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Establishment and Spread of a Single Parthenogenic Genotype of the Mediterranean arundo wasp, *Tetramesa romana*[1], In the Variable Climate of Texas

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Abstract. As part of a biological control program for the invasive weed, *Arundo donax* L., several genotypically unique populations of the parthenogenetic stem-galling wasp, *Tetramesa romana* Walker (Hymenoptera: Eurytomidae), from Spain and France were released in an infested riparian zone along the Rio Grande from Brownsville to Del Rio, TX. An adventive population of the wasp of unknown origin with limited distribution in Texas was also discovered, evaluated, and released as part of the program. More than 1.2 million wasps representing the mixture of genotypes were aerially released from 2009 to 2011. Wasps dispersed from their original release locations and now have a continuous distribution along the Rio Grande from Brownsville to Del Rio, and have dispersed throughout most of Central Texas with satellite populations as far west as San Angelo (Tom Green County), north as far as Kaufman (Kaufman County), and east to Navasota (Grimes County). The most successful genotype (#4) represented 390 of the 409 wasps recovered and matched both an imported population from the Mediterranean coast of Spain and an adventive population established in Texas before the start of the biological control program. Several other European genotypes of the wasp released in the program apparently failed to establish. This result demonstrated the benefits of evaluating and releasing the maximum genetic diversity of the biological control agent in the introduced range. Abundance of *T. romana* on the Rio Grande from Laredo to Del Rio averaged 190% more in 2013-2014 compared to a similar study in 2008-2009 before release of the European wasps. A favorability index was developed that showed that conditions from 1969 to 1977 would have been adverse to the wasp; conditions after 2009 were more favorable. Climate matching predicts the wasp will disperse throughout the southern U.S. and Mexico.

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Introduction

Arundo donax L. (Poaceae; Arundinoideae), also known as giant reed or carrizo cane, is a tall (2-10 m) perennial grass native from the Mediterranean and Caspian Basins, Arabian peninsula and east to Indian subcontinent (Hardion et al. 2014). It was likely introduced to North America from the Iberian Peninsula within the last 500 years (Tarin et al. 2013) and is now a widespread and invasive weed in the Rio Grande Basin in Texas, northern Mexico, and the southwestern U.S. In Texas, large areas of giant reed cause serious ecological impacts by displacing native vegetation (Everitt et al. 2005; Yang et al. 2009, 2011) and facilitating the invasion of cattle fever ticks from Mexico (Racelis et al. 2012). Giant reed also interferes with law enforcement activities along the international border and competes for scarce water resources in an arid region already experiencing extended drought and potential changes in rainfall patterns because of climate change (Goolsby and Moran 2009, Yang et al. 2009, Texas Commission on Environmental Quality 2012). Currently, *A. donax* is managed in limited areas by costly mechanical and chemical means (Vartanian 1998). Classical biological control may be the most cost-effective and sustainable option for management of the weed over large areas such as the Rio Grande and Nueces River Basins in Texas. A biological control program was initiated in 2005, and two insects, arundo wasp, *Tetramesa romana* Walker (Hymenoptera: Eurytomidae), and the arundo scale, *Rhizaspidiotus donacis* (Leonardi) (Homoptera: Diaspididae), have been established in Texas, with the leafsheath mining fly, *Lasioptera donacis* Coutin (Diptera: Cecidomyiidae), also being evaluated for environmental safety and efficacy (Racelis et al. 2010, Goolsby et al. 2011).

Tetramesa romana is a stem-galling wasp 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 plant growth and development (Goolsby et al. 2010). Ten European populations of *T. romana* from Granada, Las Cañas, Santa Coloma de Farners, Spain; and Ceret, Village Catalan, and St. Marie, France were imported as part of a biological control program. These European populations were host-range tested and released under appropriate permits in the U.S. and Mexico. The populations varied in their geographical distributions and came from a range of climates identified using the Köppen-Geiger climate classification system (Peel et al. 2007). The climate of coastal Las Cañas (near Almuñécar) is subtropical and Granada 80 km inland is classified as warm Mediterranean. Santa Coloma de Farners in the province of Girona, Spain has a cool, oceanic climate. Ceret, Village Catalan, and St. Marie near Perpignan in the southern province of Pyrénées-Orientales in France are classified as temperate Mediterranean climate. These collections therefore represented a range of climates and genotypes of *A. donax*. The Köppen-Geiger climate classifications in Texas included hot arid desert (Del Rio, Laredo, and San Angelo), warm temperate (Austin, College Station, Dallas, Houston, and San Antonio) and subtropical (Brownsville). The *A. donax* in Las Cañas, and Santa Coloma de Farners, Spain were closest genetic matches to the *A. donax* that invaded the Rio Grande Basin (Tarin et al. 2013). We hypothesized that *T. romana* collected from locations with similar climates to Texas and from European *A. donax* genotypes most closely related to the invasive Rio Grande Basin population would be adapted to perform best as biological control agents. In contrast, *T. romana* from Perpignan, France was from an *A. donax* genotype more distantly related to the invasive Rio Grande population, and from a climate that was not a good match for most areas of Texas, except arid areas with colder winters in western Texas. Therefore, considerable genetic and geographical diversity of the parthenogenic arundo wasp were imported, evaluated, and released in the biological control program.

More than 1.2 million *T. romana* of multiple genotypes, of approximately equal numbers, were mass reared separately and aerially released along the Rio Grande at locations approximately 25 miles apart near Brownsville, Los Indios, Hidalgo, Mission, Rio Grande City, Roma, San Ignacio, Laredo, Eagle Pass, Quemado, and Del Rio, TX from 2009 to 2011 (Racelis et al. 2010, Moran et al. 2014). None were released in other parts of Texas. However, before release of the European wasps in 2009, adventive populations of *T. romana* were detected with limited distributions near the cities of Nuevo Laredo, Mexico, and Austin, Eagle Pass, Laredo, and San Antonio, TX (Goolsby et al. 2009). Monthly surveys in 2008 along the Rio Grande found that *T. romana* was only present within the urban riparian zones of Laredo and Eagle Pass and not in the remote areas between the two locations or down river from Laredo to the Lower Rio Grande Valley (Racelis et al. 2009). From 2008 to 2009, monthly field surveys of *T. romana* on the Rio Grande documented a slow dispersal of the adventive wasp populations up and down the Rio Grande from Laredo and Del Rio. In Austin, a delimiting survey in 2008 showed the populations were limited to creeks in the central urban part of the city and not in outlying areas of Travis County (Goolsby et al. 2009). By 2009, *T. romana* was detected further up the urban watersheds in Austin, which appeared to indicate the wasp had only recently been established. In Mexico, extensive surveys were done in 2007-08 at 471 sites throughout the northern states of Chihuahua, Coahuila, Durango, Nuevo Leon, Tamaulipas, and Zacatecas, and no *T. romana* were detected (Contreras Arquieta and Goolsby, unpublished data). These studies seemed to indicate the adventive wasp had only recently established in Texas and was slowly dispersing (Racelis et al. 2010). From 2009 to 2011, multiple European *T. romana* accessions and the adventive population were mass reared and released on the Rio Grande as part of the biological control program. This paper documents the establishment and spread along the Rio Grande and across Texas 3 years after releases from the biological control program concluded. Documenting the establishment and spread of *T. romana* in Texas allows for prediction of its eventual distribution in North America and, its climatic adaptability. This study highlights the benefits of testing and releasing the maximum genetic and geographical diversity of a host-specific biological control agent in the introduced range of the weed.

Materials and Methods

Statewide Survey. Ninety-five sites with *A. donax* were sampled for *T. romana* between 31 October and 6 December 2013. Sampling in the field started in Brownsville, TX and followed the Rio Grande upstream to Dryden, TX. The survey followed the major highways, river drainages, and vegetational areas (Gould et al. 1960), including the Trans-Pecos, Rolling Plains, High Plains, Edwards Plateau, Llano Uplift, Blackland Prairie, Piney Woods, Gulf Coast Prairie, and South Texas Brush Country for a total of 3,340 miles surveyed. No *A. donax* was encountered on the High Plains, and only a few stands were located in the Piney Woods. The survey covered all Texas ecoregions known to have invasive populations of *A. donax*.

Each site was surveyed for as long as 30 minutes to determine the presence or absence of *T. romana*. Stems and lateral side shoots were visually examined for emergence holes of adults. Exit holes represented the density or cumulative emergence of *T. romana* from spring to fall of one growing season. Numbers of exit holes were evaluated at nine sites on the Rio Grande between May and October 2013 and they were positively correlated and wasp levels on the sticky traps ($n = 20$, $r = 0.57$, $p < 0.01$). Therefore exit holes are a suitable indicator of wasp density. If exit holes were found at the site, the numbers of holes were counted for 2 minutes at three locations at each site. Arundo wasp galls were collected from each site and dissected to remove *T. romana*. Larvae for genetic studies were stored in 90% ethanol in vials. Basic site characteristics were measured for all locations with *A. donax*. We used the texture-by-feel method to determine the soil type within each stand (Whiting et al. 2002). To assess stand size and quality, we measured the height of the tallest stems in the stand and numbers of live and dead shoots in a single meter-square quadrat containing the tallest stems. The quadrat with the tallest stems was selected because we assumed the area had optimal growing conditions in the stand. The mean number of exit holes at each site was regressed against the number of live and dead stems per square meter and height to determine the effect of site characteristics on density of *T. romana*, using Tibco™ Spotfire S+ 8.2, (Tibco Software Inc., Boston, MA). All count data were square root-transformed to normalize distributions before analysis.

Rio Grande Transect. Results of a survey between May 2013 and May 2014 at the nine sites along the Rio Grande from Laredo to Del Rio were compared with results of the survey at the same areas from April 2008 to June 2009 (Racelis et al. 2010). At each location, five yellow sticky traps (23 x 14 cm, unbaited Pherocon® AM, Trécé, Inc., Adair, OK) were placed along a 100-m-long trail perpendicular to the river (5-50 m from the edge of the water) to account for variations in microclimate and environment. Traps were replaced monthly, returned to the laboratory, and examined at 35X magnification to count all adult *T. romana.* Five *T. romana* galls were collected at each of the 19 sites between Brownsville to Del Rio from June 2013 to May 2014 and kept in cardboard tubes for emergence of *T. romana* and any parasitoids.

Weather Analysis. Using Laredo, TX Municipal Airport Station as the representative location, we evaluated 16 meteorological variables and found that mean monthly temperature and monthly total precipitation were most highly correlated with arundo wasp population levels, (NOAA NCDC 2014). In south Texas along the Rio Grande, the period of rapid population growth of *T. romana* is typically February through July (Racelis et al. 2009). We computed the mean, standard error of the mean (SEM), and *t*-test of the February through July period of 2008 combined with 2009, compared to the February through July period of 2012 combined with 2013. That is, we compared weather data for identical season intervals, approximately matching the survey periods of the arundo wasp. Plots were made of the product of monthly mean temperature and monthly total precipitation at the Laredo station. Based on our observation that *T. romana* in south Texas is hindered by rainfall (which may be a surrogate for days without sunshine) and benefits by moderate (versus excessive summer) temperatures, we computed the inverse of the monthly mean temperature x monthly total precipitation product to generate a *Tetramesa romana Climate Favorability Index*. To visualize changes in suitability over the variable climate of south Texas, we plotted the index from 1950 through the present (i.e., 2014), noting periods of especially adverse and beneficial weather. We superimposed on the index trend the wasp abundance surveyed in 2008-2009 and 2013-2014 to determine if weather may have played a role in the recent population expansion. To predict the potential distribution of *T. romana* in North America we used the model CLIMEX to match climate function with equal weightings for all parameters (Sutherst et al. 1999). Locations with potential suitable habitat for *T. romana* have a Composite Match Index ≥70% which indicates high similarity between climates. The index is based on rainfall, degree-days, population growth, temperature, moisture, cold stress, heat stress, drought stress, and water stress indices between all locations in North America and the home location. Weather data for the model is based on 30-year averages. We found that using the distribution of *T. romana* in Europe as the predictor of its distribution was not entirely useful because its distribution is limited by the Mediterranean Sea to the south; therefore it is not possible to know how tolerant the species might be to warmer southern climates. Instead, we used locations in the introduced range (Dallas, Del Rio, and Houston, TX) as match climate locations because they represented the western, northern, and eastern boundaries, respectively, of the known distribution of *T. romana*. Houston was substituted for College Station, TX, because the latter is not included in the CLIMEX database. However, the average yearly rainfall in College Station is 1.06m compared to 1.32m Houston so the prediction may not be as robust as the other two locations. The CLIMEX prediction using the home locations in Texas might overestimate the final distribution of *T. romana*, but is provided as a guide.

Genetic Analysis. To determine the genotype of the *T. romana*, galls were collected for dissection and removal of the larvae for genetic characterization. From each location, 30 larvae were collected and placed in 90% ethanol. Simple sequence repeats were analyzed from typically 10 larvae from each location. The patterns were compared to the original parental populations from Europe and the extant adventive population of the wasp established at a few locations before release. The genetic match locations of the individuals were evaluated to determine if the successful populations came from one region in Europe, which might suggest a climate adaptation; or conversely, the most successful populations might have come from different climates, but might be adapted to the invasive Rio Grande population of *A. donax*.

To identify a set of polymorphic microsatellites markers, also known as simple sequence repeats, DNA fragments containing SSRs were obtained from *T. romana* genomic DNA by the bioinylated-oligonucleotide capture method described previously (Terry et al. 2006) with single modification: starting genomic DNA for simple sequence repeat capture was isolated from a mixed pool of adult wasps from sample Spain 40 (2007526) using a simple mini-prep method (Pepper and Norwood 2001). The DNA sequencing methods and simple sequence repeat primer design criteria have been described previously (Burrell and Pepper 2006). Primer designs included an M13 tail to facilitate the incorporation of 6-FAM labeled ‘universal’ M13 primers into the final PCR product (M13 reference).

The simple sequence repeats from a total of 409 field-collected wasps were extracted using a Chelex method (Walsh et al. 1991). Heads of adult wasps or one-third of larvae (including head) were smashed and 100 µl of Chelex-100 solution (Bio-Rad) was added with 10 µl proteinase K (Qiagen), then heated at 56ºC for 2 hours and 99ºC for 10 minutes. For SSR genotyping, 10 µl PCR reactions included 0.5 µl extracted DNA, 1 µl 10X PCR Buffer (Biolase), 0.5 µl of 50mM $MgCl_2$, 0.08 µl of dNTP mix (Biolase), 0.5 µl of the 2 uM forward M13 tailed primer, 1.0 µl of the 2 µM reverse primer, 1.0 µl of 6-FAM dyed M13 primer, 0.05 µl of 5 units per liter of TAQ (Biolase) and water. Annealing temperature for simple sequence repeat wh10 was 55ºC, and all others (wa6, wc12, we6) were 57ºC. One-half microliter of 600 bp ladder, 9.25 µl of formamide, and 0.25 µl of PCR product were loaded into an ABI 3130 Genetic Analyzer. Simple sequence repeat fragments were visualized in GeneMapper (ABI) and fragment size was determined manually.

Results

Statewide Survey. *Tetramesa romana* was present at all the survey sites on the Rio Grande between Del Rio and Brownsville, TX (Fig. 1). It was detected for the first time 20 km up river from Amistad Reservoir at the confluence of the Pecos River. Infestations of the wasp were also found throughout central Texas, with satellite populations as far west as San Angelo (Tom Green County), north to Kaufman (Kaufman County) and east to near the city of Navasota (Grimes County). The greatest abundance of wasps occurred along the Rio Grande, especially in the Lower Rio Grande Valley and in Laredo, TX. None of the plant variables (height, number of live and dead stems per square meter) were significantly correlated to number of exit holes, although a weak, but significant correlation was found between number of exit holes and density (live stems per square meter) ($R^2 = 0.14$, $p = 0.01$). The wasp was collected from *A. donax* growing on a broad range of soil types from clays to sands.

Rio Grande Transect. In the period 2008-2009, a total of 680 *T. romana* was collected on sticky traps with peak abundance in May 2009 (Racelis et al. 2009). In comparison, 1,126 wasps were collected during the same period in 2013-2014. This represents a 190% increase between the two sample periods ($p = 0.0059$, one-tail *t*-test, assuming groups had unequal variance). It should also be noted that 2,351 wasps were collected between Falcon Dam and Brownsville in the 2013-2014 survey and none was present in that same (448 km) part of the Rio Grande during the 2008-2009 survey. Therefore, the arundo wasp expanded its population both geographically and numerically on the Rio Grande during the 5-year period from 2008-2014. The mean annual percentage of parasitism of *T. romana* was 6.4% (n = 103) and ranged from 0 to 16.2% in October and July, respectively. The parasitoids were identified as *Brasema* sp. (Eupelmidae, Eupelminae) 64.1% and *Phylloxeroxenus* near *cressoni* (Howard) (Eurytomidae, Eurytominae) 24.3%, Braconidae sp. 4.9%, and 6.8% other unknown species. Of the 1,497 *T. romana* individuals collected in the parasitism study, 5.0% were males.

Genetic Analysis. All *T. romana* sampled were homozygous at all simple sequence repeat loci examined, supporting their being parthenogenetic. Because they are homozygous, genotypes are equivalent to haplotypes. Some genotypes were collected at multiple locations in Europe, where as others appeared to have more limited distributions. One particular genotype of *T. romana* dominated collections throughout Texas (Table 1). This genotype four matched with parental material originally collected from Las Cañas, Granada, and Santa Coloma de Farners, Spain as well as the adventive population of *T. romana* present in limited areas before release of the European material. Two other European populations imported in the biological control program became established but were collected in limited numbers only on the Rio Grande where the aerial release program was done. The adventive population seems to be of Spanish origin, but is indistinguishable from the introduced genotype (#4) population from Spain. Several other unknown genotypes were also collected from Central Texas where no releases were done as part of the biological control program.

Climate Analysis. Using Laredo, TX as the standard location, the climate variables were evaluated retrospectively from 1950 to the present. Eight temperature and precipitation-related variables were examined in relation to abundance of *T. romana* and none other than mean monthly temperature and total monthly rainfall proved significantly different between the 2008-2009 and 2012-2013 periods. Arundo wasp abundance, was significantly different (p = 0.0059, one-tail t-test, assuming groups had unequal variance). Abundance in 2012-2013 averaged more than twice (190%) that of 2008-2009. The *Tetramesa romana Climate Favorability Index* changed significantly through time (Fig. 2). Conditions in 1969 through 1977 seemed especially adverse to the wasp; conditions during and after 2009 (and for the near future) show marked increase in favorable conditions.

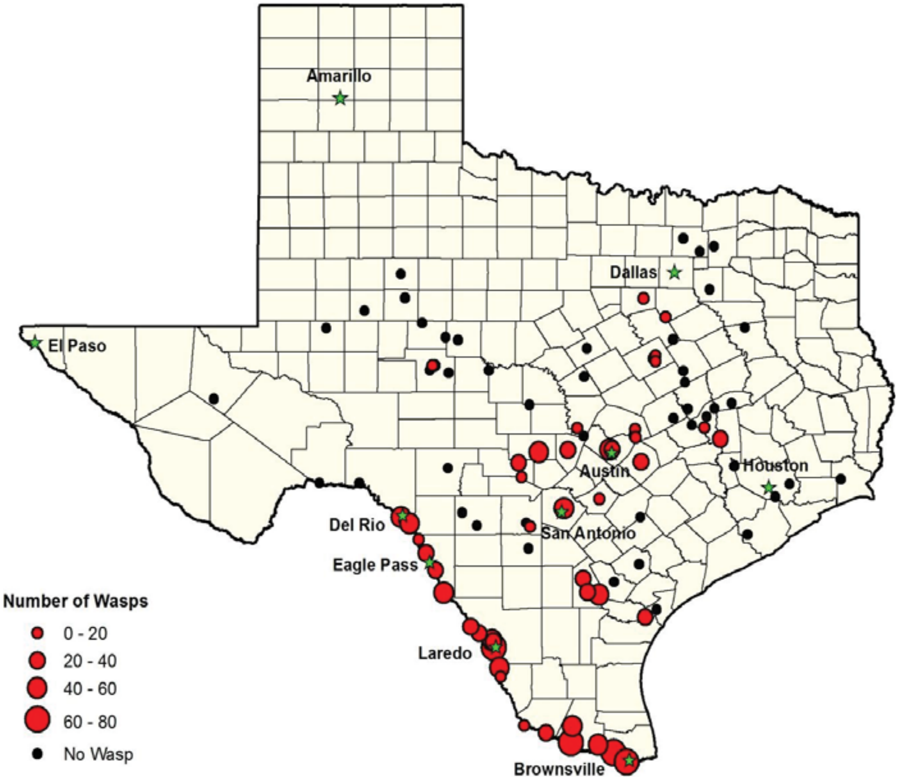

Fig. 1. Map of locations in Texas with *Arundo donax* and surveyed during November-December 2013 for the presence of the biological control agent, *Tetramesa romana*. Numbers of wasps were determined by counting the numbers of exit holes at each location per 2-minute counts.

Table 1. Origin and Abundance of *Tetramesa romana* Genotypes Collected in a Texas Survey

Collection location	European origin	Status*	Genotype	n
California, Ventura	No match	NR	1	0
None	Granada, Spain	R	2	0
None	Santa Caloma de Farners, Spain	R	3	0
Most locations, except north Central TX	Las Cañas, Granada, Santa Coloma de Farners, Spain; adventive U.S. population	R & A	4	390
Laredo, Webb Co.,	No match	A	5	3
Edinburg, Hidalgo Co.	No match	A	6	0
Brownsville, Cameron Co.	No match	A	7	0
None	Granada, Spain	R	8	0
El Indio, Maverick Co.,	Las Cañas, Spain	R	9	1
None	Ceret, Village Catalan, Saint Marie, France Santa Caloma de Farners, Spain	R	10	0
Durban, South Africa**	No match	NR	11	0
None	Village Catalan, France	R	12	0
Del Rio, Val Verde Co.	Granada, Santa Coloma de Farners, Spain	R	13	1
None	Granada, Las Cañas, Spain	R	14	0
Brownsville, Cameron Co.	No match	A	15	1
None	Santa Caloma de Farners, Spain	R	16	0
George West, Live Oak Co.	No match	A	17	1
Austin, Travis Co.	No match	A	18	3
Austin, Travis Co.	No match	A	19	1
George West, Live Oak Co.	No match	A	20	1
George West, Live Oak Co.	No match	A	21	2
Los Indios, Cameron Co.	No match	A	22	2
Laredo, Webb Co.	No match	A	23	2
Italy, Ellis Co.	No match	A	24	1
			Total	409

*NR = not released, R = released as part of biological control program, A = adventive population of unknown origin **Durban population included in table for reference only. It was not collected in the survey.

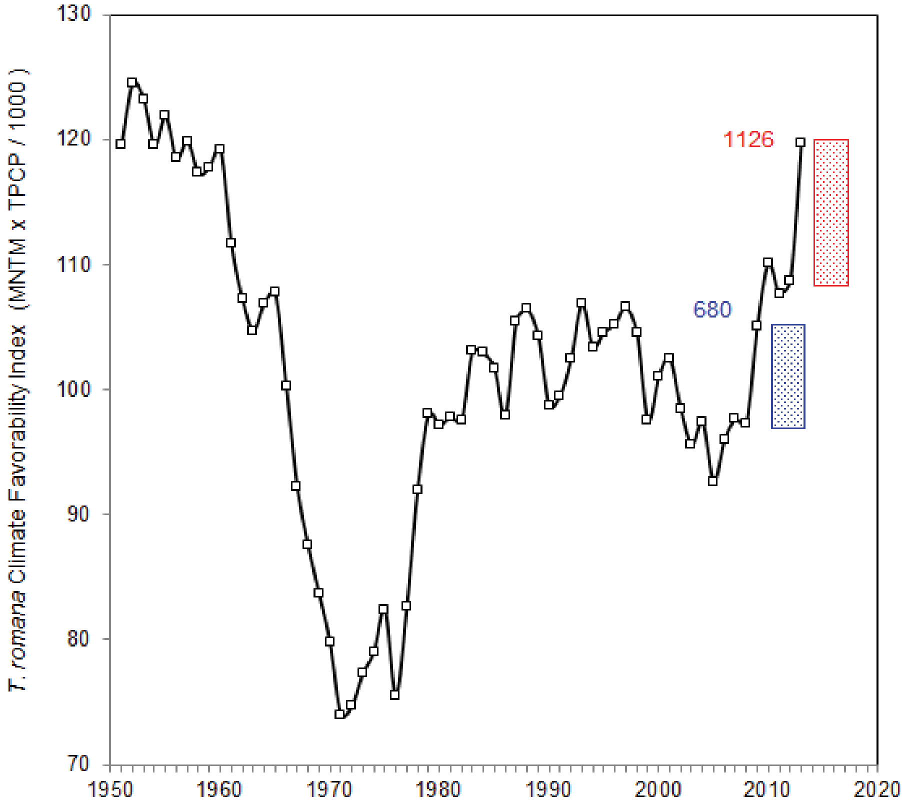

Fig. 2. Climate Favorability Index for the arundo wasp, *Tetramesa romana*, based on the inverted product of monthly mean temperature (MNTM) and monthly total precipitation (TPCP) at Laredo, Texas. Annual values shown are 1950 through latest month of 2014. Blue box indicates range of index values during first survey (2008-09); red box indicates index values over period of second survey (2013-14). Observed average monthly levels of wasp in 2008-9 and (*blue font*) 2012-13 *(red font*) period shown.

CLIMEX was used to compare the climates at the edge of the current distribution of *T. romana* to predict potential final distribution of the arundo wasp in North America. Fig. 3 shows climatic matches of Del Rio, TX and locations throughout most of the southwestern U.S. and northern Mexico. Fig. 4 shows climatic matches of Houston and most of the southeastern U.S. Fig. 5 shows the climatic match with Dallas, TX, currently the northern limit of *T. romana*. Therefore, it would seem that *T. romana* will continue to disperse throughout the area where *A. donax* is invasive.

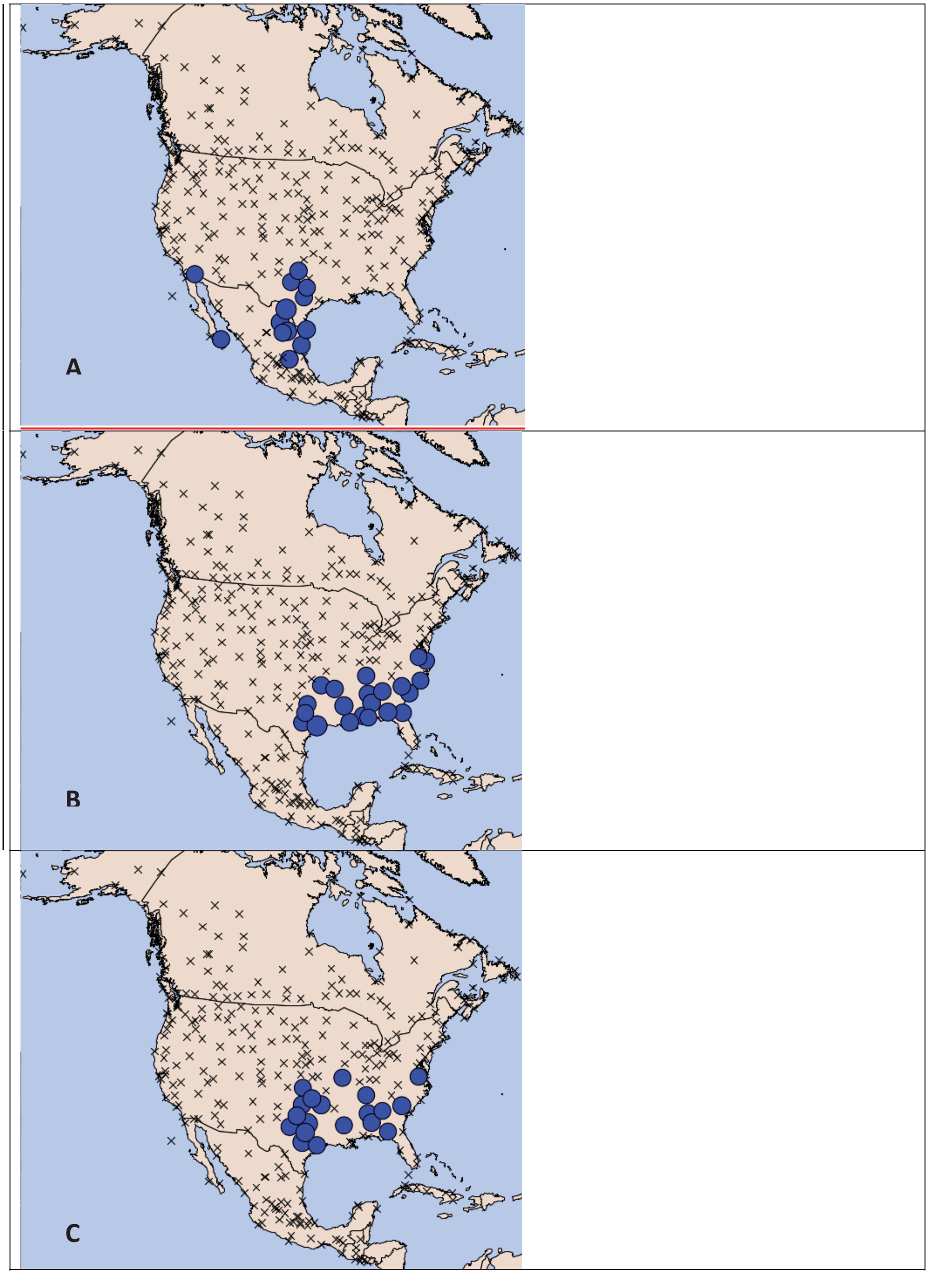

Fig. 3. Climate matching of locations in Texas with established populations of *Tetramesa romana* to predict is possible distribution in North America. Locations in with blue dots ≥70% CLIMEX Composite Match Index with (A) Del Rio, TX, (B) Houston, and (C) Dallas. Locations with x's have an index less than 70%.

Discussion

Tetramesa romana established readily along the Rio Grande and spread throughout most of southern and central Texas. One genotype of the parthenogenetic wasp (#4), of Spanish Mediterranean origin, dominated the recoveries throughout its introduced range (Table 2). This is consistent with the origin of the dominant genotype of *A. donax* in Texas which is also from Mediterranean Spain (Tarin et al. 2013). This genotype of *T. romana* might have succeeded because it was from a warm Mediterranean climate similar to the Lower Rio Grande Basin and found the matching genotype of *A. donax*. Genotype #4 is also the most widely distributed of *T. romana* genotypes in Spain, which suggests it might be tolerant of a wide range of climates. It is also possible that this genotype succeeded because it had the advantage of incumbency, being established in advance of the biological control program. In comparison, *T. romana* genotypes 10 and 12 from near Perpignan, France were released consistently for several years, but have apparently failed to establish in the introduced range. What is surprising is that 11 unknown genotypes were recovered that did not match any of the original parental populations from Europe. Seven of the 10 unknown genotypes were recovered in locations where no releases were made during the biological control program. These unknown genotypes could have been cryptic subpopulations within the original parental European populations imported as part of the biological control program and dispersed from the Rio Grande, or could have been released with the original adventive population discovered in California in 2007 and Texas in 2008 (Goolsby et al. 2009). However, the former is unlikely given the extent of the genetic characterization during the importation and evaluation phase of the biological control program.

For the past several decades, biological control practitioners have focused on importation and host-range testing of a single population of the biological control agent for release (USDA-APHIS 2014). This practice reduced the risk that a cryptic species with a different host range might be part of the insect colony being tested. However, this practice limits genetic diversity that might be important for the agent to adapt to its introduced range. For example, several releases of the tansy ragwort flea beetle, *Longitarsus jacobaeae* Waterhouse, from different climates in Europe were made in North America and hybrids of the populations have been documented (Szücs et al. 2011). The hybrid *L. jacobeae* beetles seem to be critical to the success of this agent in parts of its introduced range. We followed this practice of evaluating and releasing maximum genetic diversity in the *A. donax* biological control program. Several *T. romana* genotypes from different climates in Mediterranean Europe were released and this may have been critical for finding the most adapted parthenogenetic genotype for the southwestern U.S. and Mexico. This supports the practice of host-range testing multiple genotypes to allow for increased genetic diversity in the introduced range.

The *T. romana Climate Favorability Index* at Laredo, TX varied conspicuously during the past 64 years (Fig. 3). The index was markedly low during 1969-1977 due to higher rainfall and cooler winters, suggesting the wasp might not have established had it been introduced during this period. The period from 2009 onward showed increasing favorability and might partly explain the conspicuous expansion between the 2008-2009 and 2013-2014 surveys. During this period of time, the southwestern U.S. and northern Mexico experienced extensive drought (Texas Commission on Environmental Quality 2014). Drought conditions increased the numbers of days with sunshine and obviously reduced rainfall. The arundo wasp seems to need sunny days to perform best in the field. We know from rearing *T. romana* that light was a critical environmental factor (Moran et al. 2014). This biological attribute might limit its impact in the southeastern U.S. where there are fewer days with sunshine and more rainfall. Additionally, prolonged periods of rain from tropical depressions in the Rio Grande Basin could decrease the favorability index for the wasp and simultaneously increase the growth rate of *A. donax*.

The positive correlation between number of *T. romana* exit holes and density of live *A. donax* stems could be an indicator that stand/site quality plays a role in its dispersal. However, the wasp did establish on marginal stands, along highway ditches, seasonal creeks, and urban settings. One factor that might influence successful establishment is the use of herbicides on *A. donax*. Although *T. romana* established throughout most of the river basins of Central and South Texas it was not present on most of the upper Nueces River. The Nueces River Authority has been attempting to limit the spread of *A. donax* through targeted applications of the herbicide imazapyr. Regrowth from treated stands of *A. donax* might be unsuitable for the gall-forming wasp. This interaction should be investigated if both chemical and biological methods are to be integrated.

CLIMEX models predict the climates throughout most of the southern U.S. and northern Mexico will be suitable for the wasp to establish and spread. However the predictions are based on the current and changing distribution of the arundo wasp in Texas, which might have been influenced by drought conditions and an abundance of sunshine that favors the wasp. CLIMEX does not use numbers of days with sunshine as a variable to calculate the Composite Match Climate Index. The models show low suitability for the wasp in most of California, including the Central Valley and Sacramento-San Joaquin Delta, although a separate adventive genotype of the wasp has established is the Santa Ana and Ventura River drainages of southern California (Lambert et al. 2010) (Table 1). *Tetramesa romana* is common in Rome, Italy (J. Goolsby and A. Kirk personal observation) which has a CLIMEX match of 70% or greater with Sacramento and San Jose, CA; therefore the climate should be suitable for the wasp. Releases of *T. romana* (Genotype #4) in the Sacramento and San Joaquin valleys and the Bay Area of California are on-going.

Future Research. The spatial maps showing regions suitable to the survival of the arundo wasp indicate this species could continue to expand over large areas of Texas and across the southern U.S. While CLIMEX mapping provides a snap-shot in time of climate suitability, it does not address how suitability may change over longer periods, such as decades or on the century scale. This is not a moot consideration given the magnitude of the recent drought and shifts in temperature over the southern and central Texas region (Fig. 2). Research is currently in progress that will examine in detail how climate favorability is changing in areas of intended introduction and current expansion of *T. romana* in Texas. It will also focus on the question of why, contrary to expectations, only one genotype of the many that were introduced succeeded across a range of climates. If we had based our releases on any of our genotypes except #4, the project may have been considered a failure at this point. This research might help guide future biological control practitioners as to the benefits of assessing and releasing the maximum genetic diversity (within the range of host-specific genotypes) to improve the success of biological control programs, especially considering the potential for climate change.

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References Cited

Burrell, A. M., and A. E. Pepper. 2006. Primers for 10 polymorphic microsatellites from *Caulanthus amplexicaulis* var. *barbarae*, and cross-amplification in other species within the Streptanthoid Complex (Brassicaceae) Mol. Ecol. Notes 6: 770-772.

Everitt, J. H., C. Yang, and C. J. Deloach. 2005. Remote sensing of giant reed with QuickBird satellite imagery. J. Aquatic Plant Mgmt. 43: 81-84.

Goolsby J. A., and P. J. Moran. 2009. Host range of *Tetramesa romana* Walker (Hymenoptera: Eurytomidae), a potential biological control of giant reed, *Arundo donax* L. in North America. Biol. Control 49: 160-168.

Goolsby, J. A., P. J. Moran, J. Falk, and L. Gilbert. 2009. Distribution and spread of an adventive population of the biological control agent, *Tetramesa romana* in Austin, Texas. Southwest. Entomol. 34: 329-330.

Goolsby, J. A., D. Spencer, and L. Whitehand. 2010. Pre-release assessment of impact on *Arundo donax* by the candidate biological control agents, *Tetramesa romana* (Hymenoptera: Eurytomidae) and *Rhizaspidiotus donacis* (Homoptera: Diaspididae) under quarantine conditions. Southwest. Entomol. 34: 359-376.

Gould, F. W., G. O. Hoffman, and C. A. Rechenthin. 1960. Vegetational Areas of Texas, Texas A&M University. Texas Agricultural Experiment Station, Leaflet 492.

Gowda, P. H., J. A. Goolsby, C. Yang, S. Basu, A. Racelis, and T. A. Howell. 2011. Estimating water use by giant reed along the Rio Grande using a Large Aperture Scintillometer. Subtrop. Plant Sci. 63: 1-6.

Hardion, L., R. Verlaque, K. Saltonstall, A. Leriche, and B. Vila 2014. Origin of the invasive *Arundo donax* (Poaceae): a trans-Asian expedition in herbaria. Ann Bot. 114: 455-462.

Lambert, A. M., T. L. Dudley, and K. Saltonstall. 2010. Ecology and impacts of the large-statured invasive grasses *Arundo donax* and *Phragmites australis* in North America. Inv. Plant Sci. Manage. 3: 489-494.

Moran, P. J., and J. A. Goolsby. 2009. Biology of the galling wasp *Tetramesa romana*, a biological control agent of giant reed. Biol. Control 49: 169-179.

Moran, P. J., J. A. Goolsby, A. E. Racelis, A. C. Cohen, M. A. Ciomperlik, K. R., Summy, D. P. A. Sands, and A. A. Kirk. 2014. Mass rearing of the stem-galling wasp *Tetramesa romana*, a biological control agent of the invasive weed *Arundo donax*, pp. 163-201. *In* J. A. Morales-Ramos, M. G. Rojas, and D. I. Shapiro-Ilan [eds.], Mass Production of Beneficial Organisms: Invertebrates and Entomopathogens. Academic Press, Waltham, MA.

NOAA NCDC. 2014. Land-based Station Data, Climate Data On-Line, Data Tools: find a Station. (http://www.ncdc.noaa. gov/cdo-web/datatools/findstation; and/or http://www.ncdc.noaa.gov/cdo-web/search accessed 11 June 2014).

Peel, M. C., B. L. Finlayson, and T. A. McMahon. 2007. Updated world map of the Köppen-Geiger climate classification. Hydrol. Earth Syst. Sci. 11: 1633-1644

Pepper, A. E., and L. E. Norwood. 2001. Evolution of *Caulanthus amplexicaulis* var. *barbarae* (Brassicaceae), a rare serpentine endemic plant: a molecular phylogenetic perspective. Am. J. Bot. 88: 1479-1489.

Racelis, A. E., and J. A. Goolsby. 2013. Rapid assessment of above-ground biomass of giant reed using visibility estimates. Subtrop. Plant Sci. 64: 61-66.

Racelis, A. E., J. A. Goolsby, and P. J. Moran. 2009. Seasonality and movement of adventive populations of the arundo wasp (*Tetramesa romana*) a biological control agent of giant reed (*Arundo donax* L.) in South Texas. Southwest. Entomol. 34: 347-357.

Racelis, A. E., J. A. Goolsby, R. Penk, W. K. Jones, and T. J. Roland. 2010. The development of an inundative, aerial release technique for the arundo wasp, a biological control agent of the invasive *Arundo donax*. Southwest. Entomol. 35: 495-501.

Racelis, A. E., R. B. Davey, J. A. Goolsby, A. A. Pérez de León, K. Varner, and R. Duhaime. 2012. Facilitative ecological interactions between invasive species: *Arundo donax* (Poaceae) stands as favorable habitat for cattle ticks (Acari: Ixodidae) along the US-Mexico border. J. Med. Entomol. 49: 410-417.

Sutherst, R. W., G.F. Maywald, T. Yonow, and P.M. Stevens. 1999. CLIMEX. Predicting the Effects of Climate on Plants and Animals. CD-ROM and User Guide. CSIRO Publishing, Melbourne, Australia.

Szücs, M., M. Schwarzländer, and J. F. Gaskin. 2011. Reevaluating establishment and potential hybridization of different biotypes of the biological control agent *Longitarsus jacobaeae* using molecular tools. Biol. Control 58: 44-52.

Tarin, D., J. Manhart, A. Pepper, J. Goolsby, P. Moran, B. Contreras Arquieta, and A. Kirk. 2013. Microsatellite markers indicate multiple origins of *Arundo donax* L. in North America. Invasive Plant Sci. Manag. 6: 328-338.

Terry, M, A. E. Pepper, and J. R. Manhart. 2006. Development and characterization of microsatellite loci in endangered *Astrophytum asterias* (Cactaceae). Mol. Ecol. Notes 6: 865-866.

Texas Commission on Environmental Quality. 2014. Drought Statement. http://www.tceq.texas.gov/response/drought

USDA-APHIS. 2014. Technical Advisory Group for Biological Control of Weeds. http://nsu.aphis.usda.gov/plant_health/permits/tag/

Vartanian, V. 1998. *Arundo donax:* Giant Cane. Unpublished Report, California Exotic Pest Plant Council.

Walsh, P. S., D. A. Metzger, and R. Higuchi. 1991. Chelex-100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10: 506-513.

Whiting, D., C. Wilson, and A. Card. 2002. Colorado Master Gardener Fact Sheets #14. Colorado State University, Cooperative Extension. www.cmg.colostate.edu

Yang, C., J. A. Goolsby, and J. H. Everitt. 2009. Using QuickBird satellite imagery to estimate giant reed infestations in the Rio Grande Basin of Mexico. J. Appl. Remote Sensing 3: 033530.

Yang, C., J. A. Goolsby, J. H. Everitt, and Q. Du. 2012. Applying six classifiers to airborne hyperspectral imagery for detecting giant reed. Geocarto International 27: 413-424.

Yang, C., J. H. Everitt, and J. A. Goolsby. 2011. Using aerial photography for mapping giant reed infestations along the Texas-Mexico portion of the Rio Grande. Invasive Plant Sci. Manag. 4: 402-410.