SEMARNAT
SECRETARÍA DE MEDIO AMBIENTE Y RECURSOS NATURALES

IMTA
INSTITUTO MEXICANO DE TECNOLOGÍA DEL AGUA

Final Technical Report

Project

Investigation of the impact of *Arundo donax* in México and Evaluation of Candidate Biological Control Agents

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I.-INTRODUCTION

The arrival and spread of non-native species into new environments is a serious threat to ecosystems, that is the case of *Arundo donax* L. (*Poaceae Arundinoideae*), a perennial grass native to the western Mediterranean to India (Duke 1984). *A. donax* was introduced to North America from the Iberian Peninsula within the last 500 years and is now a widespread and invasive weed in the Rio Grande Basin, (the border line between Mexico and the United States) and in almost all the basins in Mexico. This plant is extremely invasive and damaging, affecting especially water supplies. In many parts of Mexico, precipitation and inflows periodically decline and result in a drought, for this reason water conservation programs have to consider the inclusion of control programs of this plant.

In México, *A. donax* is managed (i) by cutting the stems, which is ineffective because of prolific asexual reproduction from an extensive rhizome system, and (ii) by using herbicides, However, evidence of serious harm to health and the environment of chemical control (Steinrücken and Amrhein, 1980; Perkins et al, 2000; Soso et al, 2007) indicates that the herbicides are not desirable for it use in shorelines of water bodies where Arundo's infestations are established.

Classical biological control may be the most cost-effective and sustainable option for management of this weed over large areas such as the Rio Grande. In 2010, a Specific Cooperative Agreement for biological control of *A. donax* was initiated between México and USA. The project includes the introduction, release and evaluation as biocontrol agent a wasp: *Tetramesa romana* Walker and a scale: *Rhizaspidiotus donacis*.

Tetramesa romana is a stem-galling wasp that is host specific to the genus *Arundo* (there are no other native *Arundo* species in North or South America) and native to Mediterranean Europe (Goolsby and Moran 2009, Moran and Goolsby 2009). Females reproduce via parthenogenesis and deposit eggs into the stems and lateral shoots of giant reed. Larval development induces gall formation, impacting the plant's growth and development (Moran and Goolsby, 2009). In 2009, adventive populations of *T. romana* were detected with limited distributions near the cities of Nuevo Laredo, Mexico; Laredo, Eagle Pass, San Antonio and Austin, TX (Goolsby et al. 2009). Monthly surveys using done in 2008 along the Rio Grande and wasps were only found that *T. romana* was only present within the urban riparian zones of Laredo and Eagle Pass and not in the remote areas between these two locations or down river from Laredo to the Lower Rio Grande Valley (Racelis et al. 2009). In Mexico, extensive surveys were done in 2007-08 at 471 sites in throughout the northern states of and Chihuahua, Coahuila, Durango, Nuevo Leon, Tamaulipas, Zacatecas and Morelos and no *T. romana* were detected (Contreras Arquieta and Goolsby, unpublished data). This report documents the introduction, establishment and spread of *T. romana* along the Rio Grande and across Morelos state in Mexico, six years after releases from the biological control program (2010-2016).

II.- METHODOLOGY AND RESULTS

1.-Survey of *Arundo donax* infestation in Mexico

In order to detect *Arundo donax* infestation in México where biocontrol agents could be release, a survey was made in some states (Table 1)

Table 1. Suvey of *A. donax* infestation in Mexico

State	Municipio	Place	Coordinates		Altitude	Colector	Date
			Nort	West			
Morelos	Distrito Federal	Cuernavaca	19°24´23´´	99°11´36´´	2376	Maricela Martínez Jiménez	15/05/2008
Morelos	Cuernavaca	Tabachines	18°53´58.47´´	99°13´24.78´´	1424	Maricela Martínez Jiménez	15/05/2008
Morelos	Jiutepec	Tejalpa	18°53´34´´	99°10´4.9´´	1313	Maricela Martínez Jiménez	15/05/2008
Morelos	Jiutepec	ECACIV	18°55´55´´	99°10´20´´	1313	Maricela Martínez Jiménez	23/05/2008
Morelos	Temixco	Temixco	19°51´´16´´	99°14´19´´	1280	Maricela Martínez Jiménez	15/05/2008
Morelos	Temixco	Acatilpa	19°49´0´´	99°14´0´´	1216	Maricela Martínez Jiménez	15/05/2008
Morelos	Xochitepec	Xochitepec	20°47´23.9´´	99°13´5.6´´	1121	Maricela Martínez Jiménez	23/05/2008
Morelos	Xochitepec	Alpuyeca	21°47´30.1´´	99°131´54.8´´	1127	Maricela Martínez Jiménez	23/05/2008
Morelos	Tezoyuca	Tezoyuca	20°52´18´´	99°9´18´´	1300	Maricela Martínez Jiménez	15/05/2008
Morelos	Puente de Ixtla	Xoxocotla	22°41´6´´	99°14´38´´	1030	Maricela Martínez Jiménez	15/05/2008
Morelos	Emiliano Zapata	Emiliano Zapata	21°50´18´´	99°11´18´´	1210	Maricela Martínez Jiménez	15/05/2008
Morelos	Zacatepec	Zacatepec	23°39´16´´	99°12´56´´	1800	Maricela Martínez Jiménez	15/05/2008
Morelos	Jojutla	Jojutla de Juárez	24°31´13´´	99°9´18´´	825	Maricela Martínez Jiménez	15/05/2008
Michoacán	Pátzcuaro	Carr. A Pátzcuaro	19°33´19.9´´	10123´24´´	2097	Maricela Martínez Jiménez	15/05/2008
Michoacán	Pátzcuaro	Carr. A Pátzcuaro	19°35´32´´	101°17´46.2´´	2097	Maricela Martínez Jiménez	16/05/2008

Table 1: Continuation. Suvey of *A. donax* infestation in Mexico

State	Municipio	Place	Coordinates		Altitude	Colector	Date
			Nort	West			
Michoacán	Pátzcuaro	Carr. A Pátzcuaro	19 50´2.7´´	101°35´38.1´´	1847	Maricela Martínez Jiménez	17/05/2008
Michoacán	Pátzcuaro	Carr. A Pátzcuaro	19°50´36.7´´	101°35´35.2´´	1843	Maricela Martínez Jiménez	18/05/2008
Michoacán	Pátzcuaro	Carr. A Pátzcuaro	19°50´54.2´´	101°35´33.7	1845	Maricela Martínez Jiménez	19/05/2008
Michoacán	Pátzcuaro	Carr. A Pátzcuaro	19°51´33´´	101°35´30´´	1545	Maricela Martínez Jiménez	20/05/2008
Michoacán	Pátzcuaro	Carr. A Pátzcuaro	19°53´29´´	101°35´9.6´´	1842	Maricela Martínez Jiménez	21/05/2008
Michoacán	Pátzcuaro	Carr. A Pátzcuaro	19°33´54´´	101°35´69´´	2573	Maricela Martínez Jiménez	21/05/2008
Veracruz	Fortin de las Flores	Fortin de las Flores	18°53´57´´	97°0´10´´	1027	Maricela Martínez Jiménez	13/07/2008
Veracruz	Cordoba	Cordoba	18°53´59.4´´	96°56´13.4´´	1027	Maricela Martínez Jiménez	13/07/2208

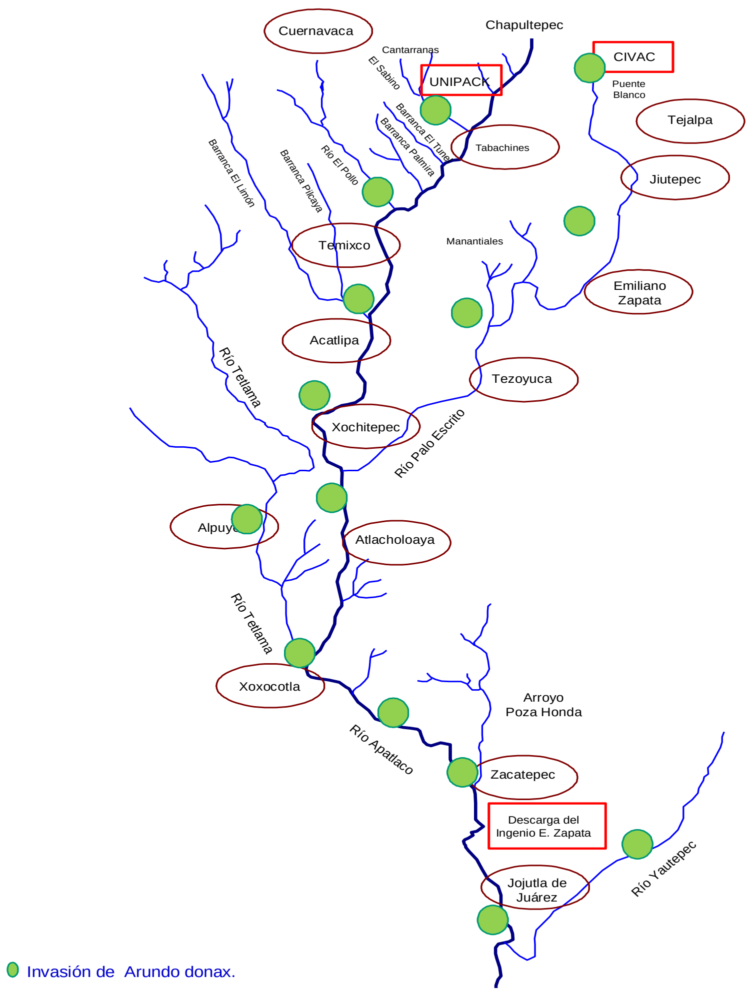
Photo 1: *A. donax* along the rod to Guadalajara, México

Photo 2: *A. donax* infestation

Photo 3: *A. donax* in Paztcuaro lake, Mexico

Photo 4: *A. donax* along the shore of Patzcuaro

In Morelos State, *A. donax* was observed in in the whole basin: Ríos Yautepec, Atoyac y Amacuzac rivers are all infested by *A. donax*



In these places, some insects were detected attacking *A. donax*: *Neoerethistes cyanipes* Champion (Coleoptera: *Curculionidae. Molytinae. Cholini*) and *Leptodictya bambusae* Drake (Hemíptera: Heteroptera, Tingidae). The taxonomy identification was made by Jens Prena and Thomas J. Henry from Enthomology departme Smithsonian Institution.

Photo 5: *N. cyanipes* feeding *A. donax stem*

Foto 6. *N.cynanipe*

Photo: 7: Nymphs of *L. bambusae*

2.-Importation and mass rearing of *Tetramesa romana*

In order to have health plants of *Arundo donax* where insects will be rearing, rhizomes of this plant were collected in Yautepec river and placed in plastic pots (20 cmm x 40 cm). The pots are watered daily and monthly fertilized with urea and osmocote alternation.

In 2009, after obtaining the permit from Mexican Plant Protection Service (SENASICA), 14,593 adults of *T. romana* were imported to Mexican Institute of Water Technology (IMTA) facilities from the USDA/ARS Beneficial Insects Research Unit in Weslaco, Texas. All insects arrived alive. To detect possible pathogen infection, a sample of 100 imported insects and from the first, second and third generations were analyzed according to procedures described by Poinar and Thomas (1984). Fourth generation of *T. romana* was used for mass production.

Plants of one month of growing, were used for wasps rearing. Each plant is placed in a covered shade made with black organza where 10 wasps will be placed for 5 days per pot. After one month, time that it takes the wasp to complete its cycle, the stems are cut and placed in acrylic boxes designed for the recovery of the wasps. Cages were checked daily and new wasp was collected in vials. The number of new *T. romana* adults emerged was recorded.

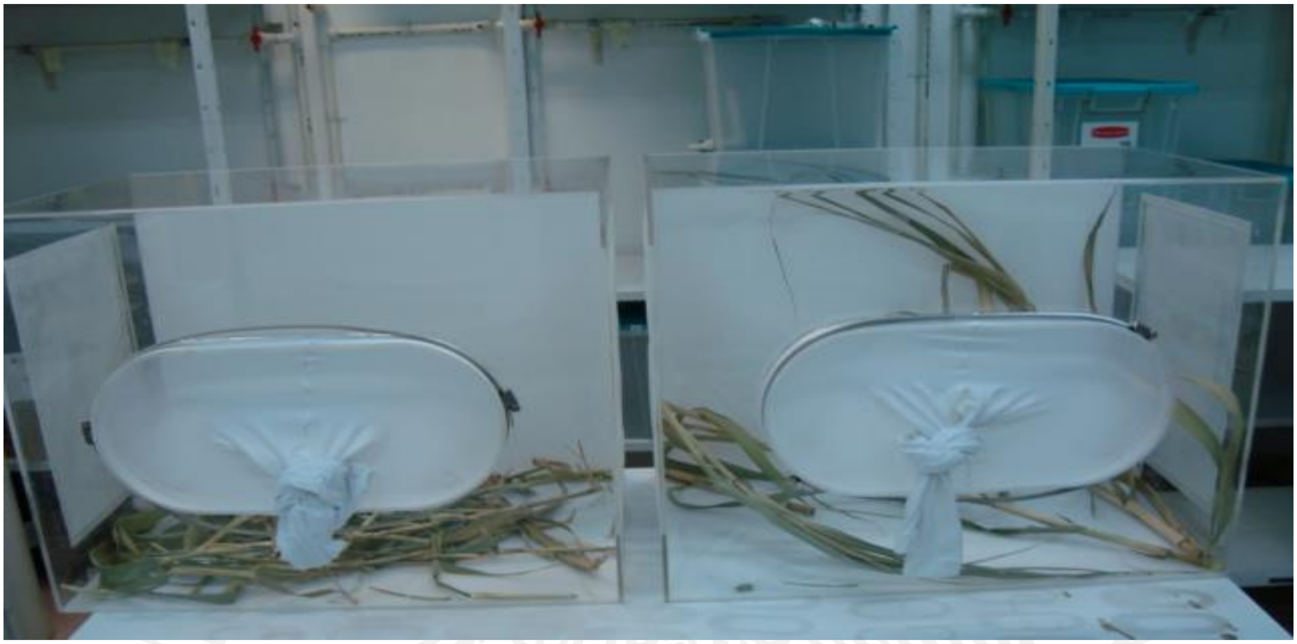


Photo 8: Acrylic boxes designed for the recovery of the wasps

2.1 Evaluation of the harvester time of new *T. romana* insects

In order to evaluate the better moment to harvester the insects, once *A. donax* stems were inoculated with *T. romana* eggs, a series of dissection of stems were made a different time during *T. romana* cycle. Results show that in a sample of 300 stems revised, a 2% presented immature larvae and 23% presented wasps that not could emerge. Therefore, we assume that it is important to let more than one month after the

inspection of the stems.

Photo 8: Dissection of *A. donax* stems

Photo 9: Diferents stages of T. romana

3.-Importation and mass rearing of *Rhizaspidioutus donacis*

In 2010, after obtaining the permit from Mexican Plant Protection Service (SENASICA), *R. donacis* scales were imported to Mexican Institute of Water Technology (IMTA) facilities from the USDA/ARS Beneficial Insects Research Unit in Weslaco, Texas. *R. donaci* was cultivated by using rhizomes of *A. donax*. The rhizomes were placed on substrate BM2 mix consisting of vermiculite, perlite and peat moss. Once the rhizomes generated a green and succulent stem, with the help of a gelatin capsule, approximately 1,600 nymphs of *R. donacis* were placed in *A. donax* stem. After 6 months, a new colony of *R. donacis* was collected and release in different places in Morelos, México.

Photo 10: Colection of *R. donacis* in USDA laboratory

Photo 11: Packaging of R. donacis to be send to Mexico

Photo 12: *R. donacis* in rizomes of *A. donax*

Photo 13: Rearing of *R. donacis*

4.-Release of Insects

Before the release of insects in the frontier zone between Mexico and the United States, a meeting for information of the scopes of the project was allocated in Nuevo Laredo, Tamaulipas at the office of Mexican Section of the International Boundary and Water Commission (IBWC). Staff of Mexican and US section of the IBWC was present and representatives of Mexican and US government.

Photo 14: Meeting in Nuevo Laredo at IBWC

Photo 15**:** Representatives of Mexican and US government.

4.1.- Release, Establishment and Dispersion of *T. romana* in México

Using methods developed by Goolsby et al. (2014), release of *T. romana* and *R. donacis* were made in México along the U.S. border with Mexico in Nuevo Laredo, and Reynosa, Tamaulipas and in the state of Morelos in central Mexico State. *T. romana* was transported in small boxes designed by USDA staff. *R. donacis* was release by using micro plants of *Arundo* were scale was growth previously.

In a survey made in February 2009 in Nuevo Laredo, Tamaulipas, adventive populations of the *Arundo* wasp w observed at this place but not in Morelos. Table. 1 shows release location in Morelos and Nuevo Laredo, Tamaulipas in México.

One square meter of each site were *T. romana* was released, was surveyed to determine the presence or absence of *T. romana*. Stems and lateral side shoots were visually examined for the presence of adult exit holes. If exit holes were found, the numbers of exit holes were counted in all square meter. Insect dispersion was evaluated by using dispersal score proposed by Van Thielen *et al,* 1994.

Photo 16: *T. romana* release at Yautepec, Mor.

Photo 17: T. romana release at Jojutla, Mor.

Photo 18: *T. romana* boxes for release in the Rio Grande

Photo 19: Transportation of *T. romana* to Rio Grande

Photo 20: Micro plants of *A. donax*

Photo 21: Microplants with R. donacis release in the Rio Grande

Photo 22: Microplants with *R. donacis* ready to be release in the Rio Grande

Photo 23: Release of *R. donacis* at place in Nuevo Laredo, Tamaulipas, Mexico

Photo 24: Infestation of Arundo donax in Nuevo Laredo, Tamaulipas, México

Photo 25: Staff of Mexican IBWC transporting *R. donacis* to the Rio Grande near Retamal dam

Photo 26: Retamal dam

Photo 27: release of R. donacis near presa Retamal

Photo 28: Release of R. donacis in Reynosa, Tamaulipas

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Table 2. Release of *Tetramesa romana* in Mexico

Release Location	Date / # wasp released	Temperature / HR / Rainfall	Altitude / Climate
Morelos: ECACIV (1)N 18° 51.9' 4.0" W 99° 10.3' 18" (2)N 18° 51' 59.0" W 99° 10' 16.4"	From May 2010 to November 2015 T=22,044	24ºC-32ºC 52-75% 6.8-60.7mm	1,335 m / Subtropical
Morelos: Alpuyeca N 18 ◦ 44.1':47'' W 99◦ 15.4':93'' N 18° 44' 7.5" W 99° 15' 27.6" N 18° 44' 11.0" W99° 15' 30.9"	From May 2010 to November 2015 T=15,028 T=10,225 T=12,348	24ºC-32ºC 52-75% 6.8-60.7mm	1,034 m / Subtropical
Morelos: Puente de Ixtla N 18° 38' 120" W 99° 19' 0.648"	From May 2010 to November 2015 T=19,380	24ºC-32ºC 52-75% 6.8-60.7mm	898 m Subtropical
Nuevo Laredo, Tamaulipas N 27°27´00.8" W 99°29´42.4" N 27°27'00.4" N W 99°29'42.2" W N 27°27´00.4" N W 99°29´42.6" W	March, May & June 2010 T=8,600 April & October 2015 T=10,000	-5 to 46ºC/24.1/0mm	114 m/ Dry steppe

Wasp Dispersal Score (WDS): 0=no wasp holes; 1=wasp holes only at the release site; 2= wasp holes 100 m from the release site; 3=wasp holes 500 m from the release site; 4 wasp holes beyond 500m

Table 3: Establishment and Dispersion of *Tetramesa romana* in México

Release Location	#exit holes/m2	WDS after 6 months	WDS after 1 year
Morelos: ECACIV (1)N 18° 51.9' 4.0" W 99° 10.3' 18" (2)N 18° 51' 59.0" W 99° 10' 16.4"	**6 months: 26** **1 year: 65**	**WDS= 3**	**WDS= 4**
Morelos: Alpuyeca (1)N 18 ◦ 44.1':47'' W 99◦ 15.4':93'' (2)N 18° 44' 7.5" W 99° 15' 27.6" (3)N 18° 44' 11.0" W99° 15' 30.9"	**6 months: 19** **1 year:74**	**WDS= 3**	**WDS= 4**
Morelos: Puente de Ixtla (1)N 18° 38' 120" W 99° 19' 0.648"	**6 months: 31** **1 year: 70**	**WDS= 3**	**WDS= 4**
Nuevo Laredo, Tamaulipas (1)N 27°27´00.8" W 99°29´42.4" (2)N 27°27'00.4" N W 99°29'42.2" W N 27°27´00.4" N W 99°29´42.6" W	**6 monts: 34** **1 year: 88**	**WDS= 4**	**WDS= 4**

Wasp Dispersal Score (WDS): 0=no wasp holes; 1=wasp holes only at the release site; 2= wasp holes 100 m from the release site; 3=wasp holes 500 m from the release site; 4 wasp holes beyond 500m

5.-Evaluation of growth and reproduction of different genotypes of *A. donax*

Since *A. donax* donax is extremely invasive and damaging, affecting especially water supplies, one of the essential tasks for effective control and management of *Arundo* is to know the growth and reproduction rate of different *A. donax* biotypes at different climates and soil conditions. The aim of this work is o evaluate the speed of spread and growth of 4 biotypes in different localities (Austin and Mac Allen (Texas, USA) and Morelos (Mexico) in order to determine the best phenological state of the plant as well as the most effective control agent.

For this evaluation, rhizomes of *A. donax* from Austin, Rio Grande Valley, Balmorhea, Texas, and Jiutepec in Morelos were planted in three different locations: the University of Austin, Texas University of Weslaco and IMTA facilities. A linear meter of rhizome of each locality was planted in a random block. Each meter was up to approximately 4 rhizomes. Three replicates of each locality were made. IMTA soil analysis revealed that it is a Vertisol composed of high compressibility and high plasticity (expansive soils). The 1st layer has a vegetative cover of about 20 cm. The 2nd layer is clay and has about 2 m deep. The 3rd layer is of vesicular basalt rock.

Photo 29: Beginning of the bioassay in México
IMTA

Photo 30: 1 m of rhizomes planted at

Photo 31: Evaluation of biomass of *Arundo*

Photo32. Evaluation of density of *Arundo*

5.1.- Results

After 1 year of cultivation, the genotype Morelos grows 277.5cm, while that genotype Austin grows 146 cm (Figure 1). The largest number of shoots produced after 1 year of cultivation, correspond to the RGV genotype (1,300), followed by Morelos (1,150), while genotype Austin, presented the lowest spread (300) [Figure 2]. The maximum values of fresh and dry weight correspond to the Morelos genotype, while Austin presented the lowest value. It is important to mention that during the year of the bioassay, not flowering was observed in any replica of the 4 genotypes of *A. donax.*

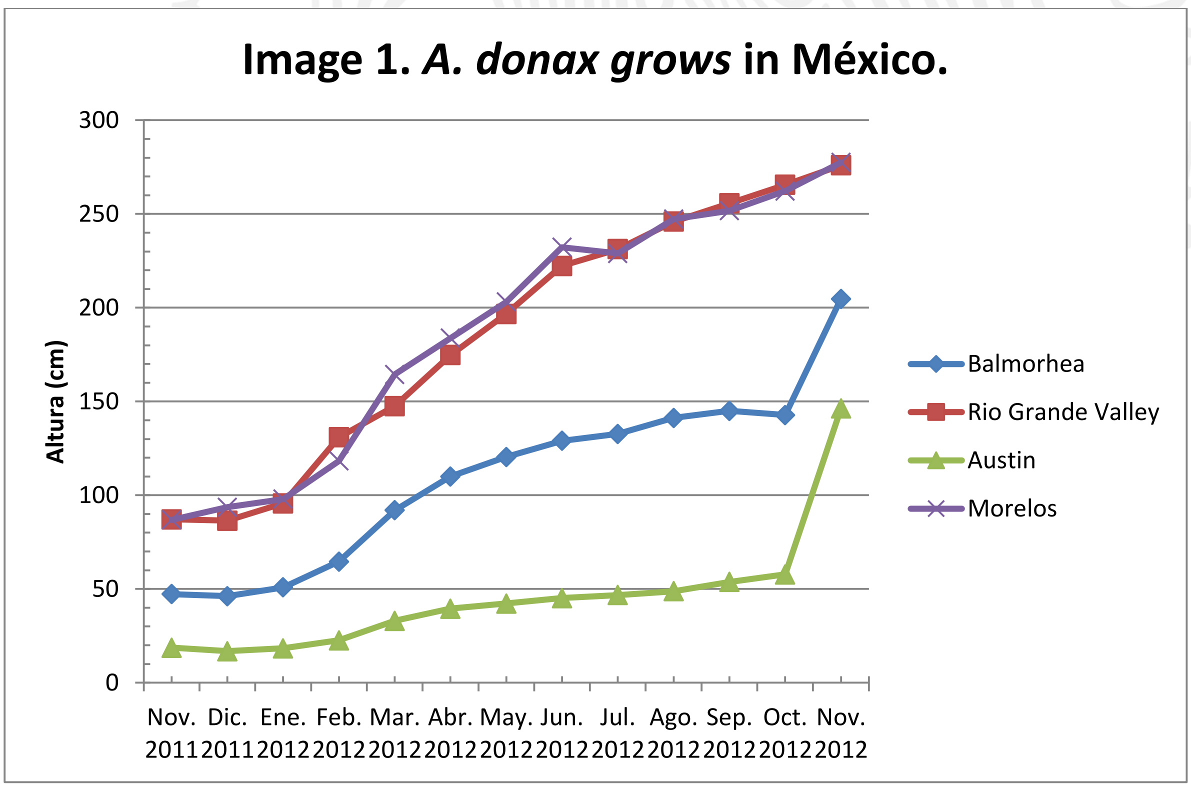


Image 1. *A. donax grows* in México.

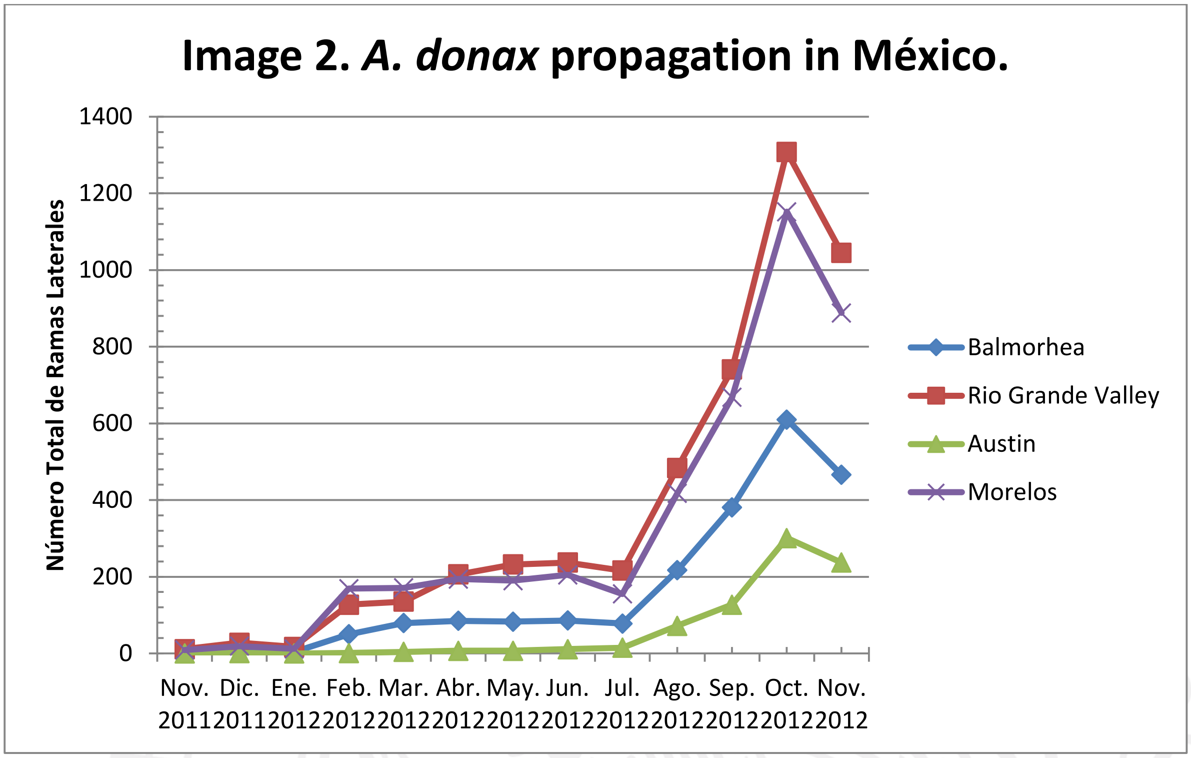
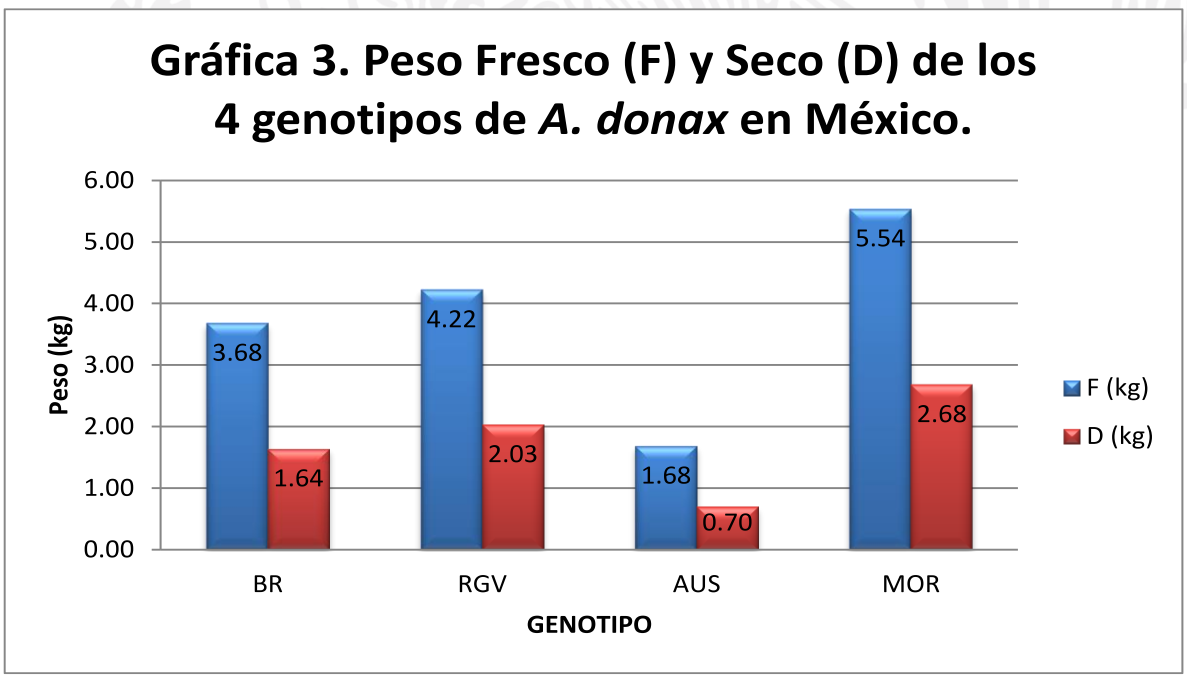


Image 2. A. donax propagation in México.
Número Total de Ramas Laterales
1400
1200
1000
800
600
400
200
0
Nov. 2011
Dic. 2011
Ene. 2012
Feb. 2012
Mar. 2012
Abr. 2012
May. 2012
Jun. 2012
Jul. 2012
Ago. 2012
Sep. 2012
Oct. 2012
Nov. 2012
Balmorhea
Rio Grande Valley
Austin
Morelos


Gráfica 3. Peso Fresco (F) y Seco (D) de los 4 genotipos de A. donax en México.
Peso (kg)
6.00
5.00
4.00
3.00
2.00
1.00
0.00
3.68
1.64
4.22
2.03
1.68
0.70
5.54
2.68
BR
RGV
AUS
MOR
GENOTIPO
F (kg)
D (kg)

From March, we observed a continuous increase of growth in the 4 genotypes, Morelos RGV genotypes accentuate its growth remarkably compares to Austin and Balmorhea. The same trend is observed in the spread of Morelos and RGV which increase at the time of the arrival of the rains. According to the results obtained, it can be concluded that actions of control of the 4 genotypes should start to later in March, since if it is postponed when the plant generates greater biomass, resources to be used will be greater and probably with little success due to the speed of propagation of this plant.

6.- CONCLUTIONS

An invasive plant is one that is introduced and successfully reproduces resulting in the establishment of a population that spreads and threatens ecosystems, habitats or species with economic or environmental harm. This is the case of *Arundo donax* one of the most important exotic and invasive weed in America affecting especially water supplies. *A. donax* is now a widespread and invasive weed in the Rio Grande Basin and in almost all the basins in Mexico. According to a survey made in Mexico (Martínez, 2018) *A. donax* is the second specie that affects the water availability, the first is water hyacinth. Conventional chemical and mechanical methods to control *A.donax* infestation are often expensive, require repeated applications and poses serious harm to health and the environment . The use of Biological Agents, usually insects or plant pathogens, have been developed that provide highly effective and environmentally control of exotic and invasive plants, through the introduction of highly host specific natural enemies that regulate the abundance of these weeds in their native areas (Coulson et al. 2000, Spafford Jacob and Briese, 2003).

For biological control of *A. donax*, the United State Department of Agriculture evaluated two specific insects, stem galler *Tetramesa romana* Walker (Hymenoptera: Eurytomidae), and rhizome-stem feeder *Rhizaspidiotus donacis* (Leonardi) (Homoptera: Diaspididae). After obtained the agreement for field release from both USA and Mexican authorities, mass rearing system was stablished at Mexican Institute of Water technology in Jiutepec, Morelos. Insects were obtained from USDA-APHIS, Mission Biological Control Containment Facility (MBCL) Moore Airbase Edinburg, TX, USA.

T. romana was released at 6 sites in Morelos and 3 in Nuevo Laredo (Table 2). In both sites, *T. romana* was well established. In Morelos, after 6 months, *T. romana* was dispersed 500 m from the release site and 1 year after, exits hole were observed more than 500m from the release site (Table 3). In Nuevo Laredo, after 6 months, *T. romana* holes were found beyond 500 m from the release site (Table3); probably populations released as part of the biological control program, were mixed with the original adventive population that was discovered in California and in Texas (Goolsby, 2009). Establishment and dispersion of *T. romana* was made in two different ecosystems: from subtropical (with 6 months of rainy season), to dry steppe, which suggests that *T. romana* may be tolerant of a wide range of climates. In addition, *R. donacis* was release in Nuevo Laredo, Reynosa Tamaulipas and Morelos. Due to the fact that his biological cycle is very long (6 months), it was difficult to determine his complete establishment in Morelos.

In summary, two biological control of *A. donax* were introduced and established in México. A substantial increase in insect population was observed. Dispersion of *T. romana* was observed in all sites were the wasp was released thereby providing control of other plants. Clonal population *of Arundo donax* expand rapidly by rhizomes grow and production of new shots. Candidates of biological control of this weed, have to have the potential for reducing rhizomes and shot production. In this respect, the stem-galling wasp *T. romana* has the ability to increase shoot mortality as result of wasps feed on stems as well as young and mature Arundo foliage (Goolsby, et al 2016). In Mexico, establishment and dispersion of *T. romana* was made in two different ecosystems: from subtropical (with 6 months of rainy season), to dry steppe, which suggests that *T. romana* may be tolerant of a wide range of climates. According to our results, after 6 month of release, *T. romana* was dispersing from the release sites, what demonstrates that a new generation took place in a new are. *T. romana* is now well established and widespread in Mexico and should be release in other parts in the country. Combinations of different biotic agents could enhance efficacy of *Arundo* control over that observed with either agent alone.

Collaboration between The United States Department of Agriculture, Agricultural Research Service (USDA-ARS) and the Instituto Mexicano de Tecnología del Agua (IMTA) represents an important step forward in terms of finding a new way to control weeds with less impact on the environment and is mutually beneficial to both countries. We look forward to continuing this collaboration.

ACKNOWLEDGMENTS

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