Biological Flora of the British Isles: *Aesculus hippocastanum*

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

Abstract

1. This account presents information on all aspects of the biology of *Aesculus hippocastanum* L. (Horse-chestnut) 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. *Aesculus hippocastanum* is a large deciduous tree native to the Balkan Peninsula. Native populations are small (<10,000 trees total) and apparently in decline, but the tree has been widely planted in gardens and streets across Europe and other temperate areas from the 17th century onwards. It was voted the UK's favourite tree in a 2017 poll. As a British neophyte it is occasionally naturalised in open wooded habitats.

3. Horse-chestnut is renowned for the beauty of its large (up to 30 cm long), upright panicles of white flowers, and for the large seeds (up to 42 g each) used in the formerly common children's game of 'conkers'. More recently, the triterpene glycosides, extractable from various plant parts but especially the seeds, have been widely used in medicine.

4. In much of Europe, Horse-chestnut is affected by chestnut bleeding canker (caused by *Pseudomonas syringae* pv. *aesculi*), the horse-chestnut leaf miner *Cameraria ohridella*, and the leaf blotch fungus *Guignardia aesculi*. The canker is likely to lead to death of <10% individuals but seeds of plants infested with the leaf miner are 40-50% smaller, which may affect long-term establishment in non-planted areas.

Horse-chestnut. Sapindaceae (formerly Hippocastanaceae). *Aesculus hippocastanum* L. (*A. asplenifolia* Loud., *A. castanea* Gilib., *A. heterophylla* hort. ex Handl., *A. ohiotensis* Lindl., *A. incisa* hort. ex Handl., *A. memmingeri* C. Koch, *A. procera* Salisb., *A. septenata* Stokes, *Esculus hipocastanea* (L.) Raf., *Hippocastanum aesculus* Cav., *Hippocastanum vulgare* Gaertn., *Pawia hippocastanum* (L.) Kuntze) is a large deciduous tree with a wide-spreading, flat-topped crown up to 20 m across, branches upswept at first, then dipping down to form low spreading limbs that turn sharply up at the ends. Height up to 25 (-39) m tall, trunk up to 190 cm diameter. Bark dark grey-brown, smooth when young, and on mature trees forming long fine scales that become detached at both ends before falling. Twigs stout, pale grey or brown, glabrous with pale lenticels. Buds large, 2.5-5 cm, ovoid, deep red-brown, resinous and very sticky. Leaves opposite, large (≤60 cm wide), with a long (7-20 cm) terete petiole, palmate with 5-7 leaflets, each (8)10-20 (25) cm long, the terminal one the largest. Leaflets sessile, obovate, long cuneate at base, usually acuminate, irregularly crenate-serrate or serrate-dentate, dark green, glabrous above, somewhat tomentose beneath when young, often glabrous at maturity, joined to the petiole by a pulvinus-like leaflet-base.

Inflorescence a large (15-30 x 10 cm), terminal, erect, conical to cylindrical panicle, andromonoecious with male flowers at the top of the panicle and hermaphrodite flowers below. Flowers zygomorphic *c.* 2 cm across; sepals 5 forming a tubular or campanulate, toothed calyx; petals 4-5, each *c.* 1 cm, white with basal spots at first yellow then pink. In Poland, Weryszko-Chmielewska, Tietze and Michońska (2012) found 56% 4-petalled and 44% 5-petalled flowers. Stamens 5-9 of variable length within the same flower, mostly longer than petals and arched downwards; pollen red. Ovary 3-celled, each cell with two ovules; single style small and simple, stigma minute. Male flowers with a small non-functional pistil and an underdeveloped ovary. Nectary a lobed disc. Only 2-5(8) flowers at the base of the panicle develop fruit. Fruit 5-8 cm diameter, subglobose, spiny with 1

(rarely 2 or 3) seeds or 'conkers'. Seed a large lustrous or glossy, glabrous, smooth, reddish-brown nut, 34-48 x 25-37 mm, ellipsoid, the radicular lobe visible as a low, broad dark ridge especially in dried nuts; hilum large, white, circular or elliptic.

Aesculus contains between 12 and19 extant species (Xiang, Crawford, Wolfe, Tang, & Depamphilis, 1998; Harris, Xiang, & Thomas, 2009) with most authors recognising 12 or 13 species (Koch, 1857; Hardin, 1960; Forest, Drouin, Charest, Brouillet, & Bruneau, 2001; Zhang, Li, & Lian, 2010). *Aesculus hippocastanum* is the only European species within Section *Aesculus* along with *A. turbinata* Blume from Japan (Zhang et al., 2010). The genus is otherwise confined to North America, and south and east Asia (Xiang et al., 1998; Forest et al., 2001).

Bean (1976) and Bellini and Nin (2005) list a number of varieties, cultivars and forms. Of these, *A. hippocastanum* 'Baumannii' is particularly common; this is a double flowered, fruitless variety (Hoar, 1927) planted where conker-hunting by children has been seen as a problem (Leathart, 1991). *Aesculus hippocastanum* 'Pyramidalis' is becoming more common; this reaches 25 m with a conical or narrowly pyramidal crown when young, becoming more ovate at maturity. Only one hybrid is known (section 8.2).

Aesculus hippocastanum is native to the Balkan Peninsula in south-east Europe but has been widely planted in temperate areas from the 17th century onwards. As a neophyte, it is widely naturalised in Europe, though only partially so in Britain where it was known from the wild by 1870 (Preston, Pearman & Dines, 2002). It is often self sown in grassy places, copses, thickets, hedges and rough ground throughout lowland Britain and into west and central Europe.

Horse-chestnut is best known as a tree planted for ornamentation and shade in parks and streets, particularly by the Victorians (Rackham, 1986), since little else can rival the sight of a horse-chestnut in full flower. Indeed, it was voted the UK's favourite tree in

2017 in a poll run by the Royal Society of Biology (Royal Society of Biology, 2017). The

Britush population is an estimated British 470,000 trees.

1 │ GEOGRAPHICAL AND ALTITUDINAL DISTRIBUTION

Aesculus hippocastanum has been extensively planted throughout the British Isles and

has continued to expand its range through naturalisation into a range of open habitats. It

is most frequent in the south and east of England, becoming less common in the wetter

areas of west Ireland, and northern Scotland (Fig. 1) and upland areas of Scotland.

Nevertheless, even in these areas it can be found planted in urban areas and gardens and

has even been planted in policy woodlands on Hebridean islands such as Rhum (Batten &

Pomeroy, 1969) although it is now rare (Pearman et al., 2008). Hill, Preston & Roy (2004)

list horse-chestnut as being present in 2186 10-km squares in Great Britain and the Isle of

Man (80%), 557 in Ireland (57%) and 12 in the Channel Islands (86%).

Aesculus hippocastanum is native to mountains of the Balkans in south-east

Europe (Fig. 2). The biggest native populations are in mainland Greece, in the central

Thessaly Mountains and northern Pindos range as well as in the southern counties of

Evrytania and Fthiotida between 40°20′ N, 21°05′ E and 38°37′ N, 22°26′ E (Avtzis, Avtzis,

Vergos, & Diamandis, 2007; Walas et al., 2018). Tsiroukis (2008) counted a total of 1,464

adult horse-chestnut trees across all 98 known populations in the mountains of Greece;

38% of all individuals were in the Pindos Mountains. Populations varied from 1-153

individuals and 63% of populations had <10 trees. Its native range also includes

populations in Albania, the Republic of Macedonia, Serbia, and eastern Bulgaria (Anchev

et al., 2009; Evstatieva, 2011, Peçi, Mullaj, & Dervishi, 2012; Gussev & Vulchev, 2015).

These populations are variable in size within and between countries. Albania has <500

individuals, with populations of <50 individuals and most containing 10-15 individuals (L.

Shuka pers. comm. 2017 cited in Allen & Khela, 2017). The Macedonian population is

probably <100 individuals and the Bulgarian populations are probably smaller (Peçi et al., 2012; Allen & Khela, 2017). The total extent of its native extant range is estimated to be 163,642 km^2, *c.* 25% of the Balkan Peninsula (Allen & Khela, 2017).

Horse-chestnut has, however, been extensively planted since the 17th century (section 10) across central Europe and south into Italy and Serbia (Fig. 2). It is also grown widely in urban areas of Iran, northern India, Asia Minor, USA, Canada (as far north as Edmonton, Alberta) and New Zealand (Loenhart, 2002; Kapusta et al., 2007, Zhang et al., 2010) and further north in the Faeroe Islands, Iceland and Norway (Højgaard, Jóhansen & Ødum, 1989), still producing fruit at 65°N in Sweden and Norway (Bellini & Nin, 2005). It was introduced into the United States most probably in 1746 in Philadelphia (Anon., 1925).

Horse-chestnut is generally found in the lowlands of Britain but reaches 505 m altitude at Ashgill (Cumberland) (Preston et al., 2002) and 1300 m in Sweden and Norway (Bellini & Nin, 2005). It occurs at 218-1,485 m in its native range in Greece and Bulgaria, and up to *c.*1,600 m in Albania (Horvart, Glavac, & Ellenberg, 1974; Leathart, 1991; Avtzis et al., 2007; Peçi et al., 2012; Walas et al., 2018). In Greece, 35% of individuals and 31% of populations are found between 900 and 1000 m, and 72% of adult trees grow between 500 and 1000m (Tsiroukis, 2008).

2 │ HABITAT

2.1 │ Climatic and topographical limitations

The mean January temperature within the 10-km squares in which horse-chestnut occurs within Britain, Ireland and the Channel Islands is 3.6 °C, and the mean July temperature 14.8 °C (Hill et al., 2004). Within the same area, the mean annual precipitation is 1014 mm. These figures are, of course, very similar to other trees that have a distribution extending over much of Britain, such as *Fraxinus excelsior* and *Acer pseudoplatanus*.

Across its range, horse-chestnut is a mesophytic tree, adapted to warm-temperate climates. Walas et al. (2018) modelled its climatic requirements over the whole of its native range in the Balkan Peninsula and concluded that the main limitations to its distribution were high precipitation in the coldest quarters of the year and low precipitation in the warmest. It is also limited by a wide range in annual temperature, preferring those areas without temperature extremes. These limitations appear to act primarily through humidity, which was seen as the main factor limiting distribution. Where humidity is low, such as in Mediterranean and urban areas, high soil moisture and low stomatal conductance (section 6.5) appear to be important in helping horse-chestnut survive (Walas et al., 2018). Certainly, the native populations in Greece rely on high air humidity resulting from the presence of open water, and undoubtedly high soil moisture. Tsiroukis (2008) found 62% of populations in ravines with constantly flowing water, 12% in humid forests, 11% in ravines with intermittent flow and only 9% in dry ravines and 6% on arid slopes by roads. It is likely that the recalcitrant nature of the seeds (section 8.4), and their intolerance of desiccation, is the main factor limiting horse-chestnut naturally to moist sites (Horvat et al., 1974), and explains why seedlings, past this vulnerable stage, can be successfully transplanted into a wide range of climatic conditions from woodlands to harsh, dry urban areas.

There appear to be few topographical limitations as horse-chestnut grows on flat urban sites and is found on steep slopes and in ravines within its native range (Thalmann, Freise, Heitland, & Bacher, 2003; Tsiroukis, 2008). In Bulgaria, it grows up onto steep slopes and scree (Horvart et al., 1974; Peçi et al., 2012; Walas et al., 2018) that reach 25–60° (Gussev & Vulchev, 2015). In Albania, it is found on limestone rocky slopes of valleys and canyons (Allen & Khela, 2017). The importance of aspect is unknown but is likely to be related to the need for moisture.

2.2 │ Substratum

The optimum soils for horse-chestnut are generally deep, siliceous, free-draining, and rather fertile soils (Tsiroukis, 2008; Fitter & Peat, 1994). The Ellenberg value for nitrogen (N) corrected for Britain is 7 (Hill et al., 2004), which is slightly higher than associated with *Fraxinus excelsior*, *Acer platanoides* and *Tilia cordata* (all 6). Similarly, the Ellenberg value for moisture (F) is 5, indicating a moist site, similar to the requirement by many deciduous trees. Moist, $CaCO_3$-rich and fertile soils produce a strong, flat root system in horse-chestnut; but poorer soils result in horse-chestnut growing a shallow but much more extensive root system (Karliński et al., 2014). Simon and Lena (2016), and Poljanšek and Lena (2016) found that radial growth of street trees in Slovenia was positively associated with soil moisture, underlining the importance of moisture.

In its native range, horse-chestnut is usually restricted to moist but well-drained stony soil (often in moist depressions). In Bulgaria and Albania, horse-chestnut grows on calcareous soils derived from limestone (Allen & Khela, 2017). These medium-deep soils are slightly acidic on the surface and more alkaline at depth (Gussev & Vulchev, 2015). Horse-chestnut will, however, cope with being planted on a range of soils from nutrient poor sand to heavy clay, from acid to alkaline although an optimum pH of 6.6 to 7.2 is recommended (Puchalski & Prusinkiewicz, 1975). The Ellenberg value for reaction (R) in Britain is 7; the commonest requirement by plants in Britain, indicating an association with weakly acidic to weakly basic conditions and never on very acid soils (Hill et al., 2004).

3 │ COMMUNITIES

In Britain, horse-chestnut is frequently planted in urban and rural settings such as in estates, parks and gardens (Whitney & Adams, 1980), churchyards, urban streets and village greens. It is also found in a large number of planted woodlands resulting "from the

Victorian forester's habit of trying everything" (Rackham, 2003). Horse-chestnut is often self-sown in open habitats, such as unmanaged scrub, waste ground or rough grassland. As such it is rarely found in semi-natural vegetation types in Britain. It does occasionally regenerate locally in woodland but is seldom naturalised in native woodland (Rackham, 2003; Preston et al., 2002). The exception is as an infrequent member of W12 *Fagus sylvatica-Mercurialis perennis* woodland (Rodwell, 1991) where it has become naturalised in some places and locally abundant.

In mainland Europe, it is planted in a wide range of urban and garden areas, and is naturalised in grassy places, copses, thickets and hedges and rough ground through west and central Europe (Łukasiewicz, 2003).

In its native range in Greece, it is found in the conifer and mixed broadleaf forests ecoregion (WWF, 2013). On dry and rocky south-facing slopes, it is found with evergreen oaks (WWF, 2013) which is common through the Middle East and into Asia. In moist mountainous valleys horse-chestnut is found with *Abies cephalonica*, *Juglans regia*, *Ostrya carpinifolia*, *Platanus orientalis* and *Alnus incana* (Leathart, 1991) and in cooler places with *Abies borisii-regis* (which forms 52% of the total tree population in native stands), *Fraxinus ornus* (41%), *Fagus sylvatica* (37%), *Platanus orientalis* (12%), *Salix alba* (11%) and *Juniperus communis* (10%) (Tsiroukis, 2008). Further north on the northeast face of Mount Ossa in central Greece horse-chestnut grows with *Tilia platyphyllos* in the formation *Aesculus hippocastanum-Tilia platyphyllos* (Raus, 1980).

In the Preslavska Mountains of the East Balkan Range in Bulgaria, horse-chestnut is present in deciduous forests that are a relict vegetation type typical of the northern Mediterranean that was more widespread in the past (Gussev & Vulchev, 2015). The stands form three associations within the formation Aesculeta hippocastani: *Aesculus hippocastanum-subnudum*, *Aesculus hippocastanum-Carpinus betulus* and more locally *Aesculus hippocastanum-Aegopodium podagraria* (Gussev & Vulchev, 2015). Horse-

chestnut is usually dominant in all these, although 10-20% of the crown may be composed of other species, particularly *Acer platanoides*, *A. pseudoplatanus*, *Fagus sylvatica* subsp. *moesiaca*, *Juglans regia*, *Sorbus torminalis*, *Tilia tomentosa* and *Ulmus glabra*. The horse-chestnuts can be up to 100 years old with a maximum DBH of 80–90 cm. Seed production is limited and reproduction is primarily by root suckers. *Cornus mas*, *C. sanguinea*, *Corylus avellana*, *Crataegus monogyna* and *Staphylea pinnata* show high constancy among the shrubs. The herbaceous layer is sparse and species-poor and in the spring is typified by *Anemone ranunculoides*, *Aremonia agrimonoides, Cardamine bulbifera*, *Corydalis bulbosa*, *C. solida*, *Erythronium dens-canis*, *Euphorbia amygdaloides*, *Isopyrum thalictroides*, *Mercurialis perennis*, *Milium effusum*, *Scilla bifolia*, *Symphytum tuberosum* and *Viola reichenbachiana*. Horse-chestnut can also be found associated with *Fagus sylvatica* in the order Fagion sylvaticae (Peçi et al., 2012).

4 │ RESPONSE TO BIOTIC FACTORS

The saplings of horse-chestnut are semi-shade tolerant with an Ellenberg value for light (L) of 5 corrected for Britain (Hill et al., 2004) and so are tolerant of some competition for light. Although most adult trees are planted in the open, or grow in open stands in its native range, it is able to persist in denser woodland communities (section 3). Indeed, Hirons and Sjöman (2018) list horse-chestnut as being suitable for shaded late-successional conditions. This supports evidence that horse-chestnut will sometimes invade established woodlands in Britain although it is likely that this only happens in fairly open conditions. In Eastern Europe, horse-chestnut is considered to be a competitive species that invades native woodlands, particularly where disturbed, growing as isolated trees or small patches (Chmura, 2004; Křivánek et al., 2006).

Horse-chestnut has a dense crown and casts a deep shade. City trees in south-east Hungary transmitted just 7 ± 10% (SD) of full sunlight in mid-summer, increasing to

25 ± 0.14% at the end of September when half leafless due to the start of autumn abscission. At this point in autumn, there was more shade below horse-chestnut than cast by *Styphnolobium japonicum* (L.) Schott (=*Sophora japonica*) (15 ± 8%) and *Tilia cordata* (12 ± 13%) which were loosing leaves, but less than *Celtis occidentalis* (7 ± 7%) that was not yet shedding leaves (Takács, Kiss, Gulyás, Tanács, & Kántor, 2016), suggesting that horse-chestnut maintains its deep shade comparatively late into autumn, increasing its competitiveness. Certainly in most urban areas around Europe little will grow beneath a group of horse-chestnut trees.

Horse-chestnut can tolerate atmospheric pollution and persists in polluted inner cities in Europe. Leaves collected in polluted areas of mainland Europe can appear small, curved and brittle, though the degree of pollution causing this is not stated (Godzik & Sassen, 1970). Pollution can alter the epidermis of leaves, particularly the adaxial surface, leading to it becoming porous or cracked with 'abnormal' stomata (Godzik & Sessen, 1970; Godzik & Halbwachs, 1986). Horse-chestnut is listed as sensitive to chlorine and moderately sensitive to hydrogen chloride by Khan and Abbasi (2000) but tolerant to sulphur dioxide and hydrogen sulphide (Velagić-Habul, Lazarev, & Custović, 1991). Salt tolerance is considered in section 5.3.

Due to its thin bark, horse-chestnut is sensitive to forest fires (Ravazzi & Caudullo, 2016). However, it will freely resprout from cut stumps and coppices well, although it is not often used in coppices since the poles grow slowly in comparison to other species such as *Fraxinus excelsior* and they are mechanically weak (Özden & Ennos, 2018).

Urban trees are tolerant of heavy pruning undertaken to maintain their aesthetic shape, and to maintain access and sight lines (Cutler & Richardson, 1989) but large wounds can be problematic. Wounds over 15-20 cm in diameter heal poorly and in Lithuania *c.* 80% of such wounds have been seen to be infected by wood and pith rot after 2-4 years (Snieskiene, Stankeviciene, Zeimavicius, & Balezentiene, 2011).

5 │ RESPONSE TO ENVIRONMENT

5.1 │ Gregariousness

In planted areas throughout Europe, Horse-chestnut is variably gregarious, obviously depending on the whims of each planting scheme. However, in many areas, groups or lines of horse-chestnuts are typically planted for visual impact or for shade. In native populations in Greece, horse-chestnut trees can grow close together, just a few metres apart, but density is variable. Walas et al. (2018) noted that the density of small trees (<1 m tall) varied from 31-33 to 1,017 trees/ha, intermediate sized trees (1.1-10.0 m) from 53 to 339 trees/ha, and for the tallest trees (≥10.1 m) from 16-20 to 565 trees/ha. There appeared to be no link between density and mean annual temperature or annual precipitation but the highest densities were found at the lowest elevations (705-915 m) compared to lowest densities at 1089-1463 m. However, the availability of soil moisture (section 2.1), topographic barriers and slope steepness, affecting the dispersal of seeds, must all also play their part in determining tree density in naturally regenerated populations.

5.2 │ Performance in various habitats

Growth in Britain is rapid when young, with trees gaining 60-90 cm in height annually, and and with growth rates of 30 cm per year sustained for at least 60 years (Leathart, 1991), particularly in the east and south-east of England. In the less optimal conditions on acidic and nutrient-poor soils of pine stands in north-east Germany, the maximum height growth of horse-chestnut was 7 cm per year, the lowest of all 13 deciduous trees tested. In comparison, *Acer pseudoplatanus* grew 0.29 m/y and *Sorbus aucuparia* 0.65 m/y (Zerbe & Kreyer, 2007). As horse-chestnut matures, height growth slows but annual increment is maintained such that in the city of Duisburg, Germany, Scholz, Hof and Schmitt (2018)

recorded horse-chestnut reaching 25 m tall at a DBH of 111 cm while *Betula pendula* at the same height had a diameter of <64 cm.

Across native populations in Greece, Walas et al. (2018) found that saplings (≤ 1m height) formed >50% of populations. They concluded that the abundance of seedlings and saplings showed that natural populations of horse-chestnut are capable of performing well and maintain themselves under favourable management (section 11).

For optimum growth, horse-chestnut requires shelter from high winds that would otherwise damage the large leaves (Bellini & Nin, 2005) and snap off branches, especially when combined with heavy, rain-soaked foliage (Mitchell, 1997). In extreme cases this damage produces a characteristic standing trunk with few remaining branches. Horse-chestnut is unusual in that its branches are comparatively stiff so branches fall with a clean break rather than buckling (Özden & Ennos, 2018). The branches are stiffer than its coppice shoots – whereas for *Acer pseudoplatanus* and *Fraxinus excelsior* it is the other way round; this may make the branches less flexible and more prone to snapping. Branches with fewer leaf nodes are stiffer and, due to the large, compound leaves, horse-chestnut has a low number of leaves per length of stem (Özden & Ennos, 2018).

Although Horse-chestnut grows well when planted in urban areas, growth is restricted in paved area. A study of 231 mature *A. hippocastanum* trees growing in Munich, Germany (mean height 16.1 m, mean DBH 63.6 cm, mean crown width 5.5 m) were growing in a mean non-paved area of 15.08 m^2. A positive linear relationship was found between non-paved area in proportion to the crown projection area and basal area increment, presumed to be due to the limited water infiltration into paved areas (Dahlhausen, Biber, Rötzer, Uhl, & Pretzsch, 2016). Mean annual ring width was 1.61 mm (range 0.32-7.91 mm), the lowest mean of all species compared in cities around the world (mean ring width of other species ranged between 1.63 to 5.30 mm). As such it was the slowest growing tree, reaching a DBH of *c.* 80 cm at 160 years old with a biomass of 3.5 t

per tree (Dahlhausen et al., 2016). They also found a positive relationship between stem diameter and height and crown radius. Performance in urban areas can be improved by injecting sucrose solution (50 g/l) into the soil around the roots. Percival, Fraser and Barnes (2004) found this to increase fine root dry mass (>4 mm diameter) from 0.24 g/m^3 to 1.17 g/m^3 five months after treatment. This was also seen to work for *Betula pendula* and *Quercus robur* but not *Prunus avium*.

Paulić, Drvodelić, Mikac, Gregurović, and Oršanić (2015) found that Horse-chestnuts in urban environments of Croatia, displayed a positive correlation of radial stem growth with average spring precipitation and a negative correlation with maximum spring air temperatures. Similarly, Wilczyński and Podlaski (2007), working with horse-chestnuts growing in a *Fraxino-Alnetum* community in south-central Poland and using tree ring width data from 1932-2003 from 15 trees without *Cameraria ohridella* infections (see 9.9.1), found a positive correlation of radial growth with air temperature of the previous winter (December to March) and of summer (August) in the growing season, and with precipitation the previous winter. However, excessive precipitation in August, which raised the already high water table, had a negative effect on radial growth. Warm year-round temperatures in a continental climate would go some way to mimicking temperatures in its native habitats, although the negative effect of high spring temperatures is likely due to drought and low humidity. Similarly, horse-chestnut favours moist habitats (section 2.2), so high rainfall short of causing flooding of roots would be beneficial. In native Greek populations, leaves with the smallest leaflets (8.6 ± 1.35 cm^2; SE, *n* unstated) were found in the most northerly population with the lowest mean annual temperature (7.3 °C). However, the precise effect of environmental parameters on performance is not always easy to disentangle. In Greece, leaves were significantly larger and longer in the Karitsa population than in the more northerly Perivoli population (69.22 ± 4.04 cm^2, 50.10 ± 2.26 cm^2, and 15.16 ± 0.48 cm, 13.48 ± 0.34 cm, respectively); Karitsa is at lower altitude

(Karitsa 705 m, Perivoli 915 m), had a higher mean annual temperature (12.4 °C, 10.5 °C, respectively) but lower precipitation (553 mm, 830 mm, respectively) (Walas et al., 2018).

5.3 │ Effect of frost, drought, etc

After the juvenile growth phase, horse-chestnut is relatively hardy and generally tolerates low winter temperatures well (Bellini & Nin, 2005). However, Wilczyński and Podlaski (2007) argue that long cold continental winters negatively affect the tree in the subsequent growing season since it "weakened the trees" and delayed the start of the growing season. But they also point out that horse-chestnut "survived through many frosty and long winters in the 20th century" and so cold is not seen as a lethal problem. Snow may help survival in continental climates, such as in Poland, by protecting roots and root collar from frosts (Wilczyński & Podlaski, 2007).

Large temperature fluctuations, particularly at the end of winter and into spring, have been seen to cause stem fissures in horse-chestnuts in open urban areas of central and eastern Europe. These fissures are invaded by the fungus *Schizophyllum commune* Fr. (Basidiomycota, Agaricales) which is saprotrophic but can become parasitic causing white surface rot (Snieskiene et al., 2011).

The wood anatomy of horse-chestnut suggests a moderate amount of drought tolerance. Jansen, Choat, and Pletsers (2009) measured the maximum diameter of pit membranes between vessels as 179 nm, with an air-seeding threshold of 1.62 ± 0.36 MPa, in a range of 0.95 MPa in *Betula pendula* to 2.8 MPa in *Laurus nobilis*. However, *A. hippocastanum* is often considered unsuitable for dry urban areas due to its moderate sensitivity to drought (Roloff, Korn, & Gillner, 2009; Hirons & Sjöman, 2018) and the hybrid *A. carnea* (section 8.2) is preferred in central European parks and gardens as it is more drought tolerant. Simon and Lena (2016) report that street trees in Ljubljana, Slovenia that were water stressed due to low May-July precipitation underwent delayed cambial activity

from the start of May to end of June, whereas in 'healthy' trees it was middle of April to middle of July. Juvenile horse-chestnuts are more sensitive to water stress than adults, due to their more restricted root spread, resulting in yellowing and falling of the leaves (Bellini & Nin, 2005). The resin on the 'sticky buds' is thought to help increase resistance to drought.

Grosse and Schröder (1985) looked at gas transport through stems of leafless 6-month-old trees as an indication of their ability to cope with flooding. They found that gas transport in horse-chestnut was 136% higher in the light compared to the dark due to temperature differentials, leading to increased gas exchange between root and shoot of flooded trees during the day. This compares to <25% increase in *Carpinus betulus*, *Acer pseudoplatanus*, *Fagus sylvatica* and *Fraxinus excelsior*, and a 314% increase in *Alnus glutinosa*. This suggests that horse-chestnut can cope moderately well with temporary flooding or anoxic soils (Hirons & Sjöman, 2018).

Horse-chestnut is generally tolerant of saline soils and urban salt spray (Chaney, 1991; Šerá, 2017) although some authors have described it as being sensitive to salt spray (Dobson, 1991). Horse-chestnut is more tolerant to saline soil and spray than *Fagus sylvatica* and *Tilia cordata* and to a lesser extent *Acer pseudoplatanus*. But Horse-chestnut does not tolerate long-term maritime exposure; Hill et al. (2004) list the Ellenberg value (corrected for Britain) for salt tolerance (S) of 0, indicating an absence from saline sites and a short life-span in coastal sites.

Direct application of saturated salt solutions to Horse-chestnut buds delayed bud break for up to 8 days (Paludan-Müller, Saxe, Pedersen, & Randrup, 2002). Street trees in Poznań, Poland exposed to de-icing salt had foliar Cl concentrations of 4.9 mg/g dry mass (Oleksyn, Kloeppel, Łukasiewicz, Karolewski, & Reich, 2007), 43% higher than the toxic level of 3.5 mg/g dry mass in sensitive trees (Marschner, 1995). In street trees in Berne,

Switzerland, Fuhrer and Erismann (1980) recorded foliar Cl of 8-14 mg/g dry mass in horse chestnut, associated with 25% leaf area necrosis.

Eckstein, Liese, and Ploessl (1978) found a significant reduction in annual ring width of Horse-chestnuts growing 0.5 m from a road edge in Freiburg, Germany, after salt was first used in the mid-1960s. Growth was so reduced that between 1970 and 1973 annual rings were virtually non-detectable, and by 1974 the trees were dead. Control trees away from salty roads were unaffected. Petersen and Eckstein (1988) recorded a similar decline following the use of salt in Hamburg. Trees declining from high salt levels showed a similar wood anatomy to drought-stressed trees; more but smaller xylem vessels, and smaller wood rays and fibres, which were replaced by parenchyma making the stems weaker and less effective in water transport (Eckstein, Liese, & Parameswaran, 1976; Petersen & Eckstein, 1988).

6 │ STRUCTURE AND PHYSIOLOGY

6.1 │ Morphology

Aesculus hippocastanum is a large tree, reaching 39 m height. The current largest tree in Britain is 36 m height and 187 cm DBH in Kettering, Northamptonshire. An exceptional tree in Andover, Hampshire has been recorded with a trunk of 304 cm DBH (The Tree Register, 2018). In urban areas it can commonly reach between 16 and 20m but can be up to 25 m in height (Cutler & Richardson, 1989). Branching is initially monopodial, extending shoot length from the apical buds. However, upon sexual maturity, flowers are borne at the apex of shoots, so subsequent growth is sympodial from lateral buds.

The wood is diffuse porous, with spiral grain (Pyszyński, 1977), close-grained and white. It has a low density (0.5 g/cm³), lower than many conifers, and the wood lacks strength and durability. The nitrogen content of wood at 0.32% is higher than many broadleaf species (Robinson, Tudor & Cooper, 2011), likely decreasing its resistance to

decay. As with most trees, there is an overall increase in vessel diameter and length from the small branches (28 μm and 208 μm, respectively) down to roots *c.* 15 mm in diameter (57 μm and 439 μm, respectively) but vessel density becomes less, reducing from 400 mm^{-2} in small branches to 53 mm^{-2} in roots (Poole, 1994). Tyloses (outgrowths of parenchyma cells into the vessels of heartwood) are not formed (Barnett, Cooper & Bonner, 1993). The bark has a small proportion of lenticels with embedded waxes (3%), compared to up to 35% in other species tested (Groh, Hübner, & Lendzian, 2002). The ratio of water vapour loss through lenticels from which waxes were extracted using chloroform compared to control lenticels was 1.7 suggesting that the waxes may reduce water loss. However, Groh et al. (2002) concluded that this did not affect water loss through the bark to a large degree.

Roots can be shallow to moderately deep, depending upon the soil type, and, as befitting a large tree, can spread a considerable distance beyond the crown. Cutler and Richardson (1989) record the maximum distance that a building has been damaged by horse-chestnut roots on clay soils as 23 m with 90% of cases being within 15 m of the tree. This is similar to *Acer pseudoplatanus* and *Ulmus* spp. and not far short of *Quercus* spp. (maximum distance 30 m, 90% of cases within 18 m). Fine root (<0.8 mm) biomass in Poland has been recorded at 223-474 g/m^2 soil, fine root volume at 615-1225 cm^3 m^{-2} soil, and number of fine root tips at 553,000 to 1.5 million/m^2 (Karliński et al., 2014).

The large terminal leaf bud consists of 7-8 pairs of cataphylls enclosing the whole of next year's shoot, usually made up of 3-4 pairs of foliage leaves, and often 1-2 pairs of scale primordia of the following year's terminal bud (Foster, 1929b). As the bud opens, there is a gradual transition from the basal cataphylls to cataphylls with a small green lamina through to normal leaves at the top (Foster, 1929a, b). Cataphylls contain chlorophyll and can photosynthesise (Solymosi, Bóka & Böddi, 2006). Specific leaf area in western Romania has been seen to change from 389 cm^2/g in April to 250 cm^2/g in

September as leaves become progressively thicker as they complete development (Ianovici, Latiş, & Rădac, 2017). This is matched by the mean ash content rising from 7.9 and 10.7%.

Horse-chestnut leaves are hypostomatous with stomata only on the abaxial (underside) leaf surface (Meidner & Mansfield, 1968; Weryszko-Chmielewska & Haratym, 2012). Stomatal density has been seen to vary across Europe from 118 to 298/mm^2. Some of this variation is due to geographical location. Cetin, Sevin, and Yigit (2018) gave a mean density of 198/mm^2 across Turkey with individual stomata 24.29 x 16.23 µm in size. However, they varied from 298/mm^2, 16.55 x 9.54 µm in the temperate ecoregion (Central Anatolia) to 177/mm^2, 37.28 x 27.02 µm in the Mediterranean ecoregion, to 118/mm^2, 19.03 x 11.30 µm in the high precipitation of the Black Sea ecoregion. Position within the crown is also important; in north Bosnia and Herzegovina, Oljača, Govedar & Hrkić (2009) recorded stomatal density of 293-372/mm^2 at edge of the crown in full light, and 169-230/mm^2 on an inner, shaded part of the crown. Similarly, Boldt & Rank (2010) found density to vary from 146/mm^2 at the base of the crown to 321/mm^2 in the middle and 322/mm^2 at the top. Boldt & Rank (2010) also found that stomata can vary in size within a leaf, with 3-10% of the stomata being 'giant' sized, reaching 25 µm to >40 µm long, mixed with those of *c.* 20 µm long. The proportion of giant stomata varied between the base of the crown (9.6%), the middle (4.7%) and top (7.5%). These large stomata are common in many woody species, particularly in the tropics, but are more pronounced in *Aesculus* than in other temperate species (Boldt & Rank, 2010). Weryszko-Chmielewska and Haratym (2012) observed that horse-chestnut leaves in Poland had a few glandular trichomes on the adaxial leaf surface with a mean length of 84 µm and head diameter of 61 µm while the abaxial surface had non-glandular trichomes 116-436 µm long.

6.2 │ Mycorrhiza

Arbuscular mycorrhizal fungi are present on the roots of Horse-chestnut (Harley & Harley, 1987) but, unlike many woody plants, there is no evidence of ectomycorrhizas. Bainard, Klironomos, and Gordon (2011) found that *c.* 40% of roots tips of horse-chestnut were mycorrhizal in rural trees and *c.* 17% in urban areas of southern Ontario (estimated from a figure), similar to many other arbuscular mycorrhizal trees. However, Karliński et al. (2014) found no difference between rural and urban sites in Poland, colonisation ranging from 54-73% of root tips. The differences may be due to Bainard et al. (2011) sampling 20-35 year-old trees in May/June while Karliński et al. (2014) sampled *c.* 100 year-old trees in November, since age and/or establishment does appear to affect mycorrhizal colonisation. Ferrini and Fini (2012) inoculated horse-chestnut trees in Milan with a mixture of arbuscular fungi growing in heavily compacted soil. One year after inoculation the frequency of arbuscular roots was not significantly different between inoculated and control newly-planted trees 6-8 cm diameter (51-59%), but in mature trees (38-51 cm diameter) colonisation was significantly higher in inoculated trees (76%) compared to control trees (63%). However, in both age groups, shoot growth in the third growing season was significantly longer in mycorrhizal trees than in controls (mature trees: mycorrhizal 8.8 cm, control 5.7 cm; young trees: mycorrhizal 13.7cm; control 12.1 cm). This supports the suggestion that mycorrhizal inoculation in urban trees is worthwhile.

Karliński et al. (2014) found 1-9% of root tips colonised by fungal endophytes, low compared to that found in other broadleaved trees (Mandyam & Jumpponen, 2005).

6.3 │ Perennation: reproduction

Phanerophyte. Reproduction is primarily by seeds but occasionally it will spread vegetatively from new shoots growing from adventitious buds on the roots of established trees up to 4-5 m from the trunk (Czekalski, 2005). Hill et al. (2004) classified horse-

chestnut as not spreading clonally but Howard (1945) noted that branches that touch the ground may root and produce new shoots. He describes such a tree at Hawkhurst Moor, Kent with a height of 27.4 m and a combined crown 27.6 m in diameter. Theoretically such a tree can keep spreading laterally by the production of new semi-autonomous stems as the central stem(s) die. Layering has been confirmed in natural populations especially on rocky and steep slopes; for example, genetic analysis of 114 trees found 94 genotypes (Walas et al., 2018 unpublished data; personal communication from Monika Dering and Grzegorz Iszkuło). Horse-chestnut will also produce new stems on cut stumps and so will coppice (Czekalski, 2005).

Horse-chestnut can be grafted onto 1- or 2-year-old seedlings using dormant apical scions in mid-winter. Budding is also successful in late summer using medium size buds from the middle of a branch (McMillan-Browse, 1971). It can also be propagated using semi hardwood cuttings (Chapman & Hoover, 1982), though this is uncommon. Horse-chestnut has been used as a rootstock in the grafting of other *Aesculus* species planted in Britain, such as *A. octandra* and *A. carnea* (Leathart, 1991).

Tissue culture has been successfully used to produce horse-chestnut plants from a variety of somatic and gametic sources including microspores (Radojević, 1978; Radojević, Marinkovic & Jevremovic, 2000), anther filaments (Jörgensen, 1989; Kiss, Heszky, Kiss, & Gyulai, 1992; Capuana, 2016), embryos (Profumo, Gastaldo, Bevilacqua, & Carli, 1991b; Troch, Werbrouck, Geelen, & Van Labeke, 2009), and leaf and stem explants (Dameri, Caffaro, Gastaldo, & Profumo, 1986; Gastaldo, Carli, & Profumo, 1994; Šedivá, Vlašínová, & Mertelík, 2013). Embryogenic tissues can also be cryopreserved for long-term storage (Jekkel, Gyulai, Kiss, Kiss, & Heszky, 1998; Wesley-Smith, Walters, Pammenter, & Berjak, 2001; Lambardi, De Carlo, & Capuana, 2005), overcoming the storage problems of recalcitrant seeds - section 8.4 (Pence, 1990). Anther and microspore culture has been used to produce haploid plants in horse-chestnut (Ćalić-Dragosavac,

Stevović & Zdravković-Korać, 2010). Up to 10-12% of the embryos produced by these methods were found to be albino, particularly when grown under short days of 8 hours light, but the proportion could be reduced by the addition of abscisic acid to the cultures (Ćalić et al., 2013). Procedures for genetically modifying *A. hippocastanum* embryos using the bacterium *Agrobacterium rhizogenes* have been devised by Zdravković-Korać, Muhovski, Druart, Ćalić, and Radojević (2004).

Flowers are first produced at 10-15 years old. Horse-chestnut normally lives for a maximum of 150-200 years (Maurizio & Grafl, 1969) but can survive for 300 and exceptionally 500 years (Leathart, 1991; Fitter & Peat 1994; Mitchell, 1997).

6.4 │ Chromosomes

2n = 40 (Hardin, 1960; Bennett, Smith & Heslop-Harrison, 1982). The hybrid, *A. carnea* (section 8.2), is a tetraploid (2n = 80) according to Hoar (1927) and Hardin (1957). A backcross between *A. carnea* and *A. hippocastanum* is reported with 2n = 60 (Upcott, 1936).

Skovsted (1929) noted that the chromosomes of *A. hippocastanum* were relatively small (*c.* 0.5 μm, estimated from a figure) while those of *A. pavia* were larger (*c.* 1 μm), and that *A. carnea* had a mix of both sizes. However, this appears to be an artefact of sample preparation since subsequent measurements have found the chromosomes in all three taxa to be similar at 1-2 μm long (Upcott, 1936; Pogan, Wcislo, & Jankun, 1980).

Aesculus hippocastanum has a comparatively small nuclear genome size at 1.22 ± 0.010 pg (2C) for a woody angiosperm, but such a small genome size is not unusual among woody species with large seeds (Krahulcová, Trávníček, Krahulec, & Rejmánek, 2017).

6.5 │ Physiological data

Horse-chestnut grows best in sunny, sheltered locations and is classified as shade-intolerant (Fitter & Peat, 1994) but it can tolerate partial shade (Jagodziński, Łukasiewicz, & Turzańska, 2003), especially as saplings. Young stems contain chlorophyll and are capable of photosynthesising (Skribanek, Apatini, Inaoka, & Böddi, 2000); the protochlorophyllide content (a precursor of chlorophyll) of horse-chestnut twigs was comparatively high at *c.* 10 µg/g fresh mass, compared to 3-5 µg/g in *Acer campestre.* In full sunlight, net photosynthetic rate has been measured in north-east Italy at 5-9 µmol [CO_2] m^{-2} s^{-1}, stomatal conductance at *c.* 105 mmol m^{-2} s^{-1}, transpiration rate at *c.* 2.4 mmol m^{-2} s^{-1} and leaf hydraulic conductance at 4.5 kg s^{-1} m^{-2} MPa^{-1} (estimated from figures in Raimondo, Ghirardelli, Nardini, & Salleo, 2003; Nardini, Raimondo, Scimone, & Salleo, 2004). Stomatal conductance in full sunlight in Gothenburg, Sweden was measured by Konarska et al. (2016) at *c.* 80-90 mmol m^{-2} s^{-1}, a similar figure to other deciduous trees tested such as *Betula pendula, Fagus sylvatica* and *Tilia × europaea*, but lower than *Quercus robur* (*c.* 200-210 mmol m^{-2} s^{-1} – estimated from figures). Horse-chestnut should not therefore be at a disadvantage in low humidity conditions compared to many native trees.

Drought tolerance of horse-chestnut can be improved by spraying trees with the triazole (fungicide) derivatives paclobutrazol, penconazole, epixiconazole and propiconazole. Percival and Noviss (2008) treated 4-year-old saplings transplanted into pots that two weeks later were exposed to a three-week drought. Spraying reduced visible leaf necrosis by 33–83% compared to drought-treated but unsprayed controls, and electrolyte leakage (a measure of cell membrane damage) by 36–64%, depending upon the fungicide used. Chlorophyll fluorescence ratio (Fv/Fm) increased 59-121% and light-induced CO_2 fixation increased by 16–137%. Leaves of treated trees also had higher concentrations of total carotenoids (29-2891%) chlorophylls (53-288%) proline concentrations (42-109%), superoxide dismutase (23-118%) and catalase (24-133%)

activities than drought-treated control trees. Treated trees also showed faster recovery after drought in the above characteristics. The triazole myclobutanil had no effect.

Horse-chestnut planted in poor soils in urban environments, responds well to nutrient addition. Oleksyn et al. (2007) investigated street trees in Poznań, Poland with a mean height of 13.6 m and 0.53 m diameter. Trees were given a mulch of organic matter (chipped tree waste stored for one year) and nutrient applications over three years of 17 g N m^{-2} $month^{-1}$ for two years, then 7 g N and 16 g S m^{-2} $month^{-1}$ for one further year. The treatments increased foliar N by 36% from 1.7 to 2.7% compared to control trees. Total phenolic concentration in treated trees was 43% lower than in control trees after 3 years of treatment (mean 256 µmol/g in control and 193 µg/g in treated) suggesting lower investment in secondary defence compounds in trees with better nutrition. Net photosynthesis per unit area and unit mass both increased by 21-30% in treated sites. Leaves became bigger (3.3 vs 1.8 g/leaf; 386 vs 248 cm^2/leaf) and thicker (specific leaf area 119 vs 141 cm^2/g). Leaves also remained on the tree longer (175 vs 130 days) and treated trees produced more seeds (>100 seeds vs <12 seeds/tree) but this was still half that of trees growing in the better soil of the nearby botanic garden.

6.6 │ Biochemical data

Various parts of *Aesculus hippocastanum* contain high levels of triterpene glycosides or saponins, including aescigenin, hippocaesculin and barringtogenol (Konoshima & Lee, 1986), the mix collectively called Aesculin, Aescin or Escin; these have medical uses (section 10.1). Horse-chestnut also contains tannins, carotenoids (including aesculaxanthin, lutein and citraurin), fatty acids (including lauric, myristic, palmitic, stearic, arachic and oleic acids), at least 10 coumarin derivatives (including esculentin), and at least 15 flavonoids, mainly glycosides of quercetin, leucocyanidine, procyanidin and kaempferol (Czeczuga, 1986; Morimoto, Nonaka, & Nishioka, 1987; Yoshikawa,

Murakami, Yamahara, & Matsuda, 1998; Deli, Matus & Tóth, 2000; Birtić & Kranner, 2006; Kapusta et al., 2007; Zhang et al., 2010; Dudek-Makuch & Matławska, 2011; Coruh & Ozdogan, 2014; Kędzierski, Kukula-Kocha, Widelski, & Głowniak, 2016; Kim et al., 2017; Turkekul, Colpan, Baykul, Ozdemir, & Erdogan, 2018) and polyprenols including undecaprenol, tridecaprenol and particularly dodecaprenol and castoprenol (Wellburn, Stevenson, Hemming, & Morton, 1967; Khidyrova & Shakhidoyatov, 2002). The highest concentration of most chemicals is in the seeds, particularly the cotyledons but they are also found in the fruit, bark, leaves and buds (Bombardelli, Morazzoni, & Griffini, 1996; Otajagić, Pinjić, Ćavar, Vidic, & Maksimović, 2012) and in embryonic callus tissue (Profumo, Caviglia, Gastaldo, & Dameri, 1991a). Abudayeh, Al Azzam, Naddaf, Karpiuk, and Kislichenko (2015) investigated seeds from Poland and found lower levels of saponins in the seed coat (0.19-0.32 g/kg) than the seed's endosperm (34.9-52.05 g/kg). The levels decreased by >30% in the endosperm and >40% in the skin when stored air-dried for two years.

The sterols and a number of monoterpene phenols (e.g. carvacrol) in bark, quercetin and kaempferol in seeds, and at least 17 phenolic compounds in leaves (Hübner, Wray & Nahrstedt, 1999; Oszmiański, Kalisz, & Aneta, 2014) are known to have antifeedant properties against insects (Eriksson, Månsson, Sjödin, & Schlyter, 2008). The highest concentration of coumarins is in the bark, particularly during summer, lower in spring and autumn (Matysik, Glowniak, Soczewiński, & Garbacka, 1994), emphasising their role as antifeedants.

The buds of horse-chestnut are renowned for being 'sticky'. The abundant resin causing the stickiness contains relatively small amounts of flavonoids (13.0%), larger amounts of triterpenoids (43.4%) but, most distinctively, high level of C_{14}–C_{22} aliphatic 3-hydroxyacids (20.1%). However, the resin had lower antimicrobial activity against gram-positive bacteria than resins of other deciduous trees, such as *Betula* spp, and *Pinus*

sylvestris (Isidorov, Bakier, Pirożnikow, Zambrzycka, & Swiecicka, 2016). Leaves have epicuticular wax containing large amounts of triterpenols and triterpenol esters including ß-amyrin, a-amyrin, lupeol, friedelanol and friedelanone (Gülz, Müller & Herrmann, 1992). Despite the abundant wax, Papierowska et al. (2018) found that, based on the angle of contact of water droplets, that the adaxial surface of horse-chestnut is 'wettable' and the abaxial 'highly wettable'. This is similar to that found in *Acer pseudoplatanus* and *Betula pendula* but in contrast to 14 of the 19 European deciduous species tested where the abaxial surface was the least wettable. Although high wettability may result in a film of water reducing gas exchange, the authors suggest that it also allows water droplets to spread out and quickly evaporate and so leaves quickly dry, reducing the time that pathogens have a moist surface to invade, and so lower the need for internal chemical defences. This may be linked to the low precipitation of its native climate where gas exchange problems would be less frequent.

Seeds contain 30-60% starch, 6-11% protein, 4-8% lipids and 8-26% saponins (Duke & Ayensu, 1985; Lemajić, Savin, Ivanić, & Lalić, 1985; Baraldi et al., 2007; Čukanović et al., 2011) but these are variable between populations. Seeds from southern Bulgaria contained 81 ± 3 g/kg (SD) of oils, including relatively high levels of unsaponifiable compounds (57 ± 1 g/kg), sterols 12 ± 2 g/kg) phospholipids (3 ± 0.1 g/kg) and tocopherols (627 ± 15 mg/kg) (Zlatanov, Antova, Angelova-Romova, & Teneva, 2012). The starch has a low amylose content (Hricovíniová & Babor, 1992). Seeds also contain sugars; thirteen compounds have been identified including glucose, sucrose, fructose, amylosaccharlde, galactosylsucrose, and fructosylsucrose (Kahl, Roszkowski & Zurowska, 1969; Kamerling & Vliegenthart, 1972; Hricovíniová & Babor, 1991).

Pollen grains of horse-chestnut contain moderate amounts of antioxidants, although total phenols (3,375 mg 100/g) flavanols (624 mg/g) and anthocyanins (183 mg/g) were found to be lower than in other woody species tested (*Robinia pseudoacacia, Malus*

domestica and *Pyrus communis*) by Leja, Mareczek, Wyżgolik, Klepacz-Baniak, and Czekońska (2007).

Atmospheric levels of heavy metals are reflected in their concentration on and in leaves and bark (Table 1). Levels in Turkey were comparatively low compared to levels in the soil (Pb, 0.81-6.75; Cd, 0.002-0.006; Zn 2.20-4.60; Cu 0.52-1.12 μg/g dry weight; Yilmaz, Sakcali, Yarci, Aksoy, & Ozturk, 2006). Levels in Turkey tend to be lower than in Bulgaria and Serbia (Table 1); similar figures for Serbia are also found in Tomašević et al. (2004) and Deljanin et al. (2016). Washing leaves reduced levels of Pb, Ca, Zn and Cu, indicating that these are primarily surface particles (Yilmaz et al., 2006). Levels of Pb, Zn and Cu were very high in New Zealand, reported in Kim and Fergusson (1994) before lead-free petrol became legally compulsory. In the same leaded-petrol era in Scotland, Guha and Mitchell (1966) found that Pb decreased towards top of crown consistent with petrol origin. Aničić, Spasić, Tomašević, Rajšić, and Tasić (2011) found that horse-chestnut street trees in Serbia accumulated more heavy metals than did *Tilia* spp. and so are considered a better species for the assessment of Pb and Cu atmospheric pollution. Some studies have shown that levels of heavy metals in leaves increase through the growing season (e.g. Kim & Fergusson, 1994) while others that they decrease (e.g. Šućur et al., 2010), depending upon the relative uptake from the soil and atmosphere, and seasonal changes in atmospheric pollution.

Uptake of radionucleoitides from the soil is generally very low; the highest recorded for horse-chestnut was ^{40}K, with a 1.3 soil:leaves transfer factor, giving a concentration of 487 Bq/kg, similar to that seen in *Tilia* spp. (Todorović, Popović, Ajtić, & Nikolić, 2013). However, one year after the Chernobyl reactor accident on 26 April 1986, horse-chestnut leaves contained the highest levels of ^{40}K of any of the land plants tested (670 ± 15 Bq/kg dry mass; SD); 2 years after the accident, levels had declined to 325 Bq/kg of ^{40}K along with 37 Bq/kg of ^{212}Pb and 11 Bq/kg of ^{208}Tl (Heinrich, Müller, Oswald, & Gries, 1989).

Indeed, horse-chestnut leaves have proved useful in tracking Pb isotopes as leaded-petrol was phased out (Tomašević et al., 2013). Yoshihara et al. (2014) have shown that Cs-137 resulting from the Fukushima reactor accident in Japan can be tracked in leaves of a variety of trees, including horse-chestnut growing nearby.

7 │ PHENOLOGY

The cambium of Horse-chestnut begins cytoplasmic activity in mid-February; the first cell divisions on the phloem are produced in early April, and the first xylem elements are formed in the middle of April (Barnett, 1992). Radial growth continues until August in Poland (Wilczyński & Podlaski, 2007). Jagiełło et al. (2017) identified that in saplings *c.* 85% of the whole-year stem volume increment occurred before the end of July.

The winter chilling requirement for buds to flush (321 degree-days above 0 °C) is similar or lower than many deciduous trees native to Britain (Laube et al., 2014). Leaf buds open in mid-April (Hutchings, Lawrence & Brunt, 2012), 2-3 weeks earlier than *Quercus robur* (Fu, Campioli, Van Oijen, Deckmyn, & Janssens, 2012). Sparks, Jeffree, and Jeffree (2000) found the mean leafing date in Britain using a 20-year record was 10 April (earliest -10 days; latest + 15 days). In Poznań Botanical Garden, Poland the mean leafing date was 5 days later (Sparks, Górska-Zajączkowska, Wójtowicz, & Tryjanowski, 2011). The spring flushing of buds in horse-chestnut is primarily controlled by February and March temperatures in Britain, and March to May temperatures in Poland (Tryjanowski, Panek & Sparks, 2006; Menzel et al., 2008) but it is also said to have a photoperiod requirement (Basler & Körner, 2012) that develops just before leaf flushing (Laube et al., 2014). Zohner and Renner (2015) found that budburst was delayed by 4 days when branches on mature trees were given 8-hour days rather than 16-hour days. This compares to a 41-day delay in *Fagus sylvatica* and no delay in *Picea abies*. Zohner and Renner (2015) concluded that the delay in budburst in horse-chestnut under short days is simply a consequence of

slower growth as a result of lower light availability rather than a photoperiod requirement itself. There appears to be no elevational change in photoperiod requirement (Basler & Körner, 2012).

Flowering in Britain usually lasts between late April and the middle of May (Tryjanowski et al., 2006). Jeffree (1960) identified mean start of flowering as 9 May +/- 5 days using a 35-year dataset, and Sparks et al. (2000) 8 May using a 58-year dataset with the earliest -20 days and the latest +16 days. In western Poland, mean first flowering date over 20 years was 6 May (Sparks et al., 2011). Pollen production (taken to indicate longevity of flowering) from five trees in Lublin, Poland between 2002 and 2009 lasted for 18 to 42 days with a mean of 28 days (Weryszko-Chmielewska et al., 2012). Anthesis is at a maximum between 13-18 °C air temperature, compared to 10.2-18 °C in *Crataegus monogyna*, and is much reduced during rain (Percival, 1955). Maximum pollen release was 6-22 May in Poland (Weryszko-Chmielewska et al., 2012).

A second flowering was seen in horse-chestnut in September 2000 in Munich and Frankfurt, Germany (Heitland & Freise, 2001) and has been noticed sporadically in Slovenia (Menzel et al., 2008). In Bulgaria a second flowering has traditionally been seen as a prediction of a severe winter (Nedelcheva & Dogan, 2011). Second flowering is probably a response to a hot, dry summer but is suggested to also be in response to damage caused by the leaf miner *Cameraria ohridella* and the fungus *Guignardia aesculi* (Menzel et al., 2008).

Saponins and flavonoid content reach their highest levels in seeds in August, 13-16 weeks after the beginning of flowering (Kędzierski et al., 2016). Seeds fall from mid-September (Farrant & Walters, 1998; Sparks et al., 2011), especially during autumn gales. In western Romania, horse-chestnut keeps its leaves for 130-175 days (Ianovici et al., 2017). Leaf colouring usually begins at the end of September (Sparks et al., 2011; Hutchings et al., 2012) but a warm May (in Germany) and June (in Slovenia) and a dry

September leads to earlier leaf colouring while a warm September delays colouring (Estrella & Menzel, 2006; Menzel et al., 2008). Leaves begin to fall in the first half of October, and leaf fall generally lasts 28 days so trees are bare of leaves by the beginning of November (Sparks et al., 2011; Hutchings et al., 2012).

7.1 | Climate change

Modeling based on the natural range of horse-chestnut suggests that the distribution and abundance of horse-chestnut should not change significantly in the Balkan Peninsula under current climate change scenarios (Walas et al., 2018). However, horse-chestnut is sensitive to spring warming and this is leading to rapid changes in spring phenology (Walther et al., 2002; Tryjanowski et al., 2006; Menzel, Estrella & Testka, 2005). Some of this is due to local changes; for example, bud burst of horse-chestnut has become earlier in central Geneva since 1808 at the rate of 0.23 days/year, attributed to the heat island effect of the growing city (Defila & Clot, 2001). Nevertheless, climate change does appear to be having an effect. Chen et al. (2018) looked at five deciduous European trees, including horse-chestnut, across Europe and found that at low altitude (<10 m) leaf opening advanced between 1951 and 2013 by *c.* 2.4 days per decade (estimated from a figure), whereas at high altitude (800-1000 m) until 1980 spring became later (+2.7 ± 0.6 days per decade, SD), and then advanced again *c.* 2.7 days per decade (estimated from a figure). But the rate of change appears to be slowing. Fu et al. (2015) looked at the number of days' advance of leaf unfolding per °C of warming across Europe and found that horse-chestnut advancement had changed from 4.2 ± 1.5 (SD) days/°C in 1980-1994 to 2.1 ± 1.5 days/°C in 1999-2013, a reduction of 2.1 days/°C, the most of the seven species tested. This was attributed at least in part to the reduced chilling the trees are getting due to shorter and milder winters, despite chilling requirements becoming shorter. The winter chilling requirement of horse-chestnut has declined across central Europe from

c. 68 days in 1980-1994 to *c.* 62 days in 1990-2013 (taken from a figure); chilling was calculated as chilling days when daily temperature was between 0 °C and 5 °C from 1 November to the average date of leaf unfolding (Fu et al., 2015).

In Germany, a 1°C increase in temperature resulted in advances of horse-chestnut flowering date by a mean of 2.6 days, particularly noticeable in areas that normally flowered early anyway (Menzel et al., 2005) and in Poland, flowering has been getting earlier at the comparatively small amount of 0.07 days per year (Jabłońska, Kwiatkowska-Falińska, Czernecki, & Walawender, 2015). Interestingly, there has been no apparent advancement in the start of fruit ripening despite leaf colouring beginning earlier (Menzel, Estrella & Fabian, 2001).

The damage to leaves caused by the horse-chestnut leaf-miner *Cameraria ohridella* has reduced the length of the growing season of horse-chestnut in Slovenia by 12 days/decade since 2000 (Menzel et al., 2008) and this is undoubtedly also occurring elsewhere in Europe. This shortening is tending to counteract the effects of climate change lengthening the growing season. Similarly, Jochner et al. (2015) stated that increased ozone pollution in cities is delaying leaf flushing and flower opening of horse-chestnut (although NO_x are not) but data are not given.

8 │ FLORAL AND SEED CHARACTERS

8.1 │ Floral biology

The large panicle has male flowers at the top and hermaphrodite flowers below. In Polish samples, 73% of flowers were male and 27% of flowers hermaphrodite (Weryszko-Chmielewska & Chwil, 2017). However, some flowers at the base of the panicle can be functionally female (Maurizio & Grafl, 1969; Ćalić-Dragosavac, Zdravković-Korać, Miljković, & Radojević, 2009). In Serbia, Ocokoljić, Vilotić, and Šijačić-Nikolić (2013) found 50% male, 28% hermaphrodite and 22% female flowers.

Aesculus hippocastanum is andromonoecious, amphimictic and normally cross pollinated (Fitter & Peat, 1994). Large bees such as *Bombus* spp. (Hymenoptera, Apidae) tend to work their way upwards on a panicle from female, to hermaphrodite to male flowers, helping to reduce self-pollination (Kevan, 1990). Hermaphrodite flowers are protogynous but the whole inflorescence is protandrous (Fitter & Peat, 1994).

Horse-chestnut is primarily pollinated by insects and is often regarded as an important bee plant as the flowers provide abundant nectar and pollen for insects (Maurizio & Grafl, 1969) and glandular trichomes on the sepals and ovary produce olfactory attractants (Chwil, Weryszko-Chmielewska, Sulborska, & Michońska, 2013). Synge (1947) lists horse-chestnut as an important source of pollen early in the year, before *Tilia* spp. flower. Pollinators are honeybees *Apis mellifera* and bumble bees (Percival, 1955; Free, 1963; Maurizio & Grafl, 1969; Weberling, 1989) but flowers are also visited by hoverflies (Kugler, 1970), solitary bees such as *Osmia* spp. (Raw, 1974) and some mining bees in *Andrena* (Hymenoptera, Apidae) (Chambers, 1968).

Nectar is found in both hermaphrodite and male flowers and is released as the buds open. A flower secretes a mean of 2.64 ± 0.94 mg (SD) of nectar in Poland (Weryszko-Chmielewska & Chwil, 2017), or *c.* 1.2 μl in Belgium, compared to *c.* 0.8 μl in *Tilia* × *europaea* and *c.* 1.7 μl in *T. cordata,* estimated from a figure (Somme et al., 2016). The nectar contains a comparatively low amount of sugar, *c.* 25-32%, similar to *Tilia tomentosa* and *A. carnea*, but low compared to >60% in *Robinia pseudoacacia* and *Tilia* × *europaea*. Sucrose makes up 90% of the sugars, which is highly attractive to bees (Percival, 1961; Somme et al., 2016; Weryszko-Chmielewska & Chwil, 2017).

An inflorescence produces a total of 1 mg of pollen at the rate of 0.5 mg day^{-1} (Percival, 1955), high compared to the other tree species tested (e.g. totals of 0.3 mg in *Ilex aquifolium* and 0.8 mg in *Crataegus monogyna*). Stamens are normally bent downwards but become erect when shedding pollen, presumably as a mechanism for

aiding pollen removal by insects. Percival (1955) classified horse-chestnut as a 'chiefly morning' flowerer, presenting pollen for honey bees from 5 am to 6 pm with the peak period at 5 am when 20% of the day's pollen was presented; 63% of its total pollen is presented by midday. Anthers in a flower dehisce over one to several days, similar to other insect-pollinated trees such as *Prunus* spp. and *Crataegus monogyna*. Horse-chestnut pollen has 39.5 ± 7.0 (SD) µg/mg of polypeptide, 331.7 ± 27.1 µg/mg amino acid content and 4.93 to 5.07 µg/mg sterol content, similar to the 8 other hardwood trees commonly grown in parks in Belgium (Somme et al., 2016). The red connective protrusions at each end of the anthers secrete droplets which contain lipids, and thus may also act as food bodies (Weryszko-Chmielewska & Chwil, 2017).

Bee deaths have been reported when fed horse-chestnut pollen and nectar (Maurizio, 1945) which may be due to the high saponin content (section 6.6). The cause may also possibly be due to the presence of mannose or nicotine (Somme et al., 2016) although Detzel and Wink (1993) have found honey bees to tolerate low concentrations of alkaloids including nicotine. Bees have been seen to prefer flowers with lower saponin content (Maurizio, 1945; Schulz-Langner, 1967).

Pollinators are attracted to flowers by yellow floral guide spots on the petals. Once the flower is pollinated these turn to red and nectar (Lunau, 1996; Willmer, 2011) and scent production is greatly reduced or stopped (Lex, 1954). The red spots are unattractive to insects (Kugler, 1936) and are presumed to be a mechanism for not wasting the bee's efforts on flowers that are already pollinated. It is suggested that this colour change occurs, rather than petals falling once pollinated, to maintain the large visual signal of a tree to attract pollinators across large distances in mountainous habitats with dispersed populations (Thomas, 2014). Both ends of the anthers have red appendages and the upper part of the style and the stigma are similarly red coloured. These markings may also act of pollinator guides (Weryszko-Chmielewska et al., 2012).

Pollen of horse-chestnut is round to oval and very distinctive with coarse spines (Pozhidaev, 1995). Size ranges from 14 to 30 µm diameter, varying with bud size and position in the inflorescence (Ćalić-Dragosavac et al., 2009; Radojević, 1989; Ćalić, Zdravković-Korać, Pemac, & Radojević, 2003-2004). In material collected from 125-year-old trees in Serbia, Ćalić and Radojević (2017) estimated there to be between 3,600 and 5,000 pollen grains per anther, depending on genotype, with viability determined by staining with fluorescein diacetate to be 56-68%, and by germination on basic medium to be 50-66%. By contrast, Kugler (1970) calculated 26,000 pollen grains per anther, 181,000 per flower and thus 42 million pollen grains from a single inflorescence.

Pollination is primarily by insects but due to the large number of pollen grains produced, wind pollination is considered a viable supplement. Certainly air-borne pollen has been detected in many European countries, averaging 8 to 69 pollen grains/m^3 of air during the flowering season (Popp et al., 1992; Biçakci, Benlioglu & Erdogan, 1999; Weryszko-Chmielewska et al., 2012). Studies across Europe have shown that horse-chestnut pollen in urban areas accounts for 0.13-1.54% of total air-borne pollen (Peternel, Čulig, Mitić, Vukušić, & Šostar, 2003; Stefanic, Rasic, Merdic, & Colacovic, 2007; Rizzi-Longo, Pizzulin-Sauli, Stravisi, & Ganis, 2010; Melgar et al., 2012), high enough densities to cause an allergic reaction in children in Vienna (Popp, et al., 1992) and presumably high enough to supplement insect pollination.

8.2 │ Hybrids

Aesculus hippocastanum is known to hybridise with the four North American species of Section *Pavia* when they are grown together - *A. pavia* L., *A. glabra* Willd., *A. flava* Sol. (=*A. octandra* Marsh.) and *A. sylvatica* L. (Hardin, 1957). The only hybrid commonly found in Europe is the red horse-chestnut, *A. carnea* Willd. (=*A. rubicunda* Lodd., *A. rubicunda* Loisel., *A. intermedia* Andre.), a hybrid of *A. hippocastanum* and *A. pavia* (note that as a

fully fertile hydrid, the species name is not preceded by 'x' by convention). This hybrid is fertile and breeds true (linked to being a tetraploid). It is often grafted onto *A. hippocastanum* for vigour. It was first grown in Britain around 1818 (Leathart, 1991) and was recorded in the wild by 1955 (Preston et al., 2002) and is now occasionally self-sown in Surrey, West Kent, and North Hampshire (Stace, 2010). A cultivar, *A. carnea* 'Briotii', produced in France in 1958, has brighter red flowers and glossier, more attractive leaves than the original hybrid, and is widely planted (Leathart, 1991). Irzykowska, Werner, Bocianowski, Karolewski, and Frużyńska-Jóźwiak (2013) found, perhaps unsurprisingly, that genetic diversity was higher in *A. hippocastanum* (mean genetic similarity of 0.55) than in *A. carnea* (0.98). The majority of the genetic variance (73.0%) was contributed by the differentiation between *A. hippocastanum* and *A. carnea*, whereas 27.0% was partitioned within species. Hardin (1960) also lists a triploid backcross between *A. carnea* and *A. hippocastanum* named *A.* × *plantierensis*.

8.3 │ Seed production and dispersal

There are typically 2-5(8) fruits per panicle, each containing one (rarely two or three) seeds (Thalmann et al., 2003). But the number of panicles is very variable, giving a seed production of from 2-3 kg to 25 kg of fresh seeds per tree (Bellini & Nin, 2005), which equates to approximately 125 to 1,600 seeds per tree. Horse-chestnut has shown masting, with large seed crops produced every two years in natural populations in Greece (Tsiroukis, 2008). This was seen to be synchronized throughout its distribution range in Greece.

Mean fruit mass has been measured at 42.14 g in natural populations in Greece (Tsiroukis, 2008). Seeds are large, each typically 13-20 g fresh mass (Daws et al., 2004; Bonner & Karrfalt, 2008), although are largest (15.3-22.6 g) in street trees in Serbia (Ocokoljić & Stojanović, 2009; Ocokoljić et al., 2013), but smaller (mean 9.9-14.5 g) in

natural populations in Greece (Daws et al., 2004; Tsiroukis, 2008) and smallest (1.2 g) at the northern end of its planted range in Scotland (Daws et al., 2004).

Seeds are primarily dispersed by gravity (barochory), with seeds falling from the fruits more or less under the crown of the mother tree. Little is known about distances moved by horse-chestnut but seeds of the closely related *A. turbinata* were found to disperse a mean of 12.2-44.7 m from the parent trees during a 3-year study in Japan, with a maximum distance of 41.5-114.5 m (Hoshizaki, Suzuki, & Nakashizuka, 1999). It is likely that dispersal distances are similar in *A. hippocastanum*. *Aesculus turbinata* seeds are known to be dispersed by rodents (Hoshizaki et al., 1999) and this is likely to happen, to some extent at least, in horse-chestnut. Seedlings have been seen far away from mature trees in Greece with rodent movement the most likely cause (personal communication from Monika Dering and Grzegorz Iszkuło). Ridley (1930) records that even the removal of one cotyledon by rats does not prevent at least initial stages to germinate such as radical elongation. In Britain, other vectors undoubtedly include grey squirrels (*Sciurus carolinensis* Gmelin), corvids (Briggs, 1989) and children collecting and ultimately discarding conkers. There is also a suggestion that seeds of horse-chestnut are secondarily dispersed by water, particularly during snow-melt, primarily based on the consistent occurrence of horse-chestnut along mountain streams and rivulets in the native Greek populations (Briggs, 1989; Tsiroukis, 2008). However, this is probably a result of dispersal by gravity and the needs of germination and early growth rather than a facet of secondary dispersal.

8.4 │ Viability of seeds: germination

Seeds grown in Britain and sown onto agar immediately after falling will germinate at between 26-36 °C (in the dark at constant temperature) within one month of sowing reaching up to 80-90% germination (Tompsett & Pritchard, 1993; Pritchard, Steadman,

Nash, & Jones, 1999), aided by the presence of heat shock proteins in the embryo

(Azarkovich & Gumilevskaya, 2012). But since British autumn temperatures are lower than

this, germination in the open is unlikely (Daws et al., 2004). However, Greek seeds can

germinate in the field at 15-19 °C and thus germination may occur in the autumn

coinciding with autumn rain. This may give seedlings an advantage in allowing

establishment and growth before summer drought the following year (Daws et al., 2004).

Variation in the temperature needed for germination appears to be linked to seed (and

particularly embryo) development. Farrant and Waters (1998) noted that in seeds collected

in Colorado, USA, the dry mass of the embryo increased from 0.5 to 4.0 mg during

development. Other work by Pammenter and Berjak (1999) showed that respiration in

developing horse-chestnut seeds remained high (3.0-5.0 nmol O_2 g dw^{-1} s^{-1}) until the seed

started drying indicating that they were still developing. Seeds from further north certainly

have a lower mass (section 8.3) and are likely less developed when shed than seeds

grown in warmer conditions. This is supported by *c.* 70% of Scottish seeds being found to

be empty or underdeveloped and nonviable (Daws et al., 2004). Indeed, British seed has

been seen to increase in dry mass right up to seed fall, and also maintain high seed

moisture content which was linked to a decrease in desiccation tolerance and germinability

(Tompsett & Pritchard, 1993). Fresh seed moisture content at time of seed fall has been

measured at 59.7 ± 0.2% (SE, $n = 150$) in Greece and 69.0 ± 1.9% in Scotland, with a

solute potential -3.0 ± 0.2 MPa in Greece and -2.1 ± 0.1 MPa in Scotland (Daws et al.,

2004).

Proteins found in the cytosol of the seed cells are mainly water-soluble albumins

which, being hydrophilic, may help prevent the seed dehydrating over winter, and may

also confer protection against cold stress (Azarkovich & Bolyakina, 2016). Seeds can be

stored when hydrated at 16°C, with more than one-third of seeds remaining germinable

after 3 years (Pritchard, Tompsett, & Manger, 1996). Nevertheless, seeds are sensitive to

desiccation and short-lived when dried (recalcitrant); as they dry to 32-40% moisture they develop dormancy and lose viability upon further drying (Tompsett & Pritchard, 1998). As with *Quercus* spp. and *Castanea sativa*, horse-chestnut viability typically declines to 50% germination over 10–24 weeks (Gosling, 2007). Higher temperatures during development and lower moisture content at seed fall result in greater desiccation tolerance and shallower dormancy (Tompsett & Pritchard, 1993, 1998; Farrant & Walters, 1998; Pritchard et al., 1999; Obroucheva & Lityagina, 2007) and helps account for variation in dormancy geographically and between years. Median water potential resulting in seed death was -5.1 ± 0.65 MPa (SE, $n = 150$) for Scottish seeds and -16.2 ± 0.83 MPa for Greek seeds (Daws et al., 2004). The physiological and morphological changes that occur in seeds as they develop, pass through dormancy and germinate are described further by Obroucheva, Lityagina, Novikova, and Sin'kevichet (2012), Obroucheva, Sinkevich, and Lityagina (2016), Obroucheva, Sinkevich, Lityagina, and Novikova (2017), and Musatenko, Generalova, Martyn, Vedenicheva, and Vasyuk (2003).

The embryo-based physiological dormancy caused by drying can be broken by stratification at -3 to 6 °C (4 °C optimum) for 8-21 weeks, longer for more northern seed (Tompsett & Pritchard, 1993; Pritchard et al., 1999; Steadman & Pritchard, 2004; Obroucheva & Lityagina, 2007; Takos et al., 2008; Azarkovich & Gumilevskaya, 2012). Stratification increases germination at temperatures from 6 to 36 °C. (Pritchard et al., 1999; Steadman & Pritchard, 2004). Germination at 2-6 °C in the dark at constant temperature is possible but takes up to 4 months and total germination is not increased by stratification (Pritchard et al., 1999). However, the minimum temperature at which germination will occur was found to be reduced at a mean rate of 0.18°C/day during stratification, with the reduction being fastest during stratification at 2 °C and slowest at 16 °C (Steadman & Pritchard, 2004).

Dormancy can also be overcome by partial drying from 50% to 32-40% moisture content (Tompsett & Pritchard, 1998). The effect of drying appeared to be interchangeable with stratification and it is likely that both aid the seed maturation process since the effect of partial drying was only seen in the relatively immature shed seeds of Scottish and English origin. Without stratification, seeds can also be induced to germinate by soaking in water from 48 hours followed by cutting away one-third of the seed at the hilum without removing the seed coat (Bellini & Nin, 2005).

8.5 │ Seedling morphology

Germination is hypogeal and usually is complete after three to four weeks (Bellini & Nin, 2005). Seedling morphology is shown in Fig. 3.

9 │ HERBIVORY AND DISEASE

9.1 │ Animal feeders or parasites

Deer and wild boar (*Sus scrofa* L.) are known to eat horse-chestnut seeds (Bratton, 1974; Bean, 1976) but there are few other records of browsing or grazing. Insects that have been recorded on horse-chestnut are given in Table 2. Pollen is used as food by hoverflies (Diptera, Syrphidae) (Kugler 1970) and it is known that *Apis mellifera* L. (Hymenoptera, Apidae) harvests resins from horse-chestnuts, but whether this is from the 'sticky buds' or elsewhere is not stated (Wilson, 2014). Rotheray et al. (2001) visited 300 Scottish woodlands, recording 31 species of saproxylic Diptera on horse-chestnut, ranking the tree 11th out of the 22 tree species investigated, similar to *Ulmus glabra* (35) and *Populus tremula* (36), compared to 74 species on *Betula pubescens.* The Diptera on horse-chestnut included the red-listed species *Systenus bipartitus* (Loew) (Diptera, Dolichopodidae) found only on horse-chestnut, and *Phaonia exoleta* (Meigen) (Diptera, Muscidae) also found on *Acer pseudoplatanus* and *Fagus sylvatica*. The larvae of a

number of rare saproxylic hoverflies (Diptera, Syrphidae) were found in Cambridgeshire on Horse-chestnut in rot-holes, including *Myathropa florea* (L.), *Callicera spinolae* Rondani and *Mallota cimbiciformis* (Fallén), and in sap runs, including *Brachyopa insensilis* (Collin), *B. scutellaris* Robineau-Desvoidy and *B. bicolor* (Fallén) by Damant (2005). He suggested that these had not be found before since Horse-chestnut has been neglected by entomologists because it is an introduced tree.

Anoplophora chinensis (Forster) (Coleoptera, Cerambycidae) will oviposit on horse-chestnut trunks but adult beetles do not feed on its twigs (Peverieri & Roversi, 2010); by contrast, horse-chestnut is very susceptible to damage by larvae and adults of *A. glabripennis* (Motschulsky) (Ravazzi & Caudullo, 2016). Cebeci & Acer (2007) list insect pests found on horse-chestnut in Turkey, Milevoj (2004) in Slovenia, and Majzlan and Fedor (2003) in Slovakia. Details of fifteen spider species (Arachnida, Areneae) forming part of the food web on on horse-chestnut in Latvia are given by Petrova, Voitkane, Jankevica, and Cera (2013).

9.9.1 | Horse-chestnut leaf miner

The horse-chestnut leaf miner *Cameraria ohridella* Deschka & Dimić (Lepidoptera, Gracillariidae) is a leaf-mining micro-moth that was first observed in the late 1970s near Lake Ohrid, Macedonia, and described in 1985 by Deschka and Dimić (1986). It has since spread rapidly through central and western Europe and into the Ukraine, Belarus and western Russia (Tomiczek & Krehan, 1998; Avtzis & Avtzis, 2002; Thalmann et al., 2003; Gilbert et al., 2005; Weryszko-Chmielewska & Haratym, 2011, 2012; Gussev & Vulchev, 2015; Pirc, Dreo, & Jurc, 2018) at a rate of approximately 50-100 km/year (Šefrová & Laštůvka, 2001) or *c.* 3 km per generation (Gilbert, Grégoire, Freise, & Heitland, 2004). It was first seen in Britain in Wimbledon in 2002 and by the end of 2010 it was in most parts of England and Wales. It was originally thought to be of exotic origin (Grabenweger et al.,

2005) but has been shown to be native on horse-chestnut in the Balkans (Kenis et al., 2005, 2006; Valade et al., 2009; Lees et al., 2011) and that just a few lineages with limited genetic diversity moved to urban areas in the second half of the 20th century (Valade et al., 2009). Grabenweger et al. (2005) found *C. ohridella* to be abundant in natural stands of horse-chestnut in Greece and Bulgaria, confirmed by Walas et al. (2018) for some populations in Greece. *Cameraria ohridella* shows a preference for *A. hippocastanum* and the closely related *A. turbinata*; larval mortality is high on *A. carnea* (Freise, Heitland, & Sturm, 2003) and other *Aesculus* species are rarely predated (Freise et al., 2004; Straw & Tilbury, 2006; Ferracini, Curir, Dolci, Lanzotti, & Alma, 2010; D'Costa, Koricheva, Straw, & Simmonds, 2013; D'Costa, Simmonds, Straw, Castagneyrol, & Koricheva, 2014) undoubtedly due to the high saponin levels in the leaves of these other species (Ferracini et al., 2010). The moths will occasionally mine *Acer pseudoplatanus* and *A. platanoides* when horse-chestnut is unavailable or as opportunistic infestations near heavily-infested horse-chestnuts (Krehan, 1995; Straw & Tilbury, 2006; Péré, Augustin, Turlings, & Kenis, 2010).

Female moths lay their eggs on the adaxial leaf surface (Weryszko-Chmielewska & Haratym, 2012) and the larvae burrow in and feed on the palisade mesophyll leaving dead, dried lines of epidermis on both sides of the leaf (Weryszko-Chmielewska & Haratym, 2011). Most often 3, but sometimes up to 5, generations are produced per year (Šefrová & Laštůvka, 2001) with the last generation overwintering as pupae in the leaf litter, probably along with a few from previous generations, to produce the first generation the following year (Skuhravý, 1998; Samek, 2003; Hněvsová et al., 2011). The first generation feeds mostly in the lower part of the crown while subsequent generations feed mainly in the upper part of the crown (Krehan, 1995). Thus, Nardini et al. (2004) observed in north-east Italy that in early May 2-3% leaf area was lost at 2 and 6 m above ground; by the end of June leaf area loss was 30% at 2 m, 18% at 6 m, 10% at 10 m and 8% at 14m;

by the end of August it was 85% at 2 m, 75% at 6 m, 65% at 10 m, 55% at 14 m. Spatial analysis in the Czech Republic showed that damage between sites was not related to the infestation of neighbouring areas, indicating that the distribution of *C. ohridella* is random (Kopačka & Zemek, 2017). However, Gilbert, Svatoš, Lehmann, and Bacher (2003) found a positive correlation in Bern, Switzerland between infestation level on a tree and the number of other horse-chestnut trees within 800 m distance, and in Brussels the proportion of green areas within 100 m and the number of other horse-chestnut trees within 2000 m.

In native Greek populations, infestation rates are apparently low with a mean of 0.08 mines per leaflet recorded by Lees et al. (2011) and <3 mines per leaflet, covering <5.3% of leaf area recorded by Walas et al. (2018) although the latter data were collected in June/July before maximum infestation. The average number of mines was negatively correlated with altitude, but there was no significant correlation between temperature, precipitation and the number of mines (Walas et al., 2018). At the highest sites investigated (1,239-1,463 m) mines were absent and at lower altitudes (<1,000 m) leaves had similar numbers of mines to 'artificial' sites where horse-chestnut has been introduced (Walas et al., 2018). This, together with herbarium specimens of branches with leaves having very few mines per leaf (Lees et al., 2011), suggests that native horse-chestnut populations have been secondarily infected by a more invasive mitochondrial DNA haplotype that has been observed in most artificial stands in Europe.

Populations of the leaf miner readily establish in planted populations of horse-chestnut (<2% of hatching larvae fail to establish a leaf mine; Gilbert et al., 2004) and leaf damage can rise dramatically in the first three years of infestation (Pocock & Evans 2014), reaching 200 mines per leaf and removing up to 75% of total leaf area in southern England (Straw & Williams, 2013) and even causing complete defoliation (Thalmann et al.,

2003) by shortening the lifespan of the leaves up to 30%, which fall from the tree beginning in July (Skuhravý, 1998).

A number of studies have shown that there is no loss of assimilation before early June in Italy and late June in Britain (Nardini et al., 2004; Percival, Barrow, Noviss, Keary, & Pennington, 2011) during which loss of leaf area is low (6-11% leaf area loss in late July in Britain). Raimondo et al. (2003) found that photosynthesis in the green parts of mined leaves was as high as in intact leaves so subsequent loss of assimilation was due to changes in leaf area only. Over the whole of the growing season, the loss in assimilation is usually no more than 30-37% even in years of heavy infestation (Nardini et al., 2004; Percival et al., 2011; Straw & Williams, 2013). This is because leaf area loss in the early part of the season is mainly in the lower part of the crown where leaves are more shaded and less productive (Straw & Williams, 2013). Moreover, loss of leaf area progressively develops through the summer and is maximal when the photosynthetic efficiency of the leaves has decreased and they are contributing less to total seasonal assimilation (Nardini et al., 2004).

Loss of assimilation reduces the growth of a tree. However, in the short-term, infestation with the leaf miner leads to greater annual wood production that is probably a reaction to maintain hydraulic conductivity. Salleo et al. (2003) in north-east Italy measured mean ring widths of 1.35 mm in the four years before infestation, and of 2.20 mm in the 4 years after, resulting in 62% more wood production after attack. Wood grown in attacked trees had its water conducting cross-sectional area increased to 32% compared to 25% in control trees sprayed with insecticide. This extra conductivity was due to a higher proportion of larger vessels 30.1-40.0 µm diameter compared to the controls (18 and 9%, respectively) and the widest vessels (>40 µm) which were only present in infested trees, forming *c.* 5% of the total or 40 vessels/mm^2. In both infested and control trees, water was taken up the outer two rings, so the higher xylem conductivity would have

helped compensate for defoliation in the lower part of the crown and overall shorter leaf lifespan by increasing water and nutrient supply to less damaged leaves higher in the crown.

One growing season after treating mature horse-chestnuts with insecticide to reduce leaf miner attack they had 33% higher root carbohydrate concentrations and 1,719% higher twig starch content than untreated controls (Percival et al., 2011). In the long-term, however, infestation reduces tree vigour and growth (Bednarz & Scheffler, 2008; Percival et al., 2011; Straw & Williams, 2013). In Kórnik, Poland, Jagiełło et al. (2018) found that trees injected with insecticide (imidacloprid), were taller 10 years after treatment than untreated controls (15 vs 13 m, estimated from figure) and had higher mean DBH (28 vs 22 cm) and higher basal area increment (125 vs 80 cm^2). But there is no compelling evidence that damage by *C. ohridella* leads to long-term health problems or tree death (Straw & Bellett-Travers, 2004).

Flowering can be reduced by the leaf miner primarily by trees producing a smaller number of functionally female flowers (Weryszko-Chmielewska et al., 2012; Franiel, Wożnica, & Orlik, 2014), although there is no evidence that the number of seeds is reduced (Thalmann et al., 2003). However, the seeds from infested trees are 40-50% smaller (Thalmann et al., 2003; Nardini et al., 2004; Takos et al., 2008; Jagiełło et al., 2017), in Italy typically with a mass of 6 g compared to 10-12 g in trees with well-developed foliage (Salleo et al., 2003). This effect is still clear in partially defoliated trees; Thalmann et al. (2003) found that heavily infested trees in Munich (>75% leaf area lost) had smaller fruits (*c.* 6g) and seeds (*c.*4.5 g) than did lightly infested trees (<25%) with fruits of >9g and seeds of >7g.

Percival et al. (2011) found in south England that seed germination was 47.6% higher in seeds from insecticide-treated trees compared to leaf-miner infested trees, presumably because the seeds were larger. However, in native populations in

Greece, Takos et al. (2008) found the opposite, that germination was significantly but marginally higher in seeds from infected trees (97.0%) compared insecticide-treated trees (92.3%), and was one week quicker. This is likely due to seeds on infested trees having a longer post-ripening period after the trees are defoliated and so are more mature (section 8.4) and ready to germinate upon falling.

Seedling survival in the first 2-3 years does not appear to be affected by the leaf miner (Raimondo, Trifilò, Salleo, & Nardini, 2005; Takos et al., 2008). Raimondo et al. (2005) noted that the leaf expansion of 3-year-old seedlings was complete before the leaves were mined, so growth of infested seedlings was similar to that of controls. However, Takos et al. (2008) saw that non-infested seedlings grew taller (*c.* 27 cm) over the first two years, compared to infested seedlings (*c.* 18 cm) and were larger (35.3 g total dry mass non-infested, 19.5 g infested). Raimondo et al. (2005) attributed slower growth in infested seedlings to lower leaf water conductance both in mined and in green areas of attacked leaves (control 160 mmol m^{-2} s^{-1}, green parts of infected leaves 130 mmol m^{-2} s^{-1}, mined areas 60 mmol m^{-2} s^{-1}) and higher hydraulic resistance.

The larval stages can be controlled by spraying or injecting trees with chitin synthesis inhibitors, such as diflubenzuron or triflumuron (Krehan, 1997; Nejmanová et al., 2006; Dzięgielewska & Kaup, 2008). Insecticides such as imidacloprid, abamectin, acetamiprid and chlotianidin have also proved effective (Ferracini & Alma, 2008; Kosibowicz & Skrzecz, 2010; Kobza, Juhásová, Adamčíková, & Onrušková, 2011; Burkhard et al., 2015) as has injection of seed extracts of the neem tree, *Azadirachta indica* A. Juss. (Pavela & Bárnet, 2005). Insecticide implants in trees, such as Acecap, have proved less useful (Krehan, 1997). Glue bands around the trunk to trap adult moths have proved effective if applied every year (Percival, 2016) but pheromone traps have not been found to reduce damage (Sukovata, Czokajlo, Kolk, Ślusarski, & Jabłoński, 2011).

Biocontrol is unlikely to be widely effective since natural rates of parasitism of the leaf miner larvae are typically <6% of a population (Skuhravý, 1998; Freise et al., 2004; Pocock & Evans, 2014) but can be up to 20% (Stojanović & Marković, 2004; Volter & Kenis, 2006) especially in sun-exposed trees (Tarwacki, Bystrowski, & Celmer-Warda, 2012). In Italy, the larvae were predated by the ant *Crematogaster scutellaris* (Olivier) (Hymenoptera Formicidae) (Radeghieri, 2004). However, the adult moths can detect infection of leaves by *Erysiphe flexuosa* and *Guignardia aesculi* (section 9.3) under laboratory conditions which results in lower egg laying (Johne, Weissbecker & Schütz, 2008) and may be useful for biocontrol in the field.

The most effective control measure for urban trees is likely to be removing fallen leaves from the ground where the pupae overwinter (Pavan, Barro, Bernardinelli, Gambon, & Zandigiacomo, 2003; Kukuła-Młynarczyk & Hurej, 2007). Adults will, however, disperse toward trees from areas where it is difficult to remove litter (Kehrli & Bacher, 2003; Straw & Bellett-Travers, 2004; Augustin et al., 2009) and local populations can rebuild (Baraniak, Walczak, Tryjanowski, & Zduniak, 2004; Gilbert et al., 2005). Moving collected litter also carries the risk of introducing the miner to new areas, so it is unlikely to be a complete solution. The collected litter can be composted; experiments burying infected litter under 15 cm of uninfected foliage or 10 cm of soil both reduced the emergence of *C. ohridella* by 96% (Kehrli & Bacher, 2004). Composting litter with sewage sludge producing temperatures >50 °C for seven days should lead to eradication of the miner (Łowiński & Dach 2006). Litter can be removed anytime before spring with the same effect (Kehrli & Bacher, 2003).

9.2 │ Plant parasites

The wood is readily decomposed and is noted for its ability to host spalting fungi, particularly *Scytalidium cuboideum* (Sacc., & Ellis) Sigler & Kang (=*Arthrographis*

cuboidea (Sacc., & Ellis) Sigler; Ascomycota, Incertae sedis) and *Ophiostoma piceae* (Münch) Syd., & P. Syd. (Ascomycota, Ophiostomatales) (Robinson, Tudor & Cooper, 2011). A number of saprophytic fungi are known on horse-chestnut, including *Kretzschmaria deusta* (Hoffm.) P.M.D. Martin (=*Ustulina deusta* (Fr .) Petrak; Ascomycota, Xylariales), *Ganoderma australe* (Fr.) Pat. (=*G. adspersum* (Schulz.) Donk; Basidiomycota, Polyporales), *G. gibbosum* (Blume & T. Nees) Pat. and *G. resinaceum* Boud. (Pearce, 1991; Guglielmo, Bergemann, Gonthier, Nicolotti, & Garbelotto, 2007; Greig, 2012). *Schizophyllum commune* Fr. (Basidiomycota, Agaricales) is saprophytic on horse-chestnut but can become parasitic causing white surface rot (Snieskiene et al., 2011). Increment cores taken from horse-chestnut resulted in extensive vertical discolouration 10 years later with a mean distance 39 cm, compared to *Tilia platyphyllos* 21 cm, *T. cordata* 16 cm, and *Betula pendula* 155 cm (Dujesiefken, Rhaesa, Eckstein, & Stobbe, 1999). Horse-chestnut is generally less good at compartmentalising decay after pruning than, for example, *Tilia* spp. (Dujesiefken, Stobbe, & Eckstein 1998).

Horse-chestnut leaves are sensitive to a number of powdery mildews such as the North American *Erysiphe flexuosa* (Peck) Barun & Takamatsu (Ascomycota, Erysiphales), introduced to Europe at the turn of the century (Ale-Agha, Braun, Feige, & Jage, 2000; Zimmermannová-Pastirčáková & Pastirčák, 2002; Kiss, Vajna, & Fischl, 2004; Stankeviciene, Snieskiene & Lugauskas, 2010; Tozlu & Demerci, 2010) including Britain (Ing & Spooner, 2002). *Erisiphe flexuosa* also affects *A. carnea* (Werner, Irzykowska, & Karolewski, 2012; Irzykowska et al., 2013). It causes small, white patches on the leaves that then expand to cover both leaf surfaces. In Poznań, Poland, infection has been found to reach up to 50% of leaf area (Irzykowska et al., 2013). Both young and old leaves are usually infected. It causes more damage on vigorous trees and pruned trees with large sprouts (Snieskiene et al., 2011) but in many cases infected trees were found next to

uninfected ones (Irzykowska et al., 2013). Trees that are more resistant to *Cameraria ohridella* (section 9.1) are also more resistant to *E. flexuosa* (Werner et al., 2012).

Guignardia leaf blotch is found in Europe, North America and in South Korea (Pastirčáková, Pastirčák, Celar, & Shin, 2009), including Britain (Hudson 1987), and affects various *Aesculus* species. The casual fungus, *Guignardia aesculi* (Peck) Stewart (=*Phyllosticta paviae* Desm.; Ascomycota, Incertae sedis: conidial anamorph *Phyllosticta sphaeropsoidea* Ellis & Everh. spermatial synanamorph *Leptodothiorella aesculicola* Höhn.; Pastirčáková et al., 2009), produces reddish or dull brown necrotic areas with bright yellow borders in horse-chestnut leaves. These 'blotches' are usually at the tips or edges of leaves. The yellow border helps distinguish this from the leaf miner *Cameraria ohridella* (section 9.1). Horse-chestnut is moderately susceptible to *Guignardia* (Ćalić et al., 2013) but it seems to cause little significant damage.

Horse-chestnut can carry many epiphytes. Of the 13 tree species investigated in central Switzerland by Ruoss (1999), horse-chestnut carried the most lichen species (114), compared to *Acer pseudoplatanus* (93) and *Fraxinus excelsior* (52). Papp, Alegro, Šegota, Šapić, and Vukelić (2013) list 11 bryophytes found on horse-chestnut in Croatia, and Seaward and Letrouit-Galinou (1991) seven species found on Paris trees. In Britain, this is somewhat lower; Bates, Proctor, Preston, Hodgetts, and Perry (1997) found <1 species of bryophyte per tetrad on Horse-chestnut along a transect acros southern England, and was ranked 20th out of a list of 52 host taxa, although this result is undoudbtedly partly due to the low density of Horse-chestnuts in this area compared to native species. Horse-chestnut is a rare host of the hemiparasite *Viscum album*. Slime moulds (Amoebozoa, Myxomycetes) associated with horse-chestnut are given in Table 3.

9.3 │ Plant diseases

The apple mosaic virus (Group 4, Bromoviridae) has been observed to cause a severe yellow mosaic disease on leaves of horse-chestnut trees (Sweet & Barbara, 1979). The Strawberry latent ringspot virus (Group 4, Picornavirales) was detected in one of six trees with a leaf vein yellows disease (Sweet & Barbara, 1979).

Fungi associated with horse-chestnut, excluding those on soil or litter below the trees, or those found solely on dead wood, are given in Table 4. *Colletotrichum acutatum* Simmonds and *C. gloeosporioides* (Ascomycota Glomerellales) have been found on horse-chestnut leaves in Norway (Talgø et al., 2012).

9.3.1 | *Phytophthora*

Since 1969, a number of *Phytophthora* spp. (Oomycota, Peronosporales) have been isolated from dead and dying roots and stems of horse-chestnut in England including *P. megasperma* var. *megasperma* Drechsler, *P. citricola* Saw., *P. cactorum* (Leb., & Cohn.) Schroet. *P. cinnamomi* Rands and possibly *P. cambivora* Petri (Brasier & Strouts, 1976; Strouts & Winter, 2000; British Mycological Society, 2018). These are known to cause small-scale bleeding cankers on horse-chestnuts and other tree species, such as *Tilia* spp. Since 2001/2, stem bleeding on *A. hippocastanum* has become more prevalent in Britain and horse-chestnut leaves have been seen to be moderately susceptible to *P. ramorum* (similar to *Quercus* spp., *Castanea sativa* and *Taxus baccata*) but more susceptible to *P. kernoviae* Brasier than most other woody plants (Brasier & Jung, 2006; Denman, Kirk, Whybrow, Orton, & Webber, 2006). *Phytophthora obscura* Grünw. & Werres, *P. cactorum*, *P. citricola* and *P. syringae* (Kleb.) Kleb. have been identified under diseased horse-chestnuts with bleeding canker in Germany (Werres, Richter, & Veser, 1995; Grünwald, Werres, Goss, Taylor, & Fieland, 2012).

9.3.2 | Chestnut bleeding canker

Bleeding cankers on horse-chestnut bark have become more common this century, partly due to *Phytophthora* spp., as described above. However, since 2001/2, bleeding cankers have become significantly more common throughout Britain, Ireland and western mainland Europe (McEvoy et al., 2016). In the majority of cankers sampled, the gram-negative bacterium *Pseudomonas syringae* pv. *aesculi* (Pae) has been identified as the cause (Webber et al., 2008). In 2007, over 70% of horse-chestnut trees surveyed in England, 42% in Scotland and 36% in Wales had symptoms of bleeding canker (Forestry Commission, 2008). Similar rates of infection have been seen in The Netherlands, with more urban trees infected than rural trees (Webber et al., 2008).

The disease is most prevalent in cool, wet climates of north-west Europe (Kennelly, Cazorla, de Vicente, Ramos, & Sundin, 2007) and the pathogen is thought to originate from the Himalayas where it infects leaves of *A. indica*. Isolates of Pae in Britain were found to be genetically virtually identical to each other and to isolates from The Netherlands and Belgium, so the epidemic in north-west Europe is likely descended from a single, recent introduction of the pathogen into western Europe, possibly from India (Green et al., 2010; McEvoy et al., 2016).

Symptoms are rust-coloured or blackened liquid oozing from necrotic lesions ('bleeding cankers') in the bark of the trunk up to small diameter branches (Webber et al., 2008; Green, Laue, Fossdal, A'Hara, & Cottrell, 2009; Green, Laue, Steele, & Nowell, 2014). The infection penetrates the cambium and phloem but there is little evidence of it penetrating the wood (Steele, Laue, MacAskill, Hendry, & Green, 2010) or of systemic spread through the vascular tissue. It spreads primarily by lateral invasion of parenchymal cells, producing local infections that spread by 70-1,000 μm day^{-1}. There may also be some local spread along phloem fibres leading to elongated necrotic areas (Bultreys, Gheysen, & Planchon, 2008). Horse-chestnut produces a number of antifungal and antibacterial proteins (Ah-AMP1 and β-1,3-glucanase and peroxidase) as a plant defence

(Osborn et al., 1995; Fant, Vranken & Borremans, 1999). However, once within the host the bacterial cells persist within a mucoid gel which may help isolate them from outside stressors or toxins (e.g. Keith & Bender, 1999) and host defences.

The disease can result in foliar discolouration and crown dieback when lesions girdle branches (Green et al., 2010) but possible resistance to the disease has been observed. For example, Pánková, Krejzar, Mertelík, and Kloudová (2015) found in the Czech Republic that 2-3 years after infection with Pae there was a natural resistance with 40% of trees appearing to be resistant, 40% tolerant (not appreciably affected by infection with small lesions) and 20% susceptible. Resistant and tolerant trees maintain healthy crowns and disease progression is slow or stops, and may even show signs of recovery with new callus development around the edge of the cankers. Trees are known to have survived for a decade or more with half of their bark area has been affected. A study in southern England found that Pae was responsible for the death or removal of 11% of *A. hippocastanum* and 27% of *A. carnea*, and surviving trees showed a decrease in growth rate by 22% between 2003 and 2012, and a decline in crown density by 4-5% (Straw & Williams, 2013). The biggest threat appears to be the cankers merging around the main trunk and girdling the tree leading to death (Steele et al., 2010). Secondary agents such as *Phytophthora* may also gain access to a diseased tree and be the ultimate cause of death.

The spores of Pae are spread mainly in wind-blown rain, possibly over long-distances at high altitude. Pae can survive for at least a year in soil even without host debris, and can survive when stored in a nutrient solution for a year at -80 °C, so is very robust (Laue, Steele, & Green, 2014). Although it is a bark pathogen it has also been found within leaves of horse-chestnut in Britain, and leaves can be artificially infected with it, but it is not known whether leaves play a role in spreading the disease (Mullett &

Webber, 2013). The bacterial cells enter branches directly through leaf scars or branch axils and lenticels (Steele et al., 2010; Laue et al., 2014).

Pae has been seen to affect trees of all ages. Koskella et al. (2017) found that both the leaf miner *Cameraria ohridella* and Pae are both associated with taller, larger trees but Pae is also more prevalent on young fast-growing horse-chestnuts while the leaf miner is most common on taller trees. This is undoubtedly a spatial preference rather than a direct interaction between the two organisms at a landscape level. Leaf miner presence does not appear to be spatially linked to Pae symptoms and it is unlikely that the leaf miner is a vector of Pae (Koskella, Meaden, Crowther, Leimu, & Metcalf, 2017). Within an individual tree, however, Pae canker size was positively correlated with leaf miner infestation, probably due to the suppression of tree defences as a result of leaf miner defoliation (Percival & Banks, 2014).

Pae infections have been successfully treated with potassium and silicon phosphites that act to prevent, and to a lesser extent, reduce the impact of, infection (Percival & Banks, 2015). Heating 4-year-old saplings at 39 °C for 48 hours kills all Pae in wounds (de Keijzer, van den Broek, Ketelaar, & van Lammeren, 2012).

10 │ HISTORY

Based on molecular phylogenetic reconstruction of the genus and fossil evidence, *Aesculus* evolved in eastern Asia at the Cretaceous/Tertiary boundary *c.* 65 MYA (Xiang et al., 1998). From there, two major lineages spread into Europe and North and Central America via the Bering Land Bridge as an element of the boreotropical flora (Hardin, 1960; Xiang et al., 1998; Manchester, 2001; Harris et al., 2009). Forest et al. (2001) suggested an American origin for *Aesculus* with a single migration to Eurasia but this now appears unlikely. The closely-related Japanese *A. turbinata* and *A. hippocastanum* split apart 15.5 ± 1.93 MYA or earlier in the middle Miocene (Xiang et al., 1998). Most of the Eurasian

lineage was lost during the Miocene and Pliocene but fossil evidence indicates that *A. hippocastanum* was widespread throughout Europe during the Miocene-Pliocene, when warm climatic conditions were optimal for this species (Mijarra, Manzaneque, & Morla, 2008) and it was distributed from North Africa and the Iberian Peninsula to northern Europe.

Pleistocene pollen records are mostly confined to the Mediterranean Basin, from Barcelona through to Turkey and the Caucasus Mountains in the east (Mijarra et al., 2008). Large amounts of *Aesculus* pollen (up to 15%) have been recorded in early Pleistocene sediments from Leffe, Italy (Ravazzi, 2003). *Aesculus hippocastanum* persisted in the Quaternary refugia of the Balkans, Italian and Iberian Peninsulas (Postigo-Mijarra, Gómez-Manzaneque, & Morla, 2008; Tsiroukis, 2008). By the end of the Middle Pleistocene, *A. hippocastanum* was restricted to its current native distribution in the Balkan Peninsula (Xiang et al., 1998; Grove & Rackham, 2001; Prada, Velloza, Toorop, & Pritchard, 2011). Gobet et al. (2017) noted an increase in fruit and fodder tree pollen, including *Aesculus*, along with crop and weed pollen in Ukraine around 6500-6000 BP suggesting the involvement of *Aesculus* in Neolithic agriculture.

When first introduced into modern cultivation, its native origins were unknown. Linnaeus (1753) suggested that it was native to the northern regions of Asia, near the Himalayas, and north India was long regarded as its original home (Bean, 1976) and as late as 1837, Loudon (1838) suggested North America. In 1795 John Hawkins almost certainly found natural stands in Greece, but these were only confirmed by Theodor von Heldreich in 1879 (Lack, 2000, 2002).

As recently as 1945, Howard (1945) thought that that horse-chestnut might have been introduced via Iran, northern India or Tibet. However, it is now believed that horse-chestnut was introduced to various parts of Europe by the Romans (Bradshaw, 2004) and that European diplomats came across the horse-chestnut in Constantinople and seeds

were sent to Prague in 1557 (Lack, 2000). These seeds were reportedly non-viable and it almost certainly reached western Europe from seedlings sent to Vienna, again from Constantinople, in 1576 (Bean, 1976; Leathart, 1991). It was first grown in central Europe, primarily Vienna, in the 16th Century before being spread throughout Europe (Lack, 2000). It reached France in 1615 and many avenues were lined with horse-chestnut trees (Loenhart, 2002). It was growing in Tradescant's Lambeth garden in 1633 and so probably arrived in England at the same time as in France (Leathart, 1991). Evelyn (1664) mentioned the fashion of planting horse-chestnut in avenues, most famously at Bushey Park, Hampton Court, and Loudon (1838) listed many specimens that were then 80-100 years old. Horse-chestnut was introduced into the USA around 1828 but has been largely surpassed by *A. carnea* (Leathart, 1991). John Gerard appreciated the tree, saying "[t]he Horse-chestnut groweth likewise to be a very great tree, spreading his great and large armes or branches far abroad, by which meanes it maketh a very good coole shadow" (Gerard, 1633).

10.1 | Uses

The horse-chestnut flower is the symbol of the city of Kiev in the Ukraine, and it is traditionally planted in Bavarian beer gardens (Loenhart, 2002). Further cultural connections include the Anne Frank Tree in Amsterdam which she mentions in her diary, and which sadly snapped in high wind in August 2010 (Gray-Block, 2010). It is locally planted as a forestry tree in, for example, the Czech Republic (Křivánek, Pyšek & Jarošík, 2006). The biggest use of horse-chestnut, however, is in medicine, reflected in the voluminous medical literature in comparison to a comparatively small ecological literature.

The common name may have come from the use of seeds to treat horses for overexertion, colic and coughs by the Turks and Ancient Greeks (Vokou, Katradi, & Kokkini, 1993; Bombardelli et al., 1996). Extracts of seeds, bark and leaves have long

been used in traditional and folk medicine (Tiffany et al., 2002). The triterpenoid saponins extracted from the seed have been used as a treatment for rheumatism, coughs, rectal complaints including haemorrhoids, bladder and gastrointestinal disorders, fever (the first written account in 1720) and leg cramps (Sirtori, 2001; Anon., 2009; Küçükkurt et al., 2010; Zhang et al., 2010). In Bosnia and Herzegovina, Redžić (2007) records that horse-chestnut 'fruits' are still carried by people who suffer from rheumatism and sciatica.

Currently, extracts from horse-chestnut seeds are widely used to treat peripheral vascular disorders including chronic venous insufficiency, haemorrhoids and post-operative oedema, (Facino, Carini, Stefani, Aldini, & Saibene, 1995; Pittler & Ernst, 1998; Dickson, Gallagher, McIntyre, Suter, & Tan, 2004; Ruffini, Belcaro, Cesarone, & Dugall, 2004; Suter, Bommer, & Rechner, 2006; Underland, Sæterdal, & Nilsen, 2012; Gurel et al., 2013; Dudek-Makuch & Studzińska-Sroka, 2015), as a preventative of dental plaque and periodontitis in toothpaste (Aravind, Lakshmi, & Arun, 2012; Kim et al., 2017) and to counter male infertility by improving sperm quality (Fang et al., 2010). Saponin extracts have been used to prevent colon cancer in rats (Szabadosova et al., 2013) and may also reduce growth of tumours in a number of cancers in humans (Geran, Greenberg, McDonald, Schumacher, & Abbott, 1972; Cheong et al., 2018; Turkekul et al., 2018). Rat and mice models have been used to show that seed extracts also relieve diabetic nephropathy and thromboses (Elmas, Erbas & Yigitturk, 2016; Ahmad et al., 2018), reduce ethanol absorption (Yoshikawa et al., 1994), reduce cholesterol in mice fed a high fat diet (Avcı, Küçükkurt, Akkol, & Yeşilada, 2010) and protect against bacterial endotoxemic injuries in mice livers (Jiang et al., 2011). Seed extracts also have anti-inflammatory and anti-oedematous properties (Matsuda, Murakami, Ninomiya, Yamahara, & Yoshikawa, 1997; Wilkinson & Brown, 1999; Sirtori, 2001; Vasiliauskas, Leonavičienė, Vaitkienė, Bradūnaitė, & Lukšienė, 2010; Dumitriu, Olariu, Nita, Zglimbe, & Rosoiu, 2013) and so have proved effective at clearing skin conditions, as an anti-wrinkle treatment and in

reducing skin ageing (Masaki, Sakaki, Atsumi, & Sakurai, 1995; Fujimura et al., 2007) and may help against cellulite and hair loss (Bellini & Nin, 2005). Extracts of leaves and the bark of young branches has also proved effective as an antioxidant and anti-inflammatory drug (Braga et al., 2012; Margină et al., 2015) and one of the most effective plant extracts in inhibiting *Candida albicans* (Tambur et al., 2018).

Perhaps not surprisingly, seeds have proved poisonous. Ingestion of seeds can cause anaphylaxis (Jaspersen-Schib, Theus, Guirguis-Oeschger, Gossweiler, & Meier-Abt, 1996; Vega et al., 2012) and there is a record of a 4-year old boy who died after eating raw horse-chestnuts (Lampe & Fagerstrom, 1968). Edem, Kahyaoğlu, and Çakar (2016) also describe a person who developed pericarditis after consuming "3 boxes of horse-chestnut paste" (no details given) over the previous 1.5 months. Saponins are poorly absorbed in the gut and are largely destroyed by heating, so roasting horse-chestnuts instead of sweet chestnuts (*Castanea sativa*) is unlikely to cause great harm. Saponins and tannins can be removed by boiling or leaching thin slices in running water for 205 days (Weiner, 1980), then grinding them to make a flour to be mixed with wheat or rye, or used as pig food. The boiling and leaching removes many of the minerals and vitamins, leaving starch which is fairly edible (Mabey, 1972). Ground seeds have been used as a coffee substitute (Loenhart, 2002) and in combination with wheat flour as a strong glue in bookbinding (Bainbridge, 1984). It is reported that giving horse-chestnut seeds to cows in moderation improves the yield and flavour of the milk (Loenhart, 2002).

The best known use of whole seeds is in playing the sadly declining children's game of conkers or 'conquerors' (Bean, 1976). Traditionally, conkers have also been used to repel spiders but this was convincingly disproved by Roselyon Primary School, Cornwall using choice experiments (Anon., 2010). Bainbridge (1984) records that children were used to collect conkers in WWI as a source of tannin for leather, and a bleaching agent for flax, hemp, silk and wool. Tannin and dye can also be obtained from the bark and fruit

shells (Zemanek, Zemanek, Harmata, Madeja, & Klepacki, 2009). Saponins can be extracted in hot water and used as a substitute for soap. An infusion of horse-chestnut seeds can be used to bring worms to the soil surface (Loenhart, 2002). Bainbridge (1984) also records accounts of conkers being used in the manufacture of TNT and fermentation to produce acetone. More recently, starch from the seeds has been used to make biodegradable thermoplastic (Castaño et al., 2014) and the ground fruit shells used as a filler in polymers to make them more biodegradable (Barczewski, Matykiewicz, Krygier, Andrzejewski, & Skórczewska, 2018).

The fruit shells of horse-chestnut have shown promise as a biosorbent of chromium and copper from aqueous solutions (Parlayıcı & Pehlivan, 2015; Parus, 2018). Nanoparticles of ZnO, 50-100 nm diameter, have also been produced from the fruit shells (Çolak, Karaköse & Duman, 2017). Leaves heated to 450 °C and so partially carbonised has been used in absorbing ions from sewage (Sapronova, Sverguzova, Sulim, Svyatchenko, & Chebotaeva, 2018).

The wood is soft and comparatively weak and has found few uses but they include kitchen utensils and dishes, brush backs, toys, prosthetic limbs and occasionally veneers (Bean, 1976; Mitchell, 1997). The wood does not burn particularly well but its charcoal has been used in making gunpowder (Leathart, 1991; Maltitz, 2003). In southern Europe the wood has been used for fruit-storing shelves as the porous nature absorbs moisture preserving the fruit (Howard, 1945).

Horse-chestnut gives good shelter as a street tree. Leuzinger, Vogt & Körner (2010) recorded that horse-chestnut crown temperatures in Basel, Switzerland were 1 °C below ambient, compared to 4 °C *above* ambient in *Acer platanoides*. This has the effect of reducing the apparent temperature below the crown by 7.5-10.0 °C (Streiling & Matzarakis, 2003; Kántor, Chen, & Gáld, 2018). In Freiburg, Germany, NO_x and O_3 levels

were reduced by 45% (down to 19 μg/m^3) and 55% (down to 37 μg/m^3), respectively, below horse-chestnut trees (Streiling & Matzarakis, 2003).

11 │ CONSERVATION

Despite the recent pests and diseases experienced by horse-chestnut (sections 9.1 and 9.3), its distribution and abundance in Britain does not appear to be declining, and it may even be expanding its range slightly. Preston et al. (2002) show a small but probably insignificant increase (+1.08) in distribution between 1930-1960 and 1987-1999, and Braithwaite, Ellis, and Preston (2006) show a similar small increase in British distribution (+38 tetrads) between 1987-88 and 2003-4, similar to *Prunus padus* and *Ligustrum ovalifolium*. Preston et al. (2002) note that Horse-chestnut was better recorded in south-west England, Wales and Ireland between 1962 and 2002, which may account for some of the increase.

The native range of Horse-chestnut (section 1) is estimated to currently be 163,642 km^2, *c.* 25% of the Balkan Peninsula (Allen & Khela, 2017), with perhaps as many as 10,000 mature trees (Allen & Khela, 2017). However, despite its abundance as a planted ornamental tree, it appears to be declining in its native range, despite the ability to regenerate freely (section 5.2). The Greek populations have satisfactory regeneration in only 6% of the areas and no regeneration in 62% (Avtzis et al., 2007; Tsiroukis, 2008). It also appears to be declining in Albania, where the population is smaller than *c.* 500 individuals, and in Macedonia where the population is probably <100 individuals (Peçi et al., 2012; Allen & Khela, 2017). Bulgarian populations are also small and limited in area (Evstatieva, 2011).

The main threats to many of the native populations of Horse-chestnut are undoubtedly the leaf miner moth *Cameraria ohridella* which impairs reproduction (section 9.1) and its limited ability to disperse to new areas (section 8.3). Other threats include

deforestation and forestry, firewood collection, forest fires, increasing water demand for irrigation, mining, overgrazing, tourism development and pollution, and population fragmentation affecting spread and microclimate (Laraus, 2004; Evstatieva, 2011; Gussev & Vulchev, 2015; Allen & Khela, 2017). Mountain tourism, ski facilities and road construction are also degrading large mountain forest ecosystems within its native range although the Pindos Mountains still host significant old-growth forest stands on inaccessible high mountain slopes and canyons (WWF, 2013). Collecting seeds for herbal medicine and larger-scale pharmaceutical use is also taking its toll on population regeneration (Tsiroukis, 2008; WWF, 2013). The potential effects of climate change on Horse-chestnut have been largely unstudied (section 7.1) but it is considered that although Horse-chestnut is regarded as sensitive to environmental changes (Łukasiewicz, 2003; Łukasiewicz & Oleksyn, 2007), the effects of current scenarios on population size and distribution will be minor (Walas et al., 2018).

As a consequence of the decline, Horse-chestnut in its native range is classified as Vulnerable or Near Threatened using IUCN criteria (Allen & Khela, 2017) and is considered as Endangered or Critically Endangered in Bulgaria and Albania (Evstatieva, 2011; Gussev & Vulchev, 2015).

Horse-chestnut is known to occur in protected areas in Albania, Greece and Bulgaria, including national parks/reserves and Natura 2000 sites (Allen & Khela, 2017) but this includes relatively few of the natural populations, particularly in Greece (Avtzis et al., 2007). Moreover, in Greece Horse-chestnut is included in the national list of protected species of the Presidential Decree 67/1981 (Walas et al., 2018), but is not in the Red List of Greece (Phoitos, Konstantinidis & Kamari, 2009). There are thus concerns about the long-term future of the small number of native populations.

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REFERENCES

Abudayeh, Z.H.M., Al Azzam, K.M., Naddaf, A., Karpiuk, U.V., & Kislichenko, V.S. (2015). Determination of four major saponins in skin and endosperm of seeds of horse-chestnut (*Aesculus hippocastanum* L.) using high performance liquid chromatography with positive confirmation by thin layer chromatography. *Advanced Pharmaceutical Bulletin, 5*, 587-591.

Ahmad, I., Sharma, S., Gupta, N., Rashid, Q., Abid, M., Ashraf, M.Z., & Jairajpuri, M.A. (2018). Antithrombotic potential of esculin 7, 3′, 4′, 5′, 6′-O-pentasulfate (EPS) for its role in thrombus reduction using rat thrombosis model. *International Journal of Biological Macromolecules, 119*, 360–368.

Ale-Agha, N., Braun, U., Feige, B., & Jage, H. (2000) A new powdery mildew disease on *Aesculus* spp. introduced in Europe. *Cryptogamie, Mycologie, 21*, 89-92.

Allen, D.J., & Khela, S. (2017). *Aesculus hippocastanum* (errata version published in 2018). The IUCN Red List of Threatened Species 2017: e.T202914A122961065. http://dx.doi.org/10.2305/IUCN.UK.2017-3.RLTS.T202914A68084249.en. Downloaded on 31 October 2018.

Anchev, M., Apostolova, I., Assyov, B., Bancheva, S., Denchev, C.M., Dimitrov, D., Dimitrova, D., Evstatieva, L., Genova, E., Georgiev, V., Goranova, V., Gussev, C., Ignatova, P., Ivanova, D., Meshinev, T., Peev, D., Petrova, A., Petrova, A.S.,

Sopotlieva, D., Stanev, S., Stoeva, M., Stoyanov, S., Tashev, A., Tosheva, A., Tsoneva, S., Tzonev, R., Vitkova, A., & Vladimirov, V. (2009). Red List of Bulgarian Vascular Plants. *Phytologia Balcanica, 15*, 63–94.

Aničić, M., Spasić, T., Tomašević, M., Rajšić, S., & Tasić, M. (2011) Trace elements accumulation and temporal trends in leaves of urban deciduous trees (*Aesculus hippocastanum* and *Tilia* spp.). *Ecological Indicators, 11*, 824–830.

Anon. (1925). Horse-chestnuts and buckeyes. *Bulletin of Popular Information, Arnold Arboretum, Harvard University, New Series, 11*, 29-30.

Anon. (2009). Aesculus hippocastanum (Horse-chestnut). Alternative Medicine Review Volume, 14, 278-283.

Anon. (2010). *Pupils Scoop Prize for Unravelling Conker Theory*. Royal Society of Chemistry, London, UK. http://www.rsc.org/AboutUs/News/PressReleases/2010/Conkerswin.asp. Viewed 20/02/2018.

Aravind, K.S., Lakshmi, T., & Arun, A.V. (2012). Role of phytomedicine against dental plaque in fixed orthodontic appliances (FOA) treatment - a literature review. *International Journal of Current Research and Review, 4*, 19-31.

Augustin, S., Guichard, S., Heitland, W., Freise, J., Svatos, A., & Gilbert, M. (2009). Monitoring and dispersal of the invading Gracillariidae *Cameraria ohridella*. Journal of Applied Entomology, 133, 58–66.

Avcı, G., Küçükkurt, I., Akkol, E.K., & Yeşilada, E., (2010). Effects of escin mixture from the seeds of *Aesculus hippocastanum* on obesity in mice fed a high fat diet. *Pharmaceutical Biology, 48*, 247–252.

Avtzis, N., & Avtzis, D. (2002). The attack of *Aesculus hippocastanum* L. by *Cameraria ohridella* Deschka and Dimić (Lepidoptera: Gracillariidae) in Greece. In: M.L. McManus & A.M. Liebhold (Eds.), *Proceedings: ecology, survey and management*

of forest insects. 2002 Sep 1–5, Kraków, Poland (pp. 1–5). USDA Forest Service, General Technical Report NE-311, PA, USA.

Avtzis, N.D., Avtzis, D.N., Vergos, S.G., & Diamandis, S. (2007). A contribution to the natural distribution of *Aesculus hippocastanum* (Hippocastanaceae) in Greece. *Phytologia Balcanica*, *13*, 183-187.

Azarkovich, M.I., & Bolyakina, Yu.P. (2016). Recalcitrant seeds of horse-chestnut lack protein bodies. *Russian Journal of Plant Physiology, 63*, 499–504.

Azarkovich, M.I., & Gumilevskaya, N.A. (2012). Response of dormant recalcitrant horse-chestnut (*Aesculus hippocastanum* L.) seeds to heat shock. *Plant Stress, 6*, 14-19.

Bainard, L.D., Klironomos, J.N., & Gordon, A.M. (2011). The mycorrhizal status and colonization of 26 tree species growing in urban and rural environments. *Mycorrhiza, 21*, 91–96.

Bainbridge, J.W. (1984). *Aesculus hippocastanum* in war and peace. *Journal of Biological Education, 18*, 65-71.

Baraldi, C., Bodecchi, L.M., Cocchi, M., Durante, C., Ferrari, G., Foca, G., Grandi, M., Marchetti, A., Tassi, L., & Ulrici, A. (2007). Chemical composition and characterisation of seeds from two varieties (pure and hybrid) of *Aesculus hippocastanum*. *Food Chemistry, 104*, 229–236.

Baraniak, E., Walczak, U., Tryjanowski, P., & Zduniak, P. (2004). Effect of distance between host trees and leaf litter removal on population density of *Cameraria ohridella* Deschka & Dimic, 1986 (Lepidoptera, Gracillariidae) – pest of chestnut (*Aesculus* sp.) trees. *Polish Journal of Ecology, 52*, 569-574.

Barczewski, M., Matykiewicz, D., Krygier, A., Andrzejewski, J., & Skórczewska, K. (2018). Characterization of poly(lactic acid) biocomposites filled with chestnut shell waste. *Journal of Material Cycles and Waste Management, 20*, 914–924.

Barnett, J.R. (1992). Reactivation of the cambium in *Aesculus hippocastanum* L.: A transmission electron microscope study. *Annals of Botany, 70*, 169-177.

Barnett, J.R., Cooper, P., & Bonner, L.J. (1993). The protective layer as an extension of the apoplast. *IAWA Bulletin, 14*, 163-171.

Basler, D., & Körner, C. (2012). Photoperiod sensitivity of bud burst in 14 temperate forest tree species. *Agricultural and Forest Meteorology, 165*, 73–81.

Bates, J.W., Proctor, M.C.F., Preston, C.D., Hodgetts, N.G., & Perry, A.R. (1997). Occurrence of epiphytic bryophytes in a 'tetrad' transect across southern Britain 1. Geographical trends in abundance and evidence of recent change, *Journal of Bryology, 19*, 685-714,

Batten, L.A., & Pomeroy, D.E. (1969). Effects of reafforestation on the birds of Rhum, Scotland. *Bird Study, 16*, 13-16.

Bean, W.J. (1976). *Trees and shrubs hardy in the British Isles* (8th ed.). London, UK: Murray.

Bednarz, B., & Scheffler, M. (2008). Wpływ żeru szrotówka kasztanowcowiaczka (*Cameraria ohridella* Deschka & Dimic) na szerokość słojów rocznych kasztanowca białego (*Aesculus hippocastanum* L.). [Effect of horse-chestnut leaf-miner (*Cameraria ohridella* Deschka & Dimic) outbreak on tree-ring widths of white horse chestnut (*Aesculus hippocastanum* L.)]. *Sylwan, 152*, 53–66.

Bellini, E., & Nin, S. (2005). Horse-chestnut: cultivation for ornamental purposes and non-food crop production. *Journal of Herbs, Spices & Medicinal Plants, 11*, 93-120.

Bennett, M.D., Smith, J.B., & Heslop-Harrison, J.S. (1982). Nuclear DNA amounts in Angiosperms. *Proceedings of the Royal Society of London, Series B, 216*, 179-199.

Biçakci, A., Benlioglu, O.N., & Erdogan, D. (1999). Airborne pollen concentration in Kütahya. *Turkish Journal of Botany, 23*, 75-81.

Birtić, S., & Kranner, I. (2006). Isolation of high-quality RNA from polyphenol-, polysaccharide- and lipid-rich seeds. *Phytochemical Analysis, 17,* 144–148.

Boldt, K.M., & Rank, B. (2010). Stomata dimorphism in dicotyledonous plants of temperate climate. *Feddes Repertorium, 121*, 167-183.

Bombardelli, E., Morazzoni, P., & Griffini, A. (1996). *Aesculus hippocastanum* L. *Fitoterapia, 67*, 483-511.

Bonner, F.T., & Karrfalt, R.P. (2008). *The woody plant seed manual.* USDA Forest Service, Agriculture Handbook 727, Washington, DC, USA.

Bradshaw, R.H.W. (2004). Past anthropogenic influence on European forests and some possible genetic consequences. *Forest Ecology and Management, 197*, 203–212.

Braga, P.C., Marabini, L., Wang, Y.Y., Lattuada, N., Calò, R., Bertelli, A., Falchi, M., Dal Sasso, M., & Bianchi T. (2012). Characterisation of the antioxidant effects of *Aesculus hippocastanum* L. bark extract on the basis of radical scavenging activity, the chemiluminescence of human neutrophil bursts and lipoperoxidation assay. *European Review for Medical and Pharmacological Sciences, 16*, 1-9.

Braithwaite, M.E., Ellis, R.W., & Preston, C.D. (2006). *Change in the British Flora 1987-2004.* London, UK: Botanical Society of the British Isles.

Brasier, C.M., & Jung, T. (2006). Recent developments in *Phytophthora* diseases of trees and natural ecosystems in Europe. *Progress in Research on Phytophthora Diseases of Forest Trees. Proceedings, 3rd Int. IUFRO Working Party, 7*, 11–17.

Brasier, C.M., & Strouts, R.G. (1976). New records of *Phytophthora* on trees in Britain. I. *Phytophthora* root rot and bleeding canker of Horse-chestnut (*Aesculus hippocastanum* L.). *European Journal of Forest Pathology, 6*, 129-136.

Briggs, M. (1989). Hon. General Secretary's notes. Enquiry replies. *Botanical Society of the British Isles News, 53*, 6.

British Mycological Society (2018). Fungal Records Database

http://www.fieldmycology.net/frdbi/frdbi.asp Accessed 22/08/2018.

Bultreys, A., Gheysen, I., & Planchon, V. (2008). Characterization of *Pseudomonas syringae* strains isolated from diseased horse-chestnut trees in Belgium. In: M. Fatmi, A. Collmer, N.S. Iacobellis, J.W. Mansfield, J. Murillo, N.W. Schaad & M. Ullrich (Eds.) *Pseudomonas syringae pathovars and pelated pathogens* (pp. 283–293). Dordrecht, The Netherlands: Springer,

Burkhard, R., Binz, H., Roux, C.A., Brunner, M., Ruesch, O., & Wyss, P. (2015). Environmental fate of emamectin benzoate after tree micro injection of Horse-chestnut trees. *Environmental Toxicology and Chemistry, 34*, 297–302.

Ćalić, D., Bohanec, B., Devrnja, N., Milojević, J., Tubić, L., Kostić, I., & Zdravković-Korać, S. (2013). Impact of abscisic acid in overcoming the problem of albinism in horse-chestnut androgenic embryos. *Trees, 27*, 755–762.

Ćalić, D., & Radojević, L. (2017). Horse-chestnut pollen quality. *Genetika, 49*, 105-115.

Ćalić, D., Zdravković-Korać, S., Pemac, D., & Radojević, L. (2003-2004). Variability and bimodal distribution of size in microspores of *Aesculus hippocastanum. Biologia Plantarum, 47*, 457-458.

Ćalić-Dragosavac, D., Stevović, S., & Zdravković-Korać, S. (2010). Impact of genotype, age of tree and environmental temperature on androgenesis induction of *Aesculus hippocastanum* L. *African Journal of Biotechnology, 9*, 4042-4049.

Ćalić-Dragosavac, D., Zdravković-Korać, S., Miljković, D., & Radojević, L. (2009). Comparative analysis of microspore size variability in the genus *Aesculus* (Hippocastanaceae). *Archives of Biological Sciences, 61*, 795-800.

Capuana, M. (2016). Somatic embryogenesis in horse-chestnut (*Aesculus hippocastanum* L.). In M.A. Germanà & M. Lambardi (Eds.), *In vitro embryogenesis in higher plants*

(pp. 431-438). Methods in Molecular Biology 1359, Springer Protocols, Springer: New York, USA,

Castaño, J., Rodríguez-Llamazares, S., Contreras, K., Carrasco, C., Pozo, C., Bouza, R., Franco, C.M.L., & Giraldo, D. (2014). Horse-chestnut (*Aesculus hippocastanum* L.) starch: Basic physico-chemical characteristics and use as thermoplastic material. *Carbohydrate Polymers*, **112**, 677–685.

Cebeci, H.H., & Acer, S. (2007). The Occurrence of some Lepidopterous species on the horse-chestnut (*Aesculus hippocastanum* L.) at Istanbul-Belgrad Forest in Turkey. *Acta Agriculturae Slovenica, 89*, 95-102.

Cetin, M., Sevik, H., & Yigit, N. (2018). Climate type-related changes in the leaf micromorphological characters of certain landscape plants. *Environmental Monitoring and Assessment, 190*, e404.

Chambers, V.H. (1968) Pollens collected by species of *Andrena* (Hymenoptera: Apidae). *Proceedings of the Royal Entomological Society of London, Series A, 43*, 155-160.

Chaney, W.R. (1991). Horse-chestnut: *Aesculus hippocastanum*. ArborAge, 15,31–32.

Chapman, D.J., &Hoover, S. (1982). Propagation of shade trees by softwood cuttings. *Combined Proceedings - International Plant Propagators' Society, 31*, 507-511.

Chen, L., Huang, J.-G., Qianqian, m., Hänninen, H., Rossi, S., Piao, S., & Bergeron, Y. (2018). Spring phenology at different altitudes is becoming more uniform under global warming in Europe. *Global Change Biology, 24*, 3969-3975.

Cheong, D.H.J., Arfuso, F., Sethi, G., Wang, L., Hui, K.M., Kumar, A.P., & Tran, T. (2018). Molecular targets and anti-cancer potential of escin. *Cancer Letters, 422*, 1-8.

Chmura, D. (2004) Penetration and naturalisation of invasive alien plant species (neophytes) in woodlands of the Silesian Upland (southern Poland). *Nature Conservation, 60*, 3-11.

Chwil, M., Weryszko-Chmielewska, E., Sulborska, A., & Michońska, M. (2013). Micromorphology of trichomes in the flowers of the horse-chestnut *Aesculus hippocastanum* L. *Acta Agrobotanica, 66*, 45–53.

Çolak, H., Karaköse, E., & Duman, F. (2017). High optoelectronic and antimicrobial performances of green synthesized ZnO nanoparticles using *Aesculus hippocastanum*. *Environmental Chemistry Letters, 15*, 547–552.

Coruh, N., & Ozdogan, N. (2014). Fluorescent coumarin components of the bark of *Aesculus hippocastanum*. *Journal of Liquid Chromatography & Related Technologies, 37*, 1334–1350.

Čukanović, J., Ninić-Todorović, J., Ognjanov, V., Mladenović, E., Ljubojević, M., & Kurjakov, A. (2011). Biochemical composition of the horse-chestnut seed (*Aesculus hippocastanum* L.). *Archives of Biological Sciences, Belgrade, 63*, 345-351.

Cutler, D.F., & Richardson, I.B.K. (1989). *Tree roots and buildings* (2nd ed.). Harlow, UK: Longman.

Czeczuga, B. (1986). Autumn carotenoids in the leaves of some trees. *Biochemical Systematics and Ecology, 14*, 203-206.

Czekalski, M. (2005). Root and stem suckers on common horsechestnut – *Aesculus hippocastanum* L. *Roczniki Akademii Rolniczej w Poznaniu, 372*, 39-41.

Dahlhausen, J., Biber, P., Rötzer, T., Uhl, E., & Pretzsch, H. (2016). Tree species and their space requirements in six urban environments worldwide. *Forests, 7*, e111.

Damant, S. (2005). Saproxylic hoverflies at Wimpole Estate. *Nature in Cambridgeshire, 47*, 3-8.

Dameri, R.M., Caffaro, I., Gastaldo, P., Profumo, P. (1986). Callus formation and embryogenesis with leaf explants of *Aesculus hippocastanum* L. *Journal of Plant Physiology, 126*, 93–96.

Daws, M.I., Lydall, E., Chmielarz, P., Leprice, O., Matthews, S., Thanos, C.A., & Pritchard, H.W. (2004). Developmental heat sum influences recalcitrant seed traits in *Aesculus hippocastanum* across Europe. *New Phytologist, 162*, 157-166.

DBIF (2018). Database of Insects and their Food Plants. http://www.brc.ac.uk/dbif/homepage.aspx Accessed 05/08/2018.

D'Costa, L., Koricheva, J., Straw, N., & Simmonds, M.S.J. (2013). Oviposition patterns and larval damage by the invasive horse-chestnut leaf miner *Cameraria ohridella* on different species of *Aesculus. Ecological Entomology, 38*, 456–462.

D'Costa, L., Simmonds, M.S.J., Straw, N., Castagneyrol, B., & Koricheva, J. (2014). Leaf traits influencing oviposition preference and larval performance of *Cameraria ohridella* on native and novel host plants. *Entomologia Experimentalis et Applicata, 152*, 157–164.

Defila, C., & Clot, B. (2001). Phytophenological trends in Switzerland. *International Journal of Biometeorology, 45*, 203–207.

de Keijzer, J., van den Broek, L.A.M., Ketelaar, T., & van Lammeren, A.A.M. (2012). Histological examination of horse-chestnut infection by *Pseudomonas syringae* pv. *aesculi* and non-destructive heat treatment to stop disease progression. *PLoS ONE, 7*, e39604.

Deli, J., Matus, Z., & Tóth, G. (2000). Comparative study on the carotenoid composition in the buds and flowers of different *Aesculus* species. *Chromatographia* (Supple.), *51*, 179-182.

Deljanin, I., Antanasijević, D., Bjelajac, A., Urošević, M.A., Nikolić, M., Perić-Grujić, A., & Ristić, M. (2016). Chemometrics in biomonitoring: Distribution and correlation of trace elements in tree leaves. *Science of the Total Environment, 545–546*, 361-371.

Denman, S., Kirk, S., Whybrow, A., Orton, E., & Webber, J.F. (2006) *Phytophthora kernoviae* and *P. ramorum*: host susceptibility and sporulation potential on foliage of susceptible trees. *EPPO Bulletin, 36*, 373-376.

Deschka, G., & Dimić, N. (1986). *Cameraria ohridella* sp. n. (Lep., Lithocolletidae) aus Mazedonien, Jugoslawien. *Acta Entomologica Jugoslavica, 22*,11–23.

Detzel, A, & Wink, M. (1993). Attraction, deterrence or intoxication of bees (*Apis mellifera*) by plant allelochemicals. *Chemoecology, 4*, 8-18.

Dickson, S., Gallagher, J., McIntyre, L., Suter, A., & Tan, J. (2004) An open study to assess the safety and efficacy of *Aesculus hippocastanum* tablets (Aesculaforce® 50 mg) in the treatment of chronic venous insufficiency. *Journal of Herbal Pharmacotherapy, 4*, 19-32.

Dobson, M.C. (1991). *De-icing salt damage to trees and shrubs*. Forestry Commission Bulletin 101. Farnham, UK: Forestry Commission.

Dudek-Makuch, M., & Studzińska-Sroka, E. (2015) Horse-chestnut – efficacy and safety in chronic venous insufficiency: an overview. *Revista Brasileira de Farmacognosia*, *25*, 533–541.

Dudek-Makuch, M., & Matławska, I. (2011). Flavonoids from the flowers of *Aesculus hippocastanum*. *Acta Poloniae Pharmaceutica, 68*, 403-408.

Dujesiefken, D., Rhaesa, A., Eckstein, D., & Stobbe, H. (1999). Tree wound reactions of differently treated boreholes. *Journal of Arboriculture, 25*, 113-123.

Dujesiefken, D., Stobbe, H., & Eckstein, D. (1998). Long-term effects of pruning on the trunk of lime and horsechestnut. *Forstwissenschaftliches Centralblatt, 117*, 305-315.

Duke, J.A., & Ayensu, E.S. (1985) *Medicinal Plants of China*. Algonac, Michigan, USA.

Dumitriu, B., Olariu, L., Nita, R., Zglimbe, L., & Rosoiu, N. (2013). Vascular anti-inflammatory effects of natural compounds from *Aesculus hippocastanum* and *Hedera helix*. *Romanian Biotechnological Letters, 18*, 7963-7974.

Dzięgielewska, M., & Kaup, G. (2008). Effectiveness of chemical control of the horse-chestnut leaf miner with insecticides from the group of chitin biosynthesis inhibitors with the use of methods of soil application and microinjection. *Ecological Chemistry and Engineering A, 15*, 253-258.

Eckstein, D., Liese, W., & Parameswaran, N. (1976). On the structural changes in wood and bark of a salt damaged horse-chestnut tree. *Holzforschung, 30*, 173-178.

Eckstein, D., Liese, W., & Ploessl, J. (1978). Histometrical studies on willows (*Salix* spp.) of different salt tolerance. *Forstwissenschaftliches Centralblatt, 97*, 335-341.

Edem. E., Kahyaoğlu, B., & Çakar, M.A. (2016). Acute effusive pericarditis due to horse-chestnut consumption. *American Journal of Case Reports, 17*, 305-308.

Elmas, O., Erbas, O., & Yigitturk, G. (2016). The efficacy of *Aesculus hippocastanum* seeds on diabetic nephropathy in a streptozotocin-induced diabetic rat model. *Biomedicine & Pharmacotherapy*, *83*, 392–396.

Eriksson, C., Månsson, P.E., Sjödin, K., & Schlyter, F. (2008). Antifeedants and feeding stimulants in bark extracts of ten woody non-host species of the pine weevil, *Hylobius abietis*. *Journal of Chemical Ecology, 34*, 1290-1297.

Estrella, N., & Menzel, A. (2006). Responses of leaf colouring in four deciduous tree species to climate and weather in Germany. *Climate Research, 32*, 253-267.

Evelyn, J. (1664). *Sylva*. London, UK: Jo. Martin & Ja. Allestry.

Evstatieva, L. (2011). *Aesculus hippocastanum*. In D. Peev, V. Vladimirov, A.S. Petrova, M. Anchev, D. Temniskova, C.M. Denchev, A. Ganeva & C. Gussev (Eds.), *Red data book of the Republic of Bulgaria: digital edition - Vol. 1 plants and fungi*, Sofia,

Bulgaria: Bulgarian Academy of Sciences & Ministry of Environment and Water. Available at: http://e-ecodb.bas.bg/rdb/en/

Facino, R.M., Carini, M., Stefani, R., Aldini, G., & Saibene, L. (1995) Anti-elastase and anti-hyaluronidase activities of saponins and sapogenins from *Hedera helix*, *Aesculus hippocastanurn*, and *Ruscus aculeatus*: factors contributing to their efficacy in the treatment of venous insufficiency. *Archiv der Pharmazi, 328*, 720-724.

Fang, Y., Zhao, L., Yan, F., Xia, X., Xu, D., & Cui, X. (2010). Escin improves sperm quality in male patients with varicocele-associated infertility. *Phytomedicine, 17*, 192–196.

Fant, F., Vranken, W.F., & Borremans, F.A.M. (1999). The three-dimensional solution structure of *Aesculus hippocastanum* antimicrobial protein 1 determined by ^{1}H nuclear magnetic resonance. *Proteins, 37*, 388–403.

Farrant, J.M., & Walters, C. (1998). Ultrastructural and biophysical changes in developing embryos of *Aesculus hippocastanum* in relation to the acquisition of tolerance to drying. *Physiologia Plantarum*, *104*, 513–524.

Ferracini, C., & Alma, A. (2008). How to preserve horse chestnut trees from *Cameraria ohridella* in the urban environment. *Crop Protection, 27*, 1251-1255.

Ferracini, C., Curir, P., Dolci, M., Lanzotti, V., & Alma, A. (2010). *Aesculus pavia* foliar saponins: defensive role against the leafminer. *Pest Management Science, 66*, 767-772.

Ferrini, F., & Fini, A. (2012). Results of a long-term project using controlled mycorrhization with specific fungal strains on different urban trees. In M. Johnston & G. Percival (Eds.), *Trees, people and the built environment*. (pp. 39-50). Edinburgh, UK: Forestry Commission,

Fitter, A.H., & Peat, H.J. (1994) Ecological Flora Database. *Journal of Ecology, 82*, 415-425. Http://ecoflora.org.uk/. Accessed 01/08/18

Forest, F., Drouin, J.N., Charest, R.C., Brouillet, L., & Bruneau, A. (2001) A morphological phylogenetic analysis of *Aesculus* L. and *Billia* Peyr. (Sapindaceae). *Canadian Journal of Botany, 79*, 154-169.

Forestry Commission (2008). *Report on the national survey to assess the presence of bleeding canker of horse-chestnut trees in Great Britain*. Edinburgh, UK: Forestry Commission, Plant Health Service.

Foster, A.S. (1929a). Investigations on the morphology and comparative history of development of foliar organs. I. The foliage leaves and cataphyllary structures in the horsechestnut (*Aesculus hippocastanum* L.). First part. *American Journal of Botany, 16*, 441-474.

Foster, A.S. (1929b). Investigations on the morphology and comparative history of development of foliar organs. I. The foliage leaves and cataphyllary structures in the horsechestnut (*Aesculus hippocastanum* L.), cont'd. *American Journal of Botany, 16*, 475-501.

Franiel, I., Woźnica, P., & Orlik, H. (2014). Zaburzenia procesu kwitnienia i owocowania *Aesculus hippocastanum* L. jako efekt żerowania *Cameraria ohridella* Deschka & Dimić. [Impact of *Cameraria ohridella* Deschka & Dimić on the disruption of the flowering and fructification processes of *Aesculus hippocastanum* L.] *Sylwan, 158*, 41-48.

Free, J.B. (1963). The flower constancy of honeybees. *Journal of Animal Ecology, 32*, 119-131.

Freise, J.F., Heitland, W., & Sturm, A. (2003). Das physiologische Wirtspflanzenspektrum der Rosskastanien-Miniermotte, *Cameraria ohridella* Deschka & Dimić (Lepidoptera: Gracillariidae) *Nachrichtenblatt des Deutschen Pfianzenschutzdienstes, 55*, 209-211.

Freise, J.F., Heitland, W., & Sturm, A. (2004) Host plant range of the horse-chestnut leaf miner, *Cameraria ohridella* (Lepidoptera: Grarillariidae), a pest of the white flowering horse-chestnut, Aesculus hippocastanum. *Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie, 14*, 351–354.

Fu, Y.H., Campioli, M., Van Oijen, M., Deckmyn, G., & Janssens, I.A. (2012). Bayesian comparison of six different temperature-based budburst models for four temperate tree species. *Ecological Modelling, 230*, 92–100.

Fu, Y.H., Zhao, H., Piao, S., Peaucelle, M., Peng, S., Zhou, G., Ciais, P., Huang, M., Menzel, A., Peñuelas, J., Song, Y., Vitasse, Y., Zeng, Z., & Janssens, I.A. (2015) Declining global warming effects on the phenology of spring leaf unfolding. *Nature, 526*, 104-107.

Fuhrer, J., & Erismann, K.H. (1980). Tolerance of *Aesculus hippocastanum* L. to foliar accumulation of chloride affected by air pollution. *Environmental Pollution (Series A), 21*, 249–254.

Fujimura, T., Tsukahara, K., Moriwaki, S., Hotta, M., Kitahara, T., & Takema, Y. (2007). A horse-chestnut extract, which induces contraction forces in fibroblasts, is a potent anti-aging ingredient. *International Journal of Cosmetic Science, 29*, 140.

Gastaldo, P., Carli, S., & Profumo, P. (1994). Somatic embryogenesis from stem explants of *Aesculus hippocastanum. Plant Cell, Tissue and Organ Culture, 39*, 97–99.

Geran, R.I., Greenberg, N.H., McDonald, M.M., Schumacher, A.M., & Abbott, B.J. (1972). Protocols for screening chemical agents and natural products against animal tumour and other biological systems. *Cancer Chemotherapy Reports, 3*, 17-19.

Gerard, J. (1633). *The herbal or general history of plants.* London, UK: A. Islip, J. Norton & R. Whitakers.

Gilbert, M., Grégoire, J.-C., Freise, J., & Heitland, W. (2004). Long-distance dispersal and human population density allow the prediction of invasive patterns in the horse-chestnut leafminer *Cameraria ohridella*. *Journal of Animal Ecology, 73*, 459–468.

Gilbert, M., Guichard, S., Freise, J., Grégorie, J.C., Heitland, W., Straw, N.C., Tilbury, C., & Augustin, S. (2005). Forecasting *Cameraria ohridella* invasion dynamics in recently invaded countries: from validation to prediction. *Journal of Applied Ecology, 42*, 805–813.

Gilbert, M., Svatoš, A., Lehmann, M., & Bacher, S. (2003). Spatial patterns and infestation processes in the horse-chestnut leafminer *Cameraria ohridella*: a tale of two cities. *Entomologia Experimentalis et Applicata, 107*, 25–37.

Gobet, E., Schwörer, C., van Leeuwen, J.F.N., Wahab, S.A., Nielsen, E.H., Kotova, N., Makhortykh, S., Kiosak, D., & Tinner, W. (2017). Vegetation shifts at the monumental Ukrainian site of Kamyana Mohyla during the neolithisation period. In S. Makhortykh & A. de Capitani (Eds), Archaeology and Palaeoecology of the Ukrainian Steppe (pp. 51-60). Kiev, Ukraine: National Academy of Sciences of Ukraine.

Godzik, S., & Halbwachs, G. (1986). Structural alterations of *Aesculus hippocastanum* leaf surface by air pollutants. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, 93*, 590-596.

Godzik, S., & Sessen, M.M.A. (1970). A scanning electron microscope examination of *Aesculus hippocastanum* L. leaves from control and air-polluted areas. *Environmental Pollution, 17*, 13-18.

Gosling, P. (2007). *Raising trees and shrubs from seed*. Edinburgh, UK: Forestry Commission.

Grabenweger, G., Avtzis, N., Girardoz, S., Hrasovec, B., Tomov, R., & Kenis, M. (2005). Parasitism of *Cameraria ohridella* (Lepidoptera, Gracillariidae) in natural and

artificial horse-chestnut stands in the Balkans. *Agricultural and Forest Entomology,* 7, 291-296.

Gray-Block, A. (2010). *Anne Frank tree falls over in heavy wind, rain*. Reuters. https://www.reuters.com/article/idUSLDE67M1DH. Accessed 22/02/18.

Green, S., Laue, B., Fossdal, C.G., A'Hara, S.W., & Cottrell, J.E. (2009). Infection of horse-chestnut (*Aesculus hippocastanum*) by *Pseudomonas syringae* pv. *aesculi* and its detection by quantitative real-time PCR. *Plant Pathology 58*, 731–744.

Green, S., Laue, B., Steele, H., & Nowell, R. (2014). *Horse-chestnut bleeding canker*. Research Note FCR017. Edinburgh, UK: Forestry Commission.

Green, S., Studholme, D.J., Laue, B.E., Dorati, F., Lovell, H., Arnold, D., Cottrell, J.E., Bridgett, S., Blaxter, M., Huitema, E., Thwaites, R., Sharp, P.M., Jackson, R.W., & Kamoun, S. (2010). Comparative genome analysis provides insights into the evolution and adaptation of *Pseudomonas syringae* pv. *aesculi* on *Aesculus hippocastanum*. *PLoS ONE, 5*, e10224.

Greig, J.W. (2012). Decay in an avenue of horse chestnut (*Aesculus hippocastanum* L) caused by *Ustulina deusta*. *Arboricultural Journal, 13*, 1-6.

Groh, B., Hübner, C., & Lendzian, K.J. (2002). Water and oxygen permeance of phellems isolated from trees: the role of waxes and lenticels. *Planta, 215*, 794-801.

Grosse, W., & Schröder, P. (1985) Aeration of the roots and chloroplast-free tissues of trees. *Berichte der Deutschen Botanischen Gesellschaft, 98*, S311-318.

Grove, A.T., & Rackham, O. (2001). *The nature of Mediterranean Europe: an ecological history*. New Haven, USA: Yale University Press.

Grünwald, N.J., Werres, S., Goss, E.M., Taylor, C.R., & Fieland, V.J. (2012). *Phytophthora obscura* sp. nov., a new species of the novel *Phytophthora* subclade 8d. *Plant Pathology, 61*, 610–622.

Guglielmo, F., Bergemann, S.E., Gonthier, P., Nicolotti, G., & Garbelotto, M. (2007). A multiplex PCR-based method for the detection and early identification of wood rotting fungi in standing trees. *Journal of Applied Microbiology, 103*, 1490–1507.

Guha, M.M., & Mitchell, R.L. (1966). The trace and major element composition of the leaves of some deciduous trees. II. Seasonal changes. *Plant and Soil, 24*, 90–112.

Gülz, P.-G., Müller, E., & Herrmann, T. (1992). Chemical composition and surface structures of epicuticular leaf waxes from *Castanea sativa* and *Aesculus hippocastanum. Zeitschrift Fur Naturforschung Section C, 47*, 661-666.

Gurel, E., Ustunova, S., Ergin, B., Tan, N., Caner, M., Tortum, O., & Demirci-Tansel, C. (2013). Herbal haemorrhoidal cream for haemorrhoids. *Chinese Journal of Physiology, 56*, 253–262.

Gussev, C., & Vulchev, V. (2015). *Forests of horse-chestnut* (*Aesculus hippocastanum*). In V. Biserkov, C. Gussev, V. Popov, G. Hibaum, V. Roussakova, I. Pandurski, Y. Uzunov, M. Dimitrov, R. Tzonev & S. Tsoneva (Eds), Red data book of the Republic of Bulgaria: digital edition – Vol. 3 natural habitats. Sofia, Bulgaria: Bulgarian Academy of Sciences & Ministry of Environment and Water. Available at: http://e-ecodb.bas.bg/rdb/en/

Hardin, J.W. (1957). Studies in the Hippocastanaceae, IV. Hybridization in *Aesculus. Rhodora, 59*, 185-203.

Hardin, J.W. (1960). Studies in the Hippocastanaceae, V. Species of the Old World. *Brittonia, 12*, 26-38.

Harley, J.L., & Harley, E.L. (1987). A check-list of mycorrhiza in the British Flora. *New Phytologist, 105* (suppl.), 1-102.

Harris, A.J., Xiang, Q.-Y., & Thomas, D.T. (2009). Phylogeny, origin and biogeographic history of *Aesculus* L. (Sapindales) – an update from combined analysis of DNA sequences, morphology and fossils. *Taxon, 58*, 108-126.

Heinrich, G., Müller, H.J., Oswald, K., & Gries, A. (1989). Natural and artificial radionuclides in selected styrian soils and plants before and after the reactor accident in Chernobyl. *Biochemie Und Physiologie Der Pflanzen, 185*, 55-67.

Heitland, W., & Freise. J. (2001). Verbreitung der Roßkastanien-Miniermotte, *Cameraria ohridella* (Lep., Gracillariidae) in Deutschland. *Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie, 13*, 131-134.

Hill, M.O., Preston, C.D., & Roy, D.B. (2004). *PLANTATT. Attributes of British and Irish plants: status, size, life history, geography and habitats.* Huntingdon, UK: Centre for Ecology and Hydrology.

Hirons, A., & Sjöman, H. (2018). *Tree species selection for green infrastructure: A guide to specifiers.* Trees & Design Action Group. Available at: https://www.myerscough.ac.uk/media/4052/hirons-and-sjoman-2018-tdag-tree-species-selection-1-1.pdf.

Hněvsová, V., Kodrík, D., & Weyda, F. (2011) Contribution to the biochemical characterization of the silk and structure characterization of the cocoons of the horse chestnut leaf miner *Cameraria ohridella* (Lepidoptera: Gracillariidae). *European Journal of Entomology, 108*, 711–715.

Hoar, C.S. (1927). Chromosome studies in Aesculus. *Botanical Gazette, 84*, 156-170.

Højgaard, A., Jóhansen, J., & Ødum, S. (1989). Træplanting í Føroyum í eina øld. [A century of tree planting on the Faroe Islands.] *Annales Societatis Scientiarum Færoensis. Supplementum, 14*. Føroya fróðskaparfelag, Tórshavn, Faroe Islands.

Horvat, I., Glavac, V., & Ellenberg, H. (1974). *Vegetation Südeuropas.* Stuttgard, Germany: Gustav Fischer Verlag.

Hoshizaki, K., Suzuki, W., & Nakashizuka, T. (1999). Evaluation of secondary dispersal in a large-seeded tree *Aesculus turbinata*: a test of directed dispersal. *Plant Ecology, 144*, 167–176.

Howard, A.L. (1945). The horse-chestnut tree (*Aesculus hippocastanum*). *Nature, 155*, 521-522.

Hricovíniová, Z., & Babor, K. (1991). Saccharide constituents of horse-chestnut (*Aesculus hippocastanum* L.) seeds. I. Monosaccharides and their isolation. *Chemical Papers, 45*, 553-558.

Hricovíniová, Z., & Babor, K. (1992). Saccharide constituents of horse-chestnut (*Aesculus hippocastanum* L.) seeds. II. Isolation and characterization of the starch. *Chemical Papers, 46*, 196-198.

Hübner, G., Wray, V., & Nahrstedt, A. (1999). Flavonol oligosaccharides from the seeds of *Aesculus hippocastanum*. *Planta Medica, 65*, 636-642.

Hudson, H.J. (1987) *Guignardia* leaf blotch of horsechestnut. *Transactions of the British Mycological Society, 89*, 400-401.

Hutchings, T., Lawrence, V., & Brunt, A. (2012). *Estimating the ecosystem services value of Edinburgh's trees.* Edinburgh, UK: Forest Research.

Ianovici, N., Latiş, A., & Rădac, A. (2017). Foliar traits of *Juglans regia, Aesculus hippocastanum* and *Tilia platyphyllos* in urban habitat. *Romanian Biotechnological Letters, 22*, 12400-12408.

Ing, B., & Spooner, B. (2002). The horse-chestnut powdery mildew *Uncinula flexuosa* in Europe (new British Record 210). *Mycologist, 16*, 112-113.

Irzykowska, L., Werner, M., Bocianowski, J., Karolewski, Z., & Frużyńska-Jóźwiak, D. (2013). Genetic variation of horse-chestnut and red horse-chestnut and trees susceptibility to *Erysiphe flexuosa* and *Cameraria ohridella*. *Biologia, 68*, 851-860.

Isidorov, V.A., Bakier, S., Pirożnikow, E., Zambrzycka, M., & Swiecicka, I. (2016). Selective behaviour of honeybees in acquiring European propolis plant precursors. *Journal of Chemical Ecology, 42*, 475-485.

Jabłońska, K., Kwiatkowska-Falińska, A., Czernecki, B., & Walawender, J.P. (2015). Changes in spring and summer phenology in Poland - responses of selected plant species to air temperature variations. *Polish Journal of Ecology, 63*, 311-319.

Jagiełło, R., Baraniak, E., Karolewski, P., Łakomy, P., Behnke-Borowczyk, J., Walczak, U., & Giertych, M.J. (2017). Ecophysiological aspects of the interaction between *Cameraria ohridella* and *Guignardia aesculi* on *Aesculus hippocastanum*. *Dendrobiology, 78*,146–156.

Jagiełło, R., Walczak, U., Iszkuło, G., Karolewski, P., Baraniak, E., & Giertych, M.J. (2018). Impact of *Cameraria ohridella* on *Aesculus hippocastanum* growth and long-term effects of trunk injection with pesticides. *International Journal of Pest Management*, DOI: 10.1080/09670874.2018.1454630.

Jagodziński, A.M., Łukasiewicz, S., & Turzańska, E. (2003). Kasztanowiec zwyczajny w środowisku życia człowieka. [Horse-chestnut in the human environment.]. *Ten Świat. Biuletyn Polskiego Klubu Ekologicznego – Okręg Wielkopolski, 3*, 6–13.

Jansen, S., Choat, B., & Pletsers, A. (2009). Morphological variation of intervessel pit membranes and implications to xylem function in angiosperms. *American Journal of Botany, 96*, 409–419.

Jaspersen-Schib, R., Theus, L., Guirguis-Oeschger, M., Gossweiler, B., & Meier-Abt, P.J. (1996). Serious plant poisonings in Switzerland 1966-1994. Case analysis from the Swiss Toxicology Information Center. *Schweizerische medizinische Wochenschrift, 126*, 1085-1098.

Jeffree, E.P. (1960). Some long-term means from the phenological reports (1891–1948) of the Royal Meteorological Society. *Quarterly Journal of the Royal Meteorological Society, 86*, 95–103.

Jekkel, Zs., Gyulai, G., Kiss, J., Kiss, E., & Heszky, L.E. (1998). Cryopreservation of horse-chestnut (*Aesculus hippocastanum* L.) somatic embryos using three different freezing methods. *Plant Cell, Tissue and Organ Culture, 52*, 193–197.

Jiang, N., Xin, W., Wang, T., Zhang, L., Fan, H., Du, Y., Li, C., & Fu, F. (2011). Protective effect of aescin from the seeds of *Aesculus hippocastanum* on liver injury induced by endotoxin in mice. *Phytomedicine, 18*, 1276– 1284.

Jochner, S., Markevych, I., Beck, I., Traidl-Hoffmann, C., Heinrich, J., & Menzel, A. (2015). The effects of short- and long-term air pollutants on plant phenology and leaf characteristics. *Environmental Pollution, 206*, 382-389.

Johne, A.B., Weissbecker, B., & Schütz, S. (2008). Approaching risk assessment of complex disease development in horse-chestnut trees: a chemical ecologist's perspective. *Journal of Applied Entomology, 132*, 349-359.

Jörgensen, J. (1989). Somatic embryogenesis in *Aesculus hippocastanum* L. by culture of filament callus. *Journal of Plant Physiology, 135*, 240–241.

Kahl, W., Roszkowski, A., & Zurowska, A. (1969). The isolation of 6-kestose from the seeds of the horse-chestnut (*Aesculus hippocastanum* L.). *Carbohydrate Research, 10*, 586-588.

Kamerling, J.P., & Vliegenthart, J.F.G. (1972). Isolation and identification of nystose from seeds of the horse-chestnut (*Aesculus hippocastanum* L.) *Carbohydrate Research, 25*, 293-297.

Kántor, N., Chen, L., & Gáld, C.V. (2018). Human-biometeorological significance of shading in urban public spaces - summertime measurements in Pécs, Hungary. *Landscape and Urban Planning, 170*, 241–255.

Kapusta, I., Janda, B., Szajwaj, B., Stochmal, A., Piacente, S., Pizza, C., Franceschi, F., Franz, C., & Oleszek, W. (2007). Flavonoids in horse-chestnut (*Aesculus*

hippocastanum) seeds and powdered waste water byproducts. *Journal of Agricultural and Food Chemistry, 55*, 8485–8490.

Karliński, L., Jagodziński, A.M., Leski, T., Butkiewicz, P., Brosz, M., & Rudawska, M. (2014). Fine root parameters and mycorrhizal colonization of horse-chestnut trees (*Aesculus hippocastanum* L.) in urban and rural environments. *Landscape and Urban Planning, 127*, 154–163.

Kędzierski, B., Kukula-Kocha, W., Widelski, J., & Głowniak, K. (2016) Impact of harvest time of *Aesculus hippocastanum* seeds on the composition, antioxidant capacity and total phenolic content. *Industrial Crops and Products, 86*, 68–72.

Kehrli, P., & Bacher, S. (2003). Date of leaf litter removal to emergence of *Cameraria ohridella* in the following spring. *Entomologia Experimentalis et Applicata, 107*, 159–162.

Kehrli, P., & Bacher, S. (2004). How to safely compost *Cameraria ohridella*-infested horse-chestnut leaf litter on private compost heaps. *Journal of Applied Entomology, 128*, 707-709.

Keilin, D. (1927). Fauna of a horse-chestnut tree (*Aesculus hippocastanum*). Dipterous larvae and their parasites. *Parasitology, 19*, 368-374.

Keith, L.M.W., & Bender, C.L. (1999). AlgT (σ^{22}) controls alginate production and tolerance to environmental stress in *Pseudomonas syringae. Journal of Bacteriology, 181*, 7176–7184.

Kenis, M., Tomov, R., Svatos, A., Schlinsog, P., Lopez-Vaamonde, C., Heitland, W., Grabenweger, G., Girardoz, S., Freise, J., & Avtzis, N. (2005). The horse-chestnut leaf miner in Europe - prospects and constraints for biological control. In M. Hoddle (Ed.), *Proceedings; Second International Symposium on Biological Control of Arthropods* (pp. 77-90). USDA Forest Service, Publication FHTET-2005-08, Morgantown. West Virginia, USA: USDA Forest Service.

Kenis, M., Girardoz, S., Freise, J., Heitland, W., Grabenweger, G., Lakatos, F., Vaamonde, C.L., & Tomov, R. (2006). Finding the area of origin of the horse-chestnut leaf miner: a challenge. In N. Kamata, A.M. Liebhold, D.T. Quiring & K.M. Clancy (Eds.), *Proccedings: IUFRO Kanazawa 2003 "Forest Insect Population Dynamics and Host Influences"* (p. 63–66). Kakuma, Japan: Kanazawa University.

Kennelly, M.M., Cazorla, F.M., de Vicente, A., Ramos, C., & Sundin, G.W. (2007). *Pseudomonas syringae* diseases of fruit trees. Progress towards understanding and control. *Plant Disease, 91*, 4–16.

Kevan, P.G. (1990) How large bees, *Bombus* and *Xylocopa* (Apoidea Hymenoptera) forage on trees: Optimality and patterns of movement in temperate and tropical climates. *Ethology Ecology and Evolution, 2*, 233-242.

Khan, F.I., & Abbasi, S.A. (2000). Cushioning the impact of toxic release from runaway industrial accidents with greenbelts. *Journal of Loss Prevention in the Process Industries, 13*, 109–124.

Khidyrova, N.K., & Shakhidoyatov, Kh.M. (2002). Plant polyprenols and their biological activity. *Chemistry of Natural Compounds, 38*, 107-121.

Kim, N.D., & Fergusson, J.E. (1994). Seasonal variations in the concentrations of cadmium, copper, lead and zinc in leaves of the horse chesnut (*Aesculus hippocastanum* L.). *Environmental Pollution, 86*, 89-97.

Kim, S.E., Kim, T.H., Park, S.A., Kim, W.T., Park, Y.W., Ahn, J.S., Jeong, M., Kim, M.-Y., & Seo, K. (2017). Efficacy of horse-chestnut leaf extract ALH-L1005 as a matrix metalloproteinase inhibitor in ligature-induced periodontitis in canine model. *Journal of Veterinary Science, 18*, 245-251.

Kiss, J., Heszky, L.E., Kiss, E., & Gyulai, G. (1992). High efficiency adventive embryogenesis on somatic embryos of anther, filament and immature proembryo

origin in horse-chestnut (*Aesculus hippocastanum* L.) tissue culture. *Plant Cell, Tissue and Organ Culture, 30*, 59-64.

Kiss, L., Vajna, L., & Fischl, G. (2004) Occurrence of *Erysiphe flexuosa* (syn. *Uncinula flexuosa*) on horse-chestnut (*Aesculus hippocastanum*) in Hungary. *Plant Pathology, 53*, 245.

Kobza, M., Juhásová, G., Adamčíková, K., & Onrušková, E. (2011). Tree injection in the management of horse-chestnut leaf miner. *Cameraria ohridella* (Lepidoptera: Gracillariidae). *Gesunde Pflanzen, 62*, 139–143.

Koch, K. (1857) Monographie du genre *Aesculus. Belgique Horticole, 7*, 309-319.

Kocić, K., Spasić, T., Urošević, M.A., & Tomašević, M. (2014). Trees as natural barriers against heavy metal pollution and their role in the protection of cultural heritage. *Journal of Cultural Heritage, 15*, 227–233.

Konarska, J., Uddling, J., Holmer, B., Lutz, M., Lindberg, F., Pleijel, H., & Thorsson, S. (2016). Transpiration of urban trees and its cooling effect in a high latitude city. *International Journal of Biometeorology, 60*, 159–172.

Konoshima, T., & Lee, K.-H. (1986). Antitumor agents, 82. Cytotoxic sapogenols from *Aesculus hippocastanum. Journal of Natural Products, 49*, 650-656.

Kopačka, M., & Zemek, R. (2017). Spatial variability in the level of infestation of the leaves of horse-chestnut by the horse-chestnut leaf miner, *Cameraria ohridella* (Lepidoptera: Gracillariidae) and in the number of adult moths and parasitoids emerging from leaf litter in an urban environment. *European Journal of Entomology, 114*, 42-52.

Kosibowicz, M., & Skrzecz, I. (2010). Wykorzystanie chlotianidyny i diflubenzuronu w ochronie kasztanowców *Aesculus hippocastanum* przed szrotówkiem kasztanowcowiaczkiem *Cameraria ohridella*. [The application of chlotianidin and

diflubenzuron for the protection of the horse−chestnut *Aesculus hippocastanum* against the horse−chestnut leaf−miner *Cameraria ohridella*.] *Sylwan, 154*, 439-449.

Koskella, B., Meaden, S., Crowther, W.J., Leimu, R., & Metcalf, C.J.E. (2017). A signature of tree health? Shifts in the microbiome and the ecological drivers of horse-chestnut bleeding canker disease. *New Phytologist, 215*, 737–746.

Krahulcová, A., Trávníček, P., Krahulec, F., & Rejmánek, M. (2017) Small genomes and large seeds: chromosome numbers, genome size and seed mass in diploid *Aesculus* species (Sapindaceae). *Annals of Botany, 119*, 957–964.

Krehan, H. (1995). Roβkastanienminiermotte - Befallssituation in Österreich. *Forstschutz-Aktuell, 16*, 8-11.

Krehan, H. (1997). Stadtbaum-Aktuell: Roβkastanienminiermotte—Vergleich der Bekämpfungsverfahren. *Forstschutz-Aktuell, 19/20*, 1-6.

Křivánek, M., Pyˇsek, P., & Jarošík, V. (2006). Planting history and propagule pressure as predictors of invasion by woody species in a temperate region. *Conservation Biology, 20*, 1487–1498.

Küçükkurt, I., Ince, S., Keleşş, H., Akkol, E.K., Avcı, G., Yeşilada, E., & Bacak, E. (2010). Beneficial effects of *Aesculus hippocastanum* L. seed extract on the body's own antioxidant defense system on subacute administration. *Journal of Ethnopharmacology, 129*, 18–22.

Kugler, H. (1936). Die Ausnutzung der Saftmalsumfärbung bei den Roβkastanienblüten durch Bienen und Hummeln. *Berichte der Deutschen Botanischen Gesellschaft, 54*, 394-399.

Kugler H. (1970). *Blütenökologie*. Stuttgart, Germany: Gustav Fisher Verlang.

Kukuła-Młynarczyk, A., & Hurej, M. (2007). Incidence, harmfulness and some elements of the horse-chestnut leafminer (*Cameraria ohridella* Deschka & Dimic) control on

white horse-chestnut (*Aesculus hippocastanum* L.). *Journal of Plant Protection Research, 47*, 53-64.

Lack, H.W. (2000). Lilac and horse-chestnut: Discovery and rediscovery. *Curtis's Botanical Magazine,17*, 109-141.

Lack, H.W. (2002). The discovery and rediscovery of the horse-chestnut. *Arnoldia, 61*,15–19.

Lampe, K.F., & Fagerstrom, R. (1968). *Plant toxicity and dermatitis.* Baltimore, USA: Williams & Wilkins.

Laraus, J. (2004). The problems of sustainable water use in the Mediterranean and research requirements for agriculture. *Annals of Applied Biology, 144*, 259–272.

Laube, J., Sparks, T.H., Estrella, N., Höfler, J., Ankerst, D.P., & Menzel, A. (2014). Chilling outweighs photoperiod in preventing precocious spring development. *Global Change Biology, 20*, 170–182.

Laue, B.E., Steele, H., & Green, S. (2014). Survival, cold tolerance and seasonality of infection of European horse-chestnut (*Aesculus hippocastanum*) by *Pseudomonas syringae* pv. *aesculi. Plant Pathology, 63*, 1417–1425.

Lambardi, M., De Carlo, A., & Capuana, M. (2005). Cryopreservation of embryogenic callus of *Aesculus hippocastanum* L. by vitrification/one-step freezing. *CryoLetters, 26*, 185–192.

Leathart, S. (1991). *Whence our trees.* London, UK: Foulsham.

Lees, D.C., Lack, H.W., Rougerie, R., Hernandez-Lopez, A., Raus, T., Avtzis, N.D., Augustin, S., & Lopez-Vaamonde, C. (2011). Tracking origins of invasive herbivores through herbaria and archival DNA: the case of the horse-chestnut leaf miner. *Frontiers in Ecology and the Environment, 9*, 322–328.

Leja, M., Mareczek, A., Wyżgolik, G., Klepacz-Baniak, J., & Czekońska, K. (2007). Antioxidative properties of bee pollen in selected plant species. *Food Chemistry, 100*, 237–240.

Lemajić, L.J., Savin, K., Ivanić, R., & Lalić, Ž. (1985). Masno ulje semena divljeg kestena. Arhiv za farmaciju, *Farmaceutski Fakultet, 36*, 295-300.

Leuzinger, S., Vogt, R., & Körner, C. (2010). Tree surface temperature in an urban environment. *Agricultural and Forest Meteorology, 150*, 56–62.

Lex, T. (1954). Duftmale an Blüten. *Zeitschrift für vergleichende Physiologie, 36*, 212-234.

Linnaeus, C. (1753). *Species Plantarum* 1-2. Holmiae.

Loenhart, K.K. (2002). *Aesculus hippocastanum*: The handsome (and useful) horse-chestnut. *Arnoldia, 61*, 20–22.

Loudon, J.C. (1838) *Arboretum et fruticetum Britannicum*. London, UK: privately published.

Łowiński, Ł., & Dach, J. (2006). Thermophilic composting of horse chestnut leaves with sewage sludge as a methode of reduction of contamination by *C. ohridella*. *Journal of Research and Applications in Agricultural Engineering, 51*, 108-111.

Łukasiewicz, S. (2003). Skład chemiczny i masa nasion na tle intensywności owocowania kasztanowca białego *Aesculus hippocastanum* L. w warunk-ach miejskich Poznania. [Chemical composition and seeds mass in relation to intensity of fruiting of white horse-chestnut *Aesculus hippocastanum* L. under urban conditions of Poznań.] *Biuletyn Ogrodów Botanicznych, 12*, 83–90.

Łukasiewicz, S., & Oleksyn, J. (2007). Zróżnicowanie przestrzenne elementów meteorologicznych i ich wpływ na rozwój kasztanowca zwyczajnego (*Aesculus hippocastanum* L.) w warunkach miejskich Poznania. [Heterogeneity of spatial meteorological traits and their effects on horsechesnut (*Aesculus hippocastanum* L.) development in urban conditions of Poznań.] *Badania Fizjograficzne nad Polską Zachodnią Seria A, 58*, 47–78.

Lunau, K. (1996) Unidirectionality of floral colour changes. *Plant Systematics and Evolution, 200*, 125–140.

Mabey, R. (1972) *Food for free.* London, UK: HarperCollins.

Majzlan, O., & Fedor, P.J. (2003). Vertical migration of beetles (Coleoptera) and other arthropods (Arthropoda) on trunks of *Aesculus hippocastanum* in Slovakia. *Bulletin de la Société des Naturalistes Luxembourgeois, 104*, 129-138.

Maltitz, I, von. (2003). *Black powder manufacturing, testing & optimizing.* Dingmans Ferry, USA: American Fireworks News.

Manchester, S.R. (2001) Leaves and Fruits of *Aesculus* (Sapindales) from the Paleocene of North America. *International Journal of Plant Sciences, 162*, 985–998.

Mandyam, K., & Jumpponen, A. (2005). Seeking the elusive function of the root-colonising dark septate endophytic fungi. *Studies in Mycology, 53*, 173–189.

Margină, D., Olaru, O.T., Ilie, M., Grădinaru, D., Guțu, C., Voicu, S., Dinischiotu, A., Spandidos, D.A., & Tsatsakis, A.M. (2015). Assessment of the potential health benefits of certain total extracts from *Vitis vinifera, Aesculus hyppocastanum* and *Curcuma longa. Experimental and Therapeutic Medicine, 10*, 1681-1688.

Marschner, H. (1995). *Mineral nutrition of higher plants* (2nd ed.). San Diego, USA: Academic Press.

Masaki, H., Sakaki, S., Atsumi, T. and Sakurai, H. (1995). Active-oxygen scavenging activity of plant extracts. *Biological and Pharmaceutical Bulletin, 18*, 162–166.

Matsuda, H., Li, Y., Murakami, T., Ninomiya, K., Yamahara, J., & Yoshikawa, M. (1997). Effects of escins Ia, Ib, IIa, and IIb from horse-chestnut, the seeds of *Aesculus hippocastanum* L., on acute inflammation in animals. *Biological and Pharmaceutical Bulletin, 20*, 1092-1095.

Matysik, G., Glowniak, K., Soczewiński, E., & Garbacka, M. (1994) Chromatography of esculin from stems and bark of *Aesculus hippocastanum* L. for consecutive vegetative periods. *Chromatographia, 38*, 766-770.

Maurizio, A. (1945). Giftige Bienenpflanzen. *Schweiz Bienenzeitung, Beiheft, 1*, 430-441.

Maurizio, A ., & Grafl, I. (1969). *Das Trachtpflanzenbuch. Nektar Und pollen die wichtigsten Nahrungsquellen der Honingbiene*. Munich, Germany: Ehrenwirth Verlag.

McEvoy, A., O'Regan, F., Fleming, C.C., Moreland, B.P., Pollock, J.A., McGuinness, B.W., & Hodkinson, T.R. (2016). Bleeding canker of horse-chestnut (*Aesculus hippocastanum*) in Ireland: incidence, severity and characterization using DNA sequences and real-time PCR. *Plant Pathology, 65*, 1419–1429.

McMillan-Browse, P.D.A. (1971). Propagation of *Aesculus*. *Plant Propagation, 2*, 4-6.

Meidner, H., & Mansfield, T.A. (1968). *Physiology of stomata*. McGraw-Hill, London, UK.

Melgar, M., Trigo, M.M., Recio, M., Docampo, S., Gracía-Sánchez, J., & Cabezudo, B. (2012). Atmospheric pollen dynamics in Münster, north-western Germany: a three-year study (2004-2006). *Aerobiologia, 28*, 423–434.

Menzel, A., Estrella, N., & Fabian, P. (2001). Spatial and temporal variability of the phenological seasons in Germany from 1951 to 1996. *Global Change Biology, 7*, 657-666.

Menzel, A., Estrella, N., Heitland, W., Susnik, A., Schleip, C., & Dose, V. (2008). Bayesian analysis of the species-specific lengthening of the growing season in two European countries and the influence of an insect pest. *International Journal of Biometeorology, 52*, 209–218.

Menzel, A., Estrella, N., & Testka, A. (2005). Temperature response rates from long-term phenological records. *Climate Research, 30*, 21-28.

Mijarra, J.M.P., Manzaneque, F.G., & Morla, C. (2008). Survival and long-term maintenance of tertiary trees in the Iberian Peninsula during the Pleistocene: first record of *Aesculus* L. (Hippocastanaceae) in Spain. *Vegetation History and Archaeobotany, 17*, 351–364.

Milejov, L. (2004). The occurence of some pests and diseases on horse-chestnut, plane tree and Indian bean tree in urban areas of Slovenia. *Acta Agriculturae Slovenica, 83*, 297-300.

Mitchell, A. (1997) *Trees of Britain and Northern Europe*. London, UK: Parkgate Books.

Morimoto, S., Nonaka, G.-I., & Nishioka, I. (1987). Tannins and related compounds. LIX. Aesculitannins, novel proanthocyanidins with doubly-bonded structures from *Aesculus hippocastanum* L. *Chemical and Pharmaceutical Bulletin, 35*, 4717-4729.

Mullett, M.S., & Webber, J.F. (2013). *Pseudomonas syringae* pv. *aesculi*: foliar infection of *Aesculus* species and temperature–growth relationships. *Forest Pathology, 43*, 371-378.

Musatenko, L.I., Generalova, V.N., Martyn, G.I., Vedenicheva, N.P., & Vasyuk, V.A. (2003). Hormonal complex and ultrastructure of maturing *Aesculus hippocastanum* seeds. *Russian Journal of Plant Physiology, 50*, 360–364.

Nardini, A., Raimondo, F., Scimone, M., & Salleo, S. (2004). Impact of the leaf miner *Cameraria ohridella* on whole-plant photosynthetic productivity of *Aesculus hippocastanum*: insights from a model. *Trees, 18*, 714–721.

Natural History Museum (2018). British Isles List of Lichens and Lichenicolous Fungi. http://www.nhm.ac.uk/our-science/data/uk-species/checklists/NHMSYS0000357024/index.html Accessed 22/08/2018.

Nedelcheva, A., & Dogan, Y. (2011). Usage of plants for weather and climate forecasting in Bulgarian folk traditions. *Indian Journal of Traditional Knowledge, 10*, 91-95.

Nejmanová, J., Cvačka, J., Hrdý, I., Kuldová, J., Mertelík, J., Muck, A., Jr, Nešněrová, P., & Svatoš, A. (2006). Residues of diflubenzuron on horse-chestnut (*Aesculus hippocastanum*) leaves and their efficacy against the horse-chestnut leafminer, *Cameraria ohridella. Pest Management Science, 62*, 274–278.

Obroucheva, N.V., & Lityagina, S.V. (2007). Dormancy release and germination in recalcitrant *Aesculus hippocastanum* seeds. *Dendrobiology, 57*, 27-33.

Obroucheva, N.V., Lityagina, S.V., Novikova, G.V., & Sin'kevich, I.A. (2012) Vacuolar status and water relations in embryonic axes of recalcitrant *Aesculus hippocastanum* seeds during stratification and early germination. *AoB Plants, 2012*, pls008.

Obroucheva, N., Sinkevich, I., & Lityagina, S. (2016) Physiological aspects of seed recalcitrance: a case study on the tree *Aesculus hippocastanum. Tree Physiology, 36*, 1127–1150.

Obroucheva, N.V., Sinkevich, I.A., Lityagina, S.V., & Novikova, G.V. (2017). Water relations in germinating seeds. *Russian Journal of Plant Physiology, 64*, 625–633.

Ocokoljić, M., & Stojanović, N. (2009). Phenotypic characteristics of trees and seeds as the base for Improvement and conservation of the horse-chestnut gene pool. *Archives of Biological Sciences, 61*, 619-622.

Ocokoljić, M., Vilotić, D., & Šijačić-Nikolić, M. (2013). Population genetic characteristics of horse-chestnut in Serbia. *Archives of Biological Sciences, 65*, 1-7.

Oleksyn, J., Kloeppel, B.D., Łukasiewicz, S., Karolewski, P., & Reich, P.B. (2007). Ecophysiology of horse-chestnut (*Aesculus hippocastanum* L.) In degraded and restored urban sites. *Polish Journal of Ecology, 55*, 245-260.

Oljača, R., Govedar, Z., & Hrkić, Z. (2009). Effects of aeropolution on stomatal density of studied wild horse-chestnut (*Aesculus hippocastanum* L.) and birch (*Betula pendula* Roth) species in the area of Banjaluka. International Scientific Conference 'Forestry

In S. Orlović (Ed.), *Achieving millennium goals', held on 50th anniversary of foundation of the Institute of Lowland Forestry and Environment, Proceedings* (pp. 117-123). Novi Sad, Serbia.

Osborn, R.W., De Samblanx, G.W., Thevissen, K., Goderis, I., Torrekens, S., Van Leuven, F., Attenborough, S., Rees, S.B., & Broekaert, W.F. (1995). Isolation and characterisation of plant defensins from seeds of Asteraceae, Fabaceae, Hippocastanaceae and Saxifragaceae. *FEBS Letters, 368*, 257-262.

Oszmiański, J., Kalisz, S., & Aneta, W. (2014). The content of phenolic compounds in leaf tissues of white (*Aesculus hippocastanum* L.) and red horse-chestnut (Aesculus carea H.) colonized by the horse-chestnut leaf miner (*Cameraria ohridella* Deschka & Dimić). *Molecules, 19*, 14625-14636.

Otajagić, S., Pinjić, Dž., Ćavar, S. Vidic, D., & Maksimović, M. (2012). Total phenolic content and antioxidant activity of ethanolic extracts of *Aesculus hippocastanum* L. *Bulletin of the Chemists and Technologists of Bosnia and Herzegovina, 38*, 35-38.

Özden, S., & Ennos, R. (2018). The mechanics and morphology of branch and coppice stems in three temperate tree species. *Trees, 32*, 933–949.

Paludan-Müller, G., Saxe, H., Pedersen, L.B., & Randrup, T.B. (2002). Differences in salt sensitivity of four deciduous tree species to soil or airborne salt. *Physiologia Plantarum, 114*, 223–230.

Pammenter, N.W., & Berjak, P. (1999). A review of recalcitrant seed physiology in relation to desiccation-tolerance mechanisms. *Revista Brasileira de Fisiologia Vegetal, 12*, 56-69.

Pánková, I., Krejzar, V., Mertelík, J., & Kloudová, K. (2015). The occurrence of lines tolerant to the causal agent of bleeding canker, *Pseudomonas syringae* pv. *aesculi*, in a natural horse-chestnut population in Central Europe. *European Journal of Plant Pathology, 142*, 37–47.

Papierowska, E., Szporak‑Wasilewska, S., Szewińska, J., Szatyłowicz, J., Debaene, G., & Utratna, M. (2018). Contact angle measurements and water drop behavior on leaf surface for several deciduous shrub and tree species from a temperate zone. *Trees, 32*, 1253-1266.

Papp, B., Alegro, A., Šegota, V., Šapić, I., & Vukelić, J. (2013) Contributions to the bryophyte flora of Croatia I. Gorski Kotar region (W Croatia). *Studia Botanica Hungarica, 44*, 193–211.

Parlayıcı, Ş., & Pehlivan, E. (2015). Natural biosorbents (garlic stem and horse chesnut shell) for removal of chromium(VI) from aqueous solutions. *Environmental Monitoring and Assessment, 187*, e763.

Parus, A. (2018). Copper(II) ions' removal from aqueous solution using green horse-chestnut shell as a low-cost adsorbent. *Chemistry and Ecology, 34*, 56-59.

Pastirčáková, K., Pastirčák, M., Celar, F., & Shin, H.-D. (2009). *Guignardia aesculi* on species of *Aesculus*: new records from Europe and Asia. *Mycotaxon, 108*, 287-296.

Paulić, V., Drvodelić, D., Mikac, S., Gregurović, G., & Oršanić, M. (2015) Arborikulturna i dendroekološka analiza stanja stabala divljeg kestena (*Aesculus hippocastanum* L.) na području grada Velike Gorice. [Arboricultural and dendroecological analysis of the condition of horse-chestnut (Aesculus hippocastanum L.) trees in the town of Velika Gorica.] *Šumarski list, 1–2*, 21–34.

Pavan, F., Barro, P., Bernardinelli, I., Gambon, N., & Zandigiacomo, P. (2003). Cultural control of *Cameraria ohridella* on horsechestnut in urban areas by removing fallen leaves in autumn. *Journal of Arboriculture, 29*, 253–258.

Pavela, R., & Bárnet, M. (2005). Systemic applications of neem in the control of *Cameraria ohridella*, a pest of horse-chestnut (*Aesculus hippocastanum*). *Phytoparasitica, 33*, 49-56.

Pavlović, M., Rakić, T., Pavlović, D., Kostić, O., Jarić, S., Mataruga, Z., Pavlović, P., & Mitrović, M. (2017). Seasonal variations of trace element contents in leaves and bark of horse-chestnut (*Aesculus hippocastanum* L.) in urban and industrial regions in Serbia. *Archives of Biological Sciences, 69*, 201-214.

Pearce, R.B. (1991). Reaction zone relics and the dynamics of fungal spread in the xylem of woody angiosperms. *Physiological and Molecular Plant Pathology, 39*, 41-55.

Pearman, D.A., Preston, C.D., Rothero, G.P., & Walker, K.L. (2008) The Flora of Rum. Truro: Published by the authors.

Peçi, D.H., Mullaj, A., & Dervishi, A. (2012). The natural distribution of horse-chestnut (*Aesculus hippocastanum* L) in Albania. *Journal of Institute Alb-Shkenca, 5*, 153–157.

Pence, V.C. (1990). Cryostorage of embryo axes of several large-seeded temperate tree species. *Cryobiology*, *27*, 212-218.

Percival, G.C. (2016). Evaluation of insect barrier glue bands and liquid glue for the management of horse chestnut leaf miner (*Cameraria ohridella*). *Arboricultural Journal, 38*, 134–142.

Percival, G.C., & Banks, J.M. (2014). Studies of the interaction between horse-chestnut leaf miner (*Cameraria ohridella*) and bacterial bleeding canker (*Pseudomonas syringae* pv. *aesculi*). *Urban Forestry and Urban Greening, 13*, 403–409.

Percival, G.C., & Banks, J.M. (2015). Phosphite-induced suppression of *Pseudomonas* bleeding canker (*Pseudomonas syringae* pv. *aesculi*) of horse-chestnut (*Aesculus hippocastanum* L.). *Arboricultural Journal, 37*, 7–20.

Percival, G.C., Barrow, I., Noviss, K., Keary, I., & Pennington, P. (2011). The impact of horse-chestnut leaf miner (*Cameraria ohridella* Deschka and Dimic; HCLM) on vitality, growth and reproduction of *Aesculus hippocastanum* L. *Urban Forestry & Urban Greening, 10*, 11-17.

Percival, G.C., Fraser, G.A., & Barnes, S. (2004) Soil injections of carbohydrates improve fine root growth of established urban trees. *Arboricultural Journal, 28*, 95-101.

Percival, G.C., & Noviss, K. (2008) Triazole induced drought tolerance in horse-chestnut (*Aesculus hippocastanum*). *Tree Physiology, 28*, 1685–1692.

Percival, M.S. (1955). The presentation of pollen in certain Angiosperms and its collection by *Apis mellifera. New Phytologist, 54*, 353-368.

Percival, M.S. (1961). Types of nectar in Angiosperms. *New Phytologist, 60*, 235–281.

Péré, C., Augustin, S., Turlings, T.C.J., & Kenis, M. (2010) The invasive alien leaf miner *Cameraria ohridella* and the native tree *Acer pseudoplatanus*: a fatal attraction? *Agricultural and Forest Entomology, 12*, 152-159.

Peternel, R., Čulig, J., Mitić, B., Vukušić, J., & Šostar, Z. (2003). Analysis of airborne pollen concentrations in Zagreb, Croatia, 2002. *Annals of Agricultural and Environmental Medicine, 10*, 107-112.

Petersen, A., & Eckstein, D. (1988). Roadside trees in Hamburg - their present situation of environmental stress and their future chance for recovery. *Arboricultural Journal 12*,109-117.

Petrova, S., Yurukova, L., & Velcheva, I. (2012) Horse-chestnut (*Aesculus hippocastanum* L.) as a biomonitor of air pollution in the town of Plovdiv (Bulgaria). *Journal of Bioscience and Biotechnology, 1*, 241-247.

Petrova, V., Voitkane, S., Jankevica, L., & Cera, I. (2013). Spider community on the horse-chestnut *Aesculus hippocastanum* L. – preliminary results. *Acta Biologica Universitatis Daugavpiliensis, 13*, 77-84.

Peverieri, G.S., & Roversi, P.F. (2010). Feeding and oviposition of *Anoplophora chinensis* on ornamental and forest trees. *Phytoparasitica, 38*, 421-428.

Phoitos, D., Konstantinidis, T., & Kamari G. (2009). *The red data book of rare and threatened plants of Greece*. Patras, Greece: Hellenic Botanical Society.

Pirc, M., Dreo, T. Jurc, D. (2018) First report of *Pseudomonas syringae* pv. *aesculi* as the causal agent of bleeding canker of horse chestnut in Slovenia. *Plant Disease*, https://doi.org/10.1094/PDIS-12-17-1868-PDN

Pittler, M.H., & Ernst, E. (1998). Horse-chestnut seed extract for chronic venous insufficiency. *Archives of Dermatology, 134*, 1356-1360.

Pocock, M.J.O., & Evans, D.M. (2014). The success of the horse-chestnut leaf-miner, *Cameraria ohridella*, in the UK revealed with hypothesis-led citizen science. *PLoS ONE, 9*, e86226.

Pogan, E., Wcislo, H., & Jankun, A. (1980). Further studies in chromosome numbers in Polish Angiosperms. Part XIII. *Acta Biologica Cracoviensia, Series Botanica, 22*, 37-69.

Poljanšek, S., & Lena, M. (2016). Radial growth response of horse chestnut (*Aesculus hippocastanum* L.) trees to climate in Ljubljana, Slovenia. *Urban Forestry & Urban Greening, 18*, 110–116.

Poole, I. (1994). "Twig"- wood anatomical characters as palaeoecological indicators. *Review of Palaeobotany and Palynology, 81*, 33-52.

Popp, W., Horak, F., Jiiger, S., Reiser, K., Wagtier, C., & Zwick, H. (1992). Horse-chestnut (*Aesculus hippocastanum*) pollen: a frequent cause of allergic sensitization in urban children. *Allergy, 47*, 380-383.

Postigo-Mijarra, J.M., Gómez-Manzaneque, F., & Morla, C. (2008). Survival and long-term maintenance of tertiary trees in the Iberian Peninsula during the Pleistocene: first record of *Aesculus* L. (Hippocastanaceae) in Spain. *Vegetation History and Archaeobotany, 17*, 351–364.

Pozhidaev, A.E. (1995). Pollen morphology of the genus *Aesculus* (Hippocastanaceae). Patterns in the variety of morphological characteristics. *Grana, 34*, 10-20.

Prada, D., Velloza, T.M., Toorop, P.E., & Pritchard, H.W. (2011). Genetic population structure in horse-chestnut (*Aesculus hippocastanum* L.): effects of human-mediated expansion across Europe. *Plant Species Biology, 26*, 43–50.

Preston, C. D., Pearman, D. A., & Dines, T. D. (2002). *New atlas of the British and Irish flora*. Oxford, UK: Oxford University Press.

Pritchard, H.W., Steadman, K.J., Nash, J.V., & Jones, C. (1999). Kinetics of dormancy release and the high temperature germination response in *Aesculus hippocastanum* seeds. *Journal of Experimental Botany, 50*, 1507-1514.

Pritchard, H.W., Tompsett, P.B., & Manger, K.R. (1996). Development of a thermal time model for the quantification of dormancy loss in *Aesculus hippocastanum* seeds. *Seed Science Research, 6*, 127-135.

Profumo, P., Caviglia, A.M., Gastaldo, P., & Dameri, R.M. (1991a) Aescin content in embryogenic callus and in embryoids from leaf explants of *Aesculus hippocastanum*. *Planta Medica, 57*, 50-52.

Profumo, P., Gastaldo, P., Bevilacqua, L., & Carli, S, (1991b). Plant regeneration from cotyledonary explants of *Aesculus hippocastanum* L. *Plant Science, 76*,139-142.

Pyszyński, W. (1977) Mechanism of formation of spiral grain in *Aesculus* stems: dissymmetry of deformation of stems caused by cyclic torsion. *Acta Societatis Botanicorum Poloniae, 46*, 501-522.

Puchalski, T., & Prusinkiewicz, Z. (1975). *Ecological basis of forest site classification*., Warsaw, Poland: Powszechne Wydawnictwo Rolnicze i Leśne.

Rackham, O. (1986). *The history of the countryside*. London, UK: Dent.

Rackham, O. (2003). *Ancient woodland: Its history, vegetation and uses in England* (2nd ed.). Dalbeattie, UK: Castlepoint Press.

Radeghieri, P. (2004). *Cameraria ohridella* (Lepidoptera Gracillariidae) predation by *Crematogaster scutellaris* (Hymenoptera Formicidae) in Northern Italy (Preliminary note). *Bulletin of Insectology, 57*, 63-64.

Radojević, L. (1978) In vitro induction of androgenic plantlets in *Aesculus hippocastanum* L. *Protoplasma, 96*, 369–374.

Radojević, L. (1989). Pollen dimorphism in *Aesculus hippocastanum* and *A. carnea*. *Archives of Biological Sciences, 41*, 137-143.

Radojević, L., Marinkovic, N., & Jevremovic, S. (2000). Influence of the sex of flowers on androgenesis in *Aesculus hippocastanum* L. anther culture. *In Vitro Cellular & Developmental Biology, 36*, 464-469.

Raimondo, F., Ghirardelli, L.A., Nardini, A., & Salleo, S. (2003). Impact of the leaf miner *Cameraria ohridella* on photosynthesis, water relations and hydraulics of *Aesculus hippocastanum* leaves. *Trees, 17*, 376–382.

Raimondo, R. Trifilò, P., Salleo, S., & Nardini, A. (2005). Seasonal changes of plant hydraulics, water relations and growth of *Aesculus hippocastanum* seedlings infested by the leafminer *Cameraria ohridella*. *Annals of Forest Science, 62*, 99-104.

Raus, T. (1980). Die Vegetation Ostthessaliens (Griechenland). III. Querco-Fagetea und azonale Gehölzgesellschaften. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie*, 101, 313-361.

Ravazzi, C. (2003) *Gli antichi bacini lacustri e i fossili di Leffe, Ranica e Piànico-Sèllere (Prealpi Lombarde). Quaderni di Geodinamica Alpina e Quaternaria.* Bergamo, Italy: Consiglio Nazionale delle Ricerche.

Ravazzi, C., & Caudullo, G. (2016). *Aesculus hippocastanum* in Europe: distribution, habitat, usage and threats. In J. San-Miguel-Ayanz, D. de Rigo, G. Caudullo, T.

Houston Durrant & A. Mauri (Eds.)*, European atlas of forest tree species.* Luxembourg: Publishing Office of the European Union.

Raw, A. (1974) Pollen Preferences of Three *Osmia* Species (Hymenoptera). *Oikos, 25*, 54-60.

Redžić, S.S. (2007). The ecological aspect of ethnobotany and ethnopharmacology of population in Bosnia and Herzegovina. *Collegium Antropologicum, 31*, 869–890.

Ridley, H.N. (1930) *The dispersal of plants throughout the world*. Ashford, UK; Reeve and Co.

Rizzi-Longo, L., Pizzulin-Sauli, M., Stravisi, F., & Ganis, P. (2010). Airborne pollen calendar for Trieste (Italy), 1990–2004. *Grana, 46*, 98–109.

Robinson, S.C., Tudor, D., & Cooper, P.A. (2011). Wood preference of spalting fungi in urban hardwood species. *International Biodeterioration & Biodegradation, 65*, 1145-1149.

Rodwell, J.S. (1991). *British plant communities. Woodlands and scrub* (Vol. I). Cambridge, UK: Cambridge University Press.

Roloff, A., Korn, S., & Gillner, S. (2009). The climate-species-matrix to select tree species for urban habitats considering climate change*. Urban Forestry and Urban Greening, 8*, 295–308.

Rotheray, G.E., Hancock, G., Hewitt, S., Horsfield, D., MacGowan, I., Robertson, D., & Watt, K. (2001). The biodiversity and conservation of saproxylic Diptera in Scotland. *Journal of Insect Conservation, 5*, 77–85.

Royal Society of Biology (2017). *Biology Week 2017: UK's Favourite Tree Species.* https://www.rsb.org.uk/get-involved/biologyweek/uk-s-favourite-tree-species. Accessed 22/02/2018.

Ruffini, I., Belcaro, G., Cesarone, M.R., & Dugall, M. (2004). Efficacy of topical treatment with aescin + essential phospholipids gel in venous insufficiency and hypertension. *Angiology, 55* (Suppl.1), S19–21.

Ruoss, E. (1999). How agriculture affects lichen vegetation in central Switzerland. *Lichenologist, 31*, 63-73.

Salleo, S., Nardini, A., Raimondo, F., Lo Gullo, M.A., Pace, F., & Giacomich, P. (2003). Effects of defoliation caused by the leaf miner *Cameraria ohridella* on wood production and efficiency in *Aesculus hippocastanum* growing in north-eastern Italy. *Trees, 17*, 367–375.

Samek, T. (2003) Diapause of *Cameraria ohridella* Deschka et Dimic and its impact on the species population dynamics. *Journal of Forest Science, 49*, 252–258.

Sapronova, Z., Sverguzova, S., Sulim, K., Svyatchenko, A., & Chebotaeva, E. (2018). Sewage treatment in megacities by modified chestnut tree waste. *IOP Conference Series: Materials Science and Engineering, 365*, e022058.

Scholz, T., Hof, A., & Schmitt, T. (2018). Cooling effects and regulating ecosystem services provided by urban trees - novel analysis approaches using urban tree cadastre data. *Sustainability, 10*, e712.

Schulz-Langner, E. (1967). Über den Trachwert der Rosskastanie (*Aesculus hippocastanum*) unter besonderer Berücksichtigung des Saponingehaltes im nektar. *Zeitschrift Bienenforsch, 9*, 49-65.

Seaward, M.R.D., & Letrouit-Galinou, M.A. (1991). Lichen recolonization of trees in the Jardin du Luxembourg, Paris. *Lichenologist, 23*, 181-186.

Šedivá, J., Vlašínová, H., & Mertelík, J. (2013). Shoot regeneration from various explants of horse-chestnut (*Aesculus hippocastanum* L.). *Scientia Horticulturae, 161*, 223–227.

Šefrová, H., & Laštůvka, Z. (2001) Dispersal of the horsechestnut leafminer, *Cameraria ohridella* Deschka & Dimiè, 1986, in Europe: its course, ways and causes (Lepidoptera: Gracillariidae). *Entomologische Zeitschrift, 111*, 194–198.

Šerá, B. (2017). Salt-tolerant trees usable for Central European cities – A review. *Horticultural Science, 44*, 43–48.

Simon, P., & Lena, M. (2016). Radial growth response of horse-chestnut (*Aesculus hippocastanum* L.) trees to climate in Ljubljana, Slovenia. *Urban Forestry and Urban Greening, 18*, 110–116.

Sirtori, C.R. (2001). Aescin: Pharmacology, pharmacokinetics and therapeutic profile. *Pharmacological Research, 44,* 183–193.

Skovsted, A. (1929). Cytological Investigations of the genus *Aesculus* L. With some observations on *Aesculus carnea* Willd., a tetraploid species arisen by hybridization *Hereditas, 12*, 64-70.

Skribanek, A., Apatini, D., Inaoka, M., & Böddi, B. (2000). Protochlorophyllide and chlorophyll forms in dark-grown stems and stem-related organs. *Journal of Photochemistry and Photobiology, B: Biology, 55*, 172–177.

Skuhravý, V., von (1998). Zur Kenntnis der Blattminen-Motte *Cameraria ohridella* Desch., & Dim. (Lep., LithocoUetidae) an *Aesculus hippocastanum* L. in der Tschechischen Republik. *Anzeiger für Schädlingskunde., Pflanzenschutz, Umweltschutz, 71*, 81-84.

Snieskiene, V., Stankeviciene, A., Zeimavicius, K., & Balezentiene, L. (2011). *Aesculus hippocastanum* L. state changes in Lithuania. *Polish Journal of Environmental Studies, 20*, 1029-1035.

Solymosi, K., Bóka, K., & Böddi, B. (2006). Transient etiolation: protochlorophyll(ide) and chlorophyll forms in differentiating plastids of closed and breaking leaf buds of horse-chestnut (*Aesculus hippocastanum*). *Tree Physiology, 26*, 1087–1096.

Somme, L., Moquet, L., Quinet, M., Vanderplanck, M., Michez, D., Lognay, G., & Jacquemart, A.-L. (2016). Food in a row: urban trees offer valuable floral resources to pollinating insects. *Urban Ecosystems, 19*, 1149–1161.

Sparks, T.H., Jeffree, E.P., & Jeffree, C.E. (2000). An examination of the relationship between flowering times and temperature at the national scale using long-term phenological records from the UK. *International Journal of Biometeorology, 44*, 82–87.

Sparks, T.H., Górska-Zajączkowska, M., Wójtowicz, W., & Tryjanowski, P. (2011) Phenological changes and reduced seasonal synchrony in western Poland. *International Journal of Biometeorology*, 55, 447-453.

Stankeviciene, A., Snieskiene, V., & Lugauskas, A. (2010). *Erysiphe flexuosa* – the new pathogen of *Aesculus hippocastanum* in Lithuania. *Phytopathologia*, *56*, 67–71.

Steadman, K.J., & Pritchard, H.W. (2004). Germination of *Aesculus hippocastanum* seeds following cold-induced dormancy loss can be described in relation to a temperature-dependent reduction in base temperature (Tb) and thermal time. *New Phytologist, 161*, 415–425.

Steele, H., Laue, B.E., MacAskill, G.A., Hendry, S.J., & Green, S. (2010). Analysis of the natural infection of European horse-chestnut (*Aesculus hippocastanum*) by *Pseudomonas syringae* pv. *aesculi. Plant Pathology, 59*, 1005–1013.

Stefanic, E., Rasic, S., Merdic, S., & Colacovic, K. (2007). Annual variation of airborne pollen in the city of Vincovci, Northeastern Croatia. *Annals of Agricultural and Environmental Medicine, 14*, 97–101.

Stojanović, A., & Marković, C. (2004). Parasitoid complex of *Cameraria ohridella* (Lepidoptera: Gracillariidae) in Serbia. *Phytoparasitica, 32*, 132-140.

Straw, N.A., & Bellett-Travers, M. (2004). Impact and management of the horse-chestnut leaf-miner (*Cameraria ohridella*). *Arboricultural Journal, 28*, 67-83.

Straw, N.A., & Tilbury, C. (2006). Host plants of the horse-chestnut leaf-miner (*Cameraria ohridella*), and the rapid spread of the moth in the UK 2002-2005. *Arboricultural Journal, 29*, 83-99.

Straw, N.A., & Williams, D.T. (2013) Impact of the leaf miner *Cameraria ohridella* (Lepidoptera: Gracillariidae) and bleeding canker disease on horse-chestnut: direct effects and interaction. *Agricultural and Forest Entomology, 15*, 321–333.

Streiling, S., & Matzarakis, A. (2003). Influence of single and small clusters of trees on the bioclimate of a city: a case study. *Journal of Arboriculture, 29*, 309-326.

Strouts, R.G., & Winter, T.G. (2000). *Diagnosis of Ill-health in Trees* (2nd ed.). Norwich, UK: The Stationery Office.

Šućur, K.M., Aničić, M.P., Tomašević, M.N., Antanasijević, D.Z., Perić-Grujić, A.A., & Ristić, M. DJ. (2010) Urban deciduous tree leaves as biomonitors of trace element (As, V and Cd) atmospheric pollution in Belgrade, Serbia. *Journal of the Serbian Chemical Society, 75*, 1453-1461.

Sukovata, L., Czokajlo, D., Kolk, A., Ślusarski, S., & Jabłoński, T. (2011). An attempt to control *Cameraria ohridella* using an attract-and-kill technique *Journal of Pest Science, 84*, 207-212.

Suter, A. Bommer, S., & Rechner, J. (2006). Treatment of patients with venous insufficiency with fresh plant horse-chestnut seed extract: A review of 5 clinical studies. *Advances in Therapy, 23*, 179–190.

Sweet, J.B., & Barbara, D.J. (1979). A yellow mosaic disease of horse-chestnut (*Aesculus* spp.) caused by apple mosaic virus. *Annals of Applied Biology, 92*, 335-341.

Synge, A.D. (1947). Pollen Collection by Honeybees (*Apis mellifera*). *Journal of Animal Ecology, 16*, 122-138.

Szabadosova, V., Hijova, E., Strojny, L., Salaj, R., Bomba, A., Cokasova, D., Pramukova, B., Zofcakova, J., Bertkova, I., Supukova, A., Soltesova, A., Brandeburova, A., &

Chmelarova, A. (2013). Effect of horse-chestnut and inulin as single supplements or in combination on chemically induced colon cancer in rats. *Veterinarni Medicina, 58*, 491–499.

Takács, Á., Kiss, M., Gulyás, Á., Tanács, E., & Kántor, N. (2016). Solar permeability of different tree species in Szeged, Hungary. *Geographica Pannonica, 20*, 32-41.

Takos, I., Varsamis, G., Avtzis, D., Galatsidas, S., Merou, T., & Avtzis, N. (2008). The effect of defoliation by *Cameraria ohridella* Deschka and Dimic (Lepidoptera: Gracillariidae) on seed germination and seedling vitality in *Aesculus hippocastanum* L. *Forest Ecology and Management, 255*, 830–835.

Talgø, V., Perminow, J.I.S., Sletten, A., Brurberg, M.B., Herrero, M.L., Strømeng, G.M., & Stensvand, A. (2012). Fungal and bacterial diseases on horse-chestnut in Norway. *Journal of Agricultural Extension and Rural Development, 4*, 256-258.

Tambur, Z., Cenić Milošević, D., Mileusnić, I., Doder, R., Marjanović, M., Miljković Selimović, B., Kulišić, Z., & Opačić, D. (2018). Inhibitory effects of different medicinal plants on *Candida albicans* growth. *Medycyna Weterynaryjna, 74*, 473-476.

Tarwacki, G., Bystrowski, C., & Celmer-Warda, K. (2012). Effect of sun-exposure of the horse-chestnut (*Aesculus hippocastanum* L.) on the occurrence and number of parasitoids of the horse-chestnut leafminer (*Cameraria ohridella* Deschka & Dimic) in central Poland in 2004– 2006. *Folia Forestalia Polonica, Series A, 54*, 56–62.

Thalmann, C., Freise, J., Heitland, W., & Bacher, S. (2003). Effects of defoliation by horse-chestnut leafminer (*Cameraria ohridella*) on reproduction in *Aesculus hippocastanum*. *Trees, 17*, 383-388.

The Tree Register. (2018). Available from http://www.treeregister.org/ Accessed 22/08/2018

Thomas, P.A. (2014). *Trees: their natural history* (2nd ed.). Cambridge, UK: Cambridge University Press.

Tiffany, N., Boon, H., Ulbricht, C., Basch, E., Bent, S., Barrette, E.P., Smith, M., Sollars, D., Dennehy, C.E., & Szapary, P. (2002). Horse-chestnut: A multidisciplinary clinical review. *Journal of Herbal Pharmacotherapy, 2*, 71–85.

Todorović, D., Popović, D., Ajtić, J., & Nikolić, J. (2013) Leaves of higher plants as biomonitors of radionuclides (^{137}Cs, ^{40}K, ^{210}Pb and ^{7}Be) in urban air. *Environmental Science and Pollution Research, 20*, 525–532.

Tomašević, M., Antanasijević, D., Aničić, M., Deljanin, I., Perić-Grujić, A., & Ristić, M. (2013) Lead concentrations and isotope ratios in urban tree leaves. *Ecological Indicators, 24*, 504–509.

Tomašević, M., Rajšić, S., Đorđević, D., Tasić, M., Krstić, J., & Novaković, V. (2004) Heavy metals accumulation in tree leaves from urban areas. *Environmental Chemistry Letters, 2*, 151–154.

Tomiczek, C., & Krehan, H. (1998). The horsechesnut leafmining moth (*Cameraria ohridella*): a new pest in Central Europe. *Journal of Arboriculture, 24*,144–148.

Tompsett, P.B., & Pritchard, H.W. (1993). Water status changes during development in relation to the germination and desiccation tolerance of *Aesculus hippocastanum* L. seeds. *Annals of Botany, 71*, 107–116.

Tompsett, P.B., & Pritchard, H.W. (1998). The effect of chilling and moisture status on the germination, desiccation tolerance and longevity of *Aesculus hippocastanum* L. seed. *Annals of Botany, 82*, 249–261.

Tozlu, E., & Demirci, E. (2010) First report of powdery mildew of *Aesculus hippocastanum* caused by *Erysiphe flexuosa* in Turkey. *Australasian Plant Disease Notes, 5*, 61–62.

Troch, V., Werbrouck, S., Geelen, D., & Van Labeke, M.-C. (2009). Optimization of horse-chestnut (*Aesculus hippocastanum* L.) somatic embryo conversion. *Plant Cell, Tissue and Organ Culture, 98*,115–123.

Tryjanowski, P., Panek, M., & Sparks, T. (2006). Phenological response of plants to temperature varies at the same latitude: case study of dog violet and horse-chestnut in England and Poland. *Climate Research, 32*, 89-93.

Tsiroukis, A. (2008). *Reproductive biology and ecology of horse-chestnut* (*Aesculus hippocastanum* L.). PhD thesis, National & Kapodistrian University of Athens, Athens, Greece.

Turkekul, K., Colpan, R.D., Baykul, T., Ozdemir, M.D., & Erdogan, S. (2018). Esculetin inhibits the survival of human prostate cancer cells by inducing apoptosis and arresting the cell cycle. *Journal of Cancer Prevention, 23*, 10-17.

Underland, V., Sæterdal, I., & Nilsen, E.S. (2012). Cochrane summary of findings: Horse-chestnut seed extract for chronic venous insufficiency. *Global Advances in Health and Medicine, 1*, 122–123.

Upcott, M. (1936). The parents and progeny of *Aesculus carnea. Journal of Genetics, 33*, 135-149.

Valade, R., Kenis, M., Hernandez-Lopez, A., Augustin, S., Mari Mena, N., Magnoux, E., Rougerie, R., Lakatos, F., Roques, A., & Lopez-Vaamonde, C. (2009). Mitochondrial and microsatellite DNA markers reveal a Balkan origin for the highly invasive horse-chestnut leaf miner *Cameraria ohridella* (Lepidoptera, Gracillariidae). *Molecular Ecology, 18*, 3458–3470.

Vasiliauskas, A., Leonavičienė, L., Vaitkienė, D., Bradūnaitė, R., & Lukšienė, A. (2010). Anti-inflammatory effects of *Aesculus hippocastanum* L. tincture and the pro-/antioxidant bodily state of rats with adjuvant arthritis. *Acta Medica Lituanica, 17*, 123–132.

Vega, S.S., Garijo, M.G., Calderon, R.R., Carreiras, M.Z., Vargas, S.C., Turrion, B.S., Martin, M.Z., Serrano, V.S., & Abejon, V.D. (2012). Anaphylactic shock due to the ingestion of chestnut from horse-chestnut (*Aesculus hippocastanum*). *Allergy, 67*, 382-383.

Velagić-Habul, E., Lazarev, V., & Custović, H. (1991), Evaluation of emission of SO_2 and occurrence of pathogenic fungi of forest tree species. *Zastita Bilja, 42*,153–164.

Vokou, D., Katradi, K., & Kokkini, S. (1993) Ethnobotanical survey of Zagori (Epirus, Greece), a renowned centre of folk medicine in the past. Journal of *Ethnopharmacology, 39*, l87-196.

Volter, L., & Kenis, M. (2006). Parasitoid complex and parasitism rates of the horse-chestnut leafminer, *Cameraria ohridella* (Lepidoptera: Gracillariidae) in the Czech Republic, Slovakia and Slovenia. *European Journal of Entomology, 103*, 365-370.

Walas, Ł., Dering, M., Ganatsas, P., Pietras, M., Pers-Kamczyc, E., & Iszkuło, G. (2018). The present status and potential distribution of relict populations of *Aesculus hippocastanum* L. in Greece and the diverse infestation by *Cameraria ohridella* Deschka & Dimić. *Plant Biosystems, 152,* 1048-1058.

Walther, G.-R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin, J.-M., Hoegh-Guldberg, O., & Bairlein, F. (2002) Ecological responses to recent climate change. *Nature, 416*, 389-395.

Webber, J.F., Parkinson, N.M., Rose, J., Stanford, H., Cook, R.T.A., & Elphinstone, J.G. (2008) Isolation and identification of *Pseudomonas syringae* pv. *aesculi* causing bleeding canker of horse-chestnut in the UK. *Plant Pathology, 57*, 368.

Weberling, F. (1989). *Morphology of flowers and inflorescences.* Cambridge, UK: Cambridge University Press.

Weiner, M.A. (1980). Earth Medicine - *Earth Food plant remedies, drugs, and natural foods of the North American Indians.* New York, USA: Collier.

Wellburn, A.R., Stevenson, J., Hemming, F.W., & Morton, R.A. (1967) The characterization and properties of castaprenol-li, -12 and -13 from the leaves of *Aesculus hippocastanum* (Horse-chestnut). *Biochemistry Journal, 102*, 313-324.

Werner, M., Irzykowska, L., & Karolewski, Z. (2012). The occurrence and harmfulness of *Erysiphe flexuosa* and *Cameraria ohridella* on *Aesculus* spp. *Phytopathologia, 65*, 5–11.

Werres, S. von, Richter, J., & Veser, I. (1995). Untersuchungen von kranken und abgestorbenen Roßkastanien (*Aesculus hippocastanum* L.) im öffentlichen Grün. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes, 47*, 81–85.

Weryszko-Chmielewska, E., & Chwil, M. (2017) Structure of floral nectaries in *Aesculus hippocastanum* L. *Acta Botanica Croatica, 76*, 41-48.

Weryszko-Chmielewska, E., & Haratym, W. (2011). Changes in leaf tissues of common horse-chestnut (*Aesculus hippocastanum* L.) colonised by the horse-chestnut leaf miner (*Cameraria ochridella* Deschaka&Dymić). *Acta Agrobotanica, 64*, 11–22.

Weryszko-Chmielewska, E., & Haratym, W. (2012). Leaf micromorphology of *Aesculus hippocastanum* L. and damage caused by leaf-mining larvae of *Cameraria ohridella* Deschka & Dymić. *Acta Agrobotanica, 65*, 25–34.

Weryszko-Chmielewska, E., Tietze, M., & Michońska, M. (2012). Ecological features of the flowers of *Aesculus hippocastanum* L. and characteristics of *Aesculus* L. pollen seasons under the conditions of central-eastern Poland. *Acta Agrobotanica, 65*, 61–68.

Wesley-Smith, J., Walters, C., Pammenter, N.W., & Berjak, P. (2001) Interactions among water content, rapid (nonequilibrium) cooling to -196°C, and survival of embryonic axes of *Aesculus hippocastanum* L. seeds. *Cryobiology, 42*, 196–206.

Whitney, G.G., & Adams, S.D. (1980). Man as a maker of new plant communities. *Journal of Applied Ecology, 17*, 431-448.

Wilczyński, S., & Podlaski, R. (2007). The effect of climate on radial growth of horse-chestnut (*Aesculus hippocastanum* L.) in the Świętokrzyski National Park in central Poland. *Journal of Forest Research, 12*, 24-33.

Wilkinson, J.A., & Brown, A.M.G. (1999). Horse-chestnut – *Aesculus hippocastanum*: potential applications in cosmetic skin-care products. *International Journal of Cosmetic Science, 21*, 437–447.

Willmer, P. (2011). *Pollination and floral ecology*. Princeton, USA: Princeton University Press.

Wilson, M.B. (2014). Origin, *Composition, and role of antimicrobial plant resins collected by honey bees, Apis mellifera*. PhD thesis, University of Minnesota, USA.

WWF (2013). *Pindus Mountains mixed forests. mediterranean forests, woodlands and scrubs*. Washington, DC, USA: World Wildlife Fund. Available at: http://worldwildlife.org/ecoregions/pa1217. Accessed: 30/07/18.

Xiang, Q.-Y., Crawford, D.J., Wolfe, A.D., Tang, Y.-C., & Depamphilis, C.W. (1998). Origin and biogeography of *Aesculus* L. (Hippcastanaceae): a molecular phylogenetic perspective. *Evolution, 52*, 988–997.

Yilmaz, Y., Sakcali, S., Yarci, C., Aksoy, A., & Ozturk, M. (2006) Use of *Aesculus hippocastanum* L. as a biomonitor of heavy metal pollution. *Pakistan Journal of Botany, 38*, 1519-1527.

Yoshihara, T., Matsumura, H. Tsuzaki, M., Wakamatsu, T., Kobayashi, T., Hashida, S., Nagaoka, T., & Goto, F. (2014). Changes in radiocesium contamination from Fukushima in foliar parts of 10 common tree species in Japan between 2011 and 2013. *Journal of Environmental Radioactivity, 138,* 220-226.

Yoshikawa, M., Harada, E., Murakami, T., Matsuda, H., Wariishi, N., Yamahara, J., Murakami, N., & Kitagawa, I. (1994) Escins-Ia, Ib, IIa, IIb, and IIIa, bioactive triterpene oligoglycosides from the seeds of *Aesculus hippocastanum* L.: Their

inhibitory effects on ethanol absorption and hypoglycemic activity on glucose tolerance test. *Chemical and Pharmaceutical Bulletin*, *42*, 1357-1359.

Yoshikawa, M., Murakami, T., Yamahara, J., & Matsuda, H. (1998). Bioactive saponins and glycosides. XII. Horse-chestnut. (2): Structures of escins IIIb, IV, V, and VI and isoescins Ia, Ib, and V, acylated polyhydroxyoleanene triterpene oligoglycosides, from the seeds of horse-chestnut tree (*Aesculus hippocastanum* L., Hippocastanaceae). *Chemical and Pharmaceutical Bulletin*, *46*, 1764-1769.

Zdravković-Korać, S., Muhovski, Y., Druart, P., Ćalić, D., & Radojević, L. (2004) *Agrobacterium rhizogenes*-mediated DNA transfer to *Aesculus hippocastanum* L. and the regeneration of transformed plants. *Plant Cell Reports, 22*, 698–704.

Zemanek, A., Zemanek, B., Harmata, K., Madeja, J., & Klepacki, P. (2009). Selected foreign plants in old Polish botanical literature, customs and art (*Acorus calamus*, *Aesculus hippocastanum, Cannabis sativa*, *Fagopyrum, Helianthus annuus, Iris*). In J.-P. Mortel & A. M. Mercuri (Eds.), *Plants and culture: seeds of the cultural heritage of Europe* (pp. 179-193). Bari, Italy: Edipuglia.

Zerbe, S., & Kreyer, D. (2007) Influence of different forest conversion strategies on ground vegetation and tree regeneration in pine (*Pinus sylvestris* L.) stands: a case study in NE Germany. *European Journal of Forest Research, 126*, 291–301.

Zhang, Z., Li, S., & Lian, X.-Y. (2010). An overview of genus *Aesculus* L.: Ethnobotany, phytochemistry, and pharmacological activities. *Pharmaceutical Crops, 1*, 24-51.

Zimmermannová-Pastirčáková, K., & Pastirčák, M. (2002). *Erysiphe flexuosa* a new species of powdery mildew for Slovakia. *Biologia, 57*, 437-440.

Zlatanov, M.D., Antova, G.A., Angelova-Romova, M.J., & Teneva. O.T. (2012). Lipid composition of *Castanea sativa* Mill. and *Aesculus hippocastanum* fruit oils. *Journal of the Science of Food and Agriculture, 93*, 661-666.

Zohner, C.M., & Renner, S.S. (2015). Perception of photoperiod in individual buds of

mature trees regulates leaf-out. *New Phytologist, 208*, 1023–1030.

TABLES

TABLE 1 Levels of heavy metals (μg/g dry mass) in various tissues of *Aesculus hippocastanum* in different geographical locations

Element	Turkey[1]			Bulgaria[2]	Serbia[3]	New Zealand[4]
	Washed Leaves	Unwashed leaves	Bark	Unwashed leaves	Washed leaves	
Pb	0.02-0.05	0.02-0.12	0.06-0.63	2.75	0.8-21.5	294
Cd	0.001-0.002	0.002-0.068	0.005-0.006	0.24		0.197
Zn	0.39-0.59	0.37-0.53	0.41-0.66		15.2-36.2	299
Cu	0.26-0.39	0.32-0.47	0.35-1.03	8.2	5.5-87.5	129
Cr				0.25	0.3-1.80	
Ni					0.41-2.38	
Sr					30-81	
As					0.11-0.35	
V				0.05	0.4-1.78	
U				0.012		

1, Yilmaz, Sakcali, Yarci, Aksoy, & Ozturk (2006); 2, Petrova, Yurukova, & Velcheva (2012); 3, Aničić, Spasić, Tomašević, Rajšić, & Tasić (2011), Kocić, Spasić, Urošević, & Tomašević (2014), Pavlonić et al. (2017); 4, Kim & Fergusson (1994).

TABLE 2 Invertebrates recorded from *Aesculus hippocastanum* in Britain. Nomenclature follows that of the Database of Insects and their Food Plants (DBIF, 2018)

Species/classification	Ecological notes	Source
Acari		
Eriophyidae		
Tegonotus carinatus (Nalepa)	Larvae and adults; leaves; *Aesculus* only	1
Vasates hippocastani (Fockeu)	Galling on leaves; *Aesculus* only	1
Tetranychidae		
Eotetranychus pruni (Oudemans)	Leaves; variety of deciduous trees	1
E. tiliarum (Hermann)	Leaves; variety of deciduous trees	1
Hemiptera		
Aphididae		
Periphyllus testudinaceus (Fernie)	Also on *Acer*	1
Cicadellidae		
Edwardsiana avellanae (Edwards, J.)	Leaves; Also on *Ulmus, Acer, Corylus*	1
E. hippocastani (Edwards, J.)	Variety of deciduous trees	1
E. lethierryi (Edwards, J.)	Leaves; variety of deciduous trees	1
Fagocyba cruenta (Herrich-Schaeffer)	Leaves; wide variety of deciduous trees	1
Alebra wahlbergi (Boheman)	Leaves; wide variety of	1

	deciduous trees	
Ossiannilssonola callosa (Then)	Leaves; also on *Alnus, Crataegus, Fagus, Tilia* and *Acer*	1
Coccidae		
Eulecanium tiliae (L.)	Stems; wide variety of deciduous trees	1
Parthenolecanium corni (Bouche)	Very wide range of trees	1
P. rufulum (Cockerell)	Also on *Quercus, Castanea, Carpinus* and shrubs	1
Pulvinaria betulae (L.)	Variety of deciduous woody plants	1
P. regalis (Canard)	Leaves, branches, trunk; variety of trees	1
Diaspididae		
Lepidosaphes ulmi (L.)	Very wide range of trees and shrubs	1
Pseudococcidae		
Phenacoccus aceris (Signoret)	Wide range of deciduous trees	1
Lepidoptera		
Cossidae		
Zeuzera pyrina (L.)	Stem miner; wide range of trees and shrubs	1
Geometridae		
Alsophila aescularia (Denis & Schiffermuller)	Leaves; variety of deciduous trees and shrubs	1
Campaea margaritata (L.)	Larvae; variety of trees and	1

	shrubs	1
Ennomos fuscantaria (Haworth)	Larvae; *Fraxinus, Ligustrum*	1
Lycia hirtaria (Clerck)	Leaves; wide variety of trees and shrubs	1
Ourapteryx sambucaria (L.)	Larvae; variety of deciduous trees and shrubs	1
Gracillariidae		
Cameraria ohridella Deschka & Dimić	Horse-chestnut leaf miner	1
Lymantriidae		
Lymantria monacha (L.)	Leaves; wide range of trees	1
Noctuidae		
Lithophane hepatica (Clerck)	Leaves; variety of deciduous trees and shrubs	1
Phalera bucephala (L.)	Leaves; wide variety of trees	1
Tortricidae		
Cacoecimorpha pronubana (Hubner)	Larvae: wide range of herbaceous plants and trees	1
Yponomeutidae		
Argyresthia glaucinella Zeller	Bark; *Betula, Quercus*	1
Coleoptera		
Attelabidae		
Attelabus nitens (Scopoli)	Leaf rolling; variety of trees	1
Curculionidae		
Mesites tardii (Curtis)	Dead bark and wood; variety of trees and shrubs	1
Rhyncolus lignarius (Marsham)	Wood; also on other woody	1

	plants	
Eucnemidae		
Hylis olexai (Palm)	Dead wood; also on *Populus, Fagus* and *Abies*	1
Lucanidae		
Lucanus cervus (L.)	Wood, roots; variety of deciduous trees	1
Melandryidae		
Abdera quadrifasciata (Curtis)	Dead wood; *Fagus, Quercus, Carpinus*	
Diptera		
Aulacigastridae		
Aulacogaster leucopeza (Meigen)	Larvae in sap exudate	2
Ceratopogonidae		
Dasyhelea flavifrons (Guérin)	Larvae in sap exudate	2
Culicidae		
Anopheles plumbeus Stephens	In wet tree hollow	2
Finlaya geniculata (Olivier)	In wet tree hollow	2
Orthopodomyia pulchripalpis (Rondani)	In wet tree hollow	2
Dolichopodidae		
Systenus pallipes (von Roser)	In wood pulp	2
S. scholtzi Loew	In wood pulp	2
Limoniidae		
Rhipidia ctenophora Loew	In wood pulp	2
Muscidae		

Phaonia cincta Zett.	In wood pulp	2
P. exoleta (Meigen)	In wood pulp	2
Mycetobiidae		
Mycetobia pallipes Meigen	Larvae in sap exudate	2

1. DBIF (2018); 2, Keilin (1927).

TABLE 3 Slime moulds (Amoebozoa, Myxomycetes) associated with *Aesculus hippocastanum*. Nomenclature follows the Fungal Records Database of Britain and Ireland (British Mycological Society 2018)

Arcyria denudata (L.) Wettst.	Live bark
A. pomiformis (Leers) Rostaf.	Live bark
Badhamia utricularis (Bull.) Berk.	Dead bark
Calomyxa metallica (Berk.) Nieuwl.	Live bark
Ceratiomyxa fruticulosa (O.F. Müll.) T. Macbr.	
Comatricha nigra (Pers.) J. Schröt.	Fallen branches
Craterium minutum (Leers) Fr.	Fallen leaves
Didymium squamulosum (Alb., & Schwein.) Fr.	Fallen twigs and leaves
Echinostelium brooksii K.D. Whitney	Live bark
E. colliculosum K.D. Whitney & H.W. Keller	Live bark
E. fragile Nann.-Bremek.	Live bark
Enerthenema papillatum (Pers.) Rostaf.	Live bark, fallen branches
Fuligo septica (L.) F.H. Wigg.	
Hemitrichia minor G. Lister	Bark
Licea bryophila Nann.-Bremek.	Live bark
L. denudescens H.W. Keller & T.E. Brooks	Live bark
L. kleistobolus G.W. Martin	Live bark
L. marginata Nann.-Bremek.	Live bark
L. minima Fr.	Live bark
L. operculata (Wingate) G.W. Martin	Live bark
L. parasitica (Zukal) G.W. Martin	Live bark
L. scyphoides T.E. Brooks & H.W. Keller	Live bark
Lycogala epidendrum (J.C. Buxb. ex L.) Fr.	Fallen, rotting wood

L. terrestre Fr.	Stumps, fallen wood
Macbrideola cornea (G. Lister & Cran) Alexop.	Live bark
Metatrichia floriformis (Schwein.) Nann.-Bremek.	Fallen wood
Mucilago crustacea P. Micheli ex F.H. Wigg.	Live and dead bark
Paradiacheopsis cribrata Nann.-Bremek.	Live bark
P. fimbriata (G. Lister & Cran) Hertel ex Nann.-Bremek.	Live bark
P. solitaria (Nann.-Bremek.) Nann.-Bremek.	Live bark
Perichaena chrysosperma (Curr.) Lister	Live bark
P. depressa Lib.	Fallen branches
Physarum cinereum (Batsch) Pers.	Dead bark
Protostelium mycophagum L.S. Olive & Stoian.	Live bark
Reticularia lycoperdon Bull.	Fallen wood
Stemonitis ferruginea Ehrenb.	
Trichia persimilis P. Karst.	Fallen branches
T. scabra Rostaf.	Fallen branches
Tubulifera arachnoidea Jacq.	Fallen trunk

TABLE 4 Fungi (by Order) directly associated with *Aesculus hippocastanum* not including those found on soil or litter below the trees, or those found solely on dead wood. Details of these can be found in the Fungal Records Database of Britain and Ireland (British Mycological Society, 2018). Nomenclature follows this database. Fungi that can be lichenised were identified from the British Isles List of Lichens and Lichenicolous Fungi (Natural History Museum, 2018)

Species/classification	Ecological notes
Oomycota	
Peronosporales	
Globisporangium intermedium (de Bary) Uzuhashi, Tojo & Kakish.	Bark
Ascomycota	
Botryosphaeriales	
Guignardia aesculi (Peck) V.B. Stewart	Necrotic spots on leaves
Capnodiales	
Ramularia endophylla Verkley & U. Braun	Fallen leaves
Septoria hippocastani Berk., & Broome	Living and fallen leaves
Diaporthales	
Diaporthe coneglanensis Sacc., & Speg.	Necrotic spots on fading leaves
Ophiognomonia setacea (Pers.) Sogonov	Petioles, dead leaves
Erysiphales	
Erysiphe flexuosa (Peck) U. Braun & S. Takam.	Leaves
Eurotiales	
Penicillium sp. Link	Old fruits
Helotiales	

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Botryotinia fuckeliana (de Bary) Whetzel	Fallen fruits
Botrytis cinerea Pers.	Live leaves
B. fascicularis (Corda) Sacc.	Fruits
Calycellina lachnobrachya (Desm.) Baral	Fruits
Crocicreas subhyalinum (Rehm) S.E. Carp.	Petioles, fruits
Gibberella baccata (Wallr.) Sacc.	Fruits
Hyaloscypha fuckelii var. *fuckelii* Nannf.	Old fruits
Hymenoscyphus albidus (Gillet) W. Phillips	Decaying petioles
H. albopunctus (Peck) Kuntze	Dead leaves
H. calyculus (Sowerby) W. Phillips	Old fruits
H. caudatus (P. Karst.) Dennis	Fallen leaves, decaying petioles
H. fructigenus (Bull.) Fr.	Old fruits
Lachnum niveum (R. Hedw.) P. Karst.	Falling leaves
L. virgineum (Batsch) P. Karst.	Falling leaves
Lanzia echinophila (Bull.) Korf	Old fruits
Leptodontidium trabinellum (P. Karst.) Baral, Platas & R. Galán	Fruits
Niptera subbiatorina Rehm	Fruits
Pezicula cinnamomea (DC.) Sacc.	Bark
Pezizella discreta (P. Karst.) Dennis	Petioles
Pseudohelotium pineti (Batsch) Fuckel	Fruits
Rutstroemia sp. P. Karst.	Old fruits
Sclerotinia sp. Fuckel	Fruits
Hypocreales	
Cylindrodendrum album Bonord.	Fruits
Fusidium griseum Ditmar	Leaves
Volutella ciliata (Alb., & Schwein.) Fr.	Live bark
Incertae sedis	

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Camposporium pellucidum (Grove) S. Hughes	Fruits
Chalara aurea (Corda) S. Hughes	Fruits
C. cylindrosperma (Corda) S. Hughes	Fruits
C. rhynchophialis Nag Raj & W.B. Kendr.	Fruits
Haplariopsis fagicola Oudem.	Fruits
Polyscytalum fecundissimum Riess	Fallen leaves
Torula herbarum (Pers.) Link	Fruits
Lecanorales	
Ramalina fraxinea (L.) Ach.	Lichen, bark
Ostropales	
Dimerella pineti (Schrad.) Vezda	Lichen, live bark
Phlyctis argena (Ach.) Flot.	Lichen, live bark
Pleosporales	
Anguillospora longissima (Sacc., & P. Syd.) Ingold	Bud scales
Clavariopsis aquatica De Wild.	Bud scales
Peltigera membranacea (Ach.) Nyl.	Lichen, live bark
Pertusariales	
Pertusaria leioplaca DC.	Lichen, bark
P. pertusa (Weigel) Tuck.	Lichen, bark
Teloschitales	
Amandinea punctata (Hoffm.) Coppins & Scheid.	Lichen, live bark
Calicium viride Pers.	Lichen, bark
Massjukiella polycarpa (Hoffm.) S.Y. Kondr., Fedorenko, S. Stenroos, Kärnefelt, Elix, J.S. Hur & A. Thel	Live bark
Xanthoria parietina (L.) Th. Fr.	Live bark, dead wood
Xylariales	
Annulohypoxylon multiforme (Fr.) Y.M. Ju, J.D. Rogers & H.M. Hsieh	Bark, dead wood

Basidiomycota	
Agaricales	
Armillaria gallica Marxm., & Romagn.	Roots, base of live trunk
A. mellea (Vahl) P. Kumm.	Roots, base of live trunk
A. ostoyae (Romagn.) Herink	Stump
Arrhenia acerosa (Fr.) Kühner	Fallen petioles
Atheliales	
Athelia epiphylla Pers.	Leaves
Clitocybe candicans (Pers.) P. Kumm.	Fallen, rotting fruit
Fistulina hepatica (Schaeff.) With.	Live trunk
Flammulina velutipes (Curtis) Singer	Live trunk, dead wood
Hemimycena lactea (Pers.) Singer	Live trunk, dead wood
Marasmius epiphyllus (Pers.) Fr.	Fallen leaves
Mycena olida Bres.	Live trunk
Pleurotus dryinus (Pers.) P. Kumm.	Live trunk, dead wood
P. ostreatus (Jacq.) P. Kumm.	Live trunk, dead wood
Resupinatus applicatus (Batsch) Gray	Fallen leaves
R. trichotis (Pers.) Singer	Fallen leaves
Volvariella bombycina (Schaeff.) Singer	Live trunk, dead wood
Atractiellales	
Phleogena faginea (Fr.) Link	Live trunk
Boletales	
Serpula himantioides (Fr.) P. Karst.	Wood of live trunk
Polyporales	
Abortiporus biennis (Bull.) Singer	Live roots, trunk and dead wood
Fomes fomentarius (L.) J.J. Kickx	Live trunk
Ganoderma applanatum (Pers.) Pat.	Wood on live trunks

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G. australe (Fr.) Pat.	Live trunk
G. lucidum (Curtis) P. Karst.	Live trunk
G. resinaceum Boud.	Live trunk, roots
Laetiporus sulphureus (Bull.) Murrill	Live trunk, dead wood
Meripilus giganteus (Pers.) P. Karst.	Live trunk, dead wood
Perenniporia fraxinea (Bull.) Ryvarden	Live trunk, dead wood
Polyporus squamosus (Huds.) Fr.	Live trunk, dead wood
Postia ptychogaster (F. Ludw.) Vesterh.	Live trunk
Rigidoporus ulmarius (Sowerby) Imazeki	Live trunk, dead wood
Trametes ochracea (Pers.) Gilb., & Ryvarden	Live branch, dead wood
T. versicolor (L.) Pilát	Live trunk, dead wood

FIGURES
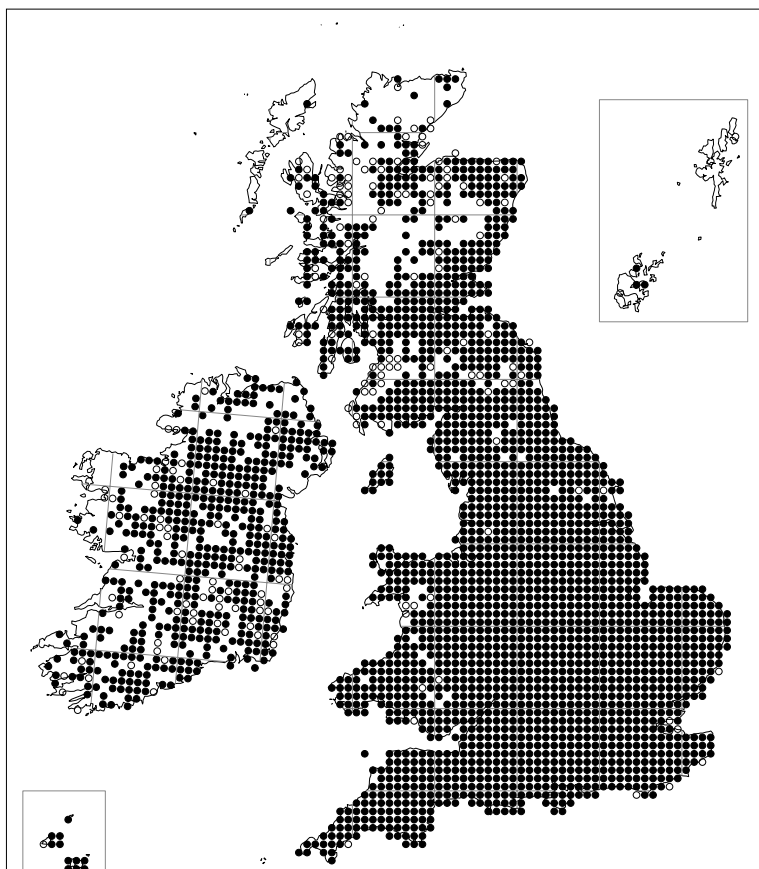
FIGURE 1 The distribution of *Aesculus hippocastanum* in the British Isles. Each dot represents at least one record in a 10-km square of the National Grid. (●) non-native 1970 onwards; (○) non-native pre 1970. Mapped by Colin Harrower, Biological Records Centre, Centre for Ecology and Hydrology, mainly from records collected by members of the Botanical Society of Britain and Ireland, using Dr A. Morton's DMAP software
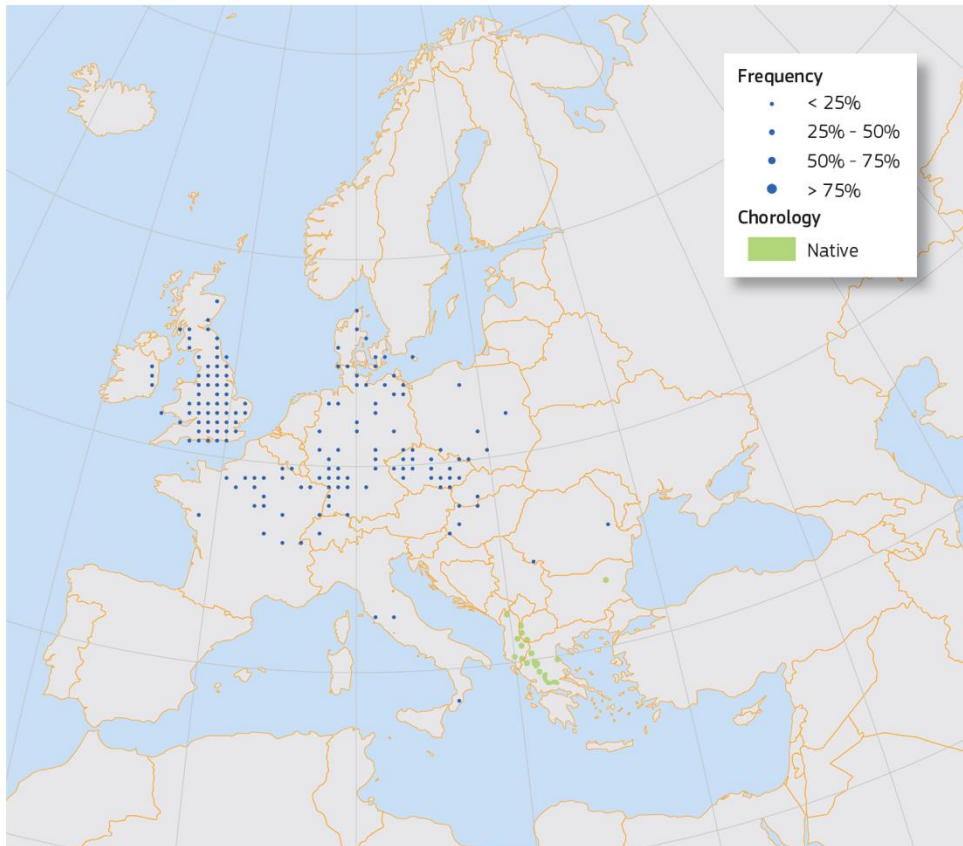
FIGURE 2 Distribution of *Aesculus hippocastanum* across Europe (small dots) and its native range (larger green dots). Frequency of occurrence are from field observations as reported by the National Forest Inventories. From: Ravazzi and Caudullo (2016), reproduced courtesy of the European Union.
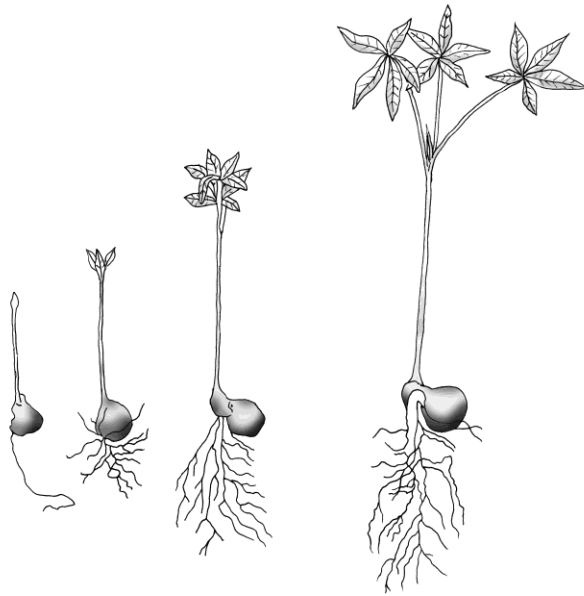
FIGURE 3 Seedlings of *Aesculus hippocastanum* at (a) 1 week, (b) 2 weeks, (c) 4 weeks and (d) 8 weeks after germination. Drawings by Omar Alhamd.

ONLINE GRAPHICAL ABSTRACT

Horse-chestnut is native to the Balkan Peninsula but is widely planted across Europe as an outstandingly attractive flowering tree, and for its large seeds used in the playground game of 'conkers' and medically for their triterpene glycosides. Horse-chestnut is threatened by a bacterial canker on the trunk, and an insect and fungus affecting the leaves. These are mostly aesthetic problems although the canker can kill trees. The leaf miner insect does not reduce the vigour of trees, despite removing up to 75% of the leaf area, but it can halve the seed size with potential problems for natural establishment.