Germination Trials and Domestication Potential of Three Native Species with Edible Sprouts: *Ruscus aculeatus* L., *Tamus communis* L. and *Smilax aspera* L.

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Keywords: Diversification, edible plants, new crops, seed propagation, vegetables

Abstract

***Ruscus aculeatus*, *Tamus communis* and *Smilax aspera* are wild species whose young spring sprouts are consumed in some areas of Italy. Although sometimes abundant in the natural vegetation, these species are not common on a country basis. Sprouts are therefore only locally commercially available. Cultivation could contribute to make them better known and increase vegetable product differentiation. Seed germination was investigated by applying the following treatments: control, concentrated H_2SO_4 for 5 or 10 minutes; GA_3 at 500 ppm coupled or not with H_2SO_4, ethrel 1 or 3 mM, KNO_3 0.2 or 0.4%, natural stratification. Germination was very low, and of no practical interest, for *S. aspera*, ranging from 0 to 65% for the two other species. The mean germination time was 140-220 days. KNO_3 and ethrel at low dose improved, GA_3 and H_2SO_4 decreased, and stratification had no effect on germination. Germination time was shorter at higher germination rates. Domestication opportunities are discussed, considering growth habit, cultural traits and consumer acceptance.**

INTRODUCTION

The present tendency towards the recovery of food traditions and the need for product diversification may offer opportunities for new crop domestication. This, however, should rely on a minimum basic knowledge. *Ruscus aculeatus* L., *Smilax aspera* L. and *Tamus communis* L., although mainly known for other characteristics, are used as edible plants in some areas of Italy, where young sprouts are consumed cooked as vegetables.

Ruscus aculeatus (*Liliaceae*) is a well known bushy plant (chamaephyte), rather common in the underwood layer of sub-mediterranean forests. The aerial part generally lives for several years, flowering and fruiting, whereas vegetative growth generally ends in the first year. The growth habit has therefore also geophytic traits, since new shoots are produced yearly from underground buds. The plant produces a dense net of rhizomes, suitable for vegetative propagation. The rhizomes and roots have been extensively studied for their active principles (mainly steroidal saponins; Elsohly et al., 1975), in pharmaceutical preparations and clinical trials. Traditional use in anti-inflammatory treatment has recently been exploited, making *R. aculeatus* a primary plant remedy for vein ailment and haemorrhoids (Oulad-Ali et al., 1996; Dunouau et al., 1996; Jager et al., 1999). *R. aculeatus* and other *Ruscus* are used as ornamentals for shaded places, and as a source of cut foliage. Fertilisers, shading, weed control and climate were studied as critical growing factors (Rumine and Bellandi, 1984; Baum and Biran, 1982; Stamps and Boone, 1992). Seed propagation has not been investigated, whereas some work was done on in vitro propagation of other *Ruscus* species (Curir et al., 1988). The use of spring sprouts as food is known in many places of Italy (Corsi and Pagni, 1979; Lonardoni and Lazzarini, 1992).

Smilax aspera (*Liliaceae*) is a climbing spiny shrub, occurring in clearings and forest margins of typical Mediterranean woodlands. It has a phanerophytic growth habit, with aerial branches elongating several meters, bearing both flower and vegetative buds, from which sprouts develop in spring. Vigorous root suckers are produced from rhizome buds as well, so that the species may occasionally behave as a geophyte (e.g. after fires or

Proc. IS on Sust. Use Of Plant Biodiv.
Eds. E. Düzyaman &Y.Tüzel
Acta Hort. 598, ISHS 2003

when the aerial part is killed by frost). Many *Smilax* species of Central and South America, collectively referred to as *sarsaparilla*, as well as east Asian species, have been investigated for the active principles and medicinal uses of their rhizomes. Food uses of *S. aspera* have been reported for Turkey (Syamoglu, 1984; Ertug, pers. commun.) and other middle-eastern countries, already since long ago (Ridolfi, 1881). In Italy, it is used in some areas and sometimes commercially harvested in Sicily (Arcidiacono et al., 1996). Uses as vegetables have been reported for two other *Smilax* species in Japan (Tazawa et al., 1990).

Tamus communis (Dioscoreaceae) is a climbing vine growing in forest margins, hedges and clearings in sub-mediterranean vegetation types. It is a typical geophyte, whose aerial parts die every year and regenerate in spring from the big tubers; spring sprouts of mature plants are very vigorous. The plant is toxic because of saponins and oxalic acid, in which the fruits are particularly rich. The tuber has been studied for its anti-inflammatory properties (Capasso et al., 1983; Mascolo et al., 1987), and as a potential source of diosgenin (Aquino et al., 1984). The plant is rather popular in some areas as a potent external remedy for traumas and distortions, having, however, a strong skin-irritating side effect. The sprouts are very popular ingredients of soups and side dishes of meat courses in central Italy. Their use is also reported for north-western France (Lieutaghi, 1974; Peyron, pers. commun.). Documented cases of toxicity have not been reported.

All three species are often abundant in their natural habitat, but typical of vegetation types not very common on a country basis, and prone to human disturbance. Cultivation may therefore represent a means of reducing the pressure on natural stands.

These species can be vegetatively propagated by means of rhizomes (*Ruscus*, *Smilax*), tubers (*Tamus*) and perhaps cuttings. However, seeds would assure faster and easier multiplication. Additionally, as indicated for *Dioscorea rotundata* (Sadik and Okereke, 1975), seed propagation is more efficient to explore and exploit biodiversity.

Experiments on seed germination are almost absent, as is information on cultivation. This paper reports the results of germination trials with seeds from Italian natural populations of the three species, and observations on early seedling growth, aimed at collecting preliminary information on cultivation and domestication opportunities.

MATERIALS AND METHODS

Ripe fruits were collected in October, 1997. *Tamus* and *Smilax* fruits were harvested in the coastal area of Montemarcello, Liguria region, northern Italy. *R. aculeatus* fruits were harvested on the western slopes of the Cimini mountains, province of Viterbo, central Italy. For the preliminary trials of 1996, fruits of all three species were harvested in the Montemarcello area. The seeds were manually extracted from the fruits, washed with water, dried with air flow at 25 °C for one week, selected for the elimination of all defective seeds, and stored in air-tight containers at 18 °C until the start of germination trials. In 1996, 50 seeds per species were subjected to the tetrazolium test.

Germination trials started in December in both years, and were carried out in three replications of 50 seeds, using sterile sand as substrate, in a climatised chamber at 20 °C and 90-95% relative humidity. No differentiated theses were compared in 1996, whereas in 1997 the following treatments were given, plus an untreated control: a) chemical scarification with concentrated H_2SO_4 for 5 or 10 minutes; b) treatment with gibberellic acid (GA_3) at 500 ppm for 1 hour; c) factorial combinations of the previous two treatments; d) treatment with ethylene (ethrel, 1 and 3 mM) for 1 hour; e) osmotic treatment with KNO_3 at 2 and 4 mg l^{-1} for 6 hours; f) stratification in humid sand under plastic tunnel for 120 days.

The seedlings were counted each 2-4 days from the beginning of germination. A seed was considered germinated when the coleoptile was fully developed. Mean germination time was calculated as: $MGT=\Sigma(d*n)/N$, where d = day from sowing; n = number of germinated seeds in each counting; N = total number of germinated seeds. The data were subjected to the analysis of variance after angular transformation.

The plantlets were transplanted individually in 5-cm, and subsequently in 20-cm Ø pots filled with 30:70 peat:sand mixture. Visual observations on growth, frost sensitivity and diseases were carried out for two years.

RESULTS

Seed Viability and Germination Trials

Germination of 1996 preliminary trials ranged from 3 % in *S. aspera* to 20-25 % in *T. communis* and *R. aculeatus*. The tetrazolium test, however, indicated over 50 % viable seeds. The 1997 results are discussed only for *R. aculeatus* and *T. communis*, since *S. aspera* germination (2-4 %) had no practical interest.

Both H_2SO_4 and GA_3 (Fig. 1) had negative effects, more markedly for *R. aculeatus*, whose germination decreased to 0 for all combinations of the two treatments. The germination of *T. communis* was not affected by sulphuric acid for seeds not treated with GA_3, whereas in the presence of GA_3 germination decreased to 0 for 10-minute acidic scarification. The shortening of the mean germination time with all treatments had no interest, being coupled with the illustrated decrease of germination. The germination of *T. communis* increased with 1 mM ethrel (Fig. 2) and decreased below the values of the control at the highest concentration, whereas that of *R. aculeatus* was not affected by this hormone. Ethrel had a slight, non-significant, effect on mean germination time, with a decrease at 1 mM and an increase at 3 mM. KNO_3 (Fig. 3) markedly increased germination of *T. communis* and decreased that of *R. aculeatus*. For this species, KNO_3 at the highest concentration also increased the mean germination time. Stratification (Fig. 4) negatively affected germination and mean germination time.

Fig. 5 shows examples of germination patterns in different conditions, compared with the control. For *T. communis*, 10-minute sulphuric acid treatment had very modest impact on the beginning of germination, whereas the marked effect of this treatment on the reduction of the mean germination time was due to the devitalisation of the later-germinating seeds. Ethrel at 1 mM accelerated germination of the whole seed population, and also promoted germination of late seeds, with, as a result, an overall reduction of the mean germination time. KNO_3 had a similar effect with respect to the beginning of germination; at 2 mg l^{-1}, it strongly promoted the germination of late seeds, so that the mean germination time was not different from the control. At 4 mg l^{-1} the effect of germination improvement on late seeds was lower, with a consequent reduction of MGT. Stratification delayed germination of the whole seed population. The two reported examples for *R. aculeatus* indicate a delay of germination of the whole seed population in both cases.

Observations on Plant Growth

Seedling survival after transplanting was rather low for *T. communis*, and good for the other two species. *R. aculeatus* and *S. aspera* developed a well established root system and vigorous aerial parts already in the first year. *S. aspera* soon developed the typical spiny and climbing habit. *R. aculeatus* produced several root suckers already in the spring of the second year, whereas no root suckers were observed for *S. aspera*, which maintained its vegetative parts throughout the winter, despite low temperatures. Plant survival after transplanting was almost 100 % for both species.

During the first year, *T. communis* devoted most resources to tuber growth, developing 1-2 leaves and a weak root system. Leaves were easily lost for any stress cause, and plant mortality was high. In the second year, no appreciable suckers were produced, and the tubers remained small. Transplanting always caused stress and plant losses. All three species, and especially *T. communis*, were severely affected by radiative early frosts during the winter of the second year. *R. aculeatus* plants almost stopped growth when kept in full sunlight during the summer.

DISCUSSION AND CONCLUSIONS

Germination of *S. aspera* was of no practical interest. Izhaki and Safriel (1990) observed very low germination percentages of intact seeds, but found that germination rose to about 50 % in seeds ingested by some birds; germination time was always above 140 days. Low germination, and germination times up to one year, were also found in *S. australis* (Conran, 1986); vegetative propagation by cuttings was successfully tested for this species. In contrast, high germination rates were obtained with *S. campestris* (Rosa et al., 1999). Further exploration may allow the identification of a better fruit harvest stage, or germination conditions to improve germination of *S. aspera*. Germination of *T. communis* was satisfactory, and prone to improvement with simple osmotic treatments. Long germination time was found in other *Dioscoreaceae* species (Sadik, 1977; Viana and Felippe, 1990), where it was shown that post-maturation after harvest can shorten dormancy. Therefore, this option could be assayed for *T. communis* too, to reduce the risks connected with a too long germination time. *R. aculeatus* germination was sufficient for practical uses, although no effect of treatments was observed; in this respect no references are available in the literature, because of the prevailing vegetative propagation of ornamental *Ruscus* species.

Post-germination observations seem to indicate that *S. aspera* and *R. aculeatus* may be easily propagated also by rhizomes, whereas the vegetative propagation of *T. communis*, although probably similar to that of yams, requires further investigation and may represent an initial problem because of the very slow tuber growth. Crop establishment seemed quite easy for *R. aculeatus* and *S. aspera*, whereas high plant mortality and slow juvenile growth may represent serious constraints for seed-propagated *T. communis*. The best option for crop management seemed to be the full exploitation of the geophytic habit of all three species. In fact, *R. aculeatus* has prickly cladodes that may present an obstacle to sprout harvest if the old aerial part is present. *S. aspera* is spiny and climbing, so it would need some support left growing free for several years. Moreover, its shoot sprouts are smaller and perhaps of lower commercial value than root suckers. Ideally, therefore, all three species should be treated like *Asparagus*, with planting followed by adequate crop establishment, removal of the residual aerial part during the rest period, sprout harvest in spring, and adequate vegetative growth during summer to restore reserves. Of course, the plant's reaction to such exploitation needs further investigation.

Other aspects should also be clarified. As an example, different tuber yields were observed in male and female plants of *Dioscorea rotundata* (Sadik and Okereke, 1975). Smaller plant size and more abundant flowering of males, and lower survival of females were noted in *Smilax herbacea* (Sawyer and Anderson, 1998). Sex-dependent variation of sprout yield potential may therefore occur also for the three dioecious species examined. The three species were damaged by frost during winter. The sensitivity of *Ruscus sp.* to radiative frosts was stressed by Stamps et al. (1994), who pointed out that net cover and mulching can attenuate this problem. In addition, *Ruscus* needs shading to reduce the adverse effect of direct radiation. Pathology may play a prominent role in the development of new crops: although literature review of this aspects is beyond the scope of this work, information is rather abundant for *Ruscus*, but still scarce for *Smilax* and, especially, for *Tamus*. Table 1 represents a tentative summary of the information collected regarding the three species with respect to the possibilities of domestication, i.e., propagation, potential crop establishment and exploitation.

A last important point is the knowledge about the use of the sprouts as vegetables, which represents the starting point for commercial exploitation. In this respect, exploration of consumers' attitude, also outside the traditional areas, may form a necessary step to start full domestication and cultivation of these interesting new food crops.

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Tables

Table 1. Scores of some characters connected to the domestication and cultivation opportunities of the three species. 1. very unfavourable; 5: very favourable.

Character	*Ruscus aculeatus*	*Smilax aspera*	*Tamus communis*
seed propagation	3	1	4
vegetative propagation	4	3	1
growth habit	4	1	2
crop establishment	3	3	1
crop management	4	1	3
climatic needs	2 (shade)	2	2
pathology	4	2	1
consumers' knowledge	2	1	3

Figures

Effects: germination	
species	**
H_2SO_4	**
GA_3	*
species x H_2SO_4	**
species x GA_3	**
H_2SO_4 x GA_3	ns
sp. x H_2SO_4 x GA_3	**
Effects: mean ger. time	
species	**
H_2SO_4	**
GA_3	*
species x H_2SO_4	**
species x GA_3	**
H_2SO_4 x GA_3	ns
sp. x H_2SO_4 x GA_3	**

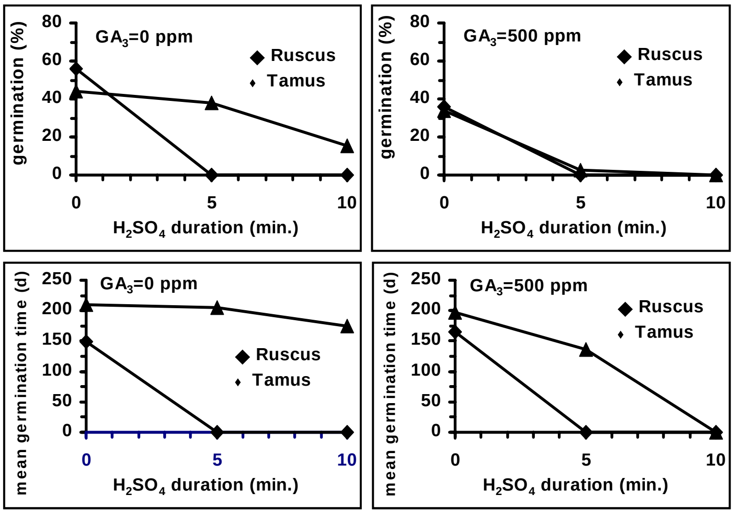

Fig. 1. Effects of sulphuric acid and gibberellic acid on germination and mean germination time (MGT). Non-significant (*ns*), statistically significant at P<= 0.05 (*) or P<= 0.01 (**).

Effects: germination	
species	*
ethrel	**
species x ethrel	*
Effects: mean ger. time	
species	**
ethrel	ns
species x ethrel	ns

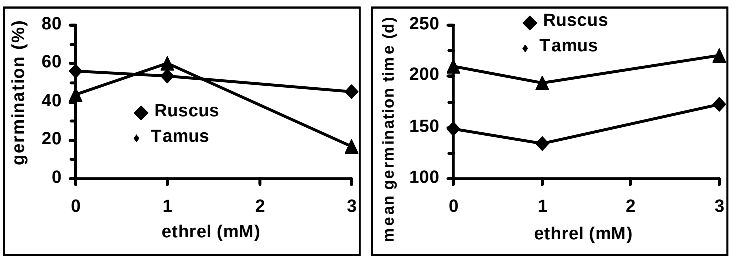

Fig. 2. Effect of ethrel on germination and mean germination time (MGT). Non-significant (*ns*), statistically significant at P<= 0.05 (*) or P<= 0.01 (**).

Effects: germination	
species	*
K_2SO_4	**
species x K_2SO_4	*
Effects: mean ger. time	
species	**
K_2SO_4	ns
species x K_2SO_4	ns

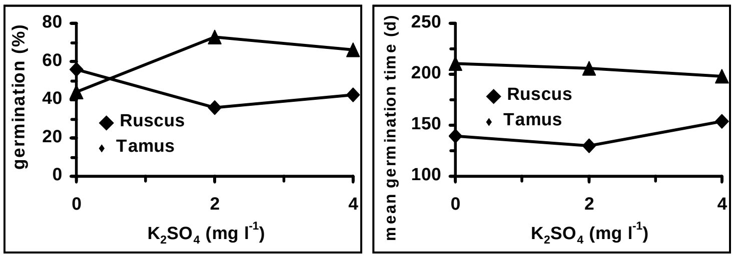

Fig. 3. Effect of potassium sulphate on germination and mean germination time (MGT). Non-significant (*ns*), statistically significant at P<= 0.05 (*) or P<= 0.01 (**).

Effects: germination	
species	*
stratification	ns
species x stratification	ns
Effects: mean ger. time	
species	**
stratification	**
species x stratification	ns

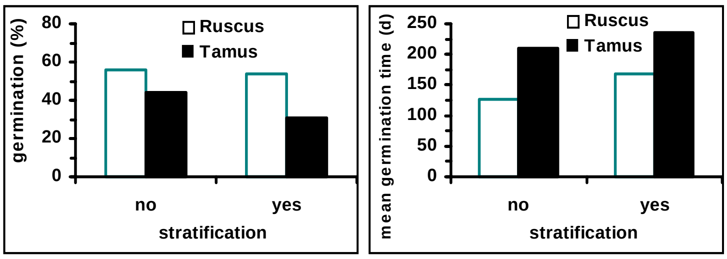

Fig. 4. Effect of stratification on germination and mean germination time (MGT). Non-significant (*ns*), statistically significant at P<= 0.05 (*) or P<= 0.01 (**).

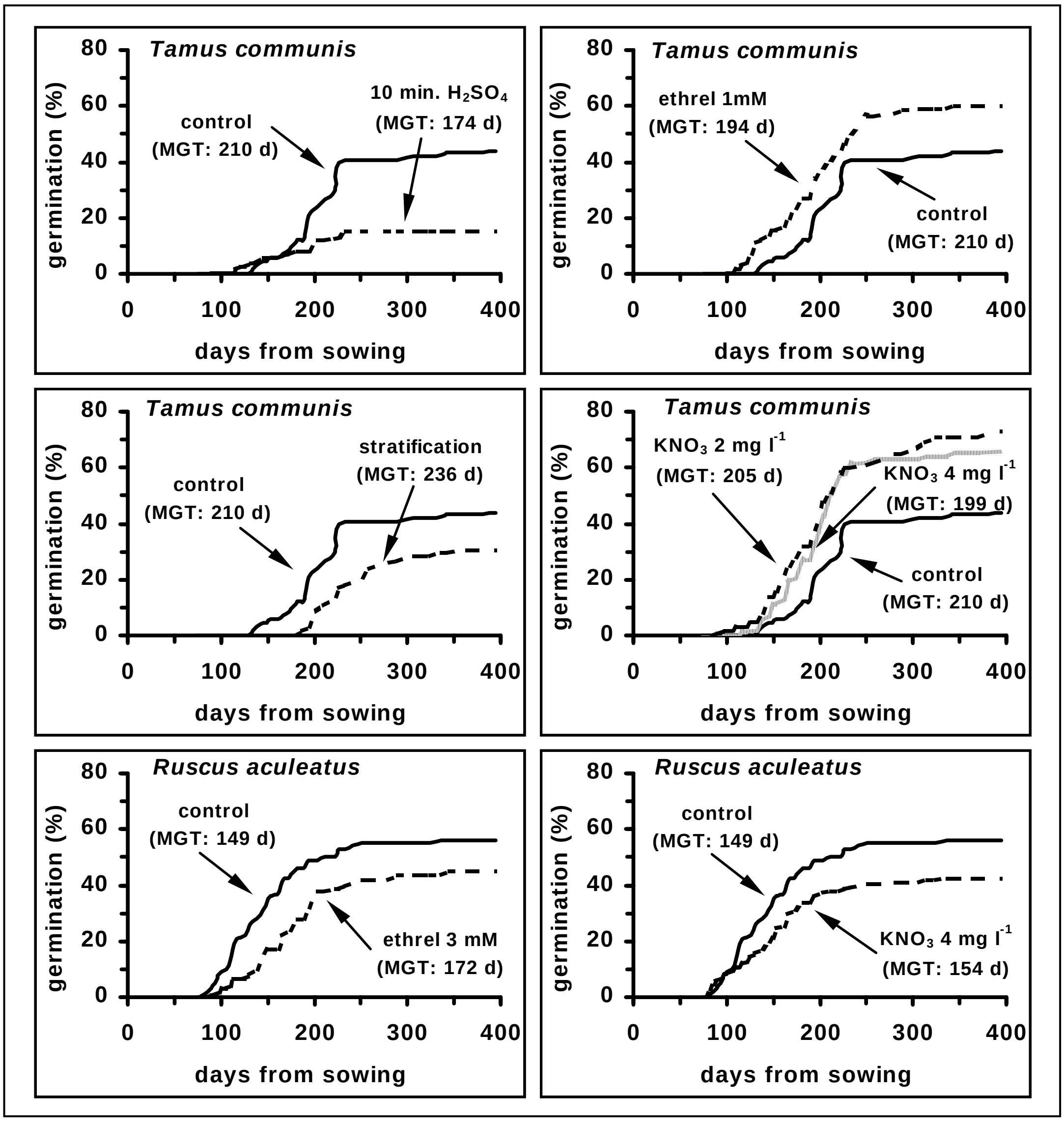

Fig. 5. Germination patterns of some selected combinations of species and experimental treatments. MGT: mean germination time.