). In 1957, *C. cactorum* was introduced into several Caribbean islands (Nevis, Montserrat, and Antigua) to control native as well as non-native *Opuntia* spp. (Simmonds & Bennett 1966). Unfortunately, the implementing agencies did not fully consider the potentially injurious environmental impacts of *C. cactorum* if this insect were to move to neighboring countries where some species of *Opuntia* are important native species and some are important commercially (Stiling et al. 2004).

The first record of *C. cactorum* in the U.S. was from Bahia Honda Key, Florida, in October 1989 (Habeck and Bennett 1990; Dickel 1991). It is uncertain how the moth arrived in Florida, but several interceptions of Caribbean ornamental *Opuntia* spp. infested with *C. cactorum* were found at ports of entry in south Florida during the 1980s and 1990s (Pemberton 1995; Zimmermann et al. 2001; Stiling 2002; Simonsen et al. 2008). Since its appearance in Florida, *C. cactorum* has become a threat to native *Opuntia* spp. in North America. Current management options include the use of Pherocon 1-C Wing traps (Trécé Incorporated, Salinas, CA) baited with a 3-component synthetic sex lure (Suterra, LLC, Bend, OR) to identify the presence of the moth, coupled with removal of infested plants to reduce *C. cactorum* populations (Bloem et al. 2005; Hight & Carpenter 2009). Complementary to the detection, monitoring, and removal efforts, implementation of the Sterile Insect Technique (SIT) is being used to slow the geographic expansion of *C. cactorum* in the U.S. (Hight et al. 2002; Bloem et al. 2005; Bloem et al. 2007). In Mexico, localized infestations of *C. cactorum* on two islands of the state of Quintana Roo were eradicated in 2008 with an integrated program using pheromone traps, host removal, and the SIT (NAPPO 2006; NAPPO 2008; NAPPO 2009).

Bennett and Habeck (1995) suggested biological control as an additional control option that should be considered for *C. cactorum*. Pemberton and Cordo (2001a) reported that several larval and pupal parasitoids attacked the cactus moth in South America, including species of Hymenoptera (Braconidae, Chalcidae, and Ichneumonidae), and one Diptera (Tachinidae). They also reported on two chalcid species [*Brachymeria ovata* (Say) and *B. pedalis* Cresson] and one unidentified egg parasitoid from the family Trichogrammatidae attacking *C. cactorum* in Florida. Logarzo et al. (2009) found the larval parasitoid *Apanteles alexanderi* Brethes (Hymenoptera: Braconidae) and the egg parasitoid

Trichogramma pretiosum Riley (Hymenoptera: Trichogrammatidae) attacking *C. cactorum* in Argentina.

Trichogrammatid egg parasitoids have been used successfully for inundative biological control against major lepidopteran pests such as corn borers [*Ostrinia sp.* and *Diatraea sp.*, Crambidae], sugarcane borers (*Diatrea* spp., Pyralidae), and cotton bollworm (*Helicoverpa* spp., Noctuidae) (Li 1994; van Lenteren 2000). Egg parasitoids are easy to mass-rear under laboratory conditions for the application of inundative releases into large areas. Biological control can be used to complement and synergize the application of SIT (Knipling 1992; Gurr & Kvedaras 2010). Studies have demonstrated that the combination of both techniques is more efficient at controlling pest populations than either technique used by itself (Bloem et al. 1998). Synergistic interactions between the SIT and fruit fly biological control using parasitoids increased the suppression of pest fruit flies, even leading to eradication (Sivinski 1996; Rendon et al. 2006). SIT and biological control have been successfully combined to combat several lepidopteran pests, including *Cydia pomonella* (L.) (Tortricidae) (Bloem et al. 1998) and painted apple moth, *Orgyia anartoides* (Walker) (Lymantriidae) (Suckling et al. 2007). Radiation doses for sterilizing *C. cactoblastis* adults have been determined to produce partially sterile but fitter males which, when mated with wild females, generate sterile offspring (Carpenter et al. 2005). The combination of egg parasitoid releases and SIT has the advantage that parasitoids manage high pest densities, while SIT works best at low pest densities. In addition, release of sterile insects provides an additional source of eggs for egg parasitoids increasing the ratio of natural enemies to adult hosts. Egg parasitoids and sterile insects are self dispersing and consequently are able to cover wide areas (Sivinski 1996).

Field surveys were conducted in order to identify egg parasitoids already established in North Florida that attack *C. cactorum*. The distribution, seasonality, and parasitism parameters of the *Trichogramma* species attacking *C. cactorum* in northern Florida are also reported upon. The number of eggs/eggstick between different flight periods and sites was compared to assess host egg resource for egg parasitoids. The effect of *C. cactorum* eggstick size on level of parasitism was evaluated by comparing number of eggs from parasitized versus un-parasitized eggsticks. These data will be beneficial in promoting discussions and action on possible implementation of biological control for the cactus moth and, in particular, assessing the potential of an inundative biological control program against *C. cactorum* in North America. The use of inundative releases of *Trichogramma* wasps in combination with SIT will also be discussed.

Materials and Methods

Field surveys were carried out at six locations (Figure 6-1) in north Florida from July 2008 to December 2009. *Cactoblastis cactorum* adults have three annual flight periods in north Florida (April-May, July-August, and October-November) (Hight et al. 2005; Hight & Carpenter 2009). The selection of study sites was based on existing records of infestations from the literature, personal observations from preliminary surveys, and information provided by experts. Female *C. cactorum* place their eggs end to end to form a chain that looks like a short “stick”, and the egg mass is referred to as an eggstick. Although no extensive field surveys were conducted from May to July 2008 at St. Marks and St. George Island, eggsticks with eggs that appeared parasitized were collected and held under laboratory conditions (25 ± 1°C, 16:8 L:D and 40–60% RH) until parasitoids emerged. At survey locations, 20 to 30 healthy *Opuntia* spp. plants were chosen with no to minor feeding damage by cactus moth larvae and an average of at least 50 pads per plant. During weekly visits throughout all three flight periods, any new eggstick was identified by plant, pad, and its general location on the plant so the eggstick could be found during subsequent checks. A mark was made on the plant at the base of the eggstick with a felt tip pen and a red tape "flag" affixed to an insect pin placed near the eggstick to aid in finding the eggstick. The flag was labeled with a unique number to identify each eggstick.

The oviposition preferences of *C. cactorum* females on host plants were recorded by classifying the attachment of the eggstick to either a glochid at an areole, to a spine, or on the fruit. Observations on plant habitat and host eggstick distribution within the surveyed site and within the selected plant were collected to provide additional information on the host finding behavior of egg parasitoids. The number of eggs/eggstick was determined either by a direct count or by a correlated estimate of eggstick length to egg number (2.62 ± 0.013 eggs/mm). The ratio of eggstick length to egg number was calculated in this study by counting the number of eggs in a segment of eggstick, replicated on 20 eggsticks. Eggstick length was estimated *in situ* by placing a plastic string next to the eggstick and cutting a piece of equivalent length. The length of the piece of string was then measured to the nearest 0.01 mm with a metric micrometer.

Measurements of eggsticks were obtained so that the number of eggs/eggstick could be estimated if the eggstick was lost before it could be collected and directly counted. The fate of each eggstick was determined by making weekly visits to each site to evaluate the status of previously tagged eggsticks. The fate of each eggstick was categorized as follows: eggstick lost; predated (visible chewing damage) eggs in the eggstick versus non predated eggs; or parasitized eggs in the eggstick (black eggs formed before *C. cactorum* larvae successfully developed). Eggsticks were collected if they were damaged during evaluation or measurement, eggs of the eggstick had hatched, or eggs appeared predated or parasitized. Eggsticks with viable eggs were collected and held in small plastic cups (30 ml) under laboratory conditions (25 ± 1°C, 16:8 L:D and 40–60% RH) to record hatch rate. Eggsticks with parasitized eggs were collected and monitored in the laboratory to determine the emergence rate, number of eggs/eggstick attacked by parasitoids, number of parasitoids emerging per parasitized egg, and to ascertain the identity of the parasitoids. Parasitoid specimens were submitted to R. Stouthamer, Department of Entomology, University of California, Riverside, for molecular identification. The sequencing of ribosomal DNA Internal Transcribed Spacer 2 (ITS 2) was used to identify the different species of egg parasitoids.

Data Analysis

The numbers of eggs/eggstick at different flight periods for each surveyed location and the average number of eggs/eggstick at each site were log transformed before analyses to satisfy the assumptions of the analysis of variance. One-way analysis of variance (PROC GLM) was applied to the log transformed data and multiple comparison of means was made with the least statistically difference (LSD) test. Comparison of number of eggs/eggstick that was parasitized versus number of eggs/eggstick not parasitized also was evaluated with a one way analysis of variance (PROC GLM). Because only a few eggsticks with parasitized eggs were collected in this study (see text below), comparisons between eggsticks with parasitized eggs were made against the same number of randomly selected eggsticks with un-parasitized eggs. Variation between the number of eggs for parasitized eggsticks and the number of eggs for the randomly selected un-parasitized eggsticks was analyzed using a folded F test (Davis 2007). Because the variances in numbers of eggs for eggsticks with parasitism and number of eggs in eggsticks without parasitism were not statistically different, means of these two groups were compared with a two sample t-test. A Pearson's Correlation Coefficient (r) was calculated to determine whether the numbers of eggs parasitized were dependent on the number of eggs/eggstick. The SAS® Statistical Software Version 9.2 (SAS Institute, Cary, North Carolina) was used to perform the statistical analyses. Estimates of central tendencies were reported as mean ± standard error of mean.

Results

Although host plant species of *Opuntia stricta* (Haworth) Haworth, *O. humifusa* (Rafinesque) Rafinesque, and *O. ficus-indica* (L.) P. Miller varied among the different geographic regions surveyed; the oviposition preferences of

C. cactorum females were similar on the different plant species (Table 6-1). In this study, parasitized eggsticks of *C. cactorum* appeared mostly on the areole/glochid structure of the pads (Table 6-1). In total 1,527 eggsticks with 91,013 *C. cactorum* eggs, not including 344 eggsticks missing from the field or lost during collection, were tagged on plants of *Opuntia* spp. (Table 6-2). Of all the eggsticks checked, 62% were collected on Okaloosa Island and had a mean of 59 ± 1.83 eggs/eggstick. The proportion of eggsticks examined in the laboratory as percentage of all eggsticks surveyed at the six field sites ranged from 53 to 100%, except for 2008 summer at St. George Island and St. Marks National Wildlife Refuge (NWR), in which only 30% and 24% respectively of the monitored eggsticks were examined (Table 6-2). The majority of the eggsticks from these two locations for this flight period were recorded as lost (Table 6-2). The cause for this high number of lost eggsticks is not clear. Several biotic and abiotic factors could have contributed to the high number of lost eggsticks. During summer 2008, 23% of eggsticks examined from St. Marks had eggs that were preyed upon compared with less than 3% in other locations. Although not directly observed at St. George Island or St. Marks, substantial predation of *C. cactorum* eggs by ants has been recorded in South Africa (Robertson 1984). Because the plants surveyed at St. Marks were located within 100 m of the waters of the Gulf of Mexico, strong winds characteristic of coastal regions could have knocked eggsticks off the plants. All other study sites were along the Gulf Coast; in none of them were the plants as close to the water as at St. Marks. In addition, heavy rainfall may have separated the eggsticks from plants, but we do not have any data on the severity of the rain storms at different study sites. *Cactoblastis cactorum* life table studies in Argentina (Logarzo et al. 2009) and South Africa (Robertson & Hoffmann 1989) identified rain and wind as major factors contributing to mortality of eggs.

Surveyed sites and oviposition periods were analyzed to evaluate their influence on number of eggs/eggstick. Eggsticks were collected for multiple oviposition periods at three sites (St. George Island, St. Marks, and Okaloosa Island) (Table 6-2). The numbers of eggs/eggsticks for the different oviposition periods were not statiscally different for St. George Island ($F = 1.84$, $df = 1$, $P = 0.18$), St. Marks ($F = 93.86$, $df = 3$, $P = 0.07$), or Okaloosa Island ($F = 0.22$, $df = 3$, $P = 0.88$). Because the numbers of eggs/eggstick for multiple oviposition periods were not different, eggsticks from all flight periods were pooled to calculate the means for those sites [St. George Island (62 ± 2.8), St. Marks (53 ± 2.8), and Okaloosa Island (59 ± 1.8)]. The pooled eggsticks were used to compare the number of eggs/eggstick between all six sites and differences were indeed found ($F = 11.44$, $df = 5$, $P < 0.0001$) (Table 6-2).

Female *C. cactorum* laid similar numbers of eggs/eggstick for each of the three oviposition periods but not at all six survey sites along the Florida panhandle. The longest eggsticks were observed at St. George Island, Pensacola Beach, and Okaloosa Island (Table 6-2). Significantly smaller eggsticks were recorded at St. Marks and Mexico Beach (Table 6-2). Panacea had significantly smaller number of eggs/eggstick than all other sites (Table 6-2). The cause of differences between eggsticks at the various sites was unclear.

Studies in South Africa identified differences in total fecundity of *C. cactorum* due to host plant species, the flight period when eggs were laid, and the temperature during oviposition (Robertson 1989). We did not distinguish eggsticks collected from different host plants (Table 6-1). While South African female *C. cactorum* had significantly higher fecundity during the summer flight (Robertson 1989), this study did not show any difference in number eggs/eggstick between flight periods in North Florida. *Cactoblastis cactorum* has a tendency to oviposit on plants with high nitrogen (Myers et al. 1981; Robertson 1987), but we have no direct measurements of plant quality at our sites.

Comparing the number of eggs/eggstick for eggsticks that were parasitized (38 ± 13.7) (Table 6-3) against un-parasitized eggsticks (61 ± 13.1) revealed there was a difference (pooled t test = 3.14, df = 12, P = 0.0085). Although the number of eggs/eggstick was highly variable, the variation of the number of eggs/eggstick for parasitized versus the randomly selected un-parasitized group was similar (folded F test = 1.10, df = 6, P = 0.91), suggesting that the difference found between the two groups was not driven by unequal or extreme variation. However, there was not a significant correlation between the number of eggs/eggstick and number of eggs parasitized by *Trichogramma* spp. (N = 7, r = -0.16, P = 0.74). Therefore, whereas female *Trichogramma* spp. parasitized eggsticks with small number of eggs, they did not parasitize more eggs as the number of eggs in an eggstick increased. The average number of eggs parasitized in an eggstick was 9 (± 5.8).

Ten eggsticks were found parasitized at three of the six sites surveyed (Pensacola Beach, St. Marks, and Okaloosa Island). Five of the parasitized eggsticks were found at Okaloosa Island. Parasitized eggsticks were found during all three oviposition periods of *C. cactorum* females: the spring flight (St. Marks and Pensacola Beach), summer flight (Pensacola Beach), and fall flight (Okaloosa Island). Of the 496 eggs in the ten parasitized eggsticks, a total of 89 eggs (or 18%) were parasitized, resulting in the emergence of 181 adult parasitoids with a sex ratio of 70% (± 14) females (Table 6-3). The level of parasitism by *Trichogramma* spp., relative to the total number of eggs examined during the different flight periods for each site, was less than 0.2% of total *C. cactorum* eggs collected (Table 6-3). We did not observe any parasitized eggsticks at St George Island, Mexico Beach, or Panacea. Two species of *Trichogramma* were reared from *C. cactorum* eggsticks in North Florida (Table 6-3) and identified by differences in IST2 sequences. *Trichogramma pretiosum* Riley was collected at St. Marks, Pensacola Beach, and Okaloosa Island, whereas *T. fuentesi* Torre was recovered only from Okaloosa Island. It was not possible to identify two collections of *Trichogramma* spp. from Okaloosa Island for two reasons: (1) because a good molecular sequence could not be obtained and (2) the sequence was not in the database and possibly represents a new species in the *T. pretiosum* group (R. Stouthamer, UC-Riverside, personnel communication).

Discussion

More than 15 million ha of agriculture and forestry worldwide are treated annually with *Trichogramma* egg parasitoids (van Lenteren 2000).

Trichogrammatid wasps have been used successfully in inundative release programs against lepidopteran pests in greenhouses and crop production worldwide (Smith 1996). Inundative releases of *Trichogramma* spp. have been implemented in Florida to control major lepidopteran pests of collards, cabbage, soybeans, bell peppers, tomatoes, corn, and tobacco (Martin et al. 1976). *Trichogramma pretiosum* is commonly found in the Western Hemisphere. This species has been released commercially against major lepidopteran pests such as cotton leafworm [*Alabama argillacea* (Hübner), Noctuidae], corn earworm [*Helicoverpa zea* (Boddie), Noctuidae], tomato pinworm [*Keiferia lycopersicella* (Walshingham), Gelechiidae], sugarcane borers (*Diatraea* spp.) (Crambidae), and cabbage looper [*Trichoplusia ni* (Hübner), Noctuidae] (Pinto et al. 1986; Hassan 1993; Li 1994; Monje et al. 1999). *Trichogramma fuentesi* has been recorded in countries in South America (Argentina, Columbia, Mexico, Peru, and Venezuela) and in the U.S. (Alabama, California, Florida, Louisiana, New Jersey, South Carolina and Texas) (Fry 1989, Pinto 1999). Its primary hosts are species from the family Noctuidae such as *H. zea* and *Heliothis virescens* (F.) and from the family Pyralidae such as *Diatrea saccharalis* (F.), *Ephestia kuehniella* Zeller, and *Ostrinia nubilalis* (Hübner) (Fry 1989; Wilson & Durant 1991; Pintureau et al. 1999; Querino & Zucchi 2003). They are also widely used for pest control in orchards (Olkowski & Zang 1990). The observed low incidence of the wasps in natural areas might be explained by unfavorable environmental factors or natural plant chemicals (Smith 1996; Romeis et al. 1997, 1999). However, contrary to other natural enemies, *Trichogramma* can be easily and cheaply mass-reared for the implementation of an augmentative biological control program.

The potential for inundative releases of *Trichogramma* spp. as a strategy against *C. cactorum* is currently being investigated using laboratory colony of *T. fuentesi* originating from insects reared from parasitized *C. cactorum* eggsticks. Biological characteristics (sex ratio, egg load, and longevity) and different behavioral mechanisms (influence of parasitoid age, density, and host age on parasitism) involved in host finding of *T. fuentesi* reared on *C. cactorum* eggs are being evaluated. Inundative releases of *T fuentesi* could be integrated into the current pest management strategy that is based on SIT and removal of infested hosts during the three flight periods of *C. cactorum*. The present field survey was useful in identifying potential biological control agents that might be integrated in a combined pest management strategy against *C. cactorum*.

Table 6-1. Sites surveyed in North Florida for egg parasitoids of *Cactoblastis cactorum* eggsticks on *Opuntia* spp. and additional information on moth oviposition preference.

Site	GPS coordinate	Dates eggsticks surveyed	Total number surveys	Species of *Opuntia* host plant	Number host plant examined	Total number eggsticks evaluated	Percent eggsticks at attachment location[a]			
							Areole/ Glochid	Spine	Fruit	Missing[b]
Pensacola Beach	N30.33083 W87.15869	Summer 2008 (Jul. 10-Sep. 10, 08)	10	*O. stricta* *O. humifusa* *O. ficus-indica*	20	120	50	34	16	0
St George Island	N29.39051 W84.53577	Summer 2008 (Jul.17-Sep.19, 08)	10	*O .stricta*	23	105	63	30	7	0
		Fall 2008 (Sep. 25, 08-Feb. 25, 09)	20		27	28	89	11	0	0
St Marks (NWR)	N30.07772 W84.18242	Summer 2008 (Jul. 15,-Sep. 12, 08)	18	*O. stricta* *O. humifusa*	30	45	80	13	7	0
		Fall 2008 (Oct. 01, 08-Feb. 25, 09)	20		30	9	78	0	22	0
		Spring 2009 (Apr. 17-Jul. 15, 09)	13		35	47	88	2	0	10
		Fall 2009 (Oct. 07, 09-Jan. 12, 10)			35	151	n/a	n/a	n/a	n/a

[a] Attachment locations of eggsticks that were not determined is indicated by “n/a”.
[b] Information about eggstick attachment failed to be recorded.

Table 6-1. Continued

Site	GPS coordinate	Dates eggsticks surveyed	Total number surveys	Species of *Opuntia* host plant	Number host plant examined	Total number eggsticks evaluated	Percent eggstick at attachment location[a]			
							Areole/ Glochid	Spine	Fruit	Missing[b]
Mexico Beach	N29.56969 W85.25250	Fall 2009 (Oct. 21-Nov.12, 09)	3	*O. ficus-indica*	n/a	29	n/a	n/a	n/a	n/a
Panacea	N30.0995 W84.22086	Fall 2009 (Oct. 21-Nov.12, 09)	3	*O. stricta* *O. ficus-indica*	n/a	65	n/a	n/a	n/a	n/a
Okaloosa Island	N30.39845 W86.59304	Fall 2008 (Oct. 08, 08-Feb. 27, 09)	21	*O. ficus-indica*	10	186	81	18.5	0.5	0
		Spring 2009 (Apr. 08-Jul.08, 09)	14		18	308	79	15	1	4
		Summer 2009 (Jul. 01-Sep. 25, 09)	18		18	280	77	19	2	2
		Fall 2009 (Sep. 18, 09-Jan. 12, 10)			25	151	n/a	n/a	n/a	n/a

[a] Attachment locations of eggsticks that were not determined is indicated by “n/a”.
[b] Information about eggstick attachment failed to be recorded.

Table 6-2. Number of *Cactoblastis cactorum* eggsticks collected, lost in the field, examined in the laboratory, and number of eggs per eggstick ± SE at different sites in North Florida for different oviposition periods.

Site	Flight period	Total number eggsticks tagged	Total number (percent) eggsticks lost	Percent eggsticks examined	Total number moth eggs examined	Mean number eggs/eggstick ±SE	Overall mean eggs/eggstick ±SE at each site[a]
Pensacola Beach	Summer 2008	120	69 (58)	42	7,402	62±1.5	62±1.5 a
St George Island	Summer 2008	105	84 (70)	30	6,685	64±2.0	62±2.8 a
	Fall 2008	28	13 (46)	53	1,614	58±3.6	
St Marks (NWR)	Summer 2008	45	35 (77)	24	3,088	68±2.9	53±2.8 b
	Fall 2008	9	4 (44)	55	513	57±3.3	
	Spring 2009	47	23 (46)	54	2,561	54±3.1	
	Fall 2009	151	0 (0)	100	6,917	46±1.4	
Mexico Beach	Fall 2009	29	0 (0)	100	1,522	52±3.0	52±3.0 b
Panacea	Fall 2009	65	0 (0)	100	2,892	45±1.9	45±1.9 c
Okaloosa Island	Fall 2008	186	61 (29)	71	11,118	60±1.4	59±1.3 a
	Spring 2009	308	3 (1)	99	20,527	61±1.2	
	Summer 2009	280	21 (8)	92	17,126	60±1.1	
	Fall 2009	151	1 (0.6)	99	8,638	57±1.2	

[a] Means with different letter are statistically different ($P < 0.05$).

Table 6-3. Location and date parasitized *Cactoblastis cactorum* eggstick was collected, identity of parasitoid species, number of eggs per eggstick, number of parasitized eggs, number of parasitoids emerged, female ratio, and parasitism level of egg parasitoids attacking *C. cactorum* in North Florida.

Site	Collection date	Flight period	*Trichogramma* sp.	Number eggs/ eggstick	Number parasitized eggs/eggstick # (%)	number parasitoids emerged	Percent females	Level of parasitism (%)
St Marks	05/15/08	Spring 08	*T. pretiosum*	73	5 (7)	8	75	n/a[a]
	05/15/08		*T. pretiosum*	78	17 (22)	34	85	
Pensacola Beach	04/22/08	Spring 08	*Trichogramma* sp.	88	19 (22)	18	77	n/a
	08/06/08	Summer 08	*T. pretiosum*	44	8 (18)	5	40	0.2
	08/13/08		*T. pretiosum*	18	6 (33)	10	70	
Okaloosa Island	10/16/08	Fall 08	*T. fuentesi*	20	2 (10)	11	73	0.1
	10/16/08		*T. pretiosum*	42	10 (24)	7	71	
	10/16/08		*T. fuentesi*	56	6 (11)	16	62	
	11/03/08		*Trichogramma* sp.	52	3 (6)	9	56	
	10/23/09	Fall 09	*T. fuentesi*	25	13 (52)	63	89	0.1

[a] Indicates that the level of parasitism was not determined.

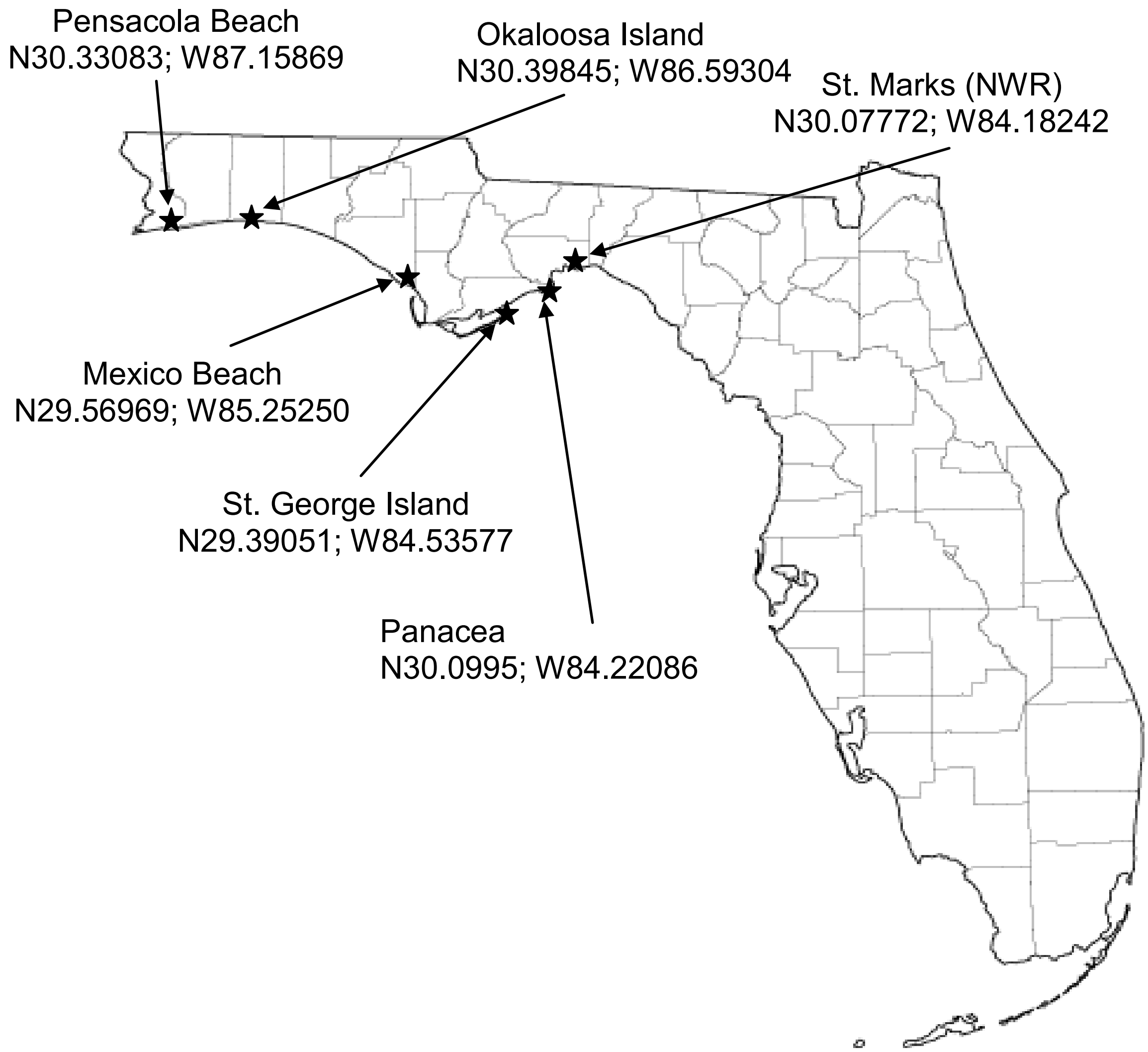


Figure 6-1. Locations surveyed for egg parasitoids of *Cactoblastis cactorum* in North Florida.

Figure 6-2. Ms. Paraiso surveying cactus plant for *Cactoblastis cactorum* eggsticks.

Figure 6-3. Parasitized *Cactoblastis cactorum* egg in an eggstick on *Opuntia* cactus pad.

CHAPTER 7
TRICHOGRAMMA FUENTESI—A NEWLY DISCOVERED POTENTIAL BIOLOGICAL CONTROL AGENT OF *CACTOBLASTIS CACTORUM*—EVALUATION OF BIOLOGICAL PARAMETERS

Previous surveys for natural enemies of *C. cactorum* in North Florida led to the discovery of *T. pretiosum* (Riley) and *T. fuentesi* Torre (Paraiso et al. 2011; Chapter 6). Inundative releases of several *Trichogramma* spp. such as *T. exiguum* Pinto & Platner against heliothine pests of cotton (Suh et al. 2000) or against the Nantucket pine tip moth [*Rhyacionia frustana* (Comstock)] in Virginia pine (Philip et al. 2005), have failed to provide an adequate level of pest suppression. Therefore, prior to field release it is important to undertake detailed studies of biological and ecological characteristics of prospective agents before release in the field (Ashley et al. 1974; van Lenteren et al. 2003; Dannon et al. 2010). Some of the important characteristics that need to be studied include: sex ratio, longevity, influence of parasitoid age, and host age on percent parasitism.

Trichogramma wasps require a source of carbohydrate to maintain basic physiological activities (Romeis et al. 2005). Generally, raisins and pure or diluted honey are used in experimental studies as a source of food to maintain *Trichogramma* colonies (Morrison 1985). The presence and nutritional quality of a supplemental source of food have been reported to increase the longevity of *Trichogramma* spp. (Laetimia et al. 1995; Oliveira et al. 2003). In addition, a source of carbohydrate can, in some cases, influence fecundity and egg resorption in parasitoids (Heimpel et al. 1997). Female parasitoid age is another biological characteristic that can affect the success of an augmentative biological control agent (Amalin et al. 2005). Studies have shown that trichogrammatids under or over a certain age are not able to parasitize their hosts

(Rajapakse 1992; Amalin et al. 2005). Host age also impacts the level of parasitism because trichogrammatid parasitoids favor young host eggs (Hagvar & Hofsvary 1986; Sequeira & Mackauer 1988; Amalin et al. 2005) and younger host age has an influence on parasitoid offspring fitness (Sequeira & Mackauer 1992, 1994).

In the present study, we examined the influence of three types of supplemental food sources on the longevity of *T. fuentesi* in the laboratory. In addition, optimum oviposition age of female *T. fuentesi* was assessed in order to obtain information for optimizing possible inundative releases against *C. cactorum*. This chapter also provides information on host age on parasitism rates, number of parasitoid progeny produced (fecundity), number of parasitoids emerged/parasitized egg (fertility), and sex ratio.

Materials and Methods

Rearing Procedures and General Methods

Experiments were conducted at the USDA, Agricultural Research Service and the Florida A&M University/Center for Biological Control Laboratory, Tallahassee, FL. *Trichogramma fuentesi* females used in the study were obtained from a colony established from field collected material. Species identity was confirmed by DNA-ITS 2 sequences performed by Dr. R. Stouthamer (Department of Entomology, University of California, Riverside, CA). *Cactoblastis cactorum* eggs derived from a colony maintained on artificial diet were used as hosts during parasitoid rearing procedures and for the experiments. To culture *T. fuentesi*, host eggsticks were glued on note card strips (4 x 2 cm) with non-toxic Elmer's® glue (Elmer's Products, Inc. Columbus, OH) (Figure 7-1). The note card strips were then placed into standard plastic petri dishes (9 x 2 cm) lined with filter paper. A fresh raisin was glued on a 1 x 1 cm note card in the center of the petri dish to provide a supplemental source of food for emerging wasps (Figure 7-2). Petri dishes were sealed with Parafilm® (Pechiney-Plastic Packaging, Menasha, WI) and arranged on plastic trays lined with moist wipes to maintain relative humidity at 60–80% (Figure 7-3). The cultures were maintained in a growth chamber at 28 ± 1°C, 16:8 L:D. Additional growth chambers maintained at 25 ± 1°C, 16:8 L:D and 60–80% RH were used for incubation of experimental units in all experiments.

Effect of Presence and Type of Diet on Female Parasitoid Longevity

We tested the effect of no supplemental source of food and presence of pure honey or raisins on the longevity of female parasitoids. Individual newly emerged (0–24 h old) *T. fuentesi* females were collected from the rearing colony and placed in a plastic petri dish (3.5 x 1 cm) lined with filter paper. A source of energy consisting of a drop of pure honey or a raisin was added to the center of the container. A set of control petri dishes did not have any supplemental source of food. Petri dishes were sealed with Parafilm® and arranged on a plastic tray lined with moist paper wipes to maintain the relative humidity. The containers were incubated until all females died. The experiment was replicated 20 times.

Influence of *T. fuentesi* Female Age on Percent Parasitism

The effect of female age on the percentage of host eggs parasitized was tested for females ranging from one to five days old. One-day old female parasitoids were isolated from the rearing colony and individually transferred to a petri dish (3.5 x 1 cm) lined with filter paper as described above. The experimental arena did not have a source of food for the wasps. Females were exposed to 60, two-day old, *C. cactorum* eggs. Petri dishes were sealed with Parafilm® and arranged on a plastic tray lined with moist paper wipes to maintain the relative humidity and then incubated. Eggsticks were replaced daily until the females were five days old (adult female parasitoids without any food live an average of four days—data from Experiment 1). The experiment was replicated 20 times. The number of parasitized eggs was determined on a daily basis.

Effect of Female Mating Status on Percent Parasitism

Newly emerged (0–24 h) females and males were collected from the rearing colony and stored in a petri dish for 24h to allow them to mate. Female wasps were distinguished from male based on antennae characteristics. Female *Trichogramma* have clubbed antennae with few short hairs on segmented flagellum while males have antennae with unsegmented flagellum and long hairs (Flanders 1965). Individual mated females were then transferred, as above, into a petri dish (3.5 x 1 cm) lined with filter paper. Single female parasitoids were exposed to 60, two-day old *C. cactorum* eggs for 24 h. The Petri dishes were sealed with Parafilm® and incubated. Female parasitoids and eggsticks were replaced daily. The experiment was replicated 20 times and the number of parasitized eggs and the percent parasitism were recorded. Rates of parasitism for these mated females were compared against unmated, 24h females used in Experiment 2.

Influence of Host Age on Percent Parasitism

Cactoblastis cactorum females lay an average of 70–90 eggs/eggstick (Zimmermann & Pérez-Sandi 2006). Eggs hatch in approximately 3 weeks (Zimmermann et al. 2001). A no-choice experimental design was used to assess the influence of ten different *C. cactorum* host age groups (1, 6, 7, 9, 11, 12, 13, 14, 15, and 20-day old) on percent parasitism. Three-day old, randomly chosen, honey-fed, mated female *T. fuentesi* were isolated from the rearing colony and each placed in an individual Petri dish (3.5 x 1 cm) lined with filter paper. In total, 60 host eggs belonging to one of the different age groups were placed in the center of the Petri dishes. Eggsticks were removed after 48 h and individually transferred into plastic cups (30 ml) for 10 days to allow all parasitoids to emerge. The experiment was replicated 20 times and the percent parasitism, total number of emerged parasitoids, number of emerged parasitoids per parasitized eggs, and sex ratio were determined.

Statistical Analysis

Data collected were subjected to analysis of variance (PROC ANOVA & PROC GLM). The SAS® Statistical Software Version 9.2 (SAS Institute, Cary, NC) was used to perform the statistical analyses. The effect of female age on the number of eggs parasitized and percent of parasitism between females that did or did not have mating experience was tested with a general linear model ANOVA followed by Tukey 's test for the separation of means (PROC ANOVA). In addition, the effect of host egg age on percent level of parasitism, number of progeny, number of successfully emerged eggs, and percentage of females produced was analyzed by polynomial regression.

Results

Effect of Presence and Type of Diet on Female Longevity

The provision of a food supplement had a significant influence on the survival of female parasitoids. Without a supplemental food source, females survived for an average of 4 ± 0.58 days ($F = 23.14$, df = 1, 12, $P < 0.001$, $R^2 = 0.85$), whereas with a raisin or honey supplement, females lived for 8 ± 0.07 days ($F = 595.03$, df = 1, 12, $P < 0.001$, $R^2 = 0.99$) and 11 ± 0.79 days ($F = 670.73$, df = 1, 12, $P < 0.001$, $R^2 = 0.99$), respectively. Statistical analysis of the data showed a difference in the number of days parasitoids survived between honey and raisin fed females ($F = 43.20$, df = 2, 57, $P <$

0.001, $R^2 = 0.60$). The maximum survival time was 16 days for a female which was fed honey. Thus, the type of food also affected the longevity of the female parasitoids.

Influence of Female Parasitoid Age and Mating Status on Percent Parasitism

Percent parasitism varied significantly with female age ($F = 15.42$, df = 4, 190, $P < 0.0001$) (Table 7-1). The number of parasitized eggs/eggstick was the highest for one and two-day-old female parasitoids and this declined rapidly thereafter (Table 7-1). In addition, the results showed that parasitism increased when females were mated prior to exposure to host ($F = 14.78$, df = 1, 190, $P < 0.001$). Unmated females had none to about 20% of parasitism after the second day. In contrast, mated females were still parasitizing cactus moth eggs after four days (Table 7-1).

Influence of Host Age on Percent Parasitism

The number of parasitized eggs and the number of emerged parasitoids/eggstick declined with host egg age. ($F = 19.53$, df = 3, 196, $P < 0.0001$; and $F = 16.53$, df = 3, 196, $P < 0.0001$, respectively) (Table 7-2). The relationship between host egg age and the number of eggs parasitized was best represented by a cubic polynomial equation ($y = 4.81 – 1.02x + 0.074x^2 – 0.0018x^3$, $R^2 = 0.23$). *Trichogramma fuentesi* displayed a preference for one-day-old host eggs; the highest level of parasitism and number of progeny produced was recorded for one-day-old host eggs. Female parasitoids did not produce progeny in host eggs older than 14 days (Table 7-2). A cubic polynomial equation also described the relationship between egg age and the number of parasitoids emerged ($y = 16.17 – 3.56x + 0.27x^2 – 0.0065x^3$, $R^2 = 0.20$). However, host egg age did not have a significant influence on the sex ratio or on the total number of parasitoids emerging/parasitized egg (Table 7-2). Although polynomial regression revealed significant relationships between host egg age and the number of parasitized eggs and the number of parasitoids/eggstick, only about 20% of the variation in the data was explained by our models (Figure 7-4). The high significance level observed indicates the influence of host egg age, but additional factors are important in explaining the variation in our data.

Discussion

This study provides information on the biological parameters of *T. fuentesi*, a naturally occurring biological control agent that might have potential application in augmentative releases against *C. cactorum*. Although there is a considerable amount of information on the biology of some *Trichogramma* spp., little is known about this particular species. *Trichogramma fuentesi* has been recorded in the neotropics, including Argentina, Colombia, Mexico, Peru, and Venezuela and in several U.S. states (Alabama, California, Florida, Louisiana, New Jersey, South Carolina and Texas) (Fry 1989; Pinto 1999). Its primary hosts are mainly noctuid species (Fry 1989; Wilson & Durant 1991; Pintureau et al. 1999; Querino & Zucchi 2003). *Trichogramma* spp. are generally pro-ovigenic parasitoids (Pak & Oatman 1982; Fleury & Boultreau 1993). However, several studies have suggested that *Trichogramma* have synovigenic tendencies, as many species develop additional eggs as female age increases (Houseweart et al. 1983, Bai & Smith 1993, Mills & Kuhlmann 2000).

Trichogramma wasps require sugar as a source of energy to sustain major physiological processes (Romeis et al. 2005). In nature, Trichogrammatids may obtain sugar from floral nectar, extrafloral nectaries, honeydew, and plant sap (Wackers 2005). Food deprived parasitoids will first search for food resources before they search for hosts (Hegazi et al. 2000). In addition, sugar intake influences overall individual flight and foraging behavior (Forsse et al. 1992; Pompanon et al. 1999; Romeis et al. 2005).

Hegazi et al. (2000) observed that honey-deprived *T. cacoeciae* Marchal only attacked one host patch whereas fed females were more likely to move to a second host egg patch.

Other studies report significant increases in the longevity of *Trichogramma* spp. adults when they are provided with a supplemental food source [e.g., *T. minutum* (Riley) (Laetemia et al. 1995); *T. galloi* Zucchi (Oliviera et al. 2003); and *T. pretiosum* (Berti & Marcano 1993)], whereas others reported no differences in longevity if adults were or were not fed [*T. demoraesi* Nagaraja (Oliviera et al. 2003)]. Female *T. fuentesi* without supplemental food never lived more than 5 days. Preferences for sucrose over fructose and glucose have been shown for Hymenoptera (Fonta et al. 1985; Cornelius et al. 1996; Koptur & Truong 1998). The main sugars in honey are the monosaccharides fructose and glucose. Additionally, various oligosaccharides such as sucrose, maltose, trehalose and turanose have also been detected (Siddiqui 1970; Doner 1977). Honey also contains proteins, enzymes and amino acids (Vela et al. 2007). As in honey, the main sugars in raisins are fructose and glucose with minimal amount of sucrose. Raisins are a good source of vitamins, minerals and phytochemicals (Williamson & Carughi 2010). In the present study, we examined the influence of the presence and the type of food source on the longevity of *T. fuentesi* females. We observed that a supplemental source of food prolonged the longevity of *T. fuentesi* by an average of 11 days when given honey and 8 days when given a raisin. Although a comparison of the nutritional quality of food sources was not done, the observed differences in longevity may be explained by the readily accessible carbohydrate in honey as compared to the raisin diet.

Various studies have shown that the parasitoid age also can affect the level of parasitism at the time of release (Hentz 1998; Honda & Kainoh 1998). Knowledge of optimal ovipositional time for *T. fuentesi* females provides information on when they are the most fecund. This information is important in deciding at what age parasitoids should be released in the field in order to obtain a higher levels of parasitism (Amalin et al. 2005). In this study, one to two-day-old mated female exhibited the highest level of parasitism (Table 7-1). This level of parasitism decreased from the third day until the death of the parasitoids. The highest level of parasitism was observed in one-day-old unmated parasitoids and one to three-day-old mated females (Table 7-1). Gregarious egg parasitoids, such as *Trichogramma* spp., can mate locally at emergence or disperse and mate later with a non-local mate (Martel & Boivin 2004). Hardy et al. (2007) reported studies showing that mating did not affect oviposition behavior of parasitoids wasps. Other studies have shown differences in oviposition behavior between virgin and mated females. Mated females oviposited immediately while unmated females laid few eggs in hosts and consequently mated with their sons (van den Assem & Visser 1976; van den Assem et al. 1982). Studies also have shown that although mating resulted in a larger number of progeny (Tagawa et al. 1987), it did not affect the number of parasitized hosts (King et al. 2000). In this study, percent parasitism did not significantly increase with mating except for two-day old females (Table 7-1). Therefore, inundative releases of two-day-old mated *T. fuentesi* females should be used against *C. cactorum* eggs to increase control levels.

Younger and older *C. cactorum* eggs coexist in the field during each flight period. *Trichogramma* spp. generally prefer younger host eggs (Pak 1986; Godin & Boivin

2000; Takada et al. 2000). For instance, *T. dendrolimi* Matsumura females spent a longer time on younger host eggs (from one-to three-days old) and did not parasitize eggs older than four-days (Takada et al. 2000). In addition, the number of progeny decreased significantly with older host eggs (Hiehata et al. 1976; Hinz & Andow 1990; Miura & Kobayashi 1998; Takada et al. 2000). In this study, the highest level of parasitism and number of parasitoid progeny was observed on one-day-old host eggs (Table 7-2). The number of eggs parasitized decreased as the host eggs age increased. *Trichogramma fuentesi* females did not parasitize *C. cactorum* eggs older than 13-days old (Figure 7-1). Older host eggs have been shown to be less suitable for *Trichogramma* egg development due to depletion of essential nutrients (Ruberson & Kring 1993). *Trichogramma* spp. are generally unable to develop from older eggs, usually because of host embryo rotation or cephalic capsule sclerotization (Guang & Oloo 1990). Therefore, young host eggs are preferantially selected for oviposition (Hagvar & Hofsvary 1986; Sequeira & Mackauer 1988; Godin & Boivin 2000). Female parasitoids generally lay a higher percentage of females in younger host eggs presumably due to their higher fitness. In this study, although sex allocation was not influenced by host age, female-biaised progeny were recovered in all experimental treatments (Table 7-2).

In summary, our results showed that a honey supplement must be provided in mass rearing programs to establish a sustainable production system of population of *T. fuentesi*. They also showed that one-to two-day-old mated *T. fuentesi* females should be released in the field to obtain a significant level of parasitism. Additionally, *T. fuentesi* displayed a preference for younger host eggs, and therefore, inundative releases should be timed to coincide with the beginning of the *C. cactorum* oviposition period or frequent releases made to attack newly layed *C. cactorum* eggs.

Table 7-1. The influence of age and mating status on number of *Cactoblastis cactorum* eggs parasitized by *Trichogramma fuentesi.*

Female *age* (Days) n = 20	Mean # of eggs parasitized (± S.E.)*	
	Mated	Unmated
1	5.35 ± 0.97 a A	4.00 ± 1.06 a A
2	5.90 ± 1.39 ab A	1.75 ± 0.78 ab B
3	2.80 ± 0.75 bc A	0.05 ± 0.05 b A
4	0.85 ± 0.65 c A	0.00 ± 0.00 b A
5	0.00 ± 0.00 c A	0.00 ± 0.00 b A

*Means with different lower case letter in columns or capital letter in rows are statistically different according to Tukey's Least Squared Means Comparison test at $P \leq 0.05$.

Table 7-2. Influence of *Cactoblastis cactorum* egg age on parasitization by *Trichogramma fuentesi*.

Age of *C. cactorum* (days)	Mean #			
Egg age	Eggs parasitized	Emerged parasitoids/eggstick	Emerged parasitoids/parasitized egg	% Females
1	3.95 ± 1.28 a	13.20 ± 4.70 a	3.42 ± 0.80	60 ± 14
6	0.50 ± 0.25 b	2.00 ± 0.89 b	5.53 ± 1.34	98 ± 2
7	0.65 ± 0.43 b	1.00 ± 0.55 b	2.00 ± 0.58	50 ± 29
9	0.80 ± 0.34 b	2.85 ± 1.43 b	3.32 ± 0.75	50 ± 0
11	0.30 ± 0.21 b	1.40 ± 0.82 b	5.83 ± 1.17	81 ± 9
12	0.30 ± 0.15 b	1.55 ± 0.87 b	6.50 ± 2.73	71 ± 5
13	0.20 ± 0.20 b	0.65 ± 0.65 b	3.25 ± 0.00	85 ± 0
14	0	0	0	0
15	0	0	0	0
20	0	0	0	0

*Means (± S.E.) with different letter are significant according to Tukey's Least Squared Means Comparison test at P ≤ 0.05. Because no significant relationship was found between age of host egg and number of parasitoids per parasitized eggstick or percent females, means were not separated.

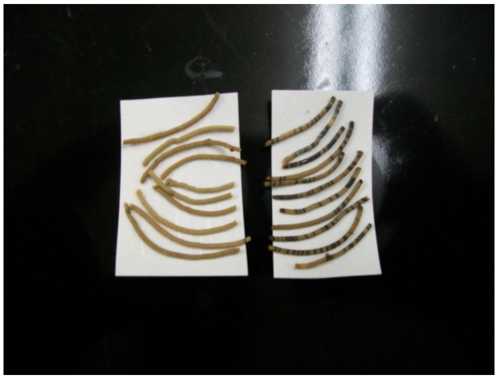


Figure 7-1. Non-parasitized (left) *Cactoblastis cactorum* eggsticks glued on paper strip next to glued parasitized eggs (right) in eggsticks by *Trichogramma fuentes*i.

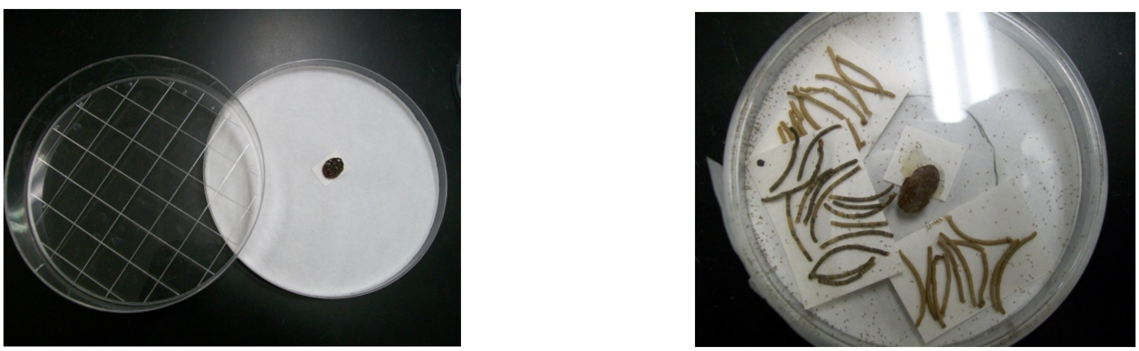


Figure 7-2. Rearing settings for *Trichogramma fuentesi* using *Cactoblastis cactorum* as host eggs.

Figure 7-3. Arrangement used to increase the relative humidity in rearing cultures of *Trichogramma fuentesi.*

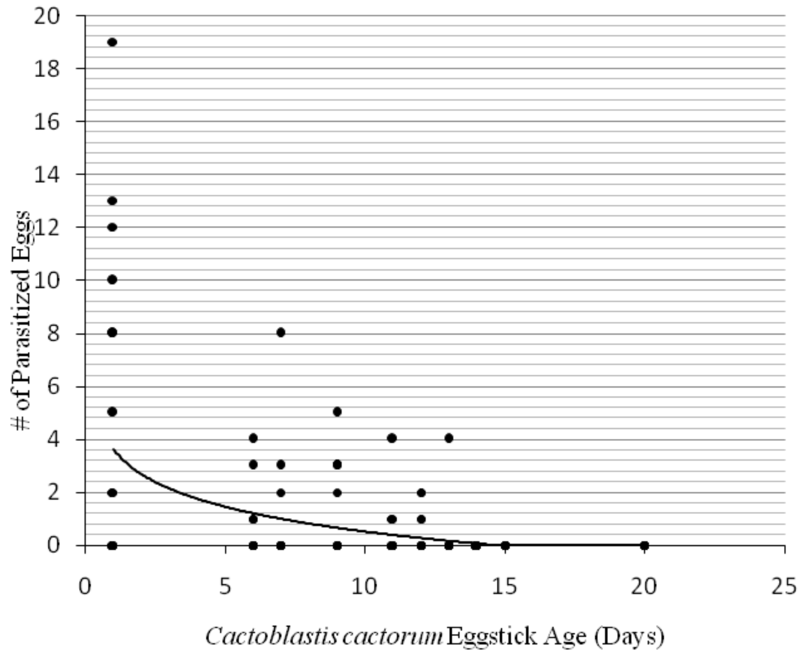


Figure 7-4. A regression showing the influence of *Cactoblastis cactorum* host egg age (1-20 days) on level of parasitism by *Trichogramma fuentesi*.

CHAPTER 8
TRICHOGRAMMA FUENTESI—EVALUATION OF FUNCTIONAL AND NUMERICAL RESPONSE

Development of an organism for augmentative biological control is usually preceded by studies to assess the biocontrol agent's potential for success. Such studies typically include an assessment of critical biological and behavioral criteria, such as dispersal ability, reproductive rate, longevity, and climatic tolerance (Kalyebi et al. 2005). Other parameters often measured are the functional and numerical responses, which provide information on natural enemy searching ability and attack rate efficiency (van Lenteren & Bakker 1976; Hassell 1978, 2000; Pandey et al. 1984; Houck & Strauss 1985; Walde & Murdoch 1988; Hugues et al. 1992; Wiedenmann & Smith 1993; Bernal et al. 1994; Kumar et al. 1994; van Alebeek et al. 1996; Berryman 1999; Fernandez-Arhex & Corley 2003). Functional response was first defined by Solomon (1949) as the relationship between the numbers of prey consumed by a predator with increasing prey density. Holling (1959) described three types of functional response: type I (linear), type II (hyperbolic), and type III (sigmoid). In type I functional response, the number of prey consumed increases linearly with host density. In a type II functional response, the number of prey attacked increases with increasing prey density and then decreases at higher host densities reaching a plateau due to prey handling time. At low prey density, a type III functional response has a similar increase in prey consumption as in type II, however attack rate decreases due to increased searching activity (Hassell 1978).

Numerical response is the other component of the host-parasitoid complex dynamic. It is described as the number of parasitoid offspring produced per host killed

(Ives & Settle 1996). In other words, the numerical response is an increase in reproduction rate due to changes in host density (Solomon 1949).

Trichogramma wasps usually display type I or type II functional responses under laboratory conditions (Smith 1996). In the context of implementing an effective inundative egg parasitoid release program, the use of species with a type II or type III functional response has been recommended (Laumann et al. 2008). Female parasitoids also react to increasing numbers of host eggs by increasing the number of progeny laid. Increase in parasitoid progeny contributes to the stability of the biological control agent population. This change in ovipositional behavior is defined as a numerical response (Podoler et al. 1978). Against this background, the functional response of *T. fuentesi* was examined to ascertain whether this species can be used in inundative program against *C. cactorum*.

Materials and Methods

Rearing Procedures

Experiments were conducted at the USDA, Agricultural Research Service and Florida A&M University, Center for Biological Control Laboratory, Tallahassee, FL. *Trichogramma fuentesi* females used in this study were isolated from a colony originating from field collected material. The identities of the wasp species were confirmed by R. Stouthamer (Department of Entomology, University of California, Riverside, CA) by differentiating DNA-ITS 2 sequences. Eggs from *C. cactorum* cultures maintained on an artificial diet were used as hosts for parasitoid rearing and as the source of experimental eggs. To culture *T. fuentesi*, host eggsticks were glued on note card strips (4 x 2 cm) with non-toxic Elmer's® glue. The note card strips were then placed into standard plastic petri dishes (9 x 2 cm) lined with filter paper. A fresh raisin was glued on a 1 x 1 cm note card in the center of the petri dish to provide a source of energy to emerging wasps. Newly emerged parasitoids were held together, with food but without hosts, for 3 days before conducting experiments to allow mating and feeding. Based on previous studies, *T. fuentesi* reared on *C. cactoblastis* eggs achieve their optimal oviposition activity 2 to 3 days after emergence (Chapter 7). Petri dishes were sealed with Parafilm® (Pechiney-Plastic Packaging, Menasha, WI) and arranged on plastic trays lined with moist wipes to increase relative humidity at 60–80%. The cultures were maintained in growth chamber at 28 ± 1°C, 16:8 L:D.

Functional and Numerical Response Experiments

The functional response of *T. fuentesi* was studied using two-day-old *C. cactorum* eggs at six different treatment densities (10, 20, 40, 60, 80, and 100 eggs). Preliminary experiments using various arena sizes (gelatin capsule size 0, glass vial 5 x 1.5 cm, plastic petri dish 3.5 x 1 cm, and plastic petri dish 9 x 2 cm) showed that the smaller size of petri dish (3.5 x 1 cm) reduced the variability in parasitoid attack rate. For each treatment a single eggstick was placed in an experimental arena lined with a filter paper (Figure 8-1). To assess the appropriate exposure time, single *T. fuentesi* females were confined with *C. cactorum* eggs for 6, 12, 24, and 48 h. A female parasitoid in contact with host eggs for 48 h exhibited the most consistent attack rate. A single three-day-old mated and fed female parasitoid was isolated from the adult colony and placed in the experimental arena for 48 h. Female parasitoids had no experience with host eggs before they were used in this experiment. Experimental eggsticks were recovered after 48h and placed in a small plastic cup (30 ml) for incubation. Cups were kept in growth chambers (28 ± 1°C, 16:8 L:D and 60–80% RH) until parasitoid emergence or for a maximum of ten days. The experiment was replicated 20 times for each treatment. The following data were recorded: The proportion of eggsticks parasitized the number of parasitized eggs, and the sex ratio for emerged parasitoids. The numerical response for *T. fuentesi* was determined by recording the number of emerged parasitoids and the number of emerged parasitoids per parasitized host egg for all treatments mentioned above.

Statistical Analysis

Data collected were analyzed using general linear models with number of host eggs as the source of variation (PROC ANOVA & PROC GLM) (SAS Institute 1999). The proportion of eggsticks parasitized, number of eggs parasitized, number of emerged parasitoids, number of emerged parasitoids per parasitized egg, and percentage of emerged parasitoids that were female were the dependent variables. When significant ($P < 0.05$) effects were detected, the relationship between number of host eggs and the dependent variable was examined using a regression model. The type of functional response was determined by performing a logistic regression of the proportion of parasitized eggs as related to their initial density. The type I functional response was estimated by the following linear equation:

(1) $$N_e = \alpha + \beta N_0$$

where N_e = number of eggs parasitized, N_0 = number of host eggs, and α and β = the intercept and slope of the prediction line, respectively.

The following curvilinear equation was used to estimate the type II functional response:

(2) $$N_e = aTN_0/(1 + aT_hN_0)$$

where N_e = number of eggs parasitized, N_0 = number of host eggs, a = instantaneous search rate (area covered by a searching parasitoid in a given amount of time), T = total time of host exposure to the parasitoid, and T_h = handling time.

In the type III functional response, the instantaneous search rate (a) was a function of host density (N_0) (Hassell et al. 1977) in the following relationship:

(3) $$a = (d + b\ N_0)/(1 = c\ N_0)$$

By substituting the value of "a" in Equation 3 to Equation 2, the following type III functional response equation was developed:

(4) $$N_e = d\ N_0T = b\ N_02T/(1 + c\ N_0 + d\ N_0T_h = b\ N_02T_h)$$

where parameters were described as above.

Results

The percentage of *C. cactorum* eggsticks parasitized by *T. fuentesi* varied from 45 to 90 but was not significantly influenced by egg density (from10 to 100 eggs per eggstick) ($F = 4.42$; df = 1, 4, $p = 0.10$) (Table 8-1). Although each female parasitoid was placed in close proximity to host eggs and enclosed in a relatively small arena (Petri dish, 3.5 x 1 cm), only 9% of the 6,000 total eggs evaluated in this study were parasitized. Host egg densities had a significant effect on the number of eggs parasitized ($F = 5.23$; df = 5, 109, $p = 0.0002$) and the number of emerged parasitoids/eggstick ($F = 2.85$; df = 5, 109, $p = 0.02$) (Table 8-1). Host egg densities also affected the number of emerged parasitoids/parasitized egg ($F = 2.62$; df = 5, 69, $p = 0.03$) but not the number of female parasitoids produced ($F = 1.13$; df = 5, 57, $p = 0.36$) (Table 8-1). The number of parasitized eggs as a function of egg density appeared to display a sigmoid curve; however, statistical analysis demonstrated that the data fit a linear equation ($N_e = 1.37 + 0.0636N_0$) (Figure 8-2). Although the number of parasitized eggs/eggstick and emerged parasitoids/eggstick (Figure 8-3) increased significantly with increasing host egg densities, these linear relationships explained very little (R^2 = 0.12 and 0.05, respectively) (Table 8-2) of the variation in the data.

Discussion

Results from functional and numerical response studies are considered key information for evaluating the likelihood of success of an introduced biological control agent (Fernandez-Arhex & Corley 2003; Lopes et al. 2008; Dannon et al. 2010). Functional response data provide information on the searching ability of a biological control agent at different host densities (Hassell 1978).

A review of experimental studies on functional response of insect parasitoids from 1959 to 2001 (36 studies) identified only one example of a parasitoid [*Eretmocerus eremicus* (Hynenoptera: Aphelinidae)] exhibiting a type I response (Fernandez-Arhex & Corley 2003). However, *Trichogramma* egg parasitoids were reported to display a type I response in more recent studies (Faria et al. 2000; Hoffmann et al. 2002; Mills & Lacan 2004). For egg parasitoids intended as augmentative biological control agents, density dependent functional response, such as a type II or III is often recommended (Laumann et al. 2008). Parasitoids displaying a type II functional response are considered even more advantageous as biological control agents because they are more efficient at low pest densities than are those with type III strategies (Lopes et al. 2008).

Ives et al. (1999) measured the behavioral response of *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae) to its host, the pea aphid *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae), on two similar host plants containing the same number of aphids. They found that the parasitoid attacked a higher number of hosts on one plant. The variability in the number of attacks reduced the foraging efficiency of the parasitoids, transforming a type II into a type I functional response (Ives et al. 1999; Fernandez-Arhex & Corley 2003). In our study, *T. fuentesi* did not show a significant variation in the attack rate of eggsticks at different host egg densities (Table 8-1), therefore the foraging efficiency was not affected when female *T. fuentesi* were presented with different numbers of host eggs. However, the percentage of the 6,000 total host eggs parasitized across the different densities was low (9%), despite the fact that the female parasitoids were released close to their host eggs (Table 8-1). Although the reasons for this low attack rate from *T. fuentesi* are unknown, later studies (Chapter 9) have shown that *C. cactorum* may not be a preferred host of *T. fuentesi*. In addition, low levels of parasitism (< 0.2%) by *Trichogramma* spp. on *C. cactorum* eggs were recorded in North Florida natural areas (Paraiso et al. 2011; Chapter 6).

A number of experimental studies have focused on host finding and parasitism behaviors of *Trichogramma* spp. on major lepidopteran crop pests (Munyaneza & Obrycki 1997; Sithanantham et al. 2001; Hommay et al. 2002). These studies showed that efficiency of host searching was increased by the parasitoids' ability to use infochemicals from its host and host plants. Conversely, little is known about parasitism mechanisms for egg parasitoids on lepidopteran pests found in natural areas. In nature, distribution of *C. cactorum* eggsticks on *Opuntia* plants is highly aggregated (Monro 1967). Field surveys conducted in North Florida showed that *Trichogramma* spp. preferentially attacked small sized *C. cactorum* eggsticks located on the areole/glochid structure of cactus pads (Paraiso et al. 2011; Chapter 6).

Trichogramma fuentesi displayed a type I functional response (Figure 8-1) to increasing numbers of *C. cactorum* eggs, suggesting that the attack rate increased linearly. Although the relationship between the number of host eggs and the attack rate was described by a linear regression curve (Figure 8-1), statistical analysis revealed that the relationship was weak. The low R^2 indicated that other factors were responsible for the main variation (Table 8-2). Such a functional response does not cause density dependent mortality for high host egg densities since the attack rate remained fairly constant. In our experiments, the search rate (i.e. the average encounters per host per unit searching time) for *T. fuentesi* among the different egg densities was low (0.063). In comparison, *T. minutum* Riley displayed a type I functional response to its preferred host, *Ephestia kuehniella* Zeller, and a search rate ranging from 0.37 to 1.75 (Mills & Lacan 2004).

A positive numerical response was considered a useful attribute of an efficient biological control agent (Huffaker 1974). In this study, the number of parasitoid offspring produced per host egg generally increased with host density (Table 8-1). A type I functional response, suggesting a lack of density dependence, was potentially compensated by the density-dependent aspect of the numerical response by *T. fuentesi*. *Trichogramma fuentesi* had the ability to change its ovipositional behavior depending on host egg density. A population of *T. fuentesi* attacking *C. cactorum* eggsticks would have its highest growth rate at high host numbers.

Trichogramma fuentesi females reproduce by arrhenotokous parthenogenesis, in other words they have the ability to reproduce without mating and can manipulate the sex ratio of their offspring. Arrhenotokous females typically produce fertilized eggs that become diploid female parasitoids whereas unfertilized eggs become haploid male parasitoids (Flanders 1965). Therefore, sex ratio of the offspring is controlled by female mating behavior (Nunney 1985; Luck et al. 1992) and the quality of the host (Charnov et al. 1981; Frank 1986). The female-biased sex ratio in *Trichogramma* contributes to reproductive success by allowing parasitoids to mate close to their host (Suzuki & Hiehata 1985). One of the advantages of local mating is that males will mate with their sisters, thereby reducing competition with brothers for mates (Hamilton 1967; Bulmer & Taylor 1980; Taylor 1981). Host size also influences sex ratio with a shift towards daughters occur in larger hosts due to increases in food resources (Charnov et al. 1981). In addition, the percentage of females produced in a patch of hosts is correlated with the fitness of the parent parasitoid (Hardy et al. 1998). Offspring production by *T. fuentesi* was characterized by a high percentage of females (Table 8-1). The mean percentage of female was not statistically different and did not increase at the various egg densities, suggesting that *C. cactorum* eggs were an acceptable host for *T. fuentesi*.

The low attack rate and type I functional response observed for *T. fuentesi* suggests that this parasitoid would not be an appropriate candidate for augmentative biological control for *C. cactorum*, particularly if the pest was present in high numbers. Although the response by *T. fuentesi* on *C. cactorum* may not be density dependent, the high rate of female to male offspring and the trend toward high numbers of parasitoids per parasitized egg suggest that *T. fuentesi* should be able to establish a stable population on *C. cactorum* eggs. However, the low level of parasitism suggests that *T. fuentesi* would not be an effective agent to use in inundative biological control programs against cactus moth.

Table 8-1. Parasitism of *C. cactorum* eggs at different densities by *T. fuentesi* (mean ± S.E.).

# eggs/eggstick	Proportion (%) eggsticks attacked (n = 20)	Mean		
		# Parasitized eggs/eggstick	# Parasitoids emerged/host egg	% emerged females
10	9 (45)	1.05 ± 0.35	2.90 ± 1.07	77 ± 10
20	14 (70)	2.95 ± 0.65	9.20 ± 2.08	68 ± 10
40	5* (33)	2.46 ± 1.60	9.80 ± 6.27	59 ± 19
60	14 (70)	8.45 ± 2.05	23.65 ± 7.07	79 ± 5
80	16 (80)	6.25 ± 1.21	13.70 ± 2.80	86 ± 3
100	18 (90)	6.40 ± 1.02	16.25 ± 3.84	80 ± 6

*n = 15

Table 8-2. Linear regressions of the proportion of *C. cactorum* eggs parasitized by *T. fuentesi* with increasing densities of eggs.

Variable	Intercept (α)				Slope (β)				R^2
	Parameter	± S.E.	t	Pr > t	Parameter	± S.E.	t	Pr > t	
Eggs parasitized	1.37	0.99	1.38	0.17	0.0636	0.02	3.92	0.0002	0.12
Parasitoids emerged	5.49	3.35	1.64	0.10	0.1381	0.05	2.54	0.0126	0.05
Parasitoids emergec per parasitized host egg	3.75	0.44	8.58	0.0001	2.830×10^{-5}	1.244×10^{-5}	2.27	0.0259	0.13

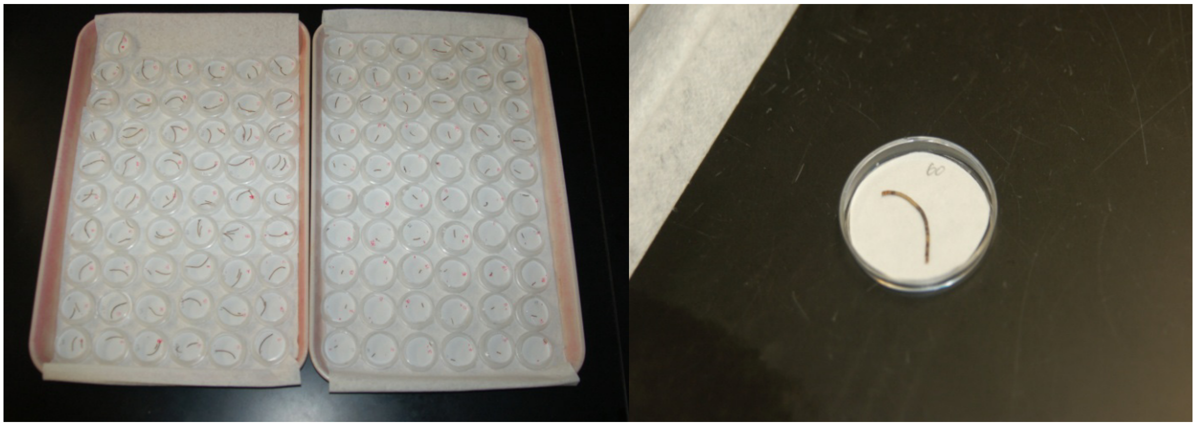


Figure 8-1. Petri dishes set-up to test functional response of individual *Trichogramma fuentesi* female parasitoids to various densities of *Cactoblastis cactorum*. *Trichogramma fuentesi* females in small Petri dishes as shown here.

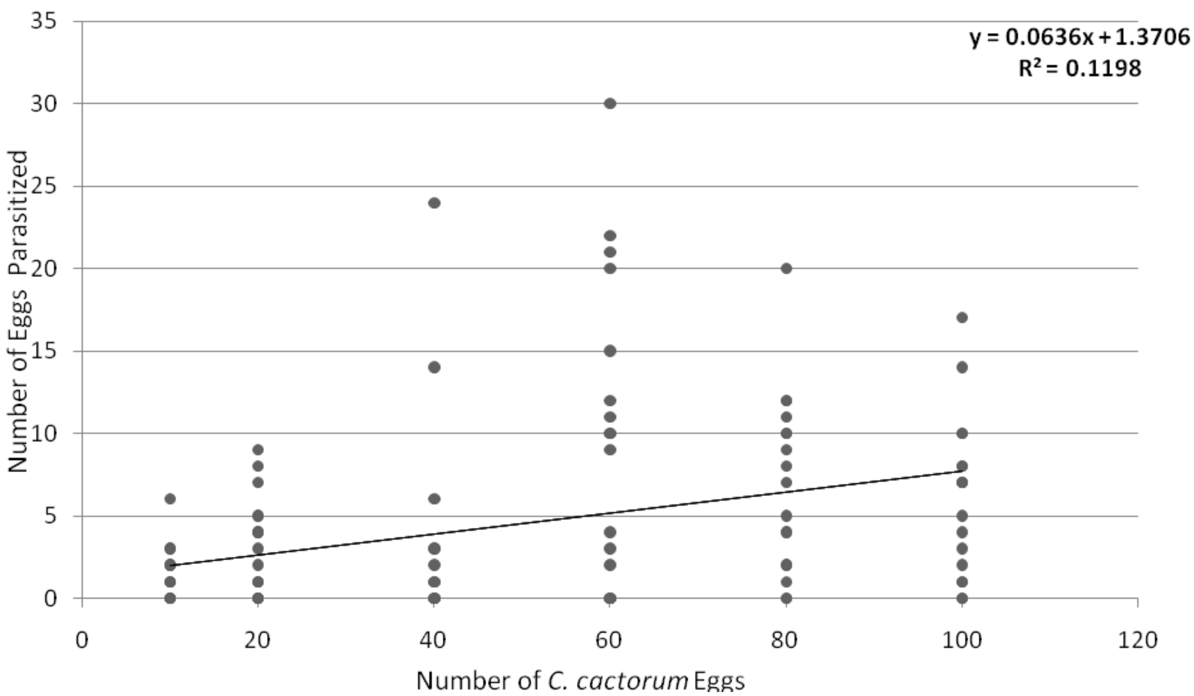


Figure 8-2. Functional response of *Trichogramma fuentesi* to different numbers of *Cactoblastis cactorum* egg densities. The continuous line is the best fit to the data represented. Regression line assumes a Type I functional response. Details of regression line and parameters are given in Table 8-2.

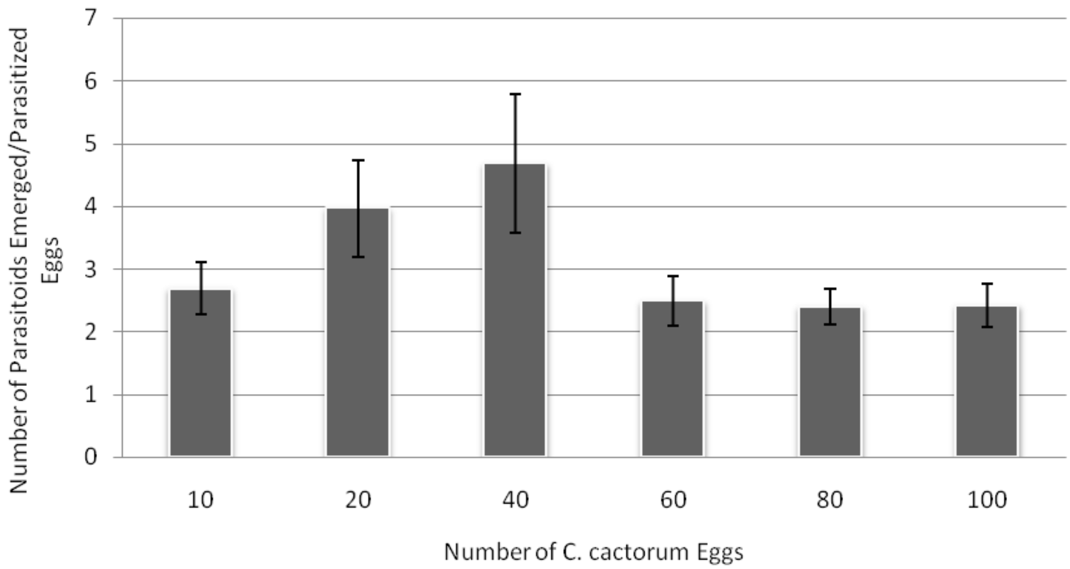


Figure 8-3. The number of *Trichogramma fuentesi* emerged per parasitized eggs (mean ± S.E.) with different *Cactoblastis cactorum* egg densities.

CHAPTER 9
TRICHOGRAMMA FUENTESI—BEHAVIORIAL NOTES AND HOST SUITABILITY

Cactoblastis cactorum (Berg) (Lepidoptera: Pyralidae) is an important pest of *Opuntia* cacti in North America (Zimmermann et al. 2001). Given its rapidly expanding geographical range, there is interest in exploring different control measures including the application of augmentative biological control. We conducted field surveys in North Florida to identify local natural enemies. These surveys led to the discovery of a naturally occurring parasitoid *Trichogramma fuentesi* Torre (Hymenoptera: Trichogrammatidae), attacking *C. cactorum* eggs (Paraiso et al. 2011; Chapter 6).

Trichogramma spp. have been used in inundative programs against major lepidopteran pests (Li 1994). Not surprisingly, much is known about their biology and ecology (Flanders 1965; Takada et al. 2000; Doyon & Boivin 2006). However, biological and ecological characteristics vary considerably across species and between populations on different hosts. Egg characteristics, such as size, chorion configuration, and egg placement can affect host quality (Corrigan & Laing 1994; Greenberg et al. 1998). As such, the efficiency of *Trichogramma* spp. released as biological control agents (BCAs) was often related to its host egg characteristics (Ram & Irulandi 1989; Baorong et al. 1992). Host finding studies demonstrated that *Trichogramma* spp. females assessed host quality based on attributes such as egg shape, size, and external or internal chemical cues (Consoli et al. 1989), and that this information influenced the females' allocation to her progeny (Schmidt & Smith 1985). The female examined a potential host by "drumming" her antennae on the host chorion for chemical cues (Schmidt & Smith 1986). Nonvolatile chemicals present on the surface of host eggs were perceived by receptors located on the antennae and tarsi of the females

(Godfray 1994). Female also walked on the host egg to assess surface area and diameter in order to estimate the host volume (Schmidt & Smith 1985, 1986). When an acceptable host egg was encountered, the female drilled into the host egg with her ovipositor which was covered in sensillae used to assess host suitability using internal chemical cues (Fisher 1971). The female laid one or more eggs depending on the host egg size (Hassan 1989). Studies showed that *Trichogramma* females attempted to parasitize any globular object between about 0.25–4.5 mm in diameter (Godfray 1994).

Because *Trichogramma* spp.are generally polyphagous, concerns have been raised about possible detrimental impacts on non-target hosts during inundative releases (Pinto et al. 1986; Andow et al. 1995; Orr et al. 2000). Host specificity tests are a crucial component in determining the non-target host assessment of the potential for entomophagous biological control agents (BCAs). Host specificity tests also help discern the potential field success of BCAs used in classical introductions or inundative releases against pests in agrosystems or natural areas (Pluke & Leibee 2006; Yong & Hoffman 2006). Results from host specificity tests help predict detrimental environmental impacts and address the safety of the proposed BCAs. Selection of non-target species for host specificity testing must be carefully considered, as the test results will give an indication on potential impacts on non-target organisms (Blossey 1995; Greathead 1995; McEvoy 1996). Kuhlmann et al. (2006) published recommendations based on a comprehensive review of existing methods on selecting host species for host range testing of parasitoids being evaluated in classical biological control programs. In the Kuhlmann's et al. (2006) method, an initial list is divided in a set of categories including ecological similarities and phylogenetic/taxonomic affinities.

Then, the list of non-target species is reduced by eliminating those with different spatial, temporal, morphological attributes, and species difficult to obtain (Kuhlmann et al. 2006). A similar process to Kuhlmann's et al. (2006), based on ecological, spatial, temporal and taxonomic similarities, was developed and used for selection of host species for entomophagous BCAs used in augmentative biological control. Although well-implemented for herbivores used in weed biological control programs, the assessment of non-target impacts for entomophagous BCAs is still in its infancy. There is no a standardized procedure for specificity testing for parasitoids (McEvoy 1996; Van Driesche & Hoddle 1997; Barratt et al. 2000). Laboratory host specificity studies have a higher possibility of giving either false positive or false negative outcomes (Kuhlmann et al. 2006). Two types of experimental designs are used to assess host specificity (McEvoy 1996; Mansfield & Mills 2004): No-choice tests assess the physiological host range which determines, in the case of parasitoids, the host species which are suitable to complete development and choice tests which evaluate the realized field or ecological host range and indicate the preference of egg parasitoids over acceptable hosts (McEvoy 1996; Mansfield & Mills 2004).

Against this background, the focus of this study was to: 1) Elucidate the developmental and reproductive biology of *T. fuentesi* on *C. cactorum* eggs; 2) Determine the key components of host finding behavior of the female parasitoids; 3) Carry out host specificity tests to evaluate the potential non-target effects on the native cactus moth and butterfly eggs in the laboratory using a no-choice test.

Materials and Methods

Trichogramma Rearing Procedures

Experiments were conducted at the USDA, Agricultural Research Service and FAMU, Center for Biological Control Laboratory, Tallahassee, FL. *Trichogramma fuentesi* females used in this study were isolated from a rearing colony which originated from field collected parasitoids in North Florida. Parasitoid identity was confirmed by R. Stouthamer (Department of Entomology, University of California, Riverside, CA). Eggs of *C. cactorum* from a mass-rearing colony maintained on artificial diet were used as hosts for rearing and also as the source for experimental eggs. To culture *T. fuentesi*, host eggsticks containing parasitized eggs were glued onto note card strips (4 x 2 cm) with non-toxic Elmer's® glue (Elmer's Products, Inc. Columbus, OH). The note card strips were then placed into standard plastic petri dishes (9 x 2 cm) lined with filter paper. A fresh raisin was glued on a 1 x 1 cm note card in the center of the petri dish to provide a source of energy to emerging wasps. Newly emerged parasitoids were provided with food and maintained without access to hosts for 3 days in order to allow mating before conducting experiments. Based on previous studies, *T. fuentesi* reared on *C. cactoblastis* eggs achieve their optimal oviposition activity 2 to 3 days after emergence (Chapter 7). Petri dishes were sealed with Parafilm® (Pechiney-Plastic Packaging, Menasha, WI) and arranged on plastic trays lined with moist wipes to increase relative humidity at 60–80% RH. The cultures were maintained in growth chamber at 28 ± 1°C, 16:8 L:D.

Developmental and Reproductive Biology

Development time of 20 randomly selected individual *T. fuentesi* females was studied using 1-d-old *C. cactorum* eggs. Twenty mated and fed females were randomly selected and isolated from the rearing colony and placed in a petri dish (9 x 2 cm) lined with filter paper containing 1-day old *C. cactorum* eggs. They were allowed to ovoposit over a 24h period. The rate of development for each stage was evaluated by observing changes in egg color at 24 h intervals. Previous studies showed that after parasitism, the host egg became tan when *Trichogramma* eggs hatched. *Trichogramma* larva fed internally and developed rapidly. During the last instar, dark melanin granules were deposited on the inner surface of the egg chorion, causing the host egg to turn black. Larvae then transformed to an inactive pupal stage. After few days, the adult wasps emerged from the pupae and escape the host egg by chewing a circular hole in the egg shell (Ruberson & Kring 1993). Parasitoid emergence rate was determined by counting the number of emergence holes from black eggs. The sex ratio was determined by sexing emerged dead adults under the microscope.

Host Finding Behavior Study

Host finding behavior of *T. fuentesi* females was studied in a plastic petri dish (30 x 10 mm) lined with filter paper under a stereoscopic microscope (Keyence-VH 5910) using online Windows Media to record all parasitoid behaviors. Behavioral events were scored using the event recording software Observer® XT version 8.0 (Noldus Information Technology, Wageningen, The Netherlands 2008). A mated and fed female parasitoid and a 1-day-old *C. cactorum* eggstick with ten eggs were placed in the petri dish for a 10 h observation period (from 9 am to 7pm). The experiment was replicated five times with different randomly chosen females. Preliminary observations showed that *T. fuentesi* females displayed six behavioral events: walking, drumming, resting, grooming, drilling, and egg laying. The females did not host feed during the observations. The walking behavior started when the female left the surface of the host egg for the filter paper and ended when she started drumming on the host egg. Drumming occurred when the female examined the host by walking on the egg surface while moving her antennae rapidly up and down (Schmidt & Smith 1989; Godfray 1994). The female occasionally became immobile which was described as resting and sometimes she would brush her antennae with her legs which corresponded to grooming. During drilling, the female created a hole in the host by twisting her ovipositor from left to right. Egg laying behavior started when the female completely inserted her ovipositor into the host egg which coincided with trembling movements of the abdomen (corresponding to the passage of the egg through the ovipositor) (Suzuki et al. 1984). The general sequence of oviposition behavior, total duration, mean duration, and timing of each behavior (the mean number of occurrences of a behavior per minute) was determined (Table 9-1).

Host Specificity Tests

Non-target host species selection

The development of a non-target host species list for *T. fuentesi* was based on recommendations developed by Kuhlmann et al. (2006). The initial list contained 22 species from 7 lepidopteran families (Table 9-2). The list was divided into six groups based on the following traits: taxonomic similarity, phylogenetic similarity, ecological affinities, endangered/threaten organisms, known weed natural enemies and other beneficial organisms, and organisms of economic value (Figure 9-1). No species were identified for group # 2, under phylogenetic similarity. At least one representative for each family in the remaining five groups was chosen. The species used in the final list were selected based on ecological, habitat, and temporal similarities of target and non-target hosts. A list of species tested can be found in Table 9-2. Several specimens of non-target species were purchased from a commercial butterfly rearing facility (Old Oak Butterfly Farm, Brooker, FL). During the development of the test species list entomologists, university researchers, federal scientists, and butterfly farm employees were consulted to ensure that all potential non-target organisms were included.

No-choice tests

Host acceptance and suitability was assessed by placing a single mated and honey-fed female *T. fuentesi* with ten eggs of only one host test species into a plastic petri dish (30 x 10 mm) lined with filter paper. Host eggs were arranged in the petri dish as single eggs and were less than 24-h old when the parasitoid was introduced into the petri dish. Petri dishes were incubated (25 ± 1°C, 16:8 L:D and 60% RH) until parasitoid emergence. The number of replications for each host test species depended on the availability of host eggs. The number of parasitized eggs (as indicated by black coloration) per female, the number of emerged parasitoids per host egg and the percentage of emerged female parasitoids was recorded.

Statistical Analysis

Data were analyzed for significant differences using analysis of variance using the SAS® Statistical Software Version 9.2 (SAS Institute, Cary, NC). To compare the percent parasitism between target and non-target hosts, a non-parametric Tukey's test was used. Estimates of central tendencies are recorded as mean ± standard error.

Results

Developmental and Reproductive Biology

The egg, larval and pupal stages of *T. fuentesi* reared on eggs of *C. cactorum* lasted an average 1 ± 0.07, 4 ± 0.05 and 5 ± 0.15 d, respectively. The sex ratio was female biased and the average percentage of females emerging from an eggstick was

74% ± 5. Adult female *T. fuentesi* provided with a source of carbohydrates lived an average of 11 days ± 0.79.

Host Finding Behavior

Female *T. fuentes*i included six types of behaviors: walking, resting, grooming, drumming, drilling, and egg laying. In general, the female wasp started the experiment by walking and searching for the host egg. Upon encountering the host, she drummed over the egg surface with her antennae, drilled through the chorion and oviposited into the host egg. Grooming was very infrequent (Table 9-1). Over a 60 min observation period, the female spent a significant portion of the time drilling (14.93 min) and laying eggs (19.66 min) (Table 9-1). Results showed that although time spent drilling (81.4 sec) was almost twice as long as the time dedicated to egg laying (49.2 sec), it was done less frequently with rates per minute (the mean number of occurrences of a behavior per minute) of 0.18 and 0.40, respectively (Table 9-1).

Host Suitability Experiment

Trichogramma fuentesi attacked all host species tested from all experimental categories tested. The percent parasitism by *T. fuentesi* on the non-target host eggs ranged from 30 to 75% (Table 9-3). The highest level of parasitism was observed for *Melitara prodenialis* Walker (Lepidoptera: Pyralidae) (75% ± 2) and *Dryas iulia* (Hübner) (Lepidoptera: Nymphalidae) (66% ± 5). The lowest level of parasitism was recorded for *Cactoblastis cactorum* (Berg) (11% ± 3.9). The statistical tests demonstrated that there was a difference in number of eggs parasitized among the various host species ($F = 8.61$, $df = 6, 142$, $P < 0.0001$). The level of parasitism for the native cactus moth (*M. prodenialis*) was significantly higher than the non-native cactus moth (*C. cactorum*). The number of progeny per host egg also was statistically different for the different hosts ($F = 11.32$, df = 6, 142, $P < 0.0001$). *Trichogramma fuentesi* exhibited gregarious tendency in most of the non-target host species with more than two parasitoids emerging per host egg. In contrast, females presented a solitary tendency for *Junonia coenia* Hübner (Lepidoptera: Nymphalidae) and *Vanessa cardui* L. (Lepidoptera: Nymphalidae) eggs. Female-biaised progeny were recovered from all experimental treatments (Table 9-3).

Discussion

The genus *Trichogramma* includes more than 100 species (Voegele et al. 1988) which vary greatly in their searching behavior, and host preferences (Hassan 1989). The general sequence of ovipositional behavior of *T. fuentesi* identified in this study differed from several other *Trichogramma* spp., such as *T. platneri* Nagarkatti, *T pretiosum* Riley, and *T. brassicae* Bezdenko (Blanché et al. 1996; Mills & Kuhlmann 2004) in that females did not host feed following oviposition. In general, the female wasp walked to a *C. cactorum* egg, drummed over the surface, drilled into the chorion and deposited an egg. Grooming and resting behaviors were observed very infrequently and host feeding was never recorded. The importance of host feeding among *Trichogramma* spp. is still unclear. Some studies suggest that the behavior does not necessarily provide nutritional resources for egg production but serves as a way to restore lost water (Blanché et al. 1996; Nurindah & Gordh 1999; Mills & Kuhlmann 2004). *Trichogramma fuentesi* behavior was similar to *T. platneri* and *T. pretiosum* (Mills & Kuhlmann 2004) in that the species failed to oviposit in some host eggs after drumming but before drilling. Host rejection is based on both chemical and physical characteristics and generally happens before completion of host examination (De Jong & Pak 1984).

When a *Trichogramma* female finds “preferred” host eggs, it will usually stay on them until all or most of them are parasitized. Less preferable host eggs may be totally rejected or the female may lay fewer eggs (Hassan 1989). Mills & Kuhlmann (2004) demonstrated that *T. pretiosum* and *T. platneri* spent a similar amount of time drumming and drilling on clusters of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs. In this study, although *T. fuentesi* spent a longer time drilling into a *C. cactorum* host egg than *T. pretiosum* and *T. platneri* on their host, the females displayed a comparable examination time on a host egg. Following the general sequence of ovipositional behavior of *T. fuentesi*, we expected to observe a similar number of behavioral events per minute (rate per minute) for drilling and egg laying. When the host was considered suitable, female *T. fuentesi* drilled into the chorion and deposited an egg. However, the rate per minute for drilling (0.18) was significantly lower than the rate per minute for egg laying (0.40) (Table 9-1). These differences in rate might be explained by females often laying additional eggs into host eggs that had already been parasitized. In fact, several studies also have confirmed that *Trichogramma* spp. require greater drilling time during superparasitism (Mills & Kuhlmann 2004).The reason for this superparasitism or lack of host discrimination behavior is unclear.

Our results suggested that, although *C. cactorum* was a suitable host it was less accepted than some of the other hosts tested. The native cactus moth, *M. prodenialis*, was a well-accepted and suitable host for *T. fuentesi*. The number of eggs parasitized was much higher for the native cactus moth (Table 9-3). *Trichogramma* spp. are believed to be much more habitat specific than host specific (Smith 1996; Pinto 1998). The native cactus moth often co-habitates in the same plant host with *C. cactorum* and both species have overlapping oviposition periods. Therefore, there is a high likelihood that the native host would be preferred during potential inundative releases of *T. fuentesi.* In addition, *T. fuentesi* parasitized a significantly higher number of butterfly eggs compared to *C. cactorum* eggs (Table 9-3). However, overall there was statistically no difference in the number of eggs laid per host egg between the non-target hosts and *C. cactorum* (Table 9-3). The high rate of parasitism for all non-target species tested and the lowest rate of parasitism for the target species suggested that *T. fuentesi* should not be considered as a BCA for inundative releases against *C. cactorum*.

Table 9-1. Time (seconds) and rate per minute allocated by female *Trichogramma fuentesi,* over a 60 min observation period, for each ovipositional related behavior when associated with *Cactoblastis cactorum* eggs.

Measure	Behavior					
	Walking	Grooming	Resting	Drumming	Drilling	Egg laying
Total duration (s)	641	101	309	663	896	1,180
Mean duration (s)	12.5	6.3	12.87	10.5	81.4	49.2
Rate/minute	0.85	0.27	0.40	1.05	0.18	0.40

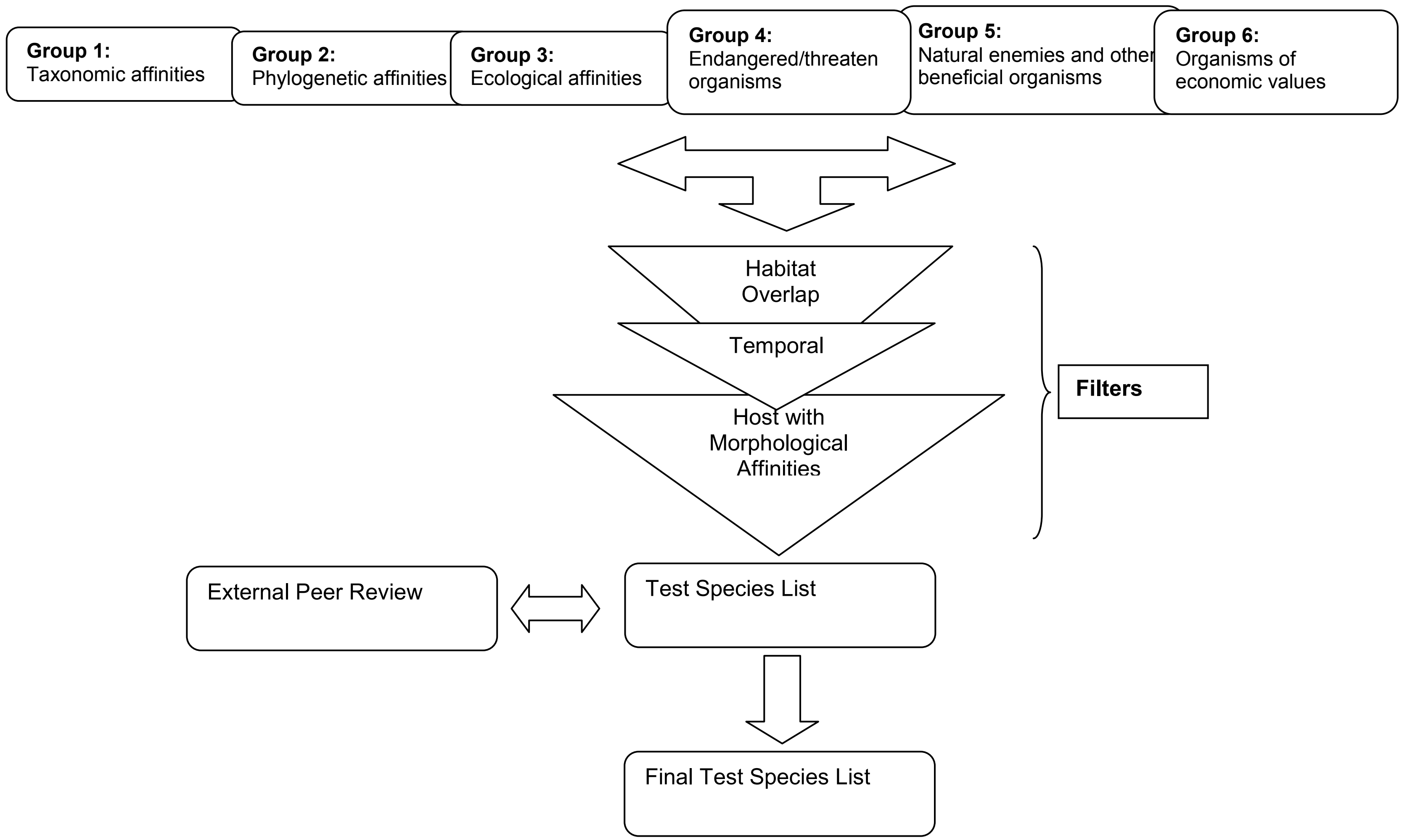


Figure 9-1. Methodology for selection of non-target species based on Kuhlmann et al. 2006.

Table 9-2. List of Lepidoptera species developed for complete host specificity testing of *Trichogramma fuentesi.* Hosts are grouped by test considerations following Kuhlmann et al. (2006).

Host	Common Name	Family	Justifications
Group 1-Taxonomic similarity			
Laetilia coccidivora (Comstock)		Pyralidae	Species taxonomically related in Florida
Melitara prodenialis Walker	Native cactus moth	Pyralidae	Species taxonomically related in Florida
Ozamia lucidalis (Walker)		Pyralidae	Species taxonomically related in Florida
Rumatha glaucatella (Hulst)		Pyralidae	Species taxonomically related in Florida
Group 3-Ecological similarity			
Anaea floridalis Johnson & Comstock	Florida Leaf-Wing butterfly	Nymphalidae	Species occurring in the same habitat
Danaus plexippus (L.)	Monarch butterfly	Danaidae	Species occurring in the same habitat
Melitara prodenialis Walker	Native cactus moth	Pyralidae	Species occurring in the same habitat
Papilio aristodemus ponceanus Schaus	Schaus swallowtail butterfly	Papilionidae	Species occurring in the same habitat
Strymon acis bartrami Johnson & Comstock	Bartram's Hairstreak butterfly	Lycaenidae	Species occurring in the same habitat
Group 4-Rare/Endangered species			
Anaea trogoglyta	Florida Leaf-Wing butterfly	Nymphalidae	Species rare or endangered in Florida
Papilio aristodemus ponceanus Schaus	Bartram's Hairstreak butterfly	Lycaenidae	Species rare or endangered in Florida
Strymon acis bartrami Johnson & Comstock	Bartram's Hairstreak butterfly	Lycaenidae	Species rare or endangered in Florida

Table 9-2. Continued

Host	Common Name	Family	Justifications
Group 5-Important herbivores			
Sameodes albiguttalis (Warren)		Pyralidae	pyralid moth used as biological control of waterhyacinth
Samea multiplicalis Guenee		Pyralidae	pyralid moth used against Salvinia and water lettuce plants
Vogtia malloi Pastrana		Pyralidae	Alligatorweed stem borer, pyralid moth used against alligatorweed
Group 6-Economic considerations			
Agraulis vanillae incarnata (Riley)	Gulf Fritillary butterfly	Nymphalidae	Commonly reared and sold in Butterfly Farms in Florida
Anartia jatrophae (L.)	Peacock butterfly	Nymphalidae	Commonly reared and sold in Butterfly Farms in Florida
Ascia monuste (L.)	Great Southern butterfly	Pieridae	Commonly reared and sold in Butterfly Farms in Florida
Danaus gilippus (Cramer)	Queen butterfly	Danaidae	Commonly reared and sold in Butterfly Farms in Florida
Danaus plexippus (L.)	Monarch butterfly	Danaidae	Commonly reared and sold in Butterfly Farms in Florida
Dryas iulia (Hübner)	Julia butterfly	Nymphalidae	Commonly reared and sold in Butterfly Farms in Florida

Table 9-2. Continued

Host	Common Name	Family	Justifications
Group 6-Economic considerations			
Heliconius charitonia (L.).	Zebra Longwing butterfly	Nymphalidae	Commonly reared and sold in Butterfly Farms in Florida
Junonia coenia (Hübner)	Buckeye butterfly	Nymphalidae	Commonly reared and sold in Butterfly Farms in Florida
Papilio cresphontes Cramer	Giant Swallowtail butterfly	Papilionidae	Commonly reared and sold in Butterfly Farms in Florida
Papilio glaucus (L.)	Eastern Tiger Swallowtail butterfly	Papilionidae	Commonly reared and sold in Butterfly Farms in Florida
Papilio polyxenes (F.)	Black Swallowtail butterfly	Papilionidae	Commonly reared and sold in Butterfly Farms in Florida
Phoebis sennae ebule (L.)	Cloudless Sulfur butterfly	Pieridae	Commonly reared and sold in Butterfly Farms in Florida
Vanessa cardui (L.)	Painted Lady butterfly	Nymphalidae	Commonly reared and sold in Butterfly Farms in Florida

Table 9-3. Parasitism (Mean ± S.E.) of potential host species by *Trichogramma fuentesi.*

Host species (10 eggs)	# replicats	% parasitism	# parasitoids/host eggs	% females
Cactoblastis cactorum	20	11 ± 3.9 a	2.9 ± 1.07 b	77 ± 10
Group 1				
Melitara prodenialis	13	75 ± 2 f	3.8 ± 0.4 c	60 ± 7
Group 3				
Danaus plexippus	9	58 ±3 d	2.3 ± 0.5 b	68 ± 4
Group 6				
Dryas iulia	26	66 ± 5 ef	3.1 ± 0.4 bc	79 ± 5
Junonia coenia	45	30 ± 7 b	1.2 ± 0.3 a	80 ± 6
Papilio glaucus	5	26 ± 5 b	3.8 ± 1.1 c	100
Papilio polyxenes	5	45 ± 2 c	3.0 ± 1.1 b	69 ± 4
Vanessa cardui	48	56 ± 6 d	1.1 ± 0.3 a	75 ± 6

*Means with different letters are statistically significant according to Tukey's Least Mean Comparison test at P ≤ 0.05.

CHAPTER 10
CONCLUSIONS

Classical biological control has been an accepted pest management tool worldwide for over a century, but its implementation is not without risks. There is currently a divergence of opinions in the U.S. between decision-makers and biological control practitioners on the appropriate way to assess the risks associated with the importation and release of entomophagous biological control agents (BCAs). The main objective of this dissertation was to improve the permitting process for the importation and release of entomophagous BCAs as implemented in 2007 in the U.S (Hunt et al. 2008). One suggestion made here for the development of an improved regulatory system was the implementation of efficient risk communication procedures during the Pest Risk Analysis (PRA). A survey was conducted on 500 biological control stakeholders among 5 professional affiliations (Federal, State, University, Private Sector, and Environmental groups) on risk communication during the permitting process for entomophagous BCAs. The objective of this survey was to characterize risk communication activities during the permitting process of entomophagous BCAs. In addition, the survey identified areas in the risk communication framework, as used in 2007, which could be improved. Results from the survey showed that the frequency of risk communication activities needs to be increased between decision-makers and permit petitioners. Survey respondents wanted additional transfer of information pertaining to the PRA process of entomophagous BCAs. In addition, respondents believed that stakeholders should be involved during the decision-making process.

The main difficulty of this study was to understand how to implement risk communication procedures within the permitting system. The proposed risk communication procedures put forward in this dissertation are needed in order to improve the efficiency of the risk assessment process without hindering the permitting procedure. Consequently, a comparative study of regulatory systems for the importation and release of entomophagous BCAs in eight countries was done to identify risk communication procedures that could be easily implemented into the U.S permitting system. The International Standard for Phytosanitary Measures (ISPM) # 3 was used to compare important criteria of PRA. This study showed that the selected countries have different approaches to risk assessment. The U.S. based their analysis on a “guilty until proven innocent” approach and the U.K. was considered highly risk adverse to the importation of BCAs. On the other hand, Australia based their assessment on a well-detailed risk criteria and ranking system. In addition, unlike the U.S., the comparative analysis showed that most of the countries used a form of participatory/collaborative process which included expert consultation and public participation. Based on these findings, a collaborative PRA process was proposed to improve the permitting process for entomophagous BCAs in the U.S.

The advantage of a collaborative-based risk analysis process is the increase of transparency during the decision-making process. In addition, this type of approach will decrease epistemic uncertainties by using recommendations from experts. In 2007, a permit (PPQ 526) was required by USDA-APHIS-PPQ for the importation, interstate movement, and environmental release of entomophagous BCAs. Information as described in the Regional Standard for Phytosanitary Measures # 12 was also required from the permit petitioner. In addition, recommendations from representatives from the North American Plant Protection Organization (NAPPO) were solicited. However, there was not a formalized review committee such as the one used for the permitting process for phytophagous BCAs so that a published decision could be made on all of the petitioners information. To increase decision-making transparency, one recommendation of this study was the development of an Environmental Assessment (EA) under NEPA (National Environmental Policy Act) which would publish all relevant information and require public comments. In addition, in the modified permitting process, an additional step for external review from an expert committee was suggested. During the course of this dissertation, several major regulatory reforms have occurred. In 2009, a proposed rule solicited public comments about the regulations related to permitting conditions as established in 7 CFR section 330 Parts 200-212 for entomophagous BCAs (CFR 2001). As of 2011, many of the regulatory changes proposed in this dissertation are currently in place. An EA is required for all permit petitioners, prior to environmental release of entomophagous BCAs (APHIS 2011). In addition, a sort of advisory committee is consulted by USDA-APHIS-PPQ during the decision-making process (APHIS 2011).

Another recommendation made in this dissertation for the development of a more efficient permitting process for entomophagous BCAs was the implementation of harmonized science-based procedures for the evaluation of risks. Several risk assessment procedures have been published attempting to estimate potential environmental and economic deleterious impacts of proposed BCAs (van Lenteren et al. 2003, van Driesche & Reardon 2004; Bigler et al. 2006). This dissertation used a real case study to assess environmental risks associated with the use of an entomophagous BCA. The cactus moth, *Cactoblastis cactorum* (Berg), is an invasive moth native to

South America attacking *Opuntia* cacti in the U.S. Field surveys conducted in Argentina identified an egg parasitoid from the family Trichogrammatidae attacking *C. cactorum* eggs. However, in 2008, because of the stringent requirements, lengthy permitting process, and time constraint inherent to this doctoral study, natural enemies already present in the U.S. were used for the evaluation. Consequently, field surveys were conducted in six locations in North Florida. Egg parasitoids from the family Trichogrammatidae, *Trichogramma pretiosum* Riley, *Trichogramma fuentesi* Torre and an unidentified species in the *T. pretiosum* group were found attacking *C. cactorum* eggs.

When implementing a classical biological control program to control an invasive pest, one major step consists of assessing possible non-target impacts. In addition, evaluation of important biological parameters is required to assess whether the entomophagous BCA is a suitable agent. Using the same rationale, this dissertation used up-to-date methods for evaluating environmental risk of *T. fuentesi* against *C. cactorum*. During risk evaluation, the best rearing conditions for the proposed BCA colony was determined to obtain accurate results. The presence, type of food source, and mating status were determined to have an influence on the fitness and percent of parasitism by *T fuentesi* on *C. cactorum*. The age range for both the egg parasitoid and target host were assessed and the optimal ages for optimal percent parasitism by *T. fuentesi* were identified. Data from the functional response experiment of *T. fuentesi* provided information on the female's searching ability and attack rate efficiency. The density independence of *T. fuentesi*'s functional response was compensated by the density dependence of the wasp's numerical response. This experiment showed that data from the numerical response also needed to be included in the assessment of this proposed BCA. For the evaluation of possible non-target impacts, step by step procedures for the development of host lists usually include species with similar biological, ecological, and taxonomic attributes to the target host. In this study, the non-target host species list used was developed using a modified version of the Kuhlmann et al. (2006) method. One important addition in the procedure was the consultation of an expert group during the development of the test species list. The group of experts was composed of entomologists, university researchers, federal scientists, and butterfly farm employees. This additional step assured that all potential non-target organisms were identified. However, the evaluation of host acceptance and suitability was limited by the availability of the hosts. In addition, the appropriate number of replications was difficult to determine. A consistent assessment of environmental impacts of a potential entomophagous BCA relied on the accurate selection of non-target species for testing, the appropriate experimental design to run host specificity tests, and the correct interpretation of results.

In 2007, the existing permitting process for the importation and release of entomophagous BCAs was considered inadequate by the biological control community. The ISPM # 3 describes responsibility and data requirements of parties involved prior to importation, shipment and release of entomophagous BCAs. However, it does not provide guidance on rigorous methods that might be used during risk assessment. This dissertation showed that to improve the efficiency of the permitting process, efforts must be given to both the regulatory system and the risk assessment process. There is a need for the development of a harmonized guidance document with robust procedures for the evaluation of potential detrimental environmental impacts from entomophagous BCAs.

APPENDIX A
INTERVIEW INITIAL LETTER

Florida Agricultural and Mechanical University
Tallahassee, Florida 32307-3100

CENTER FOR BIOLOGICAL CONTROL Telephone: (850) 412-7062
310 Perry-Paige Build. (South) Fax: (850) 561-2221

June 11th, 2007

Dr. XXXXXX
UF Department of Entomology
PO Box 110680,
Gainesville, Fl 32611-0680

Dear Dr. XXXXX,

Risk Communication in Biological Control

The Center for Biological Control at Florida A&M University is conducting research on aspects of risk analysis for entomophagous biological control agents. To this end we have a graduate student undertaking part of the research. One component of this work is focused on aspects of Risk Communication during the permitting process for entomophagous biological control agents with a view to develop an improved process.

This letter is to introduce the student, Ms. Oulimathe Paraiso, who is registered in the joint Cooperative Ph.D. Program between Florida A&M University and University of Florida, with Dr. Stephanie Bloem and myself as her major supervisors. Other members of her committee are: Drs. James Cuda, Norm Leppla, Robert McGovern and Marcia Owens.

As part of the research she will undertake a broad survey to better understand and quantify current practices and perceptions of both decision-makers and stakeholders. As the first step in this process of identifying the key issues, she plans to get the perspectives from a limited number of key decision makers and stakeholders (20 in totals) in order to help shape the survey instrument that will be distributed to broad spectrum of representatives from both groups. Because of your extensive background and knowledge of the issues, she plans to request your assistance during this first stage.

We hope you will be able to participate in this research.

With thanks.

Yours sincerely,

Moses T.K. Kairo
Director/Associate Professor

APPENDIX B
INTERVIEW PRE-NOTICE LETTER

Florida Agricultural and Mechanical University
Tallahassee, Florida 32307-3100

CENTER FOR BIOLOGICAL CONTROL
310 Perry-Paige Build. (South)

Telephone: (850) 412-7062
Fax: (850) 561-2221

June 18th, 2007

Dr. XXXXXXXXX
UF Department of Entomology
PO Box 110680,
Gainesville, Fl 32611-0680

Risk Communication in Biological Control

I am writing to follow up on the letter sent by Dr. Moses Kairo on June 11th 2007 and would like to request your assistance during this initial phase. I am specifically interested in understanding the different mental models associated with Risk Communication procedures employed during the importation of entomophagous biological control agents (BCAs). Understanding the different mental models used by decision-makers and stakeholders will contribute to the development of an improved Risk Communication framework.

Risk analysis (RA), the assessment of the likelihood of occurrence of an adverse outcome and the magnitude of the consequences of this outcome is composed of three major tasks: Risk Assessment, Risk Management, and Risk Communication. Guidelines on importation of BCAs have been developed at the international level (International Standard for Phytosanitary Measure # 3) and at the regional level (Regional Standard for Phytosanitary Measure # 12). The USDA Animal and Plant Health and Inspection Service (USDA-APHIS) in the context of safeguarding plant life and health have developed their own guidelines to conduct PRA. Implicit in these guidelines is the need for conducting a risk analysis prior to importation of BCAs. Thus an important component of the importation process is risk communication.

Mental models are representations of real or imaginary situations. They are used to anticipate an outcome. The main source of mental models is perception. Mental models shape decisions because they affect decision-maker's perceptions and beliefs. The decision-making process can be described as an interactive process between decision-makers and stakeholders. Risk Communication intends to supply decision-makers and stakeholders with the information they need to make informed independent judgments about risks. Therefore, it becomes an important tool to prevent unwanted outcomes. Recommendations for better Risk Communication are made in the ISPM #3. However, while Risk Communication is an essential component of the decision-making process it is often taken for granted.

The purpose of the current research project is to assess the mental models applied by decision-makers and stakeholders in the context of the Risk Communication procedures that take place during the entomophagous BCA permitting process. The approach used is based on methods developed by Morgan et al., 2002. This involves a series of five sequential steps as follows:

1. Create an expert model based on scientific data to determine the nature and magnitude of the problem.
2. Conduct interviews to elicit people's beliefs.
3. Create a confirmatory questionnaire to estimate the population prevalence of these beliefs
4. Use the results to determine which incorrect beliefs must be corrected.
5. Evaluate communication by testing the improved communication framework.

I am now at the second step and you have been identified as part of a carefully selected group of 20 people. All 20 persons are knowledgeable about the importation of BCAs. I have developed a short questionnaire and would also like to interview you after you have had a chance to respond. This step will essentially assist me to identify the critical issues in risk communication. Based on the information gathered during this step, I will develop a comprehensive questionnaire which will be sent to a broad range of stakeholders (1000 -1500 stakeholders).

The short questionnaire is being sent to you electronically and can be completed directly on the web. I will also mail to you a hardcopy if you prefer this medium. Please be assured that your answers to this survey will be completely confidential and they will only be released as summaries in which no individual's answers will be identifiable.

Should you have any concerns with or difficulties in responding to this survey please contact me at (850) 412-7062 or at oparaiso@ufl.edu

Sincerely,
Oulimathe Paraiso
Ph.D. Student

APPENDIX C
INTERVIEW BOOKLET

FAMU - CESTA

Center for Biological Control

Risk Communication & the Entomophagous Biological Control Agents Permitting Process

Florida A & M University

Thank you for helping with this survey of the different "mental" models used by biological control practitioners and regulators to assess and communicate risk during importation of entomophagous biological control agents.

All of the questions are open ended to enable you to fully express your opinions. Please feel free to simply make notes about your general beliefs and behaviors. Further in time you will be contacted to discuss your answers in more details. As with all surveys we conduct, your responses will be kept confidential.

After completion, please send your answers back electronically to Oulimathe Paraiso at oparaiso@ufl.edu

Should you have any difficulties in responding, please contact Dr. Moses Kairo at (850) 412-7062.

Thank you again for your participation!

Florida A &M University

1. At the international level, the International Standard for Phytosanitary Measures (ISPM) 3 “Guidelines for the Export, Shipment, Import and Release of Biological Control Agents and Other Beneficial Organisms” (see annex) requires that the importation of Biological Control Agents (BCAs) be based on a Pest Risk Analysis (PRA).

On a scale of 1 to 5 (1 being not familiar and 5 being very familiar), what is your degree of familiarity with ISPM 3?

What concerns, if any, do you have with the standard?

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Florida A & M University

2. At the regional level, the Regional Standard for Phytosanitary Measures (RSPM) 12 "Guidelines for Petition for Release of Exotic Entomophagous Agents for the Biological Control of Pests" (see annex) provides guidance on the information that should be provided when drafting a petition for release of exotic entomophagous BCAs.

On a scale of 1 to 5 (1 being not familiar and 5 being very familiar), what is your degree of familiarity with RSPM 12?

What concerns, if any, do you have with the standard?

3. What major concerns (e.g. time frame, data requirement, decision process, etc…), if any, do you have with the existing USDA-APHIS permitting process for entomophagous BCAs?

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4a. As a decision-maker, what do you think are the major *assumptions made by the stakeholders* during the permitting process of entomophagous BCAs?
(For instance: the stakeholder thinks the data requirements for the petition do not need to be extensive).

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____

____

Florida A & M University

4b. As a stakeholder, what are the major *assumptions made by the decision-makers* during the permitting process for BCAs?
(For instance: the decision-maker considers that potential BCAs should have zero non-target effect).

Florida A &M University

5. Do you use Risk Communication in the context of your occupation? If yes, please provide additional details on why and how.

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6. In your opinion which agencies or stakeholders should be contacted during Risk Communication?

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Florida A & M University

8a. Have you ever personally submitted a permit request to import or release an entomophagous BCA? If yes, please provide additional details (import vs. release, number of times, year(s), organism).

8b. Have you been involved in other aspects of the introduction process of exotic entomphagous BCAs?

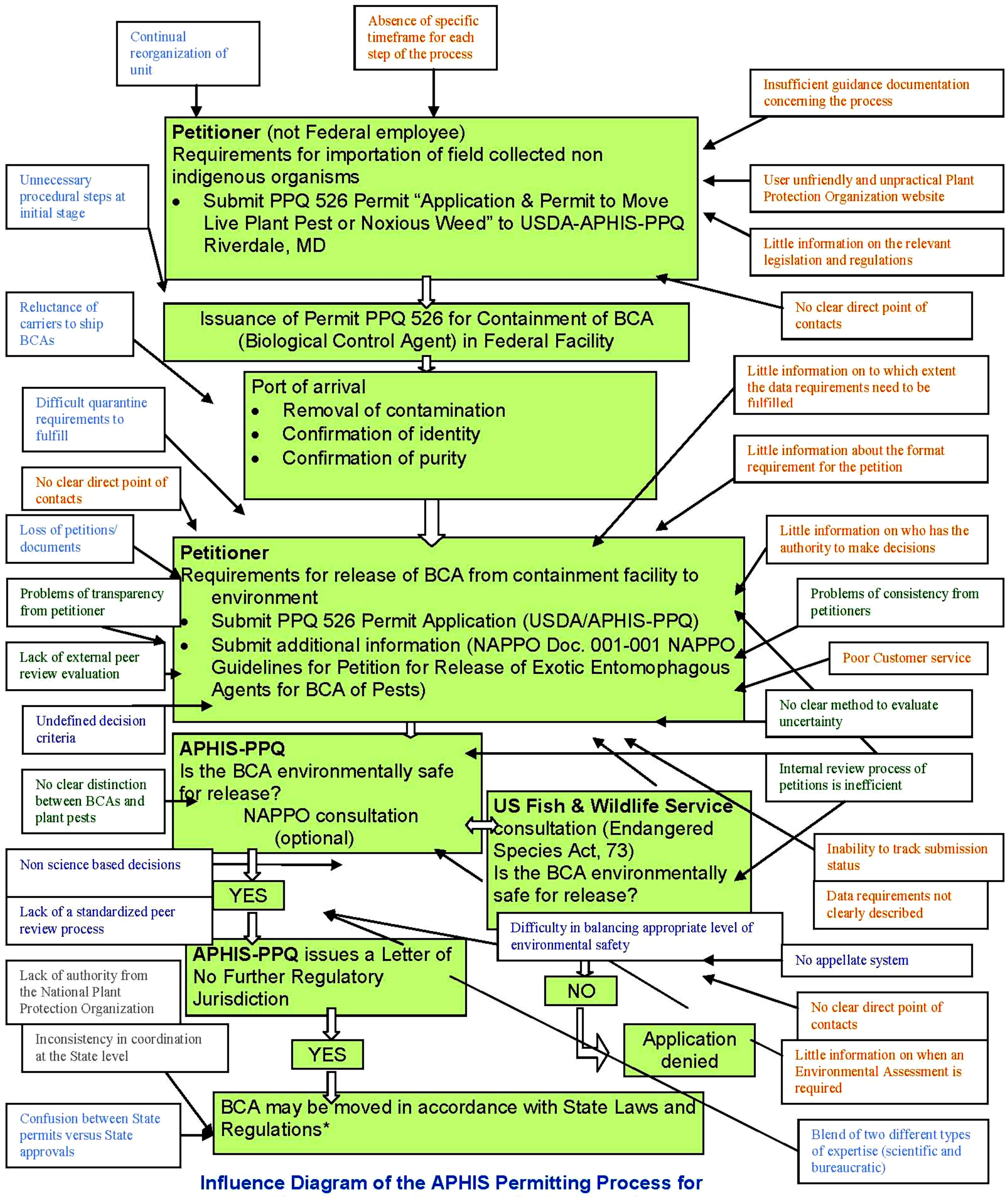
9. What is your current occupation?

Thank you for taking the time to complete this survey. Your assistance in providing this information is very much appreciated. If there is anything else you would like to tell us about this survey, please do so in the space provided below.

Comments

Florida A &M University

APPENDIX D
EXAMPLES OF MENTAL MODELS FOR THE PERMITTING PROCESS FOR ENTOMOPHAGOUS BIOLOGICAL CONTROL AGENTS IN THE U.S.



Influence Diagram of the APHIS Permitting Process for Entomophagous Biological Control Agents

Categories:
Communication
Decision making process
Legislation and regulations
Procedural system
Risk assessment process

APPENDIX E
REMINDER NOTICE

Dear Dr. XXXXXX

You were recently sent a survey concerning Risk Communication during the Permitting Process for Entomophagous Biological Control Agents. If you have already completed the survey, we would like to thank you for your time and important contribution. If you haven't had a chance to complete the survey yet, please take a moment to answer the questions at this url address:

http://is-nri.com/take?i=116471&h=TiOSnMO6tP6KD6p7VlFXWg.

Your expertise will help us document and characterize Risk Communication activities during the permitting process.
If you have any questions concerning this survey, you may contact me at (850) XXX-XXX or XXXXX@ufl.edu.

Sincerely,

Oulimathe Paraiso
Ph.D. Student
Center for Biological Control
Florida A&M University

APPENDIX F
QUESTIONNAIRE

Risk Communication & The Importation of Entomophagous Biological

1. Introduction

Thanks for helping with this survey about the Risk Communication procedures during importation of biological control agents (BCAs). Understanding the different respondents' perspectives on the various Risk Communication practices during the importation of entomophagous BCAs, will aid in the efforts to develop a more effective Risk Communication framework.

"Risk Communication can be defined as the interactive process of information and opinions among individuals, groups or institutions. It often involves messages, concerns and reactions about the nature of the risk or about the risk messages."
Ruby E. Brown

The main purpose of Risk Communication is to provide individuals with enough information to enable them to make an informed decision about a potential risk. The importation of BCAs is regulated by the USDA-Animal and Plant Health Inspection Service (APHIS). The process comprises the submission of adequate documentation concerning the BCA and the request of federal and state authorization before its release into the environment.

The survey is composed of 19 closed-ended questions (3 questions about yourself and 16 questions about Risk Communication). As with all surveys we conduct, your responses are confidential. Should you have any difficulties in responding, please call Ms. Oulimathe Paraiso at (850) 412-7059 or Dr. Moses Kairo at (850) 412 7062. If you prefer an electronic version of the survey, or need additional copies, please make a request to oparaiso@ufl.edu.

Thank you for your participation!

2.

1. About Yourself (Optional)

Name:
Company:
Address:
Address 2:
City/Town:
State:
ZIP/Postal Code:
Email Address:
Phone Number:

3.

2. In which group will you categorize yourself?

- ○ Federal
- ○ State
- ○ University
- ● Non-Governmental Agency
- ○ Private

Page 1

3. How would you categorize your involvement in Biological Control?

- Research
- Regulation
- Ethics
- Conservation
- Commercial Production
- Beneficiary (e.g.Farmers, General Public...)
- N/A

4.

4. How often do you communicate risk in the context of your profession?

- Daily
- Weekly
- Monthly
- Yearly
- Never

5.

5. Do you view Risk Communication as an important component during the importation process of entomophagous BCAs?

- Yes
- No
- Undecided

6.

6. From which entity(ies) do you receive information pertaining to risks associated with importation of BCAs and what is the relative importance of each source (percentage)?

	N/A	< 25%	26% - 49%	50% - 74%	>75%
Federal Plant Protection Agency	○	○	○	○	○
State/Local Plant Protection Agency	○	○	○	○	○
Cooperative Extension Services	○	○	○	○	○
University Researchers	○	○	○	○	○
Environmental Groups	○	○	○	○	○

7.

7. How often do you receive information about risks associated with the importation of entomophagous BCAs from USDA, APHIS- Plant Protection Quarantine?

- Daily
- Weekly
- Monthly
- Yearly
- Never (N/A)

8.

8. How would you rate your level of satisfaction with the Risk Communication information that you receive from USDA, APHIS- Plant Protection Quarantine pertaining to the importation of entomophagous BCAs?

- ◯ Very Satisfied
- ◯ Satisfied
- ◯ Somewhat Dissatisfied
- ◯ Dissatisfied
- ◯ Very Dissatisfied
- ◯ N/A

9.

9. How would you rate your level of satisfaction of the Risk Communication interactions with USDA, APHIS- Plant Protection Quarantine concerning the importation of entomophagous BCAs?

- ◯ Very Satisfied
- ◯ Satisfied
- ◯ Somewhat Dissatisfied
- ◯ Dissatisfied
- ◯ Very Dissatisfied
- ◯ N/A

10.

10. What percentage best describes the communication channel(s)through which you receive the information on risks pertaining to the importation of entomophagous BCAs?

	< 25%	26% - 49%	50% - 74%	> 75%	Never (N/A)
Television(Telecasting, Videos...)	◯	◯	◯	◯	◯
Radio	◯	◯	◯	◯	◯
Newspapers	◯	◯	◯	◯	◯
Scientific Publications	◯	◯	◯	◯	◯
Mailed Letters	◯	◯	◯	◯	◯
Pamphlets(Booklets, Brochures, Fact Sheets...)	◯	◯	◯	◯	◯
Electronic Mail (List serv, Federal Registry site, Blogs...)	◯	◯	◯	◯	◯
Telephone (Conference calls, Text messages...)	◯	◯	◯	◯	◯
Meetings (Lunch, Social or Board...)	◯	◯	◯	◯	◯
Scientific Conferences	◯	◯	◯	◯	◯

11.

11. Rank the following key goals of the Risk Communication process during the importation of entomophagous BCAs in order of importance (from 1-most important to 5 least important).

	1	2	3	4	5
Explain decisions	○	○	○	○	○
Respond to external peer review recommendations	○	○	○	○	○
Encourage good practices among BC practionners	○	○	○	○	○
Explain risks	○	○	○	○	○
Explain petition requirements	○	○	○	○	○

12.

12. How effective is USDA, APHIS- Plant Protection Quarantine in fulfilling each Risk Communication goal during the importation of entomophagous BCAs?

	Very Effective	Effective	Somewhat Effective	Ineffective	N/A
Explain decisions	○	○	○	○	○
Encourage inputs	○	○	○	○	○
Respond to inputs	○	○	○	○	○
Explain risks	○	○	○	○	○
Explain petition requirements	○	○	○	○	○

13.

13. What is your degree of familiarity with the different guidance documents pertaining to the importation of entomophagous BCAs?

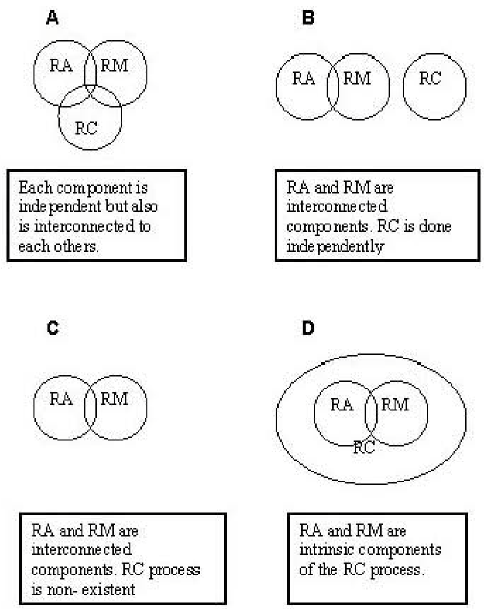
	Very Familiar	Familiar	Somewhat Familiar	Unfamiliar
ISPM #2 "Framework for Pest Risk Analysis"	○	○	○	○
ISPM #3 "Guidelines for the Export, Shipment, Import and Release of BCAs and Other Beneficial Organisms"	○	○	○	○
ISPM #11 "Pest Risk Analysis for Quarantine Pests Including Analysis of Environmental Risks and Modified Living Organisms"	○	○	○	○
RSPM #12 "Guidelines for Petition for Release of Exotic Entomophagous Agents for the BC of Pests"	○	○	○	○

14.

Risk Communication & The Importation of Entomophagous Biological

Which of these models best represent your perception of Risk Communication as it is currently incorporated during the importation process of entomophagous BCAs?

Abbreviations: RA-Risk Assessment, RM-Risk Management, RC-Risk Communication



14. Please, select one of the model choices represented above.

- A
- B
- C
- D

15.

15. Do you think there is a need for more guidance documents from USDA, APHIS-Plant Protection Quarantine concerning the importation of entomophagous BCAs?

- Yes, definitely
- Yes, somewhat
- Yes, mostly
- Not at all

16. Does your USDA, APHIS website provide you with enough explanations and guidance about importation of entomophagous BCAs?

- Yes, definitely
- Yes, somewhat
- N/A
- Yes, mostly
- Not at all

16.

17. Do you have the information (phone numbers, emails, fax number, address) of the points of contact that you can reach if you have any questions during the importation of entomophagous BCAs process?

- Yes, definitely
- Yes, somewhat
- N/A
- Yes, mostly
- Not at all

17.

18. In your opinion, is the public adequately involved in the importation of entomophagous BCAs process?

- Yes, definitely
- Yes, somewhat
- Undecided
- Yes, mostly
- Not at all

19. If there is anything else you would like to tell us about this survey, please do so in the space provided below.

18.

Risk Communication & The Importation of Entomophagous Biological

Thank you for taking the time to complete this survey. Your assistance in providing this information is very much appreciated.

APPENDIX G
INSTITUTIONAL REVIEW BOARD APPROVAL LETTER

Florida Agricultural and Mechanical University
Tallahassee, Florida 32307-3100

Excellence with Caring

CENTER FOR BIOLOGICAL CONTROL
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Date

Thank you for assisting with this survey about the risk communication procedures during importation process for biological control agents (BCAs). Understanding the different respondents' perspectives on the various risk communication practices during the importation of entomophagous BCAs, will aid efforts to develop a more effective risk communication framework.

Based on Ruby E. Brown, "risk communication can be defined as the interactive process of information and opinions among individuals, groups or institutions. It often involves messages, concerns and reactions about the nature of the risk or about the risk messages."

The main purpose of Risk Communication is to provide individuals with enough information to enable them to make an informed decision about a potential risk. The importation of BCAs is regulated by the USDA-Animal and Plant Health Inspection Service (APHIS). The process comprises the submission of adequate documentation concerning the BCA and the request of federal and state authorization before its release into the environment.

Completion of the survey is completely voluntary. The survey is composed of 19 closed-ended questions (3 questions about yourself and 16 questions about Risk Communication). As with all surveys we conduct, your responses are confidential. Should you have any difficulties in responding, please call Ms. Oulimathe Paraiso at (850) 412-7059 or Dr. Moses Kairo at (850) 412 7062. If you prefer an electronic version of the survey, or need additional copies, please make a request to oparaiso@ufl.edu.

Thank you for your participation.

Oulimathe Paraiso
Graduate student

FAMU IRB Approval
FWA 00005391
IRB Number: 011-04
From: 3-2-2011
Thru: 3-2-2012

If you have concerns about the conduct of this survey, you may also contact the Chair of the FAMU Institutional Review Board: Tel. 850-412-5246 or Email: IRB@FAMU.EDU

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BIOGRAPHICAL SKETCH

Oulimathe was born in Geneva (Switzerland). She graduated with a Bachelor in microbiology from Laval University, Quebec, (Canada) in 2002. In 2003, she was accepted in the master's program in agricultural sciences at Florida A&M University, Tallahassee (FL). Her work focused on the isolation of toxic compounds from *Xylella fastidiosa*, the causative agent of Pierce's Disease in grapevines. Following graduation in 2005, she worked for a year under her Optional Practicum Training (OPT) with the U.S. Department of Agriculture-Agricultural Research Service-Center for Medical, Agricultural and Veterinary Entomology in Tallahassee (FL). During this year she participated in the control management effort of *Cactoblastis cactorum* (Berg), a pest of *Opuntia* spp. in the U.S. and Mexico. In 2006, she started the co-operative Ph.D. program in entomology between Florida A&M University and University of Florida. Her interest focused in conceptual disciplines such as risk analysis and risk communication combined with an interest in applied entomology, particularly in the area of Classical and Augmentative Biological Control. From 2007 to 2011, she was the Florida A&M University representative on the Entomological Society of America South Eastern Branch Student Affair Committee and on the Florida Entomological Society Committee. She received her Ph.D. from the University of Florida in the spring of 2011.