

Proposed Field Release of the Salvinia Weevil, *Cyrtobagous salviniae* Calder and Sands (Curculionidae: Coleoptera) a Host-Specific Biological Control Agent of Giant Salvinia, *Salvinia molesta* D.S. Mitchell (Salviniaceae: Polypodiophyta) a Federal Noxious Weed Indiginous to Southeast Brazil

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This study was conducted at the USDA / APHIS / PPQ / CPHST / Mission Plant Protection Center (MPPC) - Arthropod Quarantine, Mission Texas. Initial releases are intended for Texas and will continue with other infested states within the United States. Releases and

monitoring in Texas will be conducted by personnel from the Mission Plant Protection Center in cooperation with Project Partners.

PETITION INTRODUCTION

Nature of the Problem: *Salvinia molesta*, also known as giant salvinia and Kariba Weed has been found in water bodies belonging to Texas, Louisiana, Alabama, Georgia, Florida, Mississippi, Arizona, California, and Hawaii (NAS 1999). Areas with economies based on aqua culture and water transportation suffer severe losses due to giant salvinia infestation (Sundaresan 1979). Massive salvinia mats halt traffic, commercial and sport fishing, block waterfowl habitat, and destroy a water-based economy in a single growing season. In addition to its direct impact, giant salvinia provides habitat for snails that are intermediate hosts for *Schistosoma* sp. (Mitchell 1981) which parasitize the human intestinal and urinary tracts. It is also an important host plant for *Mansoni* mosquitos that serve as vectors for rural filariasis.

Giant salvinia is a rapidly proliferating aquatic fern that has spread from its native habitat in southern Brazil to many other tropical countries around the world, as well as to Australia, New Guinea, New Zealand, South Africa and now to the United States (Forno et al., 1983). It may damage aquatic ecosystems by overgrowing and replacing native plants that provide food and habitat for native animals and waterfowl (Mitchell 1978). Additionally, salvinia blocks out sunlight, and decreases oxygen concentration to the detriment of fish and other aquatic species (Abassi 1986). When plant masses die, decomposition lowers dissolved oxygen still further (Kannan 1979). Blockage of waterways to traffic is common and it also imparts a foul taste to drinking water.

USDA / ARS Aquatic Weed Control Lab initiated experimental releases in June 1999 of the salvinia weevil (*Cyrtobagous salviniae*) from Florida. This weevil is adventive in Florida and was first found in the early 1960s. According to taxonomists, it is the same species as the one used by the Australians although there appear to be some genetic differences (Goolsby et al. 2000). Biological control of giant salvinia was attempted using this weevil at three sites in east Texas: a pond in the Swinney Marsh complex; another near Cow Bayou; and a beaver dam on Toledo Bend Reservoir.

Proposed Action: The proposed action is to introduce *Cyrtobagous salviniae* from Australia for field release within the United States for the control of giant salvinia, *Salvinia molesta*.

TARGET WEED INFORMATION

Taxonomy: Species-*molesta*; Genus-*Salvinia*; Family-Salviniaceae; Division-Polypodiophyta (Pteridophyta), true ferns

Common Name: giant salvinia, Kariba weed, African pyle, aquarium water moss, koi kandy (Mitchell 1981, NAS 1999)

Confirmation: Giant salvinia was first observed in Texas during 1997 at a school yard demonstration pond in Houston. Plants collected in May 1998 were determined to be *Salvinia molesta*. Later that June, giant salvinia was found in cultivation at backyard ponds and in July giant salvinia was first found naturalized, at a farm pond near Tomball. Heavy November rains spilled plants from the Tomball pond into a local creek. Farm ponds near Fulshear and Garwood, Texas were also found completely covered with giant salvinia. The pond in Garwood lies on a creek that drains the Colorado River, a tributary heavily depended upon for agricultural irrigation, into the Gulf of Mexico (NAS 1999).

Identification: *Salvinia molesta* is a free floating aquatic fern, consisting of a horizontal stem lying just below the water surface that produces a pair of ovate to oblong floating leaves and a highly dissected submerged leaf at each node (Mitchell 1981). Plants are capable of several growth forms where individual leaves can range from a few millimeters to 6 centimeters long (Mitchell 1981). During early stages, plants are smaller and leaves lay flat on the water surface. As plants grow, leaves curl at the edges in response to self competition. Eventually a vertical leaf position is attained as mature plants press into tight chains and form mats of many floating plants (Mitchell and Harley 1981). Leaf surfaces have rows of papilla (cylindrical stalks) branching into two to four hairs that rejoin at the tips to form an egg beater-like structure (Mitchell 1981). This feature distinguishes *S. molesta* from common salvinia, *S. minima*, which has branched hairs that are spreading and free at the tips (NAS 1999). Hairs on mature leaves may be damaged and not true to type, however, young, unfolding leaves will reveal intact hairs that are diagnostic for the species complex. Leaf hairs create a water repellent covering (Sundaresan 1979). Submersed leaves are filamentous, look like roots, and often bear chains of egg-shaped sporocarps (Safui 1979). Plants may demonstrate variability in the arrangement of the sporocarp chains, a function of developmental stage or a response to environmental conditions (Mitchell and Tar 1975). Mature plants can produce large quantities of sporocarps that contain many sporangia, yet plants are functionally sterile. Spores, only rarely found, are deformed and infertile (Seehra 1974), the result of improper chromosome pairing in this pentaploid species (Mitchell and Thomas 1972).

Voucher Specimens: Mission Plant Protection Center is housing voucher specimens collected on September 30, 1999 - October 1, 1999 by Lloyd E. Wendel, Robert Richard, and Marcus Hammons. Collections were made at Liberty, TX (29°55.945N 094°44.72W), Toledo Bend, TX (31°28.51N 093°45.47W, 31°27.60N 093°45.88W), Salter Creek, LA (31°27.07N 093°38.70W), and Cypress Bend, LA (31°26.06N 093°40.76W).

Description: Giant salvinia is a sterile free-floating aquatic fern with irregularly branched stems and an absence of true roots (Mitchell 1981). Its leaves, which are actually fronds, are in whorls of three; two floating and one submerged (Mitchell 1981). The opposite floating leaves are round to oblong, 20mm long and 13mm wide, with stiff, tiny water-resistant hairs above and wettable hairs beneath (Sundaresan 1979). Submerged leaves are finely dissected into linear segments, which resemble and function as modified roots (Croxdale 1981).

Distribution:

Native Range - Southeastern Brazil; between latitudes of 24 and 32 degrees S; more abundantly along coastal Brazil; extending inland to elevations of 900 m (Forno and Harley 1979).

Expected US Range - Expected range includes the Atlantic coastal plain, from southeastern Virginia to southern Florida; the Gulf coast states; central and southern California; as well as southern Arizona. It may be expected to naturalize wherever water-hyacinth persists or in areas that experience frost but not the formation of ice on fresh waters (Whiteman and Room 1991). This correlates to most regions with USDA Plant Hardiness Zones 10, 9 and 8. Giant salvinia has already overwintered along coastal North Carolina at a latitude of 34.4 degrees north (USDA Zone 8) and in Texas at 33 degrees north (USDA Zone 7b). Although top-growth died back during winter, plants resurged the following summer (NAS 1999).

World Introductions - Giant salvinia is a serious weed problem in New Guinea, Australia, Mauritius, Africa, India, Sri Lanka, Ceylon, New Zealand, and elsewhere (Forno et al., 1983). Any area that might support water hyacinth is probably at risk.

Habitat - Quiet waters of lakes and ponds, oxbows, ditches, slow flowing streams and rivers, backwater swamps, marshes and rice fields (Oliver 1993). In the United States, giant salvinia is expected to occupy habitats favorable to *S. minima*, yet predicted to extend into and colonize open water more aggressively (Oliver 1993).

Genetic Variability - Giant salvinia is not known to reproduce by spores as with other ferns. It reproduces vegetatively, that is, new plants develop as fragments break off from mature individuals (Mitchell and Harley 1981). Thus, giant salvinia is one giant clone with no significant genetic variability.

Taxonomically Related Plants: Salviniaceae, Azollaceae, and Marsileaceae are three families of true ferns. The first two listed are more closely related (Smith 1977). *Azolla pinnata* and *Marsilea drummondii* were tested by Forno et al. 1983. One species in each family is of concern and is included in the test plant list. Plants in the family Salviniaceae include *S. auriculata*, *S. biloba*, *S. herzogii*, and *S. minima*, and *S. oblongifolia* (Joy 1978). There are approximately 10 species that are related with four being members of the *S. auriculata* complex. These four are presumed to represent polyploids of a basic type. All members of this complex can be expected to be aggressive weeds and are prohibited as Federal Noxious Weeds.

Distribution of Taxonomically Related Plants: Only *S. molesta* and *S. minima* are found in the United States. Eventhough *S. minima* is not listed as a Federal Noxious Weed (NAS 1999) it has shown to be quite troublesome in the Louisiana area.

Life History: Giant salvinia reproduces very effectively through vegetative means. Stems fragment as plants mature and new plants develop from apical and lateral buds. Each node

harbors up to five serial lateral buds (Lemon and Posluszny 1997), adding to the species' high potential for growth and dormancy. Plants will withstand periods of stress, both low temperature and dewatering, as dormant buds (Mitchell and Harley 1981, NAS 1999).

Impacts: Growth factors paired with features promoting mobility make giant salvinia an aggressive, competitive species that can impact aquatic environments and local economies (Sundaresan 1979). Under favorable growth conditions, plants may double within about a week's time (Mitchell et al., 1981). In Texas and Louisiana, fresh biomass has been recorded in the range of 10-20 kg/m². Excessive growth can result in complete coverage of water surfaces which degrades natural habitats in several ways (Mitchell and Harley 1981). Heavy growth of giant salvinia competes with and shades desirable native vegetation (Abassi 1986). Mats of floating plants prevent atmospheric oxygen from entering the water (Abassi 1986), while decaying salvinia drops to the bottom, consuming dissolved oxygen needed by fish and other aquatic life (Kannan 1979). Animal habitat is most noticeably altered by the obliteration of open water. Migrating birds may not recognize or stop at water bodies covered with giant salvinia. Indeed, the resulting loss of open water in the Swinney Marsh region has prompted local waterfowl guides to question the economic benefits of renewing hunting leases. Local fishermen have found it impossible to cast into smothered lakes and are abandoning spots once fished for bass, crappie and sunfish. Dense mats of giant salvinia have caused Toledo Bend residents to resort to paddling boats out of docks after slipping clutches and stalling engines. Giant salvinia can also clog water intakes to interfere with agricultural irrigation and electrical generation (Mitchell 1981).

Alternative Management Options: Various biological characteristics must be considered before an eradication or control program can be designed for giant salvinia infestations. Methods are available; however, they must be used in combination with each other for successful eradication program.

Chemical Alternatives Tested - Diquat, Fluridone, Paraquat, Glyphosate, 2,4-D, Household Detergent, Kerosene (Mitchell and Harley 1981). The use of herbicides can be effective if a surfactant is added that destroys the ability of the cage-like hairs on the leaves to trap air, thus allowing the herbicide to penetrate the giant salvinia leaves (Miller 1989). However, when plant masses die, decomposition lowers dissolved oxygen resulting in major fish kills (Kannan 1979).

Mechanical Alternatives Tested - Hand Removal. Physical control is difficult because the plant grows rapidly. It is difficult for manual labor to stay ahead of the plant growth, but booms and nets have met with some success in confining infestations and maintaining salvinia free areas. However, booms and nets are subject to breaking when tons of windblown salvinia are pressing them (Thomas and Room 1986).

Biological Alternatives Tested - Salvinia weevil. Biological control using the salvinia weevil has been effective in reducing the biomass of salvinia by reducing the enormous growth potential of the weed (Forno et al., 1986, Thomas and Room 1986).

BIOLOGICAL CONTROL AGENT INFORMATION

Taxonomy: Species-*salviniae*; Genus-*Cyrtobagous*; Family-Curculionidae; Order-Coleoptera

Common Name: Salvinia weevil

Selection of this Agent: It is important to note that the host specificity of *C. salviniae* is well documented. Forno et al. (1983) tested 46 plant species (Table 5), but the weevils developed and reproduced only on *S. molesta*, even though the array of test species included the aquatic fern *Azolla pinnata* as well as several other fern species. Both aquatic plant species and common crops were tested. Although adults actively fed on water lettuce (*Pistia stratiotes*), they did not develop on the noxious weed. No feeding occurred on any of the other aquatics (*Marsilea drummondii*, *Sagittaria graminea*, *Azolla Eichhornia crassipes*, *Potamogeton tricarinatus*, *Typha orientalis*, *Ipomoea aquatica*, *Nasturtium officinale*, *Nymphaeae gigantea*, *Nymphoides indica*, *Ludwigia peploides*, *Polygonum lapathifolium*, *P. hydropiper*, or *P. sp.*), nor was feeding damage by *C. salviniae* ever reported on other aquatic plant species occurring in the weevil's native geographical range. One feeding scar per plant was recorded when sweet potato leaves were held in contact with the water surface, but no feeding occurred on sweet potato in a more natural terrestrial situation. Forno et al. paid particular attention to rice because of its commercial importance coupled with its being cultivated in aquatic conditions. No feeding was recorded on rhizomes, stems, or leaves of three rice varieties tested. Other crop plants tested include onion, pineapple, corn, sugar-cane, asparagus, banana, ginger, papaya, beet, spinach, lettuce, pumpkin, cauliflower, lucerne, sub-clover, cotton, strawberry, orange, lemon, mandarin, and tomato: no feeding was recorded on any of them.

A very closely related weevil has already been released and is established in Florida feeding on the closely related plant species *S. minima* (Kissinger 1966). For years the weevil in Florida was thought to be *C. singularis*, which has not been an effective biocontrol agent in other countries where it was released. However taxonomic studies by Calder and Sands (1985) revealed it to be *C. salviniae*. *C. salviniae* is the agent that has delivered effective biocontrol in Australia and other countries, but it is not controlling salvinia in Florida. In a recent molecular study Goolsby et al. (2000) detected genetic differences between *C. salviniae* collected in Florida and *C. salviniae* collected in Australia and has suggested that although they are morphologically identical, the two groups may be different species. The advance of molecular based diagnostics makes it possible to understand differences in organisms at the DNA level. Yet, we do not understand or know how these differences impact organisms at the population level in ecological and physiological studies. *C. salviniae* originating from Australia has well documented host specificity as well as a proven track record in reducing *S. molesta* infestations to tolerable levels worldwide. The *C. salviniae* we currently have in quarantine have been reared from

parental stock collected in Australia. We are petitioning for approval to release this population of weevils on *S. molesta* in the United States.

Description: *Cyrtobagous salviniae* is a small weevil ranging in length from 1.5 to 2.2 mm. It is essentially black but newly emerged individuals are often brown (photo on following page). Legs are reddish brown in coloration. The dorsal surface of the weevil is covered with numerous shallow depressions or punctures as well as yellow peltate scales. Adults typically reside on or beneath the leaves or fronds of *S. molesta*. A thin film of air adheres to the bottom of the weevil allowing for respiration during periods of submergence (Forno et al., 1983).

Identification: Steve Lingafelter, Systematic Entomology Laboratory, ARS, USDA, Beltsville, MD 20705

Voucher Specimens: Mission Plant Protection Center is housing pinned and cold storage voucher specimens collected on May 18, 2000, by M. Purcell, J. Makinson, and R. Zonneveld at the Wappa Dam in Queensland, Australia. Additional specimens were submitted to the Systematic Entomological Laboratory in Beltsville, MD.

Geographical Range: This Australian strain of *C. salviniae* originated from Joinville, Santa Catarina State, a coastal location near the northern natural limit of *S. molesta* in Brazil. This culture was used as the breeding nucleus for mass propagation when the weevil was first liberated in Australia in 1980 (Room et al., 1981).

Previous Introductions: *C. salviniae* has been introduced to at least 12 countries including India (Jayanth 1987), South Africa (Cilliers 1991), Botswana (Forno and Smith 1992), Papua New Guinea (Mitchell 1981), Australia (Calder and Sands 1985), and Sri Lanka (Doeleman 1989). In all countries, giant salvinia infestation levels have been completely and rapidly controlled by being reduced to less than 1% of their former size. No non-target effects have been seen in any of these countries despite the development of populations composed of billions of weevils. When weevil populations rise this high and *S. molesta* infestation levels decline, *C. salviniae* will starve and die rather than to switch host plants. In all reality, it just doesn't recognize other plants as food. This has been evident in all areas of introduction.

Expected Range: The Atlantic coastal plain, from south eastern Virginia to southern Florida; the Gulf coast states; central and southern California; southern Arizona. This correlates to most regions with USDA Plant Hardiness Zones 10, 9 and 8 (NAS 1999).

Known Host Range: This species feeds only on members of the genus *Salvinia* (Forno et al., 1983). Field observations and laboratory studies in Brazil and Australia showed that *C. salviniae* severely damaged *S. molesta* and that the weevil is suitable as a control agent for this weed in tropical and subtropical areas in Australia. In host-specificity studies using 46 plant species that occur in Australia, development of the immature stages took

place only on *S. molesta*. The high degree of host-specificity Color plate of *Cyrtobagous salviniae*

indicated that establishment of this weevil in Australia is without risk to nontarget plants (Forno et al., 1983). This high level of specificity has been confirmed by researchers in several other countries, at least 11 of which have introduced the weevil (Julien and Griffiths 1998) without any adverse consequences. The results of this study will also show the high degree of host-specificity of this weevil.

Life History: Eggs are laid singly in cavities in the plant formed by female feeding. The females chew a hole in which to oviposit and sometimes deposit the eggs on the surface or only partially embedded in the tissue. Eggs hatch in approximately 10 days. The larvae are white and attain lengths of 3mm. Total larval development requires 3 to 4 weeks. Larvae construct cocoons on the “roots” (root-like submersed leaves). Prepupal and pupal periods last about 2 weeks (Forno et al., 1983).

Extent of Damage to Target Weed: *C. salviniae* has completely and rapidly controlled *S. molesta* everywhere that it has been released (Julien and Griffiths 1998). In most cases, infestation levels have been reduced to less than 1% of their former size.

Extent of Damage to Nontarget Plants: This species feeds only on members of the genus *Salvinia* (Forno et al., 1983). All can be expected to be aggressive weeds and are prohibited as Federal Noxious Weeds with the exception of *S. minima*. No damage to non-target plants is known to occur anywhere in the world and none would be expected if it was released in the U.S. *Cyrtobagous salviniae* has only been found on *Salvinia minima* in Florida, despite intensive field surveys of insects associated with other aquatic plants by numerous researchers (Center, pers. comm.).

Geographical Source: This agent was collected on May 18, 2000, by M. Purcell, J. Makinson, and R. Zonneveld at the Wappa Dam in Queensland, Australia. This Australian strain originated from Joinville, Santa Catarina State, a coastal location near the northern natural limit of *S. molesta* in Brazil.

Agent Maintenance: *Cyrtobagous salviniae* was received from Australia at the Mission Plant Protection Center (MPPC) - Arthropod Quarantine on June 10, 2000. It has been maintained on *S. molesta* collected in East Texas. Ten percent of the specimens were submitted to Gerard Thomas of Consulting Diagnostic Service in Berkeley, California for pathological tests and screened for parasites and pathogens. Test results indicated the weevils were clean of pathogens and parasites. The remainder of the insects are being kept in Arthropod Quarantine and maintained on *S. molesta*.

Study Site: All host-specificity testing of this agent was conducted at USDA / APHIS / PPQ / CPHST / Mission Plant Protection Center - Arthropod Quarantine, Mission, TX.

EXPERIMENTAL METHODOLOGY AND ANALYSIS

Test Plant List: Testing of the plants in this plant list was required by the State of California as a prerequisite for consideration of a PPQ 526 form submitted in 1999. The plant list was provided by the California Department of Food and Agriculture. Due to the documentation of previously tested host plants, a list of only four plant species was generated to supplement the list of plants previously tested by Forno et al. 1983. The four plant species that were required and the reasons for being tested are as follows:

Marsilea vestita Hook and Grev., commonly known as hairy water-clover is in a related family and is fairly widespread and common in California.

Azolla filiculoides Lam., commonly known as water fern is a close relative of the *Salvinia* genera and may occupy the same habitat as giant salvinia. Also, because of an association of azolla with a blue-green algae, rice growers may use this plant as a green manure.

Zizania aquatica L., commonly known as wild rice is a minor crop in California. As such, any aquatic weed biological control agent considered for release in California must be tested on wild rice.

Sagittaria sanfordii E. Greene, commonly known as Sanford's arrowhead is a rare plant in California because its habitat is threatened by development. It was placed on the list for testing due to its growth form and habitat.

Azolla mexicana was tested because the petitioners felt that *Azolla* spp. was represented already by *Azolla filiculoides* in the required test list as well as by *Azolla pinnata* in the study conducted by Forno et al. (1983). Two genera *Lemna* and *Wolffiella* belonging to the Lemnaceae family (duckweed) could have been tested but weren't because the petitioners felt that eventhough they occupy similar habitats, they are taxonomically remote from *Salvinia*.

Design:

1. Entire plants were tested (leaves, stems, roots, buds, flowers).
2. Chris Godfrey of California Department of Food and Agriculture provided us with plant material to be tested.
3. Host specificity testing was divided into five studies. These included the following:
 - No-choice oviposition and longevity tests (240 adults, 4 replicates).
 - No-choice larval transfer tests (500 larvae, 5 replicates).
 - No-choice development tests (200 adults, 4 replicates).
 - Multiple-choice oviposition and larval development tests in closed-cages under quarantined greenhouse conditions (20 adults).
 - No-cage tests under quarantined greenhouse conditions (80 adults, 2 replicates).
4. A total of 540 adults and 500 larvae were tested.
5. Each replicate was observed daily for feeding damage, oviposition, and/or longevity.
6. Methodology was generally derived from host specificity tests conducted by Scharzlander 2000 with specific methods on the same agent and the same target weed derived from Forno et al., 1983.

Materials and Methods: *Cyrtobagous salviniae* adults used for these studies were maintained and reared in arthropod quarantine. Weevils were handled with a fine paintbrush. At the beginning of each test, all newly emerged adult weevils were sequestered for 24 hours to ensure that all females were gravid. Mating occurred at all times that visual observations were made. All of the host specificity tests were conducted in the arthropod quarantine building. A total of five plant species were tested with the agent *C. salviniae*. These test plants included; *Azolla filiculoides*, *Marsilea vestita*, *Sagittaria sanfordii*, *Zizania aquatica*, and the target plant *Salvinia molesta*.

Goals, Rationales, and Methods for Host-Specificity Tests:

Goal: Conduct No-Choice Oviposition and Longevity Tests

Rationale: To determine number of eggs oviposited, amount of feeding damage, and adult longevity.

Materials: Five pairs of weevils were kept in transparent plastic cylinders covered with a gauze lid with four replicates for each plant species. One starvation control was also setup. (10 adults x 4 replicates x 6 treatments = a total of 240 adults tested).

Method: No-choice oviposition, longevity, and adult feeding tests were conducted in 2000-2001 with *S. molesta* and four test-plant species. Five pairs of *C. salviniae* were kept in transparent cylindrical tubes. The tubes were 15 cm long and 4 cm in diameter, and covered with a gauze lid. These test cylinders (Cone-tainers) were developed at MPPC and have been repeatedly used successfully on terrestrial, aquatic, and semi-aquatic plants for over a decade. There were four replicates per each test-plant species with one starvation control setup. Whole test-plants (leaves, stems, roots) were carefully inserted into the cylinders to prevent any damage to them. Since all of the test plants are aquatic, the tubes had to be suspended in water. This was done by inserting the cylinders in a tray, then suspending the tray in a plastic container that was filled with water. *S. sanfordii*, *Z. aquatica*, and *M. vestita* required that the tubes also be filled with soil (see *S. sanfordii* and *M. vestita* in photo on following page). *S. molesta* and *A. filiculoides* required only water. The test-plants were checked daily for number of eggs laid, and feeding damage (chewing, scrapes, scratches and/or holes in plant material) that might have occurred. Mortality was also examined.

Duration: The duration of this study was 19 days (see results). The same plant material was maintained throughout the duration of this study.

Goal: Conduct No-Choice Larval Transfer Tests

Rationale: To determine the number of adults that would develop from transferred larvae on test plants.

Materials: Twenty larvae were transferred to test plants with five replicates for each plant species. (20 larvae x 5 replicates x 5 treatments = a total of 500 larvae tested).



Method: Newly hatched *C. salviniae* larvae were obtained from a breeding colony at MPPC. The larvae were then transferred with a fine paintbrush onto the *S. molesta* and the four test-plants very carefully. There were five replicates for each test-plant species. All of the test plants were kept and maintained as stated in the previous test. Observations were made for the emergence of adults.

Duration: The duration of this study was 34 days.

Goal: Conduct No-Choice Development Tests

Rationale: To determine the number of progeny to develop on each test plant.

Materials: Five pairs were kept in transparent plastic cylinders covered with a gauze lid with four replicates for each plant species. (10 adults x 4 replicates x 5 treatments = a total of 200 adults tested).

Method: No-choice development tests were conducted with *S. molesta* along with the four test-plant species. The plants were maintained in the same manner described above.

Observations were made for the emergence of adults from the new generation. Duration: The duration of this study was 55 days.

Goal: Conduct Multiple-Choice Oviposition and Larval Development Tests in Closed-Cages under Quarantined Greenhouse Conditions

Rationale: To determine the number of plants fed upon and adults emerging from test plants under greenhouse conditions.

Materials: Ten pairs of adults were released into a quarantined plexi-glass field cage (18 in x 14 in x 16 in) in a quarantined greenhouse containing five replicates of each test plant species. Two (10 in x 10 in) windows were on the sides of the cage and covered with organza to allow for air circulation. Two pairs were directly transferred to each plant species giving them the same chance to feed on all plants on the list. The plants were in the plastic tubes previously described and placed in a one-gallon aquatic tank inside the cage. Observations were made daily for feeding damage and the emergence of adults from the new generation. (A total of 20 adults were tested.)

Method: The design of this test required the plants be uncovered and arranged randomly in a block design. The plants were positioned so that the insects could easily move via leaf material from one plant species to the other. Observations were made daily for feeding damage and the emergence of adults from the new generation. Thirty-five days after testing began, each plant was isolated in order to detect the source of adult emergence.

Duration: The duration of this study was 60 days.

Goal: Conduct No-Cage Tests under Quarantined Greenhouse Conditions

Rationale: To determine the number of plants fed upon and the number of adults surviving an entire life cycle on test plants under open conditions in a quarantined greenhouse.

Materials: Open one-gallon aquatic tanks were set up with four plants (2 *S. molesta* plants and 2 test-plants). Five pairs were released directly onto the plants at each aquatic tank with two replicated tanks per test plant species. Observations were made daily for feeding damage and the emergence of adults from the new generation.

(10 adults x 2 replicates x 4 treatments = 80 adults)

Method: There were two replicated tanks per test-plant species. Observations were made daily for feeding damage and the emergence of adults from the new generation. Thirty-five days after testing began, each plant species was isolated in order to detect the source of adult emergence.

Duration: The duration of this study was 75 days.

Positive Controls: No-choice and multiple-choice tests were conducted. A control was utilized in the no-choice tests by using water and no plants (starvation) to test longevity of the weevils when no *S. molesta* is present. A small sponge (1 cm²) was used for the insects to rest on. Results for the positive controls can be seen in Figures 1 and 2.

Reason for Decision: No-choice tests / starvation tests are the most common type of host specificity tests. Results of these types of tests clearly show if the agent could possibly survive on a non-target plant. *Cyrtobagous salviniae* has an extremely voracious appetite. Typical behavior and activity of *C. salviniae* on *S. molesta*, becomes weak and sluggish in the absence *S. molesta*. Thus, the tests were conducted in this manner.

RESULTS

There was no feeding damage or oviposition observed on any of the test plants being studied, with the exception of *Salvinia molesta*, where feeding damage and oviposition were observed daily and longevity was much higher.

No-Choice Oviposition and Longevity Tests: *C. salviniae* did not survive on any of the non-target plants but did extremely well on the target plant *S. molesta*. During these experiments, the adults only fed and oviposited on *S. molesta*. No feeding damage occurred and no eggs were oviposited on the four test-plant species (Figure 1). An average of 26.2 eggs per five females were oviposited on the *S. molesta*. This experiment lasted 19 days with all the beetles dead on the 18th day. The *C. salviniae* adults were able to survive the longest on *S. molesta* (Figure 2); mean longevity for females was 16 days. The low longevity may have been the result of extenuating circumstances such as handling or testing conditions. Mortality was highest on the test-plant species; mean longevity for females was less than 6 days. Figures 1 and 2 indicate that no feeding or reproduction occurred on any plant species other than *S. molesta*. These results indicate that there is no risk to the species tested.

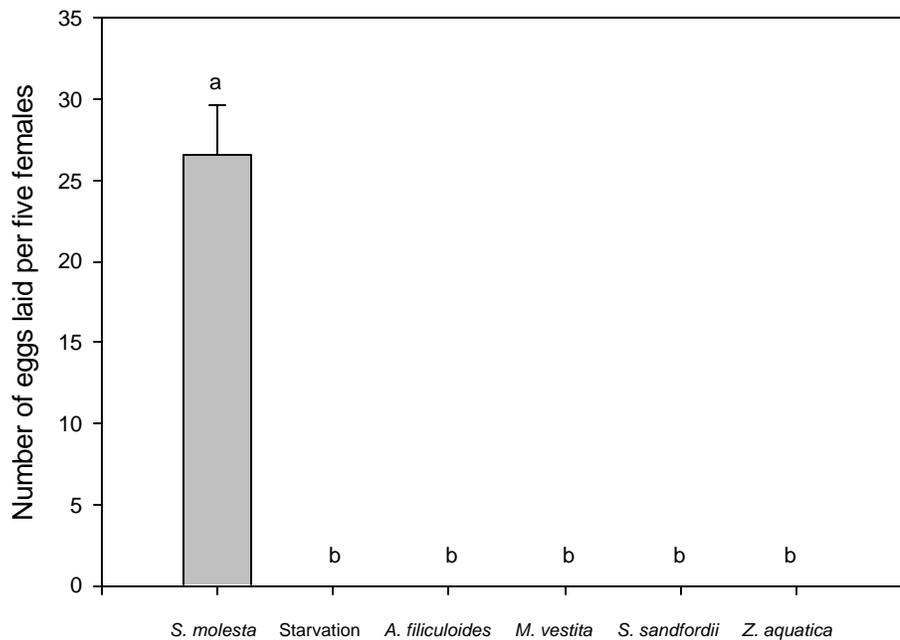


FIG. 1 Oviposition of *C. salviniae* during no-choice tests. Vertical bars, means + SE; n= 4. Bars denoted by the same letter are not significantly different, p , 0.05, by Scheffe's Multiple Comparison Test.

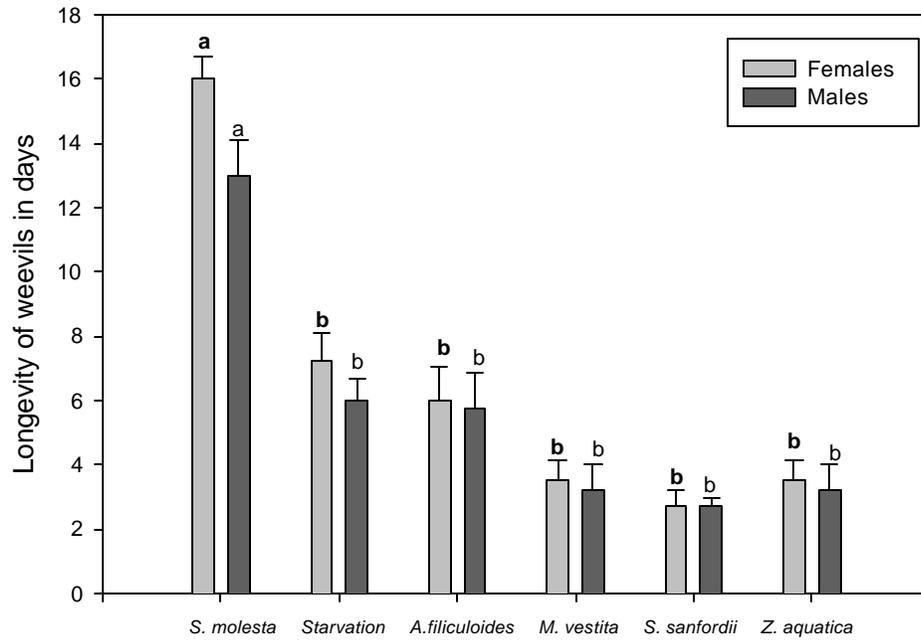


FIG 2. Mean longevity (\pm SE) of female and male *C. salviniae* during no-choice tests. Vertical bars, means \pm SE; $n = 4$; within sexes, bars denoted by the same letters are not significantly different, $P < 0.05$, by Scheffe's Multiple Comparison Test.

No-Choice Larval Transfer Tests: The complete development of *C. salviniae* larvae was only observed on the target plant *S. molesta* (Table 1) with 28% of the transferred larvae surviving. The method of dissecting the larvae from the host plant, giant salvinia, is very tedious since they are only 4 mm in length and quite fragile. Manual manipulation of these delicate larval forms can easily damage them structurally resulting in a low survivability. All larvae were handled in the same manner. The larvae that were transferred onto the test-plants died of starvation within 2 to 3 days. The larvae surviving to the adult stage on *S. molesta* emerged as adults within 24 to 31 days after being transferred as larvae. No feeding damage was observed on any test-plant except *S. molesta*. These results indicate that there is no risk to the species tested.

TABLE 1
Results of No-Choice Larval Transfer Tests

Plant species	No. replicates	No. larvae transferred/rep.	No. adults emerged	Mean % survival (\pm SE)
<i>S. molesta</i>	5	20	28	28 \pm 0.04
<i>A. filiculoides</i>	5	20	0	0 \pm 0.00
<i>M. vestita</i>	5	20	0	0 \pm 0.00
<i>S. sandfordii</i>	5	20	0	0 \pm 0.00
<i>Z. aquatica</i>	5	20	0	0 \pm 0.00

No-Choice Development Tests: The emergence of *C. salviniae* adults occurred only on the target plant, *S. molesta* (Table 2). Emergence of adults occurred within 41 to 55 days after testing began. No adults could be reared from any of the four test-plants and all were

dead within the first week. Mean percent survival (no. of progeny/ no. of original adults) of the weevils that completed an entire life cycle was 42.5% under test conditions. These results indicate that there is no risk to the species tested.

TABLE 2
Results of No-Choice Development Tests

Plant species	No. replicates	No. adults emerged	Mean % survival(\pm SE)
<i>S. molesta</i>	4	17	42.5 \pm 0.05
<i>A. filiculoides</i>	4	0	0 \pm 0.00
<i>M. vestita</i>	4	0	0 \pm 0.00
<i>S. sandfordii</i>	4	0	0 \pm 0.00
<i>Z. aquatica</i>	4	0	0 \pm 0.00

Multiple-Choice Oviposition and Larval Development Tests in Closed-Cages under Quarantined Greenhouse Conditions:

S. molesta was the only plant species showing any feeding damage by *C. salviniae*. The feeding damage was observed as tiny chewed holes in the center of the leaves. Collection and counting of adults was done 60 days after the study began. There was no feeding damage on any other plant. *C. salviniae* adults and pupae were observed only on the target plant, *S. molesta* (Table 3). An average of two adult *C. salviniae* emerged per plant and an average of 21 pupae were continuing to develop per plant. This test was the closest to simulating natural conditions, since it had the highest diversity of available plants. Since most pupae had not completed development, these results indicate that the development of *C. salviniae* can extend longer than 60 days. These results correspond to the 56 day development period reported by Forno et al. (1983). They also correspond to the development periods reported by Sands et al. at three temperatures (48 days at 31° C, 61 days at 27° C, and 93 days at 23° C). No adults could be reared from any of the four test-plants. These results indicate that there is no risk to the species tested.

TABLE 3
Results of Multiple-Choice Oviposition and Larval Development Tests in Closed-Cages under Quarantined Greenhouse Conditions

Plant species	No. of plants offered	No. of plants fed upon	Mean \pm SE no. of adults emerged at 60 days	Mean \pm SE no. of pupae
<i>S. molesta</i>	5	5	2.2 \pm 0.8	21.8 \pm 3.6
<i>A. filiculoides</i>	5	0	0 \pm 0.0	0 \pm 0.0
<i>M. vestita</i>	5	0	0 \pm 0.0	0 \pm 0.0

<i>S. sandfordii</i>	5	0	0 ± 0.0	0 ± 0.0
<i>Z. aquatica</i>	5	0	0 ± 0.0	0 ± 0.0

No-Cage Tests under Quarantined Greenhouse Conditions: Similarly to the previous test, adult *C. salviniae* only fed on *S. molesta* (Table 4). The feeding damage was observed as tiny chewed holes in the center of the leaves. Collection and counting of adults was done 75 days after the study began. There was no feeding damage on any other plant. Viable pupae were also observed on *S. molesta* “roots” but were not included in the counts. No adults emerged from any test-plant except *S. molesta*. The average number of adults to emerge from each *S. molesta* plant was 13.68. These results indicate that there is no risk to the species tested.

TABLE 4
Results of No-Cage Tests under Quarantined Greenhouse Conditions

Plant species	Replicate	Total no. adults emerging from <i>S. molesta</i>	Total no. adults emerging from test	Mean ± SE no. adults emerging from <i>S. molesta</i>	Mean ± SE no. adults emerging from test
<i>A. filiculoides</i>	1	26	0	13.75 ± 1.8	0 ± 0.0
	2	29	0		
<i>M. vestita</i>	1	35	0	13.75 ± 3.4	0 ± 0.0
	2	20	0		
<i>S. sandfordii</i>	1	23	0	10.25 ± 1.0	0 ± 0.0
	2	18	0		
<i>Z. aquatica</i>	1	26	0	17.00 ± 3.2	0 ± 0.0
	2	42	0		

DISCUSSION

Comparison of Previous and Present Host Specificity Results: During experimentation, *C. salviniae* did not feed, oviposit, or complete a full life cycle on any plant species other than *S. molesta*. The results from the host-specificity testing indicate that there exists no potential of risk to any nontarget plants that were tested. In previous host-specificity tests conducted by W. Forno in Australia, it was determined that *C. salviniae* could not survive on any host plant other than *Salvinia* spp. (Forno et al., 1983). The forty-six previously tested plants are listed in Table 5.

TABLE 5
Plants Previously Tested in Australia by Forno et al. 1983

<i>Adiantum hispidulum</i>	<i>Eichhornia crassipes</i>	<i>Gossypium hirsutum</i>
<i>Azolla pinnata</i>	<i>Potamogeton tricarlinatus</i>	<i>Nymphoides indica</i>

Pteridium esculentum
Marsilea drummondii
Schizaea dichotoma
Christella dentata
Sagittaria graminea
Allium cepa
Pistia stratiotes
Ananas comosus
Zea mays
Orzya sativa
Saccharum officinarum
Asparagus officinalis
Musa x paradisiaca

Typha orientalis
Zingiber officinale
Carica papaya
Beta vulgaris
Spinacia oleracea
Lactuca sativa
Ipomoea batatas
I. aquatica
Cucurbita maxima
Nasturtium officinale
Brassica oleracea var. botrytis
Medicago sativa
Trifolium subterraneum

Eucalyptus tereticornis
E. maculata
Nymphaea gigantea
Ludwigia peploides
Polygonum lapathifolium
P. hydropiper
Polygonum sp.
Rumex brownii
R. crispus
Fragaria x ananassa
Citrus sinensis
C. limon
C. reticulata
Lycopersicon esculentum

From all of the host-specificity tests conducted at MPPC, *C. salviniae* could not survive, develop, or reproduce on any host plant except *S. molesta*. This high degree of host-specificity indicates that the release and establishment of this weevil in the United States is without risk to nontarget plants. The high level of specificity has been confirmed by researchers in several other countries, at least 11 of which have introduced the weevil (Julien and Griffiths 1998) without any adverse consequences. The results from the host-specificity testing conducted at MPPC concur with the results from W. Forno's host-specificity tests as well as her field observations in South America.

Host Specificity with Regard to Environmental Test Conditions:

The performance of similar host-specificity tests under varying environmental conditions is an important tool for obtaining better information concerning both the realized or ecological host range and the potential or physiological host range of an insect species (Cullen, 1989; Marohasy, 1998). In addition to investigations under laboratory conditions, open-field tests (Clement and Christofarro, 1995), field-cage tests in cases in which open-field tests are not possible, or ideally both types of tests should be conducted. In this study, environmental conditions were varied by using the closed-cage versus no-cage conditions. This helps in the comparison of results to ensure that they are biologically meaningful. However, regardless of the varying conditions, the test plants remained free from attack by *C. salviniae*. In addition, it was observed that *C. salviniae* did much better under the no-cage conditions as opposed to closed-cage conditions since it is a more natural setting.

Risk of Potential Nontarget Effects: From all of the host-specificity tests conducted at MPPC as well as those conducted by Forno et al. 1983, *C. salviniae* could not survive, develop, or reproduce on any plant except *S. molesta*. The results from the host-specificity testing indicate that there exists no potential of risk to any non target plants.

Protocol for Releasing the Agent:

Culture and Identification -

Taxonomy: Species-*salviniae*; Genus-*Cyrtobagous*; Family-Curculionidae; Order-Coleoptera

Identification: Steve Lingafelter, Systematic Entomology Laboratory, ARS, USDA, Beltsville, MD 20705.

Description: *Cyrtobagous salviniae* is a small weevil ranging in length from 1.5 to 2.2 mm. Mature adults are dark brown to black in color with newly emerged individuals a lighter shade of brown. Legs are reddish brown in coloration. The dorsal surface of the weevil is covered with numerous shallow depressions or punctures as well as yellow peltate scales. Adults typically reside on or beneath the leaves or fronds of *S. molesta*. A thin film of air adheres to the bottom of the weevil allowing for respiration for periods of submergence (Forno et al., 1983).

Voucher Specimens: Mission Plant Protection Center is housing pinned and cold-storage voucher specimens collected on May 18, 2000, by M. Purcell, J. Makinson, and R. Zonneveld at the Wappa Dam in Queensland, Australia.

General Release Protocol: Once natural enemies have been approved for release and cleared through quarantine, the process of field release and establishment begins. For the past few years APHIS / PPQ has been following a 3 phase scheme for releasing and developing field insectary sites. Depending upon the rate of establishment and other factors relating to the colonization of natural enemies, some stages may be by-passed or modified. The process of establishing field insectaries needs to remain flexible to accommodate and facilitate the rapid dissemination of natural enemies.

Phase I - Initial release of *C. salviniae* into field insectary sites. These sites are limited in number depending upon availability of natural enemies being cleared through APHIS quarantine and mass production levels. APHIS / PPQ is responsible in Phase I to maintain these critical sites in order to promote natural population increases.

Phase II - The establishment of Phase II field insectary sites is a joint cooperative effort by APHIS / PPQ and state departments of agriculture, county weed districts, federal cooperators and/or research cooperators. Each site will be monitored and whenever insect populations are appropriate these populations will be made available for redistribution in Phase III. These sites may also serve as demonstration plots, showing the potential impact of the natural enemy on the targeted weed in the particular site.

Phase III - Adult weevils collected from Phase II field insectary sites will be redistributed. The collection and redistribution of the weevils will be a collaborative effort of the project partners.

Rearing Facility: Mass production of *Cyrtobagous salviniae* will be conducted at the USDA / APHIS / PPQ / CPHST / Mission Plant Protection Center, Mission Texas. These weevils will be used to establish field populations in project states.

Initial Release Sites: Initial releases are intended for East Texas (Houston, Beaumont, and Victoria Region) during the summer of 2001 and will continue with other infested states within the continental United States.

Post Release Monitoring: Releases and monitoring will be conducted by personnel from APHIS / PPQ / Mission Plant Protection Center in cooperation with Project Partners. The effectiveness of *Cyrtobagous salviniae* is dependent upon climatic conditions, such as temperature, plant nutritional status (i.e. nitrogen levels), and other biotic and abiotic conditions. Releases will be planned when these conditions are favorable. Evaluating the establishment, spread, and effectiveness of the natural enemies will be an essential and fundamental process of controlling *Salvinia molesta*. The impact that the natural enemies have on the surrounding environment either directly or indirectly are also important. Natural enemy densities will be sampled in experimental plots before and after releases are made. Treatments will be established in a randomized complete block design using four blocks, with each block having four treatments. Treatments will include (1) untreated, (2) natural enemy releases, (3) chemical application, and (4) natural enemy releases combined with chemical application. Experimental test plot dimensions for each treatment are still pending. All experimental plots will be sampled on a regular basis. Chemical applications will be made at the beginning of the experiment. Twenty plants will be sampled from each replicate weekly / biweekly. Records will be kept on populations of *C. salviniae*, phytoplankton, macrophytes, zooplankton, macroinvertebrates, game fish, water fowl, nontarget plants as well as target weed biomass, dissolved oxygen content in the waterways, and percent damage resulting from *C. salviniae*.

Benefits: The use of biological control (specifically *C. salviniae*) for management of *S. molesta* is recognized as the leading and most often used control strategy in all areas of the world due to its highly effective nature. In tropical areas, effect time is measured in terms of months instead of years, as is the typical case with most insect biological control agents. Longer effect times are observed in cooler subtropical or warm temperate areas but impact has been noted in these areas as well. It is highly cost effective since the impact is realized for years without reintroduction.

Risks: There are no reported risks associated with the release of this agent.

POTENTIAL ENVIRONMENTAL IMPACTS

Human Impacts: The utilization of biological control based management strategies eliminates adverse impacts to human, animal, and plant health. Control of *S. molesta* populations through the release of *C. salviniae* will improve and protect water quality, recreational activities, and commercial/industrial uses of infested waterways. Restoration of waterways, ponds, lakes, and other aquatic habitats to their original state will safeguard aquatic fauna, vegetation, and other resources. In addition to its direct impact, giant salvinia provides habitat for snails that are intermediate hosts for *Schistosoma* sp. which

parasitize the human intestinal and urinary tracts. It is also an important host plant for *Mansoni* mosquitos that serve as vectors for rural filariasis

Economic Impacts: Areas with economies based on aqua culture and water transportation suffer severe losses due to giant salvinia infestation (Sundaesan 1979). Massive salvinia mats halt traffic, commercial and sport fishing, block waterfowl habitat, and destroy a water-based economy in a single growing season (Mitchell 1978).

Plant Impacts: *C. salviniae* has been introduced to at least 12 countries including India (Jayanth 1987), South Africa (Cilliers 1991), Botswana (Forno and Smith 1992), Papau New Guinea (Mitchell 1981), Australia (Calder and Sands 1985), and Sri Lanka (Doeleman 1989). A high level of host-specificity has been confirmed by researchers in several other countries, at least 11 of which have introduced the weevil (Julien and Griffiths 1998) without any adverse consequences to nontarget plant species. In all countries, giant salvinia infestation levels have been completely and rapidly controlled by being reduced to less than 1% of their former size.

Nonplant Impacts: Growth factors paired with features promoting mobility make giant salvinia an aggressive, competitive species that can impact aquatic environments and local economies (Sundaesan 1979). Under favorable growth conditions, plants may double within about a week's time (Mitchell et al., 1981). Live biomass weights approach those recorded for water-hyacinth. Excessive growth can result in complete coverage of water surfaces which degrades natural habitats in several ways (Mitchell and Harley 1981). Heavy growth of giant salvinia competes with and shades desirable native vegetation (Abassi 1986). Mats of floating plants prevent atmospheric oxygen from entering the water, while decaying salvinia drops to the bottom, greatly consuming dissolved oxygen needed by fish and other aquatic life (Kannan 1976, Abassi 1986). Animal habitat is most noticeably altered by the obliteration of open water. Migrating birds may not recognize or stop at water bodies covered with giant salvinia. Indeed, the resulting loss of open water in the Swinney Marsh region has prompted local waterfowl guides to question the economic benefits of renewing hunting leases. Local fishermen have found it impossible to cast into smothered lakes and are abandoning spots once fished for bass, crappie and sunfish. Toledo Bend residents resorted to paddling boats out of docks after slipping clutches and stalling engines on packed in plants. Giant salvinia can also clog water intakes to interfere with agricultural irrigation and electrical generation (Mitchell 1981).

Proposed Methods for Mitigation: There are no undesired effects documented and/or foreseen.

Outcome of No Action: Potential impacts to human health and safety from the no action alternative would result from deleterious effects to water resources because of the unchecked spread of giant salvinia. Infestations would continue to provide the source material from which other water bodies might be contaminated. Because of salvinia's rapid rate of spread, large water bodies used for boating, fishing, waterfowl hunting, recreation, transportation, aqua culture, water consumption, agricultural irrigation would be

rendered completely inaccessible and unusable for these purposes (Mitchell 1981). People would travel further, possibly to other States, to seek preferable locations for boating and fishing (harming local economy) and, in the process, inadvertently transport viable salvinia plants, thus facilitating contamination of other water bodies (Mitchell 1981). It is highly likely that salvinia would at the very least impede uses of these water bodies, such that recreational pursuits, food fishing, water consumption, and water dependent industries, including crawfish, catfish, and rice production, would suffer serious consequences. Among the adverse effects that salvinia mats will have on the physical environment is deterioration of water quality parameters, such as light penetration, dissolved oxygen, and nutrients. Salvinia mats prevent light from penetrating into the water column resulting in various effects on aquatic plants. Emergent macrophytes, especially those in well-established stands, would likely not be affected by salvinia mats because new growth is often from underground vegetative propagative structures that can withstand reduced light and nutrients within the water column. Conversely, submersed macrophytes and phytoplankton would likely be reduced gradually as salvinia mats form and reduce the light in the water column and absorb nutrients. Zooplankton would decrease under the mats because of the decrease in phytoplankton. Benthic invertebrates would decrease as dead salvinia plants collect on the bottom and oxygen decreases (Arunachalam 1980). Most fish would avoid areas where dense, extensive mats are found and where oxygen levels are lower than in surrounding areas uncolonized by salvinia. Water reliant endangered and threatened species are found throughout the southern United States. Several species of fish and invertebrates are currently known from fewer than 10 locations, upon which they are completely reliant for their continued existence. If salvinia were to spread to and survive in those waterways, these listed species might face serious population setbacks, and possibly extinction.

PETITIONER'S CONCLUSION

In summary, it can be stated that *C. salviniae* is host specific to species within the genus *Salvinia*, with a strong preference for its field host, *S. molesta*. Test plants other than *S. molesta* were never fed upon by *C. salviniae*. In addition, there exists no evidence to indicate that *C. salviniae* can survive on any other host other than *Salvinia* spp. Currently, there are no satisfactory means of effectively controlling *S. molesta* in the United States. Measures are either expensive and/or not persistent. Biological control of giant salvinia offers the possibility of a permanent control of this weed as it has already in other countries where *C. salviniae* has been released.. Thus, this petition calls for the request to release *C. salviniae* in the field as an environmentally acceptable strategy to mitigate the presence and spread of *S. molesta* in U. S. waters where it is currently found.

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