Title

Eradicating Arundo Donax from California Ecosystems: Establishing the Most Effective Timing of Mechanical and Chemical Procedures

Author

Wijte, Antonia

Publication Date

2001-07-31

California Sea Grant Final Report

R/CZ-151

Erdicating *Arundo Donax* from California Ecosystems: Establishing the Most Effective Timing of Mechanical and Chemical Procedures

Antonia Wijte
California State University, Long Beach
Department of Biological Sciences

INTRODUCTION

Arundo donax (giant reed) is a tall grass that is native from the lower Himalayas and invaded the Mediterranean region, prior to its introduction in the America's (Rieger and Kreager, 1989; Granval, et al., 1993; Abissy and Mandi, 1999). It is suspected to first have been introduced to the United States in the 1700's, and in the Los Angeles area in the 1820's by Spanish settlers (Bell, 1993, Iverson, 1993 #1573). Its primary use was for erosion control in drainage canals. A number of other uses for *Arundo* have been identified. It is the source of reeds for single reed wind instruments such as clarinet and the saxophone (McFadden and others 1992, Popov and others 1990; Perdue 1958, Van Der Wegen and others 1991). In Europe and Morocco *Arundo* is used for waste water treatment (Abissy and Mandi, 1999), such as nutrient and heavy metal removal, and water volume evapotranspiration. The high rate of evapotranspiration by stands of this species, used as a benefit in these countries, is one of the characteristics that is detrimental in the California ecosystems invaded by *Arundo*.

By the 1990's *Arundo* has infested tens of thousands of acres in California riparian ecosystems, and these populations affect the functioning of these systems in different ways. It increases the fire hazard in the dry season (Scott, 1993). The regular fires promoted by the dense *Arundo* vegetation, are changing the nature of the ecosystem from a flood-defined to a fire-defined system (Bell, 1993). During floods, *Arundo* plant material can accumulate in large debris dams against flood control structures and bridges, and interfere with flood water control management (Frandsen and Jackson, 1993), and bridges across Southern California rivers. It can grow up to 8-9 m tall, and its large leaf surface area can cause the evapotranspiration of up to 3 x the amount of water (2.3 acre feet) that would be lost from the water table by the native, riparian vegetation (Iverson, 1993). Displacement of the native vegetation results in habitat loss for desired bird species, such as the federally endangered Least Bell's Vireo (*Vireo belli pusillus*) and the threatened Willow Flycatcher (*Empidonax trailii eximus*) (Bell, 1993; Frandsen and Jackson, 1993).

Due to the problems listed above, removal of *Arundo* from California ecosystems has been one of the priorities of a variety of organizations and agencies involved in the management of the state's natural resources, such as the California Department of Fish & Game, a number of resource conservation districts. In the practice of *Arundo* control, both mechanical and chemical methods of *Arundo* control are applied, sometimes in combination (Finn, et al., 1990; Jackson, 1993), the choice of their use depending on timing, terrain, vegetation, and funding. The risks, costs, and effects of the different control methods were listed in the most recent *Arundo* and saltcedar workshop by (Bell, 1998).

The timing of the eradication effort can be affected by a number of factors other than the biology of the target species, such as limitations due to bird nesting season, and funding availability. Ideally, the timing of any eradication effort, chemical or mechanical should be determined by the ecophysiology of the target species, in this case *Arundo donax*, rather than the calendar year. For chemical eradication, this has been recognized for a while, as stated by Nelroy Jackson of Monsanto, at the first *Arundo* workshop: "Timing of application for optimal control is important. Best results from foliar applications of Rodeo© or Roundup© are obtained when the herbicides are applied in late summer to early fall, when the rate of downward translocation of glyphosate (the active ingredient of these herbicides) would be greatest." A similar statement has not yet been made for the timing of mechanical eradication methods, nor had the effect of timing on the effectiveness of mechanical eradication been identified.

The objectives of this project were to identify patterns in those aspect of the ecophysiology of *Arundo donax* that relate to methods and effectiveness of both mechanical and chemical eradication efforts throughout the growing season, and how these patterns can be exploited to maximize the effectiveness of the eradication methods.

ASPECTS OF MECHANICAL ERADICATION

Mechanical eradication of *Arundo* can be attempted in many different manners. The most frequently used method is the cutting of the aboveground material, the plant's tall stems. Another method of mechanical eradication is digging out the underground biomass, the rhizomes.

The cutting of stems can occur before and after herbicide applications. The large amount of standing aboveground biomass, up to 45 kg/m^2 (Wijte, unpub. res.) impedes the removal of the cut material, because the costs will be too high. The costs associated with the removal of the large biomass of the stems, has led to the use of "chippers" that will cut the stems into pieces of approximately 5 - 10 cm *in situ*. After these efforts, the chipped fragments are left in place. A small fraction of the fragments left behind after chipping will contain a meristem. The stem pieces of these fragments may have been left intact, or split lengthwise. In the second case the node at which the meristem at located will have been split as well. On many pieces with a meristem, the meristem itself may still be intact. These stem fragments might sprout and regenerate into new *Arundo* plants (Boose and Holt, 1999).

If stems are not cut into small pieces, or removed after cutting, the tall, cut stems can be washed into the watershed during a flood event. This material can accumulate behind bridges and water control structures with possible consequences as described in the introduction. Meristems on the stems can also sprout, and lead to the establishment of new stands of *Arundo* at the eradication project site, or down river (Boose and Holt, 1999).

A. donax stands have a high stem density. The outer stalks of dense stands will start to lean to the outside because the leaves produced during the growing season push the stems in the stand apart. After the initial leaning due to crowding, gravity will pull the tall outside stems almost horizontal (Wijte, pers. obs.). Throughout this report these outside hanging stems will be referred to as "hanging stems". The horizontal orientation causes hormonal asymmetry in these stems. The main hormones involved are IAA (indole acetic acid), GA (gibberellic acid) and ethylene (Kaufman, et al., 1995). The unusual IAA and GA distributions cause the side shoots developing on these hanging stems, to grow vertically. IAA also plays an important role in plant root development (Bandurski, et al., 1995), and may therefore have a stimulative effect on root emergence from the adventious shoot meristem on fragments that originated from hanging stems, that would be absent in stem fragments from upright stems. In a preliminary experiment comparing root emergence between stem fragments from hanging and upright stems, 38% of the hanging stem-stem fragments developed roots, while none of the upright stem-stem fragments showed root emergence (Wijte and Peck, unpub. res.). These results indicated the need for further study into the possibility that new *A. donax* plants can regenerate from the stem fragments with shoot meristems that might be dispersed during mechanical *Arundo* removal efforts.

ASPECTS OF CHEMICAL ERADICATION

In order to apply herbicides at that time that the rate of downward translocation of photosynthates and herbicide would be greatest, this time period has to be established. Carbohydrate distribution and translocation within indeterminate plants, such as *Arundo*, results from the balance between the supply of carbon compounds to and the nitrogen concentration in the different plant tissues. Carbon

(C) and nitrogen (N) are the most important elements in plant tissues. Due to different diffusion rates of NO_3^- and NH_4^+ in soil water versus that of CO_2 in air, and differences in plant N and C uptake rates, plant growth will earlier become nitrogen limited than carbon limited. During plant development tissue nitrogen concentrations are diluted by plant growth (Fig. 1a), which is mainly based on the addition of carbohydrates to the tissues. When plant growth becomes nitrogen limited, the tissue will maintain the minimum nitrogen content needed for the nucleic acids and proteins that maintain metabolic function. At this low tissue nitrogen content, there is not enough nitrogen in an individual cell to provide the nucleic acids and proteins to support the metabolism of two cells, therefore the cells cannot divide. This means that the tissue cannot grow anymore (Fig. 1b), until it receives a new supply of nitrogen. When plant tissues cannot grow due to nitrogen limitation, they cannot incorporate or store additional carbohydrates. This lower physiological limit of tissue nitrogen content, at which no more cell division or incorporation of carbon is possible, is called the critical nitrogen content (CNC) of the tissue (Fig. 1a). The CNC is expressed on a carbon basis (g N/g C).

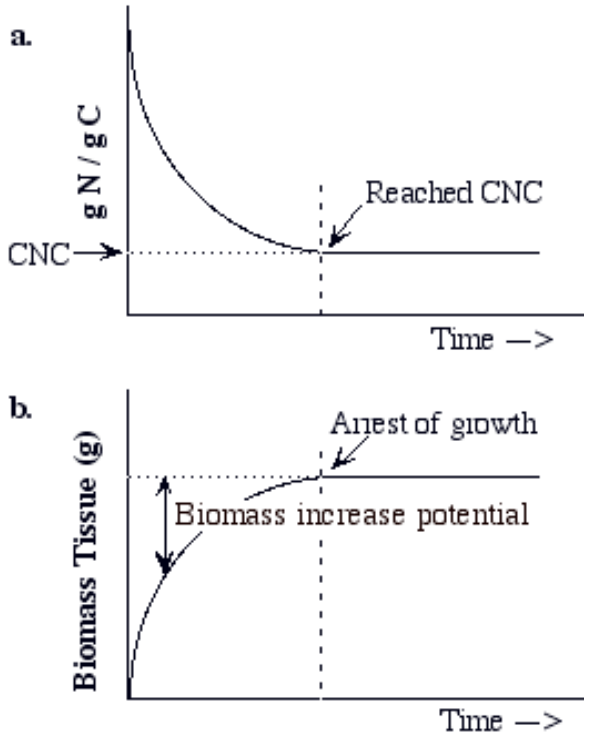


Fig. 1: Relationship between tissue nitrogen content, critical nitrogen content (CNC), and tissue growth.

The CNC can be determined for whole plants (Bradley and Morris, 1992), and for the different functional tissues of the plant. Different plant parts perform different functions, and therefore have different minimum nitrogen requirements for metabolism maintenance. In previous research with the dicotyledonous storage root perennial *Ipomoea batatas* (sweetpotato), it was determined that the most photosynthetically active tissues, the leaves, and the fibrous roots, which are involved in nutrient uptake, have the highest CNC of all vegetative plant tissues (Wijte, et al., 1997). The storage roots of *I. batatas* had a significantly lower CNC than any of the other *Ipomoea* tissues.

The difference between the actual tissue nitrogen content and the CNC determines the capacity of these different plant tissues to incorporate or store carbohydrates. Tissues with nitrogen contents that are above the CNC can still incorporate or store carbohydrates. These tissues have a positive carbon sink strength (Fig. 1a). Photosynthetically active tissues that have reached their CNC will not incorporate the produced carbohydrates, because that would dilute the nitrogen content of these tissues below the CNC, and metabolism would be impaired. Instead, the photosynthetically active tissues deposit the produced carbohydrates in the phloem, which transports them to those tissues that still have the ability to incorporate or store carbohydrates (Wijte, et al., 1997). This is how leaves, that because of their high CNC loose the ability to incorporate the photosynthates in their own tissues relatively early during the development of the plant, can still produce photosynthates and translocate them down to the reserve storage organs, such as *I. batatas* storage roots, which maintain their positive carbon sink strength, and tissue growth (Fig. 2), the longest of all plant tissues, due to their low CNC.

MATERIALS AND METHODS

Rooting of *Arundo donax* Stem Fragments Throughout the Growing Season.

Ten hanging stems and ten upright stems of *Arundo donax* were collected in the Santa Margarita basin, on the U.S. Marine Corps Base, Camp Pendleton, every six weeks. In the field the individual stems were cut into fragments that each contained an individual stem meristem. The stem fragments for each individual stem were kept together. All stem fragments were transported back to the laboratory. In the greenhouse, the stem fragment of the different stems were placed in individual containers with 5 L. of aerated rooting solution. For five hanging and five upright stems, the rooting solution was plain tap water, while for the remaining five hanging and five upright stems, the rooting solution was half strength Hoagland's nutrient solution. From the stem fragments collection of each individual stem, one (1) stem fragment was used for rooting in soil (table 1).

Table 1: Experimental design *Arundo donax* stem fragment seasonal rooting experiment, with replicate numbers.

	Stem orientation	
Rooting medium	**Hanging**	**Upright**
Water	All meristem containing fragments of 5 stems	All meristem containing fragments of 5 stems
Nutrient Solution	All meristem containing fragments of 5 stems	All meristem containing fragments of 5 stems
Soil	10 meristem containing stem fragments	10 meristem containing stem fragments

Regeneration from stem fragments starts with the growth of a new stem from the meristem. Root growth from the meristem always follows shoot growth, and not all meristems with shoots will grow roots. Therefore, rooting of the stem fragment was selected as the criteria of successful regeneration from a meristem on a stem fragment. The stem fragments were checked for rooting three times per week. Rooting success was calculated in percent of all meristems in the container. The speed with which rooting occurred was expressed is t_{50}. That is the number of days needed for 50% of the total number of meristems that eventually would root in a container, to root. When a stem fragment had rooted, the diameter of the stem at the point of the meristem was determined, to assess the effect of the relative age of the meristem on the speed with which they rooted.

Effect of temperature on rooting of *A. donax* stem fragments.

The effect of the temperature at the time of rooting was tested for hanging *A. donax* stem fragments at two times in the growing season. In April 1998, 32 stem fragments were randomly distributed over 4 containers with aerated nutrient solution each at 10 and 20 °C. The stem fragments were monitored every day for rooting. Rooting success was calculated in percent of all meristems in each individual container, and mean rooting percentages were calculated for the four replicate containers for each fragment type. The speed with which rooting occurred was expressed using t_{50}. This experiment was repeated in April 1999 at 15, 17.5, and 22.5. A repeat 20 °C treatment was included in the 1999 experiment, to allow for comparison with the 1998 experiment.

Rooting of *Arundo donax* stem fragments under controlled temperatures at different exogenous indole acetic acid (IAA) concentrations.

Hanging stems and upright stems of *A. donax* were collected along the Santa Ana River, in Riverside County, CA, every month from February, 1999 through May, 2000. Eighty meristem containing fragments of each stem type were cut and surface sterilized. Twenty replicate fragments each (n=20), were randomly assigned to a control (0 μM IAA) and three different exogenous IAA treatment levels (5, 10, and 20 μM IAA). The fragments were placed in individual culture tubes (200 x 25 mm), that had been autoclaved containing 15 ml of plant growth medium with 4.4 g/L MS salts, 2 g/L Gel Gro, and the assigned concentration of IAA (pH = 5.7). Each stem fragment was placed with its lower end in the rooting medium and its meristem submerged but near the interface of the rooting medium and the air. The upper end of the stem fragment extended above the rooting medium. The tubes were placed in a climate-controlled chamber with a temperature/light regime of 14 h of 27 °C in the light, and 10 h of 15 °C in the dark. The rooting of the fragments in the tubes was monitored daily for 30 days. Rooting success was calculated as percent of all meristems at the different IAA concentrations. The speed with which rooting occurred was expressed for each month using the t_{50} for each IAA concentration and stem type combination.

Endogenous IAA concentrations in the newly developing shoots of rooting *A. donax* stem fragments.

The remaining meristem containing fragments from the hanging and upright *A. donax* stems that were collected monthly to test the effect of exogenous IAA were stored in tap water in separate containers. The containers were placed in the growth chamber under the temperature and light regime described previously, and the water was aerated. After approximately 10 days, the meristems on the fragments had developed into shoots and roots. Three replicate samples of approximately 10 g (fresh weight) of the new shoot material were harvested from the stem

fragments of both the hanging and the upright stems, and placed on ice. For the extraction of endogenous IAA, the samples were dipped in grinding media (80 % aqueous methanol with 10 mg/L butylated hydroxytoluene, BHT), placed in glass tubes, and flash frozen in liquid nitrogen. The tissues were homogenized for 2 minutes in 20 ml grinding medium (2-3 C) with an Omnimixer (Servall). The homogenates were incubated on a wrist action shaker for 20 minutes and filtered through microcloth. The filtered solution was centrifuged at 10,000 rpm in a JA-17 rotor, and the supernatant was saved. The tissue material on the filter and the centrifugation pellet were combined and incubated again in 10 ml grinding media, and the sample was filtered and centrifuged as before. The supernatants were combined and their volume was reduced to 1 ml using a speed vacuum evaporator at 37 C. The concentrated samples were centrifuged at 13,000 rpm for 10 minutes at 4 °C. The supernatants were filtered through a 2 μm syringe filter (NalGene). The resulting tissue extracts were stored in 1.5 ml centrifuge tubes, and stored at -80 °C.

For the HPLC, the extracts were eluted from a C18 reverse phase column (SD-C18, Hewlett Packard, 46 x 250 mm; 5 micron particle diameter) with an analytical SB-18 guard column (Hewlett Packard, 46 x 12.5 mm; 5 micron particle diameter), with a gradient solution of 20-35% acetonitrile with 20 mM sodium acetate (pH = 3.5) at a flow rate of 1.5 ml/min. The samples were monitored using a spectrofluorimeter detection system (Simadzu RF-535) at an excitation wavelength of 280 ± 10 nm, and an emission wavelength of 350 ± 10 nm. The IAA peaks of the sample extracts were identified and quantified using 0.1 and 0.5 μM standard solutions.

Rooting of Cut *Arundo donax* Stem Fragments of Different Sizes

The rooting success of meristems on intact, round stem fragments, and split stem fragments of decreasing size was determined in plain water at 20 C. In one group of the split stem fragments, the size of the stem fragment was reduced to the size of the meristem itself. Additionally, the rooting of stem fragments on which the meristem had been cut was tested for both groups (table 2).

Table 2: Experimental design *Arundo donax* stem fragment size rooting experiment.

	Fragment type	
Rooting medium	**Intact, round**	**Split lengthwise**
Fragments tested (cm)	**10; 3; 1; cut meristem**	**5; 2; 0; cut meristem** (0 = meristem only)
Replicate containers (n)	4	4

One hundred thirty meristem containing *Arundo* stem fragments were collected in the Santa Margarita basin in September, 1998. The stem fragments were transported back to the laboratory. The stem fragments were randomly assigned to one of eight treatments. In a climate controlled growth chamber, 4 stem fragments each were allowed to germinate in 4 replicate containers (n = 4), in water that was aerated. The stem fragments were checked for rooting three times per week. Rooting success was calculated in percent of all meristems in each container individually, and mean rooting percentages were calculated for the four replicate containers for each fragment type.

Arundo donax Tissue Critical Nitrogen Content and Growth

The critical nitrogen content of *Arundo* leaf tissue was determined in a hydroponics experiment. One hundred *Arundo* stem fragments were collected in June 1998 from the Santa Ana River near River Road in Riverside county. In the greenhouse, the stem fragments were placed in water for 2 weeks to allow for root and shoot growth. After two weeks, 48 young plants that sprouted from the meristems on the stem fragments were randomly selected for use in the experiment. Four stems were placed in each of eleven 120-liter (L) plastic containers, that were filled with 100 L aerated, half strength Hoagland nutrient solution (Hoagland and Arnon, 1938). The sprouted stem fragments rested on a floating plastic mesh supported by a ring of plastic pipe, on the surface of each trash can's nutrient solution. A sheet of opaque white plastic was wrapped around and over each trash can to block out sunlight preventing algae growth and high temperatures in the nutrient solution.

The nutrient solutions in the containers were monitored two times per week during a 48-week growth period. Each check consisted of the following: addition of enough deionized water to bring the can's nutrient solution level up to a 100-L mark, determination of the can's electrical conductivity (YSI model 30) in full volume. A concentrated (50x) Hoagland solution was added to re-establish the conductivity of the nutrient solution to its original value. The pH was adjusted to 5.7. The harvest dates were partially determined by the growth of the plants as the experiment progressed. Harvested plants were separated into apical meristems, green leaf blades, brown leaf blades (when present), green leaf sheaths, brown leaf sheaths (when present), stems, rhizomes, and roots. Plant parts were dried to a constant weight at 60 C. Biomass of the tissue was determined and sub-samples were ground in a Wiley mill to pass a 0.5 mm mesh screen (Bradley and Morris, 1992). The nitrogen and carbon contents of the tissues were determined using an organic elemental analyzer (Exeter Analytical model CE440).

RESULTS AND DISCUSSION

MECHANICAL ERADICATION

Rooting of *Arundo donax* Stem Fragments.

Stem fragments with meristems can root and regenerate new *Arundo* plants (Fig. 2 + 3), as has been reported earlier by (Boose and Holt, 1999).

There were significant patterns in rooting success of meristems on *Arundo* stems throughout the growing season. In the winter months of November through January, rooting is low, and success percentage lies below 20%, with the exception of 28 ± 12% (mean ± S.D.) rooting for meristems from hanging stems in January (Fig. 2a + b; 3a). Nearly all meristems rooted from March through September. The speed with which meristems rooted showed a related pattern through the growing season (Fig. 2c + d; 3b). In the period with the lowest rooting success, t_{50} had the highest values, indicating the slowest rooting. Rooting was most rapid in the months of May through July, a time window that was more narrow that the period in which rooting is most successful.

The type of rooting medium does not have much influence on rooting success and speed. There are no significant differences between the results in plain water and half-Hoagland nutrient solution (two-way ANOVA, $p > 0.05$). The rooting success and speed pattern is similar in soil, but the single replicate meristems do not allow for inclusion in the two-way ANOVA.

When compared within each sampling, the rooting success of meristems from hanging stems was significantly higher than that of meristems from upright stems (paired sample t-test, $p = 0.0003$, $n = 36$). When split for rooting medium, there was no significant different in rooting success between hanging and upright meristems over time in plain water (paired sample t-test, $p = 0.076$, $n = 12$), but the differences remain significant in the nutrient solution and in soil ($p = 0.049$, and 0.041, respectively, $n = 12$). Like with rooting success, rooting speed of hanging meristems was significantly faster when compared to that of the upright meristems of the same sampling date (paired sample t-test, $p = 0.0039$, $n = 36$). When separated among rooting media, the difference in the speed with which rooting occurs was most pronounced in plain water (paired sample t-test, $p = 0.0069$, $n = 12$), but still exists in the nutrient solution and soil ($p = 0.031$ and 0.053, respectively, $n = 12$). Though these differences in rooting success and the speed of rooting may be statistically significant, they generally are too small to be ecologically significant.

Stem diameter at the node where the meristem is placed is an indicator of relative height on the stem, and the age of the meristem. Within stems, there was no relation of rooting success or speed with the diameter of the stem at the point of the meristem, so the older meristems on an *Arundo* stem do not root better or faster than the younger meristems on *Arundo* stems.

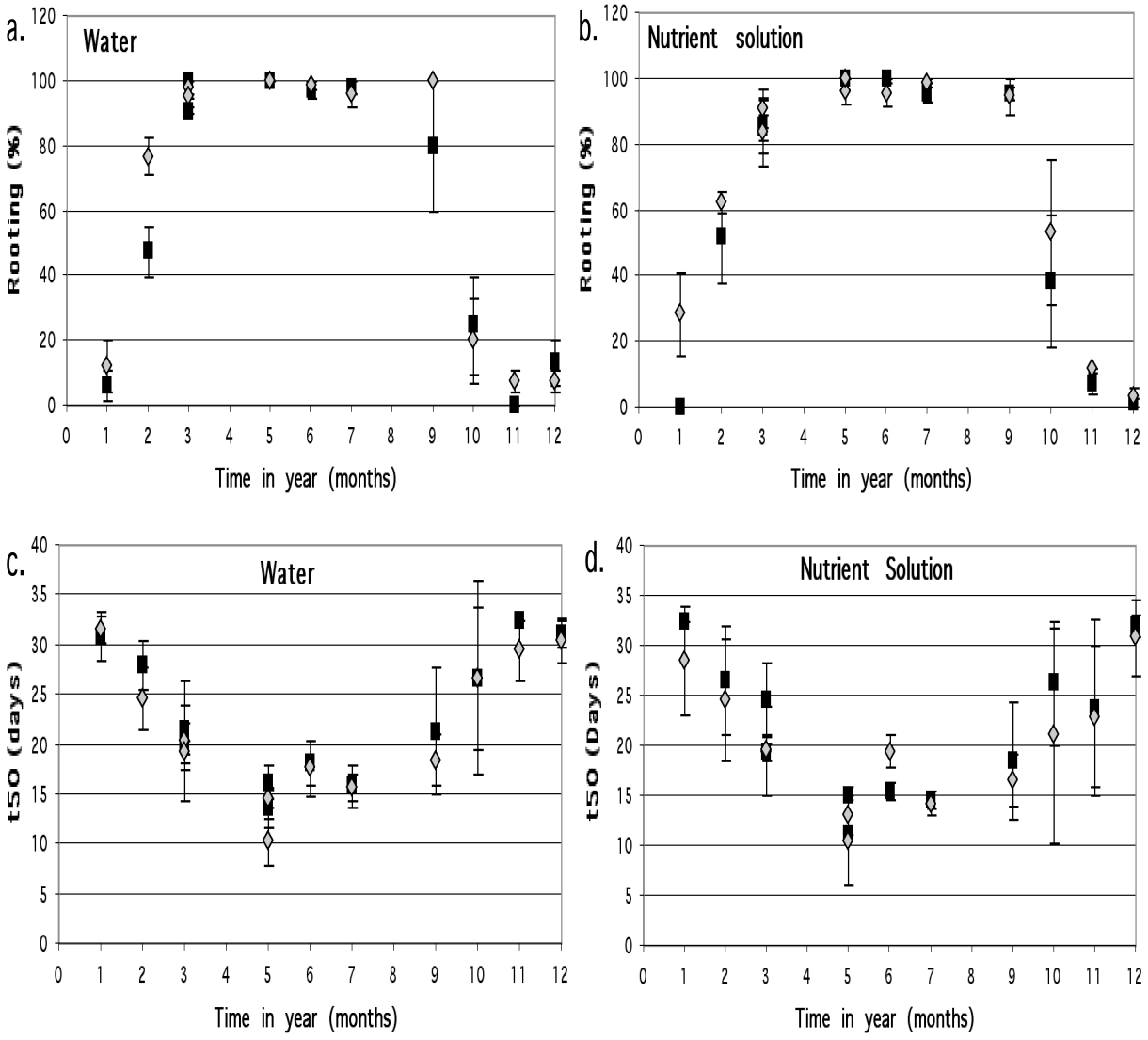


Figure 2: *Arundo donax* stem fragment meristem rooting in water and nutrient solution. a: Rooting success (%) in water; b: rooting success (%) in nutrient solution; c: speed of rooting, t_{50} (days) in water; speed of rooting, t_{50} (days) in nutrient solution. Open diamonds: meristems on hanging stems; closed squares: meristems on upright stems; Error bars represent ± 1 S.D., n = 4.

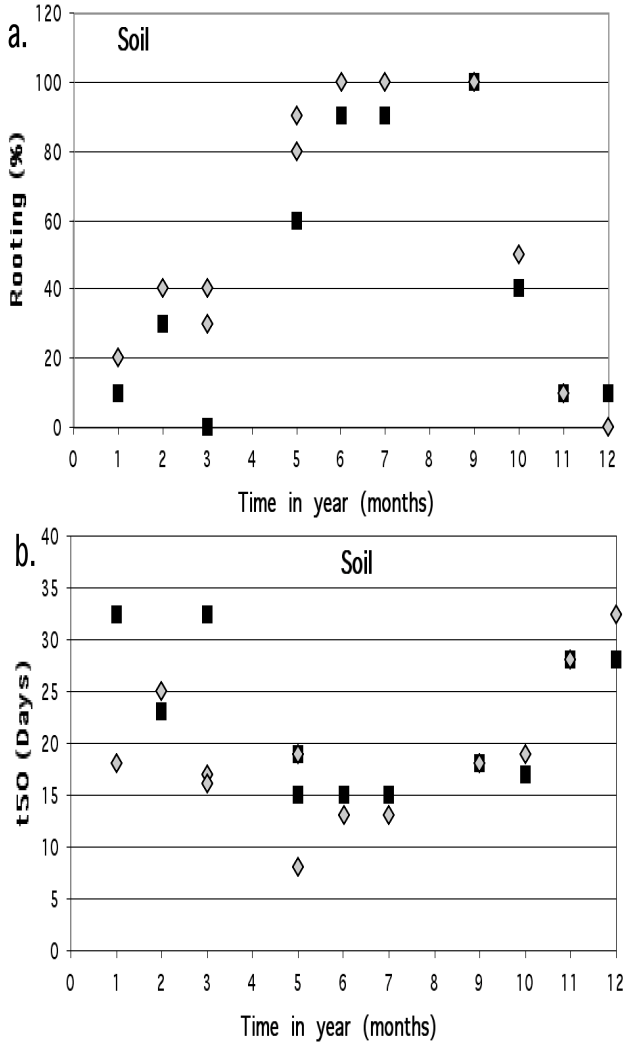
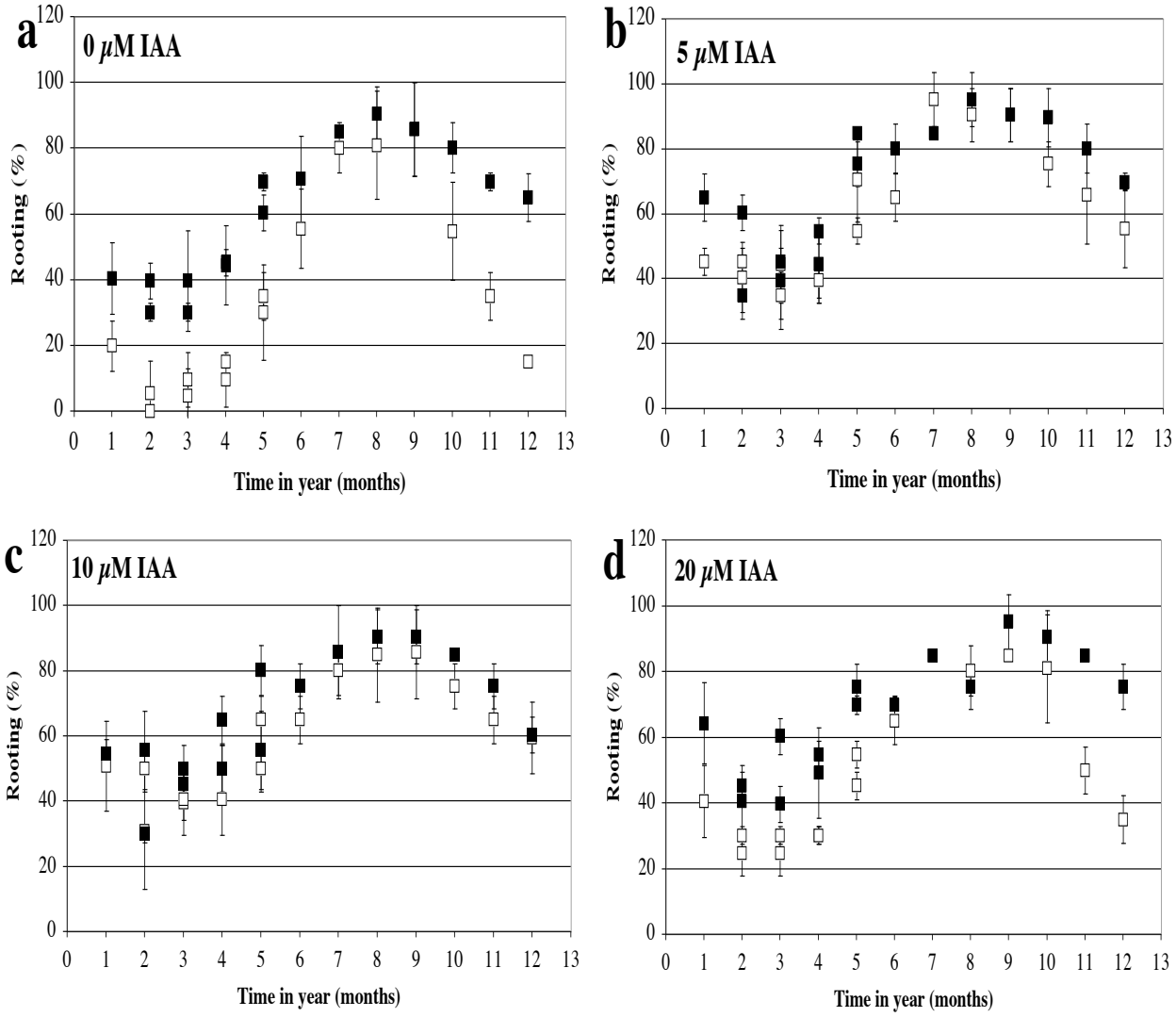


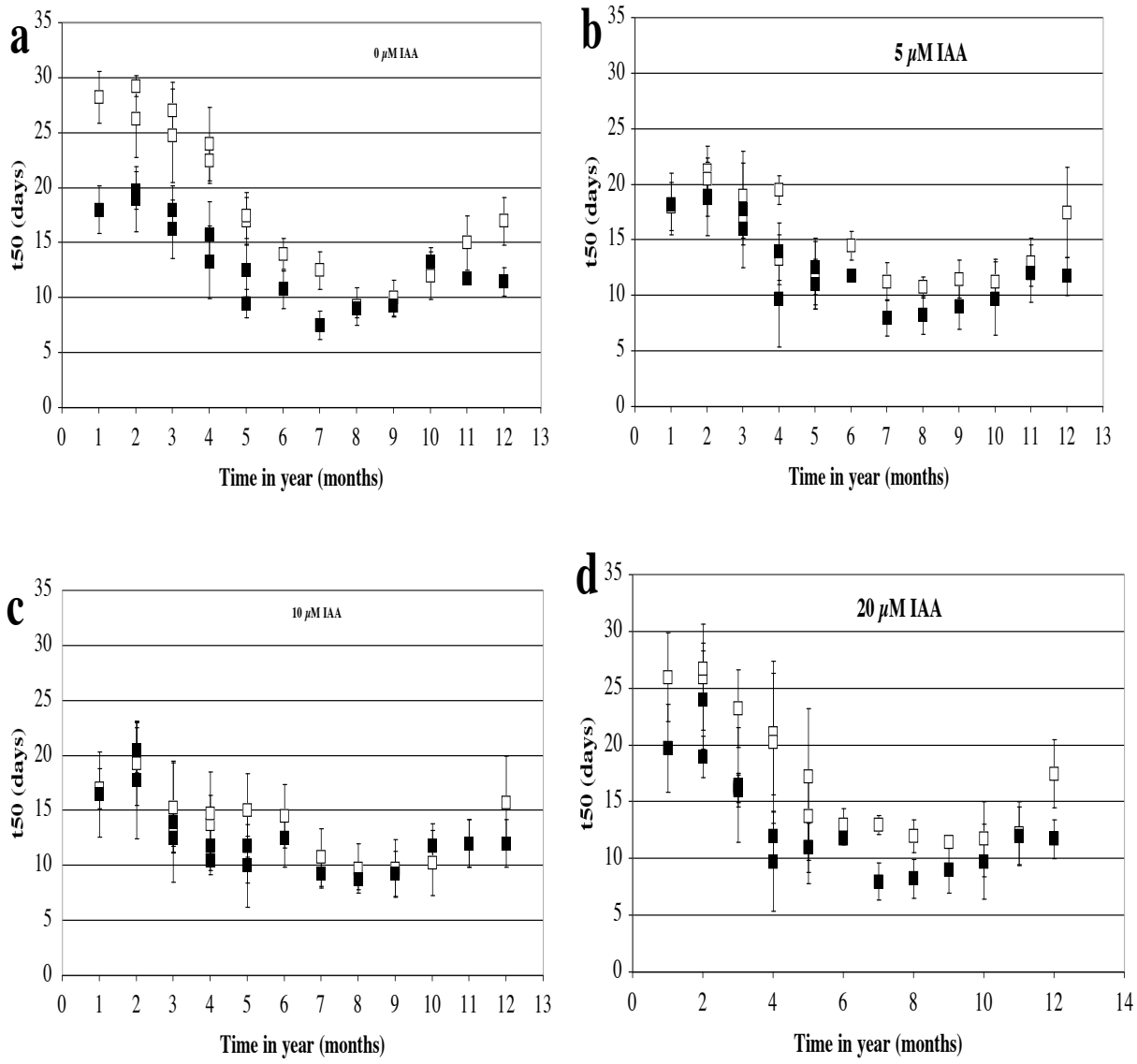
Figure 3: *Arundo donax* stem fragment meristem rooting in soil. a: Rooting success (%). b: Speed of rooting, t_{50} (days). Open diamonds: meristems on hanging stems; closed squares: meristems on upright stems; n = 1 sample of 5 stem fragments.

Seasonal rooting patterns under controlled temperature conditions.

When the temperature of the rooting environment was controlled at 28/15 °C for 14/10 h during the entire growing season the seasonal patterns of rooting success and speed remained, and differences between the seasonal rooting patterns of the fragments from hanging and upright stems emerged (Figs. 4a and 5a). The overall patterns differed slightly from those in the greenhouse experiment, with the lowest rooting by both stem type fragments in February through March. The rooting percentages increased in April, and the highest rooting rates occurred from July through September at 80 – 92% for both stem types. In October the rooting of the upright stem fragments decreased more than that of those from hanging stems. The lowest rooting rates of the stem fragments from upright stems were 0 – 10% in February and March, while the rooting rates of the hanging stem fragments only decreased to 30% (Fig. 4a). The positive influence of the seasonal effect in the months of July – September on the rooting rates of both stem types masked the difference that emerged in the Winter and Spring months. The rooting by meristems from upright stems benefits more from this seasonal effect that of meristems from hanging stems. The seasonal effect on the rooting rates of the stem types could be related to a number of environmental factors that change during the growing season, such as temperature, light intensity, and daylength. (Boose and Holt, 1999) determined that stem fragments sampled at the same time, but stored at different temperatures, displayed different sprouting percentages when potted and regenerated at the same temperature in a single greenhouse. We hypothesize that the different ambient temperatures prior to sampling in our experiment was an ecophysiological equivalent of the experimental factor "storage temperature" in the Boose and Holt study, and one of the factors involved in the seasonal pattern of *A. donax* stem fragment rooting.



Figs. 4. Rooting success (%) of meristems on *Arundo donax* stem fragments under 28/16 C, 16/8 h temperature-light regime exposed to different exogenous IAA concentrations. a. 0 μM IAA. b. 5 μM IAA. c. 10 μM IAA. d. 20 μM IAA. Open symbols: meristems on upright stems; Closed symbols: meristems on hanging stems. Error bars represent S.D.; n = 3.



Figs. 5. Speed of rooting (t_{50}; days) by meristems on *Arundo donax* stem fragments under 28/16 C, 16/8 h temperature-light regime exposed to different exogenous IAA concentrations. a. 0 μM IAA. b. 5 μM IAA. c. 10 μM IAA. d. 20 μM IAA. Lower t_{50} values indicate faster rooting. Open symbols: meristems on upright stems; Closed symbols: meristems on hanging stems. Error bars represent S.D.; n = 3.

The seasonal differences in the rooting percentages and speed between meristems from upright and hanging stems that was striking under controlled temperature conditions had been much less pronounced in the greenhouse rooting experiment. The results of the greenhouse rooting experiment show that the temperature at the time of rooting influence the effects of the seasonal factor(s). Environmental effects, such as temperature and inundation, are known to affect the success of invasive plants with either negatively or positively (Hellings and Gallagher, 1992; Morrison and Molofsky, 1999) In the greenhouse experiment, the temperatures of the rooting medium varied with the ambient temperatures and solar irradiation, while the temperature of the rooting environment in the growth chamber experiment was the same throughout the growing season. The greenhouse, the temperature of the rooting media in the winter ranged from 0.5 - 2 °C at night to 19 –21 °C during the day. In the spring and summer, solar irradiation increased these temperatures to 16 -18 and 28 - 34 °C, respectively. To test the effect of the temperature at rooting, we tested the rooting of fragments of hanging *A. donax* stems at different temperatures in April and May, a period that in the year-round temperature controlled experiment the success rates were 45 ± 10% in 1998, and 45 ± 21% in 1999. In this test using constant temperatures, no rooting occurred at 10 °C during the 40 days of the experiment (table 1). At 15 °C, rooting (25 ± 0.0%, t_{50} = 30.5 days) was better than at 10 °C , but significantly less than at 17.5, 20, and 22.5 °C (100 ± 0.0% for all, t_{50} = 15.5, 18.5, and 13.8 days, respectively). In the greenhouse experiment, the seasonal pattern of rooting success was present, but the inherent advantage of fragments of the hanging stems in the winter months was masked by the negative effect of the lower temperatures of the rooting media. The temperatures chosen for the year-round temperature controlled experiment were selected to reflect the temperature conditions in the habitats invaded by *A. donax* in Southern California in the months of April and May. From the results of this April constant temperature experiment, it appears that the lower night temperature in the 28/15 °C for 14/10 h experiment led to a reduction in rooting success from the maximum possible in that month. This reemphasizes the effect of in situ temperature on the success of stem fragment meristem rooting, and the ecological danger of the floating stem fragments in shallow waters.

The inherent seasonal pattern observed in the year round temperature controlled experiment may have resulted from cycles in the concentrations of the plant growth regulators that play a role in the growth of the side shoots, and the apical dominance of the top of the main stems. One of the growth regulators that plays a major role in the regulation of apical dominance is indole-3-acetic acid. The effect of IAA on the rooting of axillary bud on *A. donax* stem fragments throughout the growing season was tested through the use of exogenous IAA in the rooting medium, and the determination of endogenous IAA levels in the shoots that grew from the axillary buds. When the stem fragments and their axillary buds were exposed to 5 and 10 μM IAA in the rooting medium, the difference between the hanging and the upright stems disappeared. The main effect of the exogenous IAA was a significant improvement of the rooting percentage and speed of the upright stem fragments in the winter and spring periods, so that the difference between the two stem types was minimized. The exogenous IAA had little effect on the rooting success and speed of the hanging stem fragments (Fig. 5). At 20 μM exogenous IAA, the highest concentration applied, the success rate and the speed of upright stem fragment rooting decreased from the optimum observed at 5 and 10 μM, almost down to the percentages and t_{50} observed in the absence of the hormone (Fig. 5d). The IAA in the rooting medium may have reached the axillary bud through the vascular bundles of the main stem piece, directly through the cuticle of the bud itself, which was positioned immediately below the rooting medium surface, or both. In early studies into the effect of IAA on

plant growth, the growth regulator was sometimes applied to the leaf tissues, and the position of the axillary bud in the rooting medium could have resulted in a similar situation.

There is a striking similarity between the seasonal pattern of the endogenous IAA levels in the shoots that grow from the hanging and upright stem fragments and their seasonal rooting patterns. The lowest IAA levels for both stem types occurred in the spring, and the highest levels in late summer (Fig. 6).

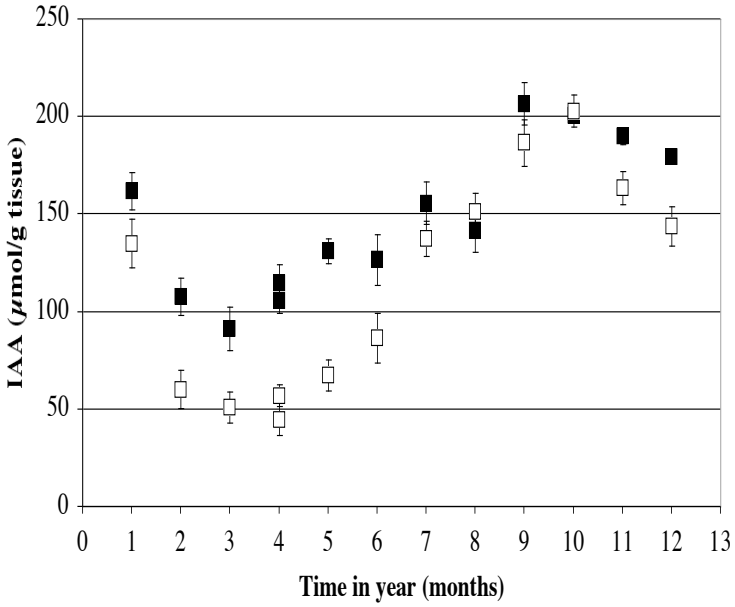


Fig. 6. Endogenous IAA concentration in new shoots grown from meristems on *Arundo donax* stem fragments, which were sampled throughout the growing season. Growth chamber conditions: 28/16 C, 16/8 h temperature-light regime. Open symbols: meristems on upright stems; Closed symbols: meristems on hanging stems. Error bars represent S.D.; n = 3.

At the time that the endogenous IAA levels are low, levels in the shoots from the upright stem fragments are significantly lower than in those from the hanging stems. As the IAA levels in the shoots increase with the progression of the growing season, the levels in the shoots from the upright stem fragments increase more than in those from the hanging stems, and the difference between the shoots of the two stem types disappears (two-way ANOVA, $p < 0.001$). The role of this IAA is unclear. Although studies have also shown more complicated mechanisms (Pearce, et al., 1995), IAA produced in the main stem apex plays an important role in the apical dominance, and the growth of side shoots. A difference between the rooting and the endogenous IAA patterns is that the seasonal pattern of endogenous IAA concentrations in the new shoots runs approximately one month behind that of the rooting success. This makes it less likely that the IAA concentrations in these new shoots, of which the growth is initiated prior to root growth, is the direct cause of the rooting of the stem fragments. It is possible that both the rooting pattern and the endogenous IAA pattern result from seasonal variation in another factor, possibly the concentrations of another plant growth regulator. Preliminary enzyme kinetics results have shown higher NADP-dependent indole-3-acetatealdehyde oxidase activity in the new shoots that developed from the hanging stems in April than in those from the upright stems (Mizutani, unpub. res.). This indicates that the IAA

measured in the new shoots may be the product of de novo synthesis in these shoots, rather than a trace of the IAA that may have been stored in the main stem fragment that was used in the experiment. IAA plays an important role in tracheary cell differentiation and xylem regeneration (Jacobs, 1998). The support for the formation of the vascular system by IAA and its promotion of the formation of adventitious roots may be related, but no causal relationship was determined in this study.

The patterns of the IAA levels that developed in less than 10 days in the new shoots on the hanging and upright stem fragments may have resulted from at least two different factors. First, we hypothesize that the overall seasonal pattern resulted from the in situ temperature conditions at the time we sampled the main stem fragment. Secondly, the difference between IAA levels in the new shoots on the hanging and the upright stem fragments may have resulted from the effect of stem orientation on the inter- and intracellular distribution of plant growth regulators in the plant tissues. The near horizontal positioning of that part of the hanging stem where the side shoots were growing from the stem caused these side shoots to grow vertically, perpendicular to the direction of the original stem. This gravitropic response of the side shoots is the result of the different inter- and intracellular distribution of plant growth regulators that resulted from the horizontal orientation of the main stem.

Rooting occurred for the intact meristems on both the intact stem fragments and those split lengthwise, with no significant difference in rooting percentages (one-way ANOVA, $p > 0.05$) (Fig. 7). Zero rooting or shoot growth occurred from meristems that had been cut.

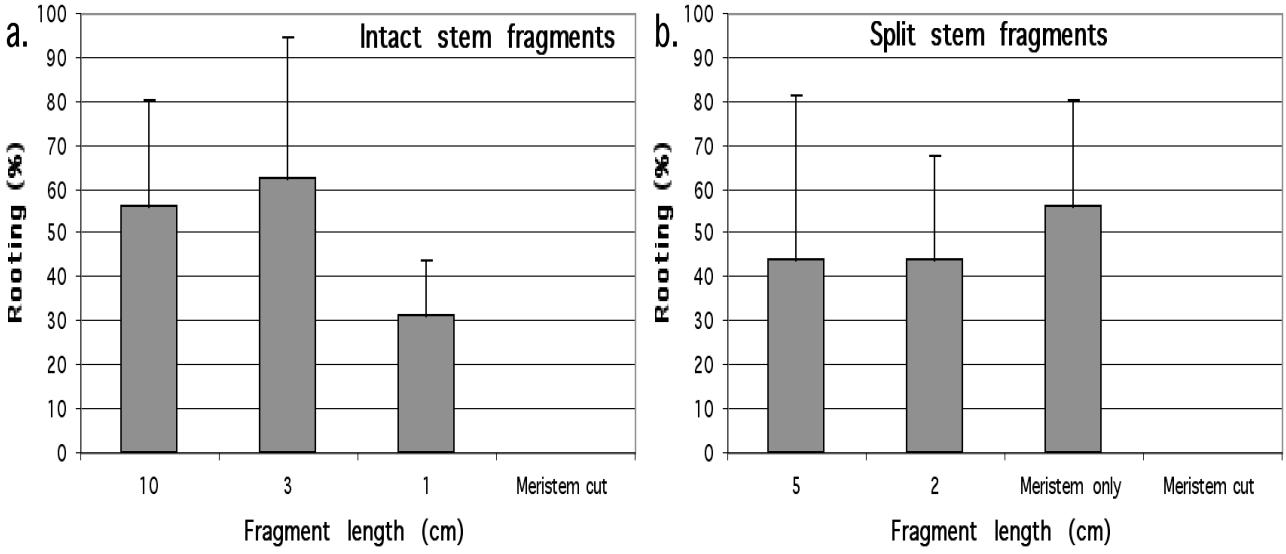


Figure 7: Rooting success (%) of *Arundo donax* stem fragment meristems. Error bars represent ± 1 S.D.; n = 4.

Therefore, only stem fragments without or with a damaged meristem will be totally harmless. Even stem fragments as small as a meristem (1.0 x 0.5 cm) can result in the establishment of a new plant, and through that, the establishment of a new stand/clone. Management personnel active in the removal of *Arundo* state that based on their observations, regeneration from stem fragments is rare, compared to regeneration from rhizome fragments. However, new *Arundo* plants that had regenerated from stem fragments were observed *in situ* during this study (Wijte and Motamed, pers. obs.). Regeneration from *Arundo* stem fragments under a series of environmental conditions was also reported by (Boose and Holt, 1999). There may be two reasons for the limited observations of *Arundo* regeneration from stem fragments in the field. If most eradication efforts that introduce stem fragments into the environment occur in the months of October through February, regeneration from these stem fragments will be inherently low (Fig. 2 and 3), and the ambient temperature will be low as well. There are limitations in place that prevent eradication activities during the breeding season of the endangered bird species that nest in the river basins that have been invaded by *Arundo*. The other reason is that after a growing season, it will be difficult to distinguish between *Arundo* plants that have grown from a stem fragment or from a rhizome fragment , because the new plant will have grown a substantial rhizome (Fig. 10d). When the a plant has regenerated from a rhizome fragment, not much of the original large rhizome fragment remains after it has supported the regeneration of a new *Arundo* plant. (Wijte and Motamed, pers. obs.).

Critical Nitrogen Content and Tissue Growth.

During the first 19 weeks in hydroponic culture, the biomass of the *A. donax* plants increased exponentially (Fig. 8). This exponential growth was followed by prolonged linear growth until the experiment was terminated after 334 days.

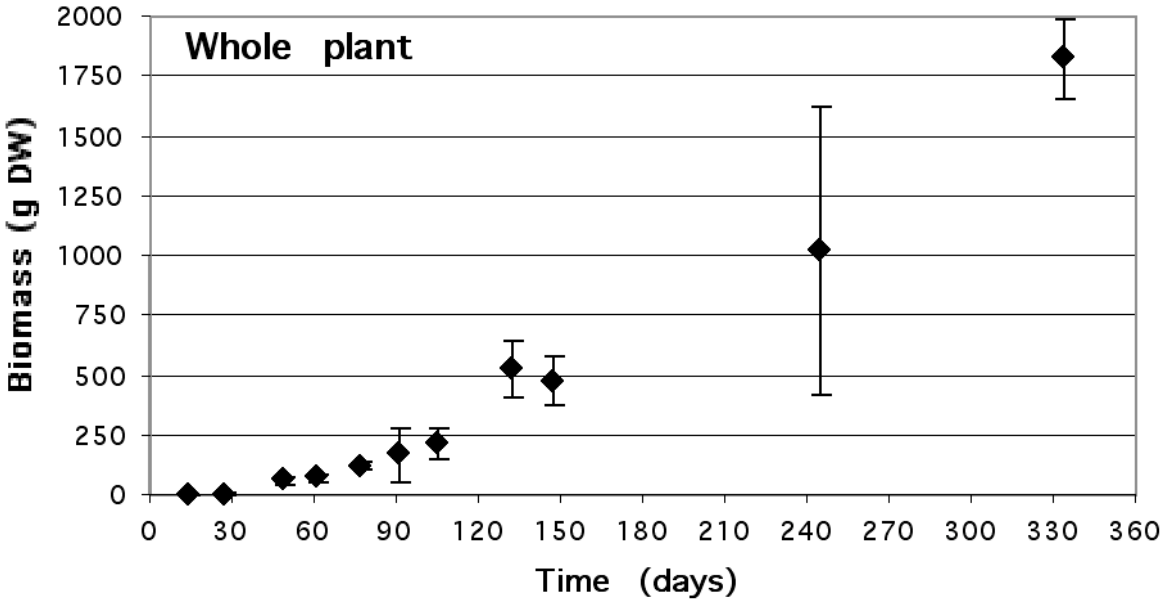


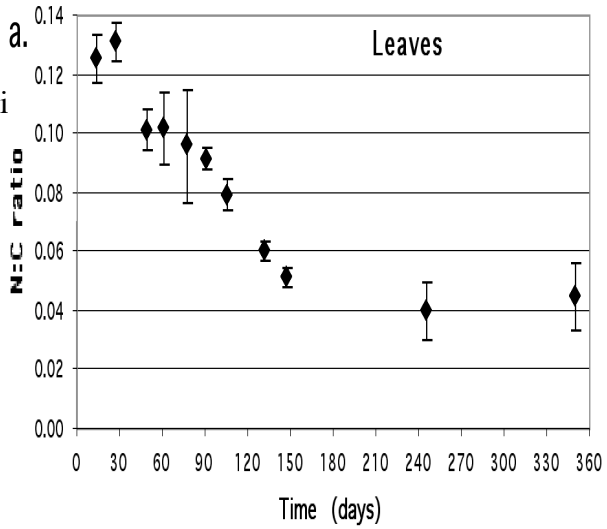
Figure 8: *Arundo donax* biomass, grown from young to mature plants in hydroponic culture. Error bars represent ± 1 S.D., n = 4.

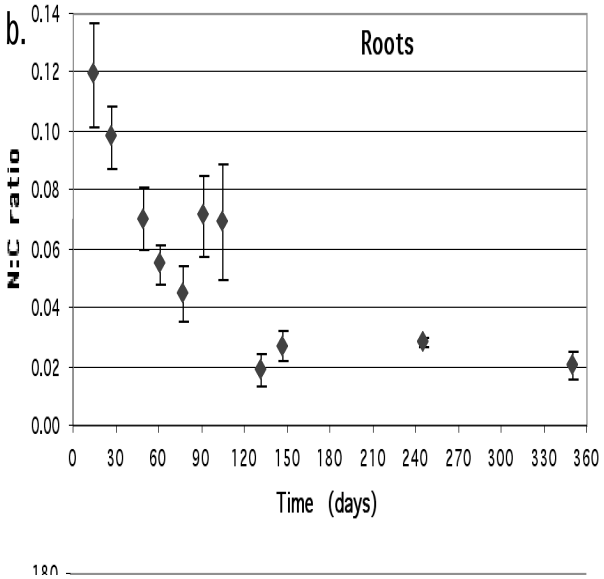
As the plants grew, the N content of each plant tissue type was diluted as described in the introduction. Carbon input through photosynthesis by the leaves outpaced nitrogen input by the roots resulting in the reduction of the N:C ratio for all tissues. 132 days after the addition of to the nutrient solution has been stopped, the N:C ratio of the leaves became constant at a minimum of 0.045 ± 0.006 g N/g C (Table 3; Fig 9a).

Table 3. Critical nitrogen content (g N/g C) for different *Arundo donax* tissues.

	Leaves	Leaf Sheaths	Stem	Rhizome	Roots	Senesced leaves	Senesced Leaf sheaths
CNC	0.045	0.021	0.013	0.030	0.024	0.031	0.022
S.D.	0.006	0.003	0.001	0.007	0.005	0.003	0.005

Fi


a.
Leaves
N:C ratio
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0 30 60 90 120 150 180 210 240 270 300 330 360
Time (days)


b.
Roots
N:C ratio
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0 30 60 90 120 150 180 210 240 270 300 330 360
Time (days)

Growth of the tissues with the highest CNC, the leaves, stopped earliest (Fig 9c), but the leaves remained green. Their continued photosynthesis supplied the carbohydrates for the continued growth of the rhizomes (Fig 10d), stems (Fig 10c), and to a lesser extent that of the roots (Fig 10d).

Both belowground tissues, the rhizome and the roots, have a lower CNC level than the leaves. For those parts of the *Arundo* that is primarily responsible for growth, the leaves and the roots, their growth patterns mirrors their internal N:C ratio. As these tissues near their CNC, their sink strength for photosynthates, and therefore their growth is reduced. Both the leaves and roots have reached their CNC approximately since day 132, and their growth started tapering off since that time. If the experiment would have been continued longer, this would have shown better for the roots. After 60 days, the root masses of the individual *A. donax* plants could not be separated, and each plant was assigned a quarter of the root biomass, to show that overall root growth was tapering off near the end of the experiment.

The tissue with the lowest CNC, was not, as expected, the rhizome which had a CNC of 0.030 g N/g C, but the stem, for which the N:C ratio went as low as 0.013 (table 3; Fig 10 a + b). Unlike the leaves, roots, and rhizome, which reached their CNC after approximately 130 days of growth, the N:C ratio did not reach its lowest levels until day 245.

The rhizome of *Arundo donax* act as a storage tissue for reserves. The reserves stored will support stem regrowth from meristems on the rhizome in the spring (Motamed and Wijte, 1999). In addition to the rhizomes, spring regrowth is also supported by the stem tissues. Unlike the common reed, *Phragmites australis*, new side shoots grow from the upper section of *Arundo* stems in the spring . Both tissues are originally stem tissues, and both play a role in the spring regrowth of the *Arundo* plant.

Both the *Arundo* tissues that support spring regrowth, stems and rhizomes, have CNC levels below that of the leaves of *Arundo*. The difference in CNC between the stems and the leaves is larger than between the rhizome and the leaves. This resulted in significantly more stem growth than rhizome growth, with a final stem biomass of 1190 ± 95 g, and a final rhizome biomass of 171 ± 79 g after 334 days of development. For *Ipomoea batatas* (sweetpotato) these patterns were the opposite, because the CNC of the reserve storage tissue, the storage roots, was significantly lower than that of the stems (Wijte, et al., 1997). The CNC of both tissues was lower than that of the *Ipomoea* leaves. The N:C ratio of the leaves was 0.045 ± 0.0014, of the stem 0.017 ± 0.000006, and that of the storage roots was 0.013 ± 0.0011. For this species, as for *Arundo*, the biomass of the tissue with the lowest CNC, the storage roots, was significantly higher at 181.8 ± 23.8 g DW, than that of the tissue with a higher CNC, the stems, which reached 28.9 ± 7.5 g DW, when the plants had matured.

<u>Provisional Implications For the Timing of Herbicide Applications.</u>

The results of this experiment indicate that the leaf N:C ratio and their CNC can be used as an indicator for good timing of systemic herbicide application, such as that of the glyphosate based Rodeo©. When the N:C ratio of the leaves has been reduced to their CNC, the only major tissues that initially continue growth are the roots, the rhizomes and the stem. The applied glyphosate will be transported to these tissues, with the flow of photosynthates in the phloem supplied by the photosynthetically active leaves. If the glyphosate accumulates in and kills the roots, this will kill the *Arundo* stand, because the uptake of soil water and nutrients will not be possible any more. This is not the most likely scenario, because root growth being reduced as they reach their own CNC. After root growth has tapered off, the roots can still function in the uptake of water and nutrients. The glyphosate will also be transported to those tissues that support spring regrowth, the

stem and the rhizomes, because their growth continues after the leaves have reached their CNC. Based on physiological studies into the amount of reserves stored in the rhizomes of *Arundo* throughout the growing season, transport of photosynthates to and storage of reserves in the rhizomes, the major underground plant structure that will support regrowth if the aboveground parts of the plant are killed or removed, starts near the end of July and continues through October/November (Motamed and Wijte, 1999). If this tissue accumulates enough glyphosate due to the use the correct herbicide concentrations and good timing of application, recovery should be reduced to a minimum.

Literature Referenced

Abissy, M. and L. Mandi. "The use of rooted aquatic plants for urban wastewater treatment: Case of *Arundo donax*." Revue des Sciences de L'Eau 12 (1999): 285-315.

Bandurski, Robert S., Jerry D. Cohen, Janet Pernise Slovin, and Dennis M. Reinecke. "Auxin biosynthesis and metabolism." In Plant Hormones., ed. Peter J. Davies. 39-65. Dordrecht, the Netherlands: Kluwer Academic Publications, 1995.

Bell, Carl E. "Risks and effects of various control methods." In Arundo And Saltcedar: The Deadly Duo. A Workshop On Combating The Threat From Arundo and Saltcedar. in Ontario, CA, University of California Cooperative Extension, Imperial County, 43 - 46, 1998.

Bell, Gary P. "Biology and growth habits of giant reed (*Arundo donax*)." In *Arundo donax* Workshop Proceedings in Ontario, California, TEAM ARUNDO, 1-6, 1993.

Boose, A.B. and J.S. Holt. "Environmental effects on asexual reproduction in *Arundo donax*." Weed Research 39 (1999): 117-127.

Bradley, P.M. and J.T. Morris. "Effect of salinity on the critical nitrogen concentration of *Spartina alterniflora* Loisel." Aquatic Botany 43 (1992): 149-161.

Finn, Monica, Harley Martin, and Dave Minnesang. "Control of giant reed grass in a southern California riparian habitat." Restoration & Management Notes 8 (1990): 53-54.

Frandsen, Paul and Nelroy Jackson. "The impact of *Arundo donax* on flood control and endangered species." In *Arundo donax* Workshop Proceedings in Ontario, California, edited by TEAM ARUNDO, , 13-16, 1993.

Granval, P., R. Aliaga, and P. Soto. "The impact of agriculture management on earthworms (Lumbricidae), commonsnipe (*Gallinago gallinago*) and the environmental value of grasslands in the Dives marshes (Calvados)." Gibier Faune Sauvage 10 (1993): 59-73.

Hoagland, D.R. and D.I. Arnon. The water culture method for growing plants without soil. Vol. Circular 347. California Agricultural Experiment Station, 1938.

Iverson, Mark E. "Effects of *Arundo donax* on water resources." In *Arundo donax* Workshop Proceedings. in Ontario, California, edited by TEAM ARUNDO, , 19-26, 1993.

Jackson, Nelroy E. "Control of *Arundo donax*: Techniques and pilot experiment." In *Arundo donax* Workshop Proceedings in Ontario, California, edited by Team Arundo, , 27-34, 1993.

Kaufman, Peter B., Liu-Lai Wu, Thomas G. Brock, and Dongherm Kim. "Hormones and the orientation of growth." In Plant Hormones., ed. Peter J. Davies. 547-571. 2nd Edition ed., Vol. Dordrecht, the Netherlands: Kluwer Academic Publishers, 1995.

McFadden, J.P., M.J. Ingram, and R.J.G. Rycroft. "Contact allergy to cane reed in clarinettist." Contact Dermititis 27 (1992): 117.

Motamed, E.R. and A.H.B.M. Wijte. "Optimizing the timing of mechanical eradication of *Arundo donax* L." In 15 Biennial International Conference of the Estuarine Research Federation in New Orleans, LA, 1999.

Perdue, R.E. "*Arundo donax* - source of musical reeds and industrial cellulose." Economic Botany 12 (1958): 368-404.

Popov, K.P., G.V. Sukhova, and K.G. Kuksov. "Anatomy of *Arundo donax* L. stems used in wind instruments." Izvestiya Akademii Nauk Turkmenskoi Ssr. Seriya Biologicheskikh Nauk 2 (1990): 70-72.

Rieger, J.P. and D.A. Kreager. Giant reed (*Arundo donax*): a climax community of the riparian zone. USDA Forest Service, 1989 General Technical Report PSW-110.

Scott, Gregory D. "Fire threat from *Arundo donax*." In *Arundo donax* Workshop Proceedings. in Ontario, California, , 17-18, 1993.

Van Der Wegen, M.H. Keijser, and D.P. Bruynzeel. "Allergy to cane reed in a saxophonist." Contact Dermatitis 25 (1991): 268-269.

Wijte, A.H.B.M., J.H. Hill, D.S. Mortley, and D.M. Douglas. "Regulation of sweetpotato development in hydroponic culture." Gravitational and Space Biology Bulletin 11 (1997): 42.