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Biotic and abiotic factors influencing infestation levels of the arundo leafminer, *Lasioptera donacis*, in its native range in Mediterranean Europe

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ABSTRACT

Lasioptera donacis is a biological control agent of *Arundo donax*, which is an invasive weed in the riparian habitats of the Rio Grande Basin of Texas and Northern Mexico. Field research was conducted in the native range of *L. donacis* in Mediterranean Europe to evaluate the biotic and abiotic factors that influence its local infestation levels. *Lasioptera donacis* feeding damage was documented on 40.4 and 67.8 % of dead and decaying leaf sheaths respectively across all sites. *Lasioptera donacis* was active in all locations including highly disturbed sites, but showed a slight preference for sites near running freshwater sources and lower infestation levels adjacent to salt water sources. The environmental preferences of *L. donacis* in Europe are similar to conditions in the Rio Grande Basin and Southwestern U.S. where *A. donax* is invasive.

Additional index words: biological control of weeds, native range studies, carrizo cane

Arundo donax L., also known as arundo, giant reed or carrizo cane, is native to the Old World from the Iberian Peninsula, across Mediterranean 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 (Bell 1997; Perdue 1958). Subsequently, it has naturalized and become invasive in many tropical, subtropical, and warm-temperate regions of the world. Genetic studies of *A. donax* indicate it was introduced into the Rio Grande Basin of Texas and Northern Mexico from Mediterranean Spain (Tarin et al. 2013). It is found throughout the southern half of the United

States from Maryland to California, but it is most invasive in the riparian habitats of the southwestern United States and northern Mexico (Di Tomaso and Healy 2003, Contreras 2007, Martinez-Jimenez et al. 2017). In the Rio Grande Basin, *A. donax* has historically dominated these habitats where it competes for scarce water resources (McGaugh et al 2006, Yang et al. 2009, Yang et al. 2011, Watts and Moore 2011, Moore et al. 2016), reduces riparian biodiversity (Racelis et al. 2012a, Rubio et al. 2014), reduces access for law enforcement (Goolsby et al. 2017a), and facilitates the invasion of cattle fever ticks (*Rhipicephalus* (= *Boophilus*) *microplus* and *Rhipicephalus* (*B.*) *annulatus*) from Mexico (Esteve-Gassent et al. 2014; Racelis et al. 2012b).

Biological control of *A. donax* with insects may be the best long-term option for managing this highly invasive weed (Tracy and DeLoach 1999). Two agents, the stem-galling wasp, *Tetramesa romana* Walker (Hymenoptera: Eurytomidae) (Goolsby and Moran 2009, Moran and Goolsby 2009) and the rhizome-feeding armored scale, *Rhizaspidiotus donacis* (Leonardi) (Homoptera: Diaspididae) (Goolsby et al. 2009; Moran and Goolsby 2010), both matched climatically and genetically to the source region in Mediterranean Spain of the dominant invasive *A. donax* genotype in the Rio Grande Basin, have been released and are having significant impacts in Texas and Mexico (Racelis et al. 2010; Goolsby et al 2014; Goolsby et al. 2017a; Martinez-Jimenez et al. 2017; Moran et al. 2017).

Lasioptera donacis Coutin (Diptera: Cecidomyiidae), the arundo leaf miner, is the third agent that has been evaluated and permitted for release in North America (Goolsby et al. 2017b). Female *L. donacis* collect spores of the cosmopolitan saprophytic fungus, *Arthrinium arundinis* (Ascomycota: Apiosporaceae), and deposit them during the oviposition process. The arundo leaf miner larvae feed and develop in the decaying tissue of the leaf sheath channels of *A. donax* (Thomas and Goolsby 2015). Damage to leaf sheaths ultimately leads to defoliation of the entire leaf. Defoliation increases light penetration through the canopy, which may accelerate the recovery of the native riparian plant community along the Rio Grande. In addition, decreased shade and humidity will make the environment less suitable for survival of cattle fever ticks, and increase within-stand visibility which improves safety and effectiveness of law enforcement personnel and cattle fever tick inspectors working along the international border in TX. For these reasons, the arundo leafminer, *L. donacis* is prioritized for release in North America.

This study investigates the biotic and abiotic factors that influence the infestation levels of *L. donacis* in its

native range in Mediterranean Europe and investigates the impact these factors may have in the Rio Grande Basin where it is being reared and released as a biological control agent of *A. donax*. The predictor variables for this study, stem height, circumference, and biomass and proximity to environmental variables such as salt water, fresh water, and human activity, were chosen due to their importance in the establishment of the *R. donacis* and *T. romana* biological control agents (Goolsby et al. 2013) and the Dipteran propensity for favoring infestation sites based on proximity to such variables (Yeates and Wiegmann 2005).

MATERIALS AND METHODS

Square Meter Samples of *A. donax*. Field studies were conducted from September to December 2017, in the native range of *L. donacis* in Greece where the insect is common and landscape provides sufficient geographical variation to evaluate the effects of multiple biotic and abiotic factors that influences its density and abundance. Samples of *A. donax* from sites near Chania, Crete, Greece (Χανιά, Κρήτη, Ελλάδα) were chosen based on three criteria: proximity to fresh water, salt water, and to regular human activity. Square meter samples of *A. donax* were selected at random along a transect and delineated using a measuring tape. All stems within the square meter were cut at the base and analyzed node by node for *L. donacis* damage. The square meter locations were chosen based on their ability to fit into the three previously mentioned criteria. Six to nine samples were taken from each site. Three samples were collected from transects nearest to salt water, fresh water, or human disturbance (Table 1); three at an outer part of the *A. donax* patch and three midway through the patch. A total of forty-five square meter samples were taken over six different sites. Data collected from the harvested *A. donax* includes stem height and circumference, total number of nodes on the stem, and number of nodes with *L. donacis* damage. Distance parameters were measured by using GPS coordinates of the square meter sampling locations in Google Earth and using the program's ruler function to establish distance to the variable in question, with the exception of human activity sites which were classified as either directly adjacent to or removed from the source of human activity. Damage to *A. donax* at human activity sites included damage to stems from cutting/abrasion, increased exposure to wind/sun, loss of leaf litter, and factors associated with construction sites and roads. Additional information on stem samples included total biomass, which was calculated using the technique established by Spencer et al. (2006): $Biomass (gDW/m^2) = Main$

Table 1. GPS coordinates of square meter sample collection sites and distances and/or proximity to environmental variables.

Table 1: GPS coordinates of square meter sample collection sites and distances and/or proximity to environmental variables.								
Site #	Sample #	Site Name	Sample Name	Latitude	Longitude	Fresh Water Distance (m)	Salt Water Distance (m)	Proximal to Human Activity
1	1	Creek	Outer 1	35°27'7.09"N	23°55'14.92"E	6.54	–	–
	2		Outer 2	35°27'7.30"N	23°55'15.38"E	19.98	–	–
	3		Near 1	35°27'13.34"N	23°55'12.98"E	1.53	–	–
	4		Near 2	35°27'13.06"N	23°55'12.94"E	2.2	–	–
	5		Near 3	35°27'14.70"N	23°55'13.67"E	2.71	–	–
	6		Outer 3	35°27'14.60"N	23°55'14.39"E	20.7	–	–
2	1	Sea	Near 1	35°31'18.31"N	23°52'45.38"E	–	42.2	–
	2		Near 2	35°31'21.97"N	23°52'34.84"E	–	20.8	–
	3		Near 3	35°31'21.40"N	23°52'36.34"E	–	40.6	–
	4		Mid 1	35°31'19.21"N	23°52'34.59"E	–	108	–
	5		Outer 1	35°31'10.68"N	23°52'31.12"E	–	320	–
	6		Mid 2	35°31'15.55"N	23°52'31.25"E	–	187	–
	7		Outer 2	35°31'11.78"N	23°52'29.96"E	–	282	–
	8		Mid 3	35°31'15.80"N	23°52'25.17"E	–	143	–
	9		Outer 3	35°31'11.82"N	23°52'20.05"E	–	275	–
3	1	Street	Adjacent 1	35°28'27.12"N	23°56'26.19"E	–	–	Yes
	2		Adjacent 2	35°28'27.07"N	23°56'26.82"E	–	–	Yes
	3		Adjacent 3	35°28'26.89"N	23°56'27.96"E	–	–	Yes
	4		Removed 1	35°28'26.14"N	23°56'26.12"E	–	–	No
	5		Removed 2	35°28'24.85"N	23°56'25.35"E	–	–	No
	6		Removed 3	35°28'23.44"N	23°56'25.85"E	–	–	No
4	1	Construction	Removed 1	35°28'42.41"N	23°56'41.48"E	–	–	No
	2		Removed 2	35°28'44.60"N	23°56'43.81"E	–	–	No
	3		Removed 3	35°28'47.02"N	23°56'46.60"E	–	–	No
	4		Adjacent 1	35°28'47.20"N	23°56'39.80"E	–	–	Yes

Table 1 (continued)

Site #	Sample Collection #	Site Name	Sample Collection Name	Latitude	Longitude	Fresh Water Distance (m)	Salt Water Distance (m)	Proximal to Human Activity
4	5	Construction	Adjacent 2	35°28'43.86"N	23°56'39.72"E	–	–	Yes
	6		Adjacent 3	35°28'45.26"N	23°56'40.39"E	–	–	Yes
5	1	Lake	Mid 1	35°28'28.90"N	23°55'57.34"E	53.2	–	–
	2		Near 1	35°28'29.02"N	23°55'59.41"E	18.9	–	–
	3		Near 2	35°28'29.87"N	23°56'4.24"E	8.63	–	–
	4		Mid 2	35°28'31.24"N	23°56'9.98"E	56.8	–	–
	5		Near 3	35°28'33.76"N	23°56'9.23"E	23.4	–	–
	6		Outer 1	35°28'27.57"N	23°55'50.12"E	51.2	–	–
	7		Outer 2	35°28'28.50"N	23°55'49.76"E	78.5	–	–
	8		Outer 3	35°28'29.03"N	23°55'49.35"E	66.2	–	–
	9		Mid 3	35°28'29.99"N	23°55'51.32"E	24.4	–	–
6	1	Port	Near 1	35°29'53.84"N	24° 3'41.12"E	–	22.9	–
	2		Near 2	35°29'46.90"N	24° 3'39.77"E	–	18	–
	3		Near 3	35°29'42.19"N	24° 3'39.01"E	–	24	–
	4		Mid 1	35°29'34.97"N	24° 3'34.50"E	–	19.9	–
	5		Mid 2	35°29'34.87"N	24° 3'29.65"E	18.3	85.3	–
	6		Mid 3	35°29'34.64"N	24° 3'29.78"E	26.3	81.3	–
	7		Outer 1	35°29'34.11"N	24° 3'25.76"E	11.6	182	–
	8		Outer 2	35°29'33.80"N	24° 3'24.49"E	1.97	215	–
	9		Outer 3	35°29'33.34"N	24° 3'23.68"E	2.84	237	–

$Shoot\ Length^2\ (m) * 14.254$. Statistical analysis was conducted using MiniTab 18 (State College, PA). A CLIMEX model was used to evaluate the degree of similarity between climatic variables in the native range of Mediterranean Europe and the introduced range of south Texas. The Composite Match Index is sensitive to the number of factors included in the anal-

ysis (minimum temperature, maximum temperature, rainfall total and rainfall pattern). The CLIMEX model is used to predict the suitability of the introduced range for the establishment of *L. donacis*.

Dissection of Infested Leaf Sheaths. Eleven samples of five stems were taken from multiple collection sites near Chania and Thessaloniki, Greece. These stems

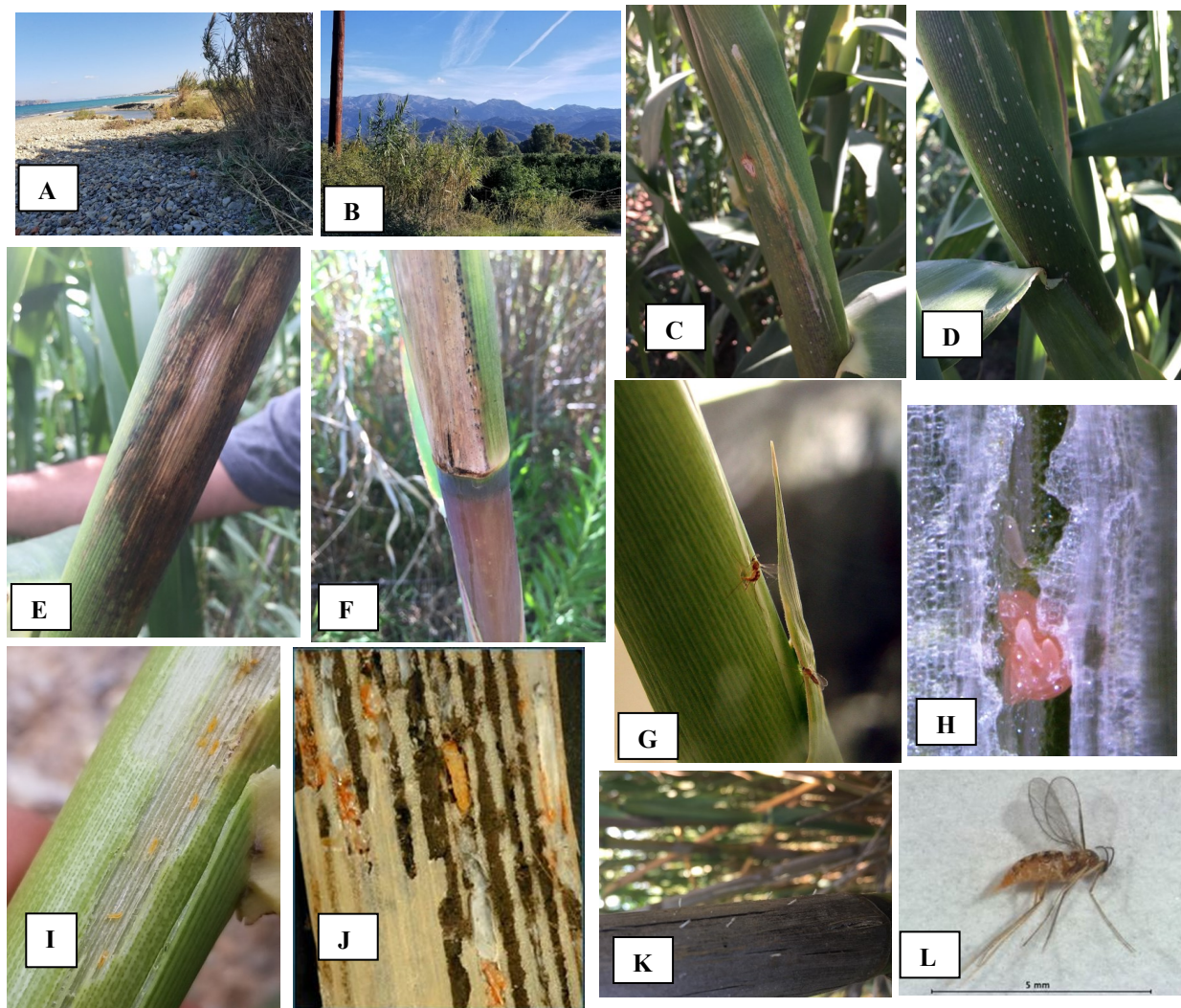


Figure 1. A) *Arundo donax* stands growing adjacent to the Mediterranean Sea in Chania, Crete, Greece. B) *Arundo donax* stands in Crete, Greece with the White Mountains in the background. C) *Cerodontha phragmitophila* damage on *Arundo donax* leaves. D) *Lasioptera donacis* ‘white spots’ created in final larval instar prior to pupation. E) *Arundo donax* leaf sheath partially dead due to *Lasioptera donacis* infestation. F) *Lasioptera donacis* infestation without *Cerodontha phragmitophila* damage. G) Adult *Lasioptera donacis* ovipositing into a leaf sheath. H) *Lasioptera donacis* egg mass in *Arundo donax* leaf sheath channel. I) 2nd instar *Lasioptera donacis* larvae feeding in leaf channels of an *Arundo donax* leaf sheath. J) 3rd instar *Lasioptera donacis* larvae and *Arthrinius arundinis* fungus inside an *Arundo donax* leaf sheath. K) *Lasioptera donacis* exuviae. L) Adult, female *Lasioptera donacis*.

were analyzed through dissection of the leaf sheath on each individual internode in order to gather more specific data on the distribution of and subsequent damage caused by *L. donacis* inside of individual *A. donax* sheaths. Dissection involved cutting off the upper epidermis and outer mesophyll layer of leaf sheath to expose the channels within which *L. donacis* larvae develop. Each node was analyzed for its height respective to the stem base, presence of *A. arundinis* black fungus inside the sheath, visual presence or absence of chlorophyll within the leaf as an indicator of whether the sheath was completely alive, partially dead/dying or dead (Figure 1 E), evidence of *L. donacis* exuviae and/or ‘white spots’ or thin areas of the outer leaf mesophyll created by the last larval instar before pupation (Figure 1 K&D), *Cerodontha phragmitophila* (Hering 1935) exit holes (Figure 1 C), presence of *L. donacis* larvae, and stages of *L. donacis* larval development (Figure 1 I&J). This damage assessment of individual nodes provides insight into the level of association between *C. phragmitophila* damage and *L. donacis* oviposition, as *C. phragmitophila* emergence holes are frequently sites for *L. donacis* oviposition (Coutin & Faivre-Amiot 1981). The damage assessments were also conducted to determine the time interval for defoliation of a leaf sheath and stem.

RESULTS

Square Meter Samples. Analysis of infestation levels in square meter samples taken from sites proximal to salt water sources showed a slight increase in *L. donacis* infestation rates at sites more distant from the salt water source ($R = 0.463$, $p = 0.001$)(Table 2). There was weak but significant correlation between

Table 2. Correlation of mean percent infestation of *Lasioptera donacis* to environmental or stem quality predictor variables.

Predictor Variables	Pearson correlation coefficient (R)	p values
Distance from Salt Water	0.463	0.001
Distance from Freshwater	-0.174	0.001
Distance from Running Freshwater	-0.514	0.001
Stem Height	-0.042	0.314
Stem Circumference	0.253	0
Stem Biomass	-0.02	0.632

fresh water proximity and *L. donacis* infestation rates ($R = -0.174$, $p = 0.001$). However comparison of data from only running freshwater sources showed a slightly higher correlation with *L. donacis* damage ($R = -0.514$, $p = 0.001$).

Histogram of Infested Leaf Sheath Heights

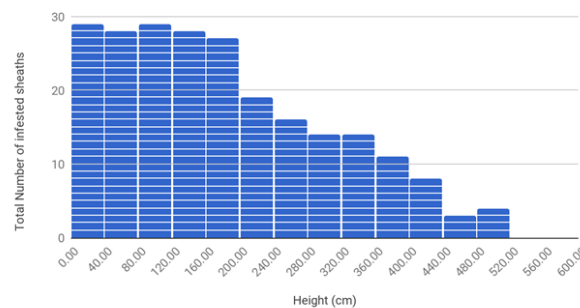


Figure 2. Collective number of *Lasioptera donacis* infested leaf sheaths on individually dissected stems relative to leaf sheath height from stem base.

Infestation levels were not strongly correlated with above ground biomass or height of stems. These variables had particularly low correlation with infestation rates, producing -0.020 and -0.042 R values respectively (Table 2). Stem circumference had a slight correlation with higher infestation levels on wider stems ($0.253=R$), though this is suspected to be due to age of stems; thinner stems were typically younger and did

Table 3. Association of *Lasioptera donacis* with leaf sheath mortality in Greece.

	Live sheaths	Partially dead/dying sheaths	Dead sheaths	Total sheaths
With <i>L. donacis</i>	9.6% (115)*	67.8% (122)	40.4% (155)	22.3% (392)
Without <i>L. donacis</i>	90.4% (1080)	32.2% (58)	59.6% (228)	77.7% (1366)

not yet have pre-formed perforations. Samples taken in stands with human activity had slightly lower population levels of *L. donacis* than those taken away from the activity with averages of 15.4 and 19.4% infestation rates respectively. At sites where cane stands had been bulldozed, regrown and sampled new green stems, the average percent infestation was 5.7%.

Dissection of Infested Leaf Sheaths. Evaluation of *L. donacis* infestations within individual stems revealed

Table 4. Stages of *Lasioptera donacis* development compared to the state of associated infested leaf sheaths.

Stage of <i>L. donacis</i> development	Live	Partially Dead/Dying	Dead
3 rd Instar and White Spots	27.00%	37.20%	35.80%
White Spots and Exuviae	3.50%	30.20%	66.30%
Exuviae Only	11.10%	0%	88.90%

an oviposition height preference for nodes below two meters with a slight decrease in percent infested nodes on the upper part of the stems (Figure 2). *Lasioptera donacis* damage was associated with 40.4% of all completely dead leaf sheaths and 67.8% of all partially dead/dying leaf sheaths as determined for the 1758 leaf sheaths dissected (Table 3). In 92.8% of all *L. donacis* infestations, a *C. phragmitophila* exit hole was found on the same leaf sheath. In total from all locations, 22.3% of all inspected leaf sheaths were either infested or had been previously infested by *L. donacis*.

The development of *L. donacis* from eggs to adults lasts twenty-five days and three instars under optimal conditions. The end of the third instar stage, before pupation, includes the formation of an “escape hatch” near which the 3rd instar larva will pupate and use to emerge as an adult (Thomas and Goolsby 2015). These escape hatches appear as white spots on the outer surface of the leaf sheath mesophyll (Figure 1 D) and occur most often between twenty to twenty-five days after oviposition. This stage, represented by line 1 in Table 4, is associated with rates of 35.8% dead leaf sheaths and 37.2% partially dead/dying leaf sheaths. After the formation of white spots, pupal exuviae appear extruded on the infested leaf sheath as adult *L. donacis* emerge (Figure 1 K). This stage most often occurs between twenty-five to thirty days after oviposition, at which point the incidence of dead and partially dead/dying leaf sheaths transitions to 66.3 and 30.2% respectively. After thirty days, under most circumstances all *L. donacis* will have emerged leaving only exuviae. On leaf sheaths with complete emergence of all *L. donacis*, a rate of 88.9% dead and 0% partially dead/dying leaf sheaths was observed.

DISCUSSION

Square Meter Samples. Infestations of *L. donacis* across sites illustrate their ability to oviposit and develop on *A. donax* under a range of environmental conditions (Figure 1 A&B). The percentage of infested

leaf sheaths may vary, based on environmental differences, but no observed variables resulted in a zero or very low percent infestation levels. However, in the south of France some small patches of *A. donax* that were very exposed to sun and wind showed no sign of *L. donacis* (A. Vacek, personal observation). *Arundo donax* is a highly successful invader in the riparian zone of the Rio Grande Basin in south Texas and expands into areas of neighboring farmland, drainage and irrigation canals. The favorability of *L. donacis* near locations with running freshwater sources in Chania ($R = -0.514$) is similar to the most common environments impacted by *A. donax* in the Rio Grande Basin. Additionally, evidence of a 15.4% success rate when exposed to sites of regular human activity suggests *L. donacis* can be successfully established into sites with common human disturbance activities in the riparian zone such as mowing, topping, road construction or farming activities. The 5.7% infestation rate on new stem regrowth after bulldozing further demonstrates the ability of *L. donacis* to re-infest stems subjected to heavy disturbance. This suggests that *A. donax* patches can be re-infested even after the destruction of mature infested stems. Therefore, *L. donacis* should be able to infest and damage *A. donax* in both disturbed and undisturbed sites where it establishes as a biological control agent.

The declining success rate of *L. donacis* in locations near salt water sources is no significant issue to the implementation of *L. donacis* as a biological control agent as *A. donax* is mostly found near freshwater sites in the United States. It is however useful to know that salt water sites are not the preferred environment for *L. donacis* establishment as this is a common environment in Mediterranean Europe and does not correspond to infested habitats in the U.S. The decline in success of infestations near salt water sources is suspected for two reasons; 1) salt water spray may act as a natural fungicide inhibiting *A. arundinis* that *L. donacis* require for reproduction and growth, and 2) exposure to coastal winds that prevent *L. donacis*, which appear to be poor fliers, from finding suitable oviposition sites in these areas.

The lack of preference by *L. donacis* for height of stems or above ground biomass of an *A. donax* stand indicates that *L. donacis* can infest most sheaths and cause significant defoliation to all but the earliest life stages of the plant; the earliest life stages being less capable of infestation due to the slight correlation between higher infestation rates on stems with larger circumference ($R=-0.253$, $p=0.001$) (Table 2), which suggests a time lag between new plant production and pre-formed leaf sheath perforation. The potential efficacy of *L. donacis* as a defoliator in the Rio Grande Basin does not appear to be significantly limited by

age or quality parameters of the stem but rather to location making all stems with green sheaths vulnerable to leafminer attack and subsequent defoliation.

In addition to a match in general environmental characteristics, the CLIMEX match climates models were used to compare Brownsville, TX in the Rio Grande Basin Europe and conversely Chania, Crete, Greece, the location of our native studies, to North America (Figure 3). In both cases, CLIMEX calculated

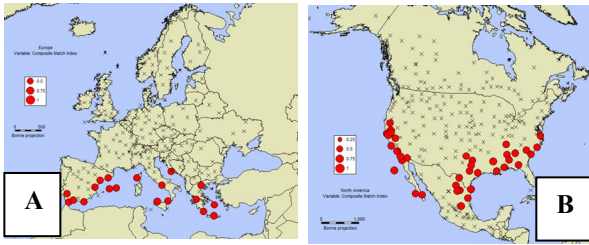


Figure 3. CLIMEX model of predicted distribution of *Lasioptera donacis* comparing A) Brownsville, TX to Europe and B) Chania, Crete, Greece to North America.

a 0.6 climatic match between locations. An index calculation of 0.6 represents a moderately good climatic match (Devorshak 2012). The most significant differences in the climates were warmer maximum summer temperatures in the Rio Grande Basin, and colder minimum winter temperatures in Greece. Rainfall amounts, rainfall patterns, relative humidity, and soil moisture were very similar. In general, the climates in the native and introduced ranges are similar and should not be a limiting factor in the establishment of *L. donacis* in the Rio Grande Basin and across the southern U.S. and Mexico.

Dissection of Infested Leaf Sheaths. The 40.4% association between the presence of *L. donacis* and dead *A. donax* leaves and 67.8% association with dying leaves shows the impact of *L. donacis* on growing stems (Table 3). Most dying leaves were associated with 3rd instar and pupal development while the dead leaves were more closely associated with exit holes and white spots. This suggests the death of an infested leaf sheath corresponds with the developmental growth of *L. donacis* larvae taking roughly 25-30 days under laboratory conditions (27°C and 80 RH) to effectively kill infested leaves. In the introduced range of *A. donax* in the Rio Grande Basin, uninfested leaves may live for up to two years and continue to provide photosynthate, which likely increases the competitiveness of the plant. Leaves also decrease light penetration through the plant canopy, which inhibits growth of native vegetation. Reducing the longevity of leaves by *L. donacis* attack should significantly increase light penetration and accelerate recovery of native riparian vegetation.

Since these data were collected in the fall in Greece, they reflect the cumulative effect of *L. donacis* over a growing season as well as the effects of natural senescence and other processes. Additionally, ambient temperature is suspected to be an important factor in the development time of *L. donacis* larvae. Since this study was carried out in the fall and not in the summer months where peak *L. donacis* activity occurs, it is possible that the 25-30 day estimate for leaf necrosis is skewed due to the dates of study.

With regards to the association of *C. phragmitophila* infestations as *L. donacis* oviposition sites, we found a 92.8% incidence of *C. phragmitophila* association with *L. donacis*. This indicates that *C. phragmitophila* exit holes provide common oviposition sites for *L. donacis* in their home environment. However, the instances wherein *L. donacis* chose an alternate oviposition site demonstrate their ability to reproduce without the presence of *C. phragmitophila* exit holes. Alternate sources of leaf sheath rupture, such as abrasion damage and side shoot growth, were shown to provide equally satisfactory oviposition sites in the remaining 7.2% of infestations (Figure 1F). It is possible that the relationship between *C. phragmitophila* and *L. donacis* oviposition exists purely out of convenience (commensalism) as these are the most common sources of leaf sheath rupture in *A. donax*. This claim can be further supported by the prevalence of *C. phragmitophila* versus *L. donacis* damage on the inspected sheaths. While 22.3% of all inspected leaf sheaths were impacted by *L. donacis* and 43.3% of all sheaths contained *C. phragmitophila* exit holes. Evidence of *L. donacis* oviposition sites without the aid of *C. phragmitophila* is significant as *C. phragmitophila* is not native to the United States and as such cannot provide the oviposition sites for the introduced biological control agents. Rupture at the base of the leaf sheath by the emerging side shoot is extremely common and should provide abundant oviposition sites on *A. donax* in North America. *Lasioptera donacis* infestations as a result of oviposition into tears caused by side shoots have been observed in quarantine studies in Texas.

In summary, studies in the native range of *L. donacis* indicate that it is adapted to a range of biotic and abiotic environmental conditions that are common in the riparian habitats of the Rio Grande Basin where *A. donax* is invasive. This agent is capable of infesting sites with varying levels of above ground biomass, sites along creeks, lakes and at all distances from these freshwater sources where *A. donax* grows. Infestations are common on *A. donax* stems of all sizes and without infestations of the commensalist leaf miner *C. phragmitophila*. *Lasioptera donacis* infestations were similar in both disturbed and undisturbed stands of *A. donax*, which indicates that it will survive in sites neighboring

farming operations and other human disturbance activities such as mowing, topping and road building that are common in the riparian habitats of Rio Grande Basin. *Lasioptera donacis* has the potential to cause significant defoliation and damage to *A. donax* throughout the Rio Grande Basin. Defoliation of *A. donax* stands by *L. donacis* may further increase light penetration of the canopy, stimulating the growth of native riparian plants, thus accelerating the decline of this invasive weed. Finally, native range studies provide valuable insights into the expected activity of a biological control agent in its introduced range.

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LITERATURE CITED

- Bell, G. P. 1997. Ecology and management of *Arundo donax* and approaches to riparian habitat restoration in southern California. In: Brock, J.H., Wade, R. Ahmad et al. / Aquatic Botany 88 (2008) 113–120 119 M., Pysek, P., Green, D. (Eds.), Plant Invasions: Studies from North America and Europe. Blackhuys Publishers, Leiden, The Netherlands, pp. 103–113.
- Contreras-Arquieta, A. and Cruz-Nieto, M., 2007. Control de la planta invasora *Arundo donax*, en Cuatro Ciénegas, un centro de gran biodiversidad de especies endémicas de flora y fauna de Norteamérica. Informe técnico final de Pronatura Noreste, AC, US Fish & Wildlife Service.
- Coutin, R. and Faivre-Amiot, A. 1981. Interrelations ecologiques et symbiotiques complexes entre insectes et végétaux, *Lasioptera donacis* n. sp. (Diptera: Cecidomyiidae) et *Arundo donax* L. Congres National des Societes Savantes Perpignan Sciences Fasc. II. 195-201.
- Devorshak, C. 2012. Plant pest risk analysis: concepts and applications. Cabi. pp 154.
- DiTomaso, J.M., & Healy, E.A. 2003. Aquatic and riparian weeds of the west (Vol. 3421). UCANR Publications.
- Esteve-Gassent, M. D., Pérez de León, A. A., Romero-Salas, D., Feria-Arroyo, T. P., Patino, R., Castro-Arellano, I., Gordillo-Pérez, G., Auclair, A., Goolsby, J., Rodriguez-Vivas, R.I and Estrada-Franco, J. G. (2014). Pathogenic landscape of transboundary zoonotic diseases in the Mexico–US border along the Rio Grande. *Frontiers in public health*, 2, 177.
- Goolsby, J.A., Gaskin, J. F., Tarin, D.V., Pepper, A.E., Henne, D.C., Auclair, A., Racelis, A.E., Summy, K.R., Moran, P.J., Thomas, D.B., Yang, C., Martínez Jiménez, M., Ciomperlik, M.J., Pérez de León, A.A., & Kirk, A.A. 2014. Establishment and spread of a single parthenogenic genotype of the Mediterranean arundo wasp, *Tetramesa romana*, in the variable climate of Texas. *Southwestern Entomologist*, 39: 675-689.
- Goolsby J.A. and Moran, P.J. 2009. Host range of *Tetramesa romana* Walker (Hymenoptera: Eurytomidae), a potential biological control of giant reed, *Arundo donax* L. in North America. *Biological Control* 49:160-168.
- Goolsby, J.A., Moran, P.J., Falk, J. and Gilbert, L. 2009. Distribution and spread of an adventive population of the biological control agent, *Tetramesa romana* in Austin, Texas. *Southwestern Entomologist* 34: 9.
- Goolsby, J.A., Racelis, A.E., Goolsby, J.B., Kirk, A.A., Cristofaro, M., Grusak, M.A., and Perez de Leon, A. 2013. Evaluation of biogeographical factors in the native range to improve the success of biological control agents in the introduced range. *Biocontrol Science and Technology*. 23:1213-1230
- Goolsby, J., Thomas, D., Perez de Leon, A., Moran, P., Kirk, A., Bon, M.C., Kashefi, J., Desurmont, G., Smith, L., Cristofaro, M., Yang, C., Gaskin, J., Gowda, P., Grusak, M., Ciomperlik, M., Racelis, A.E., Vacek, A., Landivar, J., Pepper, A., Lacewell, R., Rister, E., Martínez Jiménez, M., Marcos, M., Cortés Mendoza, E., Gilbert, L., Plowes, R., Vaughn, T., and Rubio, A. 2017. Update on Biological Control of Carrizo Cane in the Rio Grande Basin of Texas and Mexico. *Earthzine*. <https://earthzine.org/2017/06/09/update-on->

- biological-control-of-carrizo-cane-in-the-rio-grande-basin-of-texas-and-mexico/.
- Goolsby, J.A., Vacek, A.T., Salinas, C., Racelis, A.E., Moran, P.J., and Kirk, A.A. 2017. Host range of the European leaf sheath mining midge, *Lasioptera donacis* Coutin (Diptera: Cecidomyiidae), a biological control of giant reed, *Arundo donax* L. *Biocontrol Science* 27: 781-795.
- Hering, M. 1935. *Minenstudien* 15. *Zeitschrift für Pflanzenkrankheiten (Pflanzenschutz)* und *Pflanzenschutz* 45: 1-15.
- Martinez Jimenez, M., Goolsby, J., Racelis, A., Perez De Leon, A., & Negrete Arroyos, D. 2017. Introducción, Establecimiento y Dispersión en México de la avispa *Tetramesa romana*, agente de control biológico del carrizo gigante. *Southwestern Entomologist*, 42: 427-434.
- McGaugh, S., Hendrickson, D., Bell, G., Cabral, H., Lyons, K., McEachron, L., and Munoz J.O. 2006. Fighting an aggressive wetlands invader: a case study of giant reed (*Arundo donax*) and its threat to Cuatro Ciénegas, Coahuila, Mexico. *Studies of North American Desert Fishes: In honor of EP (Phil) Pister, Conservationist*. Nuevo Leon, Mexico: Universidad Autonoma de Nuevo Leon, Monterrey. pp. 100-115.
- Moore, G., Li, F., Kui, L., and West, J. 2016. Flood water legacy as a persistent source for riparian vegetation during prolonged drought: an isotopic study of *Arundo donax* in the Rio Grande. *Ecology* 9: 909-917.
- Moran, P.J., and Goolsby, J.A. 2009. Biology of the galling wasp *Tetramesa romana*, a biological control agent of giant reed. *Biological Control* 49: 169-179.
- Moran, P.J., and Goolsby, J.A. 2010. Biology of the armored scale *Rhizaspidiotus donacis* (Hemiptera: Diaspididae), a candidate agent for biological control of giant reed. *Annals of the Entomological Society of America* 103: 252-263
- Moran, P.J., Vacek, A.T., Racelis, A.E., Pratt, P.D. and Goolsby, J.A. 2017. Impact of the arundo wasp, *Tetramesa romana* (Hymenoptera: Eurytomidae), on biomass of the invasive weed, *Arundo donax* (Poaceae: Arundinoideae), and on revegetation of riparian habitat along the Rio Grande in Texas. *Biocontrol Science and Technology*, 27: 96-114.
- Perdue, R. E. (1958). *Arundo donax*—source of musical reeds and industrial cellulose. *Economic Botany*, 12: 368-404.
- Racelis, A.E., Davey, R.B., Goolsby, J.A., Pérez de León, A.A., Varner, K., and Duhaime, R. 2012. Facilitative ecological interactions between invasive species: *Arundo donax* (Poaceae) stands as favorable habitat for cattle ticks (Acari: Ixodidae) along the US-Mexico border. *Journal of Medical Entomology* 49: 410-417.
- Racelis, A.E., Goolsby, J.A., Penk, R., Jones, W.K., and Roland, T.J. 2010. The development of an inundative, aerial release technique for the arundo wasp, a biological control agent of the invasive *Arundo donax*. *Southwestern Entomologist*. 35: 495-501.
- Racelis, A., Rubio, A., Vaughan, T., and Goolsby, J. 2012. Passive restoration potential of areas invaded with giant reed in south Texas. *Ecological Restoration* 30: 112-116.
- Rubio, A., Racelis, A.E., Vaughan, T.C., and Goolsby, J.A. 2014. Riparian Soil Seed Banks and the Potential for Passive Restoration of Giant Reed Infested Areas in Webb County, Texas. *Ecological Restoration*. 32: 347-349.
- Spencer, D.F., Liow, P.S., Chan, W.K., Ksander, G.G., and Getsinger, K.D. 2006. Estimating *Arundo donax* shoot biomass. *Aquatic Botany* 84: 272-276.
- Tarin, D. Manhart, J., Pepper, A., Goolsby, J., Moran, P., Contreras Arqueta, B., and Kirk, A. 2013. Microsatellite markers indicate multiple origins of *Arundo donax* L. in North America. *Invasive Plant Science and Management* 6: 328-338.
- Thomas, D.B. and Goolsby, J. 2015. Morphology of the pre-imaginal stages of *Lasioptera donacis* Coutin (Diptera: Cecidomyiidae), a candidate biocontrol agent of giant arundo cane. *Psyche*. Available: 10.1155/2015/262678.
- Tracy, J.L. and DeLoach, C.J. 1999. Biological control of saltcedar in the United States: progress and projected ecological effects. In: Bell CE (ed.), *Arundo and saltcedar: the deadly duo*. Proceedings of the *Arundo* and Saltcedar Workshop, U.C. Cooperative Extension Service, Holtville, California. pp. 111-154.
- Watts, D.A. and Moore, G.W. 2011. Water use dynamics of an invasive reed, *Arundo donax*, from leaf to stand. *Wetlands* 31: 725-734.
- Yang, C., Goolsby, J.A. and Everitt, J.H. 2009. Using QuickBird satellite imagery to estimate giant reed infestations in the Rio Grande Basin of Mexico. *Journal of Applied Remote Sensing* 3:033530.
- Yang, C., Everitt, J.H. and Goolsby, J.A. 2011. Using aerial photography for mapping giant reed infestations along the Texas-Mexico portion of

the Rio Grande. *Invasive Plant Science and Management*. 4: 402-410.

Yeates, D.K. and Wiegmann, B.M. 2005. *The Evolutionary Biology of Flies*. Columbia University Press. Pp274-311.