

# Biological Flora of the British Isles: *Ruscus aculeatus*

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## Summary

1. This account presents information on all aspects of the biology of *Ruscus aculeatus* L. (Butcher's broom) that are relevant to understanding its ecological characteristics and behaviour. The main topics are presented within the standard framework of the *Biological Flora of the British Isles*: distribution, habitat, communities, responses to biotic factors, responses to environment, structure and physiology, phenology, floral and seed characters, herbivores and disease, history and conservation.

2. *Ruscus aculeatus* is a multistemmed monocotyledonous shrub with leaves functionally replaced by cladodes and photosynthetic stems. It is native to southern England primarily in dry shaded woodland and hedgerows (but widely planted elsewhere) often, but not exclusively, on base-rich soil. It is rarely abundant in any habitat, usually forming widely spread discrete clumps.

3. *Ruscus aculeatus* is remarkably shade tolerant and drought resistant with low water conductance and transpiration, and water storage in the cladodes. Yet unusually for a drought-tolerant stem-photosynthetic plant, it prefers shady environments.

4. The flowers have few if any pollinating mechanisms, low seed production and fruit/seed dispersal are largely ineffective, which may be a relict of its evolution in a tropical Tertiary climate. Population survival primarily depends upon vegetative spread from stout rhizomes, aided by the plant's general unpalatability.

5. Over-collecting for medicinal steroidal saponins has caused some population declines, particularly in eastern Europe, but it is otherwise facing few conservation problems.

**Key-words:** climatic limitation, communities, conservation, diseases, ecophysiology, geographical and altitudinal distribution, germination, herbivory, mycorrhiza, reproductive biology, soils

Butcher's broom. Asparagaceae. *Ruscus aculeatus* L. is a perennial, evergreen shrub with multiple stems arising from a creeping, thick, sympodially branched rhizome to form an oval, pyramidal bush. Stems striate, green, erect, much branched, 25–80 (100) cm. Leaves reduced to triangular scarious scales < 5 mm long and replaced functionally by rigid cladodes (1–4 × 0.4–1 cm), each arising from a leaf axil; cladodes ovate, entire, dark green and spine-pointed. Mostly dioecious but occasional hermaphrodite or female flowers have been reported on otherwise male plants which led Martínez-Pallé and Aronne (1999) to classify it as subandroecious. Male and female plants very similar in appearance (Yeo 1968). Flowers 1–2, arising from the axil of a small

scarious bract in the centre of the upper surface of a cladode, each with a short pedicle. Perianth greenish-white, approximately 3 mm long, in two whorls of three segments, bearing papillae. Female flowers with a cup formed from fused stamen filaments around the superior, unilocular ovary, which has a subsessile capitate stigma. Male flowers with three stamens, filaments green or violet, fused into a tube around an undeveloped ovary. Fruit a bright red globose berry, 8–14 mm with 1–4 large seeds; seed mass 163 mg.

*Ruscus* has shuttled between various families including Ruscaceae (Kim *et al.* 2010), Convallariaceae and Liliaceae (as recorded in List Vasc. Pl. Br. Isles by Kent 1992), but is currently in the Asparagaceae (Chase, Reveal & Fay 2009; Stace 2010). The genus includes approximately 7–10 species spread throughout Europe across to Iran (Yeo 1968) including the larger ornamental *R. hypoglossum* introduced into Britain from south-eastern Europe. There are several

\*Nomenclature of vascular plants follows Stace (2010) and, for non-British species, *Flora Europaea*.

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ornamental varieties, including var. *angustifolius* Boiss. with very narrow cladodes, commonest in the eastern part of its range, and var. *platyphyllus* Rouy with cladodes 5 cm long and up to 2.5 cm wide (Bean 1980). There are also a number of cultivars including: 'Lanceolatus' only female plants with very narrow cladodes five times longer than wide (Cann 2001); 'Wheeler's Variety' a heavy fruiting hermaphrodite; and 'John Redmond' and 'Christmas Berry' both dwarf hermaphrodites with short intercladode lengths. A yellow-fruited form has been recorded in woods at Heckfield, Hampshire (Anon 1866).

*Ruscus aculeatus* is the only monocotyledonous shrub native to the British Isles. It is a slow-growing, shade-tolerant shrub that occurs naturally in dry shaded woods and hedgerows in southern England, an unusual habitat for a stem-photosynthetic plant (Farmer 1918), as such species normally grow in arid, high-light environments. However, *R. aculeatus* also occurs on walls and cliffs, and rocky ground near the sea. It is also naturalized in many habitats including churchyards and near habitation, either deliberately planted or as a garden escape (Preston, Pearman & Dines 2002).

## I. Geographical and altitudinal distribution

In Britain, *Ruscus aculeatus* is native to southern England (Fig. 1) most widespread in the south-east but local across to Devon, Cornwall and possibly South Wales, and onto the Isles of Scilly. It has been much planted within this range and north of this into Scotland and west into Ireland.

In Europe, *R. aculeatus* is most widespread around the Mediterranean (Fig. 2), native to North Africa (Morocco, Algeria, Tunisia and Libya) across to eastern Europe and central Hungary (Tutin *et al.* 2002). Northwards it is found in Transylvania, southern and western Switzerland and northern France, across into the Azores (Clapham, Tutin & Moore 1987), reaching its northern European limits between 50 and 55° N (Preston 2007).

Altitudinal limits appear largely unrecorded, but it is known to reach 300 m in southern Romania (Banciu, Mitoi & Brezeanu 2009), 656 m in the deciduous forests of Southern Italy (Allen, Watts & Huntley 2000), 930 m in oak–hornbeam woodland in Slovenia (Dakskobler 2013) and 1000 m in South Anatolia, Turkey (Davis 1984).

## II. Habitat

### (A) CLIMATIC AND TOPOGRAPHICAL LIMITATIONS

*Ruscus aculeatus* is primarily a Mediterranean species but with oceanic tendencies giving it a sub-Mediterranean–sub-Atlantic distribution (Preston & Hill 1997). In north-western Spain, Retuerto and Carballeira (1992) suggest that *R. aculeatus* spans the temperate Mediterranean and into the Mediterranean maritime region where it is more abundant.

British populations grow mostly in relatively mild maritime areas (Kay & Page 1985) with warm summers (Perring 1996). Using mean values for all 10-km squares within which

*R. aculeatus* is found in Britain, Ireland and the Channel Islands, Hill, Preston and Roy (2004) record that it is found where the January mean temperature is 4.3 °C, July mean temperature is 16.4 °C with an annual precipitation of 782 mm. Similarly, Banciu, Mitoi and Brezeanu (2009) found that *R. aculeatus* in Romania grows in summer temperatures around 9–10.5 °C and 550–700 mm of precipitation. Although tolerant of low winter temperature, Salisbury (1926) found that the northern native distribution in Britain was primarily proscribed by the 8.9 °C March isotherm (except along the coast of East Anglia where it is within the 8.3 °C isotherm). Although a somewhat higher temperature than recorded by Hill, Preston and Roy (2004), Salisbury suggested that this is needed by this winter flowering species to ensure seed production and long-term success; he noted that the production of fertile seeds was rare near its northern limit except in hot summers.

In Britain, these conditions are most frequently met in woodland openings or at their edges, but in the south of its range, *R. aculeatus* tends to be found increasingly in the humidity and shelter of closed forest (Balica, Tămaş & Deliu 2005b; Banciu & Aiftimie-Păunescu 2012). For example, in southern Spain, Arista (1995) found *R. aculeatus* in 21% of 176 plots in closed *Abies pinsapo* forest and none in forest gaps. In north-western Spain, it was characterized as requiring a high minimum temperature, typical of coastal sites, and similar in temperature requirement to *Laurus nobilis*, *Erica cinerea* and *Fraxinus excelsior* (Retuerto & Carballeira 2004).

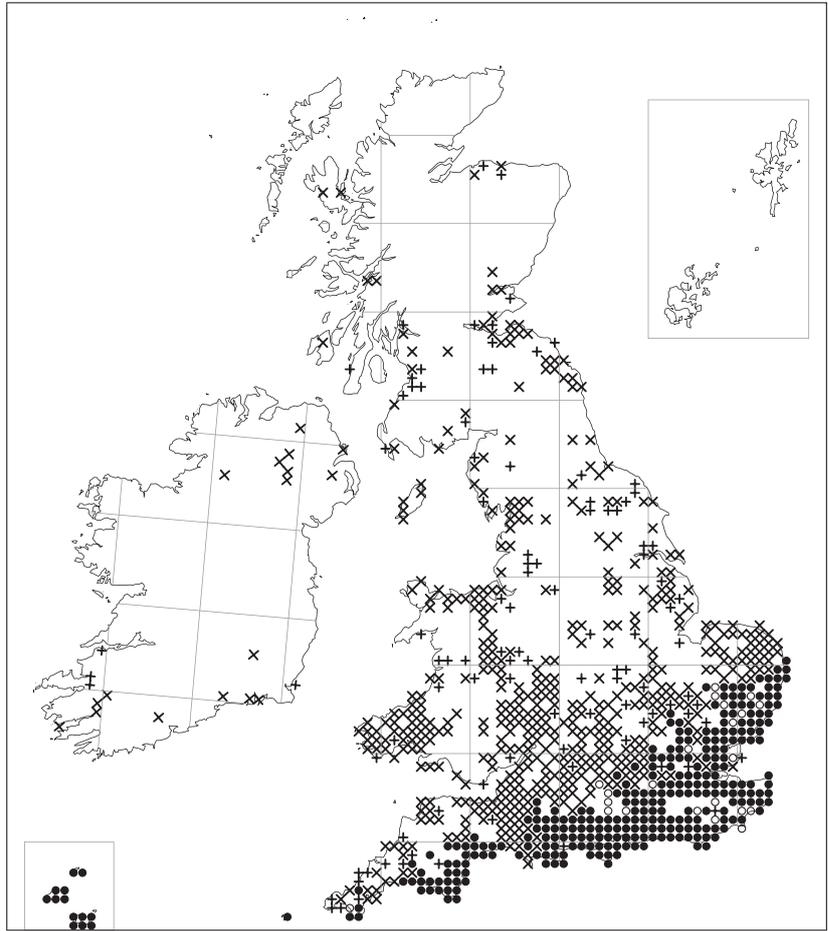
### (B) SUBSTRATUM

In Britain, *Ruscus aculeatus* is usually found on soils between pH 3 and 5, of average moisture retention, but it will grow on all soil types providing they are not too wet (Kay & Page 1985; Hill, Preston & Roy 2004). This catholic taste is shown by its ability to grow in the crevices of walls (Rishbeth 1948) and rocky ground near the sea. Fertility also appears to be unimportant in Britain and Ireland since it grows equally well on very fertile and infertile soils, although generally prefers medium fertility soils (Hill, Preston & Roy 2004). In mainland Europe, however, *R. aculeatus* is considered an indicator of poor soil (Rameau, Mansion & Dumé 1989). *Ruscus aculeatus* is quite frequent (40–60% frequency) on the serpentine (ultramafic) soils of Tuscany, Italy (Chiarucci *et al.* 1998).

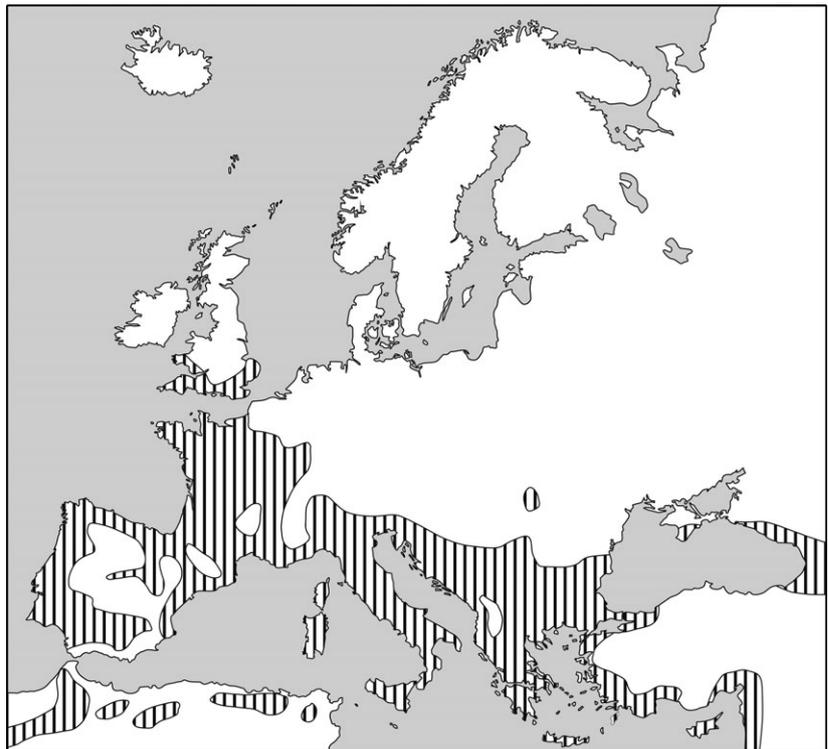
## III. Communities

Although found occasionally on walls, and cliffs, *Ruscus aculeatus* in Britain tends to be found mainly in open woodlands or scrub dominated by a wide range of tree species, including oak (*Quercus* spp.), hornbeam (*Carpinus betulus*) and beech (*Fagus sylvatica*). This varies from sea-cliff scrub in Guernsey to limestone woodland in the Gower Peninsula, Glamorgan and beechwood on chalk in southern Oxfordshire (Kay & Page 1985). *Ruscus aculeatus* is found primarily in *Fagus sylvatica* – *Rubus fruticosus* woodland (W14), and even then only as an occasional species (< 20% of samples

**Fig. 1.** The distribution of *Ruscus aculeatus* in the British Isles. Each dot represents at least one record in a 10-km square of the National Grid. (●) Native 1970 onwards; (○) native pre-1970; (+) non-native 1970 onwards; (×) non-native pre-1970. Mapped by Colin Harrower, Biological Records Centre, Centre for Ecology and Hydrology, mainly from records collected by members of the Botanical Society of the British Isles, using Dr A. Morton's DMAP software.



**Fig. 2.** The European distribution of *Ruscus aculeatus*, modified from de Bolòs and Vigo (2001).



and 1–4 on the Domin scale) mainly in open areas such as under canopy gaps and at the edges of stands (Rodwell 1991). It is also found locally, and with varying frequency, in the very base-poor, infertile soils of *Fagus sylvatica* – *Deschampsia flexuosa* woodland (W15), particularly in the New Forest.

In mid-Europe, *R. aculeatus* is found in similar woodlands, particularly the oak–hornbeam woods and calcicolous beechwoods, and also hedgerows (Burel & Bauldry 1990; Parent 2002) including the Querceto – Carpinetum serbicum aculeatetosum of Serbia (Ocokoljić, Vilotić & Šijačić-Nikolić 2013) and the Polygonato multiflori – Quercetum roboris of north-western Italy (Lonati & Lonati 2002). Further north, it is commonest in the mild, wet Atlantic oakwoods, particularly Rusco aculeati – Quercetum roboris (Izco, Amigo & Guitián 1990; González-Hernández & Silva-Pando 1999) and further south in a number of moist forests in the Pyrenees dominated by *Quercus pyrenaica* (Jongman 2000; Silva *et al.* 2011), the Endymio – Fagion beech forests of northern Spain (Dierschke 1997), and the moist *Fagus sylvatica*/*Quercus cerris* forest in the uplands of Southern Italy (Martínez-Pallé & Aronne 1999). *Ruscus aculeatus* is also common in the *Fraxinus angustifolia* woodlands (Pterocaryo pterocarpae – Fraxinetum angustifoliae) on moist alluvial soils by the Black Sea (Kutbay, Kilinç & Kandemir 1998).

In drier and warmer areas around the Mediterranean, such as Tuscany and Southern Italy, *R. aculeatus* occurs in the deep shade of *Quercus ilex* forests (Quercetea ilicis) sometimes mixed with *Pinus pinea* and *P. pinaster* (Debussche & Isenmann 1994; Grubb 1998; Kutbay, Kilinç & Kandemir 1998; Martínez-Pallé & Aronne 1999; Maremmani *et al.* 2003) often associated with *Asparagus acutifolius*, *Smilax aspera* and *Rubia peregrina* and in cooler areas by *Hedera helix* and *Ulex europaeus*. In cooler and more humid areas on calcareous soils, this changes to denser forests of *Q. ilex* including Cyclamino hederifolii – Quercetum ilicis and Festuco exaltatae – Quercetum ilicis (Biondi *et al.* 2004).

In still drier areas of Portugal and south-eastern Italy, *R. aculeatus* occurs in *Quercus suber* forests in association with shrubby plants such as *Asparagus aphyllus*, *Myrtus communis*, *Smilax aspera* and *Viburnum tinus* (Santo, Moreira & González 2005; Barrico *et al.* 2012). In semi-deciduous and evergreen woods of south-eastern Italy, *R. aculeatus* is frequent in a range of woodlands including oak woods on neutral soils (Carici halleranae – Quercetum suberis), *Quercus coccifera* scrub on more calcareous soils (Arbuto unedi – Quercetum calliprini), drier, more acidic *Q. trojana* woods (Teucro siculi – Quercetum trojanae) and the warmer wetter woods of *Q. virgiliana* (Irido collinae – Quercetum virgilianae) and Cyclamino hederifolii – Quercetum virgilianae; Biondi *et al.* 2004).

In Sardinia, *R. aculeatus* is found in a variety of mixed oak woodlands including Lonicero implexae – Quercetum virgilianae, Ornithogalo pyrenaici – Quercetum ichnusae and Glechomo sardoae – Quercetum congestae (Bacchetta *et al.* 2004). The maquis vegetation west of the Black Sea in Turkey also contains *R. aculeatus*, which is notably frequent in two low-

altitude associations: Phillyreo – Lauretum nobilis on coastal limestone and Lauro – Pinetum brutiae on mixed rock types up to 220 m, but is absent from the higher altitude deciduous woodlands (Yurdakulol, Demirörs & Yildiz 2002).

#### IV. Response to biotic factors

*Ruscus* shoots are eaten by a range of animals when young; Sack, Grubb and Marañón (2003) recorded that 10–30% of juvenile shoots showed sign of herbivore damage in southern Spain, compared to <10% damaged shoots on other Mediterranean species examined. Mature shoots, however, tend to escape damage, attributable to the physical defence from the sharply pointed cladodes but also to the poor nutritional value of the browse offered. González-Hernández and Silva-Pando (1999) suggested that the relatively high fibre content (acid detergent fibre:  $57.1 \pm 5.5\%$ ; SD) and low value of digestible organic matter ( $37.3 \pm 3.1\%$ ) make *R. aculeatus* low-quality nutritional forage despite the low silica ( $1.2 \pm 0.6\%$ ) and lignin ( $14.6 \pm 1.8\%$ ) content and the higher levels of crude protein ( $9.9 \pm 1.8\%$ ) compared to local grasses. This is clearly effective in reducing herbivory. Onaindia *et al.* (2004) found that *R. aculeatus* was significantly more abundant in grazed woodlands in northern Spain, reaching a mean cover of  $15.46 \pm 5.00$  (SE,  $n = 32$ ) compared to < 5% cover in mixed broadleaf woodland left to regenerate after clear-felling for > 30 years.

Gonçalves, Franco and Romano (2008) investigating allelopathy found that methanolic extracts ( $50 \text{ mg L}^{-1}$ ) of *R. aculeatus* completely inhibited the germination of *Lactuca sativa* (as did similar extracts of *Myrtus communis*) and that aqueous extracts (5% and 10% w/v) reduced root growth of *L. sativa* by 74% and 78%, respectively. By comparison, extracts of a number of other Mediterranean plants (such as *Olea europaea*, *Arbutus unedo* and *Pistacia lentiscus*) had no allelopathic effect.

#### V. Response to environment

##### (A) GREGARIOUSNESS

In Britain, *Ruscus aculeatus* tends to be locally abundant but usually of < 5% cover, forming well-spaced individual bushes that can spread to 2 m or more in diameter (Kay & Page 1985; Rodríguez-Loinaz, Amezaga & Onaindia 2012) but extending up to 25–50% cover (Montagnoli *et al.* 2012) or even dense carpets in shady conditions (Peltier *et al.* 1997; Bains *et al.* 2012) aided by vegetative spread from rhizomes.

##### (B) PERFORMANCE IN VARIOUS HABITATS

*Ruscus aculeatus* is slow-growing and long-lived (Kay & Page 1985), performing best in Britain in shaded and undisturbed habitats; it is usually considered a species of ancient woodland (Peterken 1974; Hermy *et al.* 1999; Rose 1999). As such it is not an early successional species, but in

Mediterranean systems tends to arrive with the mass of other shrubs (Houssard, Escarré & Bomane 1980). Patzel and Ponge (2001) noted the dense carpets of *R. aculeatus* in northern France growing under the deep shade of beech unmanaged for at least 400 years. However, even in optimal habitats, *R. aculeatus* often has very low biomass; in dense oak woodland in central Italy, Aguilar *et al.* (2012) recorded 28 kg ha<sup>-1</sup> of fresh biomass of *R. aculeatus*, the lowest mass of any woody plant they investigated.

Tansi, Karaman and Toncer (2009) found that *R. aculeatus* in Turkish oak/pine woodland (species not stated but most likely dominated by *Quercus coccifera*/*Q. cerris* and *Pinus brutia*) was almost twice the height (45.2 ± 4.69 cm; SD) compared to plants in deforested, open former pine areas (26.4 ± 1.73 cm). This was matched by plants in woodland areas having more cladodes per stem (19.8 ± 12.11 compared to 9.3 ± 1.26 in the deforested area) and a very much larger combined rhizome and root dry mass (30.6 ± 8.35 g compared to 2.2 ± 0.47 g). Above-ground dry mass was not significantly different, but the data had high standard deviations so it is likely that with more sampling, the above-ground dry mass would have been higher in the woodland; certainly, the fresh mass was significantly larger in the woodland (10.4 ± 3.07 g) compared to the cleared area (2.8 ± 0.41 g). The shade appears to be more important than lack of disturbance since in southern France the cover of *R. aculeatus* in 5- to 7-m-high *Quercus ilex* was 27%, but in nearby, more open shrublands was just 3% and completely absent in open fields (Debussche & Isenmann 1994). Similarly, in more marginal habitats such as open Mediterranean shrublands in southern Spain, Herrera (1984) recorded < 0.1% cover of *R. aculeatus*.

The apparent need for shade may also explain the differing response to grazing in different habitats. Thus, in dry, open *Quercus pubescens* forest, Debussche, Debussche and Lepart (2001) noted that *R. aculeatus* had increased in abundance in areas where coppicing or grazing and burning had been abandoned 18 years previously, presumably due to a beneficial increase in shade. By contrast, Onaindia *et al.* (2004) found that in Atlantic oak forest in northern Spain, *R. aculeatus* had its highest cover in woods clear cut approximately 30 years ago and in woodland grazed by cattle compared to older woods; presumably, the reduction in shade by cutting and grazing is outweighed by reduction in competition.

The ability to produce new shoots from large rhizomes is sometimes assumed to give *R. aculeatus* a distinct advantage in recovering from fire (Onaindia *et al.* 2004). And, indeed, there are records of it being abundant after a fire: Elia *et al.* (2012) stated that after an intense fire in oak forest in central Italy, killing 95% of canopy trees, *R. aculeatus* was amongst the 'herbs and seedlings' covering the ground in open spaces, but no specific data were given. However, detailed studies have shown that it is usually absent from immediate post-fire habitats despite being present beforehand (Schaffhauser *et al.* 2012). *Ruscus aculeatus* was present in open *Pinus halepensis* woodland in northern Greece before a fire in 1994, but did not reappear on the site for 5 months (Ganatsas *et al.* 2004).

At the end of the first growing season, the new shoots were at most 4 cm tall. Ten years after the fire, the dominant woody species had a higher shoot density than before the fire while *R. aculeatus* went from a mean stem density of 1.1 stems m<sup>-2</sup> pre-fire, to 0.2 m<sup>-2</sup> after 10 years. Similarly, dominant species were taller after 10 years than they were before the fire, whereas *R. aculeatus* was not significantly different before the fire (33.1 ± 0.04 cm tall and 1.7 ± 0.05 cm diameter, SE, *n* unstated) compared to afterwards. Úbeda, Outeiro and Sala (2006) observed that *R. aculeatus* 'disappeared completely after the fire' in *Pinus pinaster*/*Quercus suber* woodland in north-eastern Spain and after 2 years was still sparse (0.45% cover compared to 1.9% pre-fire) and then only in areas burnt at a medium intensity where the soil had least cover of litter after the fire (perhaps aiding seed germination).

The drought resistance of *R. aculeatus* does allow it to inhabit dry marginal habitats. Thus, in north-western France, it performs better in urban hedgerows than in rural woodlands and is significantly associated with the arid, impervious surfaces of urban areas (Vallet *et al.* 2008).

*Ruscus aculeatus* has been seen to affect directly the heterogeneity of both litter and earthworm populations in beech woodland (Campana, Gauvin & Ponge 2002). The stiff, spiny nature of the plant prevents beech leaf litter from reaching the ground, which in turn deprives earthworms of food, and possibly creates conditions chemically repellent to most earthworm species. This also has the probable effect of reducing carbon and nutrient availability beneath the plants.

### (C) EFFECT OF FROST, DROUGHT, ETC

The degree of frost tolerance is largely unknown. The very cold year of 1956 led to *Ruscus aculeatus* losing half of its cladodes at an altitude of 450 m at Olot, northern Spain, while still surviving (de Bolós 1956). This response was similar to other Mediterranean species.

*Ruscus aculeatus* is able to cope with a combination of extreme drought and deep shade (Grubb 1998; Pivovarov *et al.* 2013) and is similar to *Buxus sempervirens* and *Hedera helix* in this respect (Sack 2004). Coping with shade and drought is partly due to physiological mechanisms, discussed under VI (E), and partly morphological, discussed under VI (A). Using 13 species from across northern to Mediterranean Europe in a common garden experiment in Cambridge, Sack (2004) investigated growth and survival under a 20-day drought with soils reaching 18% of field capacity, at which point soils were brought back to 49% field capacity before the drought cycle began again. Drought decreased the relative growth rate of all species except for *R. aculeatus*, and it was concluded that it performed as effectively in both drying and ever-moist soil. The survival time of first year seedlings under drought was also tested; seedlings were hardened by reducing soil capacity to 37%, rewatered and then left to dry indefinitely, repeated under 3% and 30% full sunlight. Survival time of *R. aculeatus* seedlings was the highest of all 13 species tested: 65 days under 3% daylight, 59 days under 30%

daylight compared to a norm of 48 days in the others. Sack (2004) concluded that this ability to tolerate drought was attributable to water-use efficiency rather than tolerance of water loss. This fits with the findings of de Lillis and Fontanella (1992) that *R. aculeatus* in Mediterranean climates tends to limit growth to the period when water is available and stops growth once summer drought reduces water availability.

*Ruscus aculeatus* often only produces flowers and fruits in deep shade (Sack, Grubb & Marañón 2003; Sack 2004), but it will grow in less shady conditions and so has an Ellenberg indicator value for light of 4 – a semi-shade plant (Hill, Preston & Roy 2004). Despite this tolerance, Sack (2004) found that of the 13 European species tested, the growth rate of *R. aculeatus* was the most affected by shade; the relative growth rate under 3% full sunlight was 0.23 that at 30% sunlight (compared, for example, to 0.76 for *Sambucus nigra*). Yet the reduction in final mass due to shading at the end of the growing season was least in *R. aculeatus* (0.48 of the mass in 3% compared to 30% sunlight, compared to 0.05 in *Rubia peregrina*), attributed to the slow growth in both shaded and less shaded environments.

Antonellini and Mollema (2010) observed *R. aculeatus* growing in dune slacks with shrubs such as *Juniperus communis* and *Amorpha fruticosa* along the Adriatic coast of Italy. The water-table 0.45 m below the slack surface had a salinity of 16 g L<sup>-1</sup> (approximately half the salinity of sea water). However, the soil electrical conductivity below *R. aculeatus* was between 0 and 6 dS m<sup>-1</sup>; by comparison, *Pinus pinea* could tolerate 12 dS m<sup>-1</sup> and salt-tolerant plants up to 25 dS m<sup>-1</sup>, so it was concluded that *R. aculeatus* is not salt tolerant (agreeing with Hill, Preston & Roy 2004).

## VI. Structure and physiology

### (A) MORPHOLOGY

There has been argument as to whether the cladodes are derived from a branch (explaining its axillary nature and the bearing of flowers) or from a leaf on an aborted shoot, as suggested by its determinate growth and the venation (Hirsch 1977). Hirayama *et al.* (2007) looked for the expression of genes normally associated with leaf (YAB2: RaYAB2) and shoot (STM: RaSTM) growth and found both expressed in the cladode suggesting that it is a double organ derived from both. Cooney-Sovetts and Sattler (2008) agree with this dual origin.

All parts of the cladodes and stem are photosynthetic. The shoots live for several years but do not grow after the first year. New shoots are produced annually from the underground rhizome (D'Antuono & Lovato 2003; Balica, Tămaş & Deliu 2005b). The wood is typical of monocotyledons with the vascular bundles scattered through the sclerenchyma cylinder (Schweingruber 1990). Morphology of the cladodes and stem is further discussed by Arber (1924), Gilliland (1931) and Balica, Tămaş & Deliu (2005b).

Seedlings initially have a single thick root, which readily bifurcates. By the third growing season, the rhizome (up to 5 mm in diameter) develops together with coarsely branched

roots radiating out both vertically downwards and horizontally (Sack, Grubb & Marañón 2003). Rooting depth was modelled for theoretical standardized plants of 100 mg and 1 g total dry mass by Sack, Grubb and Marañón (2003) and found to be 9.6 cm (9.1–10.2 cm 95% CI) and 16.1 cm (15.0–17.2 cm 95% CI), respectively, for the two plant masses. The fine roots 5 mm from the tip have been measured at  $0.71 \pm 0.034$  mm (SE,  $n = 10$ ), the widest of any of the Mediterranean species tested by Sack, Grubb and Marañón (2003) which, other than *R. aculeatus*, ranged from 0.33 to 0.45 mm in diameter. *Ruscus aculeatus* had the highest proportion of its mass in the roots of any of the 13 species tested by Sack (2004), particularly so under 30% full sunlight (approximately 30% of dry mass in roots) compared to 3% sunlight (approximately 22%), a reflection of the relatively large fleshy roots and the short but wide (pachycaul) rhizome (Sack, Grubb & Marañón 2003). The rhizome can reach 25 cm in length and 3 cm in diameter. At the end of the growing season, the rhizome usually produces two lateral buds in the axils of the rudimentary leaf scales (Hirsch 1977).

Cladodes are 0.2–0.46 mm thick from seedling to adult plant (Balica, Tămaş & Deliu 2005b). Cladodes vary in size. Cladodes on plants grown in California were measured at  $1.8 \pm 0.06$  cm<sup>2</sup> (SE,  $n = 92$ ) with a specific leaf area (SLA) of 81.4 cm<sup>2</sup> g<sup>-1</sup> by Pivovarov *et al.* (2013), while European cladodes in full sun were measured by Sack, Grubb and Marañón (2003) to have an area of  $2.62 \pm 0.151$  cm<sup>2</sup> (SE,  $n = 10$ ) and a SLA of  $78.5 \pm 2.91$  cm<sup>2</sup> g<sup>-1</sup> (SE,  $n = 10$ ), the latter similar to *Smilax aspera* and *Rubia peregrina*. The SLA of shade cladodes of *R. aculeatus* was estimated at 134 cm<sup>2</sup> g<sup>-1</sup>, a plasticity ratio of 1.7. This was considered by Sack, Grubb and Marañón (2003) to be very low compared to other Mediterranean species. The SLA for seedlings was found to be somewhat higher by Sack (2004) at approximately 200 cm<sup>2</sup> g<sup>-1</sup> (estimated from a figure). The SLA of *R. aculeatus* is much higher than typically found in temperate evergreen plants, with a concomitant low bulk density ( $0.39 \pm 0.03$  g cm<sup>-3</sup> dry mass; SE,  $n = 10$ ) which Pivovarov *et al.* (2013) attribute to large water storage areas within the cladodes ( $10.5 \pm 1.38\%$  total leaf air/water space; SE,  $n = 5$ ), occupying a third of the cladode thickness, which aid drought resistance. Cladodes do indeed have a high water content: approximately 220–300%, estimated from a figure in Sack, Grubb and Marañón (2003). *Ruscus aculeatus* also has a high leaf water mass concentration (measured as the difference between the maximum mass of water that can be held by tissue compared to its dry mass: approximately 275%, estimated from a figure), similar to that of *Smilax aspera* and *Ceratonia siliqua* and only exceeded by *Rubia peregrina* at approximately 350% (Sack, Grubb & Marañón 2003). As stated in VI (A), the fleshy roots and rhizome also apparently store water (Antonielli, Ceccarelli & Pocceschi 1989). Withstanding shade and drought is also aided by reduced leaves, thick cladodes ( $278 \pm 3.34$  µm; SE,  $n = 5$ ) with a cuticle that is thick ( $3.52 \pm 0.22$  µm; SE,  $n = 5$ ), but similar to other evergreen species (Balica, Tămaş & Deliu 2005b; Pivovarov *et al.* 2013).

Cladodes have stomata in equal number on both sides (Dickson 1886). Stomatal density was measured at  $25.8 \pm 4.6 \text{ mm}^{-2}$  (SE,  $n$  unstated) in southern Spain (Sack, Grubb & Marañón 2003) and  $40.69 \pm 1.21 \text{ mm}^{-2}$  (SE,  $n = 18$ ) in Central Italy (Bettarini, Vaccari & Miglietta 1998). This is very low compared to dicotyledonous plants even if doubled to account for both surfaces. Stomatal density was not significantly different in plants growing beside a natural  $\text{CO}_2$  spring in Central Italy at double the atmospheric concentration, but 13 other of the 17 species investigated were also not affected (Bettarini, Vaccari & Miglietta 1998). *Ruscus aculeatus* has relatively long stomatal guard cells (approximately  $28 \mu\text{m}$ , estimated from a figure), compared to approximately  $8 \mu\text{m}$  in *Phillyrea latifolia* and  $38 \mu\text{m}$  in *Viburnum tinus* (Bettarini, Vaccari & Miglietta 1998).

#### (B) MYCORRHIZA

*Ruscus aculeatus* normally has arbuscular mycorrhiza (Harley & Harley 1986; Maremmani *et al.* 2003). Jumpponen and Trappe (1998) record colonization of roots by dark septate endophytes (Deuteromycotina, Fungi Imperfecti), which may have a mycorrhizal function.

#### (C) PERENNATION: REPRODUCTION

*Ruscus aculeatus* has geophyte properties in that it readily produces new shoots from the creeping rhizome, and this appears to be the main method of spread within a locality. D'Antuono and Lovato (2003) in Italy found that seedlings planted out (after germination in climatically controlled chambers) had already produced several new shoots from the roots when 1 year old.

There has been a lot of research on micropropagation from rhizome, stem and cladode tissue, particularly in eastern Europe (Balica, Deliu & Tămaş 2005a; Banciu, Mitoi & Brezeanu 2009; Brezeanu & Banciu 2010; Ivanova *et al.* 2011). New shoots readily develop from callus tissue (Banciu & Aiftimie-Păunescu 2012). Moreover, since the rhizomes have a large number of aerial buds, they are a particularly good source of vegetative explants for micropropagation. New plants can be produced *in vitro* from excised buds in < 4 months (Moyano *et al.* 2006) that are physiologically and structurally similar to normally produced plants except that the cladode ground parenchyma is less developed and veins are less prominent (Banciu & Aiftimie-Păunescu 2012).

#### (D) CHROMOSOMES

Chromosome number in *R. aculeatus* is  $2n = 40$  (Maude 1940).

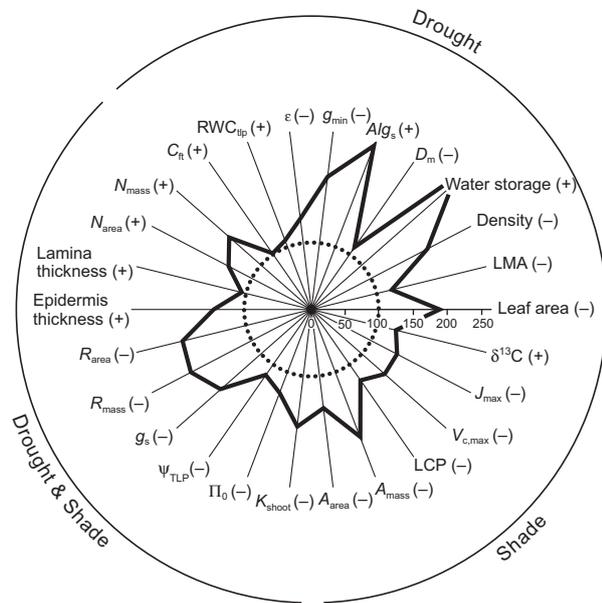
#### (E) PHYSIOLOGICAL DATA

*Ruscus aculeatus* can survive readily and grow in as little as 3–5% full sunlight (Sack, Grubb & Marañón 2003; Pivovarov *et al.* 2013). Concomitantly, it does not appear to be able to

perform well under high-light fluxes; D'Antuono and Lovato (2003) recorded that *R. aculeatus* plants in Bologna, Italy, almost stopped growth when kept in full sunlight during the summer. Light-saturated rate of photosynthesis is low, measured by Pivovarov *et al.* (2013) per unit area as  $5.22 \pm 1.33 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  (SE,  $n = 6$ ) and per unit mass as  $0.043 \pm 0.011 \text{ nmol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$  ( $n = 4$ ), associated with low maximum rate of carboxylation ( $26.6 \pm 3.18 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ,  $n = 4$ ) and maximum rate of electron transport ( $60.1 \pm 6.35 \mu\text{mol e m}^{-2} \text{ s}^{-1}$ ,  $n = 5$ ). Concomitant with withstanding shade and drought, Pivovarov *et al.* (2013) also found very low levels of respiration per unit area ( $0.044 \pm 0.009 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ,  $n = 8$ ) and per unit mass ( $3.56 \pm 0.70 \times 10^{-4} \text{ nmol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$ ,  $n = 4$ ). Sack, Grubb and Marañón (2003) found a strong relationship between chlorophyll concentration per unit area and SLA so that shade cladodes had 1.2–2.5 times the amount of chlorophyll per unit mass of cladode than sun cladodes.

The water conductance of *R. aculeatus* stems has been found to be very low. Pivovarov *et al.* (2013) measured shoot hydraulic conductance at  $2.16 \pm 0.10 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$  (SE,  $n = 10$ ). Farmer (1918) gives a figure relative to stem cross-sectional area of  $3.4 \text{ mL cm}^{-2} \text{ h}^{-1}$  at 0.04 Mpa; this compares to figures of 35 and  $36 \text{ mL cm}^{-2} \text{ h}^{-1}$  in *Ilex aquifolium* and *Ligustrum vulgare*, respectively. Similarly, Warne (1942) using identical conditions measured water conductivity as  $0.172 \pm 0.0116 \text{ mL } 100 \text{ cm}^{-2} \text{ h}^{-1}$  of cladodes (SE,  $n = 20$ ), which was the lowest of any of the 16 woody plants tested, and half that of *Buxus sempervirens*. This matched with low transpiration rates of  $0.208 \text{ mL } 100 \text{ cm}^{-2} \text{ h}^{-1}$  from cut stems of *R. aculeatus* placed in water in a laboratory in bright light with temperatures up to  $28 \text{ }^\circ\text{C}$  (Warne 1942) and the very low maximum stomatal conductance of  $33 \pm 0.007 \text{ mmol m}^{-2} \text{ s}^{-1}$  (SE,  $n = 4$ ), cladode cuticular conductance ( $0.379 \pm 0.082 \text{ mmol m}^{-2} \text{ s}^{-1}$ ,  $n = 10$ ) and stem cuticular conductance ( $0.095 \pm 0.025 \text{ mmol m}^{-2} \text{ s}^{-1}$ ,  $n = 6$ ; Pivovarov *et al.* 2013). Warne (1942) suggested that the low hydraulic conductivity is compensated for by the short distances over which this low-stature plant needs to conduct water. Moreover, Pivovarov *et al.* (2013) suggested that the low shoot conductance is aided by low stomatal conductance and the high water storage in the cladodes, enabling transpiration needs to be met.

Osmotic potential at full turgor ( $-1.28 \pm 0.10 \text{ Mpa}$ ; SE,  $n = 6$ ) and turgor loss point ( $-1.84 \pm 0.10 \text{ Mpa}$ ;  $n = 6$ ), measured in shoots progressively dried on a bench, has been found to be higher (less negative) than comparative evergreen woody species (Pivovarov *et al.* 2013). de Lillis and Fontanella (1992) found that in plants in high Maquis near Rome, Italy, water potential decreased through spring and summer reaching its lowest level (dawn value  $-1.79 \text{ Mpa}$ ) in July and August when the plants stopped growing. Water potential increased to around  $-0.8 \text{ Mpa}$  in September (a 50% increase), but no new growth was observed. These values are comparatively high (i.e. less negative) compared to trees and shrubs in the same area where water potentials  $< -3.5 \text{ Mpa}$  were common (de Lillis & Fontanella 1992). This suggests that *R. aculeatus* is not



**Fig. 3.** Radar graph of shade and drought traits, singularly or in combination, of *Ruscus aculeatus* expressed as per cent of these traits of comparative species with similar physiognomy. The inner circle represents the mean for comparative species for given traits, and the values for *R. aculeatus* are scaled as a per cent relative to this value, with a value outside the disc representing greater ability to cope with shade and/or drought; (+) and (−) indicate whether the value outside the inner circle is a higher or lower percentage than the comparative value. For traits expressed as negative values, (+) and (−) indicate more or less negative values, respectively. Symbols:  $\delta^{13}\text{C}$ , carbon isotope ratio;  $J_{\text{max}}$ , maximum rate of electron transport;  $V_{\text{c,max}}$ , maximum rate of carboxylation; LCP, light compensation point;  $A_{\text{mass}}$ ,  $A_{\text{area}}$ , light-saturated rate of photosynthesis per unit mass and per unit area;  $K_{\text{shoot}}$ , shoot hydraulic conductance;  $\pi_0$ , osmotic potential at full turgor;  $\psi_{\text{TLP}}$ , turgor loss point;  $g_{\text{s}}$ , maximum stomatal conductance per area;  $R_{\text{mass}}$ ,  $R_{\text{area}}$ , respiration rate per unit mass and per unit area;  $N_{\text{area}}$ ,  $N_{\text{mass}}$ ,  $N$  per unit area and per unit mass;  $C_{\text{ft}}$ , relative capacitance at full turgor;  $\text{RWC}_{\text{tip}}$ , relative water content at turgor loss point;  $\epsilon$ , modulus of elasticity;  $g_{\text{min}}$ , leaf cuticular conductance;  $A/g_{\text{s}}$ , intrinsic water-use efficiency;  $D_{\text{m}}$ , minimum distance from vein to epidermis; Water storage, thickness of water storage tissue in cladode; Density, density of cladodes ( $\text{g cm}^{-3}$ ); LMA, leaf (cladode) mass per area ( $\text{g m}^{-2}$ ). From: Pivovarov *et al.* (2013) courtesy of CSIRO Publishing ([www.publish.csiro.au/?paper=FP13047](http://www.publish.csiro.au/?paper=FP13047)).

drought tolerant since it limits growth to the period in spring before aridity increases. Instead, the physiology and morphology [VI (A)] of *R. aculeatus* suggests a higher degree of drought resistance through water storage. Although the degree of water storage is small compared to true succulents, since stomatal conductivity is so low, even the modest relative capacitance at full turgor of  $0.104 \pm 0.015 \text{ MPa}^{-1}$  (SE,  $n = 6$ ; Pivovarov *et al.* 2013) will enable survival for a number of weeks (Sack, Grubb & Marañón 2003; Sack 2004). The combination of traits that contribute to the overall shade tolerance and drought resistance is given in Fig. 3.

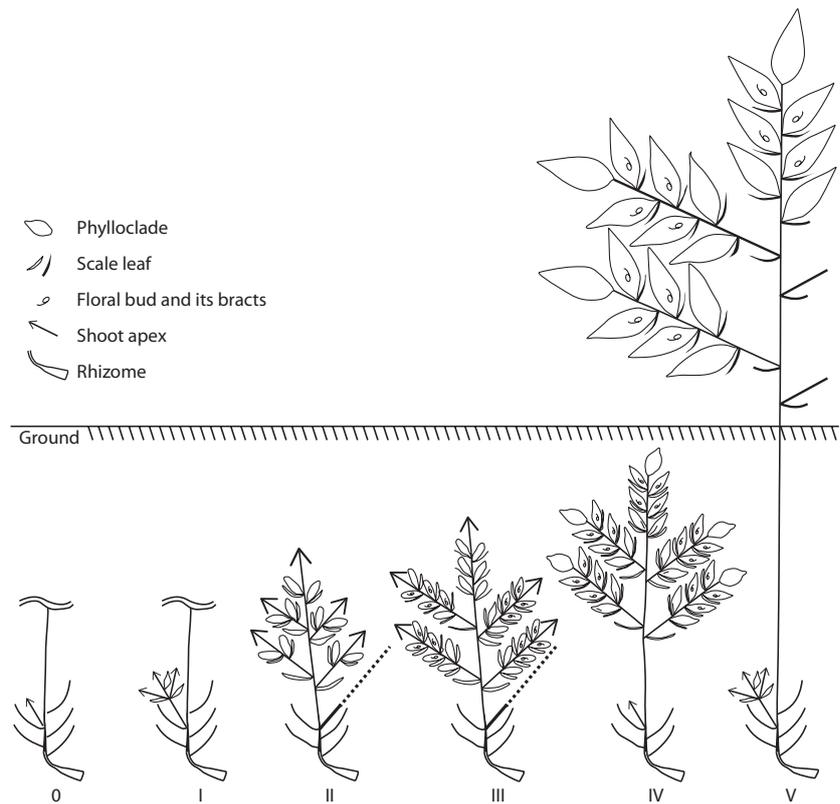
Cladode N concentration has been measured at 1.32–2.04% dry mass in northern and eastern Spain from herbarium specimens (Peñuelas & Filella 2001),  $1.94 \pm 0.06\%$  (SE,  $n = 9$ ) in plants grown in California (Pivovarov *et al.* 2013), while in semi-shaded adults in Central Italy, *R. aculeatus* N was

notably high in early spring at 3.4% (de Lillis & Fontanella 1992). Some of this variation may be due to variations during the year; de Lillis and Fontanella (1992) noted that cladode N concentration peaked in late winter (2.2%) and early spring (3.4%) before vegetative growth started and reached the lowest level (approximately 1.8%) in July. Sack, Grubb and Marañón (2003) also found levels to be higher in sun cladodes (approximately  $22 \text{ mg g}^{-1}$ ) than in shade cladodes (approximately  $16 \text{ mg g}^{-1}$ , estimated from a figure). These values are high compared to comparative broadleaf evergreen species (Pivovarov *et al.* 2013), which they suggest is consistent with adaptation to drought. de Lillis and Fontanella (1992) also found maximum cladode C concentration in August (60%) and December (52%), associated with cessation in growth, and lowest in between in October (40%). Cladode carbon : nitrogen ratio was found by Pivovarov *et al.* (2013) to be  $23.1 \pm 0.68$  (SE,  $n = 9$ ), while the carbon isotope ratio ( $\delta^{13}\text{C}$ ) was very negative ( $-33.32 \pm 0.29\text{‰}$ ;  $n = 9$ ) and typical of values normal in understory plants. The non-protein amino acid azetidine-3-carboxylic acid has been found in *R. aculeatus* (Fowden & Steward 1957).

*Ruscus aculeatus* has no known heavy metal resistance (Ecological Flora of the British Isles 2013) but was found to hyperaccumulate iron (up to  $1440 \text{ mg Fe kg}^{-1}$  dry plant mass) on a Portuguese lead mine (Pratas *et al.* 2013). Other values for heavy metal content of dried plant material were ( $\text{mg kg}^{-1}$ ) as follows: Ag, 0.1–0.17; Co, 0.2–0.63; Cr, 0.42–1.6; Cu, 3.5–6.7; Ni, 0.65–5.2; Pb 3.37–54; and Zn 32–74 (Pratas *et al.* 2013).

#### (F) BIOCHEMICAL DATA

There is a very large literature on the medicinal value of biochemical components of *Ruscus aculeatus*, including 17 steroidal saponins (characterized by spirostanol or furostanol aglycones, in particular two from the first group, ruscogenin and neoruscogenin, but also aculeosides), flavonoids, chrysophanic acid, glycolic acid, phenols and a benzofuran (Capra 1972; Elshohly *et al.* 1974, 1975; Pedersen 1994; Facino *et al.* 1995; Dunouau *et al.* 1996; Mimaki *et al.* 1998a,b, 1999; Ali-Shtayeh & Abu Ghdeib 1999; Redman 2000; de Combarieu *et al.* 2002; Mangas *et al.* 2006; Güvenç, Şatır & Coşkun 2007; De Marino *et al.* 2012; Mari *et al.* 2012; Barbič *et al.* 2013). The amount of pharmacologically active steroid saponins in plants appears to be variable but is usually highest in the rhizome and root (Longo & Vasapollo 2005). In wild plants in Romania, Balica *et al.* (2007) found the highest concentration of sapogenin in the rhizome (0.17% neoruscogenin and 0.11% ruscogenin). By contrast, Zistler *et al.* (2008) found higher levels of just ruscogenin (0.9–1.8%, estimated from a figure) in the rhizome of German material, and the *European Pharmacopoeia* states that the required pharmaceutical minimum is 1% (Council of Europe 2011). Pharmaceutical material is still primarily collected in the wild since little has been done using tissue culture sources (Moyano *et al.* 2006), and the concentration of saponins in such cultured material is low; Balica *et al.* (2007) found that in *in vi-*



**Fig. 4.** Phenology of shoot growth of *Ruscus aculeatus*. The main shoot system of the previous year is omitted in stage 0 and I, and the dashed line in stage II shows the disintegrated main shoot system of the previous year. From: Hirayama *et al.* (2007), fig. 8, p. 236. With kind permission from Springer Science and Business Media.

*tro* samples, the highest concentration was found in shoots but was low (0.075% neoruscogenin and 0.017% ruscogenin).

The Greek philosopher Theophrastus claimed *R. aculeatus* extracts stopped swelling and allowed lame people to walk, and Pliny the Elder described its use in treating varicose veins (Pedersen 1994), but its medicinal use appears to have been largely forgotten in Europe until the 1970s (Salzmann, Ehresmann & Adler 1977). The saponins are a potent venous vasoconstrictor agent with a diminishing oedema effect, acting as an agonist on adrenergic receptors of the smooth muscle of veins and reducing vascular permeability (Bouskela & Cyrino 1994; Mimaki, Kuroda & Kameyama 1998; Parrado & Buzzi 1999; Hexsel, Orlandi & Zechmeister 2005) and have been used as a diuretic and mild laxative (Berg 1990; Bouskela & Cyrino 1994; Gonçalves *et al.* 2013). They have thus been used in the therapy of cancer, circulatory problems (such as oedema) and diabetes (Tarayre & Laouressgues 1979; Cluzan *et al.* 1996; Anon 2001) since they are effective and have few side effects (Redman 2000; Sadarmin & Timperley 2013). Flavonoid and phenolic acid extracts have antimicrobial and antioxidant properties (Hadžifejzović *et al.* 2013).

The skin of the fruit has been found to contain anthocyanins pelargonidin 3-O-rutinoside (64%), pelargonidin 3-O-glucoside (16%) and pelargonidin 3-O-trans-p-coumaryl-glucoside (13%) (Longo & Vasapollo 2005).

## VII. Phenology

Hirayama *et al.* (2007) investigated the phenology of shoot growth in cultivated plants grown in Japan. New growth arose

from lateral dormant buds formed at the base of the previous year's shoots. These began to expand in February and March (stage 0 in Fig. 4) and produced new shoots in June (stage I). Over the next 5 months, cladode primordia developed in the axils of scale leaves on the developing stem (stage II), and in the following 3 months (December to early February), flower buds developed on the uppermost cladodes (stage III). From mid-February to mid-March (stage IV), the developing cladodes became progressively flattened and the next set of dormant buds (stage 0) was initiated at the base of the current shoot. The shoot appeared above-ground in late March (stage V), and, in central Italy, reached adult height within a week but were very thin and pale and continued developing until mid-July (de Lillis & Fontanella 1992; Martínez-Pallé & Aronne 1999). Cladodes are not shed individually in the autumn since the shoots grow and die as a whole, with a life span of 14–26 months (Pérez-Latorre & Cabezero 2006).

As noted by Bennett (1869), the 'normal time of flowering is almost the depth of winter'. Flower buds begin to enlarge in July, all flowers developing male and female parts until they become functionally either male or female by early September due to arrestation of either the anthers or pistil (Martínez-Pallé & Aronne 1999). Flower opening usually begins in September or October and carries on till April in mainland Europe (Herrera 1981; de Lillis & Fontanella 1992; Tansi, Karaman & Toncer 2009) sometimes extending to June in Britain (Hillman 1979). Within this period, the timing of peak flowering varies widely and has been recorded as October–November in southern Italy (Martínez-Pallé & Aronne 1999) and (November) January–April in the British Isles (Kay

& Page 1985; Clapham, Tutin & Moore 1987). Others have recorded *R. aculeatus* flowering twice in the same year (October–December and again from early February/mid-April to the end of May) in Italy (de Lillis & Fontanella 1992; Aronne & Wilcock 1997), or even flowering ‘practically all year round’ (Pérez-Latorre & Cabezudo 2006). This variation is partly due to different flower buds on the same cladode opening at different times. In Central Italy, Martínez-Pallé and Aronne (1999) noted that while many cladodes have no flower buds, others often have two flower buds. In both male and female plants, the first bud opened in October–December, while the second was delayed a number of weeks until after the main rain period, opening in January–April.

In male flowers, the anthers dehisce within the first day, and in female flowers, the stigma is receptive from flower opening. Both male and female flowers remain open for 4–10 days. Following this, all flowers fall except for a small number of female flowers that develop fruits (Martínez-Pallé & Aronne 1999).

Despite the long flowering period, fruit development usually begins only in late April (Martínez-Pallé & Aronne 2000), taking 6–8 months to mature, and so producing mature fruits usually by the end of October (Fuentes 1992; de Lillis & Fontanella 1992; Aronne & Wilcock 1997). Ripe fruits remain on the plant for long time, certainly through the winter (Herrera 1981) and often for 1–2 years (Martínez-Pallé & Aronne 1999) so that plants tend to carry fruits all year round.

### VIII. Floral and seed characters

#### (A) FLORAL BIOLOGY

Flowers are concentrated on a small number of cladodes. In southern Italy, Martínez-Pallé and Aronne (1999) recorded that on female plants, 54.4% of cladodes had no flower buds, 14.6% had one and 30.9% had two flower buds. Male plants by contrast had more flower buds: 43.3% of cladodes had no buds, while 23.9% and 32.7% had one and two flower buds, respectively. Only 10–15 flowers open simultaneously on a plant at the peak of flowering (Kay & Page 1985).

*Ruscus aculeatus* is often assumed to be entomophilous, offering pollen but no nectar or detectable scent (Kay & Page 1985; Aronne & Wilcock 1994). Indeed, Hillman (1979) found that fruit set was correlated with the number of sunny days and so concluded that it is insect-pollinated since insects are active on sunny days. Yeo (1968) also suggested that small flies that hover around *R. aculeatus* bushes might serendipitously land on open flowers. However, there is little direct evidence that insects visit the flowers. Both Kay and Page (1985) in the British Isles and Martínez-Pallé and Aronne (2000) in southern Italy made lengthy observations during the flowering period and recorded no insect visits despite the plants being covered in spider webs in winter and seeing small flies and other insects resting on cladodes (but showing no interest in the flowers). The apparent absence of pollinators in Italy belies the suggestion by Kay and Page (1985) that pollinators present in the Mediterranean region might be

absent from the British Isles. Pollen has also not been found in honeybee pollen loads in Spain and Italy, despite the plant being in the area (Diaz-Losada, Ricciardelli-d’Albore & Saa-Otero 1998). Anemophily is possible where male and female shoots intermingle, but experiments using blown air have shown that a fairly high wind speed ( $> 5 \text{ m s}^{-1}$ ) is needed for pollen removal (Martínez-Pallé & Aronne 2000). Moreover, a number of studies looking at atmospheric pollen loads near ground level have found no *R. aculeatus* pollen in samples despite the plant being present in the area (Güvensen & Öztürk 2002; Celik *et al.* 2005; Gücel *et al.* 2013). It is concluded that low pollen transport is the main reason for low seed set – see VIII (C).

The quantity of pollen produced per flower appears to be limited. In southern Italy, the number of pollen grains per anther ranged from 712 to 1316 with an average of 873 (Martínez-Pallé & Aronne 2000). Since each male flower has three anthers, an estimated 2618 pollen grains were produced per flower. Mean pollen viability was 84.9%, ranging from 71.1% to 96.3% between flowers (Martínez-Pallé & Aronne 2000). The stigmatic surface is composed of small papillae ( $20 \times 10 \mu\text{m}$ ) covered by a lipid exudate rich in calcium ions to create a ‘wet stigma’; this calcium is important in the germination and growth of pollen grains (Bednarska 1991).

*Ruscus aculeatus* has been described as dioecious (Hillman & Warren 1973; Hillman 1979), subdioecious (normally dioecious but with some exceptions) by Tutin *et al.* (2002), dioecious or andromonoecious (varying between populations) by Kay and Page (1985) and subandroecious (dioecious with occasional hermaphrodite flowers on male plants) by Martínez-Pallé and Aronne (1999). Of plants surveyed in Surrey, 104 plants were dioecious and one other plant was a male that produced a single berry (Hillman & Warren 1973; Hillman 1979). Similarly, of 164 plants sampled on the Gower Peninsula, one male plant had a solitary berry, while on Guernsey, 8.4% of plants ( $n = 347$ ) had predominantly male flowers ‘with some female or hermaphrodite flowers’ (Kay & Page 1985). In Italy, hermaphrodite flowers (4–5 flowers per plant) were found on two male plants by Martínez-Pallé and Aronne (1999). Given the presence of male flowers with occasional hermaphrodite flowers, andromonoecious would appear to be the best description of its floral biology. Being primarily dioecious, outcrossing is normally obligatory, but andromonoecious populations are capable of self-pollination (Martínez-Pallé & Aronne 1999). Despite this, neither Kay and Page (1985) nor Hillman and Warren (1973) found that andromonoecious plants produced more berries than dioecious plants, although obviously the sample sizes are small. The andromonoecious form has been exploited horticulturally to ensure that solitary plants bear fruits (Bean 1980). Kay and Page (1985) note the ‘Treseder’s Variety’ (introduced into Cornwall in the late 1950s) is andromonoecious (although described in horticultural literature as an hermaphrodite), and it may produce hermaphrodite flowers in April–May and male flowers the rest of the flowering season (P. F. Yeo, pers. comm. quoted in Kay & Page 1985).

In southern Italy, Martínez-Pallé and Aronne (2000) found the sex ratio of plants to be biased towards female plants (66% female) uniformly mixed through the population. A similar ratio was found near London (62% female) by Hillman (1979). However, Kay and Page (1985) found some populations more weighted to males (Gower: 41% female,  $n = 164$ ; Guernsey: 41%,  $n = 347$ ), while other were near equal (Oxford: 50%,  $n = 18$ ). Rottenberg (1998) found a ratio of 49% ( $n = 90+$ ) in three populations in Israel.

#### (B) HYBRIDS

No hybrids are recorded. However, closely related species are known to hybridize (*R. hypoglossum* × *R. hypophyllum* = *R. × microglossum*; Pivovarov *et al.* 2013) so *R. aculeatus* hybrids may be possible.

#### (C) SEED PRODUCTION AND DISPERSAL

Fruit production within populations tends to be very low, though variable between plants. Kay and Page (1985) found that while some plants had up to 10 fruits, many had none. Mean number of berries per plant has been recorded as 2.87 berries per female ( $n = 68$ ) on the Gower, 0.5 per female ( $n = 9$ ) in Oxfordshire (Kay & Page 1985) and 1.8 berries per female ( $n = 43$ ) in Surrey (Hillman & Warren 1973). In Italy, Martínez-Pallé and Aronne (2000) found that only 3% (one of 38) of marked flowers produced fruits in 1996 and none produced in 1997 ( $n = 130$ ) and 1998 ( $n = 410$ ). Seed output was between 1 and 5 seeds per female plant (Kay & Page 1985; Moyano *et al.* 2006).

This low fruit and seed production is primarily attributable to poor pollination. Martínez-Pallé and Aronne (2000) noted the absence of *R. aculeatus* pollen grains on the stigmatic surface of 80 sampled flowers. Moreover, hand pollination has been seen to dramatically increase fruit production, reaching 73% ( $n = 36$ ; Kay & Page 1985) and 80% ( $n = 34$ ) of flowers compared to just 3% ( $n = 38$ ) of open-pollinated developing fruits and none in unpollinated flowers ( $n = 52$ ; Martínez-Pallé & Aronne 2000). Poor pollination appears to be a mainly due to ineffective pollen movement rather than distance between male and female plants limiting pollen spread. In the study by Martínez-Pallé and Aronne (2000) described above, neighbouring plants were just 30–90 cm apart. In the British Isles, Kay and Page (1985) found that fruit number was highest (14 fruits) in the Gower population despite female plants being 24 m from the nearest male. Hillman and Warren (1973) and Hillman (1979) reported similar findings. However, distance between plants may play a small part in poor fruit set since Kay and Page (1985) also recorded that a plant 'next to male' in the Oxford population had 13.5% flowers set seed, while an isolated plant had 0.8% success. Martínez-Pallé and Aronne (2000) also found that no plant had more than four fruits when further than 130 cm away from a male plant while those closer had up to 13 fruits. Other limitations have been postulated. Low fruit number in Turkey has been attributed to a distorted sex ratio with very

few female plants (Tansi, Karaman & Toncer 2009), and Salisbury (1926) suggested that *R. aculeatus* rarely sets seed near its natural northern limit in Britain except in unusually hot summers.

Given the red fleshy fruits, it is often supposed that the seeds are spread by animals, particularly by endozoochorous animals and possibly by birds (Hermy *et al.* 1999; Debussche, Debussche & Lepart 2001; Parent 2002; Preston, Pearman & Dines 2002). However, this does not seem to be the case despite the long time of fruit retention exposing it to a wide range of fruit-eating birds (Fuentes 1992). The fruits appear to be unpalatable (Kay & Page 1985) and remain on the parent plant until they fall or are forced off by stormy weather, remaining beneath the parent plant until they rot (Martínez-Pallé & Aronne 1999). Jordano (1988) looked extensively at the diet of the blackcap *Sylvia atricapilla* (L.) and garden warbler *S. borin* (Bodd.) in southern Spain and found that although *R. aculeatus* fruit ripened at the same time as many other fruits of woody plants (including *Pistacia lentiscus*, *Myrtus communis* and *Olea europaea*), it did not form part of their diets. Herrera (1984) pointed out that *R. aculeatus* fruits, with a mean diameter of 11.9 mm, were larger than the gape width of the blackcap and garden warbler (range 7.1–8.6 mm), although this would suggest that they are capable of taking the smallest of the *R. aculeatus* fruits (range 8–14 mm). However, fruits of *R. aculeatus* were not detected in faecal samples by Herrera (1984), suggesting there is an underlying unpalatability.

Low seed production and lack of animal dispersers lead to poor seed dispersal. Debussche and Isenmann (1994) measured seed rain in Mediterranean France using seed traps with a combined area of 39.75 m<sup>2</sup> (of which 16.65 m<sup>2</sup> was in *R. aculeatus* habitat) and collected just two seeds over the 17-month study out of a total of 20 373 seeds from 38 fleshy-fruited species. Debussche and Isenmann (1994) in their survey found just three seedlings of *R. aculeatus* < 1 year old in quadrats totalling 9225 m<sup>2</sup>, and no seedlings 1–2 years old.

Fresh mass of Spanish fruits was recorded as 0.98 and 1.36 g by Herrera (1987, 1981), respectively, with a dry mass of 0.39 g (Herrera 1987). This is comparatively large compared to 70 other Mediterranean species measured in Israel, but the fruits were fairly average in their protein and mineral contents (Izhaki 2002; individual data not given). Seed mass was measured at 163 mg ( $n = 15$ ) for British material (of unknown provenance) and 174 mg for Spanish material (Herrera 1987).

#### (D) VIABILITY OF SEEDS: GERMINATION

Germination is usually very slow and often low. Trials have shown 20–80% germination on artificial media over 4–6 months or longer (D'Antuono & Lovato 2003; Banciu, Mitoi & Brezeanu 2009; Banciu & Aiftimie-Păunescu 2012). There is an element of dormancy since D'Antuono and Lovato (2003) found that tetrazolium tests indicated over 50% viable seeds, while *in vitro* germination was 20–25% despite prior removal of 'defective seeds'. *Ruscus aculeatus* has an

impervious seed coat, and germination is improved by scarification. Adding 5 ppm of gibberellic acid to artificial media has been seen to improve germination from 60% to 85% (Banciu & Aiftimie-Păunescu 2012), but treatment with 500 ppm for 1 h had little effect (D'Antuono & Lovato 2003). A combination treatment for scarification by concentrated sulphuric acid (concentration unknown) and gibberellic acid (500 ppm for 1 h) resulted in no germination. Ethylene and potassium nitrate did not affect per cent germination, but (compared to a mean germination time of 150 days in controls) 1 mM of ethylene decreased germination time to approximately 120 days (estimated from a figure), while 3 mM increased germination time to approximately 170 days (D'Antuono & Lovato 2003). Cold stratification (temperature unknown) for 120 days had little effect on germination and mean germination time (D'Antuono & Lovato 2003), but it is possible that germination may be improved by alternating periods of warm (15 °C) and cold (5 °C) conditions. Seeds can be stored under orthodox seed storage conditions of 10–15% moisture content at < 0 °C (Gosling 2007).

Martínez-Pallé and Aronne (2000) in Spain found no germination in the field over a 3-year study, although germination tests showed that 45% of tested seeds germinated in 11 months. *Ruscus aculeatus* seeds survive in the soil for <1 year and so there is no soil seed bank (Thompson, Bakker & Bekker 1997).

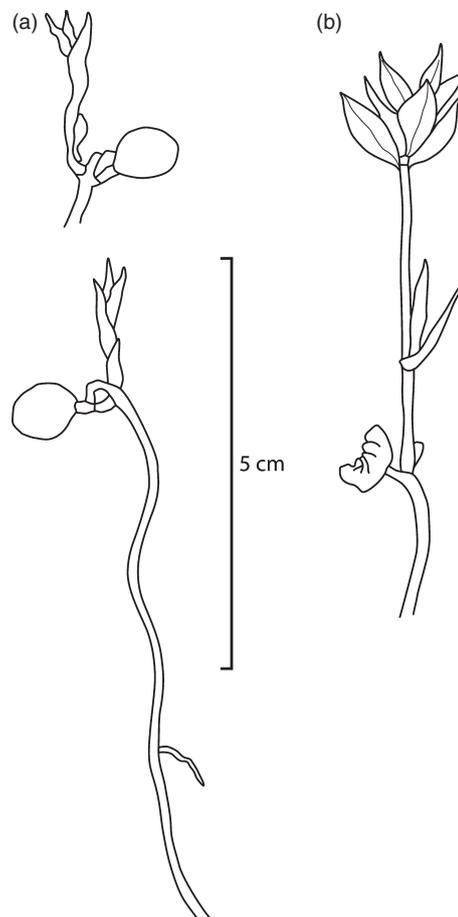
#### (E) SEEDLING MORPHOLOGY

Seedlings germinated on sterile sand and transplanted into pots rapidly develop a well-established root system and vigorous shoots in the first year. Seedlings produced several new shoots from the roots in the spring of second year (D'Antuono & Lovato 2003). Each new shoot produces scale leaves initially, the cladodes developing in their axils and overtaking the scales in size (Arber 1924). Seedling development is shown in Fig. 5.

### IX. Herbivory and disease

#### (A) ANIMAL FEEDERS OR PARASITES

Red deer (*Cervus elaphus* L.) in riparian areas within the Mediterranean scrub of Sardinia showed a strong preference for browsing on *Ruscus aculeatus* and a number of other shrubs including *Quercus ilex*, *Alnus glutinosa*, *Salix caprea*, *Myrtus communis* and *Viburnum tinus*. More than 25% of *Ruscus* individuals were browsed despite it being uncommon and the availability of other browse (Lovari *et al.* 2007). Other deer species ignore *R. aculeatus*. It was not found in the rumen contents of fallow deer (*Dama dama* L.) in the New Forest despite it being a common plant (Jackson 1977). Roe deer (*Capreolus capreolus* L.) have been found to avoid it even if starved (Pettorelli *et al.* 2003), and a density of 20 roe deer km<sup>-2</sup> in Germany led to an increase in *R. aculeatus* at the expense of palatable species such as ivy (*Hedera helix*; Cibien, Boutin & Maizeret 1988). *Ruscus aculeatus* is



**Fig. 5.** Seedling morphology of *Ruscus aculeatus*. (a) Young subterranean seedlings bearing just scale leaves, viewed from two sides. Older seedlings (b) bearing the remains of the shrunken seed, several lateral buds along the stem and with cladodes in the axils of scale leaves. From: Arber (1924) by permission of Oxford University Press.

eaten in winter by free-roaming farmed llamas (*Lama glama* L.) and alpacas (*Vicugna pacos* L.) in Italy (Aguilar *et al.* 2012).

The tortoise *Testudo hermanni hermanni* Gmelin sought out and ate the flowers and unripe fruit of *R. aculeatus* in Central Italy as they became available despite the rarity of the fruit (Del Vecchio *et al.* 2011). The authors considered these fruits to be particularly important food source for tortoises about to enter hibernation.

Comparatively, few insect feeders have been recorded on *R. aculeatus*; a small number are listed by the Biological Records Centre (2013). Two scale insects are known to feed on the cladodes of *R. aculeatus* as larvae and adults: *Dynaspidiotus britannicus* (Newstead) and *Parlatoria proteus* (Curtis) (Hemiptera, Diaspididae). The second of these is introduced. Larvae of the macromoth *Alcis repandata* (L.) (Lepidoptera, Geometridae) have been recorded feeding on *R. aculeatus* in a glasshouse. The mealybug *Ferrisia malvastrae* (Hemiptera, Coccoidea) has been found on plants in Israel (Ben-Dov 2005) along with the scale insects *Coccus hes-*

*peridum* L., *Planococcus citri* (Risso) and *Pseudococcus longispinus* (Targioni Tozzetti) (Hemiptera: Coccoidea) (Bendov 2011–2012). *Pseudococcus maritimus* (Ehrh.) (Hemiptera, Sternorrhyncha) is recorded as covering up to 50% of cladode surfaces in a Polish glasshouse (Golan & Górska-Drabik 2006).

The nematode *Longidorus helveticus* Lamberti *et al.* (Nematoda, Dorylaimida) has been isolated from *R. aculeatus* rhizomes in Serbia (Barsi & De Luca 2005) and *Rotylenchus agnetis* Szczygiel (Nematoda, Hoplolaimidae) on Italian plants (Cantalapiedra-Navarrete *et al.* 2013).

Two mites (Mesostigmata: Phytoseidae) have been found on *R. aculeatus* on Mt Carmel, Israel (Swirski & Amitai 1997): *Amblydromella crypta* (Athias-Henriot) and *Typhlodromus athiasae* (Porath & Swirski).

#### (B) PLANT PARASITES AND EPIPHYTES

Fungi associated with *Ruscus aculeatus* are given in Table 1. Ellis and Ellis (1997) record many other fungi found on living and dead *R. aculeatus* that are not specific to it.

Several authors record foliicolous lichens found on *R. aculeatus* in southern France and Spain (De Sloover & Sérusiaux 1984; Bricaud *et al.* 1993), and in Tuscany and the

humid mountains of southern Italy (Puntillo & Vězda 1994; Puntillo & Ottonello 1997).

#### (C) PLANT DISEASES

*Paraphaeosphaeria glaucopunctata* (Grev.) Shoemaker & C.E. Bab. [= *Phaeosphaeriopsis glaucopunctata* (Grev.) Câmara, Palm & Ramaley] (Ascomycota, Pleosporales) has caused leaf spot and necrosis of *Ruscus aculeatus* in Europe and Australia (Lohwag 1963; Câmara *et al.* 2001; Golzar & Wang 2012).

### X. History

Members of the former Rusaceae have been reported to occur in Laurasia in the Cretaceous (Raven & Axelrod 1974). *Ruscus aculeatus* itself is considered to be a relict species that evolved under a warm tropical climate with summer rains in the Tertiary (Martínez-Pallé & Aronne 2000; Kim *et al.* 2010). This is said to explain the breeding system of *R. aculeatus*: dioecy, small green flowers, long flowering and fruiting period, fleshy fruits with few seeds and reliance on vegetative sprouting (Aronne & Wilcock 1994). It is also likely that shade tolerance evolved before drought resistance. Subsequent climate changes during the late Pliocene and the Quaternary to the current climate have

**Table 1.** Fungi (by Order) directly associated with *Ruscus aculeatus*. Nomenclature follows the British Mycological Society (2013)

Species/classification	Ecological notes	Source
Ascomycota		
Botryosphaeriales		
<i>Guignardia istriaca</i> Bubák	Live, dead and fallen cladodes, twigs and wood; only recorded UK host	1, 3
<i>Phyllosticta ruscicola</i> Durieu & Mont.	Only recorded UK host	1, 2
<i>Phyllostictina hypoglossi</i> (Mont.) Petr. & Syd.	Stems; recorded only on <i>Ruscus</i> spp. in the United Kingdom	1, 3
Capnodiales		
<i>Mycosphaerella tassiana</i> (De Not.) Johanson [= <i>Cladosporium herbarum</i> (Pers.) Link]	Recorded mainly on a wide variety of non-woody hosts	1
Chaetosphaeriales		
<i>Menispora ciliata</i> Corda	Dead stem	1
Diaporthales		
<i>Phomopsis rusci</i> (Westend.) Grove	Dead cladodes and stems; <i>R. aculeatus</i> only British host	1, 3
Helotiales		
<i>Strossmayeria basitricha</i> (Sacc.) Dennis	Dead wood	1, 3
Hypocreales		
<i>Fusarium aquaeductum</i> (Rabenh. & Radlk.) Sacc.	Endophytic on twig	1
<i>Fusarium merismoides</i> Corda	Dead cladodes	1
<i>Gibberella baccata</i> (Wallr.) Sacc.	Endophytic on twigs and wood; wide range of woody hosts	1
<i>Nectria episphaeria</i> (Tode) Fr.		1
<i>Pycnofusarium rusci</i> D. Hawksw. & Punith.	Dead cladodes; only recorded UK host	1, 3
<i>Volutella rusci</i> Sacc.	Only recorded UK host	1
Microthyriales		
<i>Microthyrium ciliatum</i> var. <i>ciliatum</i> Gremmen & De Kam	Dead stem	1
Pleosporales		
<i>Cytoplea</i> sp. Bizz. & Sacc.	Dead wood	1
<i>Paraphaeosphaeria glaucopunctata</i> (Grev.) Shoemaker & C.E. Bab.	Live and dead cladodes; most records from <i>R. aculeatus</i>	1, 2, 3
<i>Phoma macrostoma</i> Mont.	Endophytic on twigs	1
<i>Ulocladium chartarum</i> (Preuss) E.G. Simmons	Endophytic on twigs	1

Sources: 1, British Mycological Society (2013); 2, Lohwag (1963); 3, Ellis & Ellis (1997).

resulted in conditions in which the pollination, seed production and dispersal mechanisms no longer function effectively (Martínez-Pallé & Aronne 1999, 2000).

Subfossil cladodes of *R. aculeatus* have been found in Quaternary volcanic deposits in Central Italy dating from 450 000 years ago, in a mixed conifer woodland including *Amentotaxus*, *Cephalotaxus*, *Torreya*, *Abies*, *Pinus*, *Cupressus*, *Juniperus* and *Taxus* (Tongiorgi 1938; Follieri 2010). More recently, pollen of *R. aculeatus* was noticeably abundant in Moroccan deposits dating from 9000 to 6400 BP (Morales *et al.* 2013; Zapata *et al.* 2013) where it was growing amongst maquis-type vegetation dominated by *Olea europaea* and *Pistacia lentiscus*. Herb pollen (4.2–8.3% pollen abundance) was dominated by Poaceae (1.8–3.9%) and *R. aculeatus* (0.4–1.6%).

The first British record of *Ruscus aculeatus* was published by William Turner in 1548. He reported '*Ruscus* is called ... in english buchens brome or Petigrue. Petigrue groweth in Kent wilde by hedge sydes, but it beareth no fruite as it doeth in Italy' (Britten, Jackson & Stearn 1965).

## XI. Conservation

Despite its comparative rarity, *Ruscus aculeatus* has a long and diverse history of usage in Europe. It has indeed been used as a 'butcher's broom' to clean chopping blocks (Pedersen 1994), also to make garden besoms (Bean 1980; Kızırlarlan & Özhatay 2012) and brooms for removing embers from bread ovens in southern Italy (Salerno, Guarrera & Caneva 2005). Extracts from the rhizome have been used in many dietary supplements (Mari *et al.* 2012; Di Novella *et al.* 2013; Di Sanzo *et al.* 2013; González *et al.* 2013), for treating warts, chilblains, piles and minor pains (Guarrera 2005; Chauhan, Ruby & Dwivedi 2012; Akyol & Altan 2013), and more recent medicinal uses described in VI (F). *R. aculeatus* shoots are eaten boiled or fried in spring in Sicily and Italy (Corsi & Pagni 1979; Paoletti, Dreon & Lorenzoni 1995; Lentini & Venza 2007; Laghetti *et al.* 2011; di Tizio *et al.* 2012). Extensive usage, particularly medicinal, is putting pressure on populations, especially in eastern Europe due to excessive harvesting of the roots and rhizomes (Marossy 2006; Tansi, Karaman & Toncer 2009). In Turkey, Coşkun *et al.* (2006) reported an average annual export of 900 t of dried, cleaned roots (equivalent to 4500 t fresh mass). In sandy areas, where digging machinery could be used, they noted losses of whole populations, although in stony areas, where collecting was done using hand tools, the populations, although reduced, were still extant. But it should be noted that some uses have encouraged the planting and conservation of the species. In Britain, it has been grown as cover for pheasants (Kay & Page 1985) and as an ornamental plant across Europe (Banciu, Mitoi & Brezeanu 2009; Barrico *et al.* 2012; Irmak 2013).

Preston, Pearman and Dines (2002) showed little change in recorded abundance since 1962, and more recent evidence suggests that the range and abundance of *R. aculeatus* may be expanding (JNCC 2007), primarily due to planting, and it thus has no special conservation status in the United

Kingdom. Across Europe, *R. aculeatus* is given some protection as a rare and endangered species by the Habitats Directive, listed in Annex V (plant species of community interest whose taking in the wild and exploitation may be subject to management measures). A number of eastern European countries, where harvesting is more intense, have put specific conservation measures in place. In Bulgaria, harvesting is under legal control and in Romania *R. aculeatus* is protected by law as a 'monument of nature' (Marossy 2006; Banciu & Aiftimie-Păunescu 2012). Climate change and invasive species may be detrimental to *R. aculeatus* (Vicente *et al.* 2011) in the future, but there is currently little threat to this species and it is listed as of 'least concern' by IUCN (2011).

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## References

- Aguilar, M.G., Olsen, A., Riganelli, N., Chagra, D. & Elsa, P. (2012) Studio della dieta di lama e alpaca allevati in una zona del Centro Italia, attraverso la tecnica microistologica. [Study of the diet of llamas and alpacas bred in Central Italy, through a microhistological technique.] *Sanità Pubblica Veterinaria*, **74**, 8–16.
- Akyol, Y. & Altan, Y. (2013) Ethnobotanical studies in the Maldan Village (Province Manisa, Turkey). *Marmara Pharmaceutical Journal*, **17**, 21–25.
- Ali-Shtayah, M.S. & Abu Ghdeib, S.I. (1999) Antimyzetische Aktivität von Pflanzenextrakten gegen Dermatophyten. [Antifungal activity of plant extracts against dermatophytes.] *Mycoses*, **42**, 665–672.
- Allen, J.R.M., Watts, W.A. & Huntley, B. (2000) Weichselian palynostratigraphy, palaeovegetation and palaeoenvironment; the record from Lago Grande di Monticchio, southern Italy. *Quaternary International*, **73/74**, 91–110.
- Anon (1866) Botanical notes. *Journal of Botany*, **4**, 393.
- Anon (2001) *Ruscus aculeatus* (butcher's broom) monograph. *Alternative Medicine Review*, **6**, 608–612.
- Antonellini, M. & Mollema, P.N. (2010) Impact of groundwater salinity on vegetation species richness in the coastal pine forests and wetlands of Ravenna, Italy. *Ecological Engineering*, **36**, 1201–1211.
- Antonielli, M., Ceccarelli, M. & Poceschi, N. (1989) *Rubia peregrina* L.: a stress resistant weed. *Environmental and Experimental Botany*, **29**, 467–476.
- Arber, A. (1924) Danae, *Ruscus*, and *Semele*: a morphological study. *Annals of Botany*, **38**, 229–230.
- Arista, M. (1995) The structure and dynamics of an *Abies pinsapo* forest in southern Spain. *Forest Ecology and Management*, **74**, 81–89.
- Aronne, G. & Wilcock, C.C. (1994) Reproductive characteristics and breeding system of shrubs of the Mediterranean region. *Functional Ecology*, **8**, 69–76.
- Aronne, G. & Wilcock, C. (1997) Reproductive phenology in Mediterranean macchia vegetation. *Lagascalia*, **19**, 445–454.
- Bacchetta, G., Biondi, E., Farris, E., Filigheddu, R. & Mossa, L. (2004) A phytosociological study of the deciduous oak woods of Sardinia (Italy). *Fitosociologia*, **41**, 53–65.
- Baini, F., Pitzalis, M., Taiti, S., Taglianti, A.V., Zapparoli, M. & Bologna, M.A. (2012) Effects of reforestation with *Quercus* species on selected arthropod assemblages (Isopoda Oniscidea, Chilopoda, Coleoptera Carabidae) in a Mediterranean area. *Forest Ecology and Management*, **286**, 183–191.
- Balica, G., Deliu, C. & Tămaş, M. (2005a) Applied biotechnologies to *Ruscus aculeatus* L. (Liliaceae). *Hameiul si Plantele Medicinale*, **25**, 163–166.
- Balica, G., Tămaş, M. & Deliu, C. (2005b) Contributions to the anatomy of *Ruscus aculeatus* L. (Liliaceae). *Contribuții Botanice*, **40**, 221–225.
- Balica, G., Vlase, L., Deliu, C., Tămaş, M. & Crişan, G. (2007) Comparative phytochemical study on rhizome and tissue culture of *Ruscus aculeatus* L. *Planta Medica*, **73**, P226.
- Banciu, C. & Aiftimie-Păunescu, A. (2012) *In vitro* propagation of rare species *Ruscus aculeatus* L. and histological peculiarities of the regenerants. *Analele Universității din Oradea-Fascicula Biologie*, **19**, 67–73.

- Banciu, C., Mitoi, M.E. & Brezeanu, A. (2009) Biochemical peculiarity of *in vitro* morphogenesis under conservation strategy of *Ruscus aculeatus* L. *Annals of Forest Research*, **52**, 109–116.
- Barbič, M., Willer, E.A., Rothenhöfer, M., Heilmann, J., Fürst, R. & Jürgenliemk, G. (2013) Spirostanol saponins and esculin from *Ruscus rhizoma* reduce the thrombin-induced hyperpermeability of endothelial cells. *Phytochemistry*, **90**, 106–113.
- Barrico, L., Azul, A.M., Morais, M.C., Coutinho, A.P., Freitas, H. & Castro, P. (2012) Biodiversity in urban ecosystems: plants and macromycetes as indicators for conservation planning in the city of Coimbra (Portugal). *Landscape and Urban Planning*, **106**, 88–102.
- Barsi, L. & De Luca, F. (2005) Morphological and molecular characterization of *Longidorus helveticus* (Nematoda: Dorylaimida) from Serbia. *Nematologia Mediterranea*, **33**, 41–49.
- Bean, W.J. (1980) *Trees and Shrubs Hardy in the British Isles*, 8th edn. Murray, London, UK.
- Bednarska, E. (1991) Localization of Ca<sup>2+</sup> and Ca-ATPase on wet (*Ruscus aculeatus*) and dry (*Primula officinalis*) stigma surfaces. *Acta Societatis Botanicorum Poloniae*, **60**, 285–294.
- Ben-Dov, Y. (2005) The malvastrum mealybug *Ferrisia malvastra* (Hemiptera: Coccoidea: Pseudococcidae): distribution, host plants and pest status in Israel. *Phytoparasitica*, **33**, 154–156.
- Ben-Dov, Y. (2011–2012) The scale insects (Hemiptera: Coccoidea) of Israel – checklist, host plants, zoogeographical considerations and annotations on species. *Israel Journal of Entomology*, **41–42**, 21–48.
- Bennett, A.W. (1869) On the fertilisation of winter-flowering plants. *Nature*, **1**, 11–13.
- Berg, D. (1990) Venenkonstriktion durch lokale Anwendung von Ruscusextrakt. [Venous constriction by local administration of Ruscus extract.] *Fortschritte der Medizin*, **108**, 473–476.
- Bettarini, I.A., Vaccari, F.P. & Miglietta, F. (1998) Elevated CO<sub>2</sub> concentrations and stomatal density: observations from 17 plant species growing in a CO<sub>2</sub> spring in central Italy. *Global Change Biology*, **4**, 17–22.
- Biological Records Centre (2013) *Database of Insects and Their Food Plants*. Available at <http://www.brc.ac.uk/DBIF/homepage.aspx> (accessed 1 September 2013).
- Biondi, E., Casavecchia, S., Guerra, V., Medagli, P., Beccarisi, L. & Zuccarello, V. (2004) A contribution towards the knowledge of semideciduous and evergreen woods of Apulia (southeastern Italy). *Fitosociologia*, **41**, 3–28.
- de Bolós, O. (1956) Efectos de los fríos de febrero de 1956 sobre la vegetación. *Collectanea Botánica*, **5**, 165–171.
- de Bolós, O. & Vigo, J. (2001) *Flora dels Països Catalans Vol. IV (Monocotiledònies)*. Editorial Barcino, Barcelona, Spain.
- Bouskela, E. & Cyrino, F.Z.G.A. (1994) Possible mechanisms for the effects of Ruscus extract on microvascular permeability and diameter. *Clinical Hemorheology*, **14**, S23–S36.
- Brezeanu, A. & Banciu, C. (2010) *In vivo* and *in vitro* comparative ultrastructural studies of *Ruscus aculeatus* L. cladode cells. *Romanian Journal of Biology – Plant Biology*, **55**, 27–36.
- Bricaud, O., Roux, C., Ménard, T. & Coste, C. (1993) Champignons lichénisés et lichénicoles de la France méridionale: espèces nouvelles et intéressantes (8). *Bulletin de la Société Linnéenne de Provence*, **44**, 99–110.
- British Mycological Society (2013) *Fungal Records Database of Britain and Ireland*. Available at <http://www.fieldmycology.net/GBCHKLST/gbchklst.asp> (accessed 1 September 2013).
- Britten, J., Jackson, B.D. & Stearn, W.T. (1965) *William Turner. Libellus de re herbaria 1538. The names of herbes 1548*. Facsimiles with introductory matter. Ray Society, London, UK.
- Burel, F. & Bauldry, J. (1990) Hedgerow network patterns and processes in France. *Changing Landscapes: An Ecological Perspective* (eds I.S. Zonneveld & R.T.T. Forman), pp. 99–120. Springer, New York, NY, USA.
- Câmara, M.P.S., Palm, M.E., Berkum, P.V. & Stewart, E.L. (2001) Systematics of *Paraphaeosphaeria*: a molecular and morphological approach. *Mycological Research*, **105**, 41–56.
- Campana, C., Gauvin, S. & Ponge, J.-F. (2002) Influence of ground cover on earthworm communities in an unmanaged beech forest: linear gradient studies. *European Journal of Soil Biology*, **38**, 213–224.
- Cann, D.C.G. (2001) *Ruscus aculeatus* 'Lanceolatus'. *The Plantsman*, **8**, 239–243.
- Cantalapiedra-Navarrete, C., Navas-Cortés, J.A., Liébanas, G., Vovlas, N., Subbotin, S.A., Palomares-Rius, J.E. & Castillo, P. (2013) Comparative molecular and morphological characterisations in the nematode genus *Rotylenchus*: *Rotylenchus paravitis* n. sp., an example of cryptic speciation. *Zoologischer Anzeiger*, **252**, 246–268.
- Capra, C. (1972) Pharmacology and toxicology of some components of *Ruscus aculeatus*. *Fitoterapia*, **43**, 99–113.
- Celik, A., Guvensen, A., Uysal, I. & Ozturk, M. (2005) Differences in concentrations of allergenic pollens at different heights in Denizli, Turkey. *Pakistan Journal of Botany*, **37**, 519–530.
- Chase, M.W., Reveal, J.L. & Fay, M.F. (2009) A subfamilial classification for the expanded asparagalean families Amaryllidaceae, Asparagaceae and Xanthorrhoeaceae. *Botanical Journal of the Linnean Society*, **161**, 132–136.
- Chauhan, R., Ruby, K. & Dwivedi, J. (2012) Golden herbs used in piles treatment: a concise report. *International Journal of Drug Development and Research*, **4**, 50–68.
- Chiarucci, A., Robinson, H., Bonini, A., Petit, D., Brooks, R.R. & De Dominicis, V. (1998) Vegetation of Tuscan ultramafic soils in relation to edaphic and physical factors. *Folia Geobotanica*, **33**, 113–131.
- Cibien, C., Boutin, J.M. & Maizeret, C. (1988) Einfluß des Rehwildes (*Capreolus capreolus*) auf zwei verschiedene Waldbestände in Abhängigkeit von seiner Populationsdichte. [Impact of roe deer (*Capreolus capreolus*) on vegetation in relation to population density and type of woodland.] *Zeitschrift für Jagdwissenschaft*, **34**, 232–241.
- Clapham, A.R., Tutin, T.G. & Moore, D.M. (1987) *Flora of the British Isles*, 3rd edn. Cambridge University Press, Cambridge, UK.
- Cluzan, R.V., Alliot, F., Ghabboun, S. & Pacot, M. (1996) Treatment of secondary lymphedema of the upper limb with CYCLO 3 FORT. *Lymphology*, **29**, 29–35.
- de Combarieu, E., Falzoni, M., Fuzzati, N., Gattesco, F., Giori, A., Lovati, M. & Pace, R. (2002) Identification of *Ruscus* steroidal saponins by HPLC-MS analysis. *Fitoterapia*, **73**, 583–596.
- Cooney-Sovetts, C. & Sattler, R. (2008) Cladode development in the Asparagaceae: an example of homoeosis. *Botanical Journal of the Linnean Society*, **94**, 327–371.
- Corsi, G. & Pagni, A.M. (1979) *Piante Selvatiche di uso Alimentare in Toscana*. Pacini, Pisa, Italy.
- Coşkun, M., Güvenç, A., Kiliç, C.S. & Arihan, O. (2006) *Ruscus aculeatus* trade in Turkey: is it sustainable? *Planta Medica*, **72**, P-298.
- Council of Europe (2011) *European Pharmacopoeia*, 7th edn. Council of Europe, Strasbourg, France.
- Dakskobler, I. (2013) Novosti v flori zahodne, severozahodne in osrednje Slovenije. [Novelities in the flora of western, northwestern and central Slovenia.] *Hladnikia*, **31**, 31–50.
- D'Antuono, L.F. & Lovato, A. (2003) Germination trials and domestication potential of three native species with edible sprouts: *Ruscus aculeatus* L., *Tamus communis* L. and *Smilax aspera* L. *Acta Horticulturae*, **598**, 211–218.
- Davis, P.H. (1984) *Flora of Turkey and the East Aegean Islands*, Vol. 8. Edinburgh University Press, Edinburgh.
- De Marino, S., Festa, C., Zollo, F. & Iorizzi, M. (2012) Novel steroidal components from the underground parts of *Ruscus aculeatus* L. *Molecules*, **17**, 14002–14014.
- De Sloover, J.R. & Sérusiaux, E. (1984) Une station de lichens foliicoles en Provence. *Cryptogamie, Bryologie, Lichénologie*, **5**, 291.
- Debussche, M., Debussche, G. & Lepart, J. (2001) Changes in the vegetation of *Quercus pubescens* woodland after cessation of coppicing and grazing. *Journal of Vegetation Science*, **12**, 81–92.
- Debussche, M. & Isenmann, P. (1994) Bird-dispersed seed rain and seedling establishment in patchy Mediterranean vegetation. *Oikos*, **69**, 414–426.
- Del Vecchio, S., Burke, R.L., Rugiero, L., Capula, M. & Luiselli, L. (2011) Seasonal changes in the diet of *Testudo hermanni hermanni* in central Italy. *Herpetologica*, **67**, 236–249.
- Di Novella, R., Di Novella, N., De Martino, L., Mancini, E. & De Feo, V. (2013) Traditional plant use in the National Park of Cilento and Vallo di Diano, Campania, Southern Italy. *Journal of Ethnopharmacology*, **145**, 328–342.
- Di Sanzo, P., De Martino, L., Mancini, E. & De Feo, V. (2013) Medicinal and useful plants in the tradition of Rotonda, Pollino National Park, Southern Italy. *Journal of Ethnobiology and Ethnomedicine*, **9**, article 19.
- Diaz-Losada, E., Ricciardelli-d'Albore, G. & Saa-Otero, M.P. (1998) The possible use of honeybee pollen loads in characterising vegetation. *Grana*, **37**, 155–163.
- Dickson, A. (1886) On the occurrence of foliage-leaves in *Ruscus* (*Samele androgynus*); with some structural and morphological observations. *Transactions of the Botanical Society of Edinburgh*, **16**, 130–149.
- Dierschke, H. (1997) Syntaxonomical survey of European beech forests: some general conclusions. *Annali di Botanica*, **55**, 17–26.
- Dunouau, C., Bellé, R., Oulad-Ali, A., Anton, R. & David, B. (1996) Triterpenes and sterols from *Ruscus aculeatus*. *Planta Medica*, **62**, 189–190.
- Ecological Flora of the British Isles (2013) Available at <http://www.ecoflora.co.uk/> (accessed 1 September 2013).

- Elia, M., Laforteza, R., Tarasco, E., Colangelo, G. & Sanesi, G. (2012) The spatial and temporal effects of fire on insect abundance in Mediterranean forest ecosystems. *Forest Ecology and Management*, **263**, 262–267.
- Ellis, M.B. & Ellis, J.P. (1997) *Microfungi on Land Plants*. Richmond Publishing, Slough, UK.
- Elsohly, M.A., Doorenbos, N.J., Quimby, M.W., Knapp, J.E., Slatkin, D.J. & Schiff, P.L. Jr (1974) Euparone, a new benzofuran from *Ruscus aculeatus* L. *Journal of Pharmaceutical Sciences*, **63**, 1623–1624.
- Elsohly, M.A., Knapp, J.E., Slatkin, K.F., Schiff, P.L. Jr, Doorenbos, N.J. & Quimby, M.W. (1975) Constituents of *Ruscus aculeatus*. *Lloydia*, **38**, 106–108.
- Facino, R.M., Carini, M., Stefani, R., Aldini, G. & Sibene, L. (1995) Anti-elastase and anti-hyaluronidase activities of saponins and saponinins from *Hedera helix*, *Aesculus hippocastanum*, and *Ruscus aculeatus*: factors contributing to their efficacy in the treatment of venous insufficiency. *Archiv der Pharmazie*, **328**, 720–724.
- Farmer, J.B. (1918) On the quantitative differences in the water-conductivity of the wood in trees and shrubs. Part I. The evergreens. *Proceedings of the Royal Society of London B: Biological Sciences*, **90**, 218–232.
- Follieri, M. (2010) Conifer extinction in Quaternary Italian records. *Quaternary International*, **225**, 37–43.
- Fowden, L. & Steward, F.C. (1957) Nitrogenous compounds and nitrogen metabolism in the Liliaceae. I. The occurrence of soluble nitrogenous compounds. *Annals of Botany*, **21**, 53–67.
- Fuentes, M. (1992) Latitudinal and elevational variation in fruiting phenology among western European bird-dispersed plants. *Ecography*, **15**, 177–183.
- Ganatsas, P.P., Zagas, T.D., Tsakalimi, M.N. & Tsitsoni, T.K. (2004) Postfire regeneration dynamics in a Mediterranean type ecosystem in Sithonia, northern Greece: ten years after the fire. *Proceedings 10th MEDECOS Conference, Rhodes, Greece* (eds M. Arianoutsou & V.P. Papanastasis), pp. 1–9. Millpress, Rotterdam, The Netherlands.
- Gilliland, H.B. (1931) Anomalous stem structure in *Ruscus aculeatus* Linn. *Transactions of the Botanical Society of Edinburgh*, **30**, 284–285.
- Golan, K. & Górska-Drabik, E. (2006) The scale insects (Hemiptera, Coccinea) of ornamental plants in a greenhouse of the Maria Curie Skłodowska University botanical garden in Lublin. *Journal of Plant Protection Research*, **46**, 347–352.
- Golzar, H. & Wang, C. (2012) First report of *Phaeosphaeriopsis glaucopunctata* as the cause of leaf spot and necrosis on *Ruscus aculeatus* in Australia. *Australasian Plant Disease Notes*, **7**, 13–15.
- Gonçalves, S., Franco, J. & Romano, A. (2008) Allelopathic effects of extracts from several plant species on seed germination and seedlings growth of *Lactuca sativa* L. *Planta Medica*, **74**, PE15.
- Gonçalves, S., Gomes, D., Costa, P. & Romano, A. (2013) The phenolic content and antioxidant activity of infusions from Mediterranean medicinal plants. *Industrial Crops and Products*, **43**, 465–471.
- González, J.A., García-Barriso, M., Ramírez-Rodríguez, R., Bernardos, S. & Amich, F. (2013) Ethnobotanical Resources management in the Arribes del Duero Natural Park (Central Western Iberian Peninsula): relationships between plant use and plant diversity, ecological analysis, and conservation. *Human Ecology*, **41**, 615–630.
- González-Hernández, M.P. & Silva-Pando, F.J. (1999) Nutritional attributes of understory plants known as components of deer diets. *Journal of Range Management*, **52**, 132–138.
- Gosling, P. (2007) *Raising Trees and Shrubs from Seed*. Forestry Commission Practice Guide, Forestry Commission, Edinburgh, UK.
- Grubb, P.J. (1998) A reassessment of the strategies of plants which cope with shortages of resources. *Perspectives in Plant Ecology, Evolution and Systematics*, **1**, 3–31.
- Guarrera, P.M. (2005) Traditional phytotherapy in Central Italy (Marche, Abruzzo, and Latium). *Fitoterapia*, **76**, 1–25.
- Gücel, S., Güvenses, A., Öztürk, M. & Celik, A. (2013) Analysis of airborne pollen fall in Nicosia (Cyprus). *Environmental Monitoring and Assessment*, **185**, 157–169.
- Güvenç, A., Şatır, E. & Coşkun, M. (2007) Determination of ruscogenin in Turkish *Ruscus* L. species by UPLC. *Cromatographia*, **66**, 141–145.
- Güvenses, A. & Öztürk, M. (2002) Airborne pollen calendar of Buca-Izmir, Turkey. *Aerobiologia*, **18**, 229–237.
- Hadžifežević, N., Kukić-Marković, J., Petrović, S., Soković, M., Glamočlija, J., Stojković, D. & Nahrstedt, A. (2013) Bioactivity of the extracts and compounds of *Ruscus aculeatus* L. and *Ruscus hypoglossum* L. *Industrial Crops and Products*, **49**, 407–411.
- Harley, J.L. & Harley, E.L. (1986) A check-list of mycorrhiza in the British flora. *New Phytologist*, **105**(Suppl.), 1–102.
- Hermý, M., Honnay, O., Firbank, L., Grashof-Bokdam, C. & Lawesson, J.E. (1999) An ecological comparison between ancient and other forest plant species of Europe, and the implications for forest conservation. *Biological Conservation*, **91**, 9–22.
- Herrera, C.M. (1981) Fruit variation and competition for dispersers in natural populations of *Smilax aspera*. *Oikos*, **36**, 51–58.
- Herrera, C.M. (1984) A study of avian frugivores, bird-dispersed plants, and their interaction in Mediterranean scrublands. *Ecological Monographs*, **54**, 1–23.
- Herrera, C.M. (1987) Vertebrate-dispersed plants of the Iberian Peninsula: a study of fruit characteristics. *Ecological Monographs*, **57**, 305–331.
- Hexsel, D., Orlandi, C. & Zechmeister, D. (2005) Botanical extracts used in the treatment of cellulite. *Dermatologic Surgery*, **31**, 866–872.
- Hill, M.O., Preston, C.D. & Roy, D.B. (2004) *PLANTATT – Attributes of British and Irish Plants: Status, Size, Life History, Geography and Habitats*. Centre for Ecology & Hydrology, Huntingdon, UK.
- Hillman, E.M. (1979) A study of *Ruscus aculeatus* on Bookham Common. *London Naturalist*, **58**, 44–55.
- Hillman, E.M. & Warren, A. (1973) Survey of *Ruscus aculeatus* on Bookham Common: the first two years. *London Naturalist*, **52**, 93–103.
- Hirayama, Y., Yamada, T., Oya, Y., Ito, M., Kato, M. & Imaichi, R. (2007) Expression patterns of class I KNOX and YABBY genes in *Ruscus aculeatus* (Asparagaceae) with implications for cladode homology. *Development Genes and Evolution*, **217**, 363–372.
- Hirsch, A.M. (1977) A developmental study of the cladodes of *Ruscus aculeatus* L. *Botanical Journal of the Linnean Society*, **74**, 355–365.
- Houssard, C., Escarré, J. & Bomane, F. (1980) Development of species diversity in some Mediterranean plant communities. *Vegetatio*, **43**, 59–72.
- Irmak, M.A. (2013) Use of native woody plants in urban landscapes. *Journal of Food, Agriculture & Environment*, **11**, 1305–1309.
- IUCN (2011) *IUCN Red List of Threatened Species*. International Union for Conservation of Nature and Natural Resources, Gland, Switzerland.
- Ivanova, T., Gussev, C., Bosseva, Y. & Stoeva, T. (2011) In vitro conservation of micro-propagated *Ruscus aculeatus* L. (Liliaceae) plants. *Botanica Serbica*, **35**, 61–66.
- Izco, J., Amigo, J. & Guitián, J. (1990) Los robledales galaicoseptentrionales. *Acta Botánica Malacitana*, **15**, 267–276.
- Izhaki, I. (2002) The role of fruit traits in determining fruit removal in east Mediterranean ecosystems. *Seed Dispersal and Frugivory* (eds D.J. Levey, W.R. Silva & M. Galetti), pp. 161–175. CABI Publishing, Wallingford, UK.
- Jackson, J. (1977) The annual diet of the Fallow deer (*Dama dama*) in the New Forest, Hampshire, as determined by rumen content analysis. *Journal of Zoology*, **181**, 465–473.
- JNCC (2007) *Second Report by the UK Under Article 17 on the Implementation of the Habitats Directive from January 2001 to December 2006*. Joint Nature Conservation Committee, Peterborough, UK.
- Jongman, R.H.G. (2000) The difficult relationship between biodiversity and landscape diversity. *Multifunctional Landscapes: Interdisciplinary Approaches to Landscape Research and Management* (eds J. Brandt, B. Tress & G. Tress), pp. 72–83. Conference Material for the Conference on “Multifunctional Landscapes”, Centre for Landscape Research, Roskilde, Denmark.
- Jordano, P. (1988) Diet, fruit choice and variation in body condition of frugivorous warblers in Mediterranean scrubland. *Ardea*, **76**, 193–209.
- Jumpponen, A. & Trappe, J.M. (1998) Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. *New Phytologist*, **140**, 295–310.
- Kay, Q.O.N. & Page, J. (1985) Dioecism and pollination in *Ruscus aculeatus*. *Watsonia*, **15**, 261–264.
- Kent, D.H. (1992) *List of Vascular Plants of the British Isles*. Botanical Society of the British Isles, London, UK.
- Kim, J.-H., Kim, D.-K., Forest, F., Fay, M.F. & Chase, W.M. (2010) Molecular phylogenetics of Ruscaceae *sensu lato* and related families (Asparagales) based on plastid and nuclear DNA sequences. *Annals of Botany*, **106**, 775–790.
- Kızıllarslan, Ç. & Özhatay, N. (2012) An ethnobotanical study of the useful and edible plants of İzmit. *Marmara Pharmaceutical Journal*, **16**, 134–140.
- Kutbay, H.G., Kılınç, M. & Kandemir, A. (1998) Phytosociological and ecological structure of *Fraxinus angustifolia* subsp. *oxycarpa* forests in the central Black Sea region. *Turkish Journal of Botany*, **22**, 157–162.
- Laghetta, G., Pignone, D., De Lisi, A., Cifarelli, S., Faslia, N. & Hammer, K. (2011) Collecting crop genetic resources in Italian towns of Albanian origin across the Molise, Calabria and Sicily regions. *Genetic Resources and Crop Evolution*, **58**, 139–152.
- Lentini, F. & Venza, F. (2007) Wild food plants of popular use in Sicily. *Journal of Ethnobiology and Ethnomedicine*, **3**, article 15.
- de Lillis, M. & Fontanella, A. (1992) Comparative phenology and growth in different species of the Mediterranean maquis of central Italy. *Vegetatio*, **99**–**100**, 83–96.

- Lohwag, K. (1963) Mykologische notizen aus dem belgrader wald bei Istanbul in der Türkei. *Sydowia*, **16**, 199–204.
- Lonati, M. & Lonati, S. (2002) Studio fitosociologico e selvicolturale di un Quercio-Carpinetto relitto ubicato in Valsesia (Piemonte, Vercelli). [Phytosociological and silvicultural research of a residual oak and hornbeam wood situated in Valsesia (Piedmont – North West Italy).] *Italian Journal of Forest and Mountain Environments*, **57**, 139–162.
- Longo, L. & Vasapollo, G. (2005) Determination of anthocyanins in *Ruscus aculeatus* L. berries. *Journal of Agricultural and Food Chemistry*, **53**, 475–479.
- Lovari, S., Cuccus, P., Murgia, A., Murgia, C., Soi, F. & Plantamura, G. (2007) Space use, habitat selection and browsing effects of red deer in Sardinia. *Italian Journal of Zoology*, **74**, 179–189.
- Mangas, S., Bonfill, M., Osuna, L., Moyano, E., Tortoriello, J., Cusido, R.M., Piñol, M.T. & Palazón, J. (2006) The effect of methyl jasmonate on triterpene and sterol metabolisms of *Centella asiatica*, *Ruscus aculeatus* and *Galphimia glauca* cultured plants. *Phytochemistry*, **67**, 2041–2049.
- Maremmani, A., Bedini, S., Matošević, I., Tomei, P.E. & Giovannetti, M. (2003) Type of mycorrhizal associations in two coastal nature reserves of the Mediterranean basin. *Mycorrhiza*, **13**, 33–40.
- Mari, A., Napolitano, A., Perrone, A., Pizza, C. & Piacente, S. (2012) An analytical approach to profile steroidal saponins in food supplements: the case of *Ruscus aculeatus*. *Food Chemistry*, **134**, 461–468.
- Marossy, A. (2006) Wild plant species endangered through intensive harvest. *Nature Conservation* (eds D. Gafta & J. Akeroyd), pp. 65–67. Springer, Berlin, Germany.
- Martínez-Pallé, E. & Aronne, G. (1999) Flower development and reproductive continuity in Mediterranean *Ruscus aculeatus* L. (Liliaceae). *Protoplasma*, **208**, 58–64.
- Martínez-Pallé, E. & Aronne, G. (2000) Pollination failure in mediterranean *Ruscus aculeatus* L. *Botanical Journal of the Linnean Society*, **134**, 443–452.
- Maude, P.F. (1940) Chromosome numbers in some British plants. *New Phytologist*, **39**, 17–32.
- Mimaki, Y., Kuroda, M. & Kameyama, A. (1998) New steroidal constituents of the underground parts of *Ruscus aculeatus* and their cytostatic activity on HL-60 cells. *Phytochemistry*, **48**, 485–493.
- Mimaki, Y., Kuroda, M., Kameyama, A., Yokosuka, A. & Sashida, Y. (1998a) Aculeoside B, a new bisdesmosidic spirostanol saponin from the underground parts of *Ruscus aculeatus*. *Journal of Natural Products*, **61**, 1279–1282.
- Mimaki, Y., Kuroda, M., Kameyama, A., Yokosuka, A. & Sashida, Y. (1998b) Steroidal saponins from the underground parts of *Ruscus aculeatus* and their cytostatic activity on HL-60 cells. *Phytochemistry*, **48**, 485–493.
- Mimaki, Y., Kuroda, M., Yokosuka, A. & Sashida, Y. (1999) A spirostanol saponin from the underground parts of *Ruscus aculeatus*. *Phytochemistry*, **51**, 689–692.
- Montagnoli, A., Terzaghi, M., Di Iorio, A., Scippa, G.S. & Chiantante, D. (2012) Fine-root morphological and growth traits in a Turkey-oak stand in relation to seasonal changes in soil moisture in the Southern Apennines, Italy. *Ecological Research*, **27**, 1015–1025.
- Morales, J., Pérez-Jordá, G., Peña-Chocarro, L., Zapata, L., Ruiz-Alonso, M., López-Sáez, J.A. & Linstädter, J. (2013) The origins of agriculture in North-West Africa: macro-botanical remains from Epipalaeolithic and early Neolithic levels of Ifri Oudadane (Morocco). *Journal of Archaeological Science*, **40**, 2659–2669.
- Moyano, E., Montero, M., Bonfill, M., Cusido, R.M., Palazón, J. & Piñol, M. (2006) *In vitro* micropropagation of *Ruscus aculeatus*. *Biologia Plantarum*, **50**, 441–443.
- Ookoljić, M., Vilotić, D. & Šijačić-Nikolić, M. (2013) Population genetic characteristics of horse chestnut in Serbia. *Archives of Biological Sciences*, **65**, 1–7.
- Onaindia, M., Dominguez, I., Albizu, I., Garbisu, C. & Amezcaga, I. (2004) Vegetation diversity and vertical structure as indicators of forest disturbance. *Forest Ecology and Management*, **195**, 341–354.
- Paoletti, M.G., Dreon, A.L. & Lorenzoni, G.G. (1995) *Pistic*, traditional food from Western Friuli, N.E. Italy. *Economic Botany*, **49**, 26–30.
- Parent, G.H. (2002) Etudes écologiques et chorologiques sur la flore Lorraine. Note 20. Le petit houx (*Ruscus aculeatus* L.) fait-il encore partie de la flore Lorraine? [Ecological and chorological studies on the Lorraine flora. Note 20. Is the butcher's broom (*Ruscus aculeatus* L.) still part of the Lorraine flora?] *Bulletin de l'Académie Lorraine des Sciences*, **41**, 1–2.
- Parrado, F. & Buzzi, A. (1999) A study of the efficacy and tolerability of a preparation containing *Ruscus aculeatus* in the treatment of chronic venous insufficiency of the lower limbs. *Clinical Drug Investigation*, **18**, 255–261.
- Patzel, N. & Ponge, J.-F. (2001) The heterogeneity of humus components in a virgin beech forest. *European Journal of Soil Biology*, **37**, 117–124.
- Pedersen, M. (1994) *Nutritional Herbology: A Reference Guide to Herbs*. Wendell W. Whitman, Warsaw, IN, USA.
- Peltier, A., Touzet, M.-C., Armengaud, C. & Ponge, J.-F. (1997) Establishment of *Fagus sylvatica* and *Fraxinus excelsior* in an old-growth beech forest. *Journal of Vegetation Science*, **8**, 13–20.
- Peñuelas, J. & Filella, I. (2001) Herbaria century record of increasing eutrophication in Spanish terrestrial ecosystems. *Global Change Biology*, **7**, 427–433.
- Pérez-Latorre, A.V. & Cabezedo, B. (2006) Phenomorphology and eco-morphological characters of *Rhododendron lauroid* forests in the Western Mediterranean (Iberian Peninsula, Spain). *Plant Ecology*, **187**, 227–247.
- Perring, F.H. (1996) A bridge too far – the non-Irish element in the British flora. *Watsonia*, **21**, 15–51.
- Peterken, G.F. (1974) A method of assessing woodland flora for conservation using indicator species. *Biological Conservation*, **6**, 239–245.
- Pettorelli, N., Dray, S., Gaillard, J.-M., Chessel, D., Duncan, P., Illius, A., Guillon, N., Klein, F. & Van Laere, G. (2003) Spatial variation in springtime food resources influences the winter body mass of roe deer fawns. *Oecologia*, **137**, 363–369.
- Pivovarov, A., Sharifi, R., Scoffoni, C., Sack, L. & Rundel, P. (2013) Making the best of the worst of times: traits underlying combined shade and drought tolerance of *Ruscus aculeatus* and *Ruscus microglossum* (Asparagaceae). *Functional Plant Biology*, **41**, 11–24.
- Pratas, J., Favas, P.J.C., D'Souza, R., Varun, M. & Paul, M.S. (2013) Phytoremediation assessment of flora tolerant to heavy metals in the contaminated soils of an abandoned Pb mine in Central Portugal. *Chemosphere*, **90**, 2216–2225.
- Preston, C.D. (2007) Which vascular plants are found at the northern or southern edges of their European range in the British Isles? *Watsonia*, **26**, 253–269.
- Preston, C.D. & Hill, M.O. (1997) The geographical relationships of British and Irish vascular plants. *Botanical Journal of the Linnean Society*, **124**, 1–120.
- Preston, C.D., Pearman, D.A. & Dines, T.D. (2002) *New Atlas of the British and Irish Flora*. Oxford University Press, Oxford, UK.
- Puntillo, D. & Ottonello, D. (1997) A new foliicolous lichen station in Italy. *The Lichenologist*, **29**, 388–390.
- Puntillo, D. & Vězda, A. (1994) Some foliicolous lichens new to Calabria. *Webbia*, **49**, 125–131.
- Rameau, J.C., Mansion, D. & Dumé, G. (1989) *Flore Forestière Française*. Ministère de l'Agriculture et de la Forêt, Paris, France.
- Raven, P.H. & Axelrod, D.I. (1974) Angiosperm biogeography and past continental movements. *Annals of the Missouri Botanical Garden*, **61**, 539–673.
- Redman, D.A. (2000) *Ruscus aculeatus* (butcher's broom) as a potential treatment for orthostatic hypotension, with a case report. *The Journal of Alternative and Complementary Medicine*, **6**, 539–549.
- Retuerto, R. & Carballeira, A. (1992) Use of direct gradient analysis to study the climate-vegetation relationships in Galicia, Spain. *Vegetatio*, **101**, 183–194.
- Retuerto, R. & Carballeira, A. (2004) Estimating plant responses to climate by direct gradient analysis and geographic distribution analysis. *Plant Ecology*, **170**, 185–202.
- Rishbeth, J. (1948) The flora of Cambridge walls. *Journal of Ecology*, **36**, 136–148.
- Rodríguez-Loinaz, G., Amezcaga, I. & Onaindia, M. (2012) Does forest fragmentation affect the same way all growth-forms? *Journal of Environmental Management*, **94**, 125–131.
- Rodwell, J.S. (1991) *British Plant Communities, Vol. 1. Woodlands and Scrub*. Cambridge University Press, Cambridge, UK.
- Rose, F. (1999) Indicators of ancient woodland. The use of vascular plants in evaluating ancient woods for nature conservation. *British Wildlife*, **10**, 241–251.
- Rottenberg, A. (1998) Sex ratio and gender stability in the dioecious plants of Israel. *Botanical Journal of the Linnean Society*, **128**, 137–148.
- Sack, L. (2004) Responses of temperate woody seedlings to shade and drought: do trade-offs limit potential niche differentiation? *Oikos*, **107**, 110–127.
- Sack, L., Grubb, P.J. & Marañón, T. (2003) The functional morphology of juvenile plants tolerant of strong summer drought in shaded forest understoreys in southern Spain. *Plant Ecology*, **168**, 139–163.
- Sadarmin, P.P. & Timperley, J. (2013) An unusual case of butcher's broom precipitating diabetic ketoacidosis. *The Journal of Emergency Medicine*, **45**, e63–e65.
- Salerno, G., Guarrera, P.M. & Caneva, G. (2005) Agricultural, domestic and handicraft folk uses of plants in the Tyrrhenian sector of Basilicata (Italy). *Journal of Ethnobiology and Ethnomedicine*, **1**, article 2.
- Salisbury, E.J. (1926) The geographical distribution of plants in relation to climatic factors. *Geographical Journal*, **67**, 312–335.

- Salzmann, P., Ehresmann, U. & Adler, U. (1977) *Ruscus aculeatus* L. – der Mäusedorn. [*Ruscus aculeatus* L. – a therapeutic agent in proctology.] *Fortschritte der Medizin*, **95**, 1419–1422.
- Santo, M.D.E., Moreira, I. & González, P.R. (2005) Vegetation and landscape of Serra de Montejunto. *Quercetea*, **7**, 81–92.
- Schaffhauser, A., Curt, T., Véla, E. & Taton, T. (2012) Recurrent fires and environment shape the vegetation in *Quercus suber* L. woodlands and maquis. *Comptes Rendus Biologies*, **335**, 424–434.
- Schweingruber, F.H. (1990) *Anatomy of European Woods*. Paul Haupt, Bern, Switzerland.
- Silva, M.C., Antunes, S., Gouveia, F. & de Oliveira, N.G. (2011) Using biodiversity action plans to manage high conservation value areas in Portuguese Natura 2000 Network Areas. *Fitosociologia*, **48**, 111–117.
- Stace, C. (2010) *New Flora of the British Isles*, 3rd edn. Cambridge University Press, Bern, Cambridge, UK.
- Swirski, E. & Amitai, S. (1997) Notes on phytoseiid mites (Mesostigmata: Phytosehidae) of Mt. Carmel (Israel), with descriptions of two new species. *Israel Journal of Entomology*, **31**, 1–20.
- Tansi, S., Karaman, S. & Tancer, O. (2009) Ecological and morphological variation in wild *Ruscus aculeatus* from Mediterranean region of southern Turkey. *Acta Horticulturae*, **862**, 175–183.
- Tarayre, J.P. & Laouressergues, H. (1979) Anti-edematous effect of an association of proteolytic enzymes, flavonoids, heterosides of *Ruscus aculeatus* and ascorbic acid. *Annales Pharmaceutiques Françaises*, **37**, 191–198.
- Thompson, K., Bakker, J.P. & Bekker, R.M. (1997) *The Soil Seed Banks of North West Europe*. Cambridge University Press, Cambridge, UK.
- di Tizio, A., Luczaj, Ł.J., Quave, C.L., Redžić, S. & Pieroni, A. (2012) Traditional food and herbal uses of wild plants in the ancient South-Slavic diaspora of Mundimitar/Montemitro (Southern Italy). *Journal of Ethnobiology and Ethnomedicine*, **8**, article 21.
- Tongiorgi, E. (1938) La flora fossile di Saccopastore. Suo significato nella storia della vegetazione laziale. *Rivista di Antropologia*, **32**, 237–342.
- Tutin, T.G., Heywood, V.H., Burges, N.A., Valentine, D.H., Walters, S.M. & Webb, D.A. (2002) *Flora Europaea*. Cambridge University Press, Cambridge, UK.
- Úbeda, X., Outeiro, L.R. & Sala, M. (2006) Vegetation regrowth after a differential intensity forest fire in a Mediterranean environment, Northeast Spain. *Land Degradation and Development*, **17**, 429–440.
- Vallet, J., Daniel, H., Beaujouan, V. & Rozé, F. (2008) Plant species response to urbanization: comparison of isolated woodland patches in two cities of North-Western France. *Landscape Ecology*, **23**, 1205–1217.
- Vicente, J., Randin, C.F., Gonçalves, J., Metzger, M.J., Lomba, Á., Honrado, J. & Guisan, A. (2011) Where will conflicts between alien and rare species occur after climate and land-use change? A test with a novel combined modelling approach. *Biological Invasions*, **13**, 1209–1227.
- Warne, L.G.G. (1942) The supply of water to transpiring leaves. *American Journal of Botany*, **29**, 875–884.
- Yeo, P.F. (1968) A contribution to the taxonomy of the genus *Ruscus*. *Notes of the Royal Botanic Garden Edinburgh*, **28**, 237–264.
- Yurdakulol, E., Demirörs, M. & Yildiz, A. (2002) A phytosociological study of the vegetation of the Devrekani–Inebolu–Abana area (Kastamonu, Turkey). *Israel Journal of Plant Sciences*, **50**, 293–311.
- Zapata, L., López-Sáez, J.A., Ruiz-Alonso, M., Linstädter, J., Pérez-Jordà, G., Morales, J., Kehl, M. & Peña-Chocarro, L. (2013) Holocene environmental change and human impact in NE Morocco: Palaeobotanical evidence from Ifri Oudadane. *The Holocene*, **23**, 1286–1296.
- Zistler, C., Krafka, O., Kranvogel, A. & Sonnenschein, M. (2008) Anbauversuche mit Mäusedorn (*Ruscus aculeatus* L.). [Cultivation experiments with butcher's broom (*Ruscus aculeatus* L.).] *Zeitschrift für Arznei- und Gewürzpflanzen*, **13**, 114–116.