Field Release of *Cecidochares* (*Procecidochares*) *connexa* Macquart (Diptera:Tephritidae), a non-indigenous, gall-making fly for control of Siam weed, *Chromolaena odorata* (L.) King and Robinson (Asteraceae) in Guam and the Northern Mariana Islands

Environmental Assessment

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Proposed Action:	The U. S. Department of Agriculture (USDA), Animal and Plant Health Insepction Service (APHIS) is proposing to issue a permit for the release of the nonindigenous fly, *Cecidochares* (*Procecidochares*) *connexa* Macquart (Diptera:Tephritidae). The agent would be used by the applicant for the biological control of Siam weed, *Chromolaena odorata* (Asteraceae), in Guam and the Northern Mariana Islands .
Type of Statement:	Environmental Assessment
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1. Purpose and Need for Action

1.1 The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) is proposing to issue a permit for release of a nonindigenous fly, *Cecidochares* (*Procecidochares*) *connexa* Macquart (Diptera: Tephritidae). The agent would be used by the applicant for the biological control of Siam weed, *Chromolaena odorata* (L.) King and Robinson, (Asteraceae) in Guam and the Northern Mariana Islands.

C. connexa is a gall forming fly. Adults live for up to 14 days and are active in the morning, mating on Siam weed and then ovipositing in the buds. The ovipositor is inserted through the bud leaves and masses of 5 to 20 eggs are laid in the bud tip or between the bud leaves. The eggs hatch in 4-5 days and tunnel into the stem tissue. The first visible swelling of the plant tissue occurs in 15 days and the gall develops steadily until the larvae are grown after 45 to 60 days, with 4-10 larvae per gall (Sipayung and Desmier de Chenon 1994). Larvae feed in curved tunnels inside the gall tissue. Mature larvae pupate within the tunnel and adults emerge through an epidermal window formed by the larva. The galls formed slow and distort but do not arrest further growth of the stem.

Voucher specimens of *C. connexa* have been deposited in the collections of the University of Guam and the U.S. National Museum, Washington, DC. Dr. G. Steyskal of the U.S. National Museum (USNM) in Washington, DC, made the initial species identification on the basis of specimens collected in Bolivia and Trinidad. Dr. Allen Norrbom, a specialist on Tephritidae at USDA, Agricultural Research Service, Systematic Entomology Laboratory, has confirmed the identification.

The applicant's purpose for releasing *C. connexa* is to reduce the severity and extent of infestations of Siam weed on Guam and the Mariana Islands. Siam weed is a perennial shrub native to South and Central America that thrives in warm, humid, low altitude areas below 1000 m. In its native habitat, it is common but not a serious weed because it is kept in check by competing plants and effective natural enemies. However, in recent decades, it has become an invasive weed in much of tropical Asia, Africa, and the western Pacific by outcompeting native plants and overrunning cultivated farmlands, grazing lands, and young forest plantations. Siam weed flowers from November through December in the northern hemisphere and the seeds are dispersed by wind during February and March. It is allelopathic, suppressing native vegetation and preventing the natural re-seeding of forest trees. It is very competitive in tropical wet-dry climates, becoming a fire hazard during the dry season and surviving to regrow rapidly during the wet season (Cruttwell-McFadyen 1999). The tangled thickets of this weed interfere with wildlife movement in forests and are toxic to livestock.

Siam weed prefers well-drained soils although it grows in many soil types. It does not tolerate shade but thrives in open areas. The seed is dispersed by wind but may also cling to hair and clothing. The seeds can occur as a contaminant in imported grass seed. Habitat disturbance is required for the weed to become established. This weed is not a problem in annual crops where fields are cultivated frequently, but is a serious weed in plantation crops such as rubber, oil palm, coffee, cocoa, teak, cashew and coconut. It becomes dominant vegetation in abandoned fields, vacant lands, disturbed forests and roadsides.

It was accidentally introduced to the Marianas, first becoming a problem on Rota in 1980 and later on other islands including Saipan, Tinian, Aguijan and Guam (Muniappan and Marutani 2000). This weed has become a difficult problem for the following reasons:

- *Interferes with the cultivation of plantation crops.* Siam weed grows in dense thickets that interfere with the cultivation of crops including coconut, oil palm, citrus, rubber, cocoa and teak.

- *Toxic to livestock and humans.* Livestock that consume its toxic leaves are poisoned due to the plant's high nitrate concentration (Waterhouse 1994). In addition, it can cause skin rashes and allergies to humans.

- *Invasive.* It infests vacant lots, roadsides, pastures, disturbed forests and natural reserves (Holm et al. 1977). Infestation of pastures is a particular problem due to the poisonous nature of the weed.

- *Results in negative effects on the ecosystem.* Siam weed disrupts natural communities in forests and natural reserves (Holm et al. 1977), restricts the movement of wildlife by forming tangled thickets (Waterhouse 1994), and prevents the natural reseeding of native trees (Cruttwell-McFadyen 1999).

- *Constitutes a fire hazard during the dry season.* During the rainy season after it is burned, it rapidly regrows to produce another fire hazard in the same place during the succeeding dry season.

R. Muniappan (Univ. Guam, personal communication) estimates that over 50,000 acres are now infested on Guam, and infestations continue to spread.

A previously released tiger moth (*Pareuchaetes pseudoinsulata*) that has established throughout Micronesia has been very effective in controlling large, dense stands of Siam weed but is less effective in controlling the weed in areas of lower density. However, *C. connexa* has the ability to locate and establish within patchy distributions of Siam weed and the researcher expects it to complement *P. pseudoinsulata*. Successful control of this weed has occurred in northern Sumatra with the release of the combination of both insects (Cruttwell McFayden 1999).

Before a permit is issued for the release of *C. connexa*, APHIS needs to analyze the potential effects of the release of this agent into Guam and the Northern Mariana Islands.

1.2 APHIS must decide among the following options:

A. To deny the permit application (no action)

B. To issue the permit as submitted
C. To issue the permit with management constraints or mitigation measures.

1.3 Issues arising from the field release of *C. connexa* are:
A. Will *C. connexa* attack non-target plants within and outside the area infested with Siam weed?
B. Will *C. connexa* affect any federally listed threatened or endangered species?

1.4 The pending application for release of this biocontrol agent into the environment was submitted in accordance with the Plant Protection Act of 2000 (7 USC 7701 *et seq.*). This environmental assessment (EA) was prepared by APHIS in compliance with the National Environmental Policy Act (NEPA) (42 USC 43421 *et seq.*) as described in the implementing regulations adopted by the Council on Environmental Quality (40 CFR 1500-1509), by USDA (7 CFR 1b) and by APHIS (7 CFR 372).

2. Alternatives Including the Proposed Action

2.1 This chapter will explain the alternatives available to APHIS. Although APHIS' alternatives are limited to a decision whether to issue a permit for release of *C. connexa*, other methods available for the control of Siam weed are also described. These control methods are not decisions to be made by APHIS and may continue whether or not a permit is issued for environmental release of *C. connexa*. These are methods presently being used to control Siam weed by public and private concerns and are presented to provide information to the reader.

2.2 Description of the alternatives.

2.2.1 Alternative 1 - No Action: Under this alternative, APHIS would not issue a permit to the University of Guam for the release of *C. connexa* for the control of Siam weed on Guam and the Northern Mariana Islands. The release of the biological control agent would not take place.

2.2.2 Alternative 2 - Issue the Permit: Under this alternative, APHIS would issue a permit for the field release of *C. connexa* for the control of Siam weed on Guam and the Northern Mariana Islands. This permit would contain no special provisions or requirements concerning release procedures or mitigating measures.

2.2.3 Alternative 3 - Issue the Permit with Specific Management Constraints and Mitigating Measures: Under this alternative, APHIS would issue a permit for the field release of *C. connexa* for the control of Siam weed on Guam and the Northern Mariana Islands. However, the permit would contain special provisions or requirements concerning release procedures or mitigating measures.

2.3 The following methods are presently being used to control Siam weed by public and private concerns on Guam and the Northern Mariana Islands. These controls will continue under the "No Action" alternative but may continue whether or not the permit is issued for release of *C. connexa.*

2.3.1 Chemical control: This alternative would include the continued use of herbicides to control Siam weed. Most chemical control experiments on Siam weed were conducted in the Philippines, Indonesia, India, West Africa and South Africa. Chemical control is practiced mostly in high value plantation crops. In the Philippines, 2,4-D, Gramoxone and Tordon were recommended (Madrid 1974, Tumaliuan and Halos 1979, Castillo et al. 1980). In Indonesia, Picloram, 2,4-D and Triclopyr were recommended (Risdiono 1975, Soerjani et al. 1975, Tjitrosemito et al. 1986). In India, 2,4-D, Gramoxone and Fennoxone were recommended (George 1968, Nair 1973, Rai 1976, Mathew et al. 1977, Borthakur 1977). In West Africa, Glyphosate, 2,4-D and Picloram were recommended (Sheldrick 1968, Ivens 1974, Durfour et al. 1979). Metasulfuron methyl, Sulfosate, Glyphosate and Triclopyr as a foliar spray, Triclopyr and Imazapyr for stump application and Tebuthiuron for soil application were recommended in South Africa (Goodall and Erasmus 1995). On Guam, Glyphosate is the most commonly used herbicide to control Siam weed where it occurs in disturbed areas such as roadsides and vacant lots (R. Muniappan, personal communication). The continued use of chemical controls would be a result of APHIS choosing the "No Action" alternative.

2.3.2 Mechanical control: Hand weeding is a common practice in many countries. In cocoa plantations, slashing 4 times a year is recommended (Are and Folarin 1970). A combination of slashing, ring weeding and mulching has been suggested in Nigeria (Komolafe 1976). Slashing the top growth and uprooting the subterranean portion is advised in South Africa (Erasmus 1991). On Guam, slashing is the main control used to clear an area infested by Siam weed (R. Muniappan, personal communication). The continued use of mechanical controls would be a result of APHIS choosing the "No Action" alternative.

2.3.3 Biological control: If *C. connexa* becomes established on Guam and the Northern Mariana Islands, it will be the second biological control agent to become established there for the control of Siam weed. The first agent is the tiger moth, *Pareuchaetes pseudoinsulata*. The larval stage of this insect damages Siam weed by defoliating plants. The introduction and establishment of *P. pseudoinsulata* on Guam in 1985 has resulted in the suppression of dense thickets of Siam weed but has been less effective in controlling areas with lower weed densities (Marutani and Muniappan, 1991). However, *C. connexa* has the ability to locate and establish even within patchy distributions of host plants (Cruttwell McFayden 1999). *C. connexa* attacks the terminal and axillary shoots of Siam weed by forming galls, reducing the growth of shoots. Galls produced on the tip shoots reduce flowering and eventual seed production. Reduction in seed production is an important factor in suppressing Siam weed since plants produce a large seed bank for new infestations to emerge each year. The ability of *C. connexa* to locate small infestations of Siam weed as well as its gall-making behavior are expected to complement the activity of *P. pseudoinsulata*. Successful control of Siam weed has occurred in northern Sumatra with the release of the combination of *C. connexa* and *P. pseudoinsulata* (Cruttwell McFayden

1999).

Three other insects have been released on Guam to control Siam weed including an Apionid beetle, *Apion brunneonigrum* Béguin Billecocq, in 1984, an Agromyzid fly, *Melanagromyza eupatoriella* Spencer, in 1986, and a Pyralid moth, *Mescinia* nr. *parvula* Zeller, in 1984 (Julien and Griffiths 1998). None of these insects established.

2.4 Summary of Consequences

Table 1. Summary of Consequences

Consequences	No Action	Issue Permit	Issue Permit with conditions
Effects on non-target organisms	Use of non-selective herbicides would cause harm to native plants and cause water quality to be threatened.	None expected	None expected
Effects on threatened and endangered species	Would expose T&E species to the effects of herbicides and disturbance of critical habitat from mechanical controls.	None expected	None expected

3. Affected Environment

3.3 Evidence of host specificity of *C. connexa* .
Indonesia: Both "choice" (test plant and *C. odorata* exposed to *C. connexa*) and "no choice" (only the test plant exposed to *C. connexa*) tests were conducted with 55 species of plants belonging to 15 different families. These plants are listed in Appendix 2. *C. connexa* deposited no eggs on any test plant in "choice" tests. In “no choice” tests, it deposited eggs on *Austroeupatorium inulaefolium* and *Ageratum conyzoides*, but larvae did not develop and no galls were formed (Sipayung and Desmier de Chenon 1994). Based on these results, the government of Indonesia issued a permit to release *C. connexa* in the field. In 1995 *C. connexa* was released and established in Sumatra, Java, Irian Jaya, Timor and Sulawesi.

Guam: In 1998 five species of Asteraceae were tested with 198 adults of *C. connexa*: Ageratum (*Ageratum conyzoides*), bidens (*Bidens pilosa*), cosmos (*Cosmos sulfureus*), mikania (*Mikania scandens*) and sunflower (*Helianthus annuus*). Other plants tested were bean, *Phaseolus* sp.

(Fabaceae), cabbage, *Brassica oleracea* (Cruciferae), corn, *Zea mays* (Poaceae), lime, *Citrus aurantifolia* (Rutaceae), okra, *Abelmoschus esculentus* (Malvaceae), pepper *Capsicum annuum* (Solanaceae) and watermelon, *Citrullus lanatus* (Cucurbitaceae). The genus *Austroeupatorium*, included in the host specificity tests in Indonesia, does not occur on Guam and thus was not tested. Both "choice" and "no choice" tests were conducted. For discussion of experimental procedures of Guam host specificity tests, see Appendix 3.

No galls developed on beans, *B. pilosa*, cabbage, citrus, corn, cosmos*, A. conyzoides, M. scandens*, okra, pepper, sunflower and watermelon in either "no choice" or "choice" tests (Table 1). Table 1. *Cecidochares connexa* host specificity choice testing. Plants within cages were exposed to flies for one month. Mean ± SEM are reported. Four replications of each pair were conducted. Guam 1998.

Pair	Test Plants	No. galls	No. of flies emerged		
			Males	Females	Total
1	Cosmos	0	0	0	0
	Chromolaena	11.8 ± 5.5	7.0 ± 3.5	7.8 ± 3.2	14.8 ± 6.2
2	Bidens	0	0	0	0
	Chromolaena	9.3 ± 8.3	5.5 ± 5.2	6.3 ± 5.3	11.8 ± 10.5
3	Corn	0	0	0	0
	Chromolaena	23.3 ± 2.3	14.8 ± 2.6	18.8 ± 3.5	33.5 ± 6.0
4	Citrus	0	0	0	0
	Chromolaena	20.5 ± 6.8	15.8 ± 6.8	18.3 ± 9.0	34.0 ± 15.03
5	Mikania	0	0	0	0
	Chromolaena	12.3 ± 4.6	6.0 ± 2.0	4.5 ± 1.9	10.5 ± 3.9
6	Sunflower	0	0	0	0
	Chromolaena	17.0 ± 5.3	16.5 ± 3.7	13.3 ± 5.1	29.8 ± 7.5
7	Pepper	0	0	0	0
	Chromolaena	18.0 ± 3.2	10.8 ± 3.6	11.0 ± 3.3	21.8 ± 6.5
8	Watermelon	0	0	0	0
	Chromolaena	8.5 ± 2.9	5.3 ± 2.7	8.8 ± 4.3	14.0 ± 6.9
9	Okra	0	0	0	0
	Chromolaena	8.5 ± 4.8	10.8 ± 6.1	11.8 ± 7.3	22.5 ± 13.4
10	Cabbage	0	0	0	0
	Chromolaena	7.0 ± 4.1	4.3 ± 2.9	3.3 ± 3.3	7.5 ± 5.5
11	Bean	0	0	0	0
	Chromolaena	20 ± 2.9	10.5 ± 2.9	7.3 ± 2.3	17.8 ± 4.9
12	Ageratum	0	0	0	0
	Chromolaena	13.0 ± 6.9	20.0 ± 12.5	22.3 ± 13.9	42.3 ± 26.0

These results indicate that *C. connexa* is host specific to Siam weed and corroborate the host specificity tests conducted in Indonesia. Since galls formed by *C. connexa* significantly reduce flower and seed production in Siam weed, field release of this fly will complement the effect of *P. pseudoinsulata* that has already been demonstrated on Guam.

3.3.1 Endangered and threatened species are a special concern because they are protected by the Endangered Species Act. One endangered plant (*Serianthes nelsonii*) and three plants proposed for endangered listing occur on Guam and the Northern Mariana Islands (*Tabernaemontana rotensis*, *Nesogenes rotensis* and *Osmoxylon mariannense*). Only *Tabernaemontana rotensis* (Apocynaceae), an edge species that inhabits roadsides, occurs within habitats occupied by Siam weed.

3.4 No minority, low income populations, or children should be negatively impacted due to the proposed action. Potential reductions in herbicide usage to control Siam weed may even be beneficial to human populations.

4. Environmental Consequences

4.1 This chapter will analyze the potential environmental consequences of each alternative on the resources described in Chapter 3.

4.2 Effects of Alternative 1 - No Action

4.2.1 Effects on Non-Target Organisms: In the absence of successful control agents, Siam weed will continue to expand its range, displacing the native flora, blocking movements of wildlife, and poisoning livestock. The biological control agent *Pareuchaetes pseudoinsulata* has been introduced into Guam where it effectively defoliates pure stands of Siam weed. However, it is less successful in scattered plants and patches. Chemical control is effective but expensive. It poses some environmental concerns such as soil contamination, affecting nontarget species and causing health hazards. It is practiced only in plantation crops and not in other ecosystems wherein *Chromolaena* is a problem. Mechanical control includes labor intensive hand weeding, digging and uprooting or use of machinery such as brush cutters, mowers, tillers, plows and tractor drawn equipment. This method provides short-term control, is expensive and use of tractor drawn equipment is limited to areas that are successful. Mulching is done mostly in plantation crops around the bases of trees. It is also labor intensive, expensive and the availability of mulching materials limits its use. Planting cover crops is also practiced only in plantation crops.

4.2.2 Effects on Threatened and Endangered Species: The small tree *Tabernaemontana rotensis* (Apocynaceae), a species proposed for the endangered species list, is an edge species and inhabits roadsides. This species is threatened by wildfires which have increased in Guam over the past decades **(**USDI 2000**)**. If no action is taken, Siam weed, which also inhabits roadsides, may contribute to the decline of this species due to its potential as a fire hazard. In addition, the non-selective sprays and slashing used to control Siam weed may also negatively impact this species.

4.3 Effects of Alternative 2 - Issue Permit

4.3.1 Several lines of evidence indicate that *C. connexa* is highly host-specific and will not have direct negative impacts on native plant species:

Evidence of host specificity from museum specimens.
The following host plants were recorded on labels of specimens of *C. connexa* in the collection of the U. S. National Museum in Washington, DC: *Chromolaena odorata* in Northern Argentina (galls); *C. odorata* in Trinidad (flowers); *C. laevigata* in Santa Cruz, Bolivia and Curitiba, Brazil (galls); and *Chromolaena* sp. in Panama (galls). These records seem to indicate that *C. connexa* attacks both *C. odorata* and *C. laevigata.* However, this may not be the case (see section on "scientific literature").

Evidence of host specificity from the scientific literature.
The family Asteraceae is represented by 32 genera in Guam. Only 3 are considered indigenous while another 15 genera are naturalized weeds. The remaining 14 genera are found under cultivation or are weedy but rare (Stone 1970). Species found on Guam belonging to the tribe Eupatorieae are *Adenostemma lavenia*, *Ageratum conyzoides*, *C. odorata* and *Mikania scandens*. *C. odorata* is the only species of the genus *Chromolaena* in Guam.

The genus *Cecidochares* includes about 13 known species, all native to North and South America. All known species of *Cecidochares* form stem or flower galls or feed on flowers of plants in the family Asteraceae. They are believed to be highly host-specific, and many are specific to a single plant species or perhaps even to certain biotypes of one species. Zachariades *et al.*, (1998) reported that *C.* (*P.*) *connexa* collected on *C. odorata* in Indonesia would not multiply on the South African form of the weed.

Although museum records seem to indicate that *C. connexa* attacks both *C. odorata* and *C. laevigata*, this may not be the case. *C. connexa* reared from *C. laevigata* in Bolivia did not accept *C. odorata* as a host (Cruttwell McFayden, 1988). This might mean that the specimens of *C. connexa* used in the test were highly host-specialized biotypes, or it might mean that two different species were involved (*Cecidochares* species are extremely similar morphologically).

A closely related species, *Procecidochares utilis*, was introduced and established in Hawaii, New Zealand, Australia, South Africa, China, Nepal and India to control *Ageratina adenophora* (*Eupatorium adenophorum*) (Asteraceae) (Bess and Haramoto, 1958, 1959,1972; Kapoor and Malla, 1978; Zhang*, et al.,* 1988; Dodd, 1961; Hill, 1989; Kluge, 1991). Another related species, *Procecidochares alani* was introduced to Hawaii for the control of mistflower, *Ageratina riparia* in 1974 (Nakao and Funasaki, 1976). The scientific literature contains no reports of adverse environmental effects from any of these introductions.

The intended environmental impact of the proposed action is a reduction in the severity of Siam weed infestations on Guam with consequent regrowth and reestablishment of forage plants in pastures and native vegetation in natural areas. However, a post-release monitoring plan will be conducted to monitor the spread and effect of *C. connexa* on Siam weed and to determine any unanticipated results of this release on native plants including *Adenostemma lavenia*, *Glossogyne tenuifolia* and *Wollastonia* (*Wedelia*) *biflora*(Appendix 4).

4.3.2 No negative effect is expected to occur on *Serianthes nelsonii* (Fabaceae), the endangered plant currently located on Guam and Rota. Fifteen plants within the family Fabaceae were included in host specificity tests conducted in Indonesia. No eggs were deposited or galls formed on any plants tested. In addition, this plant does not occur in habitats infested by Siam weed. Although the proposed plant species *Tabernaemontana rotensis* may occur within the same habitat as Siam weed, host specificity tests on the surrogate species, *Tabernaemontana divaricata,* have indicated that this plant is not a host for *C. connexa* (see Appendix 3 for test methods). In choice and no choice tests conducted on Guam in 2001, no galls were formed on *T. divaricata.* Removal of the fire hazard associated with areas infested with Siam weed may have a beneficial impact on *T. rotensis* since increased frequency of wildfires is considered a threat to this species (USDI 2000). *Nesogenes rotensis* (Verbenaceae) does not occupy the same habitat as Siam weed and from host specificity testing conducted on *Lantana camara* (Verbenaceae), the release of *C. connexa* is not likely to negatively impact this species. Although no host specificity tests were conducted on plants within the Araliaceae, the family to which the proposed plant species *Osmoxylon mariannense* belongs, host specificity testing and records from the literature clearly indicate the specificity of *C. connexa.* In addition, *O. mariannense* does not occur within habitats infested by Siam weed. Informal consultation under Section 7 of the Endangered Species Act resulted in a concurrence from the U.S. Fish and Wildlife Service that release of *C. connexa* is not likely to adversely affect endangered or proposed endangered plants in Guam or the Northern Mariana Islands (Appendix 5).

No listed endangered, threatened, proposed or candidate animals (including mammals, birds, insects, reptiles or snails) utilize Siam weed and none are expected to be adversely affected by releases of *C. connexa.*

4.4 Effects of Alternative 3 - Issue the Permit with Specific Management Constraints and Mitigating Measures

4.4.1 No specific management constraints or mitigating measures have been recommended for this species. Therefore, under this alternative, impacts on non-target organisms would be identical to those described in 4.3.1.

4.4.2 No specific management constraints or mitigating measures have been recommended for this species. Therefore, under this alternative, impacts on threatened and endangered organisms would be identical to those described in 4.3.2.

4.5 No disproportionate effects are expected to impact low income or minority populations or pose undue risks for children.

4.6 An unavoidable effect of the proposed action would be the lack of complete control of the target pest. The success rate of biological control of weeds is approximately 30%. Should the proposed action be unsuccessful, the present chemical, mechanical, and biological control activities would continue. Siam weed would continue to infest disturbed habitats.

4.7 Once a biological control agent such as *C. connexa* is released into the environment and it becomes established, it could move from the target plant to non-target plants and itself become a pest. If a host shift does take place, the resulting effects could result in environmental impacts not easily reversed. Biological control agents such as *C. connexa* generally spread without the agency of man. In principle therefore, release at even one site must be considered equivalent to release over the entire area in which potential host plants occur and in which the climate is suitable for reproduction and survival.

5. List of Preparers

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6. List of Agencies Consulted

Paul Henson, Field Supervisor, U.S. Fish and Wildlife Service, Pacific Islands Fish and Wildlife Office, Honolulu, Hawaii, was consulted under Section 7 of the Endangered Species Act.

7. List of Reviewers

This environmental assessment was reviewed by **Charles Bare,** Senior Staff Officer, APHIS, Riverdale, MD, **Robert Flanders,** Containment Branch Chief**,** APHIS, Riverdale, MD, **Michael Firko,** Assistant Director, Plant Health Programs, APHIS, Riverdale, MD

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Appendix 2. List of plants used in host-specificity tests conducted in Indonesia (Sipayung, A. and R. Desmier de Chenon 1994). *C. connexa* deposited no eggs on any test plant in "choice" tests. In no choice tests, it deposited eggs on *Austroeupatorium inulaefolium* and *Ageratum conyzoides*, but larvae did not develop and no galls were formed.

Family

Species

Amaranthaceae

Amaranthus tricolor

Asteraceae

Ageratum conyzoides
Aster spp.
Austrocupatorium inulaefolium
Chrysanthemum morilifolium
Clibadium surinamense
Cosmos caudatus
Dahlia pinnata
Gerbera jamesonii
Gynura aurantica
Helianthus annuus
Pluchea indica
Tithonia diversifolia
Zinnia elegans

Convolvulaceae

Ipomaea aquatic
Ipomaea batatas

Cucurbitaceae

Citrullus lanatus
Cucumis melo
Cucumis sativus
Cucurbita moschata

Euphorbiaceae

Hevea brasiliensis
Manihot esculenta
Ricinus communis

Fabaceae

Albizia falcataria
Arachis hypogaea
Caesalpinia pulcherrima
Calliandra haematocephala
Crotalaria juncea
Desmodium heterocarpon
Dolichos lablab

Flemingia strobilifera
Gliricidia sepium
Glycine max
Leucanena glauca
Pachyrhizas erosus
Psophocarpus tetragonolobus
Sesbania grandiflora
Vigna unguiculata

Liliaceae

Allium sativum

Malvaceae

Hibiscus rosasinensis
Gossypium obtusifolium

Myrtaceae

Eugenia aquea
Eugenia caryophyllus
Psidium guajava

Poaceae

Zea mays
Oryza sativa

Rubiaceae

Coffea robusta

Rutaceae

Citrus nobilis

Solanaceae

Capsicum annuum
Lycopersicum esculentum
Nicotiana tabacum
Solanum melongena
Solanum tuberosum

Sterculiaceae

Theabraoma cacao

Verbenaceae

Lantana camara

Appendix 3. Host-specificity test methods conducted in Guam in 1998 and 2001.

Host specificity testing of *Ageratum conyzoides, Bidens pilosa, Mikania scandens* and *C. odorata*. Guam 1998.

All plants were raised in 12-inch dia. plastic pots filled with soil. Beans, corn, okra, sunflower and watermelon were directly seeded in the pots. Seedlings of cabbage and cosmos were transplanted to the pots. Young plants of *Ageratum conyzoides*, *Bidens pilosa*, *C. odorata*, and *Mikania scandens* were field-collected and transplanted to the pots. Citrus plants were obtained from a nursery. All test plants were initially one foot in height when used in the experiments except for *C. odorata* and cabbage, which were 18" and 6" respectively. Test plants were kept in the quarantine laboratory and were covered with a muslin cloth cage supported by a cylindrical wire mesh frame. Newly emerged adult *C. connexa* were fed a dilute honey solution and maintained in a container for 24 hours to ensure mating before releasing into test cages. Plants were kept inside the cages for one month from the time of release of the flies and then the cages were removed. All shoots on the plants with galls were individually covered with muslin cloth sleeve bags. These bags were examined daily for fly emergence. Emerged flies were collected in test tubes and the sex determined.

Host specificity testing of the surrogate plant for the proposed species *Tabernaemontana rotensis, Tabernaemontana divaricata.* Guam 2001.

Four replications were used for both “Choice” and “No Choice” tests. All plants were raised in 12” diameter plastic pots filled with soil. *Chromolaena odorata* plants, approximately 12" tall, were collected from the field and transplanted in the pots. These plants were kept in the plant nursery for two months and were about 18” in height before using them for the tests. *Tabernaemontana divaricata* cuttings of about 12” in height were allowed to root in a mist bed and then were transplanted into the pots. These plants were about 18” in height when used for host specificity testing. Test plants were kept in the quarantine laboratory and were covered with a muslin cloth cage supported by cylindrical wire mesh frame. Newly emerged adult *C. connexa* were fed a dilute honey solution and maintained in a container for 24 hours to ensure mating before releasing into test cages. Plants were kept inside the cages for one month from the time of release of the flies and then the cages were removed. All shoots on the plants with galls were individually covered with muslin cloth sleeve bags. These bags were examined daily for fly emergence. Emerged flies were collected in test tubes and the sex determined.

Appendix 4. Post-release monitoring protocol for *C. connexa* on Guam

A. Monitoring the spread of *C. connexa*: A known number of adults will be released in a field cage in a field heavily infested with Siam weed near the Naval Communication Station (NCS) area. Gall formation and development of *C. connexa* inside the cage will be monitored once a week. After gall formation on plants (a month after release of the flies in the cage) the cage will be removed. Two months after cage removal, weekly observations will continue until complete information is obtained on the spatial and temporal spread of the fly throughout Guam.

B. Monitoring the effect of *C. connexa* on Siam weed: To observe the effect of *C. connexa* on Siam weed growth, 50 plants will be tagged at different parts of the island. Measurements on height, width, number of branches, number of galls, size of galls, number of inflorescences and number of flowers per inflorescence in each plant will be recorded every three months for a period of 2 to 3 years.

C. Monitoring plant succession: To monitor the effect of *C. connexa* on Siam weed and other vegetation, three 5x5m permanent quadrats will be set up on different areas of the isalnd. Within each quadrat, number of individuals of each plant species, average height of plant species and canopy cover will be measured. To determine average height of a plant species, 10 individual plants of the species will be randomly sampled. If less than 10 plants are found, then heights of all plants present will be measured. To meaure the canopy cover of a species within a quadrat, a one square meter grid containing 100-10cm x 10cm units will be used. The maximal area of the species in the quadrat will be $25m^2$. Relative Importance Value (%) of a species will be calculated by:

(Relative density + Relative height + Relative canopy cover)/3 where:
Relative density (%) = (no. of individuals of a species/total no. of plants in a quadrat) x 100
Relative height (%) = (avg. height of a species/sum of avg. height of al species) x 100
Relative canopy cover (%) = (canopy cover of a species/total canopy of all species present in a quadrat) x 100

Observations will be made once every three months for a period of 2 years or more if needed. The quadrats will be examined every month to remove any caterpillars of *P. pseudoinsulata* infestation.

D. Effect of *C. connexa* on non-target plants: After field release of *C. connexa* in Guam, monthly monitoring of endangered and proposed endangered plants including *Serianthes nelsonii* and *Tabernaemontana rotensis* and indigenous plants belonging to the Asteraceae including *Adenostemma lavenia*, *Glossogyne tenuifolia*, and *Wollastonia* (*Wedelia*) *biflora*, will be monitored by observing at least 10 plants monthly for gall formation. This observation will continue for 2 years.

Decision and Finding of No Significant Impact

for Field Release of *Cecidochares* (*Procecidochares*) *connexa* Macquart (Diptera: Tephritidae), a nonindigenous, gall-making fly for control of Siam weed, *Chromolaena odorata* (L.) King and Robinson (Asteraceae) in Guam and the Northern Mariana Islands

Environmental Assessment

April 2002

The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), is proposing to issue a permit to a researcher at the University of Guam for the field release of a nonindigenous, gall-making fly (*Cecidochares* (*Procecidochares*) *connexa*). The agent would be used by the applicant for the biological control of Siam weed (*Chromolaena odorata*) in Guam.

The alternatives available to APHIS are No Action, Issue Permit, and Issue Permit with Management Constraints or Mitigating Measures. Because of the action being proposed by APHIS, the Issue Permit and the Issue Permit with Management Constraints or Mitigating Measures alternatives will result in the release of the biological control agent into the environment. APHIS has therefore analyzed the potential effects of the release of the agent into the environment. The No Action alternative, as described in the environmental assessment (EA), would result in the continued use at the current level of chemical, mechanical and existing biological control methods for the management of Siam weed. These control methods described are not alternatives for decisions to be made by APHIS, but are presently being used to control Siam weed on Guam and the Northern Mariana Islands and may continue regardless of issuance of a permit for field release for *C. connexa*.

I have decided to issue the permit for the field release of *C. connexa* without management constraints or mitigating measures. The reasons for my decision are:

- This biological control agent is sufficiently host specific and poses little, if any, threat to the biological resources of Guam or the Northern Mariana Islands.
- This species will not disproportionately affect minority or low- income populations, nor will they disproportionately affect children or result in any environmental health risks or safety risks to children.
- *C. connexa* poses no threat to the health of humans or wild or domestic animals.
- *C. connexa* is not likely to adversely affect endangered or threatened species or their habitat. The U.S. Fish and Wildlife Service has concurred with this conclusion.
- While there is not total assurance that the release of *C. connexa* into the environment will be reversible, there is no evidence that this organism will cause any adverse environmental effects.

Based on the analysis found in the EA, I find that issuance of a permit for the field release of *C. connexa* without management constraints or mitigating measures will not have a significant impact on the quality of the human environment.

__________________________________ ____________________

Michael J. Firko Date

Assistant Director

APHIS Plant Health Programs

Plant Protection and Quarantine