Environmental Assessment (EA) for Release of the Biological Agent, Zygogramma (*Zygogramma bicolorata L.)* to Control the Invasive Weed, Parthenium (*Parthenium hysterophorus L.*) in Ethiopia

Submitted by the Management Entity of the Integrated Pest Management Collaborative Research Support Program (IPM CRSP)

on behalf of
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Acronyms

Ag- Agriculture
ARC-PPRI- Agricultural Research Council- Plant Protection Research Institute- South Africa
EIAR- Ethiopian Institute of Agriculture Research
EA-Environmental Assessment
IPM CRSP- Integrated Pest Management Collaborative Research Support Program
EGAT- Economic Growth, Agriculture, and Trade
NGO- Non Governmental Organization
USAID- United States Agency for International Development

1. Summary of Host-Specificity Evaluations of the Leaf-Feeding Beetle, *Zygogramma bicolorata* in Ethiopia, South Africa, Australia and India

Parthenium (*Parthenium hysterophorus* L. Asteraceae) commonly known as parthenium, congress weed, carrot weed, and false ragweed, is an invasive weed species in Africa, Australia and Asia. It is a native of tropical and sub-tropical South and North America. The lightness of the seed, prolific seed production, adaptability to wide range of habitats, drought tolerance, its ability to release toxic chemicals against other plants, and its high growth rate allow it to colonize new areas quickly and extensively. In eastern and southern Africa, parthenium reduces the yield of all major crops. It competes with preferred pasture species, reducing pasture carrying capacity by up to 90% and when consumed by domestic animals, parthenium taints their milk and meat, reducing their value. Parthenium also causes human health problems such as severe contact dermatitis, hay fever and respiratory stress. Biological control of parthenium is the most cost-effective, environmentally safe and ecologically viable method available. One of these effective biological agents against parthenium introduced from Mexico to Australia and India in the 1980s is the leaf-feeding beetle, *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae). *Zygogramma* has been under evaluation in Ethiopia for the control of parthenium with the support of a USAID funded-project. This report summarizes the host-range evaluation conducted on *Zygogramma bicolorata* in Ethiopia and South Africa under quarantine and compares these results with work done earlier in Australia and India.

Ethiopia

The safety of *Zygogramma bicolorata* to non-target plants was tested on a total of 17 crop species and 10 non-crop species under quarantine in Ethiopia. In addition, the beetle was tested on 5 niger seed (*Guizotia abyssinica*) varieties, on teff (*Eragrostis teff)* and on 2 sunflower (*Helianthus annuus*) cultivars in Ethiopia. Like parthenium, both niger seed and sunflower belong to the family of Asteraceae but teff is in the family of Poaceae. Niger seed and teff are important oil seed and staple crops, respectively, in Ethiopia. Sunflower is not a major crop in Ethiopia. Host range testing conducted under quarantine in Ethiopia on the above economically important crop species and varieties and indigenous species, established that *Zygogramma* is safe for release against parthenium. There was some egg-laying and nibbling of leaves of sunflower varieties by *Zygogramma* under no-choice test conditions. But the feeding was minimal and the beetle did not complete its life cycle on sunflower varieties. Based on these results of the quarantine evaluations, the Ethiopian government has given permission for the release of *Zygogramma* in parthenium infested areas (Appendix C). An advertisement on an Ethiopian news paper, Ethiopian Herlad, asking for public comments on the release of Zygogramma for the control of parthenium (Appendix D) was also placed and there was no objection from the general public on the release (Appendix E). This application is submitted to USAID as part of IEE/EA requirement for funded projects.

South Africa

Zygogramma bicolorata has been tested in no-choice and choice tests on 41 indigenous, exotic and economically important species (including 12 varieties of sunflowers, 3 varieties of teff, 2 varieties of niger seed) that are closely related to parthenium in South Africa. Larval development and feeding were observed on 6 sunflower varieties. However, subsequent analyses suggests that the risks of non-target sunflower varieties suffering feeding damage in the field are extremely low (<0.2%). In addition, the reproductive risk calculations indicated that the six non-target varieties of sunflower showed a very low (<0.151%) risk of supporting viable populations of *Z. bicolorata* in the field. Based on years of studies under quarantine in South Africa, scientists have concluded that *Zygogramma bicolorata* is suitably host-specific and does not pose danger to non-target plants. Presently, scientists are preparing an application for a permit to release *Zygogramma* for the control of parthenium in South Africa (personal communication with Dr. Andrew McConnachie).

Australia

Australia initiated an extensive biocontrol of parthenium in 1977. Since then, nine species of insects and one fungal pathogen have been introduced (McFadyen, 1992; Dhileepan et al., 1996; Dhileepan and McFadyen, 1997). Among these agents, *Zygogramma bicolorata* is the most prominent agent. *Z. bicolorata* was first introduced to Australia from Mexico in 1980 (McFadyen and McClay, 1981). Host-range testing of *Zygogramma* was conducted in Australia on 69 species (25 Asteraceae) in 14 families: 3 species, including sunflower. It was reported that under no-choice test, there was slight adult feeding of some sunflower varieties that was restricted to the young leaves and did not support survival of larvae or of newly emerged adults beyond 7 days of age. Since 1990 when *Zygogramma* became abundant in Australia, there was no reported damage by this insect to non-target plants, including sunflower. Thus, in Australia, despite the release of *Z. bicolorata* over 20 years ago in areas of extensive sunflower cultivation, there have been no reported instances of beetle attack on the crop. Instead, *Zygogramma* has been an effective agent in controlling parthenium in Australia.

India

Zygogramma bicolorata from Mexico was introduced into India in 1983, for the control of parthenium. From India, *Zygogramma* has also spread to Pakistan. Host-specificity tests were conducted on *Zygogramma* under quarantine conditions on 40 plants belonging to 27 families in India. The beetle was released after results proved that the insect is capable of feeding and reproducing only on parthenium (Jayanth and Nagarkatti, 1987). In subsequent years, there was a report of *Zygogramma* feeding on tender leaves of sunflower. However, research showed that such feeding was induced by parthenium pollen deposited on sunflower leaves (Jayanth, et al., 1993). In India in some localities *Zygogramma* has been credited for up to 99.5% in parthenium population decline and reversing the decline of biodiversity (Jayanth & Visalakshy, 1996).

Conclusion

Parthenium is an invasive weed that originated in tropical America and now has spread to Africa, Asia and Australia. In eastern and southern Africa, it has adversely affected food security, biodiversity, human and livestock health. Experience in the last twenty years in Australia and India showed that a leaf-feeding insect, *Zygogramma bicolorata*, imported from Mexico can control parthenium without affecting non-target plants. *Zygogramma* oviposited and fed on young leaves of some sunflower varieties in Australia and later in the field in India. Similar results were obtained in Ethiopia and South Africa under quarantine where the beetle was safe to all non-target plants except a few sunflower varieties. In all cases, the observed feedings on sunflower were minor, and were confined to a few varieities. So, based on twenty-years of field experiences in Australia and India and recent evaluations in Ethiopia and South Africa, the chances of *Zygogramma* becoming a pest of sunflower or other non-target plants in Ethiopia is very remote.

Project Title: Abating the Weed Parthenium (*Parthenium hysterophorus* L.) Damage in Eastern Africa Using Integrated Cultural and Biological Control Measures

Country/Region: Ethiopia

2. The IPM CRSP and USAID Strategic Objectives

The overall purpose of the IPM CRSP is to develop and implement a replicable approach to IPM that will help reduce: (a) agricultural losses due to pests, (b) damage to natural ecosystems including loss of biodiversity, and (c) pollution and contamination of food and water supplies. By combining strong regional IPM programs with work on critical global cross-cutting themes, the goals of the IPM CRSP are to measurably reduce crop and animal losses due to pests, increase farmer income, reduce pesticide use, reduce residues on export crops, improve IPM research and education program capabilities, improve ability to monitor pests, and increase the ability of women in IPM decision making and program design. By reaching these goals, the results of the IPM CRSP program will directly contribute to the Strategic Objective of the Land Resources Management Team (LRMT) of EGAT/NRM to increase the capacity of USAID and its partners to advance land resource management practices that provide long term social, economic, and environmental benefits.

The IPM CRSP's global theme on invasive species was awarded competitively to Dr. Wondi Mersie of Virginia State University by recommendation of an ad hoc external proposal evaluation panel. The program proposed to develop a biological control solution for management of the invasive weed parthenium in eastern and southern Africa, with principal effort focused on Ethiopia. Classical biological control was the proposed approach. Invasive weeds often become so because, in their exotic habitat, they are released from pressure by natural enemies living in their native habitat. In classical biological control of an invasive weed, a species-specific natural enemy from the target plants native home range is sought whose introduction to the invaded environment will reduce reproduction of the target weed population (Medal et al., 2002). Classical biocontrol agents of weeds are most often insects. They interfere with the target weed's reproduction through herbivory. Depending on insect species' habits it may defoliate the target plant or attack its roots, thereby weakening it or killing it outright; it may destroy flowers or the shoots from which flowers bloom, thus preventing the setting of seed; or it may directly destroy seeds. A key benefit of a classical biocontrol agent is that it is a self perpetuating technique requiring little additional effort once the agent is established throughout the desired range. In this way, classical biological control, when successful, is typically cost-effective and has essentially no technology transfer barrier because adoption by farmers is not required.

Successful implementation of a biological control program against parthenium is consistent with USAID goals to strengthen agriculture's contribution to broad-based economic growth, better health, and effective natural resource management and increase the capacity of USAID and its partners to advance land resource management practices that provide long-term social, economic, and environmental benefits.

This global theme on invasive species contributes to USAID/EGAT's efforts to strengthen agriculture's contribution to broad-based economic growth, better health, and effective natural resource management. Successful completion of this global theme program will exemplify USAID/EGAT's efforts to advance land resource management practices that provide long-term social, economic, and environmental benefits.

3. Description of Project – Purpose and Need

Parthenium hysterophorus L. (Asteraceae) commonly known as parthenium, congress weed, carrot weed, and false ragweed was not ranked among the world's worst weeds when they were cataloged in 1977 (Holm et al., 1977). However, within the last ten years it has become one of the seven most damaging weeds of the world (Evans, 1997) because it has spread fast and extensively. The lightness of the seed, prolific seed production, adaptability to wide range of habitats, drought tolerance, its ability to release toxic chemicals against other plants, and its high growth rate allow it to colonize new areas quickly and extensively. In eastern and southern Africa, parthenium reduces the yield of all major crops. It competes with preferred pasture species, drastically reducing pasture carrying capacity by up to 90% (Nath, 1988). When consumed by domestic animals, parthenium taints their milk and meat, reducing their value. Parthenium also causes human health problems such as severe contact dermatitis, hay fever and respiratory stress (Kololgi et al., 1997). In addition, because of its ability to release plant-inhibiting toxic chemicals parthenium replaces natural vegetation and thus it is a threat to one of the world's richest regions of biodiversity, Eastern Africa. In African small-scale subsistence farming, parthenium is controlled by hand weeding. This task is primarily done by women and school-age children. Any parthenium management system that can control parthenium will reduce the workload on women and school-age children to allow them to engage in other productive activities. Ethiopia was chosen as the site for focusing the work of the IPM CRSP Global Theme on Invasive Species because parthenium is a well recognized problem and it is degrading the environment.

<u>Goal and objectives of the project funded from October 1, 2005 to September 30, 2009</u>

The goal of the project was to develop an integrated weed management system that reduces the adverse impact of parthenium on humans, crops, livestock and plant biodiversity. The project has four objectives; 1) collect accurate information on the distribution and spread of parthenium in eastern and southern Africa and assess its socio-economic impact in Ethiopia; 2) determine the effect of parthenium on plant diversity; 3) evaluate and release insect agents for the control of parthenium; and 4) evaluate and demonstrate pasture management systems for the control of parthenium.

<u>Goal and objectives of the project funded from October 1, 2009 to September 30, 2014</u>

The newly funded project entitled, "Abating the Weed Parthenium (*Parthenium hysterophorus* L.) Damage in Eastern Africa Using Integrated Cultural and Biological Control Measures" has the goal of developing an integrated weed management system that reduces the adverse impact of parthenium on humans, crops, livestock and plant biodiversity in the whole region of eastern Africa. The specific objectives of the new phase are to: 1) collect accurate information on the distribution and spread of parthenium in Kenya and Tanzania with a follow-up in Ethiopia and Uganda; 2) evaluate and demonstrate best management practices for the control of parthenium; 3) evaluate parthenium biocontrol agents for their safety to non-target plant species; and 4) release and evaluate the impact of approved biocontrol agents for the control of parthenium. Under objective 3, host-range testing of the stem-boring weevil *Listronotus setosipennis* will be conducted under quarantine whereas under objective 4, *Zygogramma bicolorata* will be released once a permit is obtained from USAID through this IEE application.

4. Accomplishments of the Completed Project (2005-2009)

Under objective 1a**,** Surveys of parthenium have been conducted in Ethiopia, Uganda, Botswana, Swaziland, and South Africa. No parthenium has been recorded in Botswana.
CLIMEX modeling indicated that Ethiopia, Kenya, Somalia, Tanzania and Uganda in Eastern Africa and South Africa, Swaziland, and Mozambique in Southern Africa, as well as the south of Madagascar are ecoclimatically suitable for the favorable growth of *P. hysterophorus.* Distribution survey data for Ethiopia and South Africa were compiled into a database and mapped and the weed was shown to be much more widespread than previously recorded. Actual distributions determined during road surveys concurred with CLIMEX predictions, validating the model. There are still areas that are highly suitable for parthenium growth that have not yet been surveyed. A parthenium distribution map has been developed for Ethiopia. The availability of such a map is important for a nationwide parthenium control strategy.

Under objective 1b**,** a socioeconomic survey was conducted in three locations (Dugda Bora, Kobo and Jijiga) representing different farming systems in Ethiopia. Results revealed that all surveyed farmers were aware of parthenium and its distribution. However, time of awareness and their perception on means of parthenium dissemination differed. Parthenium negatively impacts farmers' well-being and their productivity. Parthenium could reduce crop yield by 50%, negatively impact livestock and livestock products, and cause human health problems. All farmers expressed their willingness to cooperate in parthenium eradication programs.

Under objective 2**, t**he effect of parthenium on biodiversity and soil seed bank was investigated in Somali rangelands and in grazing lands and sorghum fields of Eastern Amhara, Ethiopia. Species diversity and evenness declined with increasing density of parthenium in both locations. The average seedling density over all infestation levels indicated that parthenium accounted for 61% of the total seedlings germinated from the soil seed bank samples taken from sorghum fields and grazing lands in the Amhara region. In Somali rangelands 85-90% of the soil seed bank was dominated by parthenium.

In objective 3**,** an IEE for import of two potential bio-control agents, the stem-boring weevil *Listronotus setosipennis* (Coleoptera: Curculionidae) and leaf-feeding beetle *Zygogramma bicolorata* (Coleoptera: Chrysomelidae) to Ethiopia was prepared and submitted to USAID in 2007. A permit to import *Z. bicolorata* was received from the Ethiopian government (Appendix A) followed by a USAID approval for an IEE submission (Appendix B) in 2007. After these approvals, *Z. bicolorata* was imported, cultured, and tested for its safety on a number of crop and non-crop plant species under quarantine. Host range testing done in South Africa and Ethiopia on a number of native and economically important crop species and varieties, and indigenous non-crop species, has established that *Z. bicolorata* is safe for release. Thus, a release permit has been granted by the Ethiopian Ministry of Agriculture and Rural Development (Appendix C). The stem-boring weevil *L. setosipennis* was imported to Ethiopia in September 2009 and is being evaluated under quarantine conditions. Similar work is being done in South Africa and so far 20 species have been tested and no *L. setosipennis* progeny were produced on these 20 plant species.

***In objective 4*,** a study in a greenhouse in Eastern Ethiopia identified grass species that can be used to rehabilitate parthenium fields under high, medium and low parthenium infestation levels but a suggestion has been made to confirm this result in field trials and by including legumes. In a field trial in the north where six forage species were evaluated under mowing and burning of pasture/grazing field, parthenium was found to be dominant in all treatments except mowing where *Amaranthus* was the dominant species. Mowing followed by planting *Amaranthus* may help reduce the negative impact of parthenium.

Capacity building was an integral component of the project. The project has assisted in converting a greenhouse into a quarantine facility in Ethiopia. Two separate quarantine rooms have been established to house the agents. The first facility inaugurated in October 2007 has an area of 46 sq m and is being used to test *Zygogramma*. The second facility, which became operational in September 2009, has an area of 73 sq m. This facility houses *Listronotus* and, because of its large size, it can also be used to evaluate another bioagent once an application is made and appropriate permits are received. Three senior Ethiopian researchers and two laboratory technicians, one of them female, have been trained in South Africa on weed biological control and quarantine facility management. Having the first biological control facility in the country, the Ambo Plant Protection Research Center is now serving as the only training center for students and researchers. So far 75 students and researchers, 11 of them female, have been trained at Ambo on various aspects of biological control.

The project provided an opportunity for four Ethiopian students, one of them a female, to study at a Masters Degree level. All of them did their research on parthenium related topics and successfully graduated. They are now all gainfully employed. Furthermore, through annual workshops and planning meetings, a total of 132 people, 11 of them female, have been trained on parthenium weed management.

Presentations (oral and poster) and articles related to the parthenium research project were produced during the reporting period. Posters to create awareness on the health impact of parthenium were published in English and major Ethiopian languages namely, Amharic, Oromiffa, Tigrigna, and Somali. These posters are being distributed throughout the country.

5. Leaf-feeding Beetle, *Zygogramma bicolorata* (Coleoptera: Chrysomelidae)

During the more than 200-year history of biological weed control, beetles as a group have been relatively successful candidates (Crawley, 1989). This project has now evaluated the leaf-feeding beetle *Zygogramma* and has reached the conclusion that it is safe to be released in Ethiopia. *Zygogramma* belongs to the leaf beetle family Chrysomelidae, subfamily Chrysomelinae. The genus contains about 100 species in North and South America. *Zygogramma* is a New World genus; it doesn't occur in Africa. Given the lack of close relatives of *Zygogramma* in Africa, there is no possibility of hybridization with native insect species in Ethiopia. *Zygogramma* is present as an introduced biocontrol agent of parthenium in eastern Australia and India. It has a narrow host range within the Abrosiinae (ragweed) subfamily of the family Asteraceae (sunflower/daisy) (Withers, 1998) and has demonstrated the ability to reduce parthenium seedling production from 73-90% in the field (Dhileepan et al., 2000). Its preferred host range is restricted to the

subfamily in which parthenium occurs (Withers, 1998).
Zygogramma adults measure 5-6 mm thick in length and both adults and larvae feed on parthenium leaves. Adults lay eggs either singly or in groups on the leaves, flower heads, stem surfaces and on terminal and auxiliary buds. The emerging larvae feed on young leaves and then the larvae enters in to the soil to pupate. The pupal stage lasts two weeks and the whole life cycle takes 6-8 weeks and there may be up to 4 generations/year depending on rainfall and food availability. Adults diapause in the soil and adults emerge in response to rainfall and increased temperature. Adult beetles can live up to 2 years.

6. Justification for Proposed Release of *Zygogramma*

Surveys conducted in different parts of Ethiopia during the last few years, through this project, have shown that parthenium is present in most of the areas surveyed and there are still areas that are suitable for the growth of parthenium but not yet infested. There have been few instances in which farmers have attempted to control parthenium, including by hand weeding, but with very little success. There is pressure from farmers on the government to stop parthenium. Attempts to control the weed through a campaign in both the urban and rural areas, by uprooting and burning, did not bring the desired outcome. Consequently, there is strong support from the government side to release *Zygogramma*.

Host range tests in Ethiopia and South Africa have revealed that *Zygogramma* is restricted to parthenium with no risk to non-target plants which is also the case in Australia and India. The project has a number of partners (Haramaya and Mekele Universities, Ethiopian Institute of Agricultural Research, two regional bureaus of agriculture and NGOs) who are prepared to facilitate the release program. Haramaya University, in collaboration with the Regional Bureau of Agriculture, is ready to play a major role in mass rearing, distribution and conduct follow-up studies to determine the impact of *Zygogramma*. The safety of *Zygogramma* on non-target plants, increasing negative effect of the weed and existence of the above favorable conditions justifies the release of *Zygogramma*. In Australia and India, biological control of parthenium has been shown to be the most effective, environmentally safe and ecologically viable providing long-term and sustainable control (please see Appendix Ffor answers on frequently asked questions about weed biocontrol agents). The first step to achieve such control shall start with the release of *Zygogramma*.

7. Alternatives

According to findings of study made by this project some farmers hand weed and burn to control the weed but most farmers do not practice any control measure against parthenium in pastoral range lands and agro pastoral areas such as in the eastern part of the country. The effort to control the weed through an eradication campaign has also not been successful. Control measures such as burning and slashing down have been largely ineffective by themselves because of the high seed production, wide range of adaptability, and the allelopathic effect of parthenium.

With respect to alternative techniques for large-scale control of parthenium, there are no examples of bringing a widespread invasive weed under control other than classical biological control (e.g. water hyacinth, nodding thistle, salvinia). Once an invasive species has established itself in a new habitat, there is only a very brief time when chemical control or manual removal could be used to exterminate the species. Manual weeding is only practical on small parcels of highly valuable land, such as agricultural fields and garden plots. Widespread spraying with chemical herbicides may not be economical in Ethiopia.

Twenty five years of experience with *Zygogramma* provides a wide margin of safety with respect to unexpected consequences, making it one of the best alternatives according to the biocontrol experts consulted and the literature.

It is, therefore, the opinion of the principal investigator and the Management Entity of the IPM CRSP that there are no alternatives other than classical biological control that have a reasonable likelihood of success in reducing parthenium populations in Ethiopia.

8. Host Specificity Tests

8.1 Selection of test plants

In Ethiopia, the selection of non-target species for host preference testing was made by a taxonomist from Addis Ababa University (Figure 1) following the centrifugal phylogenetic method (Wapshere, 1974). Testing starts in the center of the circle and proceeds to the outside in the direction of the arrows (Figure 1). Test plants were identified by the Taxonomist after making an analysis of relationships of Taxa of the family Asteraceae (Compositae), to which parthenium belongs. Emphasis was placed on testing of species in the family Asteraceae. The test plant species were identified based on how closely related they are to parthenium, strict endemic, near endemic, cultivated and indigenous they are to Ethiopia, while others were selected based on how much similar ecological preference and overlapping distribution they have with parthenium. The number of crop and plant species tested under this host range test were more than enough, because current host-specificity test lists have progressed from long lists of crop plants unrelated to the normal host to targeted lists of plants closely related to the weed and including native species (Fornasari and Turner, 1995; McFadyen and Marohasy, 1990, Wapshere 1989). The aim of the host range test is to determine the potential host range of the agent and, therefore, which plants, if any, will be at risk in the field (Blossey, 1995, Cullen, 1990, McClay, 1996). This is why emphasis was given to crops and other plant species that are closely related to parthenium, crops of economic importance and also on native species. As indicated by the taxonomist, there is no native Parthenium species in Ethiopia.

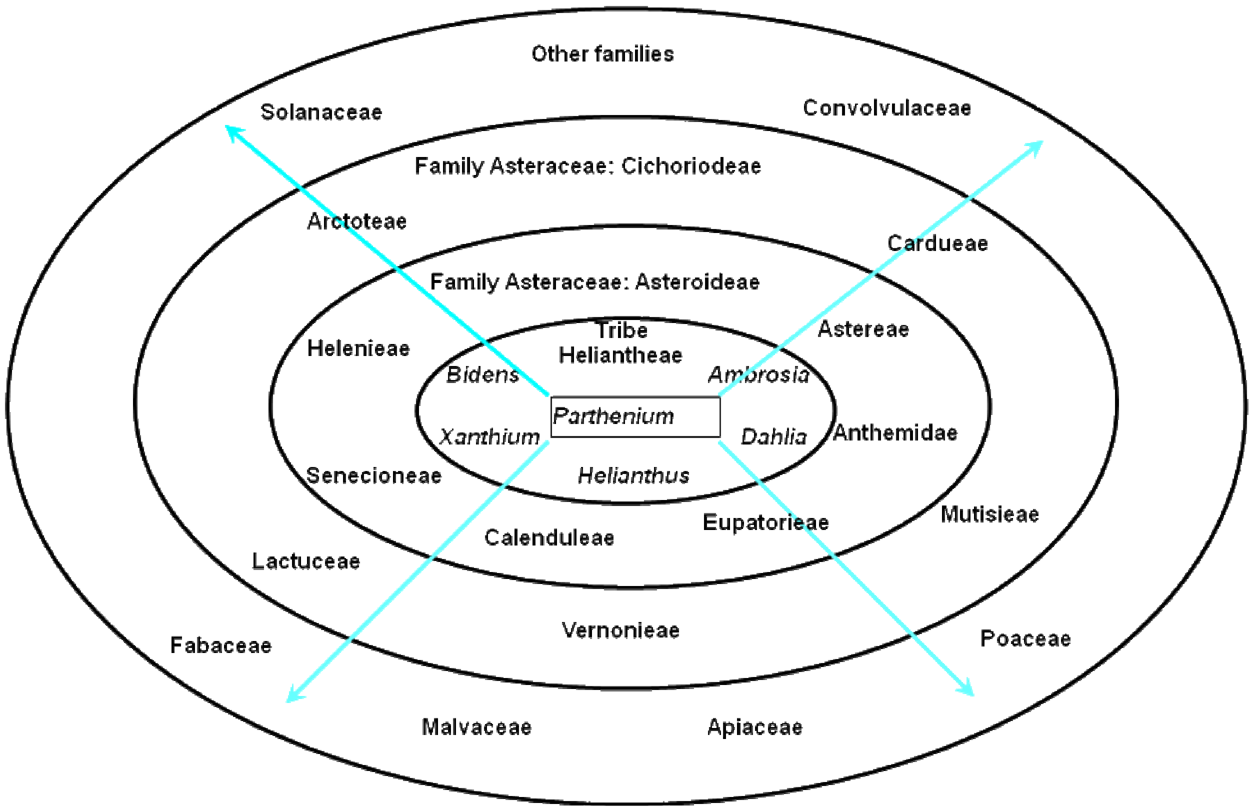


Figure 1. Diagrammatic Representation of The Tribe Heliantheae and Some Crop Plants (adapted by Lorraine Strathie from Wapshere, A. J. 1974).

8.2 Evaluation of* Zygogramma *safety in India and Australia.

Biocontrol of parthenium was first initiated in Australia in 1977 and, since then, nine species of insects and one fungal pathogen have been introduced, of which at least six species of insects and a pathogen are known to have established in the field. *Zygogramma*, intended for release in Ethiopia is one of them, and has successfully established itself and is now being widely used without any harm to crop species or to other plants except parthenium, in both Australia (McFadyen, 1992; Dhileepan et al., 1996; Dhileepan and McFadyen, 1997) and India (Jayanth, 1987). Australia and India have proved that biological control of parthenium is possible and effective (Dhileepan, 2001).

In India, *Zygogramma* became abundant within three years after introduction, resulting in a significant reduction in parthenium density in local areas (Jayanth and Bali, 1994; Jayanth and Visalakshy, 1996).

A study in Australia has shown reduction of parthenium in plant height (by 18-65%), plant biomass (by 55-89%) and flower production (by 75-100%) (Dhileepan et al., 2000) while in India, a single adult *Z. bicolorata* per plant caused 85–100% defoliation within six to eight weeks, depending on the stage of plant growth (Jayanth and Bali, 1994). In field-cage trial, *Z. bicolorata* caused 92% defoliation in about 90 days and reductions in plant height by 27%, root length by 56%, root biomass by 69%, shoot biomass by 81%, flower production by 83% and soil seed-bank by 73% (Dhileepan et al., 2000).

The body of literature on host range testing and release of parthenium biocontrol agents, in Australia and India, were used to select *Zygogramma* for efficacy and host range tests in Ethiopia under quarantine.

8.3 Quarantine facility and host range testing procedure

Establishment of a quarantine facility was a prerequisite for import of the insect and maintaining of the bioagent until release. The 46 sq m facility was renovated and then inspected and approved as meeting international standards. Researchers and technicians were also provided training in South Africa and Ethiopia that has enabled them to handle the facility and carry out tests without needing outside help. It has been possible to multiply the bioagent and later use it for host range tests with no risk of release of the agent to the outside environment.

Crop and non-crop test plants are required to go through a no-choice test (where insects are placed on one test plant only) to narrow the range of plants that are suitable for agent oviposition, feeding or development. No further test will be carried if no feeding symptom is observed during this test but are tested under choice test (test plants are kept with host plant) if feeding symptom or oviposition is observed. Choice test is designed to test whether the bio-agent preferred to feed, lay egg, and develop on a test plant in the presence of parthenium or it avoided the test plant in preference to parthenium weed. Series of tests would continue to determine if the bioagent can complete its life cycle before declaring the safety of the agent to the test plant.

In this project, ten healthy pairs (10 females + 10 males) of adult *Zygogramma* are placed on a potted plant. After 10 days of exposure all adults are removed from each test plant and put in a freezer and the number of adults that survived, died, and/or missed are recorded. The number of eggs or larvae present on the plant is also counted. The extent of feeding is assessed by determining the percentage of the total number of leaves that demonstrated feeding symptoms. The range of feeding damage is categorized by a scale of 0-5 (0 = 0%, 1 = <10%, 2 =<20%, 3 = 40%, 4= 60% and 5 = > 60% leaves with feeding symptoms).

8.4. Results from Ethiopia quarantine tests

8.4.1 Evaluation of major crops

Fifteen major crop species, mostly used as food and others for export, that are not related to parthenium, were tested under no choice test and *Zygogramma* fed on none of them (Table 1). The test included, teff (*Eragrostis teff*), an important endemic food grain crop in Ethiopia.

Table 1. Adult feeding symptoms and range of feeding of *Zygogramma* on major crops in no-choice tests at Ambo Plant Protection Research Centre, Ethiopia. There were three replications of each species.

No	**Scientific Name**	**Common name**	**Leaves with feeding symptoms/pot**	**Range of feeding***
	Parthenium hysterophorous (control)	partehnium	112	5
1	*Vicia faba*	fababean	0	0
2	*Capsicum sp.*	pepper	0	0
3	*Zea mays*	maize	0	0
4	*Solanum lycopersicum*	tomato	0	0
5	*Phaseolus vulgaris*	haricot bean	0	0
6	*Lens cultinaris*	lentil	0	0
7	*Solanum tuberosum*	potato	0	0
8	*Triticum aestivum*	wheat	0	0
9	*Cicer arietinum*	chick pea	0	0
10	*Pisum sativum L.*	field peas	0	0
11	*Eragrostis teff*	teff	0	0
12	*Sesamum indicum*	sesame	0	0
13	*Lathyrus sativus*	rough pea	0	0
14	*Brassica sp.*	cabbage	0	0
15	*Sorghum bicolor*	sorghum	0	0

*(0 =0%, 1=<10%, 2 =<20%, 3 = =40%, 4= =60% and 5 = > 60% leaves/pot with feeding symptoms).

8.4.2 Evaluation of species related to parthenium

Crops and other plant species, belonging to the family Asteraceae, were tested under no-choice test in the quarantine facility at Ambo, Ethiopia. The test plants consisted of five economically important Asteraceae plants: Vernonia (*Vernonia galamensis*), a potential source of industrial oil, lettuce (*Lactuca sativa*), *Bidens pilosa* (weed and potential medicinal plant), *B. pachyloma* (used as ornamental during Meskel and Ethiopian new year festivals), *B. ghedoensis* (forage for animal as well as weed). Four Asteraceae weeds: *Guizotia scabra*, *Flaveria trinervia*, *Tagetes minuta,* and *Conyza bonariensis* were also tested. The result on feeding of *Zygogramma* on the test plants is shown on Table 2. The no-choice test result indicated that none showed any feeding symptoms.

Table 2. Adult feeding and oviposition, and larvae development of *Zygogramma* on plants related to parthenium in no-choice tests at Ambo Plant Protection Research Centre, Ethiopia. There were three replications of each species. 0 = no feeding/eggs, + = weak positive response (nibbling of leaves at the edges, ++ = strong positive response (defoliation/extensive feeding).

Species/variety	**Common name**	**Feeding***	**Oviposition**	**Larvae**
Bidens pilosa		0	0	0
Bidens ghedoensis		0	0	0
Bidens pachyloma		0	0	0
Flaveria trinervia		0	+	+
Galinsoga parviflora		0	0	0
Conyza bonariensis		0	+	+
Tagetes minuta		0	+	+
Lactuca sativa	lettuce	0	+	+
Vernonia galamensis		0	+	+
Guizotia scabra		0	0	0
Carthamus tinctorius	saflower	0	0	0
Parthenium hysterophorus	parthenium	5	++	++

*(0 =0%, 1=<10%, 2 =<20%, 3 = =40%, 4= =60% and 5 = > 60% leaves with feeding symptoms).

Adults laid eggs on some of the species and died before feeding. Similarly, the larva produced on some of the above species under no-choice tests died within a week of hatching from eggs.

8.5.3 Evaluation of niger seed and sunflower varieties

Five varieties of niger seed and two varieties of sunflower were tested under no-choice test. There were three replications of each species/variety.

Table 3. Adult feeding and oviposition and larvae development of *Zygogramma* on two compositea crops in no-choice tests at Ambo Plant Protection Research Centre, Ethiopia. 0 = no feeding /eggs + = weak positive response (nibbling of leaves at the edges, ++ = strong positive response (defoliation/extensive feeding).

Species/variety	Common name	Feeding*	Oviposition	Larvae
Guizotia abyssinica (Local)	niger seed	2	+	0
G. abyssinica (Fogera)	niger seed	0	0	0
G. abyssinica (ESTE)	niger seed	1	+	0
G. abyssinica (Kuyu)	niger seed	1	+	0
G. abyssinica (Shambu)	niger seed	1	+	0
Helianthus annuus (oissa)	sunflower	3	+	+
Helianthus annuus (R.B)	sunflower	2	+	+
Parthenium hysterophorus	parthenium	5	++	++

*(0 =0%, 1=<10%, 2 =<20%, 3 = =40%, 4= =60% and 5 = > 60% leaves with feeding symptoms).

Zygogramma nibbled leaves and oviposited on four of the niger seed varieties. But the eggs did not hatch to larva and feedings were minor. The local niger seed variety that showed 20% (2) feeding was further tested under choice test (Table 4). Also, some feeding symptoms were observed on the two varieties of sunflower. These sunflower varieties were then tested in a choice trial to further examine the host range of the agent (Tables 4 and 5).

Table 4. Range of feeding of *Zygogramma* adults under choice test plant species that showed feeding symptoms under no-choice test at Ambo Plant Protection Research Centre, Ethiopia.

Test plant species	Mean total number of leaves/pot	Mean total leaves with feeding symptoms	Range of feeding *(0-5 scale)
G. abyssinica (niger seed) - local	135	0	0
Sunflower -Oissa	98	24	2
Sunflower –R-black	92	29	2
Parthenium (Control)	141	107	5

*(0 =0%, 1≤ 10%, 2 ≤ 20%, 3 = =40%, 4= =60% and 5 = > 60% leaves with feeding symptoms).

There was no feeding or oviposition on niger seed, a major crop in Ethiopia under a choice test . Similar results were obtained in India where studies showed no damage on niger seed by *Zygogramma*. But two eggs on "Oissa" and four eggs on "R-black" varieties of sunflower were laid but none hatched into larva under choice tests (Table 5).

Table 5. Number of eggs and larvae recorded on sunflower varieties ten days after under choice test.

No.	Test plants under choice test	Total Number of eggs or larvae in 10 days of testing	
		Eggs	**Larvae**
1	Sunflower- Oissa	2	0
2	Sunflower –R-black	4	0
3	Parthenium (Control)	332	20

In all the above host range tests, none of the test plants except parthenium have enabled the bioagent to complete its life cycle, thereby indicating that they are not the true hosts. However, two varieties of sunflower had to go through choice test twice before declaring them safe. The nibbling by *Zygogramma* of sunflower is explained by the fact that *Zygogramma* adults do not recognize their host by initial contact, but only after sampling it (i.e. breaking the leaf surface by biting) (Withers, 1999). This finding explains the feeding on sunflower by *Zygogramma*.

Zygogramma failed to feed and complete its life cycle on plants taxonomically related to parthenium.

8.4 Results from South Africa quarantine tests (from an annual report)
Zygogramma bicolorata has been tested in no-choice tests on 41 indigenous, exotic and economically important species (including 12 varieties of sunflowers) that are closely related to parthenium in South Africa (Table 6). Feeding was recorded on 13 of these species and oviposition on 14. In all cases, the relative amounts of feeding/oviposition were significantly less than that were recorded on *P. hysterophorus*. All sunflower varieties that tested were fed on, and some received eggs, in no-choice tests. All of these species were included in choice trials to further examine the host range of *Z. bicolorata* (Table 6).

Choice tests resolved results from no-choice tests with the exception of feeding on six varieties of sunflower (Table 6). However, far fewer eggs were deposited on test plants than on the control plants (parthenium). Larval development trials were initiated to further resolve these results. In larval development trials, 20 one-day old eggs were placed onto each test or control plant. After 21 days (based on H. King's (King, 2008) Z. *bicolorata* thermal physiology results that larvae would have reached 5th instar by this stage at the study temperature), all stages of all larvae found were assessed, and any feeding damage was scored. The experiment was replicated three times.

Table 6: Summary results of *Zygogramma* adult feeding and oviposition in no-choice tests in South Africa. 0 = no feeding /eggs + = weak positive response (nibbling of leaves at the edges, ++ = strong positive response (defoliation/extensive feeding). * = one of three replicates, ** = two of three replicates, *** = all three replicates

Family Tribe	Species (variety)	No-choice tests		Choice tests	
		feeding	oviposition	feeding	oviposition
Asteraceae: Asteroideae					
Heliantheae	***Parthenium hysterophorous***	**++**	**++**	++	++
	Blainvillea gayana	+	+	0	0
	Aspilia natalensis	+	0	0	+
	Spilanthes mauritiana	+	0	0	+
	Bidens schimperii	+	0	0	0
	Bidens pilosa	0	0	0	0
	Melanthera scandens	0	0	0	0
	Zinnia angustifolia (orange, light orange & white)	+	0	0	0
	Coreopsis grandiflora (early sunrise)	0	0	0	0
	Dahlia rosea (red skin mixed)	0	0	0	0
	Guizotia abyssinica (Kuyu)	0	0	0	0
	Guizotia abyssinica (Shambu)	0	0	0	0
	Helianthus annuus (PAN 7351)	+	0	0	0
	H. annuus (HYSUN 333)	+	0	0	0
	H. annuus (HYSUN 345)	+	0	0	0
	H. annuus (AFG 271)	+	0	0	+
	H. annuus (AGSUN 5671)	+	0	0	+
	H. annuus (AGSUN 8251)	+	0	+	+
	H. annuus (AGSUN 5383)	+	0	+	+
	H. annuus (PAN 7034)	+	+	0	+
	H. annuus (PAN 7049)	+	+	0	+

	H. annuus (PAN 7050)	+	+	0	0
	H. annuus (DK4040)	+	0	0	0
	H. annuus (Sirena)	+	+	0	0
	Helianthus tuberosus	+	+	0	0
	Wedelia trilobata	0	0	0	0
	Xanthium strumarium	+	0	0	0
Helenieae	*Tagetes patula*	0	0	0	0
Eupatorieae	*Mikania capensis*	+	0	0	0
	Mikania natalensis	+	+	0	0
	Adenostemma viscosum	0	0	0	0
	Adenostemma caffrum	0	0	0	0
	Ageratina adenophora	0	0	0	0
	Ageratina riparia	0	0	0	0
	Ageratum conyzoides	0	0	0	0
	Campuloclinium macrocephalum	+	+	0	0
	Chromolaena odorata	+	0	0	0
Senecioneae	*Senecio tamoides*	0	0	0	0
	Senecio deltoides	0	+	0	0
	Delairea odorata	0	+	0	0
	Senecio macroglossus	0	0	0	0
	Senecio oxydontus	0	+	0	0
	Senecio pleistocephalus	0	+	0	0
Anthemidae	*Aster novi-belgii*	0	+	0	0
	Osteospermum muricatum subsp. muricatum	0	+	0	0
	Dendranthema grandiflorum	0	0	0	0
	Microglossa mespilifolia	0	0	0	0
	Schistostephium flabelliforme	+	+	0	0
	Schistostephium heptalobum	0	+	0	0
	Argyranthemum frutescens	0	0	0	0
Gnaphalieae	*Athrixia phylicoides*	0	0	0	0

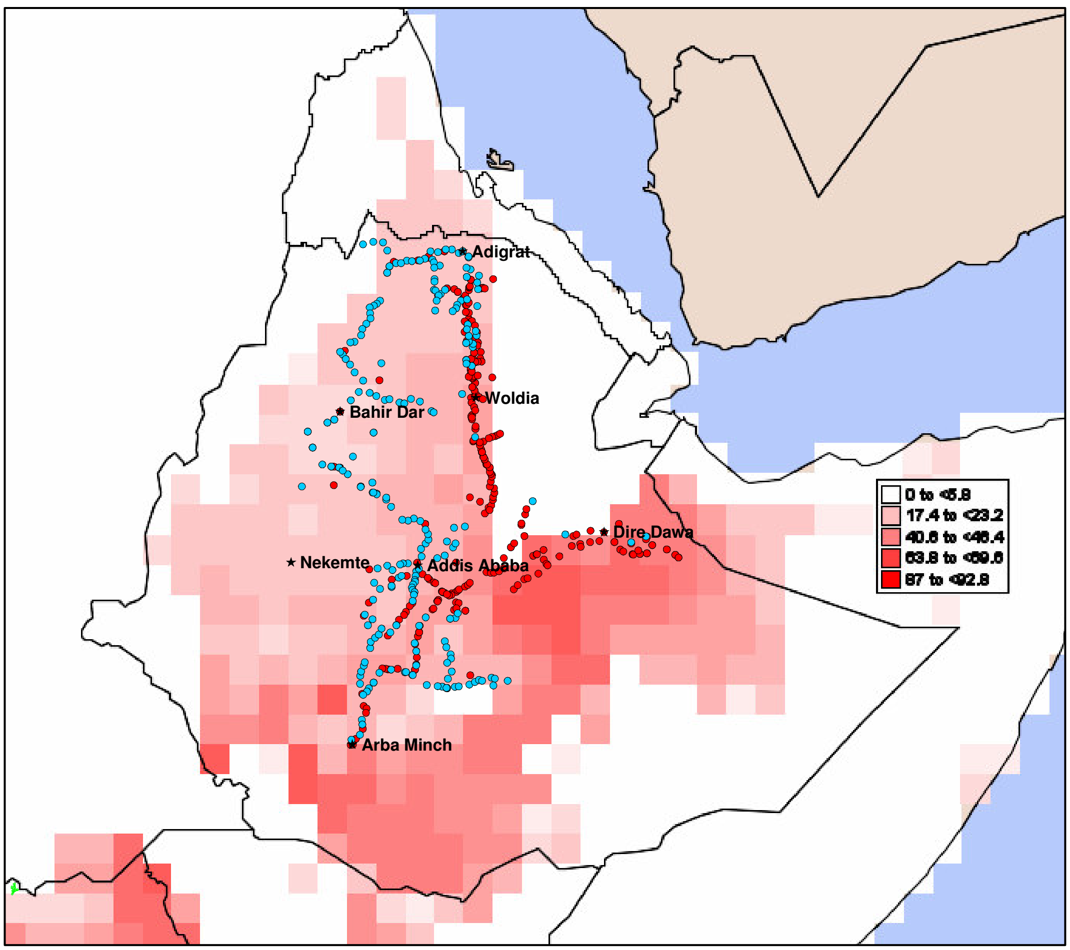
	Callilepis laureola	0	0	0	0
Calenduleae	*Garuleum sonchifolium*	0	0	0	0
Asteraceae: Cichorioidae					
Arctotidae	*Cynara scolymus*	0	0	0	0
Arctoteae	*Arctotheca poss. calendula* (green)	0	0	0	0
	Arctotheca poss. arctotoides (grey)	0	+	0	0
Vernonieae	*Ethulia conyzoides*	0	0	0	0
Lactucaceae	*Cichorium intybus*	0	0	0	0
	Lactuca sativa	0	0	0	0
Poaceae	*Eragrostis teff (D2-01-974)*	0	0	0	0
	Eragrostis teff (D2-01-2053)	0	0	0	0
	Eragrostis teff (D2-01-1278)	0	0	0	0

9. Selection of release sites in Ethiopia based on the distribution of parthenium and suitability to the development of Zygogramma.

CLIMEX model prediction and actual survey results parthenium distribution in Ethiopia.

Maps have been generated using CLIMEX model that show the relative climatic suitability for parthenium in Ethiopia. Colours of the squares (ecoclimatic index) depict the suitability of each location (the darker the shade of red, the more suitable) (Figure 2).

Figure 2. CLIMEX generated map of the relative climatic suitability of Ethiopia for parthenium and actual survey results. Colours of the circles (ecoclimatic index) depict the suitability of each location (the more reddish the more suitable). Distribution map of *Parthenium hysterophorus* in Ethiopia, with previously known records (green dots) and distribution records from 2006 survey (blue dots) and 2007 survey (red dots).



CLIMEX model parameters were obtained from the Queensland Department of Natural Resources and Mines (QDNR&M), Australia. These parameters were developed using known thermal characteristics of parthenium (from the literature) and mapped distributions of the weed in its native range. The parameters were entered into a new species template in CLIMEX program (ver. 2) and the model was run. Actual distributions determined during road surveys concurred with CLIMEX predictions, validating the model. Based on this CLIMEX model and actual results obtained from surveys carried out in Ethiopia, sites in the east (around Haramaya University), north (Alamata area) and central (Wilinchite area) have been selected for the earliest release.

CLIMEX model prediction of regions suitable for Zygogramma rearing in Ethiopia
Based on CLIMEX model, parthenium infested areas that are suitable for the growth of *Zygogramma* have been identified (Figure 3). Accordingly, three sites, one each from the East, North and Central part of the country have been selected as the initial release sites. The site in the North is in Tigray regional state (encompassing Woldia and Adigrat in figure 3) while the other two are in Oromiya regional state (at Haramaya near Dire Dawa and in the rift valley about 90 km south east of Addis Ababa). Other release sites will be identified in due course. Other factors which have influenced site selection are costs (start-up and maintenance), safety and hygiene, gender empowerment, and site protection for mass rearing.

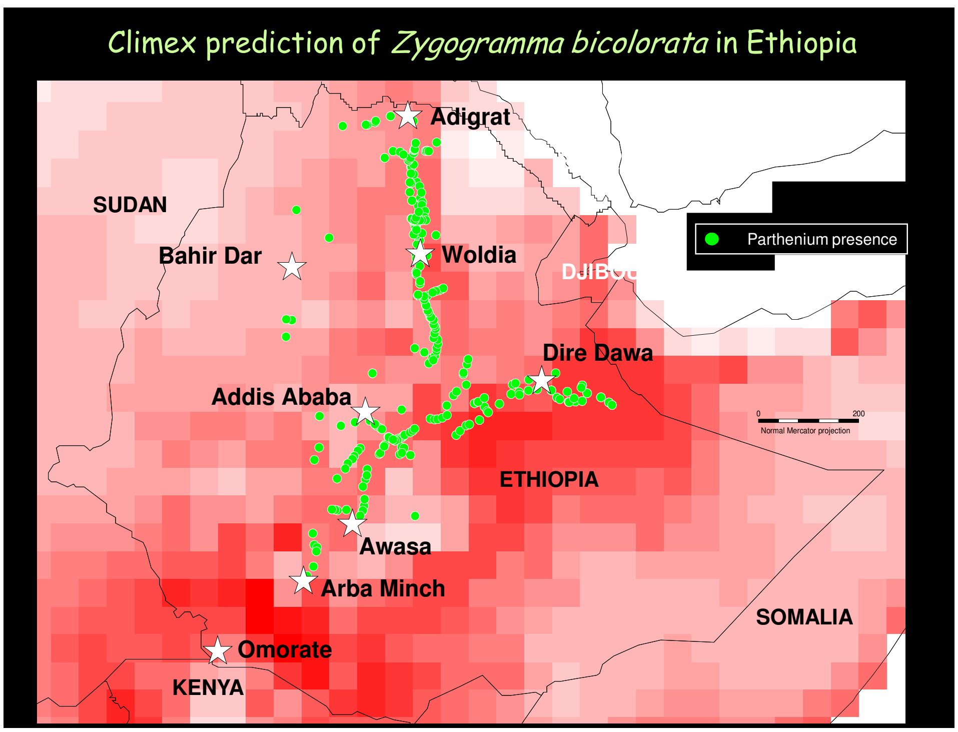


Figure 3. CLIMEX model of regions suitable for *Zygogramma* rearing (the more reddish the more suitable for *Zygogramma*).

The mass rearing center will be the source of insects to be released. It is arranged in such a way that there will be a continuous supply of insects. The rearing experience at the quarantine will be applied to multiply the agent. Populations that are mature and well adapted to external conditions will be used. Approximately 1,000 individuals may be considered as an initial minimum release size but smaller or higher numbers may also be considered. They will be transported within cages and released during late afternoon when it is neither cold nor hot. The first release is planned to take place around Haramaya University whose management body has agreed to take the responsibility of release in collaboration with Oromiya Bureau of Agriculture and other interested stakeholders, possibly NGOs.

10. Rearing

To ensure there are adequate numbers to conduct field releases of agents, mass-rearing cultures need to be set up appropriately. A mass rearing centre similar to the one used in Australia has been proposed. The centre will have a length of 30 m and width of 15 m (Figure 4). There will be six compartments, three on each side and a corridor in between. Five of the six compartments are breeding cages while the other one is a nursery plot. The five cages each measure 5 m by 7 m while the nursery is 10 m long and 5 m wide. Within the corridor are a toilet/washing facility and shed-tools/food area. Most of the materials required to build the nursery are locally available. There will also be a water tanker with a capacity of 10,000 L to supply water for the rearing centre. Healthy and pest-free parthenium plants will be used to rear *Zygogramma*. As *Zygogramma* is a leaf-feeding insect, plants will be well watered and fertilized so that they have lush leaves. The existing experience in rearing the agent will be applied to mass rearing.

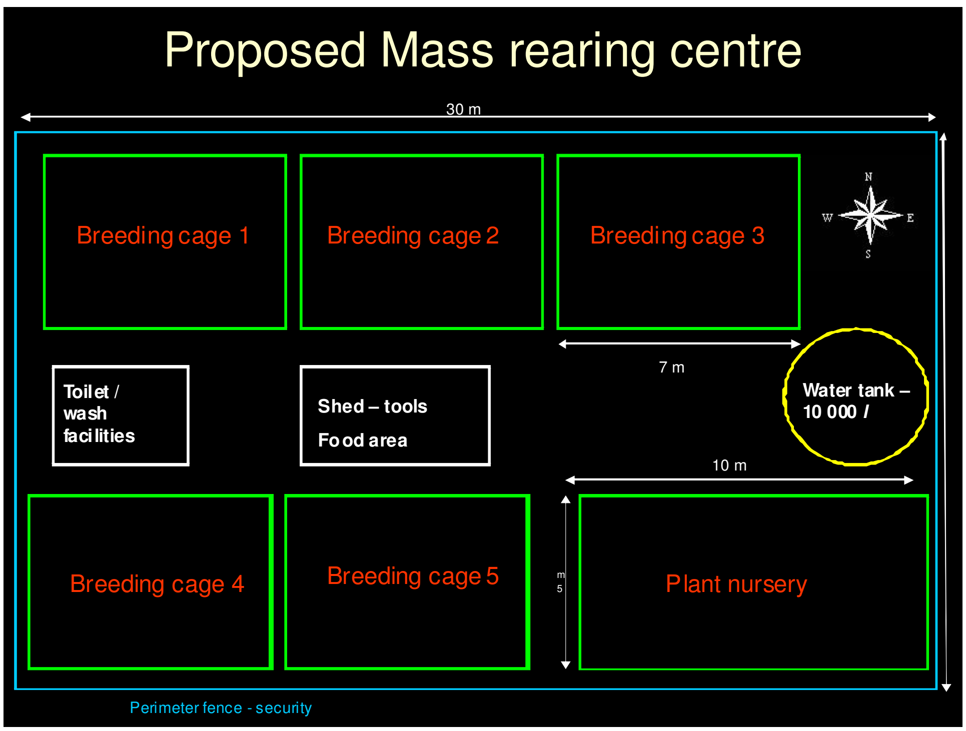


Figure 4. Schematic of proposed mass rearing center.

11. Training

Training will be conducted in order to get the active involvement of landholders and other community members for the release program and further follow up. A training of trainers will be organized at each site. Agricultural extension personnel and selected farmers will be trained and provided with supporting literature in local languages that clearly explains the techniques and processes involved, including managing sites. These trainees are expected to train other potential stakeholders within the release area. Training provided through practical displays, such as demonstrating agent release techniques in a field situation, can be effective in transmitting the message. When community members receive hands-on experience, they often feel more confident to continue without much further assistance. The benefits of community participation can be enormous as potentially many more releases can be conducted, thereby speeding up the delivery of biological control to end users. In addition, the community gains a greater understanding of biological control and a sense of ownership of the program through direct involvement in its implementation. One needs to establish an effective extension system for release and redistribution which has been recognized, in Australia, as the weak link in many weed biological control programs (Briese and McLaren, 1997).

12. National Committee and Monitoring

A national committee is being established to oversee the release program. It will be the central entity for soliciting public comment and stakeholder input as part of a future risk/benefit analysis for release of the agent. It is composed of representatives from Bureaus of Agriculture of the two regional states (Oromiya and Tigray), Director of AgriService Ethiopia, a local NGO, Ethiopian Institute of Agricultural Research, Mekele and Haramaya Universities (Table 7). Officers and members of farmer associations from the three release sites as well as from the national office will be included on the committee. The table below shows the nucleus of the committee that will be expanded once the permit is obtained from USAID.

Table 7. List of members of the national committee overseeing release program

Name	Institution	Responsibility/Profession
Mr. Getachew Worku	AgriService Ethiopia, NGO	Director
Dr. Samuel Assefa	Oromiya Bureau of Agric.	Crop Protection
Mr. Girmay Shinun	Tigray Bureau of Agric	Head, plant Health Clinic
Dr. Kassahun Zewdie	EIAR	Senior Weed Scientist
Dr. Mulugetta Negeri	Ambo Univ.	Senior Entomologist
Dr. Lisanework Nigatu	Haramaya Univ.	Botanist
Dr. Ibrahim Fitwe	Mekele Univ.	Senior Entomologist
Mr. Million Abebe	Parthenium project	Entomologist

Duties of the committee, among others, include: assisting in the selection of sites; securing resources for rearing sites; mobilization of farmers to take part in the release, organization of training programs; give press releases; and organize an awareness creation forum for farmers, extension workers and the public. Committee members will take the responsibility of organizing the release program in their respective regions and localities. The committee also directs and receives information on the progress of release and impact data.

13. Impact Assessment

The effectiveness and impact of the release will be assessed periodically. The assessment includes how the agent has affected the target weed and how this, in turn, has benefited other plant communities, pastures and ecosystems and even the society and the economy at large. Measurements will typically include agent survival, weed numbers and density, and the size of the weed seed bank, but they will also include native species diversity, crop or pasture yields, and other ecosystem characteristics (Evans and Landis, 2007; Yates and Murphy, 2008). Most importantly, evaluating the effectiveness of agents using a range of ecological, economic and social indicators provides data to assess whether and to what extent the agents have successfully suppressed weed populations and contributed to biodiversity, economic benefits and human health (Syrett et al., 2000).

The different methodologies, for impact assessment, described below are taken from a document entitled "Best Practice Guide- Impact Evaluation of Weed Biological Control Agents" of CRC for Australian weed management. The first two methods, included below, have been used for impact evaluation of *Zygogramma* on parthenium in Australia and were found effective.

13.1 Before and after release assessment – photo points

Pictures of parthenium infested field will be taken, just before or soon after release, and compared with a series of pictures, of the same field, taken at a regular interval. This will give a visual impression of the impact of the agent but this shall be supported by a subsequent quantitative impact data.

13.2. Comparing plots with and without agents

Three parthenium sites will be surveyed for two years, to be extended to three years if need be. At each site parthenium patches will be selected based on whether or not the agent is present and defoliating plants. Within each patch, ten 0.25 m^2 quadrants will be randomly selected and data on plant height, plant density, flower production, total plant biomass, viable soil seed bank and defoliation level by *Zygogramma* will be recorded. Each year measurements will be made in different parthenium patches. Control plots will be excluded with cages to prevent infestation of plants.

13.3. Comparing quantitative ecological data

Quantitative data collected on selected target weed populations, co-existing plant communities, and/or other ecosystem parameters some years before the release of an agent, can be compared with similar data gathered after its release (McClay, 1995; Blossey, 1999; Carson et al., 2008). This method can be applied in areas where some data has been collected. These areas include sites in the north and eastern part of the country where such information has been gathered by Mekele and Haramaya Universities, respectively.

14. Expected Results from use of the Biological Agent, *Zygogramma bicolorata*, (benefits, beneficiaries)

It is anticipated that the use of the biological agent as part of an integrated pest management program will significantly reduce the infestation and further spread of the weed parthenium. As a result, in line with IPMCRSP goals:

- -Crop yield loss will be reduced
- -Pasture carrying capacity will be improved
- -Biodiversity will be maintained
- -Negative health effects such as asthma, allergic skin reaction etc. will be minimized

-Quality of animal products such as milk, meat, honey that are negatively affected by parthenium will be improved
-The productivity of the farming community will be improved as children and women do not have to spend as much time in the field pulling out the weed

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Appendix A. Permit received from the Ethiopian government to import the bioagents *Zygogramma bicolorata* and *Listronotus setosipennis* for evaluation under quarantine.

The Federal Democratic Republic of Ethiopia
Ministry of Agriculture and Rural Dev,t
Plant Quarantine Service
Plant Import permit

Permit No: - 2070
Date Issued APRIL19,2007

Permission is hereby granted to:-DR. MULUGETA NEGRI,PPRC P.0.BOX 37 AMBO ETHIOPIA TEL 0911-866296
(Name and address of the application)

to import from:-SOUTH AFRICA
the following plants/seeds/plant parts:

"NOT VALID FOR CUSTOMS CLEARANCE"

Common name	Botanical name/Scientific name.	Quantity/ (KGS)
1.LEAF FEEDING BEETLE	*Zygogramma bicolorata*	
2.STEM BORER WEEVIL	*Listronotus stotosipennis*	*1200 INDIVIDUALS*

Through the ports (s) of: -BOLE INTERNATIONAL AIRPORT
Subject to the following conditions:

1. Country and place of origin:-SOUTH AFRICA(Central queensland and Argentina)
2. The consignment is subjected to inspection on arrival and if necessary to treatment or the destruction or return to the country of the origin, as the case warrants.
3. Plants or plant parts must be entirely free of soil.
4. phytosanitary certificate (p) from the country of origin together with one of this permit shall accompany each consignment.
5. The following materials are prohibited entry as packing materials:______________
6. Additional Declarations:- PCendorsed that the bio control agents are free from hyperparasites and other insects.

Note A/ This permit is valid for 6(six) months from the date of issue, but may be Cancelled at any time by plant quarantine division.
B/ This permit is drawn up in triplicate: two copies are delivered to the importer who should send one copy to the supplier:

Ministry of Agriculture-Plant Quarantine, A.A. The Federal Democratic Republic of Ethiopia

19/04/07

MERID KUMSA
Head,crop. Prot.Lab. & Quarantine Division

Appendix B. Communication from USAID and the first IEE submission for the importation of the biological control agents for parthenium, *Zygogramma bicolorata* and *Listronotus setosipennis*.

Original Message -----
From: Hedlund, Robert(EGAT/NRM/LRM)
To: Wondimkun, Yacob (ADDIS/BEAT) ; Hirsch, Joe (ADDIS/BEAT) ; Demissie, Belay (ADDIS/BEAT)
Cc: Jatko, Joyce(EGAT/ESP/MPC) ; ipm-dir@vt.edu ; Wondi Mersie ; Hirsch, Brian(AFR/SD)
Sent: Wednesday, October 10, 2007 4:20 PM
Subject: IEE for evaluation of biological control organisms for parthenium

Greetings Joe and others,

I have attached a copy of the IEE for evaluation of biological control organisms for the weed, *Parthenium*, for which we expect approval by Joyce A. Jatko, EGAT BEO, this week. I want to thank you for your interest in this program and for meeting with both Wondi Mersie, the program leader for this activity; Muni Muniappan the IPM CRSP Director and with the members of the external evaluation panel who visited Ethiopia last month.

I was pleased to learn that you will be participating next week in the dedication of the quarantine facility where these bio-control organisms will be evaluated. Mission interest and support for CRSP activities is always appreciated. Please let me know of any ideas you may have for IPM CRSP support of Mission goals.

Bob

Robert C. Hedlund, Ph.D.
USAID/EGAT/NRM
Integrated Pest Management
Natural Resources Management
Rm. 2.11-91, Ronald Reagan Building
1300 Pennsylvania Ave. NW
Washington, D.C. 20523-2110

TEL: (202) 712-4188
FAX: (202) 216-3010
Email: rhedlund@usaid.gov

INITIAL ENVIRONMENTAL EXAMINATION

PROGRAM/ACTIVITY DATA

Program/Project Title: Importation of Two Biological Control Agents for Contained

Experiments against an Invasive Weed

Program/Project Number: IPM CRSP, EPP-A-00-04-00016-00, Global theme on invasive species

Project Country(ies): Ethiopia

Funding Period: 9/30/04-9/30/09

Life of Activity Funding: $12,000,000

IEE Amendment Yes _X_ No___ If yes, date of original IEE: _May 5, 2004____________

IEE Prepared by:__IPM CRSP Management Entity _9/27/07________
Name/Office Date

ENVIRONMENTAL ACTION RECOMMENDED(check all that apply)**:**

Categorical Exclusion ___________ Negative Determination ______________________
Positive Determination___________ Negative Determination w/ Conditions ___X______
Deferral___________

Recommended By: ____
____
EGAT/AG, **John Thomas** Date

Concurrence: ____
____
Joyce A. Jatko Date
EGAT Bureau Environmental Officer

Approved: ______

Disapproved: ______

Clearances:

RHedlund: CTO IPM CRSP_____________________________________
____
Date

BHirsch: AFR/SD BEO ____
____
Date

Appendix C. Permit received from the Ministry of Agriculture and Rural Development to Release the Bioagent, *Zygogramma bicolorata*, in Ethiopia.

Federal Democratic Republic of Ethiopia
MINISTRY OF AGRICULTURE AND
RURAL DEVELOPMENT

No 21/132/2/24
Date 26/08/2009

Ethiopian Institute of Agricultural Research

Addis Ababa

Subject:- **Release of bio-agent (Zygogramma bicolorata)**

Reference is made to your letter Ref. No. 1597/2001/143/1 dated 22 Aug., 2009 which requests the release of bio-agent, Zygogramma bicolorata for the control of parthenium weed, which have been under post entry quarantine supervision for one year at Ambo Plant Protection Research Center.

A team of experts from the Ministry of Agriculture and Rural Development, Animal and Plant Health Regulatory Directorate visited the quarantine facilities and activities carried out with regard to the bio-agent, and submitted a report or minutes of agreement of the visit expressing its satisfaction with the testing and safety procedures followed and thereby agreeing upon the release of the bio-agent in whilinchiti area.

Therefore, permit is hereby granted for the initial release of the bio-agent Zygogramma bicolorata, in the above specified area, as per the recommendation.

Regards,

FIKRE MARKOS
Deputy Director, Animal & Plant Health
Regulatory Directorate

Fax No 51 15 43
62347
3138
518840
517354
517194
517383
517394
Ethiopia - Addis Ababa
Telex No
Please quote Our Ref.
When replying

Appendix D. Advertisement in an Ethiopian news paper, Ethiopian Herlad, asking for public comments on the release of Zygogramma for the control of parthenium.

The Ethiopian Herald

Vol.LXVI No 281	Wednesday 4 August 2010 Hamle 28, 2002	Price birr 2.00

Only short listed candidates will be not

Solicitation of Comments for the Release of a Leaf-Feeding Beetle to Control the Weed Parthenium in Ethiopia

Parthenium (Parthenium hysterophorus L. Asteraceae) is believed to have been introduced to Ethiopia in late 1970 and now has spread to all regions of the county. Parthenium reduces the yield of crops and pastures and when consumed by domestic animals, it taints their milk and meat, reducing their value, Parthenium also causes human health problems such as severe contact dermatitis, hay fever and respiratory stress. A project lead by Virginia State University and funded by the USAID through the Integrated Pest Management Collaborative Research Support Program (IMP CRSP) has been in operation in Ethiopia since 2005 to develop an integrated weed management system that reduces the adverse impact of parthenium on humans, crops, livestock and plant biodiversity. Parthenium has been successfully controlled in Australia and India using biological agents such as the leaf-feeding beetle, Zygogramma bicolorata. As a prerequisite for field release, host range tests were conducted under quarantine at Ambo Research Center of Ethiopia's Institute of Agricultural Research to assess the safety of this beetle on major crops and plant species taxonomically related to parthenium. The safety of Zygogramma was tested on a total of 17 crop species and 52 non-crop species. In addition, the beetle was tested on 7 noog seed (Guizotia abyssinica) varieties, on 3 vartieties of teff (Eragrostis teff) and on 15 sunflower (Helianthus annuus) cultivars.

The tests under quarantine on the above economically important crop species and varieties, and indigenous species, established that Zygogramma is safe for release against parthenium.

We are soliciting comments on the release document for Zygogramma in Ethiopia from the public. The document can be found at **www.eiar.gov.et** & **www.moard.gov.et** website under basic documents link or hard copies can be read at Ethiopian Institute of Agricultural Research Library and Ministry of Agriculture and Rural Development HQ block-b floor -2 room 2-1 office number.

The public comment period will end fifteen days after the appearance of this advertisement on the Newspaper. Send your comments at **ergbaberhanu@yahoo.com** or **Kassahunzewdie@yahoo.com**

Appendix E. A letter from Ethiopian Ministry of Agriculture on lack of public comment on the release of *Zygogramma bicolorata* for the control of parthenium.

August31, 2010

Wondi Mersie
Associate Dean and Director of Research
Director of Parthenium Project
Virginia State University
Petersburg,
VA 23806
USA

Dear Dr. Mersie:

Parthenium (*Parthenium hysterophorus L.* Asteracese) reduces the yield of crops and pastures and when consumed by domestic animals, it taints their milk and meat, reducing their value. *Parthenium* also causes human health problems such as severe contact dermatitis, hay fever and respiratory stress. A project lead by Virginia State University and funded by the USAID through the Integrated Pest Management Collaborative Research Support Program (IPM CRSP) has been in operation in Ethiopia since 2005 to develop an integrated weed management system that reduces the adverse impact of *Parthenium* on humans, crops, livestock and plant biodiversity. *Parthnium* has been successfully controlled in Australia and India using biological agents such as the leaf-feeding beetle, *Zygogramma bicolorata*.

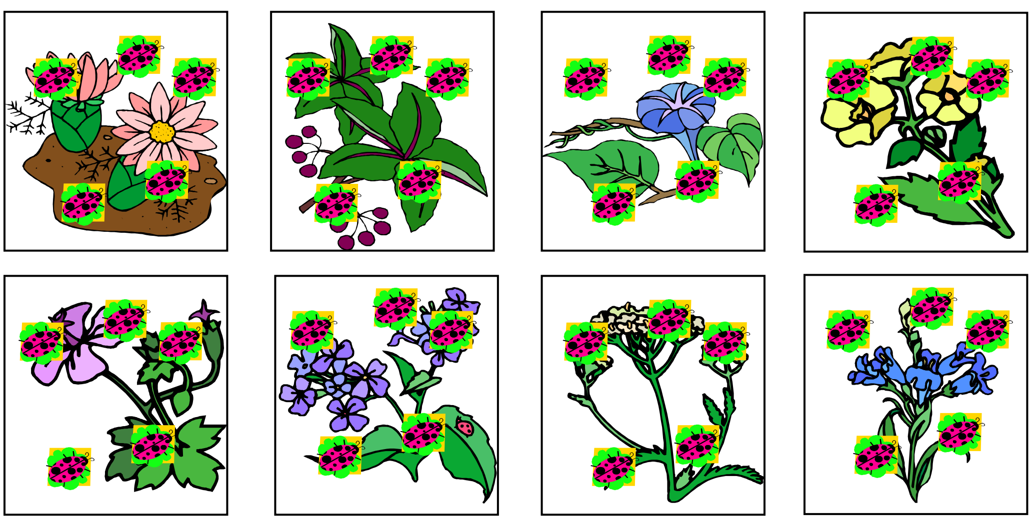
In Ethiopia the host range of this leaf-feeding beetle was evaluated under quarantine at Ambo Research Center of Ethiopia's Institute of Agricultural Research to assess the safety of this beetle on major crops and plant species taxonomically related to Parthenium. The safety of *Zygogramma* was tested on a total of 17 crop species and 52 non-crop species.In addition, the beetle was tested on 7 noog seed (*Guizotia abyssinica*) varieties, on 3 varieties of teff (*Eragrostis teff*) and on 15 sunflower (*Helianthus annuus*) cultivars. The tests under quarantine on the above economically important crop species and varieties, and indigenous species, established that Zygogramma is safe for release against Parthenium. Based on the data collected from these tests, the Ethiopian Ministry of Agriculture and Rural Development has issued a permit to release *Zygogramma bicolorata* for the control of *Parthenium*.

An application for a permit to release Parthenium in Ethiopia was submitted to USAID. As a part of the application USAID requested for a public comment in Ethiopia on the release of Zygogramma bicolorata. Pursuant to this request from USAID, an advertisement was placed on one of the widely distributed and read English Newspaper in Ethiopia, The Ethiopian Herald. As you see attached the solicitation for comment on the application to release *Zygogramma bicolorata* in Ethiopia was printed on the Newspaper, Vol. LXVI No 281 Wednesday,

Appendix F. Frequently Asked Questions About Weed Biocontrol Agents.

Compiled by Arne Witt, ARC-PPRI Weeds Division, Pretoria, South Africa. May 2007.

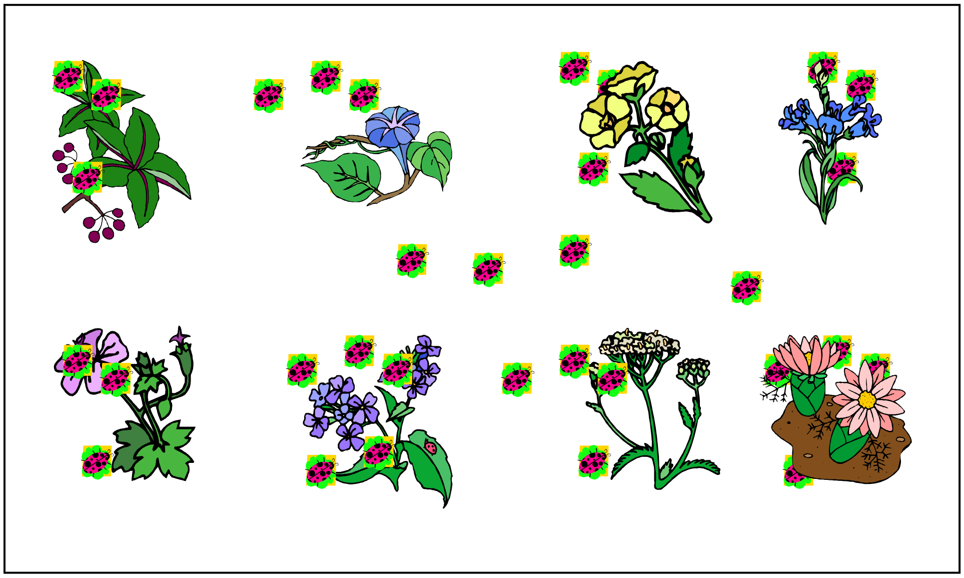
One of the key elements in weed biocontrol research is to prove that a potential biocontrol agent is host specific (monophagous) ie. it will only feed and produce viable offspring on the target species. In some cases oligophagous (feed on more than one species in the same genus) species may also be acceptable provided that there are no indigenous species or crops within that particular genus. Host range trials or host specificity tests are generally always undertaken in quarantine glasshouses where the agents and the test plants are exposed to natural light. These trials or tests normally take the form of no-choice, paired-choice or multiple-choice trials. In no-choice trials the potential agent is only exposed to one plant species at a time (Figure 1). Researchers will place the potential agent (10 adults for example) in one cage on one plant and monitor its survival, damage and its ability to reproduce on the target or non-target species. The results of these trials are often rather conservative – they often indicate a wider host range than what would occur naturally in the field because the experimental design is not in any way similar to what is found in the field. The non-target species used in these trials are not selected randomly, as is often thought, but are selected based on their taxonomic relationship with the target species with more closely related than distantly related plants being tested. Most indigenous plants within the same genus are tested while fewer species in more distantly related families are used in trials. Distantly related species that are similar morphologically to the target species are often also used in trials including crop plants.



Choice trials are a more accurate reflection of the potential agents host range as they mirror more accurately the natural situation in the field – the female can make a decision as to which plant she favours for oviposition. In paired-choice trials one plant of the target species (natural host) and one plant of a non-target species is placed in each cage (Figure 2). For example, ten adults will then be placed in the cage and the damage, number of eggs, surviving nymphs/larvae etc. on each plant will be recorded after a certain time period. If the agent is extremely host specific there will only be feeding damage, oviposition and nymphal/larval survival on the host/target plant. Multiple-choice trials are an attempt to replicate as close as possible to what the potential agent may find in the field – it will be exposed to a number of different species and will have to select one specific species to feed and develop on (Figure 3). In this case we place a large number of plants of different species including the agents natural host in a large walk-in cage. We then release a large number of agents into the cage.

Figure 2: An example of a paired-choice trial – the agents are exposed to the host plant and a non-target plant in the same cage.

Figure 3: An example of a multiple-choice trial – the agents are exposed to the host plant and a number of plants of non-target species in the same cage.



Frequently asked questions:
Question: What happens to the agents when the host plant is eradicated?
Answer: The agent never eradicates its host but controls it to the extent that it reduces plant vigour and/or seed production. The agent populations will track those of its host plant, when the host plant is abundant the agent's population will also increase with populations crashing when the food source becomes scarce.
Question: Will the agent eat my maize?
Answer: The host range of the agents has been determined in host-range trials using the various experimental designs as explained above. In addition, extensive literature searches are done to determine if the agent is a pest of any agricultural crop in its country of origin or elsewhere. If the agent in question is exposed to similar crop species as in South Africa and has never been recorded as a pest on those species then it is additional information that can be used to support its release.
Question: Won't the agent "evolve" the ability to feed on other plant species?
Answer: Agents have evolved with their host plants over millions of years and are adapted to only feed or develop on that particular species. The extinction of one plant species will therefore have cascade effects on other species, particularly host-specific phytophagous species, which cannot feed on other plants. If all phytophagous insects were generalists the extinction of plant species would not be of concern to invertebrate conservation because they could merely feed on other surviving plants. Further proof of this is the fact that most introduced plant species have only acquired very few indigenous pests, most of them generalists. Despite a huge food resource available in crop monocultures only 765 and 3359 indigenous insect and mite species have been recorded as pests on indigenous and alien cultivated plants in South Africa, respectively (Moran, 1983). Of these insect and mite species, 78% are polyphagous. If one considers that approximately 630 phytophagous insect species were collected on *Acacia nilotica* in South Africa alone then the pool of potential pest species present in southern Africa that could attack these crop species is exceptionally high. It has been estimated by various

taxonomists that there are between 500000 and 1 million phytophagous insect species in southern Africa which means that only 0.003 – 0.006% of all insect species present are "pre-adapted" to feed on introduced cultivated plants. Surely this is an indication that most phytophagous insect and mite species have a limited host range and that despite their natural hosts being reduced in distribution and abundance none of them have "evolved" the ability to feed on crop species which dominate the landscape in most regions of South Africa.

PERCEIVED RISKS OF BIOCONTROL

i) Potential of proposed biological control agents to hybridise with indigenous species:

"Hybridization" is generally known as interbreeding of individuals from what are believed to be genetically distinct populations, regardless of the taxonomic status of such populations. According to Rhymer and Simberloff (1996) "hybridization" most commonly refers to mating by heterospecific individuals but has also been applied to mating by individuals of different subspecies and even of populations that, though not taxonomically distinguished, differ genetically. Others like Arnold et al. (1991) suggest restricting "hybrid" to matings between species and using "integrade" for matings between subspecies and "cross" or "interbreed" for matings between populations of geographically distinct populations. The definition of hybridization has specific bearing on the species concept or definition of what a species really is.

According to Gordh and Headrick (2000 – A Dictionary of Entomology) a species is, "the primary biological unit, debatably an actual thing or a purely subjective concept; a static component in the continuum of life; an aggregation of individuals similar in appearance and structure, mating freely and producing young that themselves mate freely and bear fertile offspring resembling each other and their parents, including all varieties and races." The debate surrounding the definition of a species has given rise to various "species concepts." The most commonly quoted one is probably Mayr's (1963) "biological species concept" which develops the notion that species are interbreeding natural populations that are reproductively isolated from other similar, natural populations. The "evolutionary species concept" maintains that a species is a genetical lineage evolving independently of other genetical lineages and which displays its own unitary evolutionary role and tendencies (Simpson, 1961). Then we also have the "phylogenetic species concept", the "recognition species concept" and others like the "cohesion species concept." Paterson's "recognition species concept" argues that species are cohesive wholes as a result of pre-zygotic sexual signaling within species, rather than due to isolating mechanisms between species. Paterson replaced the concept of isolating mechanisms with specific mate recognition systems. Unfortunately, the word "system" has many group-benefit connotations, and for this reason the "recognition concept" of species has not gained universal acceptance.

Isolating mechanisms are particularly important in the "biological species concept", in which species of sexual organisms are defined by "reproductive isolation", i.e. an absence of gene mixing. There are two broad kinds of isolating mechanisms between species, pre-mating and post-mating isolating mechanisms. Pre-mating isolating mechanisms are probably more important from an ecological point of view because matings between members of different species are a waste of reproductive resources for both partners. The waste for females is generally greater than for males, because females produce large, heavily resourced gametes, while males produce small gametes containing little more than a nucleus and a "tail". However, the cost to males is not negligible. Not only does a male that mates with a female of another species waste gametes, he wastes energy in mating, he wastes time that may be used in other more productive ways such as feeding, resting or courting more appropriate females, and, as he is less mobile while copulating, he may be more prone to predation. He also runs the risk of contracting disease from his mating partner. Females are also liable to these latter costs. Note should also be taken of the fact that unlike many vertebrate species where absence of mating between two "species" is governed largely by behavioural differences, in insects and other invertebrates there are a number of additional factors or "mechanisms" which inhibit or prevent mating. Anyway, pre-mating isolating mechanisms are those, which cause species to only mate with their own kind (assortative mating):

i) **Temporal isolation:** Individuals of different species do not mate because they are active at different times of the day or in different seasons;

ii) **Ecological isolation:** Individuals mate in their preferred habitat, and therefore do not meet individuals of other species with different ecological preferences;

iii) **Behavioural isolation:** Potential mates meet, but choose members of their own species;

iv) **Mechanical isolation:** Copulation is attempted, but transfer of sperm does not take place. Genital morphology of insects with internal fertilization has long been recognized by taxonomists as an important characteristic for distinguishing closely related species. The observation of intricate matching between male and female genitalia has led to suggestions that differences in genital morphology may be important in maintaining reproductive isolation between closely related species. Proper morphological matching between male and female genitalia may be necessary for efficient fertilization, and so mismatched genitalia may reduce the fitness of copulating individuals in both intraspecific and interspecific copulations. Prezygotic reproductive isolation at the stage of copulation may result from:

a) rejection of heterospecific genitalia or impossibility of genital coupling,
b) reduced efficiency of sperm transfer, and
c) direct physical cost on copulating individuals (injury of reproductive organs and resultant mortality). This "lock-and-key" hypothesis of species-specific genitalia has been criticized repeatedly because of the lack of evidence that differences in genitalic morphology actually affect copulatory success between closely related species.

<u>Example 1:</u> Experiments were conducted in the field on males of five species and females of ten species of damselflies to determine the relative importance of visual and reproductive isolating mechanisms. When the males attempted to mate with females of other species, they were usually prevented from doing so because their abdominal appendages were unable to secure a firm grip on the appropriate thoracic structures in the females (mechanical isolation) (Paulson, 1974).

<u>Example 2:</u> The apple-feeding "host race" of the tephritid fruit fly (*Rhagoletis pomonella*) differs from the hawthorn-feeding race in that the apple race not only emerges earlier in the year (temporal isolation) but each "host race" preferentially chooses to rest, lay eggs and mate on its own host (ecological isolation) (Calow, 1998). In a similar study on *Rhagoletis pomonella*, Feder *et al*. (1994) confirmed the tendency of an insect to reproduce on the same host species that it used in earlier life-history stages. This behaviour restricts gene flow between sympatric apple- and hawthorn-infesting races of *R. pomonella* to ~ 6% per generation. Genetically based differences in host preference, adult eclosion under the "correct" host species, and allochronic isolation contribute to host fidelity in various degrees in the races. **The results verify that host-associated adaptation can produce reproductive isolation.**

Example 3: Differences in the courtship behaviour in the sympatric and morphologically similar Hawaiian fruitflies (*Drosophila heteroneura* and *D. simulans*) results in females only responding to behaviours characteristic of her own species (behavioural isolation) (http://www.micro.utexas.edu/courses/levin/bio304/evolution/repriso.html)

Example 4: The impact of habitat choice was investigated as a factor in reducing gene exchange between sympatric populations of the pea aphid (*Acyrthosiphon pisum*), one which predominates on alfafa and the other on red clover. Because mating occurs on the host plant, habitat choice leads to assortative mating and is therefore a major cause of reproductive isolation between the sympatric pea aphid populations on alfafa and clover (ecological isolation) (Vea, 1999).

Example 5: The sexual behaviour of phyophagous insects is often integrated in a variety of ways with their host plants. Certain insects sequester or acquire host plant compounds and use them as sex pheromones or sex pheromone precursors. Other insects produce or release sex pheromones in response to particular host plant cues. Chemicals from host plants often synergize or otherwise enhance insect responses to sex pheromones. By these means, host plants may be used by insects to regulate or mediate sexual communication.

For many species of insects, host plant influences on insect sex pheromone communication may be important aspects of the formation of feeding and mating aggregations, of insect strategies to locate both hosts and mates, of behavioural reproductive isolation among sibling species, and of the regulation of reproduction to coincide with the availability of food and oviposition sites (Landolt and Phillips, 1997). In other words host-specific biocontrol agents are more likely to mate or come into contact with those species feeding on the same plant if the agents in question sequester plant compounds and use them as sex pheromones. This phenomenon can be seen as a combination of ecological and behavioural isolation.

Example 6: Males and females of *Prokelisia marginata* (Delphacidae) and *P. dolus* communicate through substrate-transmitted vibrations. The acoustic signals (attraction and courtship calls) of these planthoppers are effective in mate location, attraction, and mate choice. Attraction calls are structurally distinct for both species and differ in pulse type, pulse repetition rate, and pulse duration. Using playback of prerecorded calls, individuals discriminated between conspecific and heterospecific signals. Depending on the sex and species, response calls were produced three to eight times more frequently to conspecifics than to heterospecifics. However, acoustic signals alone did not explain reproductive isolation and hybridization failure in these two congeners. Some heterospecific pairs called, courted, and attempted to join genitalia, but no connections were successful and no progeny were produced. In other words, other courtship behaviours and possibly morphological differences in genitalia also contributed to their isolation (behavioural isolation) (Heady and Denno, 1991).

Example 7: To test the hypothesis that *Rhagoletis mendax* (Tephritidae) and *R. pomonella* have evolved viability differences on alternate hosts which can contribute to the restriction of gene flow between them, researchers measured the larval-to-adult viability of *R. mendax*, *R. pomonella* and F_1 interspecific hybrid progeny reared on naturally-growing, highbush blueberry and apple plants in the field. The results indicate that genetic changes associated with the adaptation of these species to distinct host plants could also cause reduced fitness of interspecific hybrids, and thereby restrict interspecific gene flow. Fewer interspecific hybrids survived to adulthood than either *R. pomonella* progeny reared on apples or *R. mendax* progeny reared on blueberries. These differences in the viability of progeny from hybrid versus conspecific crosses can substantially restrict gene flow between *R. mendax* and *R. pomonella* flies, and may be an important factor influencing their reproductive isolation (ecological isolation) (Bierbaum and Bush, 1990).

Example 8: Responses of a biological control agent, the ragwort flea beetle (*Longitarsus jacobaeae*), to cues associated with conspecific beetles were examined using two-choice tests in the laboratory and in the field. The results from two sets of experiments using, respectively, ragwort leaves and filter paper as substrates suggest that male beetles responded to cues associated with female beetles rather than to host plants. These results were confirmed in the field where ragwort leaves, which had been previously exposed to female beetles attracted more male beetles than clean leaves without female-associated cues. All of the evidence suggests that *L. jacobaeae* females emit a sex pheromone that is attractive to male beetles and persists in areas formerly occupied by females. The presence of a female-produced sex pheromone has been documented in over 18 families of the order Coleoptera and can be seen as a mechanism to inhibit hybridization (Zhang and McEvoy, 1994).

Post-mating isolating mechanisms are those, which cause genomic incompatibility, hybrid inviability or sterility:

i) **Gametic incompatability:** Sperm transfer takes place, but egg is not fertilized;
ii) **Zygotic mortality:** Egg is fertilized, but zygote does not develop;
iii) **Hybrid inviability:** Hybrid embryo forms, but of reduced viability;
iv) **Hybrid sterility:** Hybrid is viable, but resulting adult is sterile;
v) **Hybrid breakdown:** First generation (F1) hybrids are viable and fertile, but further hybrid generations (F2 and backcrosses) may be inviable or sterile.

Example 1: A good example of post-mating isolation is that between various species in the *Drosophila simulans* species complex. Researchers allowed females to mate only once, observed and timed all copulations, dissected a subset of the females to track the storage and retention of sperm, examined the number and hatchability of eggs laid after insemination, counted all progeny produced, and measured the longevity of mated females. When *D. simulans* females mate with *D. sechellia* males, few heterospecific sperm are transferred, even during long copulations. In contrast, copulations of *D. simulans* females with *D. mauritiana* males are often too short to allow sperm transfer. Those that are long enough to allow insemination, however, involve the transfer of many sperm, but only a fraction of these heterospecific sperm are stored by females, who also lay fewer eggs than do *D. simulans* females mated with conspecific males. Finally, when *D. mauritiana* females mate with *D. simulans* males, sperm are transferred and stored in abundance, but are lost rapidly from the reproductive tract and are therefore used inefficiently (Price *et al.*, 2001)

Example 2: In laboratory trials, *Chrysopa quadripunctata* (Neuroptera) females failed to lay eggs when crossed with *C. slossonae* males, but invariably produced viable *C. quadripunctata* offspring (no hybrids) within one day after the heterospecific male was replaced with a conspecific one. The barrier to hybridization may involve the females ability to (a) distinguish between heterospecific and conspecific sperm and (b) allow the transfer of only conspecific sperm to the spermatheca (Albuquerque *et al.*, 1996)

It is clear from the examples given above that there are many "mechanisms" in place to prevent hybridization from occurring between different insect species and that these isolating "mechanisms" will be enhanced in situations where two species have evolved separately over millions of years on different continents and on different plant species. In addition, the intended agents have no close relatives in Africa which makes hybridization even less likely. In cases where there are possibly native congeners, host specific phytophagous insects on particular weed species are unlikely to ever come into contact with congeners which feed on other plants and are therefore ecologically isolated from each other (pre-zygotic isolating mechanism).

ii) Potentially harmful organisms (parasites/parasitoids) associated with the biological control agents and the mitigation measures to prevent their release into the environment:

Insect parasitoids are generally only associated with the immature stages of potential biocontrol agents with very few to no natural enemies associated with the adults. Any immature stages of biocontrol agents brought into quarantine may be parasitized. In order to establish a culture in quarantine it is critical that all organisms, which may affect population growth, be eliminated. To that end researchers ensure that over many generations, through a process of elimination, all parasitoids are removed from the population. It is not possible to undertake research on a particular agent if some of the individuals are parasitized so it is in the best interest of researchers to remove all potentially harmful organisms. An agent released with its parasitoids will not be very effective which is why all detrimental organisms associated with a potential agent are eliminated prior to release.

iii) Monitoring plans/programmes and environmental plans for each application agents:

It is essential to determine the impact and potential non-target effects of any agent once it is released. This requires long-term monitoring of release sites because the impact of the agents on target plants can often only be determined after the release of thousands of agents over a long period of time.

iv) Control measures for biological control agents, should they become problematic.

More than 90 agents for the biological control of invasive plants have been released in South Africa with no recorded non-target effects. These agents were deemed to be safe for release based on extensive host range trials, which indicated that they were host specific. The results of these trials were further supported by extensive literature surveys, which indicated that none of these agents had ever been recorded as pests of any crop within their countries of origin. The same applies to the agents which we hope to release provided that laboratory trials demonstrate their host specificity and they have not been recorded as pests of any agricultural crop anywhere in the world. There is therefore no need to consider any measures to control these agents as they will not become problematic.

It is unrealistic to expect anyone to exterminate agents once they have been released unless they have an inability to move away from a specific release site. In cases where they can be confined to a specific site they can possibly be exterminated by using a pesticide which would also eliminate all other invertebrates in a specific area. However, biocontrol agents can only be confined to a specific area by placing them in a large cage in the field. By doing this one will, to a large extent, merely be replicating a laboratory multiple-choice trial which is usually undertaken in large walk-in cages; there will be nothing different other than the environmental conditions and the fact that the agents will be exposed to fewer potential hosts in a cage in the field. The decision to release agents therefore has to be taken on the basis of laboratory trials and current literature which indicates that none of the agents have been recorded as agricultural pests anywhere in the world and pose no threat to indigenous fauna or flora.

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