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Agamic propagation of giant reed (*Arundo donax* L.) in semi-arid Mediterranean environment

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Abstract

This review describes giant reed propagation methods taking into account propagation organs and transplanting times. Field results of researches carried out in semi-arid Mediterranean environment are presented and discussed with the aim to help producers to make decisions on the most suitable establishment method and season of transplant. Temperature and soil water availability are limiting factors constraining optimal establishment of giant reed in Mediterranean semi-arid environment. Days with maximum temperatures of 17°C (T max) and minimum temperatures over 7.5°C (T min) coupled with good soil water availability were found as suitable for stems sprouting. Rhizomes optimal transplanting time resulted in spring, while horizontal stem cuttings in autumn. Vertical stem cutting showed worst results in stem density and biomass yield in every transplanting time. Rhizomes of big size showed greater biomass dry matter yield both at the first year and in the subsequent years after establishment. Stem cuttings biomass yield level off in the subsequent years after establishment. The irrigation supplied during the establishment showed a beneficial effect in all transplanting times and propagation methods.

Introduction

The use of dedicated bioenergy crops for biomass production is augmenting in favor of perennial grasses. Indeed, perennial grasses show several advantages as compared to annual crops, namely reduction of greenhouse gas emission (Cosentino *et al*., 2005a; Rettenmaier *et al.*, 2010) and positive energy balance (Mantineo *et al*., 2009; Fernando *et al*., 2010). Moreover, perennial crops have the potentialities to be cultivated in marginal lands as compared to traditional crops (Tilman *et al*., 2006).

In warm temperate environment giant reed (*Arundo donax* L.) seems to be a promising dedicated biomass crop, since it is widespread and well adapted in these environments and show appreciable biomass yield as compared to other perennial biomass crops (Lewandowski *et al*., 2003; Cosentino *et al*., 2005b; Angelini *et al*., 2009). It has been reported to be very interesting for second-generation biofuel, thanks to its high biomass yield and structural polysaccharide compositions (Cosentino *et al*., 2006; Scordia *et al*., 2011, 2012, 2013).

However, the use of this species as a new crop for bioenergy production requires specific agronomic and crop management issues before its introduction at farm scale. One of the most important is related to its propagation. As known giant reed does not produce viable seeds, at least in environments where it is naturalized, therefore the agamic propagation of this species is the only way to establish giant reed (Lewandowski *et al*., 2003). Amongst agamic propagation strategies, rhizomes, stems cuttings and in vitro culture have been studied (Copani *et al*., 2003; Cosentino *et al*., 2009; Takahashi *et al*., 2010).

The rhizome is a creeping stem that grows just below the ground surface; it is branched and composed by nodes and internodes. Several buds provide the shoot extension and its emergence above the soil (Onofry, 1940).

The aboveground stems can reach up to 500 cm in height when fully developed; an average basal stem diameter of 1-4 cm, up to 30-40 nodes and 16-20 cm long internodes have been usually reported; the internodes gradually reduce its length from the bottom upwards (Cosentino *et al*., 2006).

One-year-old stems (young stems) do not branch, while the older one (stems of previous year) due to apical dominance phenomena start to develop branches from the upper nodes (Cosentino *et al*., 2009). Nodes enclose dormant buds from which, at maturity and with appropriate conditions, develop roots and shoots.

Semi-arid Mediterranean environment is characterized by mild, rainy winters with hot and dry summers, leading several constraints to the techniques of vegetative propagation of giant reed that in turn must be carefully evaluated in order to identify the most cost effective and efficient solution.

In view of the thermal requirements of the species, the useful window to allow a successful rooting and shoot extension can be identified in early spring, when usually widespread giant reed buds start to shoot from rhizomes after winter stasis.

Indeed, in this period, rising temperatures and adequate soil water availability conditions make favorable stems sprouting by rhizomes. Other factors to be taken into account are related to the size of the rhizomes and the interaction with water availability and the transplanting time.

A piece of rhizome suitable for transplantation can have a weight ranging from a few hundred up to thousand grams, containing from

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few to several numbers of already differentiated buds, representing the potential stems. For planting a hectare of giant reed at least ten thousand pieces of rhizomes are needed (Lewandowski *et al*., 2003). However, since the cost of establishment is generally high, the size of propagation material should be kept as small as possible in order to reduce the establishment cost (Lewandowski *et al*., 2003).

The use of stem cuttings, instead of rhizome pieces, show several advantages related mostly to the availability and abundance of propagation material and to the transplanting cost. In this case, using stem cuttings with lateral branches can greatly improve uniformity and vigor of the juvenile giant reed stand (Ceotto and Di Candilo, 2010). Stem cuttings are generally buried at about 10-15 cm depth in rows 80-100 cm spaced (Boose and Holt, 1999; Copani *et al*., 2010; Ceotto and Di Candilo, 2010).

According to the transplanting time, early spring is the best season since rhizomes retain the highest nutrient reserves and due to the favorable temperatures, as also reported for other perennial grasses as *Miscanthus* (Atkinson, 2009).

However, in the semi-arid Mediterranean environment the water deficit usually occurs from the month of April in the hill areas, while it starts about a month earlier in lowland areas. Therefore, transplanting time in spring basically requires certain soil water availability to achieve good establishment. In order to reduce input, as irrigation water, transplanting time in autumn looks a promising option. However, while water availability in spring might constrain the success of the establishment, temperatures play a key role in this season.

This review describes giant reed propagation methods taking into account propagation organs and transplanting times. Field results of researches carried out in semi-arid Mediterranean environment are presented and discussed with the aim to help producers to make decisions on the most suitable establishment method and season of transplant.

Propagation organ

Rhizome size

In order to assess the impact of the size of the rhizome on survival rate and subsequently on biomass production, Cosentino and Copani (2010) compared, in a typical semi-arid Mediterranean environment (south of Italy), four different size and weight of rhizomes of a local giant reed clone. Size and weight ranged between 135 g and 699 g, enclosing three main buds (R3), 1 main bud (R1), ten cm length with no visible buds (T10) and five cm length with no visible buds (T5).

During the establishment year, under conditions of good soil water availability (seasonal irrigation volume of 240 mm), the rhizome of smallest size (T5) showed a lower survival rate (75%) and a lower number of differentiated stems (3.9 stems m^{-2}). The highest survival rate was recorded in R3 (100%), while no statistical difference concerning stem density was observed between R3, R1 and T10 (6.2 stems m^{-2} in the average)

R3 was the fastest in shoot emissions (14 days after transplant), while T5 the slowest (20.5 days after transplant). No differences were observed between R1 and T10.

However, compensation between number, weight and height of stems resulted in a final biomass yield not statistically different among the rhizome size and weight in the first year harvest (between 2.0 and 2.9 t DM ha^{-1}) (Table 1).

Stem cuttings

The same authors, in the same environment, year and season of trial, compared three different stem cuttings of 100 cm length, namely basal (CB), median (CM), apical (CA) cuttings and the whole plant (CI). Cuttings were buried at 15-20 cm depth, overlapping 2 stems per row with a distance between rows of 100 cm. The seasonal irrigation volume was 240 mm.

Internodes length of the different treatments lead to a different number of buried nodes, with CA and CI showing the highest (32 and 38 node m^{-2}, respectively), while CB and CM the lowest (about 10 node m^{-2}).

Despite CB and CM showed the lowest number of buried node m^{-2}, the percentage of stem sprouted was the highest (13 and 10%, respectively) than CI (3.3%) and CA (2.8%). However, no statistical difference was observed in the stem density at the end of vegetative growth (4.3 stem m^{-2}, in the average).

Even though CB showed the largest stem height (128.4 cm), than CM (118.2 cm), CA and CI (105.1 and 104.7 cm, respectively), the biomass yield was not different among the stem cutting and whole plant treatments (between 0.24 and 0.57 t DM ha^{-1}) in the year of transplant, as shown in Table 2.

Productivity of rhizomes and stem cuttings

The subsequent years, both stem cuttings (CA, CB, CM and CI) and rhizome size (R3, R1, T10 and T5) derived establishment showed a greater biomass DM yield than the first year, as generally observed for perennial grasses (Lewandowski *et al*., 2003). Aboveground biomass yield increased from 2.6 to 31.3 t DM ha^{-1} in the average of rhizomes size and from 0.4 to 28.1 t DM ha^{-1} in the average of stem cutting treatments, both at the third year after establishment. Regardless the size of rhi-

Table 1. Rhizome weight, percentage of rhizomes survival at the end of growing period, days from planting to emergence, stem density, stem height, aboveground biomass yield recorded in Catania during the first growing cycle (2007) in relation to the different type of rhizomes (Modified from? Cosentino and Copani, 2010).

Treatments	Rhizome weight (g)	Survival rate (%)	Emergences (DAT)	Stem density (n m^{-2})	Stem height (cm)	D.M. yield (t ha^{-1})
R3	699.3^{a}	100.0^{a}	14.0^{b}	6.6^{a}	176.5^{a}	2.7^{a}
R1	250.9^{b}	81.3^{c}	17.0^{ab}	5.6^{a}	145.6^{a}	2.0^{a}
T10	233.1^{b}	93.8^{b}	17.0^{ab}	6.3^{a}	163.0^{a}	2.6^{a}
T5	135.1^{c}	75.0^{d}	20.5^{a}	3.9^{b}	168.8^{a}	2.9^{a}
Average	329.6	88.8	17.8	5.6	163.5	2.6

DAT, days after transplant; D.M., dry matter?; R3, rhizome with three main buds; R1, rhizome with one main bud; T10, rhizome segment 10 cm long with no visible buds; T5, rhizome segment 5 cm long with no visible buds. a,b,c,dValues with the same letter do not significantly differ at $P \leq 0.05$ (Student-Newman-Keuls test).

zomes, the biomass yield was always higher than stem cuttings at first and second year harvest (85 and 55% respectively), however the gap reduced at the third year harvest to 11% (Cosentino and Copani, 2010). As far as the rhizome size is concerned, R3 showed the highest biomass yield than the other rhizomes size tested, with 21.5 t DM ha^{-1} in the average of three-year harvests and a maximum of 43.7 DM t ha^{-1} at the third year. Stem cuttings, on the other hand, did not show any difference among the part of stems used (CB, CM, CA and CI) following the year after establishment and an average of 11.5 t DM ha^{-1} was observed. Stem cuttings and rhizomes sizes, except R3, showed a comparable biomass DM yield at the third harvest after establishment (Table 3).

Transplanting time

Spring time

Copani *et al*. (2009) carried out a field experiment in an inland hills of Sicily (south of Italy), between the end of winter and spring, using rhizomes and stem cuttings transplanted in six different dates (between 25 February and 23 April). In this trial, 124 mm irrigation water from transplant to settlement of cuttings (May to June) were supplied. The beginning of the emergence of new shoots was recorded from April 5 (rhizomes) and 15 April (stem cuttings), while the end of the emergence on May 14 (rhizomes) and May 30 (stem cuttings). Significant differences were recorded in relation to the propagation system: emergence occurred averagely 31 days after transplant with rhizomes, while the same interval was 15 days longer (46.5 days after transplanting) with stem cuttings (Table 4). Planting-emergence interval progressively decreased from the first planting date in February to the last one in April, ranging from 40 to 21 days (rhizome cuttings) and from 50 to 37 days (stem cuttings).

The thermal time required for emergence was 385°C and 631°C respectively for rhizomes and stem cuttings (Table 4). Authors calculated the thermal threshold, defining as the minimum temperatures necessary for the emergence of new stems; days with maximum temperatures of 17°C (T max) and minimum temperatures over 7.5°C (T min) were found as suitable for sprouting (Figure 1). The same minimum threshold was proposed by Spencer and Ksander (2006) in estimating giant reed stem emergence in relation to temperatures and nitrate in

Table 2. Number of buried nodes, days from planting to emergence, stem density, nodes sprouted, stem height, aboveground biomass yield recorded in Catania during the first growing cycle (2007) in relation to the different type of stems (Modified from? Cosentino and Copani, 2010).

Treatments	Buried nodes (n m^{-2})	Emergences (DAT)	Stem density (n m^{-2})	Stem sprouted (% of nodes buried)	Stem sprouted (n m^{-2})	Stem height (cm)	D.M. yield (t ha^{-1})
CI	38.1^{a}	35^{a}	3.9^{a}	3.3^{b}	1.3^{a}	104.7^{b}	0.57^{a}
CB	10.1^{b}	38^{a}	3.9^{a}	13.0^{a}	1.3^{a}	128.4^{a}	0.42^{a}
CM	12.9^{b}	37^{a}	5.2^{a}	10.1^{a}	1.3^{a}	118.2^{ab}	0.34^{a}
CA	32.2^{a}	37^{a}	4.3^{a}	2.8^{b}	0.9^{a}	105.1^{b}	0.24^{a}
Average	23.3	36.8	4.3	7.3	1.2	114.1	0.39

DAT, days after transplant; D.M., dry matter?; CI, whole stems; CB, basal stem cuttings; CM, median stem cuttings; CA, apical stem cuttings. ^{a,b,c,d}Values with the same letter do not significantly differ at P≤0.05 (Student-Newman-Keuls test).

Table 3. Aboveground dry biomass yield recoded in Catania during the three years period (2007-2010) in relation to the different studied treatments (Modified from? Cosentino and Copani, 2010).

	Rhizome					Stem				
Years	R3	R1	T10	T5	Average	CI	CB	CM	CA	Average
2007-2008	2.7	2.0	2.6	2.9	2.6c	0.6	0.4	0.3	0.2	0.4c
2008-2009	18.9	9.5	15.4	12.0	13.9^{b}	6.0	6.4	5.8	7.1	6.3^{b}
2009-2010	43.7	20.3	28.9	32.3	31.3^{a}	33.5	25.9	25.1	26.8	27.8^{a}
Average	21.8^{a}	10.6^{c}	15.6^{b}	15.7^{b}	15.9	13.4a	10.9^{a}	10.4^{a}	11.4^{a}	11.5

R3, rhizome with three main buds; R1, rhizome with one main bud; T10, rhizome segment 10 cm long with no visible buds; T5, rhizome segment 5 cm long with no visible buds; CI, whole stems; CB, basal stem cuttings; CM, median stem cuttings; CA, apical stem cuttings. ^{a,b,c}For each propagation materials values with the same letter do not significantly differ at P≤0.05 (Student-Newman-Keuls test).

Table 4. Date of transplant, days from planting to emergence, thermal sum, stem density and aboveground dry biomass yield during the first growing cycle in relation to the different studied factors (Modified from? Copani *et al*., 2009).

Transplanting date	Emergences (date)		Transplant-emergence (days)		Thermal sum (°C)		Stem density (n m^{-2})		D.M. yield (t ha^{-1})	
	R	C	R	C	R	C	R	C	R	C
25/02/08	05/04/08	15/04/08	40	50	405	589	5.8	6.6	2.9	1.5
05/03/08	10/04/08	30/04/08	36	56	379	662	6.7	8.3	4.4	4.9
17/03/08	15/04/08	05/05/08	29	49	330	631	6.7	7.2	5.3	4.2
31/03/08	30/04/08	14/05/08	30	44	405	622	6.3	6.4	3.8	3.6
12/04/08	10/05/08	25/05/08	28	43	408	662	5.3	3.6	3.5	2.1
23/04/08	14/05/08	30/05/08	21	37	383	618	5.3	5.7	4.0	2.4
Average			30.7^{b}	46.5^{a}	385^{b}	631^{a}	6.0^{a}	6.3^{a}	4.0^{a}	3.1^{a}

D.M., dry matter?; R, rhizome; C, stem cutting. ^{a,b}Values with the same letter do not significantly differ at P≤0.05 (Student-Newman-Keuls test).

the soil solution.

According to the stem density, no differences were observed with respect to propagation organs in the average of transplanting date (in the average 6.0 with rhizomes and 6.3 stem m^{-2} with stem cuttings), as well as for aboveground biomass yield (in the average 4.0 t DM ha^{-1} with rhizomes and 3.1 t DM ha^{-1} with stem cuttings).

Even though no statistical differences were observed between propagation organs in term of biomass yield, transplanting from early (stem cuttings) to mid-March (rhizomes) resulted in significantly higher values than the other transplanting dates.

Autumn time

In order to compare autumn with spring transplanting time (following the trial of inland hills of Sicily discussed above), Copani *et al*. (2010) carried out a field experiment in lowland site (average temperature higher than 2°C than inland hill). Three different transplanting times (05 November, 17 March and 30 April) and propagation organs (rhizomes, horizontal stem cuttings and vertical stem cuttings) were used. In this experiment, two irrigation levels have been also tested, namely rainfed (irrigation of 23 mm of water only in spring transplant) and irrigated (250 mm of water in both seasons).

Stem emergence occurred, in the average of propagation methods, after 173 days from transplanting in November, after 50 days from transplanting in March and after 40 days from transplanting in April (Figure 2).

Autumn transplant heavily penalized the shoot sprouted by rhizomes that in the average of the irrigation treatment (rainfed and irrigated), showed 3.8 stems m^{-2} against 8.2 and 7.5 stems m^{-2} detected in the transplant of March and April, respectively. On the other hand, the horizontal stem cuttings showed the best result at the autumn transplant (10.6 stems m^{-2}) against 5.0 and 1.7 stems m^{-2} in the two spring transplant dates, in average of the irrigation treatment. Vertical stem cuttings resulted in lowest stem densities in all transplanting times.

The irrigation effect

As part of the experiment described above (lowland site), an important role was played by the irrigation (Cosentino and Copani, 2011). The increased availability of water resulted, in the average, in improved number of new stems sprouted (63%), but with substantial differences in relation to the propagation organ and to the transplant time.

Regarding stem cuttings transplanted in autumn its gap was 39% (8.0 against 13.3 stems m^{-2} for rainfed and irrigated, respectively), while the gap increased to 69% in the transplant of March (4.4 against 14.3 stems m^{-2} for rainfed and irrigated, respectively) and 82% in the transplant of April (1.3 against 7.5 stems m^{-2} for rainfed and irrigated, respectively). It is worth to note how the water availability influence stem sprouting from stem cutting method; the higher the water deficit the lower the stem emissions.

Rhizomes behaved differently respect to stem cuttings method being affected by only 30% gap between rainfed and irrigated treatments in the spring transplanting.

In autumn transplanting time, by contrast, a marked difference between rainfed and irrigated (1.2 compared to 6.4 stems m^{-2}, representing a reduction of 80%) was shown, which might be attributed to the weakening of the rhizome during the long winter stasis (more than 160 days between transplant and the emergence).

Vertical stem cuttings showed the lowest stem density in all transplanting times. Within this method best results were observed in the transplanting time of March (~2.0 stem m^{-2}, in the average of irrigated and rainfed treatment). Biomass yield, in the establishment year, has reflected faithfully enough the stem density achieved by the different propagation methods. As far as the transplanting time is concerned, March was the best in terms of biomass yield, irrespective of propagation method (5.8 t DM ha^{-1} in the average of propagation methods) followed by the transplant time of April and November (4.2 and 2.9 t DM ha^{-1}, respectively). Rhizomes showed the significantly highest yield in the spring transplanting times (8.7 and 8.5 t DM ha^{-1} in March and April). Horizontal stem cuttings produced the significantly highest biomass yield than rhizomes and vertical stem cuttings in the autumn transplanting time (5.7, 2.2 and 0.8 t DM ha^{-1}, respectively), however the gap between rainfed and irrigated was always higher in horizontal stem cuttings than rhizomes propagation method in all transplanting times.

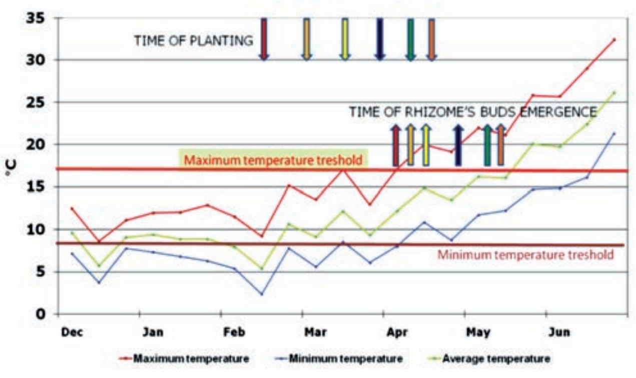

Figure 1. Minimum and maximum temperature threshold for stem emission in giant reed (Arundo donax L.), according to/modified from? Copani *et al.*, 2009.

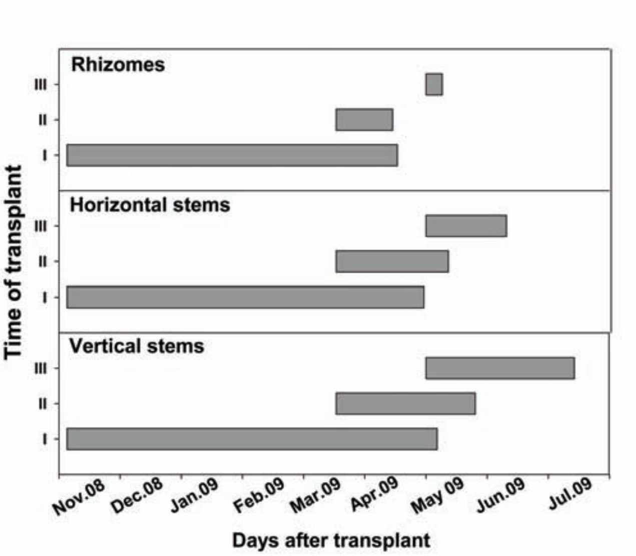

Figure 2. Days from planting to emergence of different propagation methods and transplanting time of giant reed (Arundo donax L.), according to/modified from? Copani *et al.*, 2010.

In the subsequent growing cycle the irrigation was no longer used, however the conditions established previously were significantly kept. Horizontal stem cuttings transplanted in autumn overyielded rhizomes transplanted at the same time (13.4 and 9.0 t DM ha^{-1}). Rhizomes yielded more with the spring than autumn transplanting time (11.8 and 13.1 t DM ha^{-1} in March and April; 9.0 t DM ha^{-1} in November) compared to horizontal and vertical stem cuttings, with this latter showing the lowest values in all transplanting dates (Table 5).

Basically, the transplant in autumn was more favorable for horizontal stem cuttings propagation method, while spring transplant for rhizome method; the irrigation shown beneficial effect in all cases.

Other stem cuttings pretreatment effect

In order to attempt faster root/shoot extension, field trials comparing two different transplanting times, 02 and 24 March and stem cutting pretreatment was carried out. Stem cuttings were pretreated either with (NAA explain abbreviation) or hydration.

Hydration time ranged between 0 and 4 weeks, while NAA treatment was applied 24 h before hydration or not applied. Stems were buried in row 80 cm distant at a depth of 15 cm.

Stem density was measured about two months after transplant, before summer. Significant differences were recorded between the transplanting time and hydration pretreatment, while NAA did not show any statistical difference. Three weeks hydration showed best values (1.76 stems m^{-2} in the average of NAA and transplanting dates), while in the second transplanting time a faster shoot emission and higher stem density was observed (1.10 and 1.83 stem m^{-2} for first and second transplanting time, respectively) (Table 6). Higher temperatures encountered during the second transplanting time coupled with good soil water availability might have positively affected the stem emission. However, these stem density values are comparably lower than what reported by Cosentino and Copani (2010) or Copani *et al.* (2009). It is worth to note that in the present experiment only one stem cutting was buried and not overlapping two stems in the same row as shown to be beneficial in order to increase buried nodes and therefore stem density.

Discussions and conclusions

Seed production of giant reed populations widespread in Mediterranean areas is apparently absent. Johnson *et al*. (2006) examined more than 36,000 florets in Californian giant reed populations and found only five ovules that may have been viable.

There is molecular evidence that naturalized populations of giant reed in the USA and Europe are a single genetic clone (Perdue, 1958).

This confirms that dispersal is vegetative and suggests that a single genetic clone has been cultivated in multiple regions of the world (Ahmad *et al*., 2008). Fragmentation and dispersal of vegetative propagules usually occurs during winter floods (Bell, 1997) leading to rooting and establishment of new populations and field invasions (Dudley, 2000).

Invasion of giant reed can be seen by fragmentation, as means by which giant reed propagules invade a new site in the flood zone, rhizomes expansion with the aim to maintain clumps, and stem layering is the means by which giant reed spreads quickly and episodically within the flood zone (Boland, 2006). Outside the flood zone invasion is very slow either by rhizomes and stems layering. Studies have shown that virtually any segment of stem or rhizome, even if split sideways, can sprout and grow into a new plant if it possesses an axillary bud (Boose and Holt, 1999; Wijte *et al*., 2005).

However, environmental effects play a key role in new propagules development, either by rhizomes or by stem cuttings.

Boose and Holt (1999) conducted a study on stem and rhizomes storage, size, soil moisture and depth of transplant on sprouting. Stem sprouting was affected by storage duration, temperature and moisture, whereas only storage duration and moisture affected rhizome sprouting. Over 90% of stem and rhizome pieces with at least one node sprouted. Stem pieces, irrespective of stem length, without any node

Table 5. Aboveground dry biomass yield in the first and second growing cycle in relation to the studied treatments (propagation materials and irrigation) (Modified from? Cosentino and Copani, 2011).

	I year			II year		
	I transplanting date (05/11/2008)					
	Rainfed	Irrigated	Average	Rainfed	Irrigated	Average
Horizontal stem	2.6	8.8	5.7^{a}	7.5	19.4	13.4^{a}
Vertical stem	0.4	1.3	0.8^{c}	3.4	2.5	2.9^{c}
Rhizome	1.2	3.3	2.2^{b}	9.6	8.4	9.0^{b}
Average	1.4^{b}	4.5^{a}	2.9	6.8^{b}	10.1^{a}	8.4
	II transplanting date (17/03/2009)					
Horizontal stem	2.4	11.0	6.7a	5.1	14.0	9.6^{b}
Vertical stem	1.5	2.5	2.0c	2.9	4.4	3.7^{c}
Rhizome	6.6	10.8	8.7a	8.0	15.5	11.8^{a}
Average	3.5^{b}	8.1^{a}	5.8	5.4^{b}	11.3^{a}	8.3
	III transplanting date (30/04/2009)					
Horizontal stem	0.6	5.8	3.2^{b}	3.0	15.7	9.3b
Vertical stem	0.2	1.4	0.8^{c}	0.1	4.0	2.1^{c}
Rhizome	4.2	12.8	8.5^{a}	8.6	17.7	13.1^{a}
Average	1.7^{b}	6.7^{a}	4.2	3.9^{b}	12.4^{a}	8.2

^{a,b,c}Within each transplanting date, values with the same letter do not significantly differ at $P \leq 0.05$ (Student-Newman-Keuls test).

did not sprout. A positive correlation was found between length of stem and time taken to sprout. For rhizomes no correlation was found between length, size and volume on time of sprouting. Rhizomes with visible buds sprouted 100% while only 8% with no visible buds.

Copani *et al*. (2009) found similar survival rates when used rhizomes with visible buds; however satisfactorily results were also shown with rhizomes of 5 cm length with no visible buds (75% survival rates) compared to what reported by Boose and Holt (1999).

It has been reported that rhizomes display high regeneration potential year round (Decruyenaere and Holt, 2001), while stems show a seasonal depression in sprouting potential when temperatures fall below approximately 17°C (Boose and Holt, 1999; Wijte *et al*., 2005).

In a temperature controlled experiment (16 h at 28°C and 8 h at 16°C) Wijte *et al*. (2005) found successful regeneration (30-80%) from stem fragments with lengths ranging from 1 to 10 cm, even if split lengthwise, but only when the axillary bud was present and intact. In an unheated greenhouse the same authors reported 0-20% regeneration in the winter and 90-100% in the summer time.

Controlled experiments indicate that temperature, type of propagation organ, soil moisture, storage method of propagation materials, among others, impact on the success of giant reed establishment by means of vegetative organs.

However, field experiments would better describe the behavior of the vegetative organs and their interaction with climatic conditions on shoot emission and most importantly on aboveground biomass yield over years.

In field experiments temperature and soil water availability are limiting factors constraining optimal establishment of giant reed in Mediterranean semi-arid environment (Copani *et al*., 2009; Cosentino and Copani, 2011). According to the temperature threshold, optimal transplanting time should be carried out in early spring time, however, in Mediterranean semi-arid environment, water shortage could affect stem emission and therefore the establishment of giant reed. In order to overcome water shortage, giant reed could be established in autumn, taking advantage of rainfall. However, in this case minimum temperature threshold could affect or postpone stem emission.

Spencer and Ksander (2006) carried out outdoor experiments in northern California showing that during the year of establishment stems emerged when average soil temperature of the proceedings week was 11.8°C (26 March) and continued to sprout until autumn when the temperature was still at 15.6°C (4 November). In the second year, stem sprouting was observed earlier in the season, when the temperature was 10.8°C (15 February) and continued through early December when temperature was 5.2°C.

The subsequent year, stem started to sprout when soil temperature was 6.9°C (mid-February). Similarly, Decruyenaere and Holt (2001) reported stem emergence in southern California mostly in May, June and July.

In a field experiment in two different location in northern Italy (sandy and loam-silty-soil, respectively), Ceotto and Di Candilo (2010) transplanted stem cuttings with lateral branches in July, using weekly irrigation volume of 35 mm and 20 mm (sandy and loam-silty-soil, respectively) through August, and found and excellent and uniform crop establishment both in terms of stem density and plant height.

Giant reed propagation field experiments carried out in Mediterranean semi-arid environment (southern Italy) and discussed in this work allow drawing some key remarks.

In autumn or spring transplanting time, the propagation method showed a different behavior. Rhizomes resulted most suitable in spring time, while horizontal stem cuttings in autumn transplant. Vertical stem cuttings showed worst results in stem density and biomass yield in every transplanting time.

When using rhizomes as propagation material, its size plays a key role. Indeed, a survival rate of 100% and highest biomass yield in the subsequent years after establishment were reported with rhizomes of weight of about 700 g. Stem cuttings from different part of the stem (whole plant, basal, median and apical cutting) did not show any difference in terms of biomass yield, both at first and subsequent years after establishment.

The irrigation supplied during the establishment showed a beneficial effect in all transplanting times and propagation methods. Results indicate that the autumn transplant favors the stem cuttings even under rainfed condition, while the spring transplant benefits the rhizome; stem cuttings transplanted in spring requires water supply.

Table 6. Stem density in relation to stem cutting pretreatment.

Hydration (week)	NAA	Transplanting date	Stem m^{-2}
4	Yes	I	1.20
4	No	I	1.41
3	Yes	I	1.72
3	No	I	1.15
0	Yes	I	0.57
0	No	I	0.57
4	Yes	II	1.20
4	No	II	2.29
3	Yes	II	2.14
3	No	II	2.03
0	Yes	II	2.14
0	No	II	1.20
	Transplanting date	I	1.10^{b}
	Transplanting date	II	1.83^{a}
	Hydration	4	1.52^{b}
	Hydration	3	1.76^{a}
	Hydration	0	1.12^{c}
	NAA	Yes	1.49^{a}
	NAA	No	1.44^{a}

^{a,b,c}Within each studied factor values with the same letter do not significantly differ at P≤0.05 (Student-Newman-Keuls test). NAA, ... please explain abbreviation.

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