Seed production in *Arundo donax?*

Maile Johnson[1], Tom Dudley[1] and Casey Burns[2]. [1]Marine Science Institute, University of California, Santa Barbara, CA 93106-6150; [2]USDA

The invasion of California riparian areas by *Arundo donax* (arundo, or giant reed) continues despite efforts to control its spread, and there remains some uncertainty as to how it is able to do so. Arundo can establish monocultural stands from fragmented rhizomes distributed by flooding with many well-known impacts to ecosystems and biodiversity (Else 1996, Decruyeneare & Holt 2005), but the question of whether seed production plays any role in this invasion is frequently cited, yet not adequately answered. No viable seed production has been observed and no seedling has ever been reported here (Bell 1997, Dudley 2000, Boose and Holt 1998, Else 1996, Spencer 2005), but no study has definitively confirmed this statement nor addressed the issue of sexual reproduction in arundo. For this reason, we conducted a study of reproductive structures from arundo found in California and other infested regions, to determine whether seed production and viability could be documented.

Arundo produces flowering cymes (plumes) toward the end of the growing season, from approximately mid-autumn through late winter. Their presence and abundance vary widely among regions and stands, and the specific environmental or biological cue to promote flower growth has yet to be identified, but there does seem to be a general pattern in North America of increased flowering as one moves from north to south. The plume is typically borne on mature stalks, its size ranging from about 20 to 45 cm or more in length. Plumes contain many hundreds of spikelets, each containing two to six florets. In each floret and enclosed within the lemma and palea we observed all the essential reproductive structures: one ovule, two stigmata and three anthers, all distinct characteristics of the Poaceae family.

Arundo donax along the Santa Clara River. *Photo: Tom Dudley*

Approach and Findings

To provide comprehensive coverage across the region, we requested that co-operators collect plumes at the appropriate time in their areas. Co-operators provided a total of 244 plumes from late September to early February, from 31 collection sites representing California, Nevada, Colorado, New Mexico, Texas, Nuevo Leon (northern Mexico), Georgia and Washington D.C.; 22 of these were from northern and southern California. At some sites plumes were collected periodically throughout the flowering season to increase the chance of finding seed in a mature state, while other sites yielded only one collection date. Immature flowers were still suitable for identifying developing ovules.

From each plume approximately 200 florets were scanned under a dissecting microscope to establish presence of any developing structures. We gently peeled back the lemma and the palea with forceps to expose the ovule, and if there was any evidence of seed or ovule development the samples were placed into a Petri dish and stored at 4ºC. The criteria we used to distinguish an inactive ovule from a developing one were the following: if it showed an obvious increase in size, if it looked swollen, if it appeared to have a hard, or hardening, seed coat, or if an embryo was visible. We observed a total of roughly 36,666 florets, from which 43 ovules were suspected as possibly being developing ovules. These originated from only three sites within the Los Angeles River watershed (Los Angeles River near Griffith Park, and nearby from the San Gabriel River), collected between 17 November and 18 December, 2005.

Laboratory Testing

The putative developing ovules were tested with tetrazolium to determine whether actively metabolizing embryo tissue was present. The tetrazolium test (TZ test) is a standardized procedure for evaluating seed viability for a wide range of plant types, including grasses. The TZ test detects live tissue by staining a red/pink color on contact with hydrogen derived from enzymatic activity associated with embryo respiration (Garay 2002). Ovules were exposed to a 0.5% tetrazolium salt solution for 24 hours, and evidence of staining was monitored at one hour, five, 12 and 24 hour intervals (Peters 2000).

Five ovules displayed distinctive staining patterns suggesting the presence of dehydrogenase activity. These may have been viable embryos with the potential to develop into seed, although no true seed was ever found. Generally, the TZ test is used on mature seeds to determine percent viability of the samples, but given the extremely limited ovule sample size we only report absolute numbers. Remaining chaff that potentially held additional developing 'seed' was placed onto moist sand in nursery flats (covered with clear plastic to retain moisture) to test whether any germination would occur, but no germination was observed.

Conclusion and Interpretation

If the TZ staining correctly indicated developing tissue, a total of five developing ovules were derived from a single general location. In the previous year there was suggestion of possible seed production from a single location in Ventura County (D. Kanthack and D. Dyer, pers. comm.), although that site showed no evidence of viable reproductive tissue during this study. This may indicate that the environmental conditions necessary to stimulate reproduction, if reproduction is occurring at all, may be highly specific and not frequently encountered. The 2005 season was unusually wet in California, which may have reduced the tendency to produce flowering structures by arundo because there is anecdotal evidence that low soil moisture plays a role in stimulating flower production.

This study indicates that arundo may be capable of producing seed, albeit in very low numbers and the results cannot be considered definitive. Whether hypothetical 'seeds' could germinate and survive in a natural habitat also remains an open question, but this and other ecological questions can only be addressed if viable seed production can be documented in the future. Therefore, targeted studies will continue in 2006 to better establish whether fertilization and seed development are possible. Of equal interest will be to identify the physiological mechanisms that cause plants to be sterile in most, if not all, cases (we did not have sufficient material to evaluate whether fertilization preceded ovule production). Pollen production appeared to be low, so it is possible that male sterility is a factor limiting fertilization.

The extreme rarity of ovule development in *Arundo donax* can be interpreted to mean it is unlikely to be of ecological significance. Other invasive plants are known to maintain and expand populations based largely on asexual reproductive tactics, such as water hyacinth and other aquatic species which form daughter plants that disperse and form large masses, but these generally produce seed as well. The capacity for multiple reproductive tactics is a common trait for successful invaders (Reichard and Hamilton 1997), so it is unusual for a weed as successful as arundo to effectively have no sexual reproduction.

The essential lack of sexual reproduction also means that genetic diversity, or variation available for natural selection of invasive traits, should be low, as Khudamrongsawa et al. (2004) verified in arundo at the Santa Ana River. Apomixis (diploid seed produced without fertilization) is a means of asexual reproduction that allows rapid production of propagules by some invasive plants (e.g. dandelions), but the lack of genetic variability may limit invasive potential, as indicated by the greater success of out-crossing *Cortaderia selloana* compared with asexual *Cortaderia jubata* (Lambrinos 2001).

Thus, arundo remains one of the few cases in which a serious invader may not depend on sexual reproduction nor seed production at all. Its continuing invasion relies on exceptionally robust rhizomes for population re-distribution and expansion. Despite the fact that no seedlings have yet been found in the field, there is now a slight suggestion that new populations could be established from seed dispersal. The case for seed production may not be fully closed, but the remarkable success of this plant certainly depends on other factors (e.g. nutrient augmentation, altered hydrology, lack of natural controls) that warrant further investigation.

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Contact Tom Dudley at tdudley@msi.ucsb.edu.