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in

Ricciardi L. (ed.), Myrta A. (ed.), De Castro F. (ed.).  
Italo-Albanian cooperation for the enhancement of plant biodiversity

Bari : CIHEAM

Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 47

2001

pages 103-115

Article available on line / Article disponible en ligne à l'adresse :

<http://om.ciheam.org/article.php?IDPDF=2001550>

To cite this article / Pour citer cet article

Cocozza Talia M.A., La Viola A.M.F. **Identification of propagation methods for some species common to Albania and Southern Italy.** In : Ricciardi L. (ed.), Myrta A. (ed.), De Castro F. (ed.). *Italo-Albanian cooperation for the enhancement of plant biodiversity.* Bari : CIHEAM, 2001. p. 103-115 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 47)



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# Identification of propagation methods for some species common to Albania and Southern Italy

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## Summary

Some preliminary results are given of a research carried out in order to develop the techniques of sexual and asexual propagation of species common to both countries such as: *Fraxinus excelsior* L., *Myrtus communis* L., *Phlomis fruticosa* L., *Rosmarinus officinalis* L., *Sambucus nigra* L. and *Viburnum tinus* L.. As for sexual propagation, several sowing periods were compared (from February to June) as well as pre-sowing treatments at a temperature of 5°C for different periods (from 0 to 6 days). Pertaining to the asexual propagation, a study was conducted on the impact of the date of cutting collection (from February to May), the type of cutting (herbaceous, semihardwood, hardwood); for each type, apical, central and basal cuttings were confronted. Results show that the sowing period impacts only the germination rate, whereas low temperature treatments increase the germination percentage. As to the asexual propagation, the various species gave a different response according to the period and type of cutting.

**Key words:** *in vivo* propagation techniques, Mediterranean plants.

## 1. Introduction

The Mediterranean vegetation features a high floristic richness (Pignatti, 1994). The high number of species determines its considerable value and characterises numerous habitats. Over the last years, several species have been used for ornamental reasons, as cut flowers, for the design of Mediterranean gardens or for the rehabilitation of degraded areas.

The scarcity of propagated material, especially the local one, and the lack of propagation protocols which might promote a wider-scale cultivation fostered a preliminary investigation into some species common to Italy and Albania that could be exploited in potential commercial sectors. Later on, propagation techniques were developed.

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## 2. Materials and methods

The species studied in the work are: *Fraxinus excelsior* L., *Myrtus communis* L., *Phlomis fruticosa* L., *Rosmarinus officinalis* L., *Sambucus nigra* L. and *Viburnum tinus* L.

*Fraxinus excelsior* L (family Oleaceae) is a hardy tree with pinnate leaves, rapid growth, fit for any type of soil; it resists winds and polluted environments. It is usually cultivated for the production of timber and as ornamental plant. Following Hartmann and Kester (1990), seeds germinate if they are stratified for two to four months at about 4°C; according to Zocca (1999), the species is sexually propagated with outdoor sowing in autumn and germination occurring the following spring.

*Myrtus communis* (family Myrtaceae) is an evergreen shrub, smooth, aromatic, 2-4 m high, with numerous ramifications, leaves opposite, persistent, close, leathery, shortly petioled. Flowers, whose perfume is the same as that of the leaves, are single with white petals and several stamens develop in the summer. Fruits are globose berries, black to blue in colour. It is a hardy species, diffused in the woodland and in the maquis along the coast and is fit for any type of soil. It prefers dry and sunny places. It is cultivated as ornamental plant, for medicinal purposes and in the processing industry thanks to its high content in essential oils. It can also be used as cut frond with both its flowers and berries. Some cultivars located in Sardinia have a high ornamental value (Mulas, 2000). Furthermore, this species is used for the rehabilitation of Mediterranean degraded coastal areas (Adversi and Marras, 1994). The literature reports that the myrtle may be propagated in the summer by shoot cuttings with partially mature wood (Hartmann and Kester, 1990) and by hardwood cuttings in places with mild winters (Zocca, 1999). No specific treatments are envisaged for autumn sowing in cold box when berries reach maturity (Zocca, 1999).

*Phlomis fruticosa* L. (family Labiatae) is a perennial shrub, 1.30 m high with flowers gathered in verticillasters. It is diffused in the garigue and rangelands and used for ornamental purposes. Propagation methods are not reported in the literature.

*Rosmarinus officinalis* L. (family Labiatae) is an evergreen shrub, with brown twigs and a strong aromatic perfume. At the leaf axil, some small flower clusters develop with light blue corollas. It is both an aromatic and ornamental plant; varieties have an upright, pyramidal and prostrate habit which may cover the soil. It withstands low temperatures if moisture is not high and is fit for warm and dry climates. It is diffused in garigues, maquis and loose woodlands. The literature reports that shoot and herbaceous

(apical and middle) cuttings are used for its asexual propagation (Hartmann and Kester, 1990; Ruta *et al.*, 1997).

*Sambucus nigra* (family Caprifoliaceae) is a big shrub or small tree, with pinnate leaves and serrate leaflets. Flowers are white, perfumed, gathered in flattened tops, sprout in June followed by black globose berries. It is very hardy, prefers cool places and calcareous soils. In order to promote the production of big flowers and of the foliage, side twigs can be pruned in March a few centimetres apart from the old wood; these plants are ideal for a wild garden. Following Hartmann and Kester (1990), sexual propagation is hard because of the seed double dormancy (integuments and embryo); asexual propagation is easier with herbaceous cuttings taken from spring to summer.

*Viburnum tinus* L. (family Caprifoliaceae) is an evergreen shrub, richly ramified with oval leathery leaves, dark green in colour. Its white flowers are gathered in flat bundles which appear continuously from end-autumn to early spring followed by blue and then black globose fruits. It is one of the most widespread evergreens, medium to large in size, with a dense and bushy habit. It grows in shadowed areas, maquis and evergreen woods as well as along the coast. Hartmann and Kester (1990) report that it is mainly propagated asexually by shoot cuttings; Ferrini *et al.* (1998) state that etiolation-inducing treatments favour the development of a higher number of roots which is a basic feature for the survival of plantlets.

This preliminary research was designed to set up *in vivo* propagation protocols by seed or plant organ, by identifying both the propagation modes (sexual/asexual) and the best period for the sampling of the material of the above said species.

In particular, *Fraxinus excelsior* L. was submitted only to the sexual propagation; *Myrtus communis* L. to both sexual and asexual propagation, whereas *Phlomis fruticosa* L., *Rosmarinus officinalis* L., *Sambucus nigra* L. and *Viburnum tinus* L. were submitted just to asexual propagation trials.

Concerning sexual propagation, we tried to evaluate the effect on germination of different sowing periods and techniques of seed storage (hot water treatments or pre-cooling), as reported for the individual species.

As for asexual propagation, we tried to estimate the effect on rhizogenesis of the cutting type by comparing herbaceous, semihardwood and hardwood cuttings and different sampling dates. Cuttings were prepared by removing basal leaves, leaving just 4 apical leaves with a reduced surface. Before planting, the cut surface was disinfected into a fungicidal

solution; rooting was also stimulated by treating cuttings with an alpha-naphthalenetic acid-based rooting hormone powder.

The propagating material was allowed to germinate and/or root in polystyrene trays using a substrate made up of a 70:30 universal soil mix and perlite.

Trials were carried out between February and June 2000, in the Campus of the Faculty of Agriculture, University of Bari, (Italy).

### 3. Results and discussion

#### *Fraxinus excelsior* L.

Sowing was made during the third decade of May by comparing the untreated control with the seed taken from a fruit pre-cooled at 5°C for one week.

Fig. 1 reports that germination occurred 15 days in all the trials; as for the germination percentage, a considerable difference is reported between the control (6%) and the pre-cooled seed (44%).

It seems that pre-cooling has a positive repercussion only on the germination rate at least in so far as the sowing date is concerned. The same study will be repeated for other periods and, eventually, the pre-cooling period will be prolonged to check to what extent it can increase the germination rate.

#### *Myrtus communis* L.

##### First trial

For sexual propagation, five sowing periods were compared once a month starting from the first decade of February.

In the first period, the untreated control was compared with the seed taken from fruits dipped in water at 100°C. For the other four periods, the untreated control was compared with the seed taken from fruits stored at 5°C for 24, 48 and 72h.

Tab. 1 and 2 show that for the first sowing period emergence took place only for the untreated control (20%) with a mean germination time of about 30 days. In the following sowings, the germination rate for the control increased steadily from 20% in the first period to 43% in the fifth period. The same trend was recorded with low-temperature pre-sowing treatments. Anyhow, a higher germination rate was recorded with the June sowing. Tab. 2 highlights that the mean germination time was shorter with

late sowings. The trial will be repeated in other sowing periods and the pre-cooling duration will be increased.

Tab. 1 - *Myrtus communis* L., influence of sowing time and treatments on germination percentage (%).

Sowing times	Test	Hot water	Treatments at 5°C		
			24 hrs	48 hrs	72 hrs
1 <sup>st</sup> decade of February	20	0	*	*	*
1 <sup>st</sup> decade of March	25	*	4	7	12
1 <sup>st</sup> decade of April	30	*	12	22	22
1 <sup>st</sup> decade of May	35	*	21	37	34
1 <sup>st</sup> decade of June	43	*	57	58	63

(\*) no treatment.

Tab. 1 - *Myrtus communis* L., influence of sowing time and treatments on the mean germination time (%)

Sowing times	Test	Hot Water	Treatments at 5°C		
			24 hrs	48 hrs	72 hrs
1 <sup>st</sup> decade of February	30	0	*	*	*
1 <sup>st</sup> decade of March	20	*	20	20	20
1 <sup>st</sup> decade of April	15	*	15	15	15
1 <sup>st</sup> decade of May	12	*	12	12	12
1 <sup>st</sup> decade of June	13	*	13	13	13

(\*) no treatment.

## Second trial

For asexual propagation, two sampling periods were compared: the first decade of April and the first decade of May. In the first period, two types of hardwood cuttings were used: apical and basal, 10 cm long with 10 nodes. In the second period, the comparison was made between herbaceous, semihardwood and hardwood cuttings, 7 cm long with 7 nodes.

Fig. 2 shows that in the first period, the rooting percentage was 63% and 100% for apical and basal cuttings respectively. For the second period, rooting took place only for the hardwood cuttings with a mean value of 45%.

It seems that cuttings should be taken in autumn when the plant tissues contain storage substances.

### *Phlomis fruticosa* L.

Cuttings were taken in four different periods once a month starting from the first decade of February. For the first two periods, apical semihardwood cuttings were used 10 cm in length with 4 nodes; for the others, herbaceous (shoot and apical), semihardwood and hardwood cuttings were compared, 7 cm in length with 5 nodes.

Fig. 3 shows that for the first two periods, the rooting percentage was rather low (6%) with semihardwood cuttings; in the other two periods, only herbaceous cuttings rooted with a mean value of 25%; in particular, the mean value equalled 38% for the shoot cuttings and 10% for the apical ones (Fig. 4).

Since Mediterranean species usually show two phases of growing activity (autumn and spring), it would be advisable to extend the sampling period of cuttings in order to assess their rooting capacity and growing activity.

### *Rosmarinus officinalis* L.

Asexual propagation was carried out by taking cuttings in three different periods once a month starting from the first decade of March; herbaceous, semihardwood and hardwood cuttings were taken for the three dates, with a mean length of 7 cm with 7 nodes.

Fig. 5 shows that for all the dates, the mean rooting value of cuttings equalled 45% and, in general, for all the periods, semihardwood cuttings gave the best results.

It would be advisable also for this species to extend the sampling period of cuttings and to assess their rooting ability.

### *Sambucus nigra* L.

Asexual propagation was tried by taking cuttings in four periods, one month apart starting from the first decade of February; for the first two periods, herbaceous and hardwood cuttings were compared, 15 cm long with 4 nodes. For the other two periods, herbaceous, semihardwood and hardwood cuttings were compared, 10 cm long with 4 nodes.

Figure 6 shows that for the first two periods, the rooting percentage was rather low for all the types of cuttings with values between 12 and 16%; for the following ones a considerable increase was recorded (82%). As to the cutting type (Fig. 7), in the first two periods herbaceous cuttings gave mean values of 10% whereas for hardwood cuttings 15%. For the other periods, herbaceous cuttings showed a higher rooting ability with mean values of 100%; semihardwood cuttings 60% and hardwood cuttings did not root at all.

### *Viburnum tinus* L.

For this species two sampling periods were compared: the first decade of April and the first decade of May.

For both periods, herbaceous and semihardwood cuttings were used, 14 cm in length with 5 nodes.

Fig. 8 shows that for the first sampling period, the mean rooting equalled 20% (18% for herbaceous cuttings and 22% for semihardwood cuttings). For the second period, the rate was lower, on the average 14% (10% for herbaceous cuttings and 19% for the semihardwood ones).

It would be advisable to extend the cutting sampling period in order to assess the best rooting period.

## Conclusions

In conclusion, for sexually propagated species, as to *F. excelsior* L. pre-cooling induced a considerable increase in the germination rate; as for *Myrtus communis* the increase was induced only by the June sowings.

For asexually propagated species (Fig. 9) (*Myrtus communis* L., *Phlomis fruticosa* L., *Rosmarinus officinalis* L., *Sambucus nigra* L., and *Viburnum tinus* L.), the highest rooting rate was reported for *Myrtus* and *Sambucus* followed by *Rosmarinus* whereas the rooting percentage of *Viburnum* was very low. In particular, as for the impact of the sampling period, the highest rooting rate was achieved with cuttings taken in April for myrtle, rosemary and phlomis; sambucus showed the highest rooting rate in May whereas viburnum a low rooting ability at least during the tested periods.

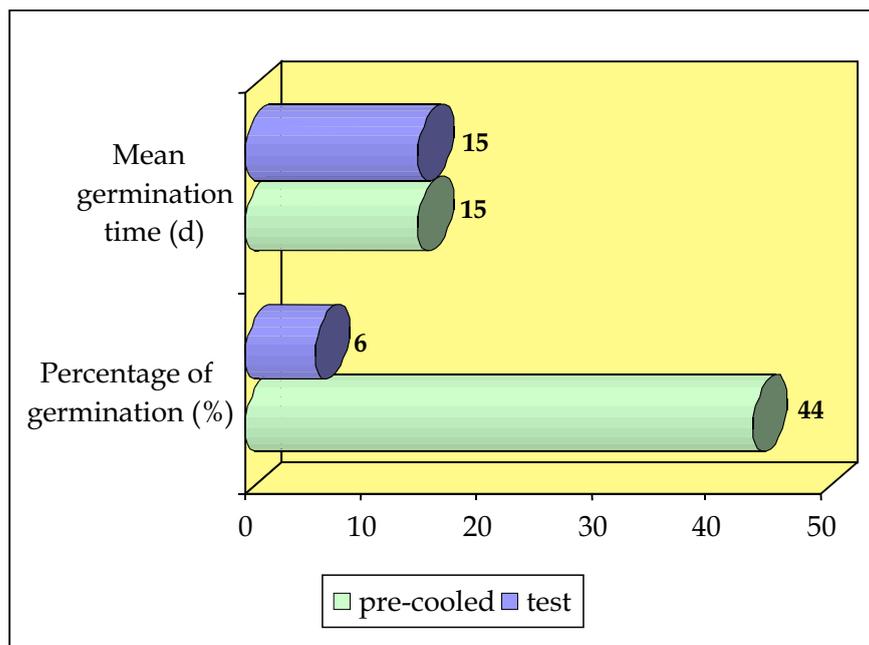


Fig. 1 - Influence of treatments on the percentage and mean time of germination.

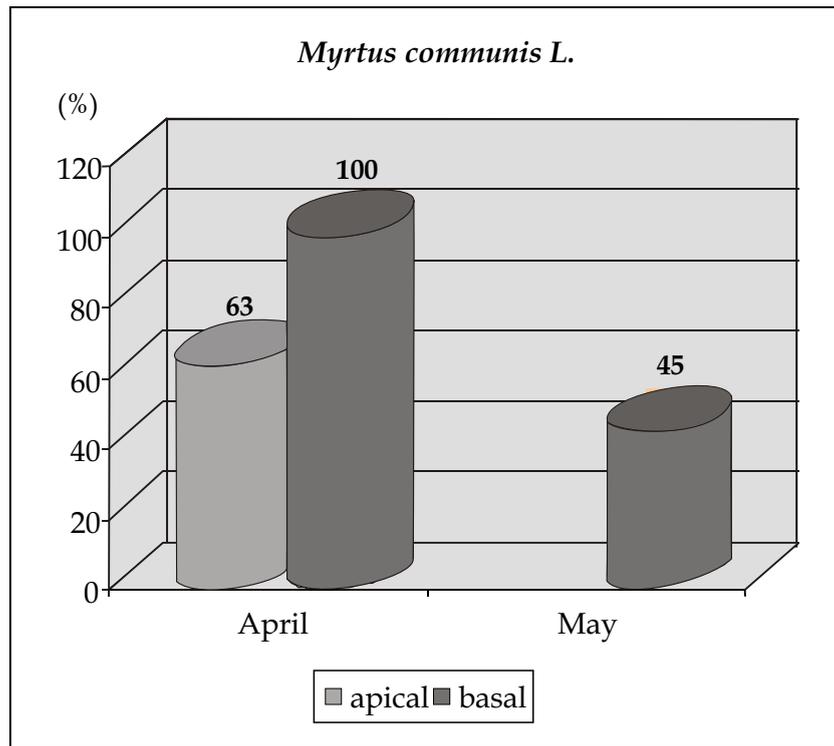


Fig. 2 Influence of sampling time on the rooting percentage of woody cuttings.

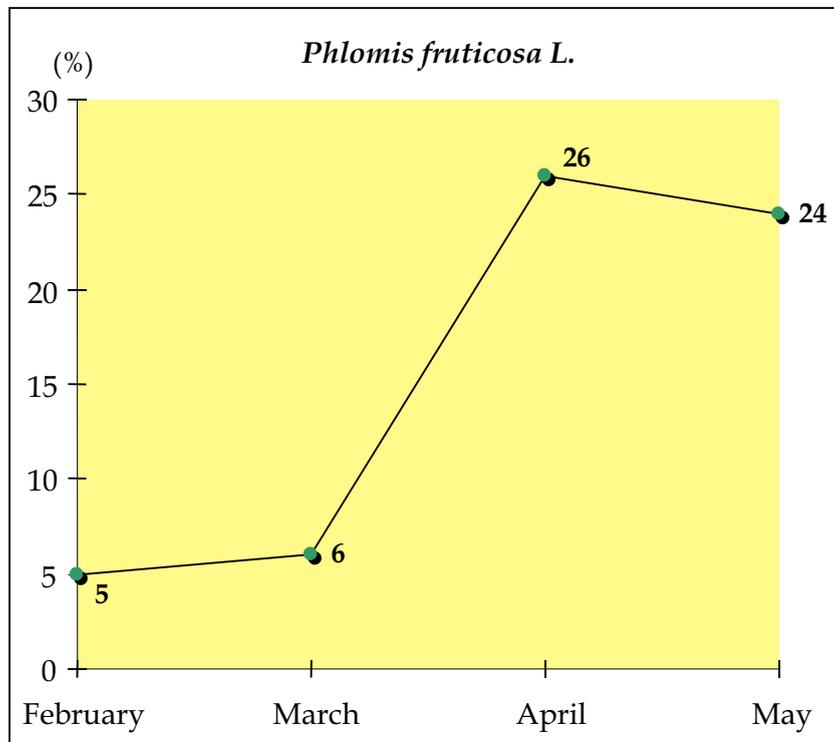


Fig. 3 Evolution of the rooting percentage for different cutting sampling times.

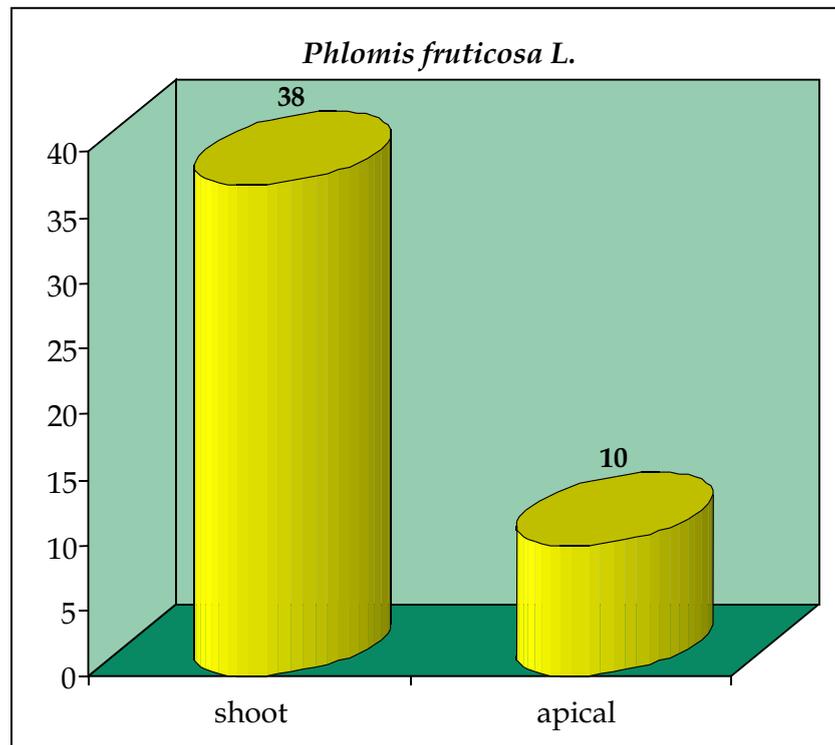


Fig. 4 Influence of different types of herbaceous cuttings on rooting percentage.

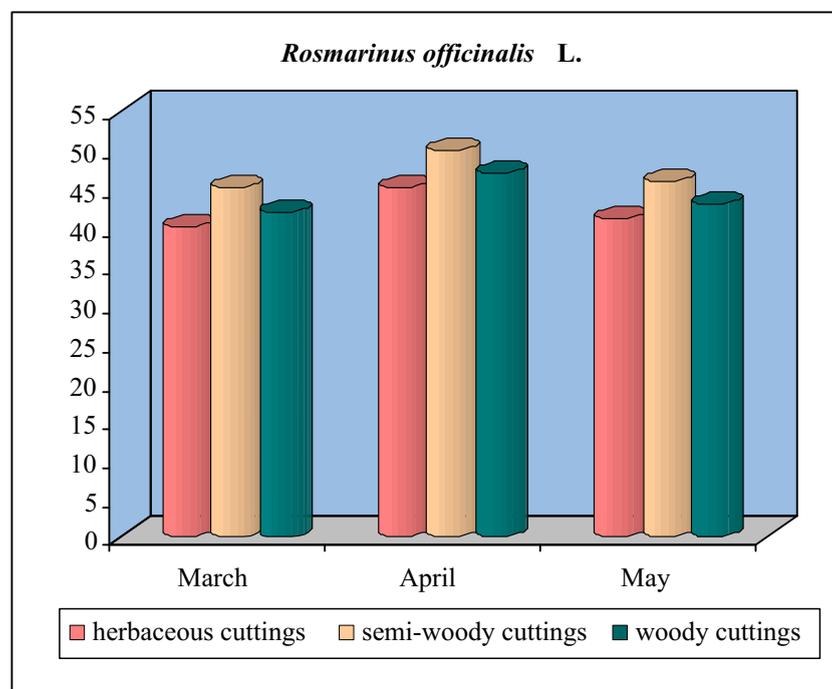


Fig. 5 - Interaction of the sampling time and type of cutting on rooting percentage.

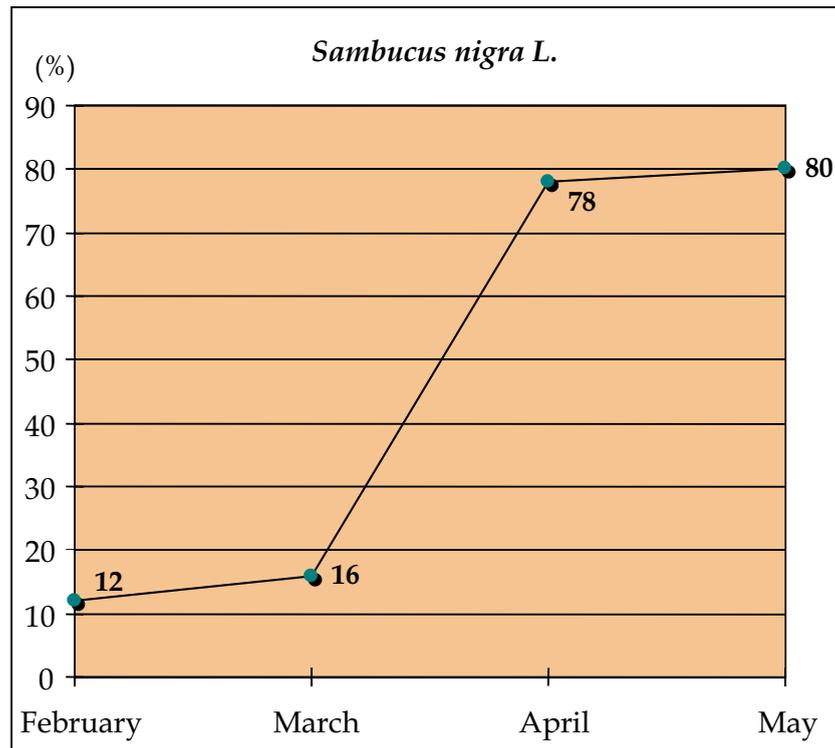


Fig. 6 Evolution of rooting percentage for different cutting sampling times.

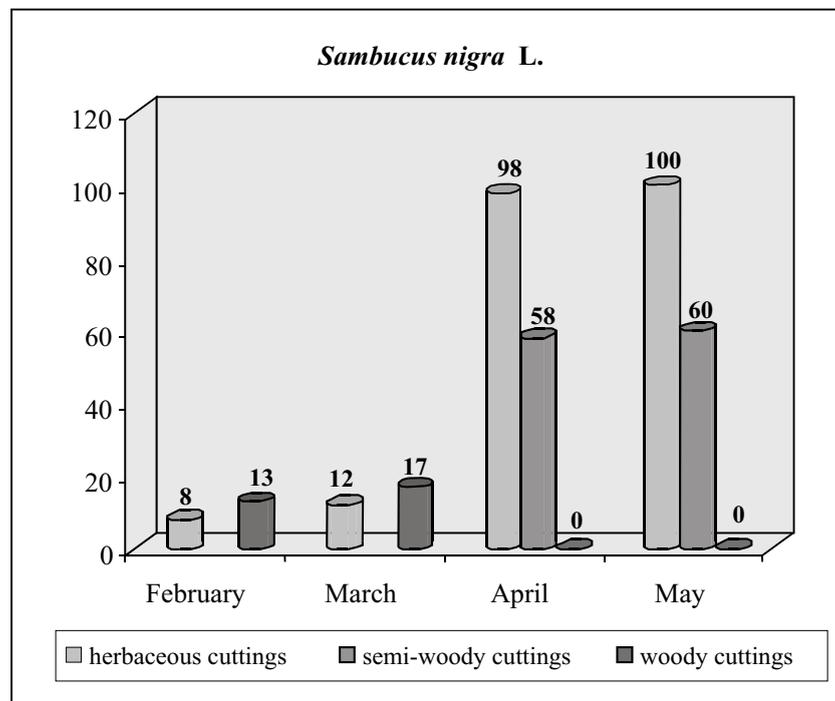


Fig. 7 Rooting percentage for sampling times and different cutting types.

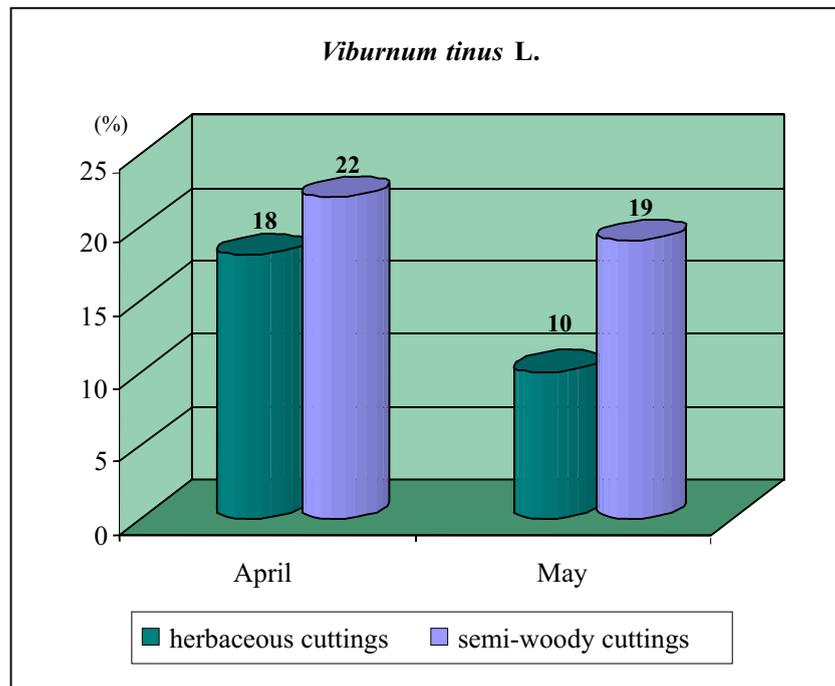


Fig. 8 Interaction of sampling time and type of cutting on rooting percentage.

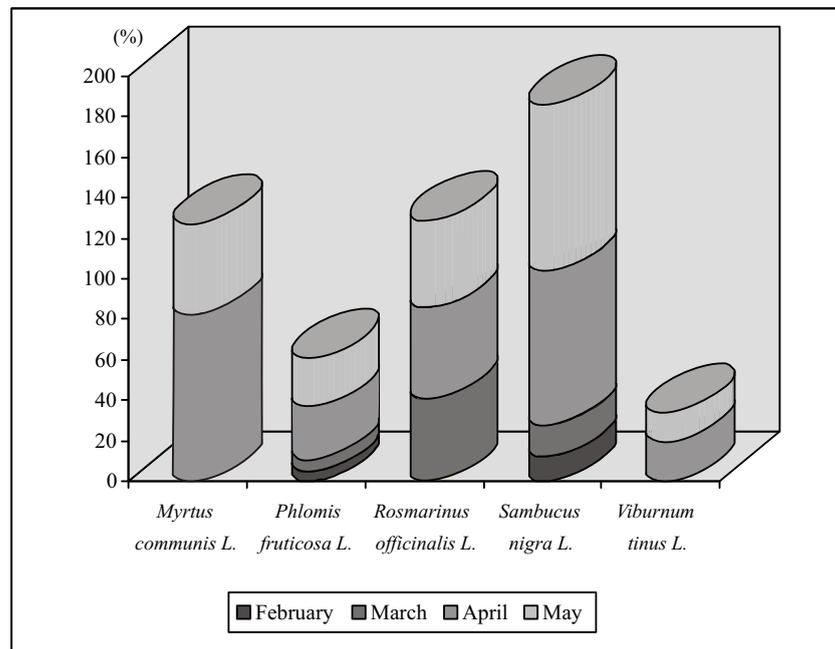


Fig. 9 Comparison of rooting percentage for different species and different cutting sampling times.

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