Biology of the galling wasp *Tetramesa romana*, a biological control agent of giant reed

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A R T I C L E   I N F O

Article history:
Received 6 August 2008
Accepted 28 January 2009
Available online 5 February 2009

Keywords:
Galling wasp
Eurytomidae
Development
Reproduction
Oviposition behavior
Exotic invasive grass

A B S T R A C T

The biology of the gall-forming wasp *Tetramesa romana* Walker (Hymenoptera: Eurytomidae) from southern France and Spain was studied to determine its suitability as a biological control agent of giant reed (*Arundo donax* L.), an exotic and invasive riparian weed in the US and Mexico. Females produced eggs parthenogenetically and deposited them into shoot tips. Eggs were 1.26 mm long and hatched within 5 days of oviposition. The larvae completed three instars within 23–27 days, with the first and second instars lasting 5 days each at 27 °C and the third instar lasting 12 days. Larvae increased in length from 0.9 mm to 4.1 mm between the first and last instars. The total generation time averaged 33 days. Adults were 6.8 mm long with antennae and lived 3.7 days. Spanish wasps lived 1.3 days longer than French wasps. Most (80%) parthenogenetic reproduction involved wasps that were less than 5 days old. A total of 36% of French wasps tested and 65% of Spanish wasps produced offspring. Individual Spanish wasps produced an average of 26 (up to 66) progeny, displaying an intrinsic capacity for increase of 0.081 and a population doubling time of 9.2 days. Over 90% of exit holes made by emerging wasps were located within two nodes of the shoot tip. Reproductive wasps made 2.5-fold more probes than did wasps that failed to gall stems, and probing behavior was 13% more prevalent in the afternoon than in the morning. The Spanish genotypes of *T. romana* are particularly suitable for mass production to support field releases.

1. Introduction

1.1. Invasion of giant reed in North America

Giant reed (*Arundo donax* L., Poaceae), along with many other food and fiber plants, was introduced to North America ca. 400 years ago from Mediterranean Europe (Dunmire, 2004), with possible introductions from other native areas, including northern Africa, the Middle East, the Arabian Peninsula, India and Nepal (Perdue, 1958). Giant reed has invaded native riparian habitats in the southern US and northern Mexico (Bell, 1997), forming dense infestations along 600 river-miles of the Lower Rio Grande in Texas (Everitt et al., 2005), with additional populations throughout the Lower Rio Grande Basin (LRGB), which includes Texas and five Mexican states. Giant reed consumes water from the Rio Grande and its tributaries that is needed for agricultural and domestic use. Large thickets alter stream flow patterns, increase erosion and flood damage (Frandsen and Jackson, 1994), fuel wildfires along rivers (Rieger and Kreager, 1989), limit river access (Hughes and Mickey, 1993) and displace populations of native plants and animals (Bell, 1997; Herrera and Dudley, 2003), leading, for example, to the possible extinction of a Mexican fish species (*Etheostoma segrex* Norris & Minckley) (McCaugh et al., 2006). Infestations of giant reed cause similar problems in southern California (Bell, 1997; Dudley, 2000; Quin and Holt, 2008) and in the Sacramento-San Joaquin Delta in northern California (Spencer et al., 2006). Giant reed was brought to the New World for use in thatch housing and fences, to make windwood musical instruments (Perdue, 1958), and for erosion control (Tracy and DeLoach, 1999). Several *A. donax* cultivars and other *Arundo* species are occasionally used as ornamental grasses and as forage for exotic mammals in the southern US (Greenlee, 1992; Oakes, 1993). Giant reed is a source of industrial cellulose (Perdue, 1958) and a potential biofuel (Szabo et al., 1996). However, the economic and ecological damage caused by giant reed outweighs the potential benefits of this species. Physical (burning), mechanical (mowing or mulching) and chemical control methods are available (Tracy and DeLoach, 1999), but may not have sufficient impact, in part because giant reed regrows vigorously from its well-provisioned rhizomes (Decruyenaere and Holt, 2001; Spencer and Ksander, 2006).

1.2. Potential for biological control

Biological control may provide sustainable control of giant reed in North America and in other areas, such as South Africa (Ross...
et al., 1998). Although grasses have been considered difficult targets for biological control with arthropods (Evans, 1991) because of their relatively simple architecture and reduced loads of chemical deterrents, toxins, and stimulants (Strong et al., 1984), monophagy and oligophagy are in fact common among endophagous herbivores such as shoot-borers feeding on perennial grasses (Tscharntke and Greiller, 1995). Evaluations of potential non-native agents (some adventive) of another invasive perennial grass, the Eurasian form of common reed, Phragmites australis (Cav.) Trin ex. Steudel, are ongoing (Tewksbury et al., 2002; Häßiger et al., 2005, 2006). In the case of giant reed, 21 mostly polyphagous insect herbivores were identified (Tracy and DeLoach, 1999), with oligo- or monophagous species expected among the endophagous fauna. More recent surveys have focused on Mediterranean Europe as a source for candidate agents (Kirk et al., 2003), and a classical biological control program for A. donax is in development.

1.3. Selection of Tetramesa romana for biological control

Biological control of non-native weeds using exotic arthropod herbivores has led to partial or complete control of over 30 invasive weeds in North America since 1946 (Nichols et al., 1995; Coulson et al., 2000). The decision to release an agent into the field must be grounded on a thorough understanding of its biology (development, feeding, survival, and reproduction) in order to predict the agent’s role in introduced environments (Price, 2000). Biological and ecological information from the native range can be used to prioritize agents (Myers, 1987; van Klinken and Raghu, 2006). Suitable agents must meet modern host specificity requirements, which are based on a thorough understanding of agent host location, feeding behavior and immature and mature survival (Heard, 2000; Sheppard et al., 2005). Candidate agents must show efficacy on the target weed, at least when protected from natural enemies (McFadzen, 1998; Pearson and Callaway, 2003; Sheppard and Raghu, 2005; McClay and Balciunas, 2005; Goolsby et al., 2006), and they should be compatible with climatic conditions in the highest-priority ecoregions of the weed’s invasive range (Briesse, 2004), as well as with the genotypes and demographics of the target weed that are prevalent in these ecoregions (Müller-Schärer and Schaffner, 2008). In the case of giant reed, one of the agents selected for evaluation on the basis of safety criteria, broad geographic distribution and abundance (Kirk et al., 2003) is the stem-galling wasp Tetramesa romana Walker (Hymenoptera: Eurytomidae).

The wasp T. romana has been reported from southern France (Steffan, 1956), Italy and Egypt (Claridge, 1961), Spain, Greece, Turkey and the northern coast of Africa (Kirk et al., 2003). Other members of the genus Tetramesa are known to be highly host-specific (Phillips, 1936; Claridge, 1961), restricted to a single host species or genus in the family Poaceae, and they have developed associations with inquilines and parasitoids that show similar levels of specificity (Dawah et al., 1995, 2002; Dubbert et al., 1998; Matusumo and Saigusa, 2001). Gall induction by Tetramesa spp. wasps can cause damage leading to economic losses in crops. Lodging and yield loss occur in North America and Spain in wheat (Triticum aestivum L.) galled by the wheat jointworm T. tritici (Fitch) and the wheat strawworm T. grandis (Riley), barley (Hordeum vulgare L.) galled by the barley jointworm T. hordei (Fitch), and rye (Secale cereale L.) galled by the rye jointworm T. secale (Fitch) (Davis, 1918; USDA, 1954; Holmes and Blakeley, 1971; Sterling, 1976; Martin and Harvey, 1982; Cantero-Martinez et al., 2003; Shanower and Waters, 2006). No other Tetramesa species has been evaluated for biological weed control, although two species in the genus Eurytoma have been examined (Kleinjan and Edwards, 2006; Neser, 2008), and one species (Eurytoma attiva Burks) has contributed to control of Cordia curassavica (Jacq.) R. & S. in Malaysia (Simmonds, 1980).

1.4. Biology of T. romana

Little information is available about the biology of Tetramesa species, in particular the number and duration of immature stages, and the fecundity and longevity of adults. Female reproduction likely involves thelytokous parthenogenesis, as in many gall wasps in the Cynipidae (Askew, 1984), since males are rare or unknown in most Tetramesa species (Phillips, 1936; Claridge, 1961). A recent DNA analysis indicated that geographic populations of T. romana are genetically variable (D. Tarin, A. Pepper, J. Manhart, Texas A&M University, College Station, Texas, unpublished data), and could therefore differ in life history parameters. Tetramesa romana may sometimes cause no visible gall (Steffan, 1956; Claridge, 1961). However, galls are visible as swollen protuberances on side shoots in southwestern France and in Spain (Kirk et al., 2003). New adult wasps emerge by chewing exit holes from the gall chamber to the outer surface of the stem. Field observations (Kirk et al., 2003; Dudley et al., 2006) indicate that galls are found at shoot tips. Past studies of other Tetramesa spp. (Dubbert et al., 1998) and an adventive population of T. romana in California, USA (Dudley et al., 2006) suggest that females perceive external cues that provide information about stem diameter. Ovipositor probing behavior in stems likely exposes females to plant species-specific chemical cues (Askew, 1984). Wasp oviposition may vary according to light quantity or daylength, as in the case of other herbivores evaluated for biological control, such as the saltc-edar leaf beetle Diorhabda elongata Brulle subspecies deserticola Chen Desbrochers (Bean et al., 2007). Past studies on temperate Tetramesa species indicated that wasps complete only one or two generations per year (Phillips, 1936; Claridge, 1961). The objective of this study was to characterize the immature development, generation time, and adult survival, reproductive output and oviposition behavior of T. romana from France and Spain under laboratory conditions.

2. Materials and methods

2.1. Experimental organisms

2.1.1. Arundo donax

Arundo donax is a rhizomatous perennial grass, up to 8 m tall, that thrives in southern temperate and subtropical North America in riparian zones along river systems, reservoirs, and canals, forming impenetrable thickets in floodplains (Everitt et al., 2005). It can grow on wide range of soils, from loose gravelly and sandy substrates to heavy clays and river sediments (Perdue, 1958; Spencer et al., 2008), of varying fertility (Quinn et al., 2007; Spencer and Ksander, 2006), and can tolerate a broad range of environments (ossa et al., 1998; Decruyenaere and Holt, 2001; Quinn and Holt, 2008).

New shoots emerge throughout the year in warm climates such as southern Spain and the LRGB of Texas and Mexico. Both rainfall and temperature influence shoot production by rhizomes, which often constitute over half of total biomass (Spencer et al., 2006; Thorby et al., 2007; Spencer et al., 2008). Clonal populations expand rapidly as rhizomes grow and produce new shoots (Thorby et al., 2007). Rooting from plagiotropic (horizontally layered) stem canes and broken stem fragments is also common (Wijte et al., 2005; Boland, 2006). Rhizomes and canes establish new populations after being uprooted and dispersed by flooding (Bell, 1997). Shoots become woody within the first year of growth and produce many axillary shoots. Giant reed produces large (30–60 cm) pani-
cles from main shoots and occasionally side shoots throughout the year, but in the invaded range the seeds are sterile (DiTomaso and Healy, 2003). Panicle formation leads to shoot senescence. Senescing and dead shoots remain standing in many cases. Fire kills shoots and breaks down dead biomass, but also favors generation of new shoots (Rieger and Kreager, 1989).

Despite the clonal nature of the giant reed invasion of North America, multiple introductions may have occurred, and candidate natural enemies that are most likely to be adapted to local genotypes should be selected (Briese, 2004; Goolsby et al., 2006). A population genetics analysis (D. Tarin, A. Pepper, J. Manhart, unpublished data) is underway to determine the geographic points of origin of the small number of A. donax genotypes present in the LRGB. Initial results indicated points of origin within Spain. Tetramesa romana was therefore collected from Spanish locations, as well as from a population near the USDA-ARS, European Biological Control Laboratory (EBCL) in coastal southern France.

2.1.2. Tetramesa romana

The wasp T. romana (Fig. 1A) is in the family Eurytomidae (Claridge, 1961). Noyes (2004) describes the Family Eurytomidae, focusing mostly on Palearctic fauna. The subfamily Eurytominae includes Tetramesa (Lotfalizadeh et al., 2007). Claridge (1961), Noyes (2004), and Lotfalizadeh et al. (2007) provide morphological features to separate the genus Tetramesa from other eurytomid genera. Illustrations of the adult stage may be found in Zerova (1976). Diagnostic information for the adult stage was provided by G. Delvare (CIRAD, Montferrier-sur-Lez, France, unpublished data) based on examinations of a lectotype in the Natural History Museum (London) and adults collected from France, Spain and Morocco. Observations on T. romana larval morphology and feeding behavior were made by A. Cohen, Insect Diet and Rearing Research, Raleigh, North Carolina (personal communication). Fig. 1 shows photographs of the adult female at rest and exhibiting abdomen-curling indicative of oviposition probing behavior, and also shows the egg, larval and pupal stages of T. romana.

Our native range collections (Kirk et al., 2003) and laboratory host range studies (Goolsby and Moran, 2009) indicate that T. romana is host-specific to Arundo spp. Stimuli provided by the parent wasp or by eggs lead to the development of gall tissues (Price, 2008), as the gall begins to form before the larvae hatch. Extra-oral digestion may occur, as the larvae appear to secrete liquids onto the gall tissue and then use their mandibles to rasp and collect gall tissue (A. Cohen, personal communication). At least two parasitoids attack T. romana in the Mediterranean region: the pteromalid Gugolzia harmolitae Delucchi & Steffan and the eurytomid Eurytoma steffani Claridge (Steffan, 1956).

2.2. Plant and insect cultures

2.2.1. Plants

Arundo donax rhizomes were collected in Laredo and San Juan, Texas, at sites adjacent to the Rio Grande. The majority (70%) of plants used in developmental and reproduction studies were from Laredo, while observations of oviposition behavior involved plants from San Juan. Rhizomes of A. donax were either potted immediately or stored at 10 °C at the USDA Animal and Plant Health Inspection Service (APHIS)-Pest Detection, Diagnostics and Management Laboratory (PDDML) in Edinburg, Texas. Rhizomes were potted in 4-L pots containing 80% peat moss soil with perlite (‘Sunshine Mix No. 1’, Sun-Gro Horticulture, Bellevue, WA). Pots were placed in a greenhouse regulated between 25–32 °C and 70–90% relative humidity (RH) with ambient light (12–14 h daylight per day). Pots containing one to three A. donax shoots were transferred to the quarantine laboratory at the PDDML for exposure to T. romana after 4–6 weeks of growth, at which time shoots were 0.5–1.0-m tall with 0.7–1.3 cm thick main shoots and no lateral shoots.

Fig. 1. Tetramesa romana biology. (A) Adult female (bar = 5.33 mm). (B) Abdomen-curling behavior indicative of stem probing and oviposition. (C) Swollen A. donax shoot tip indicative of galling with exit holes made by emerging adults. (D) Ovaries dissected from newly emerged female showing numerous eggs with stalk-like pedicels (bar = 1 mm). (E) Third-instar larva (bar = 5.95 mm). (F) Pupa (bar = 6.05 mm).
2.2.2. Wasps

_Tetramesa romana_ wasps from Perpignan, France (accession number M06008), and the area surrounding Granada, Spain (accession numbers M07064 and M07074) were reared on _A. donax_ by confining 2–4 newly emerged females in cylindrical cages (40 cm long by 12 cm wide) made of black silk organza with a metal wire frame, secured to the _A. donax_ shoot at the top and bottom with twine. Two cotton balls, each saturated with purified water and one drop (ca. 0.5 g) of honey, were placed in leaf blade axils inside the cage as an adult food source. Wasps were held on shoots for 1–3 days in a quarantined greenhouse regulated between 20–30 °C and 60–80% RH with ambient light (400–500 µmol m⁻² s⁻¹ at ca. 2 m above the ground at noon) supplemented between November and March by sodium halide lights (total 14:10 L:D cycle). The cages were lightly misted with water once per day. After wasp and cage removal, shoots were held in a growth chamber set to a constant 27 °C, 70% RH and 14:10 L:D cycle. Cages were placed on galled shoots 25 days after parent wasp removal. Newly emerged wasps were collected daily for use in studies of _T. romana_ development, adult survival, reproductive output and behavior.

2.3. Development and generation time of _T. romana_

To determine the size and duration of the life stages of _T. romana_, _A. donax_ shoots (0.5–1 m tall) were each infested with 2–4 female wasps as for rearing, except that females were exposed to shoots for only 1 day. The approximate time from oviposition to egg hatch was deduced from the appearance of larvae in dissected shoots 4–5 days after oviposition. Shoots were dissected daily from the 4th to the 33rd day after parent wasp removal (1–9 galled plants dissected each day, average (±SE) of 4 ± 2 plants per day, total of 155 plants dissected). Larval and pupal length (at 12.5 × or 25 ×) and larval head capsule width (at 50 ×) were measured with an ocular micrometer installed in the right-hand lens of a Leica MZ16 dissecting microscope, or with a digital micrometer and Leica Application Suite software (Version 2.7.1) (Leica Microsystems, New York, NY). Four to eighty larvae were measured per day of development (average of 30 ± 4 larvae per day), and a total of 707 larvae were measured. Two to sixty-four pupae were measured per day, beginning on the 17th day after oviposition (average of 19 ± 3 pupae per day) and a total of 286 pupae were measured. On separate shoots that were left undissected, the time from release of the parent wasp onto the shoot to emergence of new adults was recorded to determine total generation time. The length of 34 adult females with and without antennae were measured with the digital micrometer, as were five eggs proximal to the oviducts that were dissected from each of 10 newly emerged adult females. The total number of eggs in the ovaries was counted.

2.4. Adult female longevity, reproductive output, and gall position

Newly emerged adult female _T. romana_ wasps were caged individually as for wasp development tests and were transferred to new _A. donax_ shoots daily. Adult males (recognizable by their long, setose antennae) were rarely collected from quarantine colonies; when available they were paired with individual females in cages. A total of 229 Perpignan (French) females and 69 Granada (Spanish) females were caged. The longevity (in days) of each female was noted. The shoot tip of each _A. donax_ plant exposed to a wasp was marked with a felt tip pen immediately after female removal. Shoots were maintained in a growth chamber at 27 °C and were re-caged after 25 days. New adults were collected from galled shoots daily. About 45–50 days after parent wasp removal, the shoots were dissected and larvae, pupae, and non-emerged live adults were counted. Exit holes in the stem were counted, and their position noted relative to the shoot tip as marked at the time of parent wasp removal.

2.5. Oviposition behavior

To examine relationships between wasp oviposition behavior and reproductive success or time of day (morning or afternoon), individual _T. romana_ wasps from Perpignan, France were observed in cylindrical cages in a greenhouse maintained as above for up to 8 h per day for up to the first 5 days of their adult lives. Adults were transferred to new shoots daily, prior to the start of the next daily observation period. The number of abdomen-curling events indicative of probing were counted, and time (minutes) spent in probing behavior (Fig. 1B), resting on the stem, resting on the leaf, resting on the cage, and resting on a cotton plug containing honey and water were summed across the wasp’s lifetime, and converted to proportions by dividing the total time of observation for each wasp. Wasps were observed for a total of 177 h in the morning (08:00–12:00) (mean ± SE, 4.8 ± 0.5 h per wasp over its lifetime) and 267 h in the afternoon (13:00–17:00) (6.1 ± 0.5 h per wasp).

2.6. Data analysis

All analyses used SAS software (Version 9.1.3) (SAS Institute, 2004). Larval and pupal morphological measurements were averaged by day and graphed. Decreases in average larval head capsule width and larval length, which interrupted the general upward trends over time, were used to infer larval transition times and the duration of instars, based on the assumption that apolysis and ecdysis during molting interrupt larval growth in the pharate larva of the next instar (Chapman, 1982). Head capsule widths and larval lengths were compared across instars with multivariate and univariate analyses of variance (ANOVA), using measurements of 74 first instars, 113 second instars, and 520 third instars. Wilk’s lambda was used to infer multivariate significance with normal data distribution assumptions. The mean number of days from female parent wasp removal to collection of pupae from shoots was used to infer the larval–pupal transition time. The number of days post-oviposition at which individual pupae were collected as well as generation time (oviposition to new adult), were compared between French and Spanish wasp populations with ANOVA.

Adult longevity was determined from tests with individual wasps in cages by counting the number of days between wasp emergence and death, and was compared between French and Spanish wasps using ANOVA. Wasp survival over time was compared between populations with SAS PROC LIFETEST using an estimated log-rank _χ²_ test. The percentage of wasps that produced offspring was compared between French and Spanish wasps and among wasp ages with maximum-likelihood _χ²_ tests (SAS PROC FREQ). The reproductive output of individual wasps was compared between countries of origin using ANOVA. The intrinsic reproductive rate _r_ was determined by the equation _r = ln(R0/T_ (Begon et al., 1990)), where _R0_ is average reproduction per female (including all females that failed to produce) and _T_ is generation time. Population doubling time (_T_d_) was determined by the equation _T_d = T × (log(2))/log(q1/q0)) where _q1_ is size of the parent wasp cohort and _q0_ is total number of wasps produced. This equation assumes a constant net population growth rate. The distributions of wasp exit holes on the stem, relative to the location of the shoot tip at the time of female parent removal, were compared between French and Spanish wasps using a Kolmogorov–Smirnov test (SAS PROC NPAR1WAY).

Wasp behavior was compared between 23 French wasps that were reproductively successful at least once in their lives and 21 non-productive wasps. Most (92%) observations involved wasps that were 3 days old or less. Differences in counts of probing events
and in proportional ‘time budgets’ were assessed using PROC GLIMMIX in SAS with a Poisson (probe count) or binomial (time budget) data distribution specification, and a random residual term (time budget data only). A similar approach was used to compare lifetime wasp behavior between morning and afternoon observation periods. A total of 37 wasps were observed in both periods, with seven additional wasps observed only in the afternoon. To present both the reproductively successful/non-productive and morning/afternoon comparisons (Table 1), the number of probes and minutes spent in the five behaviors were summed across all wasps observed in each category, and time measurements were then divided by the total observation time for all wasps in that category.

3. Results

3.1. Characteristics of T. romana

3.1.1. Immature stages

Adult T. romana females (Fig. 1A) deposited eggs into the stem wall or cavity by curling their abdomen (Fig. 1B) and inserting eggs with their needle-like ovipositor, leading to the formation of gall tissue and shoot distortion (Fig. 1C) within 2 weeks. The eggs of T. romana were creamy-white, with a hook-like pedicle on one end (Fig. 1D) and a short hair on the micropylar end. The eggs without the pedicle averaged (±SE) 0.39 ± 0.07 mm long, and the total egg length with pedicle was 1.26 ± 0.08 mm long (range 1.05–1.39 mm, 47 eggs measured). The larvae hatched 4–5 days after oviposition, and gall tissue was already forming around the eggs and larvae at that time. Gall tissue was found at the shoot apical meristem, a white band of tissue located directly below the leaf primordia, or within 3 cm below the meristem. Young gall tissue was white, while older material (observed more than 2 weeks after oviposition) was yellow. Gall tissue filled the stem cavity, causing maturity shoot distortion within 2 weeks of oviposition (Fig. 1C). However, on main shoots greater than ca. 1 cm in diameter, gall formation often occurred with no visible shoot distortion. The larvae (Fig. 1E) were white-yellow and partially translucent, averaging 3.65 ± 0.06 mm long (range 0.4–8.6 mm, 707 larvae measured) and had head capsules averaging 0.42 ± 0.004 mm in width (range 0.10–0.64 mm) with poorly developed bidentate mandibles. The larvae were apodous and moved by undulations of the body wall. In dissections, the gut appeared blind, with no connection between the hindgut and the presumptive anal area. The head is globose in dorsal view, with long temples. The distance between the lateral ocelli is small in comparison to other Holarctic species of this genus. The flagellum is filiform with elongate segments, quite longer than broad. The funicle is five-segmented. The head and pro- and mesonotum have long suberect pilosity. The pronaular collar and midlobe of the mesoscutum are coarsely rugose-punctured. The pronotal collar is somewhat depressed anteriorly in the middle. The pronotum is long and shoulder-like anteriorly. The nota Eli, located on the mesoscutum, are deep, wide, and crenulate. The metasoma (gaster) is subelliptical, elongated and has an acute apex. In colonies, males were readily distinguished from females by their approximately 2-fold longer setose antenna. Adult female wasps averaged 5.38 ± 0.55 mm long without antennae and 6.77 ± 0.06 mm long with antennae (range 5.14–7.73 mm, 34 Spanish wasps measured). The female: male sex ratio for wasps produced over several generations from a colony of French wasps was 9:1, while for Spanish colony progeny the sex ratio was 26:1. Newly emerged and 1-day-old females, never exposed to A. donax, contained an average (±SE) of 40 ± 3.1 eggs (range 1–58 eggs, 20 Spanish females dissected).

3.2. Immature development

The development study combined data for larvae reared from French and Spanish wasps. The number of larval instars was inferred by inflections (declines or plateaus) in daily mean head capsule width, and the pattern of increase of larval length (Fig. 2A) paralleled that of head capsule width (Fig. 2B). The first instar lasted 5 days (days 4–8 inclusive post-oviposition), the second instar 5 days (days 9–13), and the third instar 12 days (days 14–25). Larvae were classified into instars on the basis of these time ranges, and length and head capsule width measurements differed significantly across the three instars in a multivariate ANOVA (W = 0.376, F = 221.7, df = 4, 1406, P < 0.0001) (Fig. 3A). The measurements for some of the larvae classified on the basis of time as third instars grouped closely with the measurements on first and second instars (Fig. 3A). In univariate analyses, mean larval...
length varied significantly across instars ($F = 282.2$, $df = 2, 704$, $P < 0.0001$) (Fig. 3B), as did mean head capsule width ($F = 534.4$, $df = 2, 704$, $P < 0.0001$) (Fig. 3B). The average time to pupation was 26.0 ± 0.2 days (median 27 days, 286 pupae observed), although pupae were observed as early as the 17th day after oviposition (Fig. 2A). Spanish larvae reached pupation 23.6 ± 3.6 days after oviposition (median 23 days), 4 days faster than French larvae, which required 27.3 ± 2.3 days (median 27 days) ($F = 107.2$, $df = 1, 284$, $P < 0.0001$). The pupal development time for wasps from both countries was estimated to be 7 days, on the basis of a total larval development time of 26 days and a total generation time (see below) of 33 days.

3.3. Generation time

The average time from the day of parent wasp oviposition to emergence of adults from galls was 33.3 ± 0.1 days (2133 wasps emerged, median time 33 days). However, generation time varied between a minimum of 25 and a maximum of 50 days. Spanish wasps had a marginally longer generation time (33.9 ± 0.1 days, median 33 days; 878 wasps reared) than did French larvae, which required 27.3 ± 2.3 days (median 27 days) ($F = 107.2$, $df = 1, 284$, $P < 0.0001$). The pupal development time for wasps from both countries was estimated to be 7 days, on the basis of a total larval development time of 26 days and a total generation time (see below) of 33 days.

Fig. 2. Daily averages (±SD) of Tetramesa romana (A) larval and pupal length and (B) larval head capsule width, in galls dissected 4–33 days after oviposition. Brackets indicate inferred time durations of instars.

Fig. 3. (A) Association between larval length and head capsule width of T. romana, with observations grouped into three instars on the basis of larval age at the time of dissection. (B) Average (±SE) length and head capsule width compared between instars. Means with different letters for each measurement are significantly different in analyses of variance ($P < 0.0001$).

3.4. Adult longevity, reproduction, and gall position

3.4.1. Longevity

Adult wasps confined in cylinder cages on A. donax shoots and transferred daily lived an average (±SE) of 3.7 ± 0.1 days (median 3 days, maximum of 10 days) (260 wasps observed). Wasps reared from Spanish populations lived 1.3 days longer (4.7 ± 0.3 days, median 4 days, 69 wasps observed) than French wasps (3.4 ± 0.1 days, median 3 days, 191 wasps observed) ($F = 19.9$, $df = 1, 259$, $P < 0.0001$). French wasps had only a 13% probability of being alive 6 days after emergence, while Spanish wasps had a 32% chance of surviving 6 days. The survival function for Spanish wasps was significantly different from that of French wasps (log-rank test, $\chi^2 = 16.4$, $df = 1$, $P < 0.0001$) (Fig. 4).

3.4.2. Frequency of reproductive success

Across all 298 T. romana wasps isolated individually on A. donax beginning on the day of emergence, 43% produced offspring at least once. A greater proportion of Spanish wasps (65%, $n = 69$) were reproductive than were French wasps (35.8%, $n = 229$) ($\chi^2 = 18.7$, $df = 1$, $P < 0.0001$). Twenty-four of the 82 reproductive French wasps produced offspring on more than one shoot, 10.5% of all French wasps tested. Out of 45 reproductive Spanish wasps, 25 produced offspring on more than one shoot, 36.2% of all Spanish wasps tested. On separate A. donax shoots, two to four French or Spanish females were isolated inside each cage. The reproductive success rate differed significantly between 41 shoots caged with two wasps, of which 34% developed galls, 148 shoots caged with three wasps, of which 65.5% developed galls, and 90 shoots caged...
with four wasps, of which 48.9% developed galls ($\chi^2 = 15.3, df = 2, P = 0.0005$). Wasp age also influenced reproduction ($\chi^2 = 46.6, df = 10, P < 0.0001$). Of 573 A. donax shoots exposed to French or Spanish wasps that were 4 days old or less, 182 (31.8%) developed galls and yielded wasp progeny, while only 16 of 153 shoots (10.5%) exposed to wasps that were 5 days old or older yielded progeny. The total offspring produced by the colonies declined by 40% or more each day beginning with 3-day-old wasps (Fig. 5).

### 3.4.3. Reproduction by individual wasps

The mean (±SE) lifetime reproductive output of reproductively successful wasps was 21.2 ± 1.2 offspring, including both adult wasps and larvae/pupae still in the stem at the time of dissection. A typical French wasp was exposed to three shoots in its lifetime and a typical Spanish wasp to four shoots, beginning on the day of emergence. The average reproductive output per shoot in a total of 182 tests involving successful female wasps from both countries exposed to new shoots daily within the first 4 days of their lifetimes was 14.1 ± 0.7 offspring (maximum = 42). Reproductively successful Spanish wasps ($n = 45$) produced more adult and larval offspring over their lifetimes (26.0 ± 2.4, maximum = 66) than did French wasps ($n = 82$) (18.7 ± 1.2, maximum = 55) ($F = 9.13, df = 1, 125, P = 0.003$) and the same was true when only adult offspring were considered (Spanish wasps, 23.4 ± 2.4; French wasps, 16.6 ± 1.2; $F = 7.67, df = 1, 125, P = 0.007$). The number of exit holes did not correspond exactly to the number of adult offspring collected (Spanish wasps, 20.2 ± 2.1 holes; French wasps, 15.0 ± 1.2 holes), suggesting that more than one wasp used the same exit hole in a few cases. Considering both reproductively successful and unsuccessful wasps, lifetime output was 6.7 ± 0.7 offspring per French wasp and 17.4 ± 2.2 offspring per Spanish wasp. The intrinsic rate of increase $r$ for the entire cohort of 229 French wasps was 0.054 and the population doubling time (DT) was 13.4 days. For the cohort of 69 Spanish wasps, $r = 0.081$ and the doubling time was 9.2 days. Eighty percent of the offspring of French and Spanish wasps combined were produced within the first 4 days, beginning on the day of emergence (Fig. 5), and the number of offspring per female (both successful and non-productive wasps included) varied significantly according to wasp age ($F = 3.81, df = 8, 711, P = 0.0002$) (Fig. 5). However, three Spanish wasps produced a total of 50 offspring when they were 9 days old.

### 3.4.4. Position of galls on shoots

Across 186 A. donax shoots that had been exposed to French (104 shoots) or Spanish (82 shoots) wasps and were then dissected after progeny emerged, 82% of the 2075 exit holes indicative of gall formation were found at the apical node marked at the time of parent wasp removal, or within two nodes above the tip. The distribution of exit holes across nodes differed between French and Spanish wasps (Kolmogorov–Smirnov test, adjusted KS = 4.27, $P < 0.0001$) (Fig. 6). Spanish T. romana wasp offspring produced exit holes 19% more frequently at the shoot position located one or more new nodes above the apical node at oviposition than did French wasps (Fig. 6).

### 3.5. Oviposition behavior

Abdomen-curling behavior, suggestive of ovipositor insertion into the stem (Fig. 1B), was frequently observed in T. romana females confined on A. donax stems in cages. Wasps spent the remainder of their time resting on the stem, leaf blade, cage material, or cotton plug containing honey, with short (1–2 s) bouts of...
flight. Wasps that reproduced at least once in their lifetime made 2.5-fold more probes than did non-productive wasps \( (F = 181.1, df = 1, 42, P < 0.0001) \), but did not differ from non-reproductive wasps in proportion of time budgeted to probing or other behaviors \( (P > 0.38) \) (Table 1). The mean length of individual probing events did not differ on the basis of reproductive success (successful wasps, \( 3.3 \pm 0.4 \) min, non-productive wasps, \( 3.3 \pm 0.2 \) min, \( F = 0.04, df = 1, 1129, P = 0.834 \)). Wasp observed in the afternoon made 4.3-fold more probes \( (F = 283.6, df = 1, 79, P < 0.0001) \) and spent 13% more time probing \( (F = 17.2, df = 1, 79, P < 0.0001) \) and 9% more time resting on the stem \( (F = 14.0, df = 1, 79, P = 0.0003) \), than did the same wasps in the morning (Table 1). Wasp spent 21% less time resting on leaf blades in the afternoon \( (F = 9.35, df = 1, 19, P = 0.003) \) while time spent resting on the cage or on a honey plug did not differ between observation periods \( (P > 0.15) \).

4. Discussion

4.1. Characteristics of \( T. \) romana

Our diagnosis of the adult stage of \( T. \) romana is consistent with but builds upon past descriptions of this species (Steffan, 1956; Claridge, 1961; Zerova, 1978). Little prior information was available on immature stages of \( T. \) romana. The egg pedicle, a structure 2- to 3-fold longer than the egg itself (Holmes and Blakeley, 1971), could serve as a respiratory conduit between the stem surface and egg during egg development, as in cynipid gall-forming wasps (Askew, 1984) or may facilitate oviposition (Várdal et al., 2003). The larvae of \( Tetramesa \) spp. have few features to separate species (Roskam, 1982), but high host specificity is expressed in larval feeding (Claridge, 1961; Dawah, 1987). The large size of the adult female of \( T. \) romana (well over 5 mm) relative to other Palearctic \( Tetramesa \) spp. was noted by Steffan (1956). The female-biased sex ratios and fully developed eggs we observed in newly emerged French and Spanish \( T. \) romana agree with past findings (Phillips, 1936; Claridge, 1961) that reproduction by \( T. \) romana occurs via thelytokous parthenogenesis (Askew, 1984), with diploid females developing from unfertilized eggs. The addition of occasional \( T. \) romana males to cages containing females did not alter female-biased sex ratios in progeny, suggesting that males are sterile.

4.2. Development of \( T. \) romana

The identification of three larval instars in \( T. \) romana is the simplest explanation for the observed patterns of increase in larval head capsule width and body length. No prior information of comparable detail is available to validate our conclusions about the number and duration of the instars and of the pupal stage (Phillips, 1936; Claridge, 1961; Al-Barrak et al., 2004). The wasp \( Eurytoma tumoris \) Bugbee galling Scots pine \( (Pinus sylvestris L.) \) was reported to have four instars (Stark and Koehler, 1964), and larvae of a \( Eurytoma \) sp. leafminer evaluated for control of mother-of-millions weed \( (Bryophyllum dela Gomez) \) (Ecklon and Zeyher)) completed five instars (Witt et al., 2004). Some \( T. \) romana larvae classified as third instars, based solely on the number of days between oviposition and dissection, may have in fact been second instars developing more slowly than normal, due perhaps to inferior tissue quality or quantity at the edges of gall. The more than 2-fold longer duration of the third as compared to the first and second instar of \( T. \) romana may facilitate developmental plasticity in responses to environmental cues. Under field conditions, final larval instar may develop rapidly during the long warm season of Mediterranean Europe and the LRGB, where summer daily temperatures average 25-35 °C, and where winter temperatures may be high enough to permit year-round wasp activity. In contrast, the penultimate instar or the pupa is the overwintering stage in temperate pest \( T. \) romana in North America (Davis, 1918; USDA, 1954; Salt, 1971), and the third instar may serve the same role in \( T. \) romana in temperate regions such as southern France.

4.3. Generation time of \( T. \) romana

The 33-day generation time of \( T. \) romana wasps maintained at 27 °C suggests that the number of generations per year in the field after release are likely to be more than the one to two generations per year reported for other \( Tetramesa \) spp. in Britain (Claridge, 1961; Dawah et al., 1995) and temperate North America (Davis, 1918; Knowlton and Janes, 1933; USDA, 1954; Shanower and Waters, 2006). In the LRGB, average daily temperatures are likely to be near or above 27 °C for at least 4–5 months of the year, suggesting that \( T. \) romana will complete at least five generations per year under field conditions. The variable generation time in laboratory-reared wasps (ranging from 17 and 50 days after oviposition) likely reflected variable development rates of third-instar larvae. In the field, variation in development times may buffer \( T. \) romana against environmental extremes. Galled \( A. donax \) shoots hosting an adventive population of \( T. \) romana in Austin, Texas cut under winter conditions (average daily temperature 5–15 °C) and maintained at 25 °C yielded adult wasps up to 2 months after cutting (J. Goolsby and P. Moran, unpublished data), suggesting that reduced development rates of the final instar will characterize the life cycle of \( T. \) romana in the mild winter climate of the LRGB.

4.4. Adult longevity of \( T. \) romana

The survival duration of French and Spanish \( T. \) romana reared under growth chamber and greenhouse conditions (25–30 °C) is short relative to those reported from field studies on other \( Tetramesa \) species (USDA, 1954; Claridge, 1961). However, survival times for these species were based on observations of populations, rather than individuals. Daily laboratory handling may have shortened adult female wasp survival relative to what could be expected in the field. Resource provisioning may have also influenced survival. The dietary requirements of adult \( Tetramesa \) are unknown (Claridge, 1961), but could involve floral nectar as a carbohydrate source. Honey-water was provided in this study. For mass-rearing purposes, additional resources such as yeast lysate may extend adult lifetimes (Cohen, 2004) and thereby increase reproduction, as well-developed eggs were found inside female \( T. \) romana that had died within 4 days of emergence. The longer survival time of Spanish as compared to French wasps suggests that Spanish populations will be more suitable for mass-rearing.

4.5. Reproduction of \( T. \) romana

4.5.1. Frequency of reproduction

Despite the fact that reproduction was parthenogenetic, females varied in their ability to reproduce. Almost twice as many Spanish females reproduced as French females, again suggesting that the Spanish population is better-suited to large-scale rearing. The cause of reproductive failure is uncertain. Almost all newly emerged females contained eggs, though some eggs may have contained a non-viable zygote. Variation in stem diameter, which can influence oviposition (Dubbert et al., 1998) may have affected success, if non-reproductive wasps, which made fewer probes than reproductive wasps, were confined on thinner stems. Alternatively, non-reproductive females may have deposited eggs into stem locations that were unsuitable for gall formation. The fact that galling rates differed but were not consistently lower on shoots exposed to groups of 2–4 Spanish wasps compared to a single wasp suggests that inductive releases will not lead to competitive interference. About one-third of the Spanish wasps demonstrated an ability to
induce gall formation on more than one shoot during their short lifetimes, an important attribute since both primary and axillary shoot production by giant reed is profuse. The declines in galling ability in long-lived wasps (more than 5-days old) could reflect egg depletion, insufficient egg provisioning due to poor adult nutrition, or age-related reduced muscular capacity to penetrate the shoots of A. donax.

4.5.2. Reproductive output

A single reproductive T. romana wasp produced 20–30 offspring parthenogenetically, showing the potential to increase population size 20-fold or more, even if a female galls only one shoot in its lifetime. Even taking unsuccessful wasps into account, the intrinsic rate of increase and doubling time of the Spanish T. romana population are comparable to those of the saltcedar leaf beetle (D. elongata deserticola) (Lewis et al., 2003) which has been released successfully in the arid western US to control saltcedar (Tamarix spp.; Tamaricaceae), exotic invasive trees which often co-occur with A. donax in the LRGB. The reproductive output of T. romana is lower than the fecundity, expressed as production of 70 eggs per female, reported for wheat jointworm (T. tritici) (USDA, 1954), but production of adults is a more reliable measure since it is likely that not all eggs survive. When provided a new A. donax shoot daily, the average Spanish wasp produced 40% more adults than did the average French wasp, in contrast to host range tests (Goolsby and Moran, 2009), in which wasps were confined on one shoot from emergence until death.

4.5.3. Position of galls on shoots

Tetramesa romana females, especially those from Spain, strongly preferred shoot tips for oviposition, suggesting that these tissues are uniquely susceptible to gall induction. Other Tetramesa species show a similar preference, including T. petiotata (Al-Barrak, 2006) on Deschampia cespitosa (L.) P. Beauv., as well as the wheat jointworm T. tritici (USDA, 1954) and the rye jointworm T. secale (Holmes and Blakeley, 1971). Attack on shoot meristematic tissues could enhance the biological control impact of T. romana (Tracy and DeLoach, 1999), as in the case of a ceceomyiid gall-making fly (Rhopalomyia n. sp.) targeting scentless chamomile (Tripleurospermum perforatum (Merat) Lainz) (Hinz, 1998).

4.6. Oviposition behavior

Reproductive wasps made more probes of A. donax stems than non-reproductive wasps, but did not differ in overall time allocation to probing or in length of probing events. Reproductive wasps may have been more sensitive to plant-derived physical or chemical cues that stimulated at least some of the elements of probing. Some probes may have served to sample host tissues rather than to deposit eggs. The mean length of probing events and proportion of time budgeted to probing did not differ on the basis of reproductive success, suggesting that the extra probes made by successful wasps were not costly in terms of time. Variation in probing behavior tied to reproductive success could reflect variation in reproductive structures or host perception, perhaps tied to the quality/quantity of gall tissues available to the adults when they were larvae (Awmack and Leather, 2002). Relatively large females reared on large galls had the highest fecundity in the cynipid wasp Aphelonyx grandulifera (Monzen) (Ito and Hijii, 2004), and T. romana females varied in size by about 35% around the mean value. Adult nutrition was not likely involved, as reproductive and non-reproductive females did not differ in time spent on honey-provisioned cotton. Wasps spent more time in probing and stem-resting behaviors in afternoon than in morning observations, perhaps as a sensory response to variable ambient light, temperature, or moisture levels.

5. Conclusions

The rapid immature development, short generation time and prolific asexual reproductive capacity of the stem-galling wasp T. romana support its candidacy for field release as a biological control agent against giant reed (McCly and Balciunas, 2005; van Klinken and Raghu, 2006), in combination with the wasp’s high degree of host specificity (Goolsby and Moran, 2009), its abundance and wide distribution in its native range (Kirk et al., 2003) and its ability to increase shoot mortality in quarantine tests (J.A. Goolsby, unpublished data). Establishment and efficacy predictions for weed biological control agents require knowledge of plant demography (Müller-Schärer and Schaffner, 2008). The life history traits of T. romana, as revealed here on young shoot tips, in combination with the perennial vegetative development of giant reed populations in California (Spencer and Ksander, 2006) and the LRGB, suggest that conditions for wasp establishment will be favorable. Plasticity in the life cycle of T. romana, mediated in part by variable immature development time, may allow the wasp to establish under variable temperature regimes, as shown by the existence of adventive T. romana populations in both southern California (Dudley et al., 2006) and in Austin and Laredo, Texas (J.A. Goolsby, unpublished data). The longer adult longevity and greater reproductive output of wasps from Spain as compared to France suggests that Spanish wasps are more suitable for the mass-rearing that will be necessary for field releases in the Lower Rio Grande Basin of the US and Mexico.

Acknowledgments

We thank Alan and Gerhild Kirk for conducting surveys in over 15 countries for T. romana and for collection of the wasp from France and Spain. We thank Crystal Salinas, Ann Vacek and Sandra Espinoza for technical assistance, and Joe Balciunas, Michael Gates, and two anonymous reviewers for critical reviews. This work was supported in part by a grant from the US Department of Homeland Security, Science and Technology Directorate, and by the Lower Rio Grande Valley Development Council.

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