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**Field release of the European
leaf sheath mining midge,
Lasioptera donacis Coutin
(Diptera: Cecidomyiidae), for
biological control of giant reed,
Arundo donax L. (Poales:
Poaceae) in the Contiguous
United States.**

**Environmental Assessment,
October 2016**

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**Environmental Assessment,
October 2016**

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I. Purpose and Need for the Proposed Action

The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), Pests, Pathogens, and Biocontrol Permits (PPBP) is proposing to issue permits for release of the European leaf sheath mining midge, *Lasioptera donacis* Coutin (Diptera: Cecidomyiidae). The agent would be used by the permit applicant for the biological control of giant reed, *Arundo donax* L. (Poales: Poaceae), in the contiguous United States.

This environmental assessment¹ (EA) has been prepared, consistent with USDA, APHIS' National Environmental Policy Act of 1969 (NEPA) implementing procedures (Title 7 of the Code of Federal Regulations (CFR), part 372). It examines the potential effects on the quality of the human environment that may be associated with the release of *L. donacis* to control infestations of *A. donax* within the contiguous United States. This EA considers the potential effects of the proposed action and its alternatives, including no action.

APHIS has the authority to regulate biological control organisms under the Plant Protection Act of 2000 (Title IV of Pub. L. 106–224). Applicants who wish to study and release biological control organisms into the United States must receive PPQ Form 526 permits for such activities. The PPBP received a permit application requesting environmental release of a leaf-sheath mining midge, *L. donacis*, from Europe, and the PPBP is proposing to issue permits for this action. Before permits are issued, the PPBP must analyze the potential impacts of the release of this agent into the contiguous United States.

The applicant's purpose for releasing *L. donacis* is to reduce the severity of infestations of *A. donax* in the United States. It is an extremely invasive weed of riparian habitats (wetlands adjacent to rivers and streams) and irrigation canals of the Rio Grande River Basin and the Southwestern United States. *Arundo donax* is native to the Old World from the Iberian Peninsula of Europe to south Asia, including North Africa and the Arabian Peninsula. It has been cultivated in the Old World for thousands of years and has been widely introduced around the world as an ornamental and for its fiber uses. It was introduced into North America in the early 1500s by the Spanish for its fiber uses and quickly became naturalized. It is now found throughout the southern half of the United States from Maryland to

¹ Regulations implementing the National Environmental Policy Act of 1969 (42 United States Code 4321 et seq.) provide that an environmental assessment "shall include brief discussions of the need for the proposal, of alternatives as required by section 102(2)(E), of the environmental impacts of the proposed action and alternatives, and a listing of agencies and persons consulted." 40 CFR § 1508.9.

California; however, it is most invasive along muddy banks of creeks and rivers in the southwestern United States.

Arundo donax infestations in riparian habitats lead to loss of biodiversity, stream bank erosion, altered channel morphology, damage to bridges, increased costs for chemical and mechanical control along transportation corridors, and impediment of law enforcement activities on the international border. Additionally, this invasive weed competes for water resources in an arid region where these resources are critical to the environment, agriculture, and municipal users. *Arundo donax* is a severe threat to riparian areas where it displaces native plants and animals by forming massive stands that pose a wildfire threat (Frandsen and Jackson, 1994). It may reduce stream navigability (Dudley, 2000). It consumes excessive amounts of water and competes for water resources in an arid region prone to perennial droughts. Under optimum conditions, it can attain growth rates of 0.7 meters (m) per week or 10 centimeters (cm) per day, putting it among the fastest growing plants (Perdue, 1958; Bell, 1997). Under ideal growth conditions, *A. donax* can produce more than 20 metric tons of above-ground dry mass per hectare (Perdue, 1958).

Existing chemical and mechanical management options for *A. donax* are expensive, temporary, ineffective, and can have nontarget impacts. In addition, there is a need to release an agent that will work in concert with biological control agents previously released for biological control of *A. donax*. For these reasons, the applicant has a need to release *L. donacis*, a host-specific, biological control organism for the control of *A. donax*, into the environment.

II. Alternatives

This section will explain the two alternatives available to the PPB—no action and issuance of permits for environmental release of *L. donacis*. Although the PPB's alternatives are limited to a decision on whether to issue permits for release of *L. donacis*, other methods available for control of *A. donax* are also described. These control methods are not decisions to be made by the PPB, and their use is likely to continue whether or not permits are issued for environmental release of *L. donacis*, depending on the efficacy of *L. donacis* to control *A. donax*. These are methods presently being used to control *A. donacis* by public and private concerns.

A third alternative was considered, but will not be analyzed further. Under this third alternative, the PPB would have issued permits for the field release of *L. donacis*; however, the permits would contain special provisions or requirements concerning release procedures or mitigating measures. No issues have been raised that would indicate special provisions or requirements are necessary.

A. No Action

Under the no action alternative, the PPB would not issue permits for the field release of *L. donacis* for the control of *A. donax*. The release of this biological control agent would not take place. The following methods are presently being used to control *A. donax*; these methods will continue under the “No Action” alternative and will likely continue even if permits are issued for release of *L. donacis*, depending on the efficacy of the organism to control *A. donax*.

1. Chemical Control

Arundo donax may be controlled using herbicides. Glyphosate is a broad-spectrum herbicide that is commonly used on a variety of wetland and aquatic plants, such as water hyacinth (*Eichhornia crassipes*), giant salvinia (*Salvinia molesta*), saltcedar (*Tamarix spp.*), and others, including *A. donax*. Glyphosate has proven to be effective against *A. donax* (Finn and Minnesang, 1990; USDA-FS, 1993). One of the reasons for its effectiveness is that glyphosate is a systemic herbicide and, when used at appropriate times, it is translocated to the roots, killing the entire plant. A number of techniques were developed for its use, including 1) use as a foliar spray, 2) cutting plant stems and spraying, or painting the herbicide on the surface of the cut, and 3) cutting stems, letting plants re-sprout, and treating the re-sprouts with herbicide.

Additionally, an herbicide (Habitat[®]) with another active ingredient, imazapyr, has been developed and registered for use on *A. donax*. In general, Habitat[®] requires one to two applications and control may be achieved for several years. Removal of dead canes may be necessary if stem densities are great enough to inhibit recovery of native vegetation after treatment.

2. Mechanical Control

Mechanical methods of *A. donax* control include use of prescribed fire, heavy machinery (e.g. bulldozer, Hydro-axe), hand-cutting, chipper, etc.). Removal of dead canes may be necessary if there is a possibility that cut vegetation might create a flood hazard during high water events or if biomass density is great enough to inhibit recovery of native vegetation. Burning is a cost-effective way of removing biomass if it does not threaten native vegetation. Another, but more costly, means of removal is chipping. Equipment and labor are expensive relative to other forms of removal; however, the small dry chips that are produced pose little threat in terms of regeneration, and they do not form debris dams. Biomass removal by vehicle is expensive and, generally, not preferred due to its lack of cost-effectiveness. The use of heavy machinery, such as the Hydro-axe, is extremely expensive and slow, cutting only about 3 to 4 acres per day (Bell, 1997).

3. Biological Control

Two biological control agents, the stem-galling wasp, *Tetramesa romana* Walker (Hymenoptera: Eurytomidae) and the rhizome feeding armored scale, *Rhizaspidotus donacis* (Leonardi) (Homoptera: Diaspididae) have been released against *A. donax* in Texas, California, and in Mexico. When both *T. romana* and *R. donacis* are present, *A. donax* plants become severely stressed with extreme stunting and virtually no leaf production. These agents are beginning to have an impact on the invasive populations of *A. donax*, but additional host specific and damaging agents that target different parts of the plant are needed to manage this highly invasive weed.

B. Issue Permits for Environmental Release of *L. donacis*

Under this alternative, the PPB would issue permits for the field release of the leaf sheath mining midge, *L. donacis*, for the control of *A. donax*. These permits would contain no special provisions or requirements concerning release procedures or mitigating measures.

Biological Control Agent Information

1. Taxonomy

Common name: Arundo leafminer
Scientific name: *Lasioptera donacis* Coutin
Synonyms: None

Order: Diptera
Family: Cecidomyiidae
Tribe: Lasiopterini
Genus: *Lasioptera*
Species: *L. donacis* Coutin

2. Description of *L. donacis*

The genus *Lasioptera* belongs to the tribe Lasiopterini, the so-called “short-horned gall midges” because of their foreshortened antennae. *Lasioptera* is also the type-genus for the subtribe Lasiopterina (Gagné, 1976) which is distinguished by characteristics of the female ovipositor. The setae (hairs) on the dorsal area of the cerci are enlarged and curved, and cluster to form a comb or rasp. Voucher specimens have been deposited in the USDA, Agricultural Research Service (ARS), Systematic Entomology Laboratory, Beltsville, Maryland.

3. Geographical Range of *L. donacis*

a. Native Range

Based on the results of extensive foreign exploration, *L. donacis* has a western Mediterranean distribution including Portugal, Spain, France,

Italy, Bulgaria, and Greece. In Spain and Greece (Crete) it occurs on both *A. donax* and *Arundo plinii*.

b. Expected Attainable Range of *L. donacis* in North America

Distribution of *L. donacis* does not appear to be limited by high or low temperatures. It is the most cold-hardy of the *A. donax* specialists and is able to overwinter as a third instar larva in dry *A. donax* leaf sheaths and emerge after winter to infest spring re-growth. *Lasioptera donacis* populations are highest in the high rainfall areas of the Mediterranean such as Rome, Italy. It is not known whether *L. donacis* will be able to establish throughout the range of *A. donax* in the United States, but it is likely to be a large part of this range. It appears likely that *L. donacis* could establish as far north as Zone 7a including most of the southern United States. (Fig. 1, Table 1).

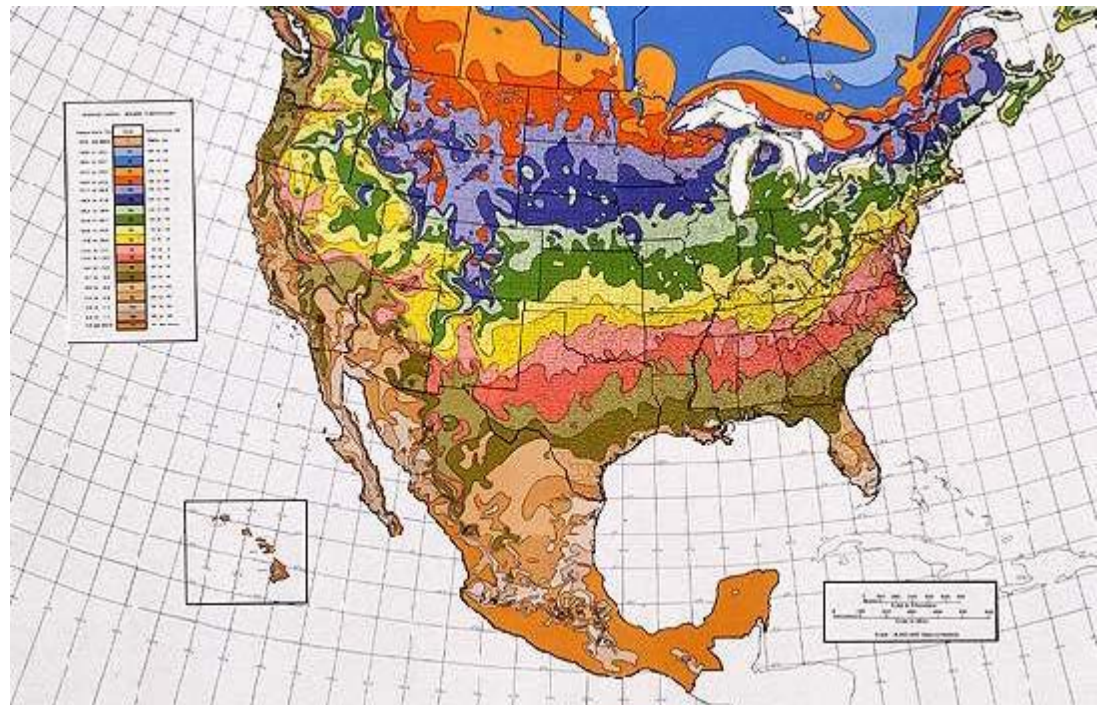


Fig. 1. Map of USDA Cold hardiness zones in North America (USDA-ARS, 2012).

Zone T	Fahrenheit (F)	Celsius (C)	Example Cities
4b	-25 to -20 F	-28.9 to -31.6 C	Northwood, Iowa; Nebraska
5a	-20 to -15 F	-26.2 to -28.8 C	Des Moines, Iowa; Illinois
5b	-15 to -10 F	-23.4 to -26.1 C	Columbia, Missouri; Mansfield, Pennsylvania
6a	-10 to -5 F	-20.6 to -23.3 C	St. Louis, Missouri; Lebanon, Pennsylvania
6b	-5 to 0 F	-17.8 to -20.5 C	McMinnville, Tennessee; Branson, Missouri
7a	0 to 5 F	-15.0 to -17.7 C	Birmingham, Alabama; South Boston, Virginia
7b	5 to 10 F	-12.3 to -14.9 C	Little Rock, Arkansas; Atlanta, Georgia
8a	10 to 15 F	-9.5 to -12.2 C	Tifton, Georgia; Dallas, Texas
8b	15 to 20 F	-6.7 to -9.4 C	Gainesville, Florida; Charleston, South Carolina
9a	20 to 25 F	-3.9 to -6.6 C	Houston, Texas; St. Augustine, Florida
9b	25 to 30 F	-1.2 to -3.8 C	Brownsville, Texas; Fort Pierce, Florida
10a	30 to 35 F	1.6 to -1.1 C	Naples, Florida; Victorville, California
10b	35 to 40 F	4.4 to 1.7 C	Miami, Florida; Coral Gables, Florida
11	above 40 F	above 4.5 C	Honolulu, Hawaii; Mazatlán, Mexico

Table 1. USDA Cold Hardiness Zones and their associated average minimum temperatures.

3. Life History of *L. donacis*

Adult *L. donacis* males are considerably smaller than females and males emerge several days earlier than the females. Mating occurs shortly after female emergence and usually occurs on the underside of the leaf. Females begin searching for the saprophytic fungus *Arthrrium arundinis* shortly after mating. A saprophytic fungus is one that derives its nourishment from dead or decaying organic matter. Females have specialized structures on their egg-laying organ (ovipositor) for detection, acquisition, and storage of *A. arundinis* spores/conidia. *Arundo donax* leaf sheaths can have a mix of fungal species yet the female chooses *A. arundinis* for egg laying (oviposition). Females slowly drag their ovipositor over the blister-like fruiting bodies of the fungus to acquire conidia. After successful acquisition, the conidia are mixed with the eggs at oviposition. *Lasioptera donacis* females seek pre-existing holes or wounds in the leaf-sheath for oviposition. According to Coutin and Faivre-

Amiot (1981) these are typically the emergence holes from a leaf-mining agromyzid fly of the genus *Cerodontha*. These holes may also be created by splits in the leaf sheath as the stem grows or from feeding by other insects.

Lasioptera donacis has three larval instars that are spent entirely within the inner (mesophyll) layer of the stem leaf sheaths of *A. donax*. The larvae are usually colonial, feeding in clusters of about the same stage of development, and presumably from the same clutch of eggs, but at times in larger aggregations that can include two instars likely from successive ovipositions. The first instars are free-living for at least several days after hatch because it typically requires about a week after inoculation for the dark mass of fungal mycelia (threads that are the vegetative form of the fungus) to accumulate. Invariably the second and third instar larvae were found feeding on the mycelial mass. The third instar has a feeding and then a pre-pupal, non-feeding stage. At the end of the feeding stage the third instar larva spins a silken cocoon in which it will eventually pupate, still occupying the fungus filled channels of the inner leaf sheath. Just prior to pupation the larva cuts an escape hatch in the leaf wall from which the adult will eventually emerge. When the adult emerges it leaves the remains of the pupal shell protruding from the exit hole in the leaf.

III. Affected Environment

Arundo donax is a bamboo-like perennial that grows to 8 meters (m) tall. It reproduces vegetatively from rhizomes and stem fragments. A rhizome is a plant stem that grows horizontally under or along the ground and often sends out roots and shoots. Intact rhizomes buried under about 1 to 3 m of silt can develop new shoots. Stem fragments disperse with water, mud, and human activities. Under optimal conditions, plants grow and spread rapidly during the warm season. Viable seed has not been observed in North America or in the native range (DiTomaso and Healy, 2003).

A. Taxonomy of the Target Weed

Common name: Carrizo cane, giant reed, carrizo gigante, bamboo reed, donax reed, elephant grass, reed cane, reed grass, Spanish reed, giant cane, Georgia cane, wild cane.

Scientific name: *Arundo donax* L.

Synonyms: *Arundo glauca* Bubani, *Arundo latifolia* Salisb., *Arundo sativa* Lam., *Donax arundinaceus* P. Beauv., *Scolochloa arundinacea* (P. Beauv.) Mert. & Koch, *Scolochloa donax* (L.) Gaudin, *Cynodon donax* (L.) Raspail. A cultivated variety with variegated leaves is often called *Arundo*

donax L. var. *versicolor* (P. Mill.) Stokes, synonym: *Arundo versicolor* P. Mill.

Class: Liliopsida
Subclass: Commelinidae
Order: Cyperales
Family: Poaceae
Genus: *Arundo*
Species: *donax* L.

A. Areas Affected by *A. donax*

1. Native and Introduced Range of *A. donax*

Arundo donax is native to Europe from the central Atlantic coast of Portugal, inland along the major rivers of the Iberian Peninsula, along the Mediterranean coast from Spain to Greece, including the warmer parts of the Adriatic coast. Figure 2 shows the areas within the native range which are most suitable to *A. donax* ecoclimatically using the climate matching program CLIMEX. Note that large areas of North Africa are suitable if there is sufficient water. In North Africa along the Mediterranean, populations are discontinuous from the Western Sahara, Morocco, and Algeria to the Arabian Peninsula. Remote populations are known far inland into the Sahara in stable oases. Populations in China are not considered to be native. In addition to *A. donax* L., other *Arundo* species are native to the Mediterranean including *Arundo plinii* Turra, *Arundo collina* Tenore and *Arundo mediterranea* (Danin et al., 2002; Danin, 2004; Danin et al., 2006). The only other known *Arundo* spp. outside of the Mediterranean is *Arundo formosana* in Taiwan.

Arundo donax has a nearly worldwide distribution in tropical to warm-temperate regions. In the United States, it is invasive from northern California across the Southwestern and Southeastern United States to Maryland. It is widely distributed in Mexico, and Central and South America. The most severe infestations in the United States are in Arizona, California, and Texas, especially the Santa Ana River Basin and Rio Grande Basin.

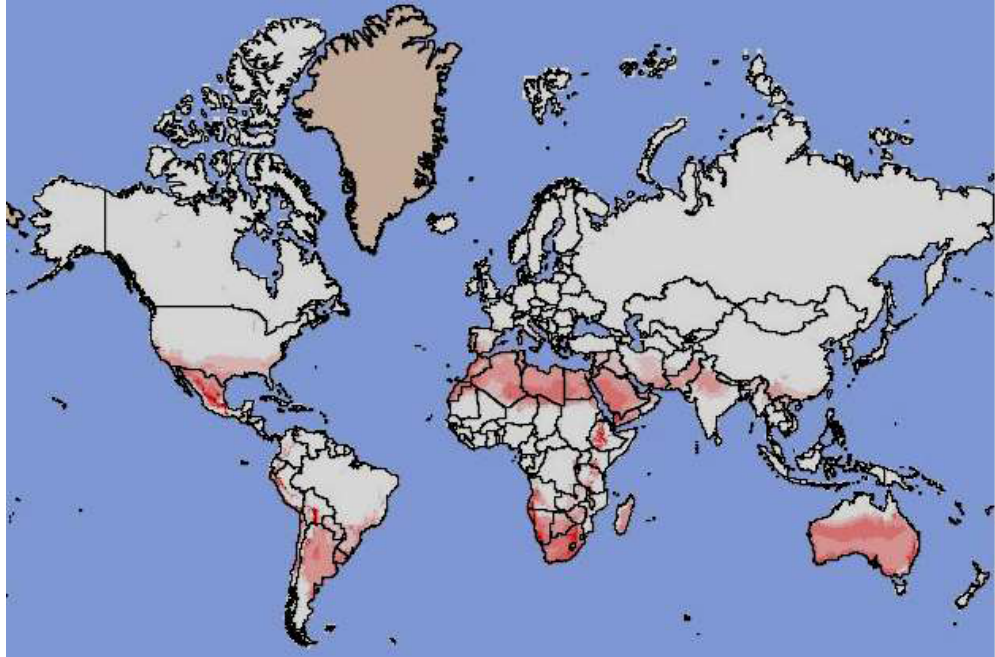


Figure 2. Native and predicted range of *Arundo donax* based on CLIMEX. The areas shaded in red are suitable for *Arundo donax* given adequate soil moisture (from Goolsby, 2014).

2. Present and Potential Distribution in North America

Arundo donax is well established in North America, although it continues to spread into new areas. Figure 3 shows the areas that are climatically suitable based on CLIMEX parameters from Europe. While the predicted CLIMEX distribution broadly agrees with the actual distribution, *A. donax* has naturalized further north. It has been documented in South Bend, Indiana, and Coeur'd'alene, Idaho.

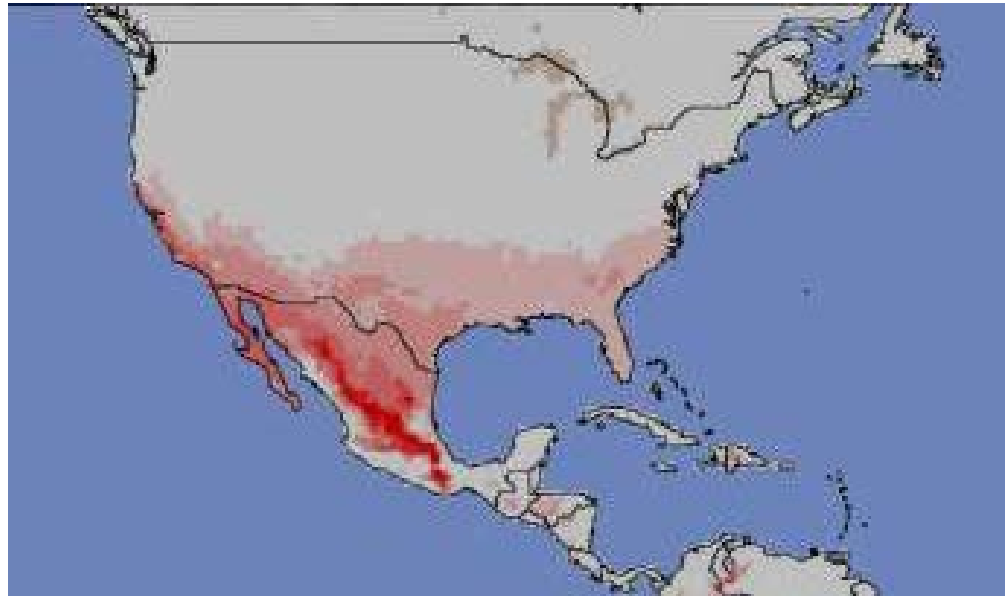


Figure 3. Predicted range of *Arundo donax* in North America based on CLIMEX. The areas shaded in red are suitable for *Arundo donax* given adequate soil moisture. Darker red indicates a higher ecoclimatic suitability rating, based on climate parameters from the native range in Europe (from Goolsby, 2014).

Some of the most severely infested areas are in the Rio Grande Basin and in the coastal rivers of southern California. A continuous stand of *A. donax* occurs from just south of Laredo to Del Rio, Texas. The swath of *A. donax* is nearly 0.5 miles wide along this stretch of the Rio Grande River. Further upriver, near Big Bend National Park, stands of *A. donax* are increasing in size and density. Heavy rains during the summer of 2007 stimulated new growth, and flood waters distributed propagules downstream. Aerial surveys conducted by USDA researchers in the fall of 2007 revealed much more *A. donax* than had been previously seen in the 2002 surveys (Goolsby, 2014). Goolsby (2014) estimates that the size of the *A. donax*-infested area in the Texas and Mexican portions of the Rio Grande Basin is 11,135 hectares (27,515 acres).

The spread of *A. donax* into new areas appears to be from earthmoving equipment and roadway mowers (Goolsby, 2014). Once established in a watershed, rhizomes and canes move downstream during flood events to establish new stands. It appears to be increasing density over time below reservoirs in riparian habitats (Goolsby, 2014). Conveyance flows from dams stimulate year round production of *A. donax*, giving it a competitive edge over the native vegetation that is adapted to seasonal flows. Because many of the dams in the Rio Grande/Rio Bravo Basin are recent (1980s-90s), the level of infestation may increase dramatically over the next decade.

3. Habitat

Arundo donax typically grows on sites with a low slope in riparian areas, floodplains, ditches, and irrigation canals. In the eastern United States, with average rainfall above 30 inches, it can grow in upland sites, such as windbreaks or in ornamental settings. *Arundo donax* occurs in a wide range of soils types with variable fertility, but grows best in well-drained, moist soils, although it tolerates periodic flooding. Plants tolerate some salinity and extended periods of drought; however, they do not survive in areas with prolonged or regular periods of freezing temperatures (DiTomaso and Healy, 2003).

C. Plants Related to *A. donax* and Their Distribution

Plants in North America are discussed below that meet the following criteria: *Arundo* species in North America, species in related genera in the subfamily Arundinoideae; species in related subfamilies that are either native to the introduced range of *A. donax* and/or similar in appearance to *A. donax*; species in related orders of monocots (plants having a single cotyledon in the seed); economically important grasses; and habitat associates. Information regarding plants taxonomically related is included in this document because plant species that are related to *A. donax* have the most potential to be attacked by *L. donacis*. The plants listed below were used to test the host specificity of *L. donacis* to *A. donax*. These tests are discussed later in this document.

Phragmites australis (Cav.) Trin. ex Steud. (common reed) is similar in appearance and habitat to *A. donax* but lacks the light colored wedge and auricles at the base of the leaf of *A. donax*. *Phragmites australis* is found nearly worldwide in temperate and tropical wet habitats.

Molinia caerulea (L.) Moench (purple moorgrass) is a perennial bunch grass native to temperate areas of Eurasia. It has been introduced as an ornamental to northeastern Canada and the United States where it has invaded damp areas.

Hakonechloa macra (Munro) Makino (Hakone grass) is endemic to Japan where it grows along rivers. It is grown as an ornamental in temperate areas of North America.

Aristida purpurea Nutt. var. *longiseta* (Steud.) Vasey (red threeawn) is native from western Canada to northern Mexico and grows in well-drained soils.

Chasmanthium latifolium (Michx.) Yates (inland sea oats) is native from the Middle Atlantic States of the United States west to Texas and grows along waterways and in moist woods.

Cynodon dactylon (L.) Pers. (Bermuda grass) is a pasture and turf grass native to Africa that now grows worldwide except in the coldest and driest areas.

Spartina alterniflora Loisel. (smooth cordgrass) is an obligate wetland species native to saltmarshes along the Atlantic Coast of Canada, the Atlantic and Gulf coasts of the United States, the Caribbean, and northern South America to Uruguay. It is an introduced invasive along the Pacific Coast of California, Oregon, and Washington as well as in western Europe and New Zealand.

Spartina spartinae (Trin.) Merr. ex Hitchc. (Gulf cordgrass) is a native bunch grass that grows mainly along the Atlantic Coast of Florida, the Gulf Coast of the United States and Mexico to Costa Rica. In South America it is native to Venezuela, Argentina, and Paraguay.

Uniola paniculata L. (sea oats) is a native that grows on sand dunes along the coast from Maryland to Veracruz, Mexico as well as in the Bahamas and Cuba.

Leptochloa fusca (L.) Kunth subsp. *uninervia* (J. Presl) N. Snow (= *L. uninervia*) (red sprangletop) is native to the southern half of the United States and much of Mexico, the Caribbean, and Central and South America. This species is now an invasive species in rice fields in Spain and Italy.

Bouteloua trifida Thurb. (red grama) is a bunchgrass native to the southwestern United States and into central Mexico.

Eragrostis intermedia Hitchc. (plains lovegrass) is a native grass ranging from the southern United States to Costa Rica.

Eragrostis spectabilis (Pursh) Steud. (purple lovegrass) is native to southeastern Canada, the eastern two-thirds of the United States and south to Belize.

Muhlenbergia capillaris (Lam.) Trim. (hairawn muhly) is a bunchgrass native to the southeastern United States and the Bahamas.

Cortaderia selloana (Schult. & Schult. f.) Asch. & Graebn. (pampas grass) is an ornamental grass native to Brazil and the southern part of South America. Pampas grass has been planted in the southeast and southwest of the United States and is invasive in some areas.

Panicum amarum Elliot. (bitter panicgrass) is native to coastal dunes

along the Atlantic and Gulf coasts of the United States and the Gulf Coast of northern Mexico as well as in swamp edges and wet sandy soils in this range.

Panicum virgatum L. (switchgrass) is a native grass that grows mainly east of the Rocky Mountains from southern Canada through Central America and in Cuba.

Sorghum bicolor (L.) Moench (sorghum) is a native of Africa that is grown through much of the world.

Megathrysus infestus (= *Urochloa maxima*, *Panicum maximum*) (Jacq.) B.K. Simon & S.W.L. Jacobs is an introduced grass from Africa. It is invasive in the southern part of the United States and in Mexico.

Zea mays L. (corn) is native to Mexico but is grown through much of the world.

Alopecurus pratensis L. (meadow foxtail) is native to temperate Europe and Asia. It was introduced to North America as a pasture grass and has become naturalized.

Saccharum officinarum L. (sugarcane) is grown in the southeastern United States as well as in other tropical/subtropical regions throughout the world. It is native to tropical Asia and Oceania.

Andropogon glomeratus (Walter) Britton et al. (bushy bluestem) is native to the southern United States and north to New York and south to northern South America.

Pennisetum ciliare (L.) Link (buffelgrass) is native to Africa, western Asia, and India. It has been introduced to and become highly invasive in the southern United States and Mexico and elsewhere as a forage crop.

Schizachyrium scoparium (Michx.) Nash (little bluestem) is native to most of Canada and the United States and northern Mexico.

Tripsacum dactyloides (L.) L. (eastern gamagrass) is native to the eastern and central United States and south through Mexico to northern South America.

Sporobolus wrightii Munro ex Scribn. (alkali sacaton) is a native growing in Texas and Oklahoma and west to California and south to central Mexico.

Elymus virginicus L. (Virginia wildrye) is native throughout most of

Canada and the United States.

Arundinaria gigantea (Walter) Muhl. (giant cane) is native to the southeastern United States.

Typha latifolia L. (broadleaf cattail) is an obligate wetland plant native to the Americas, Europe, Asia, and Africa.

Sabal mexicana Mart. (Rio Grande palmetto) is native to Texas, Mexico, and Central America.

Carya illinoensis (Wangenh.) K. Koch (pecan) is a native that grows in the southeastern United States and south into central Mexico.

Baccharis halimifolia L. (eastern baccharis) is native to the eastern and southeastern United States and eastern Mexico from Nuevo Leon to Veracruz.

IV. Environmental Consequences

A. No Action

1. Impact of *A. donax*

a. Native Plants

Non-target plants growing in riparian areas are severely impacted by *A. donax* throughout North America. *Arundo donax* grows in dense stands that restrict light thus preventing the establishment or growth of other plants. If *A. donax* stands are killed by herbicides or cutting they are not easily removed and will remain for long periods as thick mats preventing regrowth of native vegetation. In many areas, *A. donax* is burned yearly to keep standing vegetation to a minimum. In other areas, accidental wildfires enter riparian zones infested with *A. donax* damaging riparian plants (Goolsby, 2014.). In both cases native plants, especially trees that are not fire adapted, are killed by the hot fires. *Arundo donax* survives the wildfires due to its extensive below ground rhizomes. It regrows quickly after fires, shading out emerging seedlings, thus increasing its dominance over native riparian vegetation.

b. Effect on ecosystem function

Widespread effects of *A. donax* on ecosystems have been documented on several continents including, Australia, North America, Oceania, and Africa. *Arundo donax* can increase sediment deposition in natural and man-made channels resulting in reduced channel depth and greater flooding (Frandsen and Jackson, 1994). In addition, during flooding, debris dams of *A. donax* may collect adjacent to flood control structures,

bridges, and culverts exacerbating flooding (Frandsen and Jackson, 1994). *Arundo donax* produces profuse quantities of biomass (Perdue, 1958; Sharma et al., 1998; Spencer et al., 2006) that are quite flammable at the end of the growing season. As a result, it has changed control of ecosystem processes in some Californian riparian zones from flood-regulated to fire-regulated (Rieger and Kreager, 1989). Thus, it may be considered a transformer species in North America (Richardson et al., 2000).

c. Beneficial uses

Arundo donax is grown for woodwind reeds, although there is currently no commercial production in North America (Perdue, 1958; Obataya and Norimoto, 1995). The highest quality reeds come from the native range in Europe. Currently, the most significant use of this plant is its proposed use as biofuel (Duke, 1984; Szabo et al., 1996). There are a few small-scale research plantings of *A. donax* in Texas and Georgia. Social, recreational uses. *Arundo donax* has no social or recreational uses.

2. Impact from Use of Other Control Methods

The continued use of chemical herbicides, and mechanical and biological controls at current levels would be a result if the “no action” alternative is chosen).

a. Chemical Control

The most common herbicide used for *A. donax* is glyphosate which may require continued application for 3 to 5 years for local control (Newhouser et al., 1999; Dudley, 2000). The herbicide imazapyr is also used for control along ditches and canals. However, chemical control methods are not feasible for large-scale infestations covering hundreds of river miles, such as the infestation in the Rio Grande Basin. Broadcast applications of herbicides could have adverse impacts on nontarget vegetation if not carefully applied.

b. Mechanical Control

Mechanical methods of *A. donax* control include use of prescribed fire, heavy machinery (e.g. bulldozer, Hydro-axe,), hand-cutting, chipper, etc. Biomass removal may be necessary if there is a possibility that cut vegetation might create a flood hazard during high water events. Chipping is a costly method of removal. Equipment and labor are expensive relative to other forms of removal; however, the small dry chips that are produced pose little threat in terms of regeneration, and they do not form debris dams. Biomass removal by vehicle is expensive and generally not preferred due to its lack of cost-effectiveness. The use of heavy

machinery, such as the Hydro-axe, is extremely expensive and slow, cutting only about 3 to 4 acres per day (Bell, 1997). Mechanical eradication with a backhoe has been ineffective because the rhizome fragments buried under the soil will readily re-sprout. Prescribed burning has not been successful because it cannot kill the rhizomes, and generally promotes *A. donax* regeneration over native riparian species.

c. Biological Control

The Arundo wasp, *Tetramesa romana* Walker (Hymenoptera: Eurytomidae) was approved for release in April 2009. It currently occurs in Texas and California. It is not expected that *Tetramesa romana* (*T. romana*) alone will completely control *A. donax*. However, the stem galling of *A. donax* caused by *T. romana* results in shortened internodes, stunted stems, and sometimes death of the stems. The Arundo scale, *Rhizaspidotus donacis* (Leonnardi) (Hemiptera: Diaspididae), was approved for release in the United States in December 2010, and is one of the most damaging insects to *A. donax* in its native range. The scale attacks the rhizome and developing underground buds by feeding on plants cells that carry out photosynthesis and cellular respiration and can store food for the plant. Damage symptoms include side shoot distortion with thin, brittle, short canes. Crawler feeding often causes distortion and a witch's broom effect (an abnormal brushlike growth of weak, closely clustered shoots). Other effects over time include gradual thinning, leaf reduction, and a sickly, yellowish-clouded appearance of canes. The overall effect is diminished vigor with *A. donax* stands characterized by thin, brittle, naked canes. These two agents are beginning to have an impact on the invasive populations of *A. donax*, but additional host specific and damaging agents that target different parts of the plant are needed to manage this highly invasive weed.

These environmental consequences may occur even with the implementation of the biological control alternative, depending on the efficacy of *R. donacis* to reduce *A. donax* in the continental United States.

B. Issue Permits for Environmental Release of *L. donacis*.

1. Impact of *L. donacis* on Nontarget Plants

Host specificity of *L. donacis* to *A. donax* has been demonstrated through scientific literature, field observations, and host specificity testing. If an insect species only attacks one or a few closely related plant species, the insect is considered to be very host-specific. Host specificity to the target weed is an essential trait for a biological control organism proposed for environmental release.

a. Scientific Literature

Lasioptera donacis Coutin, is only found on *A. donax* in its native range in Europe (Coutin and Faivre-Amiot, 1981).

b. Field Observations

Extensive surveys by Alan Kirk (USDA-ARS, EBCL, Montpellier, France) throughout the western Mediterranean, including Morocco, confirmed the widespread presence of *L. donacis* on *A. donax*. Once, this insect was found on *Arundo plinii* in Spain.

c. Host Specificity Testing

Host specificity tests are tests to determine how many plant species *L. donacis* attacks, and whether nontarget species in North America may be at risk. See appendix 3 for information regarding host specificity testing methods.

(1) Site of Quarantine Studies

Host specificity testing was conducted at USDA-APHIS, Mission Biological Control Containment Facility (MBCL) in Edinburg, Texas.

(2) Test Plant List

The list of plant species used for host-specificity testing of *L. donacis* is shown in appendix 1. The strategy used for selecting plants for testing is based on the phylogenetic approach, where closely related species are theorized to be at greater risk of attack than are distantly related species (Wapshere, 1974).

(3) Discussion of Host Specificity Testing

No-choice tests were used in the host specificity testing of *L. donacis* to determine its fundamental host range (*sensu* Van Klinken and Edwards, 2002). No-choice tests are those where the biological control agent selection is restricted to a single plant species. The U.S. accession of the fungus, *Arthrinium arundinis* was manually applied to leaf sheaths on each test plant to demonstrate that the saprophytic fungus could not infect the host without the damage caused by *L. donacis* larval feeding. *Arthrinium arundinis* is a cosmopolitan, worldwide, saprophytic fungus. See Appendix 2 for full results of host specificity testing and Appendix 3 for more information about host specificity test methods.

In the no-choice testing, *L. donacis* only completed its life cycle on the target host *A. donax*. None of the non-target plants tested were utilized by

the leafminer. Additional replicates of corn, *Zea mays*, were tested because this plant is an important food plant native to Mexico in areas where *A. donax* is invasive. A local variety of sugarcane, *Saccharum officinarum*, was also tested. No development of any kind was found on corn or any of the economic grasses. In addition to testing closely related grasses, grasses similar in appearance to *A. donax*, with large stems and leaf sheaths such as *Cortaderia selloana* (pampas grass), *Panicum amarum* (bitter panic grass), and *Arundinaria gigantea* (giant cane), were also tested. Several important ecologically important ‘riparian cornerstone species’ such as *Typha latifolia* (cattail), *Sabal Mexicana* (Texas palmetto), *Carya illinoensis* (pecan), *Baccharis halimifolia* (eastern baccharis/groundsel bush) were tested, but no feeding or development was observed. In all of the testing, minor larval development was noted on one single replicate of *Phragmites australis*. Eleven dead second and third instar larvae were found in the leaf sheath when it was dissected. Additional *P. australis* testing was conducted on both the Gulf Coast and European ecotypes, but no larval development of any kind was observed. The insect fauna of *P. australis* has been extensively studied in Europe and North America, but no record exists of *L. donacis* developing on *P. australis* despite the presence of *L. donacis* in the same habitat in Europe (Gagné and Jaschhof, 2014; Tschardtke, 1999; Tewksbury et al., 2002). It is likely that the limited larval development on one *P. australis* plant was a quarantine artifact. *Lasioptera donacis* is specific to *A. donax*.

The saprophytic fungus, *Arthrinium arundinis* only developed on *A. donax* despite being systematically applied to wounds of leaf sheaths of all plants used in testing. *Arthrinium arundinis* requires active larval feeding to grow and develop. This confirms that *A. arundinis* is truly a saprophyte and not a primary pathogen.

Release strategy and post-release monitoring of *L. donacis* are described in Appendix 4.

2. Impact of *L. donacis* on *A. donax*

In the area of its origin in the Mediterranean, *L. donacis* causes extensive defoliation of *A. donax* stands (Goolsby, 2014). In general, populations of *L. donacis* are highest in parts of the Mediterranean that receive summer rainfall, including France and Italy (Goolsby, 2014). The potential impact of *L. donacis* comes from comparison of plant demographics between *A. donax* plots in Rome (where *L. donacis* occurs) and Weslaco (where *L. donacis* is not present). In Rome, 27 percent of leaves per cane were dead and 11 percent of the nodes were infested with *L. donacis*. In comparison, the Weslaco plots had less than 8 percent dead leaves, none of the nodes were infested (as expected), and *A. donax* in Weslaco was denser than in Rome plots where *L. donacis* was present (Goolsby, 2014).

Lasioptera donacis is one of the most widespread and damaging arthropods associated with *A. donax*. It has been collected throughout the native range of *A. donax*. *Lasioptera donacis* has the greatest impact on *A. donax* when it occurs with the Arundo wasp, *Tetramesa romana*, most notably in the western Mediterranean (Portugal, Spain, and southwestern France). *Lasioptera donacis* is only known from *Arundo* spp. in western Europe. In its native range, it is host specific and damaging to *A. donax*.

3. Impact of biological control agents on *A. donax* grown as a biofuel

Because *A. donax* is being considered as a biofuel in some states, potential impacts of the three biological control agents (including the two previously released agents, *Tetramesa romana* and *Rhizaspidotus donacis*) on *A. donax* where it is grown for harvest were investigated (Goolsby, 2014). The biological control agents were highly affected by disturbance such as repeated cutting of the plant for a biofuel harvest (Goolsby et al., 2013). One of the most common disturbances in the native range is mowing. Mowing destroys the above ground developing insects, including the proposed biological control agent *L. donacis*, and the two previously released agents, *T. romana* and *R. donacis*, effectively removing a large portion of the local population. Simultaneously, mowing stimulates new stem growth which is not likely to be infested by these specialist insect because the stems emerge out of synchrony with their normal life cycle. The new stems grow with little or no impacts from their specialist herbivore insects. Normally, these insects emerge in late spring just as the plant produces new stems in its natural Mediterranean habitat. Therefore, breaking the natural growth cycle of *A. donax* with mowing and/or supplemental irrigation leads to impacts to the lifecycle of the three insects. Intensive disturbance from repeated mowing and irrigation may even lead to localized elimination of *R. donacis*. This scenario explains why *A. donax* can appear to be very robust and even invasive in some settings, usually in heavily cropped areas. However, where *A. donax* is largely undisturbed, the biological control agents build to high densities that cause causing extensive damage to *A. donax*. Along the Rio Grande in riparian settings, *A. donax* is typically not disturbed; therefore, the biological control agents can build to damaging populations and reduce the invasiveness of the plant. Periodic fires may be the exception and should be avoided to achieve the full impact of the biological control agents.

In agricultural areas where *A. donax* is grown for biofuels, repeated disturbance (harvesting) is expected to greatly reduce the impact of the agents even if they should colonize these areas. *Arundo donax* grown as biofuel in Italy, Spain, and Greece has no significant pests even though *T. romana* (and other potential biological control agents) occur throughout these production areas. Cultural practices used in growing of *A. donax* do not appear to allow for populations of the specialist herbivore insects to reach levels that are damaging to the plant where it is grown as a biofuel.

4. Uncertainties Regarding the Environmental Release of *L. donacis* Once a biological control agent such as *L. donacis* is released into the environment and becomes established, there is a slight possibility that it could move from the target plant (*A. donax*) to attack nontarget plants. Host shifts by introduced weed biological control agents to unrelated plants are rare (Pemberton, 2000). Native species that are closely related to the target species are the most likely to be attacked (Louda et al., 2003). If other plant species were to be attacked by *L. donacis*, the resulting effects could be environmental impacts that may not be easily reversed. Biological control agents such as *L. donacis* generally spread without intervention by man. In principle, therefore, release of this biological control agent at even one site must be considered equivalent to release over the entire area in which potential hosts occur, and in which the climate is suitable for reproduction and survival. However, significant non-target impacts on plant populations from previous releases of weed biological control agents are unusual (Suckling and Sforza, 2014).

In addition, this agent may not be successful in reducing *A. donax* populations in the contiguous United States. Worldwide, biological weed control programs have had an overall success rate of 33 percent; success rates have been considerably higher for programs in individual countries (Culliney, 2005). Actual impacts on *A. donax* by *L. donacis* will not be known until after release occurs and post-release monitoring has been conducted. However, it is expected that *L. donacis*, in concert with the other previously released biological control agents *T. romana*, and *R. donacis*, will reduce invasive *A. donax* populations in the United States.

5. Cumulative Impacts “Cumulative impacts are defined as the impact on the environment which results from the incremental impact of the action when added to other past, present and reasonably foreseeable future actions regardless of what agencies or person undertakes such other actions” (40 CFR 1508.7). Many Federal and State agencies, as well as private entities, conduct programs to manage *A. donax*, as well as other invasive weeds. Chemical, biological control, and mechanical methods, as described previously in this document, are used in a wide range of habitats. Some of these control programs are listed below.

Dept. of Homeland Security, Customs and Border Protection: The Border Patrol is using mechanical and chemical methods to control *A. donax* along the United States and Mexican border in Webb County, Texas, to assist in law enforcement activities associated with illegal border crossings.

Dept. of State, International Boundary and Water Commission (IBWC), El Paso, Texas: The IBWC uses annual mowing along the sections of the Rio Grande to manage access to the River.

U.S. Fish and Wildlife Service, International Services: Chemical control is used to stop the spread of *A. donax* at the Cuatro Cienegas nature preserve in Coahuila, Mexico.

U.S. Dept. of Interior, National Park Service, Big Bend National Park, Texas: Park staff use a combination of fire and herbicides to manage *A. donax*.

Texas Dept. of Parks and Wildlife, Bentsen State Park, Mission, Texas: Park staff use herbicides to control *A. donax* and *Phragmites* growing in the alternate river channels.

Lower Rio Grande Valley Irrigation and Drainage Districts, Brownsville, Harlingen, Mercedes, McAllen, and La Hoya, Texas: All of the irrigation districts report that they use mechanical control, shredders, and backhoes for control of *A. donax* along irrigation canals and drainage ditches.

Maverick Irrigation District, Eagle Pass, Texas: The district reports the use of mechanical and chemical control to manage *A. donax* along irrigation canals and drainage ditches.

Texas Dept. of Transportation (TXDOT), Austin, Texas: The State vegetation coordinator reports that TXDOT uses mechanical and chemical control to maintain populations of *A. donax* growing along roadsides. The problem is most severe in south-central Texas near College Station.

Team Arundo Del Norte, California: A consortium of homeowner associations, municipalities, and the State of California combine their resources to use chemical control, mechanical removal, and revegetation to restore ecologically sensitive rivers and creeks in northern California.

Team Arundo Del Sur, California: A consortium of homeowner associations, municipalities, and the State of California combine their resources to use chemical control, mechanical removal and revegetation to restore ecologically sensitive rivers and creeks in southern California.

California Dept. of Transportation (CalDOT), Sacramento, California: CalDOT uses mechanical and chemical control to manage *A. donax* along highways and bridges in the State.

Private landowners throughout the southern tier of the United States use a variety of methods to control *A. donax* where it has become invasive on private land.

Santa Ana Watershed Association (SAWA), California: SAWA has removed over 2,000 acres of *A. donax* from the Santa Ana watershed to restore habitat for native species, including the southwestern willow flycatcher.

USDA, Agriculture Research Service is conducting releases of the previously released biological control agents *T. romana* and *R. donacis* in the vicinity of Laredo, Texas.

Release of *L. donacis* is not expected to have any negative cumulative impacts in the continental United States because of its host specificity to *A. donax*. Effective biological control of *A. donax* is expected to have beneficial effects for weed management programs, and may result in a long-term, non-damaging method to assist in the control of *A. donax*, and prevent its spread into other areas potentially at risk from invasion.

6. Endangered Species Act

Section 7 of the Endangered Species Act (ESA) and ESA's implementing regulations require Federal agencies to ensure that their actions are not likely to jeopardize the continued existence of federally listed threatened and endangered species or result in the destruction or adverse modification of critical habitat.

APHIS has determined that release of *L. donacis* may affect, but is not likely to adversely affect the ocelot, jaguarundi, southwestern willow flycatcher and its critical habitat, least Bell's vireo and its critical habitat, yellow-billed cuckoo and its proposed critical habitat (beneficial), western snowy plover and its critical habitat (beneficial), Arroyo toad and its critical habitat (beneficial), and unarmored three-spine stickleback (beneficial). Release of *L. donacis* may affect, but is not likely to adversely affect the following listed grasses: *Neostapfia colusana* (Colusa grass), *Orcuttia californica* (California Orcutt grass), *Orcuttia inaequalis* (San Joaquin Orcutt grass), *Orcuttia pilosa* (hairy Orcutt grass), *Orcuttia tenuis* (slender Orcutt grass), *Orcuttia viscida* (Sacramento Orcutt grass), *Tuctoria greenei* (Greene's tuctoria), or *Tuctoria mucronata* (Solano grass), *Swallenia alexandrae* (Eureka dunegrass). There will be no effect on the critical habitat of these grasses. APHIS requested concurrence from the U.S. Fish and Wildlife Service (FWS) on these determinations, and received a concurrence letter dated April 27, 2016, and is located in the administrative record for this action. The biological assessment submitted to FWS (prepared by T. Willard, USDA-APHIS, Dec. 16, 2015) is also in the administrative record.

V. Other Issues

Consistent with Executive Order (EO) 12898, “Federal Actions to Address Environmental Justice in Minority Populations and Low-income Populations,” APHIS considered the potential for disproportionately high and adverse human health or environmental effects on any minority populations and low-income populations. There are no adverse environmental or human health effects from the field release of *L. donacis* and will not have disproportionate adverse effects to any minority or low-income populations.

Consistent with EO 13045, “Protection of Children from Environmental Health Risks and Safety Risks,” APHIS considered the potential for disproportionately high and adverse environmental health and safety risks to children. No circumstances that would trigger the need for special environmental reviews are involved in implementing the preferred alternative. Therefore, it is expected that no disproportionate effects on children are anticipated as a consequence of the field release of *L. donacis*.

EO 13175, “Consultation and Coordination with Indian Tribal Governments,” was issued to ensure that there would be “meaningful consultation and collaboration with tribal officials in the development of Federal policies that have tribal implications....”

APHIS is consulting and collaborating with Indian tribal officials in Arizona, California, and Texas to ensure that they are well-informed and represented in policy and program decisions that may impact their agricultural interests in accordance with EO 13175. APHIS sent letters to all Tribes in the three States regarding this proposed action and received no comments from them.

VI. Agencies, Organizations, and Individuals Consulted

The Technical Advisory Group for the Biological Control Agents of Weeds (TAG) recommended the release of *L. donacis* on April 2, 2015 (Petition 14-03). TAG members that reviewed the release petition (Goolsby, 2014) included USDA representatives from the U.S. Forest Service, National Institute of Food and Agriculture, Animal and Plant Health Inspection Service; U.S. Department of Interior’s U.S. Fish and Wildlife Service, and Bureau of Land Management; Environmental Protection Agency; U.S. Army Corps of Engineers; and representatives from SAGARPA-SENASICA-DGSV (Mexico), Agriculture and Agri-Food Canada, and the National Plant Board. The petition 14-03 (Goolsby, 2014) is included in the administrative record for this action.

This EA was prepared by personnel at USDA, APHIS and USDA ARS. The addresses of participating APHIS units, cooperators, and consultants follow.

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USDA-FS—see U.S. Department of Agriculture, Forest Service.

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Appendix 1. Host plant test list for *Lasioptera donacis* (Goolsby, 2014).

Order	Family	Sub-family	Scientific name	Common name	TAG Category	Indigenous to US	Indigenous to Mexico	Grain/ Forage	Ornamental	Habitat Associate
Cyperales	Poaceae	Arundinoideae	<i>Arundo donax</i> L.	giant reed, carrizo cane	1	No	No	No	Yes	-
“	“	“	<i>Phragmites australis</i> (Cav.) Trin. ex Steud. (European ecotype)	common reed	3	No	No	No	No	No
“	“	“	<i>Phragmites australis</i> (Cav.) Trin. ex Steud. San Benito TX	common reed	3	Yes	Yes	No	No	Yes
“	“	“	<i>Molinia caerulea</i> (L.) Moench	purple moorgrass	3	No	No	No	Yes	No
“	“	“	<i>Hakonechloa macra</i> (Munro) Makino	Hakone grass	3	No	No	No	Yes	No
“	“	Aristidoideae	<i>Aristida purpurea</i> Nutt. var. <i>longiseta</i> (Steud.) Vasey	red threeawn grass*	3	Yes	Yes	No	No	No
“	“	Centothecoideae	<i>Chasmanthium latifolium</i> (Michx.) Yates	inland sea oats	3	Yes	Yes	No	Yes	Yes
“	“	Chloridoideae	<i>Bouteloua trifida</i> Thurb.	red grama*	3	Yes	Yes	Yes	No	No
“	“	“	<i>Cynodon dactylon</i> (L.) Pers.	bermuda grass	3	No	No	Yes	Yes	Yes
“	“	“	<i>Eragrostis intermedia</i> Hitchc.	plains lovegrass*	3	Yes	Yes	Yes	No	No
“	“	“	<i>Eragrostis</i>	purple	3	Yes	Yes	Yes	Yes	No

Appendix 1. Host plant test list for *Lasioptera donacis* (Goolsby, 2014).

			<i>spectabilis</i> (Pursh) Steud.	lovegrass*						
“	“	“	<i>Leptochloa fusca</i> (L.) Kunth subsp. <i>uminervia</i> (J. Presl) N. Snow	Mexican sprangletop	3	Yes	Yes	No	No	No
“	“	“	<i>Muhlenbergia capillaris</i> (Lam.) Trim.	hairawn muhly*	3	Yes	Yes	Yes	Yes	No
“	“	“	<i>Spartina alterniflora</i> Loisel.	smooth cordgrass	3	Yes	No?	No	No	Yes
“	“	“	<i>Spartina spartinae</i> (Trin.) Merr. ex Hitchc.	Gulf cordgrass	3	Yes	Yes	No	No	Yes
“	“	“	<i>Uniola paniculata</i> L.	sea oats	3	Yes	Yes	No	No	Yes
“	“	Danthonioideae	<i>Cortaderia selloana</i> (Schult. & Schult. f.) Asch. & Graebn.	pampas grass	3	No	No	No	Yes	No
“	“	Panicoideae	<i>Andropogon glomeratus</i> (Walter) Britton <i>et al.</i>	bushy bluestem*	3	Yes	Yes	Yes	Yes	Yes
“	“	“	<i>Megathyrsus infestus</i> Simon and Jacobs = <i>Panicum maximum</i>	Guineagrass	3	No	No	Yes	No	Yes
“	“	“	<i>Panicum amarum</i> Elliot.	bitter panicgrass*	3	Yes	Yes	Yes	No	No
“	“	“	<i>Panicum virgatum</i> L.	Switchgrass*	3	Yes	Yes	Yes	Yes	No
“	“	“	<i>Pennisetum ciliare</i> (L.)	buffelgrass	3	No	No	Yes	No	Yes

Appendix 1. Host plant test list for *Lasioptera donacis* (Goolsby, 2014).

			Link							
“	“	“	<i>Saccharum officinarum</i> L.	sugarcane	3	No	No	Yes	No	No
“	“	“	<i>Schizachyrium scoparium</i> (Michx.) Nash	little bluestem grass*	3	Yes	Yes	Yes	Yes	Yes
“	“	“	<i>Sorghum bicolor</i> (L.) Moench	sorghum	3	No	No	Yes	No	No
“	“	“	<i>Tripsacum dactyloides</i> (L.) L.	eastern gamagrass *	3	Yes	Yes	Yes	No	Yes
“	“	“	<i>Zea mays</i> L.	corn	3	No	No	Yes	No	No
“	“	<u>Ehrhartoideae</u>	<i>Zizania texana</i> Hitchc.	TX wild rice ⁺	3	Yes	No	No	No	Yes
“	“	Pooideae	<i>Alopecurus pratensis</i> L.	meadow foxtail *	3	No	No	Yes	No	Yes
“	“		<i>Elymus virginicus</i> L.	Virginia wildrye*	3	Yes	No	Yes	No	Yes
“	“	“	<i>Sporobolus wrightii</i> Munro ex Scribn.	alkalai sacaton *	3	Yes	Yes	Yes	No	Yes
“	“	Bambusoideae	<i>Arundinaria gigantea</i> (Walter) Muhl.	giant cane	3	Yes	No	No	Yes	Yes
Typhales	Typhaceae	----	<i>Typha latifolia</i> L.	broadleaf cattail	5	Yes	Yes	No	No	Yes
Arecales	Arecaceae	----	<i>Sabal mexicana</i> Mart.	Rio Grande palmetto	5	Yes	Yes	No	Yes	Yes
Juglandales	Juglandaceae	----	<i>Carya illinoensis</i> (Wangenh.) K. Koch	pecan	6	Yes	Yes	Yes	Yes	Yes
Asterales	Asteraceae	----	<i>Baccharis halimifolia</i> L.	Eastern Baccharis	6	Yes	Yes	No	No	Yes

Note: * indicates congener of state or federal threatened or endangered species; ⁺ indicates actual endangered species tested

Appendix 2. Results of no-choice larval development testing for *Lasioptera donacis* on the target weeds and test plants (Goolsby, 2014).

Table 1. Results of no-choice host range tests for *Lasioptera donacis*. Values are mean numbers of adults produced per test replicate.

Test Plant Species	Reps	Reps with Adult Emergence	Adults
			Mean ± SE
<i>Arundo donax</i>	62	52	113.6 ± 16.1
<i>Phragmites australis</i> (European ecotype)	8	0	0
<i>Phragmites australis</i> (Gulf Coast ecotype)	6	0	0
<i>Molinia caerulea</i>	3	0	0
<i>Hakonechloa macra</i>	3	0	0
<i>Aristida purpurea</i> var. <i>longiseta</i>	3	0	0
<i>Chasmanthium latifolium</i>	3	0	0
<i>Bouteloua trifida</i>	3	0	0
<i>Cynodon dactylon</i>	3	0	0
<i>Eragrostis spectabilis</i>	3	0	0
<i>Leptochloa fusca</i> subsp. <i>uninervia</i>	6	0	0
<i>Muhlenbergia capillaris</i>	3	0	0
<i>Spartina alterniflora</i>	6	0	0
<i>Spartina spartinae</i>	3	0	0
<i>Uniola paniculata</i>	5	0	0
<i>Cortaderia selloana</i>	3	0	0
<i>Andropogon glomeratus</i>	3	0	0
<i>Megathyrsus infestus</i> = <i>Panicum maximum</i>	3	0	0
<i>Panicum amarum</i>	3	0	0
<i>Panicum virgatum</i>	3	0	0
<i>Pennisetum ciliare</i>	6	0	0
<i>Saccharum officinarum</i>	6	0	0
<i>Schizachyrium scoparium</i>	3	0	0
<i>Sorghum bicolor</i>	3	0	0
<i>Tripsacum dactyloides</i>	3	0	0
<i>Zea mays</i> var. silver queen	10	0	0
<i>Zizania texana</i>	6	0	0
<i>Alopecurus pratensis</i>	6	0	0
<i>Elymus virginicus</i>	3	0	0
<i>Sporobolus wrightii</i>	3	0	0

<i>Arundinaria gigantea</i>	3	0	0
<i>Typha latifolia</i>	3	0	0
<i>Sabal mexicana</i>	3	0	0
<i>Carya illinoensis</i>	3	0	0
<i>Baccharis halimifolia</i>	3	0	0

Table 2. Results of no-choice host range tests for French *Lasioptera donacis*. Values are mean numbers of *L. donacis* adults produced per plant in which development occurred.

Test Plant Species	Reps	Reps with Adult Emergence	Adults
			Mean ±SE
<i>Arundo donax</i>	11	11	74.4 ± 19.9
<i>Phragmites australis</i> (Gulf Coast ecotype)	6	0	0
<i>Bouteloua trifida</i>	3	0	0
<i>Eragrostis spectabilis</i>	3	0	0
<i>Spartina alterniflora</i>	6	0	0
<i>Uniola paniculata</i>	2	0	0
<i>Saccharum officinarum</i>	5	0	0
<i>Sporobolus wrightii</i>	3	0	0
<i>Carya illinoensis</i>	3	0	0

Table 3. Results of no-choice host range tests for Italian *Lasioptera donacis*. Values are mean numbers of *L. donacis* adults produced per plant in which development occurred.

Test Plant Species	Reps	Reps with Adult Emergence	Adults
			Mean ±SE
<i>Arundo donax</i>	6	5	61 ± 26.6
<i>Pennisetum ciliare</i>	3	0	0
<i>Saccharum officinarum</i>	1	0	0
<i>Megathyrsus infestus</i> = <i>Panicum maxima</i>	3	0	0
<i>Sabal mexicana</i>	3	0	0
<i>Zizania texana</i>	6	0	0

Table 4. Results of no-choice host range tests for Greek *Lasioptera donacis*. Values are mean numbers of *L. donacis* individuals produced per plant in which development occurred.

Test Plant Species	Reps	Reps with Adult Emergence	Adults
			Mean ±SE
<i>Arundo donax</i>	6	5	61 ± 26.6
<i>Pennisetum ciliare</i>	3	0	0
<i>Saccharum officinarum</i>	1	0	0
<i>Megathyrsus infestus</i> = <i>Panicum maxima</i>	3	0	0
<i>Sabal mexicana</i>	3	0	0
<i>Zizania texana</i>	6	0	0

Appendix 3. Host-specificity testing methods (Goolsby, 2014).

Stage of plants tested

Arundo donax rhizomes were field dug near Weslaco, Texas and planted in 23 centimeter (cm) plastic pots. Pots are held in a warm greenhouse with overhead irrigation until stems were 2 feet tall. Plants were then moved to an open air shade house for 3-6 weeks to harden off and grow to height of 4-6 feet. Plant selection for optimal *Lasioptera donacis* preference was based on node length, thickness, and sheath and stem quality. Plants were selected that had at least 2 fully extended internodes. The stems were at least 1 cm thick. The stems were tough, but the leaf sheaths were still green at the lower internodes. Once selected, plants were allowed to dry for 1-3 days inside the greenhouse in order to limit the amount of sap produced once the stem is pricked. Optimal pots were transferred to the quarantine laboratory for inoculation with *L. donacis* and *Arthrinium arundinis* and topped to fit inside cages.

Sources of plants tested

Arundo donax was collected from along the Rio Grande, near Weslaco, Texas. Genotyping of the *A. donax* in the Rio Grande Basin revealed one dominant genotype that matched with populations near Barcelona and Almuñécar, Spain. Other genotypes of *A. donax* from outside of Texas were not introduced due to legal constraints regarding movement of noxious weeds. However, DNA samples were obtained from California, Mexico, and Argentina for the genetics study.

Non-target host plants were obtained and or grown from seed by Mike Heep of Heep's Nursery in Harlingen, Texas. Mr. Heep is a botanist and a grass specialist. He supplies other Federal and State agencies with native plant material for revegetation programs. Grass and broadleaf species used in the testing were obtained locally. *Panicum virgatum* was supplied by the U.S. Fish and Wildlife Service because the population tested represented a local relict population near Kenedy, Texas. Some species such as *Molinia* and *Hakonechloa*, which are exotic to North America, but closely related to *A. donax*, were imported from ornamental nursery stock in California.

No-Choice host specificity testing

No-choice tests were used because they provided the clearest prediction of the fundamental host range (*sensu van Klinken and Edwards, 2002*) of *L. donacis*. The fungus, *Arthrinium arundinis*, was manually applied to leaf sheaths on each test plant to demonstrate that the saprophytic fungus could not infect the host without the damage caused by larval feeding.

Plants

Arundo donax plants were housed in a greenhouse for 3 to 6 weeks, regulated between 25-32 °C and 70 to 90 percent Relative Humidity (RH) with ambient light for 12 to 14 hours per day. Plant selection for optimal *L. donacis* preference was based on internode length, thickness, and sheath and stem quality. Plants were selected that had at least two fully extended internodes. The stems were at least 1 cm thick. The stems were tough, but sheaths on the lower internodes were still green. Once selected, plants were allowed to dry for 1 to 3 days inside the greenhouse in order to reduce over-hydration that interferes with larval development. Optimal plants were transferred to the quarantine laboratory for inoculation with *L. donacis* and *A. arundinis*.

Fungus

Arthrimum arundinis is a U.S. fungus obtained from a collection at the American Type Culture Collection (ATCC) facility in Arlington, Virginia. *A. arundinis* was grown on oatmeal agar. After two days, *A. arundinis* spores were added to the plates and grown for 2 to 5 weeks. Plates were kept in an incubator on a 12 hours light/12 hours dark, cool white light cycle at 25 °C. Once the plates were covered with black sporulated hyphae, the hyphae and spores were scraped from the agar and added to 1.5 ml sterile water in a plastic centrifuge tube. The tube was vigorously shaken until spores mixed evenly into a homogenous solution. A concentration of approximately 1 million spores/ml H₂O was used for all inoculations.

Infestation

Sheaths were smeared with a 1 million spores/ml of H₂O suspension of ATCC *A. arundinis*. The suspension was allowed to dry for a few minutes. Then, each plant was pricked (10-12 times per sheath) with forceps to provide oviposition sites that in nature would be provided by damage by other insects, sheath breakage due to emerging side shoots, or sheath damage due to wind breakage. Plants were set up in one of four different types of cages: 1) a Plexiglas box (15.5 x 15.5 x 22 cm) with holes cut out of the sides and sealed with silk organza fabric to provide air ventilation; 2) aluminum framed cages with top, bottom, and 2 sides covered with mesh and 2 sides covered with plastic film; 3) Bugdorms (mesh and clear plastic sides); or 4) metal framed cages with 2 glass sides and mesh on the top, bottom, and 2 sides. The cages were housed in an environmental growth chamber maintained at 25 to 27 °C and 50 to 60 percent RH. Approximately 50 *L. donacis* adults that had emerged from European *A. donax* canes were released in each cage containing a test plant. After 7 days, plants were removed from the cage and placed in a greenhouse at 27°C and 40 to 60 percent RH. Sheaths were monitored for symptoms of *L. donacis*, blotchy black areas due to fungal growth inside the sheath.

Dissections

After about one month stems of *A. donax* were harvested and put in small plastic boxes with mesh-covered lids until they could be dissected. Upon dissection, numbers of *L. donacis* adults, pupae, third and second instar larvae were counted. Stems of non-*Arundo* species were grown for up to two

months before harvest and dissection.

Positive Controls

Arundo donax controls were used for every individual test conducted over time. Each test normally involved multiple test plant species. This method assured that both the *L. donacis* adults and fungus were viable at the time of testing. This more stringent method was necessary due to the tri-trophic interactions of the plant, insect, and fungus. Therefore, more than 50 replicates without corresponding positive controls were not included in the results section. Due to space limitations in quarantine, only 12 test plant species could be set up per week.

Appendix 4. Release and Monitoring Strategy for *Lasioptera donacis* (Goolsby, 2014).

Release protocol

Adults will be removed from quarantine populations for release. *Lasioptera donacis* colonies used for releases will be intensively sampled to determine they are free of parasitic nematodes and pathogens. Adults will be initially colonized in greenhouses and outdoor plots for mass rearing. Initial field releases will take place on the Rio Grande near Los Indios, Texas in Cameron, County.

Rearing or culturing facility: Rearing will be conducted at the USDA-APHIS Mission Biological Control Laboratory, Moore Airbase, Edinburg, Texas and/or the Texas A&M Kingsville Citrus Center facilities operated by USDA-ARS in Weslaco, Texas.

Release details: The leafminer will be released as soon as APHIS issues a permit. Initial field releases will take place on the Rio Grande near Los Indios, Texas in Cameron, County. Subsequent field releases will take place on the Rio Grande between Brownsville and Del Rio, Texas. Material will be provided to researchers at the Instituto Mexicano de Tecnología (IMTA), Jiutepec, Morelos, Mexico for field release in Morelos.

Post Release Monitoring

Monitoring Group: Researchers at USDA-ARS, Edinburg, Texas and researchers at IMTA, Jiutepec, Morelos, Mexico will conduct monitoring when the agent is released in Mexico. IMTA is a formal collaborator in the biological control program and they are also rearing and releasing the arundo wasp, *T. romana* and arundo scale, *R. donacis*.

Monitoring Establishment: Initial monitoring will be conducted at the study site in Los Indios, Texas. We will survey for the presence of leaf sheath infections of *A. arundinis* and infestations of *L. donacis*. *Lasioptera donacis* leaves behind the everted pupal case attached to the leaf sheath as the adults emerge. These visible tubes are an excellent indicator of reproduction and establishment.

Monitoring Techniques: Field sampling will be used to determine the impact of *L. donacis* on *A. donax* in the United States and Mexico. To collect sufficient data across time and space, 19 sites approximately every 25 miles along the Rio Grande will be sampled from subtropical Brownsville (near mouth of river) to Del Rio which is 350 miles inland in the Chihuahuan Desert. The sites represent the full range of conditions where giant reed occurs in the riparian zone of the Rio Grande. Cut stems of *A. donax* will be collected and returned to the laboratory to determine numbers of *L. donacis* per stem and to check for the presence of parasitoids. Field sites will be established with multiple quadrats at each release location to measure the changes in stem length, side shoot production, leaf length, stem diameter, standing biomass and within stand visibility (Spencer et al., 2006; Racelis and Goolsby, 2013). Some sites will receive only *L. donacis* to compare sites with

both the *R. donacis* and *T. romana*. Quadrats will be surveyed for the presence, regrowth or colonization of native and exotic plants. Plant inventories will be conducted over time to assess changes in plant communities following the establishment of the arundo leafminer and interactions with the other agents. Selected locations may receive chemical and/or mechanical control. In these locations, we will make the same measurements as above and assess synergy between the control methods and the agents. In addition to ground sampling using standard techniques, we will use remote sensing to look for local and regional changes in *A. donax* density and health. Non-target plants will be regularly monitored for utilization by *L. donacis*. However, there are no closely related plant species in the riparian habitat where *A. donax* is invasive other than *P. australis*.