Completion Report: Biological control of invasive giant reed (*Arundo donax)*

For: U.S. Fish & wildlife Service/Santa Clara River Trustee Council, 31 December 2007

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EXECUTIVE SUMMARY

1. Three non-indigenous insects, the stem-boring wasp *Tetramesa romana*, the shoot-fly *Cryptonevra* sp., and an aphid, *Melanaphis donacis*, were common on *Arundo donax* in the Santa Clara River system and other *Arundo*-infested sites throughout much of southern California and into northern Mexico.
2. Feeding by these three herbivores causes moderate, but significant damage to *A. donax* and *T. romana*, in particular, appears to inflict sufficient damage to young shoots and secondary branching stems that it has a substantial negative effect on target weed production and biomass. Controlled experimental studies are underway at UCSB to quantitatively document the magnitude of herbivory by *T. romana.*
3. No utilization of native or economically beneficial hosts *T. romana* was documented, except for a minor horticultural plant from the same genus, indicating a high level of specificity by this herbivore and safety with respect to potential non-target effects.
4. Populations of *T. romana* can be established in new locations and regional redistribution studies are on-going; therefore, we recommend that this insect be transferred, with appropriate County Agriculture Commission authority, to Arundo infestations in the State of California.
5. Preliminary genetic studies indicate that there is little differentiation between non-indigenous North America *T. romana* and European populations, and variation in impacts observed among southern Californian sites suggest that environmental, not genetic factors are responsible for differences in impact; therefore, we do not recommend that this insect be imported because the regulatory procedures for re-distribution of existing insects are less complex and more readily met than those necessary for importation of insects from outside North America. Current populations can potentially be augmented to provide greater impact, but we believe that reduction of the negative effects of *Arundo* invasion using biological control will rely on development and importation of new organisms (herbivores and pathogens) to enhance the impact of existing herbivores.
6. Ecological studies indicated that *A. donax* does not propagate by seed production, but does negatively affect abundance of native riparian vegetation at the Santa Clara River, and its litter is inadequate to support aquatic insects important to native fish. These results provide further evidence of the negative consequences of A donax invasion, and justify its control using biological or other methods.

Biological control of invasive giant reed (*Arundo donax)*

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With Appendix of SCRTC-supported research by Alan A. Kirk
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Approach and Summary

The basic goal of this research program was to determine the ecological impact of *Arundo donax* on coastal riparian ecosystems and evaluate the potential for biological control to be an effective tool for the control of this invasive weed. This is an continuing, multi-agency effort with the intent of facilitating the development and subsequent implementation of *A. donax* biocontrol in the western U.S. We used the Santa Clara River as our focal study system, but also worked with riparian systems throughout California and adjacent states. Our objectives were:

1) Quantify level of herbivore damage to the target plants, including plant mortality;
2) Verify that agents do not feed on nor infect native and economically important plants;
3) Evaluate potential for large-scale rearing, transport and introduction into infested field locations;
4 Conduct baseline vegetation analyses to document impacts to target plants and ecosystem responses following eventual release of biocontrol agents;
5 Develop scientific and public information to facilitate public acceptance of, and participation in, the biocontrol and other habitat restoration actions.
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To provide a fuller picture of existing herbivores, both native and non-indigenous, of North American *Arundo*, sampling was conducted in riparian ecosystems of the major watersheds of southern California, with particular focus on the coastal counties (Santa Barbara, Ventura, Los Angeles, Orange, and San Diego) and spot sampling in other counties throughout

California and adjoining states. These surveys have yielded three insect taxa that are characteristically associated with *Arundo*, all exotic in origin. These cause significant, if not dramatic, damage to the host plant suggesting that their wider dissemination could potentially provide some suppression of *Arundo*, particularly in combination with additional specialist natural enemies and/or pathogens to enhance target impact.

Two of these insects, a stem-boring herbivorous wasp, *Tetramesa romana*, and a shootfly, *Cryptonevra* sp., are currently under evaluation by the United States Department of Agriculture (USDA - Weslaco, TX) for possible release. Our field collections suggest that both only attack *Arundo*, and experimental tests with *T. romana* indicate it is host specific. Our recommendation is that further testing with foreign material may be unproductive given the current existence of these taxa in North America. In particular, testing with foreign *T. romana* could be terminated because genetic tests indicate the California insect is the same as that proposed for introduction. Results from studies (supported, in part, from SCRTC funds) by our USDA-ARS collaborator and Co-Principal Investigator, Dr. Alan Kirk, at the European Biological Control Laboratory (EBCL) are summarized in Appendix 1.

We have also sampled *Arundo* and associated vegetation intensively in the Santa Clara and Ventura Rivers (sampling and study sites illustrated in Fig. 1) to better understand its growth dynamics, to evaluate the impacts of herbivory, and to document its community relationships where it infests native riparian areas. Documentation of *Arundo* growth parameters include measures of stem densities and diameters, areal (percent) cover and distribution of secondary shoots. In experimental plots in the Santa Clara River, we are evaluating plant diversity, decomposition rates, soil moisture, and light availability in *Arundo*-infested and uninfested areas. Preliminary analysis shows that *Arundo* has significant negative effects on all of the parameters measured, as well as providing a poor food resource for aquatic organisms (Herrera and Dudley 2003), justifying efforts to reduce its abundance and vigor, including through development of biological control.

In addition, a large-scale program to characterize riparian vegetation for the floodplains of the Santa Clara River and its tributaries downstream of Los Angeles County was conducted by Stillwater Sciences, URS, and our UCSB research team. This study was funded by the SCR Trustee Council and the California Coastal Conservancy. The results of this study were released in August of this year (results not described here, but can be found at http://www.santaclarariverparkway.org/newswkb/scrvegmap). Two research assistants hired on our SCRTC grant (Todd Lemein and Keegan Kennedy) performed critical roles in this mapping project, both in field assessment and GIS data entry and analysis phases.

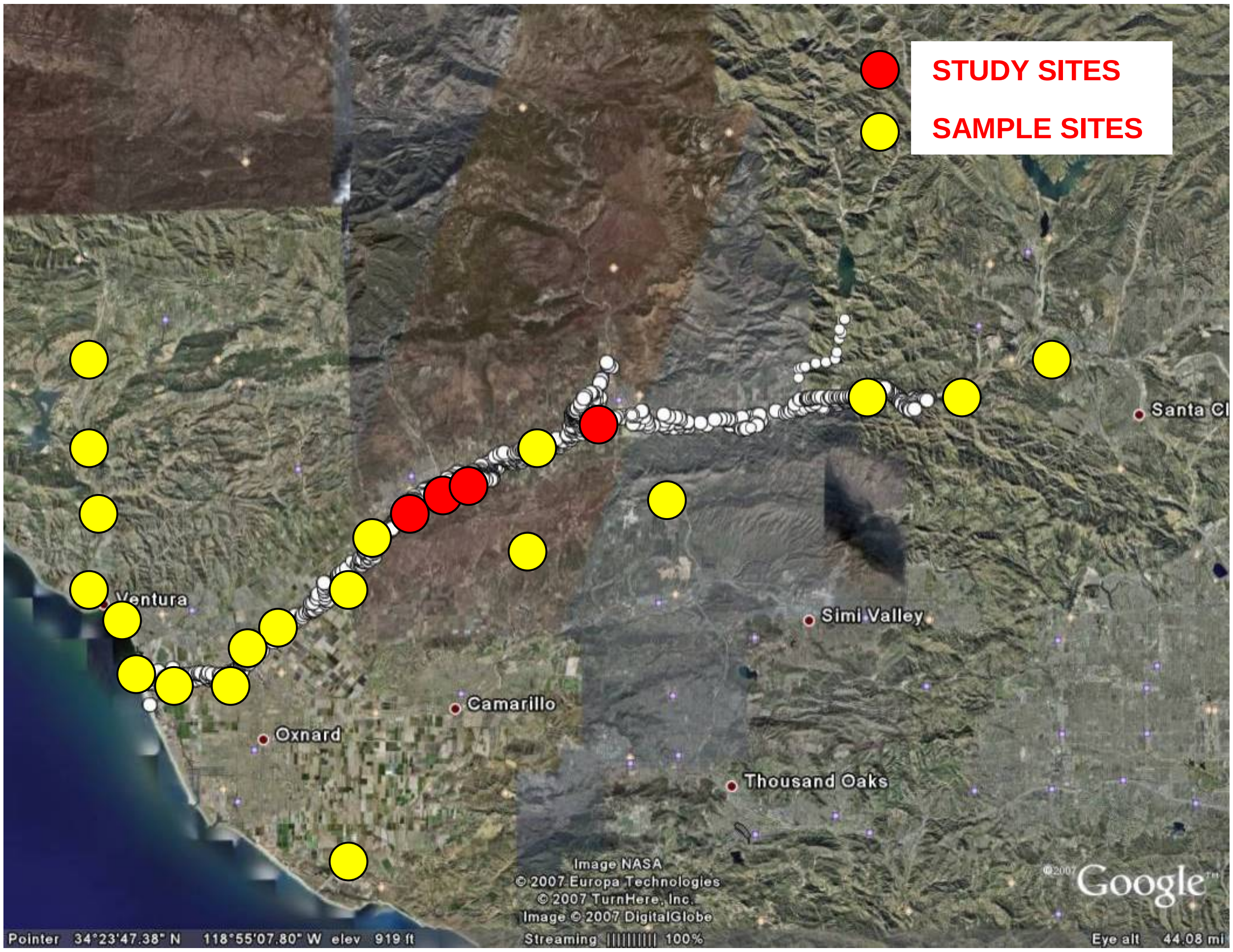

Figure 1. Sites in Ventura County and adjacent Los Angeles County where herbivore sampling was done (Sample Sites) and where ecological studies are being conducted. The study sites are areas under conservation protection, including Briggs Rd., Peto/Hallock St., and Valley View/Hedrick Ranch, and an unnamed site near Fillmore. Map from Stillwater Sci. (2007).

Insect Surveys

Several exotic insect herbivores have been discovered feeding on *Arundo* in California (Table 1). Sampling locations, insect herbivores recovered and feeding damage for *Arundo* populations specifically sampled in the Santa Clara River are given in Table 2. Three in particular, the stem-boring wasp (Eurytomidae: *Tetramesa romana*), a stem-feeding shootfly (Chloropidae: *Cryptonevra* sp.), and an aphid (Aphididae: *Melanaphis donacis*) are present in the

Santa Clara watershed. These can co-occur on a single plant, and effectively partition the *Arundo* plant by feeding in different microhabitats (Fig. 2). A single herbivore or biocontrol agent is

Table 1. Sampling locations and *Arundo*-feeding insects recorded in regional surveys.

County	# Sampling Sites	Insects
Humboldt	2	None
Imperial	2	None
Inyo	2	None
Kern	1	None
Los Angeles	4	Aphids, Shoot Flies, Stem-boring wasps
Monterey	5	Aphids
Orange	2	Aphids, Shoot Flies, Stem-boring wasps
San Bernardino	3	Stem-boring wasps (one site)
San Diego	5	Aphids, Shoot Flies, Stem-boring wasps
San Luis Obispo	2	Aphids
Santa Barbara	7	Aphids, Shoot Flies, Stem-boring wasps
Ventura	8	Aphids, Shoot Flies, Stem-boring wasps

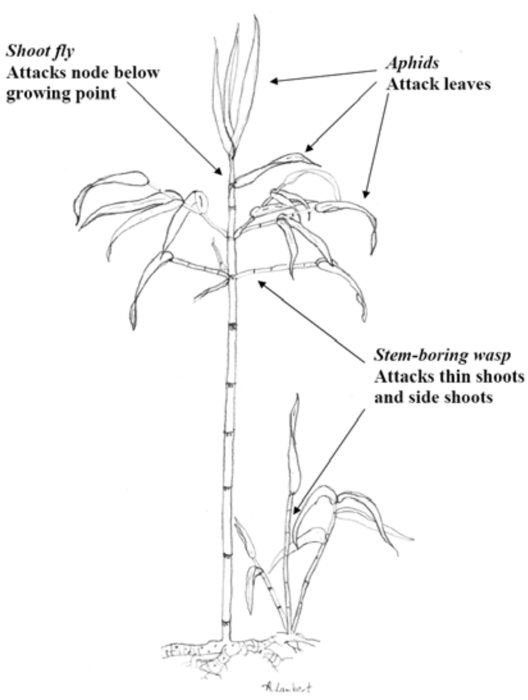

Figure 2. Microhabitats or feeding Locations of specialist herbivores on *Arundo*.

Table 2. Sampling locations, insects recorded and qualitative plant damage caused by herbivores in the Santa Clara River Watershed.

Town	Site	Insects	Damage to *Arundo*
Castaic	Highway 126	Aphids, Stem-boring wasps	Young shoots stunted/killed
Camulos	Highway 126	Aphids, Stem-boring wasps	Young shoots stunted/killed
Camulos	Highway 126	Aphids, Stem-boring wasps	Young shoots stunted/killed
Fillmore	Ojai Street	Aphids, Stem-boring wasps	Mature stems killed
Fillmore	Sespe Creek	Aphids, Stem-boring wasps	Young shoots stunted/killed
Santa Paula	Hallock Drive	Aphids, Shoot Flies, Stem-boring wasps	Young shoots stunted, top of mature stems killed
Santa Paula	Briggs Road	Aphids, Shoot Flies, Stem-boring wasps	Mortality of side-shots
Ventura	Harbor Blvd.	Stem-boring wasps	Young shoots stunted/killed
Ventura	Ventura Beach	Aphids, Shoot Flies, Stem-boring wasps	Young shoots stunted, top of mature stems killed
Ventura	Victoria Ave.	Aphids	None
Ventura	Ventura Avenue	Aphids, Shoot Flies, Stem-boring wasps	Young shoots stunted/killed

rarely able to exert a high degree of control on target plant, so typically multiple agents are necessary in order to provide adequate suppression of an invasive weed (Denoth et al. 2002). The benefit of herbivores that feed on different plant parts is that there is potential for interactive or synergistic effects, in that stress on one plant part can enhance the damage caused by another natural enemy (insect or pathogen). The long-range goal of the *Arundo* biocontrol program is to introduce such a suite of organisms to provide control (Kirk et al. 2003, Kirk and Widmer 2004). Details of the biological control program and European insects being evaluated are discussed by A. Kirk in Appendix 1.

Regarding the impact that any or all of these herbivores are currently having on *Arundo* performance, this is difficult to do quantitatively in a correlational study, such as represented by our extensive surveying exercises. Even though there is some spatial segregation by these insects, when they co-occur one cannot readily assign proportionate damage to each, nor is it feasible to differentiate between subtle direct insect impacts and other environmental factors that influence plant growth, other than observation of damage. Such information would come from experimental studies in which ecological factors can be kept constant other than the manipulation of insect presence, and such studies are underway in our field plots, as noted below.

Individual herbivores: *Tetramesa romana*

The most important of these specialist herbivores, *T. romana*, is the primary biological agent in quarantine testing in Texas. This species is also already present in California, making host specificity testing in the field possible and redistribution of organisms easier and less risky *Tetramesa romana* is present in many drainages in southern California below 34° N latitude (Fig. 3), including the western Mojave Desert (Mojave River). It is particularly common in the Santa Clara River system, based on its presence in appropriate-sized material at most sites we have sampled. Species identification was verified as *T. romana* by Commonwealth Scientific and Industrial Research Organisation (CSIRO), Europe. Its biology and distribution are not well known in its native range, but *T. romana* is known to be oligophagous on *A. donax* based on collection records in the Palearctic (Kirk et al. 2003, Tracy and DeLoach 1999). Several species

Figure 3. Distribution of *Tetramesa romana* in California.

of *Tetramesa* specialize on various grass species (Al Barrak et al. 2004), including one (*T. phragmitis*) on *Phragmites australis* (common reed). *Phragmites* is structurally and taxonomically similar to *A. donax*, and is native in California, including rare populations in the upper Santa Clara system. It is a primary non-target plant included in our host range testing.

The relatively limited distribution of *T. romana* in North America (with the caveat that Texas *Arundo* populations have not been adequately sampled) may suggest that either it is a fairly recent, unintentional introduction into North America and hasn't dispersed yet to occupy its full potential range, or that it was brought to the continent with original plant material and environmental factors (including possible poor dispersal ability) restrict its occupation to certain regions. We hope to answer this question because it bears on the potential for augmentation or re-distribution of the population to enhance its impact to target plants more broadly. We have collected this insect in South Africa as well, where *Arundo* is also invasive and *T. romana* is presumably an unintentional hitch-hiker (T. Dudley, unpublished data).

Female wasps oviposit multiple eggs into young main shoots and side shoots (Fig. 4), and prefer stems with narrower diameters (Fig. 5). Multiple larvae can feed and develop within a stems, and once fully developed, each adult will bore a hole in the stem wall to exit the plant.

Figure 4. *Tetramesa romana*, a non-native herbivorous wasp discovered in *Arundo* stands in the Santa Clara River. Wasps oviposit in young stems and side shoots; larvae feed within stems and shoots causing galleries and plant deformities.

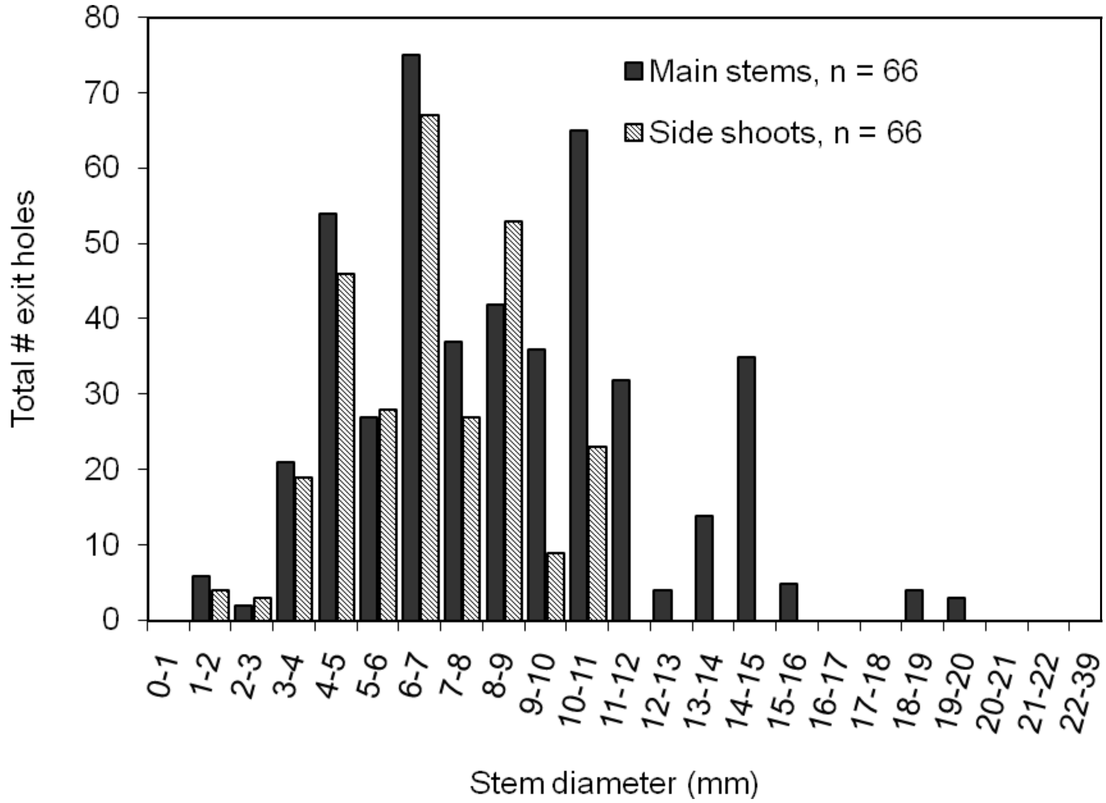

Figure 5. Total number of exit holes observed in *Arundo* stems collected in the Santa Clara River, indicating stem diameter preference.

This wasp can have locally high population densities with as many as 20 larvae per 10 cm of stem, but usually fewer, and cause significant mortality to new, smaller diameter shoots and side shoots.

We destructively sampled stems from 31 plots at the SCR study sites to determine wasp infestation rates in *Arundo* populations (Table 2). At each site, 100m transects were run through *Arundo* infested areas in the active river channel, at the river channel/terrace transition zone, and on the river terrace. Every 10m a 0.5m X 1m sampling frame was placed parallel to the transect line. All *Arundo* stems within the frame were cut at the soil surface, packaged together, and transported back to the laboratory for dissection.

Frequency of stem colonization was moderately high in the lower, river bottom habitats (33.4% of stems colonized), highest at the terrace margin just above the active channel (97.4% colonization), and substantially lower on the high and drier terraces a greater distance from the channel (6.1% of stems occupied). Although not experimentally verified during the timeframe of this study, it appears that wasp occupation induces proliferation of additional side shoots, re-directing growth or draining energy from apical parts of the plant, and the leaf surface area of individual culms appears to be reduced by this damage. Many of these secondary stems are subsequently killed by wasp infection, given its preference for small-diameter stems.

Variation in plant growth and other confounding variables (environmental differences among sites) reduce our ability to provide quantitative evidence that *T. romana* does indeed reduce growth and fitness of *Arundo* populations. We are currently using controlled greenhouse and common garden experiments to experimentally determine how wasp infestation affects *Arundo* growth parameters. *Arundo* plants are grown using a systemic insecticide (imidacloprid; Merit™) to exclude wasps from plants. Similar-sized plants (Fig. 6) from each treatment (with vs. without insecticide) are paired and female wasps are released onto plants to allow colonization. We expect that we will be able unequivocally determine the effects of wasps on infested plants using these methods. Follow-up studies are proposed (to DWR which did not fund in 2007; re-submission will be done in 2008) using sap-flow gauge technology (microthermisters to estimate water transport through stems) to estimate water loss from whole plants, which can be extrapolated to landscape level estimates of water savings resulting from biocontrol.   We expect that reduced leaf area caused by *T. romana* (and other potential biocontrol agents) will translate into reduced transpiration.

Figure 6. Herbivore impact experiment (left and middle photos) manipulating *Tetramesa* presence and absence using imidoclorprid insecticide to exclude wasps, September 2007. Photo on right shows student researches assisting in related study of native and non-native *Phragmites* interactions, including host range tests.

We are continuing investigations of wasp impacts on *Arundo* populations in the Santa Clara River and in the Mediterranean region (in collaboration with A. Kirk, USDA-ARS European Biological Control Lab - EBCL; Appendix 1), the apparent origin of this species. *T. romana* is being tested by USDA-ARS at the EBCL and under quarantine conditions at their Weslaco, TX laboratory for possible introduction to Texas and California from Mediterranean

Europe. To determine the precise origin of the California insect material, we procured *Arundo* shoots from river systems throughout the southern part of the state. We held these stems in 3-meter long mesh bags and collected newly emerging *T. romana* wasps. These wasps are being compared morphologically to wasps collected throughout the Mediterranean by Dr. Kirk to determine if they are the same, and to calculate variability in European and North American insect populations. This information is useful in predicting biotypes or genetic forms of the species that would be most effective for biocontrol in infested regions of California and other states.

Molecular analysis of genetic variation and relatedness using Amplified Fragment Length Polymorphism (AFLP), was conducted by collaborator D. Kazmer (USDA-ARS, Sydney, MT), which indicated that there is very low genetic variation in the California material and that it does not appear differentiated from *T. romana* in the western Mediterranean region. This lack of variability suggests that *T. romana* is essentially identical to material being considered for overseas introduction, and thus, that California material is appropriate as an alternative source for re-introduction elsewhere. Using California insects as source material greatly reduces the regulatory difficulties in implementing biocontrol elsewhere in North America, given the complexity of approving international transfers of live organisms, particularly as its current range extends into Mexico. It is anticipated that these results will allow USDA to shift resources to other efforts involving candidate agents not currently present in North America [a recent $1.5 million grant to USDA from the Dept. of Homeland Security is focused on evaluation of *T. romana* and two other species].

Studies in our common garden research plots near UCSB and in the associated greenhouse were conducted to verify that *T. romana* is specific to *A. donax* and will not have non-target effects on native or economically important plants. As noted earlier, *Tetramesa* spp. are known to be grass specialists (and each insect species a specialist on only one or a few closely-related grass species), so our testing was only done with members of the grass family and other monocots; USDA-ARS evaluation includes other co-occuring plants, such as *Salix* spp. and economic plants expected in the vicinity of *Arundo* infestations (J. Goolsby, personal communication).

Our studies consisted of 'no-choice' trials in which fine-mesh sleeves or cages were placed over potential host plants and the wasps introduced within to observe oviposition behavior and to document colonization based indications of larval use. These trials were replicated using a minimum of 5 cages or sleeves for each non-target species (Fig 7). Other 'choice' tests were similarly conducted with non-target plants either enclosed with *Arundo*, or in closed greenhouse conditions where full-sized *Arundo* plants were 'permanently' established (in the ground, not in pots) and were already heavily colonized by *T. romana*. Choice tests involved a minimum of 5 plants, and on important taxa typically 12-20+ individual plants were included. While wasps were emerging from the host *Arundo* plants, we monitored their behavior to observe any use of non-targets, and dissected plants later to verify that uninfested *Arundo* were colonized, and that non-target plants did not contain larvae. Of all tests plants used, *Arundo* was always colonized, while no wasp was ever observed showing oviposition behavior on a non-target plant nor was there any indication of any utilization of non-target plant species, with the exception of *Arundo formosana*, a horticultural plant originating in Taiwan and used infrequently for horticultural purposes (Table 3). This is useful corroboration of the specificity of *T. romana*, most likely indicating that a chemical stimulus common to the genus *Arundo* is important as an oviposition cue, and that utilization is not dependent on structural traits of the host plants alone.

Figure 7. Illustrating host range testing. Left to right: *A. donax* and *Arundo formosana*; A. donax and 'lemon grass'; Phragmites australis and Elymus condensatus with Arundo in background; 'sugar cane' with Arundo in background; lower is seven P. australis haplotypes, one native and others exotic, near end of growth season. Mesh sleeves for retaining wasps.

We also collected a minimum of 20 specimens of naturally occurring grasses and other monocots within 100 m of *Tetramesa*-colonized Santa Clara River field sites. These were dissected in the laboratory to determine if any plants in close proximity to high wasp densities were utilized. In some cases fewer than 20 individual plants were available (e.g. *Miscanthus*, an ornamental plant found in some nearby residential areas), but a minimum of 20 stems were collected in such cases. No evidence of *Tetramesa* presence was ever found (Table 3).

Table 3. Non-target host plants tested for potential utilization by *T. romana*. Type of test indicated as C = Choice, NC = No-Choice, F = Field sampling.

Potential Host	Type of test	Adult use	Larval evidence
Arundo donax	C, NC	Yes	Yes
Arundo formosana	C, NC	Yes	Yes
Phragmites australis (common reed)			
Native genotype	C, NC	No	No
Invasive genotypes (5)	C, NC	No	No
Elymus condensatus (giant wild rye, native)	C, NC, F	No	No
Elymus, leymus – wayne?	C, NC	No	No
Leymus triticoides (creeping wildrye, native)	F	No	No
Piptatherum miliaceum (smilograss, exotic)	F	No	No
Phalaris aquatica (Harding grass, exotic)	F	No	No
Polypogon monspeliensis (rabbitfoot grass, exotic)	F	No	No
Pennisetum setaceum (fountaingrass, exotic/horticult)	F	No	No
Muhlenbergia rigens (deergrass, native/horticult)	F	No	No
Zea mays (corn/maize, 2 varieties)	C, NC	No	No
Cymbopogon sp. (lemongrass)	C	No	No
Saccharum sp. (sugarcane)	C, NC	No	No
Cortedaria jubata (pampas grass, exotic)	F	No	No
Miscanthus sinensis (silvergrass, exotic/horticult)	F	No	No
Typha latifolia/domingensis (cattail, native)	C, F	No	No
Cyperus eragrostis (umbrella sedge, native?)	F	No	No
Scirpus sp./*S. acutus*? (bulrush)	F	No	No
Juncus balticus? (wire sedge, native?)	F	No	No
Eleocharis macrostachya (spike rush)	F	No	No
Equisetum arvense (horsetail)	F	No	No

Trials in the UCSB common garden plots in 2005 and 2006 showed that *T. romana* readily colonizes previously uninfested clumps or stands of *Arundo*, and may be amenable to redistribution to other sites in the state where wasps are absent. No evidence of *Tetramesa* has been found in the Santa Ynez River in northern Santa Barbara County, so this system was used to determine if wasps can be introduced into and maintain stable populations in previously uncolonized stands. Because it is within a county in which the insect is naturally established, no regulatory approvals are necessary; however, the trials were informally approved by David Chang, Weed Coordinator, Santa Barbara County Agriculture Commission. In late July 2007, using both caged and open release methods, we released 155 female *T. romana* in Santa Ynez River *Arundo* stands in the vicinity of Lompoc, Santa Barbara County (Fig. 8). Two other uninfected populations of *Arundo* on the UCSB campus were also inoculated in August with wasps in fine-mesh sleeves to verify that field introductions are feasible, and to monitor herbivore population dynamics under controlled conditions. Results, which require destructive sampling of trial plants, will not be available until the end of the 2007 field season, and we will continue to monitor these open release sites for two years.

Figure 8. Redistribution of *T. romana* to uncolonized Arundo plants that the Santa Ynez River, Santa Barbara County; Adam Lambert shown.

***Cryptonevra* sp.**

A shoot-fly (Chloropidae) currently identified as a non-indigenous *Cryptonevra* sp. has been recovered from *Arundo* shoots in Santa Barbara and Ventura Counties (Fig. 9). Some *Cryptonevra* species are partly saprophagous within damaged/decaying areal shoots, but observations suggest that this fly is likely the primary feeder causing the observed damage (Fig. 6). This insect is being identified by the USDA-ARS EBCL, where researchers are conducting their own tests with what appear to be three species of *Cryptonevra*. Although it is not clear yet that the California insect is the same species as any of those, a shoot-fly from France has also been imported into quarantine in Weslaco, Texas, for evaluation as a candidate for biocontrol

Figure 9. Characteristic 'hourglass' stem damage by *Cryptonevra* sp., its pupa dissected from an *Arundo* stem, and the adult of *Cryptonevra*.

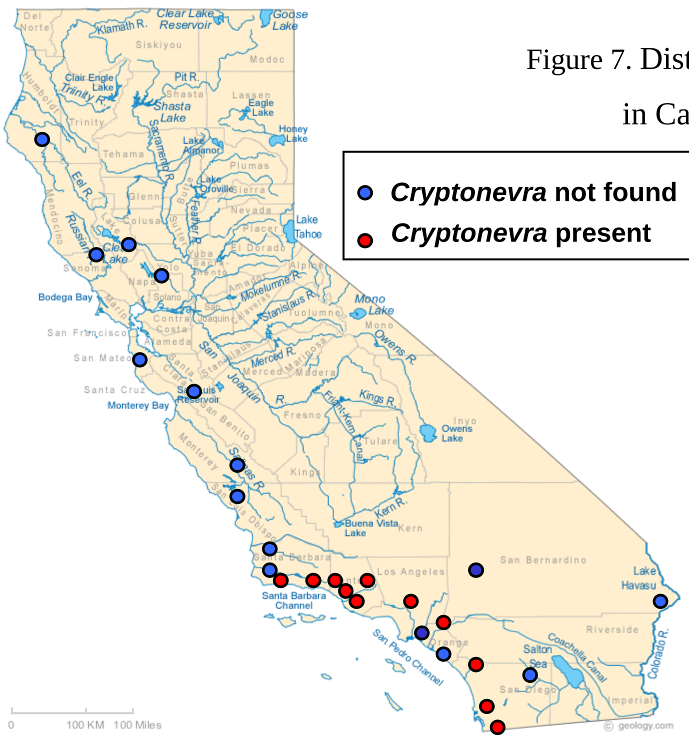

Figure 7. Distribution of *Cryptonevra sp.*

in California.

release. We suspect that the California *Cryptonevra* is the same based on similarity of damage to that observed by Dr. Kirk at EBCL, and thus recommend that these identities be verified and/or efficacies be compared before expanding quarantine testing with this candidate agent. Again, insect material from California would be more practical for re-distribution to *Arundo*-infested areas where *Cryptonevra* is not currently present in California and Texas.

Qualitative estimates suggest that frequencies and infection densities of *Cryptonevra* sp. are substantially lower than those of *T. romana*, and have not been fully quantified yet as our emphasis has been on the wasp because it is easier to handle under experimental conditions. Nonetheless, the extent of damage does suggest that *Cryptonevra* sp. has significant potential for inflicting damage to target plants.

Melanaphis donacis

The Old World aphid, *Melanaphis donacis* (Fig. 10), has been found throughout the sampling range and appears to have relatively minor impact to *Arundo* plants, primarily occasional yellowing of colonized tissue. This aphid can attain very high densities in excess of 500 individuals on a single leaf blade, and prefer feeding on the younger leaves. The are often attended by various ant species (Fig. 8) that utilize egested carbohydrates ('honeydew'), presumably in exchange for protection from predatory arthropods, and which may allow high aphid densities to establish. Impacts are potentially greater if attack is in combination with damage by other agents, including those still being tested by USDA.

Figure 10. Dense population of *Melanaphis donacis* infesting the apical leaves of an *Arundo* stem, and a formicine ant tending individual aphids.

Ecological effects of *Arundo donax* on the native plant community.

To document differences in the plant community in *Arundo*-infested vs. uninfested areas, we set up a series of 80 1-m^2 plots in infested and adjacent uninfested areas at the Santa Paula and Fillmore study sites noted in Fig. 1, both on the river terrace and in the active river channel, and monitored them for one year. Plots were chosen at uniform intervals along transect running parallel to the active river channel and stratified to equally represent the two vegetated conditions (infested vs. uninfested), although sampling intervals varied between transects. We measured species richness (diversity) and percent cover of all plant species in May, August, and November 2006, and in May 2007. We also measured soil moisture using time-domain refractometry (TDR) and light penetration to the soil surface with a linear-integrated light meter (light bar) in these plots on the same sampling dates.

To evaluate effects of *Arundo* on organic material nutrient cycling, native leaf litter (*Salix exigua, S. laevigata*, *Baccharis salicifolia*) and *Arundo* leaf litter were collected from plants in April 2006. Leaf material was selected that was fully or mostly senesced, but not dead, still attached to plants so that prior exposure to soil microbiota would be minimized. Three grams of native litter (one gram of each species) and three grams of *Arundo* litter were placed, separately, in 15 cm fiberglass mesh bags and two bags of each litter type were placed on the ground outside the corner of each plot. Plots were at the Briggs Rd. study site. One bag of each litter type was collected in August and November. Carbon and nitrogen from the initial litter collection in May and litter bags collected in August and November was analyzed to determine differences in nutrient content and decomposition between *Arundo* infested and uninfested areas.

Results

On the terrace, plant diversity was 57% lower in *Arundo* infested areas than in uninfested areas, and native percent cover was four times greater in plots without *Arundo*. In the active river channel, plant diversity was 41% lower in *Arundo* infested areas than in uninfested areas. Soil moisture and light availability at the soil surface were also negatively associated with the presence of *Arundo* (Fig 11). We are currently analyzing the decomposition data; however, preliminary analyses indicate that *Arundo* has a significantly higher C:N ratio than native litter in both infested and uninfested areas. A high C:N ratio typically indicates that plant tissues are high in lignins and cellulose, and that nitrogen may be bound up in compounds, potentially making it less available to decomposers and subsequently, to higher trophic levels.

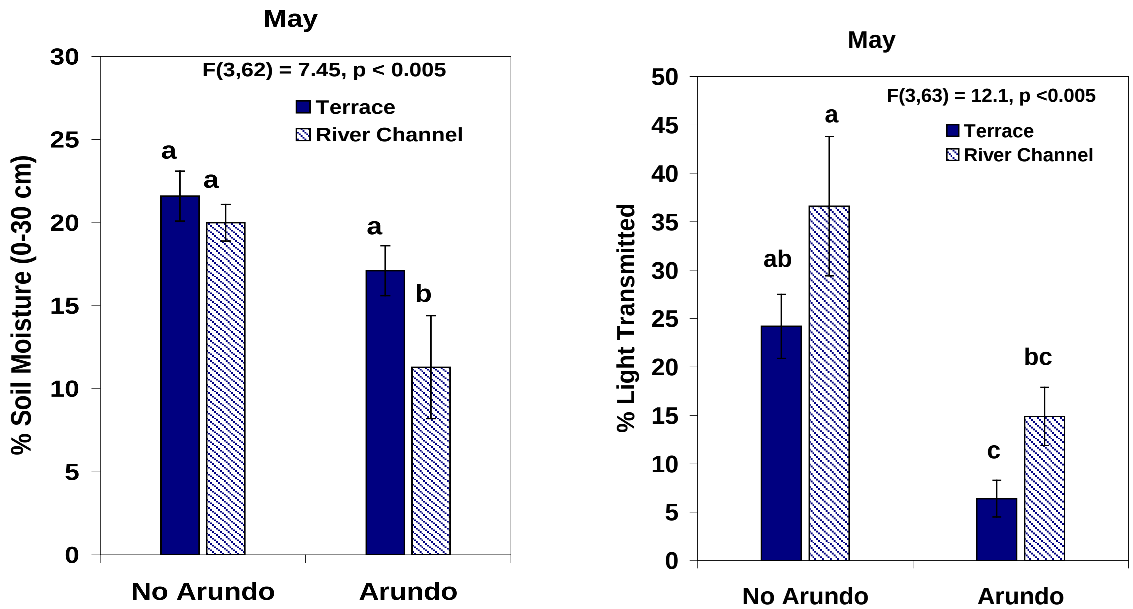

Figure 11. Means for percent soil moisture in *Arundo* infested and uninfested plots (left). Means for percent of light transmitted from above the vegetation to the soil surface in *Arundo* infested and uninfested plots (right). Bars with different letters above them (a,b,c) are significantly different from each other (Tukey's test).

Evaluation of seed production by *Arundo donax*

Many anecdotal reports statewide postulate that *A. donax* is propagated not only by rhizomes transported to new sites (by flood flows, equipment), but may also produce viable seed. The USDA-NRCS and the Ventura County Watershed Protection District were particularly concerned that new establishment may be occurring, based on preliminary suggestion that viable seed was present in the Ventura River system [D. Kanthack (VCWPD), D. Dyer & C. Burns (NRCS)], so we co-ordinated serial collections from plants observed to be producing flower plumes throughout California and from 7 other states, including northern Mexico to determine if viable seed could be detected.

Flower material was examined under a dissecting scope (200 florets per plume) with a total of approx. 36,666 individual florets observed. Of these, we found that 43 ovules appeared to have potential metabolic activity, al from the Los Angeles/San Gabriel River system (Johnson et al. 2006). These were tested for metabolic activity using a standard tetrazolium test, and we determined that 5 of these ovules may have been developing tissue, but there was no indication that any would ever achieve maturity. No evidence of metabolic activity was found in any of the Santa Clara nor Ventura River samples. Representative samples of plume/flower materials were also spread onto seed germination trays in the greenhouse to verify whether residual material had any germination activity. Subsequent collections have indicated that, while healthy stamens are always present, no pollen is produced. Thus, rare observed ovule development is considered to be either apomictic germ production or extraneous tissue elaboration unlikely to produce viable propagules. We concluded that *Arundo* reproduction via seeding is certainly insignificant, and most likely non-existent in its life cycle.

Ecological effects of *Arundo donax* on aquatic ecosystems

Arundo invasion can have effects on aquatic ecosystem processes as well as terrestrial, as indicated by a study culturing leaf-feeding aquatic insects on *Arundo* litter compared with native leaf material, as well as tamarisk (Going and Dudley, in press). Litter was collected from plants in riparian areas on the Santa Clara and Ventura Rivers, and 'conditioned' in large (1-m long) mesh bags for 2 weeks in a smaller stream to inoculate material with decomposer organisms that can enrich such resources for 'leaf-shredding' insects. This material was then returned to the lab and refrigerated, and introduced as needed into experimental containers. The ubiquitous caddisfly, *Lepidostoma unicolor* - an aquatic leaf-shredding insect, was used in lab trials to compare larval growth and survival rates of insects fed exclusively on diets comprised of native litter and litter from non-native plants. Twenty first-instar larvae were placed into each of 6 aerated 20 X 40 cm plastic dishes for each litter type, and the food was replaced regularly to maintain constant litter condition.

We found that survival was very high (ca. 90%) on the native litter (two treatments: alder (*Alnus rhombifolia*) and a Salicaceae mix (willows – *Salix* spp., cottonwood – *Populus fremontii*), as well as on tamarisk litter (*Tamarix ramosissima*). However, *Lepidostoma* survival was poor when fed exclusively *Arundo*. When mortality of *Arundo*-fed larvae reached about 80% we were forced to terminate the experiment in order to make biomass measurements. Growth was also greatly reduced when fed *Arundo* (Fig. 12), in part because nitrogen content was much lower than the other litter types (< 1% N vs. 2.5 – 5% N for alder and tamarisk), even after enrichment by microbial colonization.

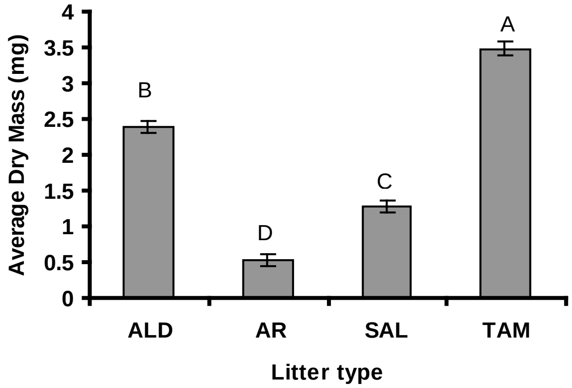

Figure 12. Biomass achieved by *L. unicolor* fed native and non-native litter types; ALD = alder, AR = *Arundo*, SAL = cottonwood/willow mix, TAM = tamarisk.

Caddis larvae and resulting terrestrial adult insects can be important food resources for other aquatic species, particularly insect-dependent salmonids, so increasing dominance of waterways by *Arundo* can potentially harm populations of endangered rainbow/steelhead trout.

Outreach & Education

We have created and continue to update a website dedicated to *Arundo* information (http://rivrlab.msi.ucsb.edu) and other target invasive species in California riparian areas. The website is intended for dissemination of ecological and control information for scientists, resource managers, and the general public. It contains information on the effects of *Arundo* invasion, current state of the *Arundo* biocontrol program, and descriptions of our research and findings from insect sampling and vegetation monitoring. We also published an article in the February 2007 Santa Clara River - Watershed Times (U.C. Cooperative Extension; http://celosangeles.ucdavis.edu/newsletterfiles/Santa_Clara_River_Watershed_Times10398.pdf) on the presence of *T. romana* in this river system and the potential of this wasp to contribute to weed suppression. This outlet reaches conservation managers, farmers, and local citizens and has increased awareness of the *Arundo* problem and interest in the potential for biological control to be a sustainable and environmentally beneficial approach to *Arundo* control. A similar article for the lay public was presented in the UCSB Center for Biodiversity and Ecological Restoration newsletter: http://ccber.lifesci.ucsb.edu/newsletter/CCBERVolume2/vol002_page_12.php .

We have initiated a process for establishing a field station for the U.C. Natural Reserves System (NRS) which is anticipated to contribute enormously to the information base and conservation planning for the Santa Clara River watershed. Unique within the UC research station systems, this is proposed also to be part of the Agricultural Experiment Station, as well as NRS. This would be a research station jointly administered between UCSB and UCLA, the latter of which is developing a companion field station on the Tejon Ranch in the upper watershed; UC Riverside ecological and agricultural scientists have also expressed support for the proposal. We envision these two permanent stations with facilities for faculty and student research, information technology, and a series of undeveloped, controlled-access research sites to provide an integrated approach to understanding ecosystem processes and facilitating restoration actions through the watershed. The research unit would also have linkages with the public through the activities of the U.C. Hansen Agricultural Learning Center (Faulkner Farm), and there are excellent opportunities to provide a framework for agro-ecosystem research that is, unfortunately, declining in increasingly urbanized regions such as those served by U.C. Riverside.

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Students Supported

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Publications

Dudley, T.L., A.M. Lambert, and A. Kirk. 2006. Augmentation biological control of *Arundo donax*, pp. 141-144. *In*: Proceedings of the California Conference on Biological Control, V (M. Hoddle and M. Johnson, eds.). Riverside, CA.

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Invited Seminars/Presentations

2007 Non-Native Reed Invasion in North America: Causes, Impacts, and Potential for Biological Control. E. Connecticut State University, Willimantic, CT (Lambert).

Potential for Biological Control of Giant Reed (*Arundo donax*) in California. Entomological Association of So. California, Pasadena, CA (Lambert & Dudley).

Calif. Invasive Plant Council.

2006 Ecology and Biological Control of *Arundo donax* in the Southwestern United States. USDA European Biological Control Lab., Montpellier, France (Lambert et al.).

Invasive Reeds in the U.S.: ecology, management, and potential for biological control. So. California Wetlands Recovery Project, Ventura, CA (Dudley & Lambert).

Biological Control of Perennial Weeds in Riparian Ecosystems. Joint U.S.-India Forum on Invasive Plant Management, Ramnagar, India (Dudley).

Biological Control of Grasses: *Arundo donax* (giant reed) as a Case History, Weed Society of Western Australia, Perth, W. Australia (Dudley).

Potential for Augmentation Biological Control of *Arundo donax* in California. California Invasive Plant Council, Sonoma, CA (Lambert & Dudley).

Invasive species and riparian ecosystem linkages in the Santa Clara watershed. South Coast Long Term Ecological Research Symposium, Santa Barbara, CA (Dudley, Lambert & Dalin).

Augmentation Biological Control of *Arundo donax*. U.C. Invasive Pests & Diseases Research Program Workshop, Riverside, CA (Lambert, Dudley & Kirk).

Appendix 1.

Biological control of Giant Reed *(Arundo donax)* an invasive plant species in the USA: Preliminary results of foreign exploration for natural enemies.

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Objectives:

The overall goal of this work is the development of an environmentally sound method to alter the competitiveness, invasiveness, and abundance of *Arundo donax* in the USA. The use of a biological control agent that is self-sustaining and self-dispersing is one viable option in the management of this weed.

The specific objectives are:
1. Collecting and rearing out the natural enemies on *Arundo donax* in its native range.
2. Identifying the agents that have the greatest impact on growth and spread of *A. donax* and discovering new potential agents.
3. Selection of the most promising agents.
4. Determination of the geographical origin of *Arundo donax* in Texas and California using molecular techniques

Key Words: *Arundo donax*, Biological control, Giant reed, Natural enemies, Weeds

Abstract:

Arundo donax, Giant Reed, is a widespread invasive weed in California and south western U.S. Outside the U.S., it ranges from the Canaries to northern Myanmar. It is a biocontrol target because of its intransigence to cultural and chemical control and its disastrous impact on the local ecology. Foreign exploration in Nepal and India revealed little sign of natural, control whereas collections made around the Mediterranean resulted in 3 Hymenoptera, 5 Diptera, a scale species and several plant pathogens, which cause death of shoots, tips and dormant buds. On the average, 56% of an *Arundo* stand is dead in the region around Montpellier France. Preliminary results suggest mortality may be attributed to Diptera (38%), and scale insects (14%), with Hymenoptera and fungal pathogens also playing a role. Infested areas of California and parts of the Mediterranean basin are excellent climatic matches. These results suggest that the Mediterranean is likely to be a profitable area to explore for natural enemies. . In addition, characterization of *Arundo* samples from Europe, Africa, Asia, North America and Australia has shown that the origin of the *Arundo* stands in the U.S. is Spain.
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Introduction:

Arundo donax, Giant reed, is a tall, 2-10 m perennial grass found naturally from the western Mediterranean to India (Duke, 1984). The potential distribution of *Arundo* in the U.S. would include nine States from East to West. Giant reed tolerates salinity and is drought and inundation tolerant (Perdue, 1958). Dudley and Collins (1995) note that the densest growth of *Arundo* occurs along the coastal rivers of California. In California, large areas of giant reed constitute serious ecological and flood management difficulties by displacing native vegetation (Vartanian, 1998, Dudley, 2000). Currently, *Arundo* is managed by costly mechanical and chemical means (Vartanian, 1998).

The damage caused by giant reed in California far outweighs the small economically beneficial uses. It has a growth rate up to five times faster than native plants (e.g. willows), in the same habitats on southern California coastal rivers (Rieger & Kreager, 1989). *Arundo* is flammable but the rhizomes are resistant and sprout after fires, while native plants are much slower to recover (Bell, 1997). Extensive areas of giant reed pose serious ecological problems, such as evapotranspiration/water loss, flood and debris risks, fire risks, erosion from undercutting, excess sediment retention, and biodiversity threats due to loss of critical riparian habitat. Generally, large areas are removed by expensive mechanical and chemical controls. Because of high costs and environmental concerns with the use of conventional control measures, alternative control measures, such as implementing classical biological control agents are actively being sought.

Results obtained from this study will be of direct use in the future to resource management agencies where giant reed is a problem and current control strategies are inadequate and costly. It will also contribute to the development of a comprehensive IPM program. The major *Arundo* control method is the use of broad spectrum herbicides such as Roundup. Costs of this method are from $22000-$46000/hectare based on removal costs in an eradication program in the Los Angeles County Flood Control District and along the Rio Hondo. (Vartanian, 1998). Such costs may prove unacceptable when it is considered that along the Santa Ana River there are 4000 hectares of *Arundo*, which at $20000/ hectare would reach $80 million. Classical biological control of weeds has been used against 35 invasive weed species in the U.S., and against many weed targets in 70 countries worldwide; about 33% have been successfully controlled (Julien 1999). Classical biological control of weeds has a fine safety record; more than 350 natural enemy species have been utilized over the last 100 years and only eight of these have been documented as causing minor damage to non-target plants (Julien, 1999). Several major water weeds considered unpromising targets initially have been successfully controlled; ultimately the same may be true for grassy weeds (Julien, 1999). Tracy and DeLoach (1999) and Cummins, (1971) list 21 insect species, five mite species, one nematode species and 21 fungal pathogens from *Arundo.* Of these one insect species is considered monophagous, zero mites and nematodes, and four fungal pathogens; the others are oligophagous or polyphagous.

Methods

Collections

All overseas work was conducted at the European Biological Control Laboratory (EBCL) which is a USDA/ARS-funded laboratory located in Montferrier sur Lez, France. EBCL works exclusively on biological control and has numerous clients within the U.S. at the national and state level. The region of France where EBCL is located has stands of *A. donax*, growing along ditches and waterways.

Based on climatic matching with Arundo infested areas in California (using CLIMEX), surveys have been made in appropriate areas of France, Spain, Portugal, Canary Isles, Italy, Greece Turkey ,North Africa and Australia. Initial surveys were in fall, second year in summer and the third year in spring to cover the most important growth periods. Site details, locality, altitude, GPS position were recorded. Giant reed rhizomes were unearthed and dissected at each site for natural enemies. Lengths of rhizome and cut stems and leaves were placed in moisture absorbent bags, cooled, and returned to the EBCL quarantine for emergence. As many sites as possible were surveyed during 10 day trips to each area. Fifty cm square quadrats have been taken from *Arundo* stands each week for 15 weeks (starting May 5 2003 in 2004 and 2005) in the Montpellier and Perpignan areas of southern France. All *Arundo* within the quadrats was cut, taken back to the laboratory, examined, dissected and documented.

Organisms found were where possible reared and adults passed on to appropriate taxonomists. However, several agents, already identified and known to attack *A. donax*, were selected for an in-depth study of their impact on *A. donax*. These agents were *Nigrospora oryzae* (isolates collected from France, Cyprus, and Crete), *Phoma* sp., Chloropid and Cecidomyid fly species and scale insects. These agents have been found abundantly in *Arundo* patches in Europe. Field collections of *Arundo* shoots will be made. This material will be stored for emergence of insects that will give an idea of field infestation and for the emergence of hyperparasites. This is important as insects introduced into the U.S. in the absence of their hyperparasites might lead to heavier infestation and damage to *Arundo*. If an insect and pathogen both look promising, studies will be conducted to follow the impact of both agents for potential synergistic effects. This will be done by inoculating the *A donax* shoots in an insect cage with the pathogen, under the optimal conditions, and releasing the insects. Comparisons will be made with shoots exposed to the pathogen and insect alone and to nonexposed control shoots.

Genetic variability.

Determining the origins of an invasive organism and understanding the effect of its natural enemies in its native habitat are essential to the design of a control program (Roderick, 2004). To this end *Arundo* germplasm has been collected in 17 countries and will be used to trace the origins of *Arundo*. Samples of Arundo material placed over silica gel will be characterized and compared to *Arundo* populations in California and Texas in order to trace the center of origin of *Arundo* stands in North America. In addition plant samples will be put in tubes over silica gel and stored for future genetic characterization.

The origins of *A. donax* will be investigated using cp DNA sequence data and its genetic population structure in North America and Europe using SSRS markers (microsatellites). An enriched library for SSRS markers has been produced already. Additional work is needed to develop the suitable SSRS markers. Once the sequence data has been completed, the data will be analzyed with suitable computer software to develop phylogenetic trees and sequence comparisons.

Results and Discussion:

Giant reed survives winters with temperatures as low as -8°C.Surveys for *Arundo* in Europe showed current limits in France at about the latitude of Lyon to Bordeaux; to about 400m altitude in the Pyrenees; just north of Udine in Italy; in Sicily up to about 800m; not in Austria

Bulgaria, or Romania; but at the border between Greece and Bulgaria on the road from Thessaloniki to Sofia; up to about 1000m in Crete. In Nepal *Arundo* was found up to about 1400m altitude in the Katmandou valley. There remain large areas in the south western and southern USA which *Arundo* could invade.

Preliminary characterization of *Arundo donax* material collected outside the USA shows that Arundo from Spain and Italy is the closest genetically to Arundo in Texas and California respectively. Further work is continuing .

EBCL has collected fungal pathogens from *Arundo* rhizomes in southern France. Buds were often observed to be decaying before opening. Fungal pathogens of *Arundo* leaves have also been collected in France, Greece, Nepal and South Africa (preliminary collections). However the effects of fungal pathogens on giant reed are unknown. Further surveys for pathogens of *Arundo* in Europe are needed if the full potential of natural enemies for biocontrol is to be realized.

At least 3 Diptera: Chloropidae species probably of the genus *Cryptonevra* (currently being studied by taxonomists) have been reared from dead or dying *Arundo* shoot tips, developing canes and cane tips. These are first records of Chloropidae from *Arundo (*Tschirnhaus pers. comm.. J. Tracy). The flies oviposit into the tight whorl of leaves of new shoots which develop into mature canes and descend to the growing base. This is damaged by the larvae feeding along the fibres. The shoot yellows, and eventually becomes a dried brown husk. (Image 1) A fungus *Nigrospora* sp. is associated with this damage and probably with the *Cryptonevra* larvae.

More than 80% of *Arundo* canes from the 0-100cm class are dead in *Arundo* stands in southern France, (Table 1). Total mortality within stands is 56%; mortality within the 0-100cm class contributing 38% of total mortality. The effect of this mortality is to remove canes from within a stand. These are replaced inside the stand by new canes thereby reducing invasion out of and expansion of the stands.

Mortality within classes from 101-300cm is mainly attributable to damage caused by an armored scale (species as yet unknown) which results in these mature canes dying. The witches broom effect on these canes results from pad like deformations caused by intensive feeding. Effects on the *Arundo* rhizome are probably severe and the stressed canes eventually die.

Tetramesa romana of the Hymenoptera Family Eurytomidae is recorded only from giant reed in Mediterranean Europe. It causes elongate galls around side shoots and induces internodal shortening. (Image 2) A new species of *Tetramesa* has been collected by EBCL scientists from *Arundo* in southern France (as yet unidentified). It induces elongated galls which coalesce into large swollen patches; internode length is reduced also. Studies on the impact of *Tetramesa spp.* on giant reed are needed as this genus of Hymenoptera is the only monophagous insect currently known.

A Diptera: Cecidomyidae species which massively attacks and destroys leaf sheaths of canes (Image 3) and opens up the understory to light and the possibility for native plant seeds to germinate.

The most damaging insect is the *Arundo* scale *Rhizaspidiouts donacis* which attacks the rhizome and developing underground buds. (Image 4). In addition during the growing season many crawlers invade the canes and settle on shoots causing distortion and eventually shortening and thinning of the canes. (Image 5).

When 2 or more of these insects are present with the scale, the *Arundo* plants become severely stressed and a light yellow colour before dying.

Summary of *Arundo donax* insects with potential for biocontrol of *A. donax* in the US.

DIPTERA
CHLOROPIDAE
Chloropinae
Cryptonevra spp. Possibly 3 or 4 species, primary invaders on *Arundo donax*.

DAMAGE: Stops shoot from telescoping, attacks buds and shoots up to about 75cm in length; maybe associated with a fungus/bacterium. Shoot browns dries and dies. Of primary importance. (Image 1)

HYMENOPTERA

EURYTOMIDAE
Tetramesa romana (Walker)

DAMAGE: Forms extensive galls on side shoots, developing side shoots and main canes of thin size. Seems to be most effective when scale sp. *Rhizaspidiotus donacis* is present. Of primary importance Image 2

CECIDOMYIIDAE
Lasioptera donacis Coutin. Large colonies in leaf sheaths of Arundo; associated with a *Phoma f*ungus
DAMAGE: Yellowing and death of attacked leaves Image 3

HOMOPTERA

DIASPIDIDAE
Rhizaspidiotus donacis (Leonardi) Attached to rhizome at base of developing and developed canes. Crawlers migrate upwards and/or are transported by ants to developing side shoots.

DAMAGE: Heavy infestations result in distorted side shoots and witchs brooming, resulting in thin brittle often shorter than average canes. Of primary importance Images 4 & 5

Table 1. Percent mortality of *Arundo donax* in southern France. Results based on 15 50X50cm quadrats

Class length (cm)	0-100	101-200	201-300	301-400	401-500	501-600
No. Canes/class	217	44	120	87	21	1
No. Canes (dead-alive)	187-30	29-15	40-80	13-74	6-15	0-1
% mortality in class	86	66	33	15	29	0
Total % mortality	56					

Class contribution to % mortality	38	6	8	3	1	0

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Images of impact of insects on Arundo donax in the Mediterranean area

Image 1. Impact of Cryptonevra sp. on young culms in Spain.

Image 2: Arundo cane galled and shortened by *Tetramesa* attack in France.

Image 3: Arundo leaves killed by Cecidomyid sp. in Spain.

Image 4: *Rhizaspidiotus donacis* females on *Arundo donax* rhizome in Spain.

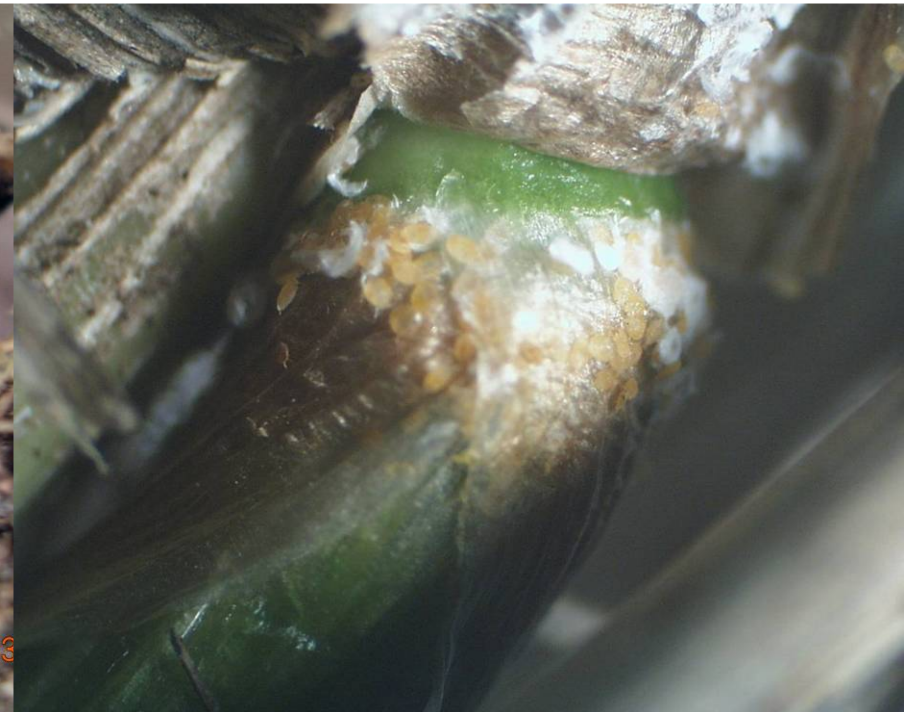

Image 5: *Arundo* shoot twisted by *R. donacis* crawlers in Spain.

Appendix 2.
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Herbivores associated with *Arundo donax* in California

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Summary

The Old World grass, *Arundo donax* L. (giant reed), is a serious invader of California riparian areas, and its purported ecosystem impacts led to its consideration as a target for biological control development. However, the herbivore complex in the *Arundo* adventive range has not been characterized, so there is little information regarding insects that may hinder biological control efforts by interfering with the release of new agents, or that could be promoted as augmentative biological control agents if they have a substantial impact on the target weed. Here we report the results of surveys in California, with emphasis on three presumably non-indigenous insects that inflict significant damage to the host. One is a shoot-boring wasp, *Tetramesa romana* (Walker) (Hymenoptera: Eurytomidae), with a range limited to southern California and that damages shoots generally less than 1 cm in diameter. A shoot fly, *Cryptonevra* sp., is also associated with shoot damage and often mortality of secondary stems. A third herbivore, the aphid *Melanaphis donacis* (Passerini), is widespread in the southern and central parts of the state, but has less apparent impact to the host. *Tetramesa romana* and *Cryptonevra* sp. are currently candidates for biological control development and introduction from overseas locations. Their established presence in California suggests that efforts could be revised to focus on documentation of host-ranges and impacts under field, rather than in quarantine conditions, in anticipation of future re-distribution in North America.

Keywords: Biological control, *Cryptonevra***,** *Melanaphis donacis*, *Tetramesa romana***,** giant reed, herbivore

Introduction

Arundo donax L. (giant reed) may be the most destructive invader of California riparian areas, displacing native vegetation, transpiring excessive groundwater, posing erosion and wildfire risks, and providing poor wildlife habitat (Bell, 1994; Dudley, 2000; Kisner, 2004). Few arthropods appear to be associated with *Arundo* in California (Herrera and Dudley, 2003; Kirk et al., 2003), and most are using it as opportunistic structural habitat rather than as a food source. A variety of herbivores is found in the region of origin (from the Mediterranean Basin and across southern Asia), and the level of herbivore impacts is considered to be much greater than in California or other areas where *Arundo* is invasive (Kirk et al., 2003). Classical biological control is being developed using several candidate agents from Eurasia (Kirk and Widmer, 2004), but implementation is not anticipated for at least several years.

A standard element of a biological control programme should involve documentation of herbivores attacking the target weed in its adventive range to determine if new agents are needed or if effects of existing herbivores can be enhanced, as well as to evaluate the potential for interference with introduction of new agents (Harris, 1975; Olckers and Hulley, 1995). As part of the programme to build the ecological framework for justifying *Arundo* biological control, we are characterizing and comparing *Arundo* herbivores and associated plant condition in California and southern Europe. Early investigations indicated that several non-indigenous insects, including a shoot-boring wasp of the family Eurytomidae, are present in California. The wasp was subsequently identified as *Tetramesa romana* (Walker), a widespread Mediterranean species and a primary candidate in the *Arundo* biological control programme. Members of this genus are highly host specific (Al-Barrak et al., 2004), which makes them particularly suitable for biological control.

Our initial objective is to determine the geographic extent of this and other current *Arundo* herbivores in California, and to quantify their efficacy against this host weed. The larger objective is to increase the impact of existing herbivores through augmentative measures and/or to distribute them more widely to provide more extensive host suppression. We are currently evaluating their host specificity and appropriateness for mass rearing and redistribution.

Materials and methods

To examine *Arundo* herbivore distributions in California, we conducted monthly insect surveys of *Arundo* stands on the Santa Clara River and less frequent (once or twice during study period), extensive surveys of *Arundo*-infested areas throughout the southwestern US (Table 1). Line transects 100 m long were established within *Arundo* vegetation and samples were collected from 0.5-meter square quadrats placed at 10 meter intervals along transect lines. As of April 2007, a total of 994 *Arundo* shoots within all plots surveyed were cut at the soil surface and bundled together for transport back to the laboratory, where they were stored at 8°C until processed. Primary or main shoots, and side shoots or secondary shoots were examined separately. Shoot lengths and diameters were recorded, and shoots were visually examined for herbivores and evidence of herbivore damage. These were then split lengthwise and examined for internal feeders. After dissection, shoots were dried (2 d. at 55° C.) to determine dry weight biomass. All recovered insects were sent to the USDA European Biological Control Laboratory in Montpellier, France for identification and voucher specimens were deposited in the Santa Barbara Museum of Natural History, Santa Barbara, California. Plots were also used to determine shoot density and biomass per unit area for analysis of plant growth differences between native and introduced ranges and evaluation of impacts of biological control agents after release and establishment.

Plant use by *T. romana* (oviposition, feeding and pupation sites) and potential impacts of infestation on *Arundo* were evaluated. We focused on this species because it is one of the primary agents being tested as a potential biological control (Kirk and Widmer, 2004). Shoot length, basal diameter, and biomass of shoots and side shoots infested with *T. romana*, were compared with those of uninfested plants. Main shoot and side shoot data were analyzed separately using student's T-test.

Results and Discussion

No native insect herbivores were found using *Arundo* as a significant food source, in contrast to prior surveys that show numerous arthropods using this plant for non-consumptive purposes (Herrera and Dudley, 2003). Two non-native insects and another unidentified (but potentially non-native) insect were recovered from *Arundo* shoots during sampling. An aphid, *Melanaphis donacis* (Passerini), was found throughout the sampling range (except very northern California) with greatest abundance in coastal *Arundo* populations. Aphids feed primarily on the apical shoots and less mature, distal leaves, and although reaching high population densities in early spring in some locations, only minor damage was observed on plants in one location. Coccinellids were abundant on *Arundo* shoots several weeks after peak aphid densities and may have been responsible for the decrease in aphids by late May - aphids were often not present during sampling in July through December. We did not quantify aphid densities during surveys for several reasons; a lack of any visible aphid feeding effect, aphids were often dislodged from stems during stem sampling and collection, aphid movement reduced accurate measurements, and the time and labor intensive nature of counting aphids would have substantially reduced our ability to adequately sample *Arundo* populations throughout the state.

Dipteran larvae were recovered from over 80% of *Arundo* shoots in one area on the floodplain terrace in the Santa Clara River, Ventura County, CA. We infrequently found similar damage throughout this river system and the nearby Ventura River. Several larvae feed together in the upper nodes on main shoots of plants and feeding damage resulted in atypical 'witches broom' shoot growth with 25% stem mortality in infested shoots (Fig. 1). The 'witches broom' shoot growth was evident in most shoots covering a two hectare radius. We did not examine these damaged shoots for bacterial disease, which can cause this type of deformity; however, we assume that feeding damage on the primary shoot promoted secondary shoot production, as similarly occurs in stems with wind-damaged primary shoots. The gregarious larvae were associated with 'hour glass' shoot damage (Fig. 2), and may be an inquiline species that feeds on microbes that colonize damage from another fly species (A. Kirk, personal observations). Chloropid flies (*Cryptonevra* sp.) in the Mediterranean region primarily attack developing canes up to about 75 cm in length, and cause similar 'hour glass' damage to what we observed. In addition, it or another Chloropidae sp. attacks side shoots and/or leading shoots on taller canes and are often followed by several inquiline species - up to 18 inquiline spp. have been recorded from southern France (A. Kirk, unpublished data). Damage prevents shoots from elongating during growth and results in stunted shoots and a 'witches broom' appearance from increased side shoot production (Fig. 1). We are focusing our sampling efforts during the spring growing period when herbivores producing the initial shoot damage may be present. *Cryptonevra* sp. is a candidate for potential biological control introduction (Tracy and Deloach, 1998), so further documentation of its distribution and impacts in North America, and verification that the species present here is the same as the Mediterranean taxon being tested by USDA-ARS, are essential for future agent selection.

The shoot-boring wasp, *T. romana*, was collected from *Arundo* populations throughout southern California (Fig. 3). Its exit holes and gall-like formations produced during larval feeding are evident on primary and secondary shoots. We have observed oviposition on shoots both in greenhouse cultivation and at field sites (Fig. 4). Shoot infestation was variable (range: 0 to 80%) with a mean of 23.1 ± 4.4% (± SE) shoots infested across all sites sampled (n = 994). However, in March 2007, we observed about 2.5 hectares of an *Arundo* stand near the Santa Clara River (Ventura Co.) with ca. 99% of side shoots infested and killed. A recent survey at this same location suggests that infestation levels of current-year shoots are still high.

Wasp densities are highest on smaller diameter main shoots (primarily new shoots) and side shoots of larger, mature shoots (Fig. 5). Wasp densities can exceed 35 individuals on a main shoot and six individuals on a side shoot, but average 4.9 ± 3.6 individuals per main shoot and 3.4 ± 1.9 per side shoot. Infested main shoots were shorter and smaller diameter than uninfested shoots (shoot height - $t = 3.02$, d.f. = 315, $p = 0.003$; shoot diameter – $t = 3.93$, d.f. = 315, $p < 0.001$), but biomass was not significantly different between infested and uninfested shoots. Infested side shoots were thinner than herbivore free shoots ($t = 2.0$, d.f. =197, $p=0.05$). Side shoot height and biomass were not significantly affected by wasp infestation. In France, *T. romana* infests shoots over a broader diameter range (A. Kirk, personal observation); *Arundo* shoots are also, on average, thinner and shorter than in North American populations (A. Lambert, unpublished data).

Biomass reduction in infested shoots could not be inferred from these data for two reasons: 1) extreme variability in measures of morphological character (shoot length, diameter, and biomass) even with substantial replication, and 2) wasps colonize primarily new shoots and secondary shoots, which have lower biomass than mature and main shoots, respectively (Spencer et al., 2006). Therefore, wasp impact may be less noticeable on young shoots relative to that of an herbivore attacking the larger mature shoots. The frequency of damage, but lack of substantial impact, of *T. romana* to *Arundo* populations in our surveys does not necessarily reflect an absence of impact for this species. It is anticipated that this herbivore, in association with other agents such as the rhizome-feeding scale insect, *Rhizaspidiotus donacis* (Leonardi), may inflict greater damage to host plants (A. Kirk, personal observations).

Further surveys are necessary to accurately determine the full extent and limits of the *T. romana* distribution and its potential to infest a greater proportion of shoots. For example, as our sampling size increases the relationship between wasp density and main and side shoot mortality becomes stronger. We are currently evaluating above and belowground biomass relationships of infested and uninfested plants in a common garden experiment to elucidate the effect of herbivory on resource allocation. Experiments are also being conducted by the USDA-ARS to determine the impact of *T. romana* on water use by *Arundo* (J. Goolsby, USDA, Weslaco, TX, personal communication).

Genetic comparisons are underway in collaboration with D. Kazmer and W. Jones, USDA-ARS, Sidney, Montana and EBCL, using newly eclosed females collected throughout California, and matching with wasps collected in the Mediterranean region to determine the origin of California *T. romana*. It is unclear at this time whether this wasp is a recent introduction in California and is still in its establishment phase, in which case its potential (future) range may be much greater. Au contraire, if *T. romana* has been established for a long period then dispersal may have achieved the full extent of its potential range and the likelihood for redistribution is poorer. Wasps have been found throughout the watersheds of Ventura County, whereas stands in other counties, particularly at the northern and southern extents of its apparent range, are often colonized more heterogeneously with uninfected streams in moderately close (e.g. <10 km) proximity to colonized stands. This may indicate that the colonization process is, as yet, incomplete. Patchy distributions could also represent reduced physiological compatibility with climate regimes at the limits of the *T. romana* distribution. Such a distribution in the warm- temperate coastal region of California is not dissimilar to other organisms introduced for biological control of other California pests, e.g. parasitoid *Aphytis* spp. against the citrus red scale (Rosen and DeBach 1978, 1979). Furthermore, it is not yet clear whether there may have been the co-introduction of *T. romana* parasitoids (A. Kirk, unpublished data), and subsequent population regulation, which could also explain the variation in attack rate observed over *T. romana*'s distribution in California.

The unintentional introduction of *T. romana* and other apparent specialist herbivores to North America provides the opportunity to conduct host-specificity trials and other experimental studies in the field, which will provide instructive ecological information less easily obtained under quarantine conditions. Plant growth and insect behavior are often constrained or atypical in highly controlled environments (Blossey et al. 1994a, 1994b), so results in open field settings are expected to be more representative of natural responses. We suggest that an *Arundo* biological control programme based on development of 'California' *T. romana* and *Cryptonevra* sp. as augmentative biological control agents for local population enhancement and/or re-distribution in North America should be carried out in parallel with foreign importation and further quarantine testing. Host range testing should be continued to validate host specificity prior to agent movement, but conducting this work with insects already established in North America would enhance the ecological validity of the tests and conserve financial resources. This survey of California *Arundo* herbivores also validates the advice of Harris (1975) and others that biological control be developed as a logical progression, with evaluation of weed ecology and associated herbivores in the invasive range before importing new organisms.

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Table 1. Sampling locations (river systems) by county, sampling intensity, and insects recorded in California.

County	# Sampling Sites	Insects
Alameda	1	*Melanaphis donacis* (Passerini)
Humboldt	2	None
Imperial	2	*M. donacis*
Inyo	2	None
Kern	1	None
Los Angeles	4	*M. donacis*, *Tetramesa romana* (Walker)
Mendocino	2	None
Monterey	5	*M. donacis*
Orange	2	*M. donacis*, *T. romana*
Riverside	3	*M. donacis*, *T. romana*
San Bernardino	3	*T. romana* (one site)
San Diego	5	*M. donacis*, *T. romana*
San Luis Obispo	2	*M. donacis*
Santa Barbara	7	*M. donacis*, *Cryptonevra* sp., *T. romana*
Santa Clara	1	*M. donacis*
Ventura	8	*M. donacis*, *Cryptonevra* sp., *T. romana*
Yolo	1	*M. donacis*

Figure 1. *Arundo donax* L. shoot infested with *Cryptonevra* sp. in the Santa Clara River, CA (arrow). Note the atypical 'witches broom' appearance of the infested shoot relative to the other, uninfested shoots.

Figure 2. *Arundo donax* L. shoot damage caused by *Cryptonevra* sp. feeding. The arrow points to the characteristic 'hourglass' damage.

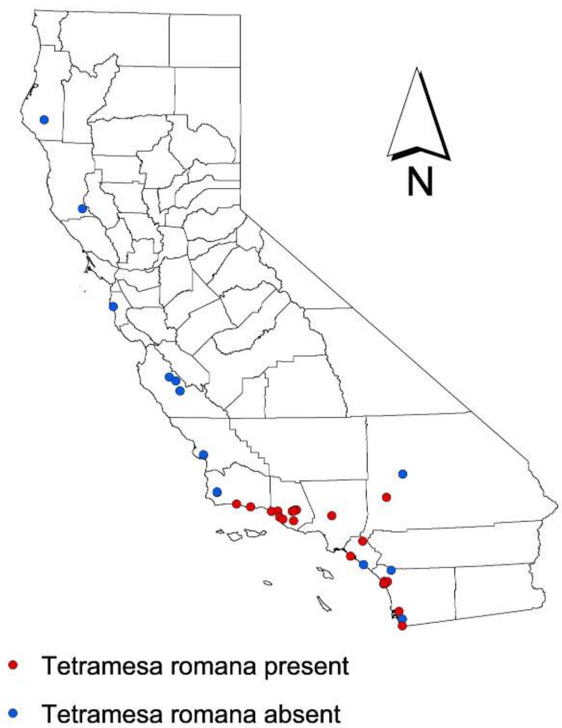

Figure 3. Distribution of *Tetramesa romana* (Walker) (Hymenoptera: Eurytomidae) in California as of April 2007.

Figure 4. *Tetramesa romana* (Walker) (Hymenoptera: Eurytomidae) ovipositing in an *Arundo* side shoot. Bar represents 2mm.

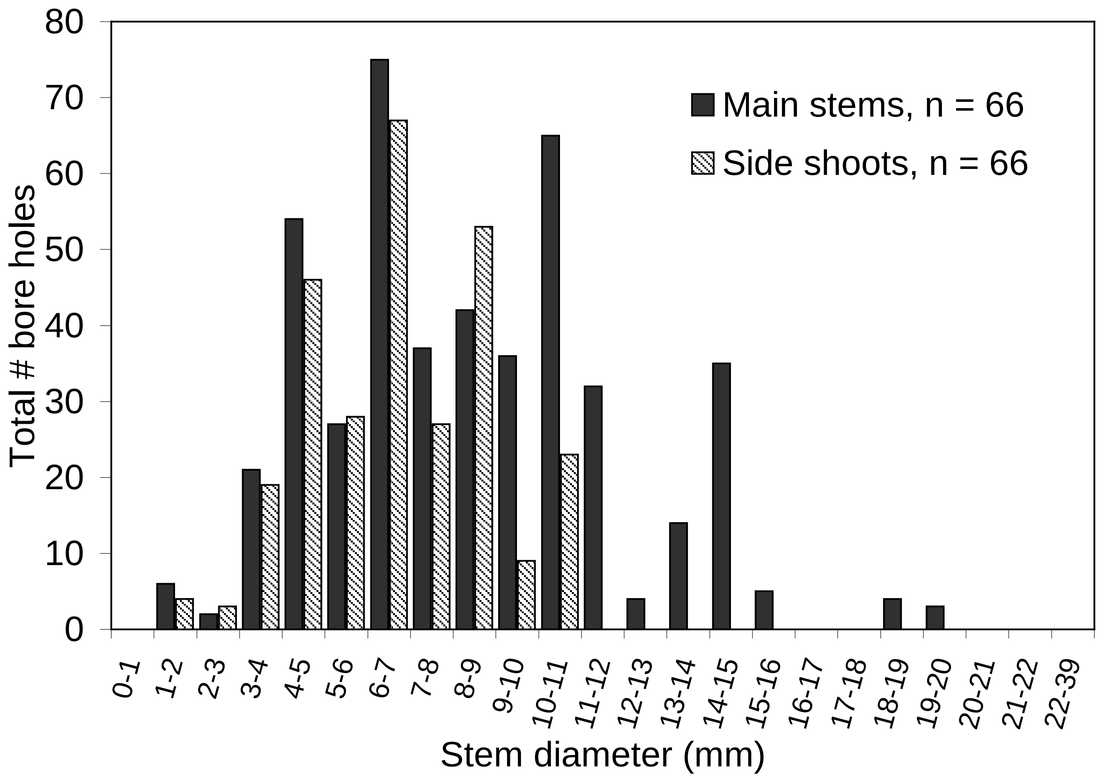

Figure 5. Distribution of emergence holes of *Tetramesa romana* (Walker) over the stem density range (mm) of *Arundo donax* L. main and side shoots.