

Complex Combination of Seed Dormancy and Seedling Development Determine Emergence of *Viburnum tinus* (Caprifoliaceae)

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• **Background and Aims** The shrub *Viburnum tinus* is widely distributed in matorral vegetation of the Mediterranean basin. The purpose of the present study was to classify the seed dormancy type and examine the requirements for embryo growth, root protrusion and shoot emergence.

• **Methods** Overwintered fruits were collected in western Spain in April 2001 and prepared in three ways: entire pericarp was removed, exocarp and mesocarp were removed or fruits were left intact. Fruits treated in these three ways were subjected to artificial annual temperature cycles or to constant temperature regimes for 1.5 years.

• **Key Results** Removal of exocarp and mesocarp was necessary for embryo growth and germination. High temperature favoured dormancy alleviation and embryo growth, intermediate to low temperatures favoured root protrusion, and intermediate temperature shoot emergence. There was substantial germination at constant temperature regimes, indicating an overlap between temperature intervals suitable for the different stages of embryo and seedling development. Functionally, *V. tinus* has the same root and shoot emergence pattern that is described for other *Viburnum* species considered to have epicotyl dormancy. However, the requirement for high and low temperatures for radicle protrusion and epicotyl emergence, respectively, was missing in *V. tinus*; these characters are the foundation for the epicotyl dormancy classification.

• **Conclusions** It is concluded that *V. tinus* does not have epicotyl dormancy. Instead, there is a combination of a weak morphophysiological dormancy and a slow germination process, where different temperatures during an annual cycle favour different development stages. The present study suggests that the first complete seedlings would emerge in the field 1.5 years after fruit maturation in October, i.e. seed dispersal during winter, embryo growth during the first summer, root protrusion and establishment during the second autumn and winter, and cotyledon emergence during the second spring.

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Key words: Adoxaceae, Caprifoliaceae, epicotyl dormancy, matorral, morphophysiological dormancy, pericarp, persistent fruits, ruminant endosperm, seedling emergence, shrub, Viburnaceae, *Viburnum tinus*.

INTRODUCTION

Viburnum tinus is a large shrub with a wide distribution in the Mediterranean basin (Dirr, 1998), and it is a characteristic or dominant species in sclerophyllous shrubland (matorral) vegetation (Le Houérou, 1981; Tomaselli, 1981). It has horticultural (Dirr, 1998; Nobre *et al.*, 2000) as well as medicinal properties (Cometa *et al.*, 1998). The fruit is a one-seeded drupe consisting of a fleshy exocarp and mesocarp and a hard endocarp that is united with the seed coat (cf. Ferguson, 1966). The drupes are metallic blue that later turn blue-black to black and ripen in October (Debussche *et al.*, 1987; Dirr, 1998). They are relatively dry (Debussche *et al.*, 1987; Herrera, 1987) with 53 % water content in pulp (Debussche *et al.*, 1987), and are removed from plants mainly by birds during winter (Thebaud and Debussche, 1992; Herrera, 1998). The embryo is surrounded by ruminant endosperm (Dragone-Testi, 1933), a feature also known to occur in other species of the genus (Rehder, 1940). *Viburnum tinus* is evergreen (Cornelissen *et al.*, 1997), which is unusual within this predominantly deciduous genus. This species is placed in the section *Tinus*, along with five to six other taxa that occur mostly in China (Rehder, 1940; Krüssmann, 1986).

The genus *Viburnum* (Caprifoliaceae, Adoxaceae or Viburnaceae) consists of about 150 species of small trees and shrubs distributed primarily in the northern hemisphere but extending south in the mountains of south-east Asia and South America (Krüssmann, 1986; Mabberley, 1997; Donoghue *et al.*, 2004). Seeds of *Viburnum* species have underdeveloped embryos (Martin, 1946; Gill and Pogge, 1974). Species containing embryos described as underdeveloped are dormant at maturity, and may need warm (≥ 15 °C) and/or cold (0–10 °C) stratification before embryo growth and germination can take place (Nikolaeva, 1977; Baskin and Baskin, 1998). This class of dormancy is called morphophysiological, and eight types are distinguished (Baskin and Baskin, 2004). In the case of deep simple epicotyl morphophysiological dormancy, the shoot does not immediately emerge after the radicle. Instead, both the radicle and epicotyl are dormant and require different temperature treatments to overcome dormancy; warm temperatures alleviate radicle dormancy, whereupon cold temperatures alleviate epicotyl dormancy in those seeds where the radicle has already emerged (Barton, 1933; Nikolaeva, 1977; Baskin and Baskin, 1998). The inhibitory mechanism of shoot growth appears to be in the cotyledons and not in the epicotyl *per se* (Knowles and Zalik, 1958).

Epicotyl dormancy has been reported in nine *Viburnum* taxa growing in northern and central North America

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[*V. acerifolium* (Giersbach, 1937; Hidayati *et al.*, in press), *V. dentatum*, *V. dilatatum*, *V. lentago*, *V. opulus*, *V. prunifolium*, *V. pubescens* (Giersbach, 1937), *V. alnifolium* (Gould, 1966) and *V. trilobum* (Fedec and Knowles 1973)], one species in the southern United States [*V. rufidulum* (Giersbach, 1937)] and one species in Russia [*V. opulus* (Zolobova, 1970; Nikolaeva *et al.*, 1985)]. In contrast, *V. nudum* and *V. scabrellum* in southern United States are reported to have morphologically dormant seeds, i.e. roots and shoots readily emerged without any treatments (Giersbach, 1937). Chien *et al.* (2002) demonstrated that cold stratification alone overcame dormancy in seeds of a Taiwanese species, *V. odoratissimum*, in the section *Solenotinus* (Thyrsoma). Based on these results, members of sections *Lentago* and *Odontotinus* principally have epicotyl dormancy, with the exception of one species in each section with morphological dormancy; two members in the section *Opulus* and one in *Pseudotinus* have epicotyl dormancy. The phylogeny of 42 *Viburnum* species was investigated recently (Donoghue *et al.*, 2004); in general, the traditional classification in sections was supported, but *Lentago* was paraphyletic, and *Odontotinus* non-monophyletic.

It has been proposed that epicotyl dormancy, in the case of *Viburnum*, might be an adaptation to temperate climates (Kollmann and Grubb, 2002). One way of determining whether a particular character is an adaptation requires the examination of patterns in nature among relatives. The issue that needs to be resolved is whether the observed trait is an adaptation or merely a phylogenetically conservative one (Westoby, 1999). On the other hand, groups of related species inhabiting similar environments tend to be homogeneous, referred to as phylogenetic niche conservatism, due to stabilizing selection or developmental constraints (Lord *et al.*, 1995; Webb *et al.*, 2002). Species growing in different vegetation types and on different continents offer prospects to explore adaptations, and begin to clarify evolutionary factors, such as constraints or conservatism, that influence traits (Hidayati and Walck, 2002).

When comparing dormancy and germination patterns between species it is important to use a consistent dormancy classification. Otherwise, there is a substantial possibility of grouping species together just because they, for example, germinate the same time of the year, even if different environmental cues alleviated their seed dormancy. In this research, the seed dormancy definitions stated by Vleeshouwers *et al.* (1995) and Baskin and Baskin (1998), in which dormancy is an innate attribute of the seed and is not related to the particular environment the seed happens to be in, were followed.

The purpose of the present study was to classify the nature of seed dormancy exhibited by *V. tinus* and, if possible, compare it with the dormancy known to occur in congeneric species primarily found in the temperate deciduous forest of North America. *Viburnum tinus* grows in a vegetation type and is classified in a section of the genus in which epicotyl dormancy has not been investigated before. Understanding the kind of dormancy found in *V. tinus* would fill a gap in our knowledge, especially with regards

to the adaptive importance of epicotyl dormancy in the temperate region. Specifically, the requirements for embryo growth, root protrusion and shoot emergence, and the influence of the pericarp on dormancy and germination for *V. tinus* were examined. Using the results of this study, a conceptual model for the timing of germination in the field could be produced.

MATERIALS AND METHODS

Seed collection, pre-treatment and experimental set-up

Fruits were collected on 28 Apr. 2001 at Las Batuecas (in Sierra de Francia), approx. 65 km south-west of Salamanca, in western Spain. The fruits had remained on the plants over the winter and were relatively hard and dry. There was no indication that animals had removed any part of the seed crop. The fruits were kept dry for approx. 1 month at room temperature (approx. 20 °C) before initiation of the studies.

Two thousand fruits were prepared in each of three ways: (1) 'seed'—the entire pericarp was removed from dry fruits; (2) 'stone'—the fruits were soaked in water for 3–5 h, the pulp (exocarp and mesocarp) were carefully removed from the stone with the aid of a scalpel, and the stones allowed to dry again (for at least 48 h); and (3) 'drupe'—the fruits were left intact. Hereafter, the collective of the three different preparations is referred to as 'fruits'.

Experiments were conducted in three temperature- and light-controlled incubators (Rubarth Apparatebau, Laatzen, Germany) and in a cold room. The incubators were set at alternating 12/12 h (day/night) temperatures of 15/5, 20/10 or 25/15 °C, with a 2-h linear transition between the maximum and minimum temperature. The cold room, equipped with shelving and lighting similar to the incubators, was set at a constant temperature of 5 °C. These four temperature regimes approximate the mean monthly temperatures in Salamanca (Weather Channel, 2004a) and the mean monthly minimum and maximum temperatures in Madrid, approx. 170 km from Salamanca (Weather Channel, 2004b): 5 °C, December–February; 15/5 °C, March and November; 20/10 °C, May, April and October; and 25/15 °C, June–September.

Fruits were exposed to a photoperiod of 12 h per day in both the incubators and room. Warm white fluorescent light tubes were used as the light source in the incubators (two tubes at 18 or 20 W) and in the room (two tubes at 36 W and one at 20 W). The photon flux density (400–700 nm) in the incubators and room was 22–59 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (photometer: SKP 200, sensor SKP 215, Skye Instruments Ltd, Wales, measure precision 1 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and the 660/730 nm photon fluence ratio was 9.4–9.5 during the first 53 weeks and 2.6–3.5 during the remaining time (photometer: SKR 100, sensor SKR 110, Skye Instruments Ltd, Wales).

On 1 Jun., 2001, fruits were placed on two filter papers (Munktel 1003) in 9-cm-diameter Petri dishes and moistened with 6 mL of deionized water. The dishes were wrapped with parafilm to reduce water loss during incubation. They were rearranged randomly on the shelves of the incubators and in the room every second week. Water was

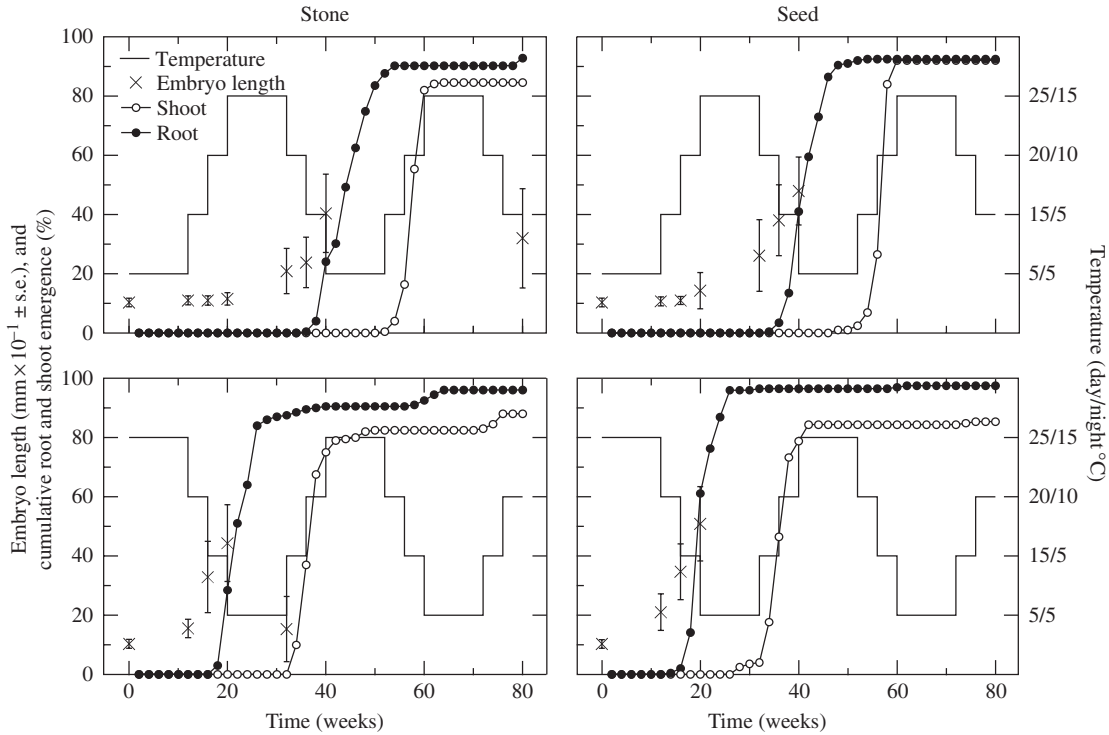


FIG. 1. Mean (\pm s.d.) embryo length on different occasions, and cumulative root protrusion and shoot emergence of *Viburnum tinus* fruits subjected to annual temperature cycles starting at a winter (5 °C) or summer (25/15 °C) regime. The fruits were treated in two ways before the start of the experiment: the entire pericarp was removed (seeds) or the pulp was removed (stones). In each annual cycle, 200 seeds or stones were used. Embryo lengths were measured in viable fruits remaining in an embryonic stage.

added to the dishes as needed to keep the filter papers at a near-constant moisture level.

Requirements for root protrusion and shoot emergence

Four or five Petri dishes of 40 fruits per dish were used in each treatment. Fruits were exposed to two types of treatments: continuous temperature regime or annual temperature cycle. The four continuous temperature regimes corresponded approximately to winter (5 °C), early spring/late autumn (15/5 °C), late spring/early autumn (20/10 °C) and summer (25/15 °C). The annual temperature cycles simulated a natural sequence of the seasonal temperatures above, either beginning at summer or at winter regime (Fig. 1). Each artificial year, fruits were subjected to 12 weeks of summer and winter, and 4 weeks of both spring and autumn temperature levels. Experiments with seeds and with stones lasted for 80 weeks (i.e. for two annual temperature cycles), while those with drupes were terminated after 60 weeks.

Roots were determined to have protruded when the radicle had split the endosperm at which point it was clearly visible. Shoots were judged to have emerged when at least one cotyledon was completely exposed from the seed coat. At this stage, both the cotyledons and epicotyls (if visible) were green. Fruits with radicles were counted and removed from the dishes every 2 weeks. When a root had protruded, the fruit was removed from the dish and planted in a small pot (65 mm diameter, 70 mm deep), filled with approx. 20 mm moist sand (90 % gneiss and/or granite, 10 % other minerals, 0.2–0.6 mm grain size) and sealed with

parafilm on top. Germinated fruits from the same dish were planted in the same pot and a small stake placed beside each one indicated the day of root protrusion. The pots were checked for shoots every 2 weeks. Once a shoot had emerged, it was counted and the plant discarded (during the first 32 weeks, plants at 15/5 and 20/10 °C were retained until the emergence of the first true leaves). Fruits from which radicles did not protrude were checked to determine if the embryos were white and firm, indicating they were viable, or if the embryos were brown and soft, indicating they were nonviable. Percentages of root protrusion and shoot emergence are based on number of viable seeds at the end of the experiment.

Requirements for embryo growth

Lengths of embryos were measured at the beginning of the experiment and at various times. The point in development used to define the end of the embryonic stage was root protrusion. Embryos were excised using a scalpel, and their lengths measured under a dissecting microscope equipped with a micrometer. At the beginning of the experiment, 40 drupes were placed for 24 h on moist filter paper at room temperature before embryos were removed and measured.

One dish of 40 fruits was used on each occasion when measurements were made, using fruits still in the embryonic stage. In each of the continuous temperature regimes, measurements were taken after 60 weeks and for seeds and stones also at 80 weeks. In the annual temperature

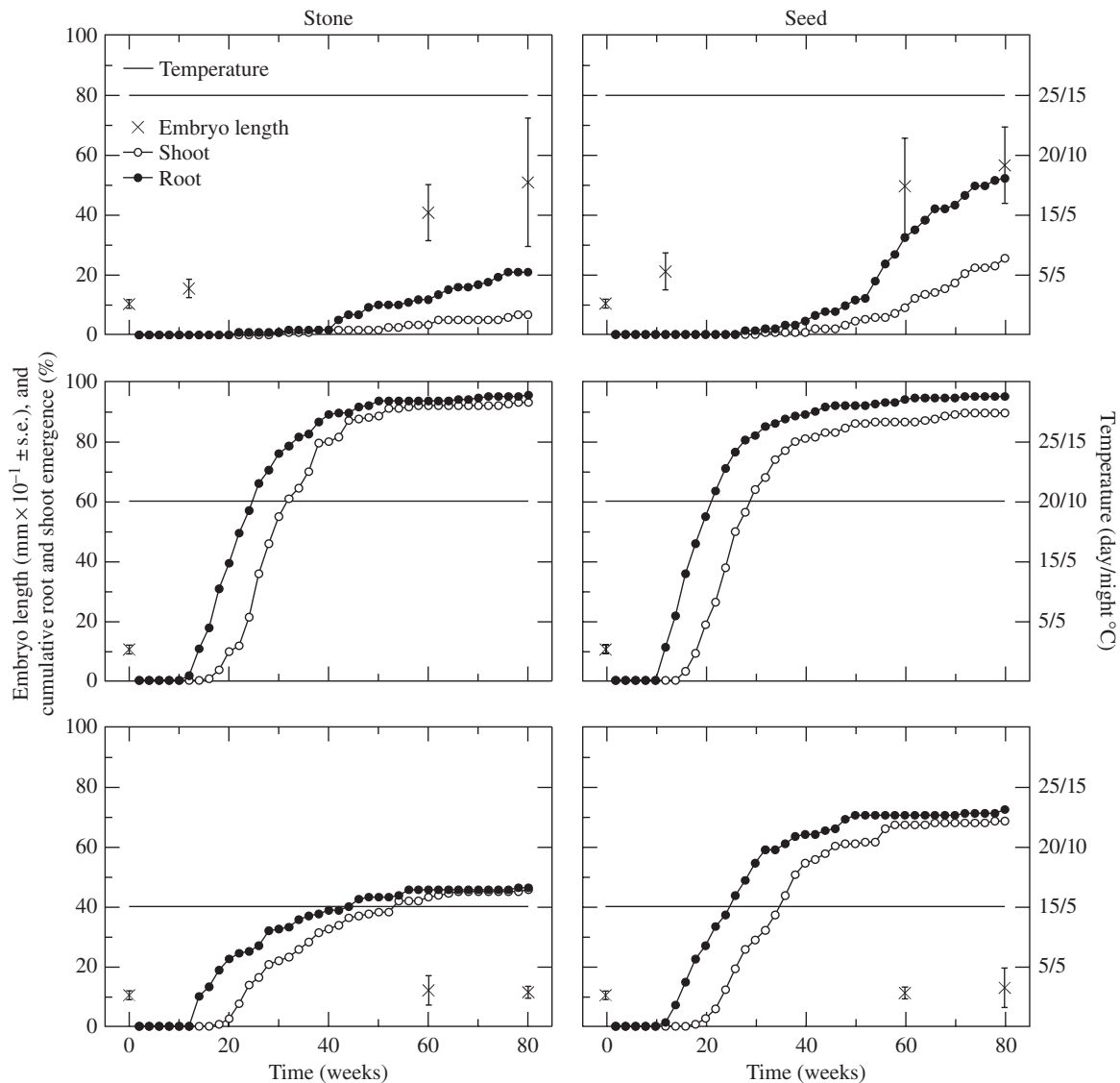


FIG. 2. Mean (\pm s.d.) embryo length on different occasions, and cumulative root protrusion and shoot emergence of *Viburnum tinus* fruits subjected to continuous temperature regimes (15/5, 20/10 or 25/15 °C day/night, indicated by the line on each graph). The fruits were treated in two ways before the start of the experiment: the entire pericarp was removed (seeds) or the pulp was removed (stones). In each temperature regime, 160–200 seeds or stones were used. Embryo lengths were measured in viable fruits remaining in an embryonic stage.

cycles, embryos were measured each time dishes were moved to a new temperature regime (Fig. 1). At 5 °C and 25/15 °C continuous temperature regimes, embryo lengths were recorded after 12 weeks; the data were obtained from fruits in the annual temperature cycle treatment after the first 12-week period at the winter or summer regimes, respectively. Embryo lengths were measured if ≥ 8 (20 %) of the fruits in one dish remained in embryonic stage, but on the last occasion (after 60 or 80 weeks) they were measured if in total ≥ 8 fruits (4–5 %) in the treatment remained in embryonic stage. Only fruits in which embryo and endosperm were white and firm, indicating viability, were used for measurements. Means and standard deviations were calculated each time measurements were obtained.

RESULTS

Effects of pericarp removal

In general, embryos did not grow and germination did not occur in drupes. Of 686 embryos from drupes, only two, both in the annual cycle with summer start, were considerably longer (3.33 and 4.05 mm, respectively) than in fresh drupes (0.76–1.29 mm). Roots protruded from two (one in the annual cycle with summer start, one at continuous 20/10 °C) of 1159 drupes, and no shoot emergence occurred from drupes. In contrast, embryos grew and roots and shoots emerged from fruits in which the pulp was removed ('stones') and the entire pericarp was removed ('seeds') (Figs 1 and 2). Percentages of root protrusion and shoot

TABLE 1. Time between root protrusion and shoot emergence for *Viburnum tinus* seeds (pericarp removed from fruits) and stones (pulp removed from fruits) subjected either to annual temperature cycles or to continuous temperature regimes (15/5, 20/10 or 25/15 °C day/night)

Treatment	Condition		Mean time [weeks (s.d.)]	No. of shoots	Dead fruits (%)	Remaining fruits (%)
Annual temperature cycles	Starting at a winter regime	Seed	15.2 (2.6)	184	2.1	0.0
		Stone	13.7 (3.2)	165	2.8	4.5
	Starting at a summer regime	Seed	15.7 (2.3)	170	6.1	0.0
		Stone	14.5 (3.3)	174	7.9	0.0
Continuous temperature regimes	15/5 °C	Seed	8.8 (1.3)	107	2.7	1.8
		Stone	9.0 (2.8)	73	0.0	0.0
	20/10 °C	Seed	6.5 (2.0)	178	4.8	0.0
		Stone	6.9 (2.4)	186	2.1	1.0
	25/15 °C	Seed	6.4 (2.2)	40	45.1	6.1
		Stone	6.3 (1.3)	8	64.0	4.0

In each treatment 160–200 seeds or stones were used.

'Dead fruits' produced roots but died before producing shoots.

'Remaining fruits' had roots but not shoots when the experiment was terminated after 80 weeks.

emergence were higher for seeds than for stones at continuous 25/15 and 15/5 °C, but were similar at 20/10 °C (Fig. 2).

Requirements for embryo growth

Embryos in fresh (untreated) drupes were 1.03 mm (s.d. 0.14) long, approx. 14 % the length of the seed. In seeds and stones that had been subjected to summer temperature followed by autumn in the annual cycles, lengths of embryos were 8.5–9.1 mm ($n = 3$) when the radicles were very close to protrusion. In the annual temperature cycles, embryos of seeds and stones started to increase their lengths at 25/15 °C and they continued to do so when transferred to 20/10 and 15/5 °C (Fig. 1). At continuous temperatures, embryos in seeds and stones did not differ (t -test, $P > 0.15$) from embryos in fresh drupes after 80 weeks of incubation at 5 °C, but growth of embryos occurred in seeds and stones at 15/5 °C and higher temperatures (Fig. 2).

Requirements for root protrusion and shoot emergence

In the annual temperature cycles, roots protruded and shoots emerged from 85–97 % of stones and seeds regardless of whether they were subjected to winter or summer temperatures first (Fig. 1). Only after stones and seeds had been exposed to the summer temperature did roots protrude. Root protrusion occurred during late autumn (15/5 °C) and winter (5 °C) (Fig. 1). Shoots emerged 13.7–15.7 weeks after root protrusion (Table 1). Shoot emergence began at the end of the winter temperature period after root protrusion (Fig. 1).

In the continuous temperature regimes, neither roots protruded nor shoots emerged from 160 stones or 160 seeds during 80 weeks of incubation at 5 °C. The highest percentage of root protrusion and shoot emergence for stones and seeds (≥ 90 %) occurred at 20/10 °C, followed by 15/5 °C (46 % for stones, 69–72 % for seeds) and then 25/15 °C (7–21 % for stones, 25–52 % for seeds) (Fig. 2). The time lag between root protrusion and shoot emergence in the

continuous temperature regimes was much shorter than that in the annual temperature cycles, being 6.3–6.9 weeks at 20/10 and 25/15 °C and 8.8–9.0 weeks at 15/5 °C (Table 1). Once cotyledons had emerged, the development of true leaves proceeded without further delay (data not shown).

In general, only a small percentage of the stones and seeds produced roots and then died during the experiment, or produced roots but failed to produce shoots before the experiment was terminated (Table 1). However, the percentage of seeds and stones that produced roots but died before shoots emerged was high (45 and 64 %, respectively) in the 25/15 °C continuous temperature regime (Table 1).

DISCUSSION

Removal of the exocarp and mesocarp was necessary for embryo growth and germination in *V. tinus* seeds, and removal of the entire pericarp improved germination (Fig. 2). Endocarp removal or exocarp/mesocarp (pulp) removal enhanced germination in *V. trilobum* (Knowles and Zalik, 1958) and *V. dentatum* (Meyer and Witmer, 1998). Pulp affects germination by preventing imbibition due to its imperviousness, its high osmotic pressure from dissolved sugars, or by containing germination inhibitors (Mayer and Poljakoff-Mayber, 1989). Meyer and Witmer (1998) found no differences in germination between seeds manually cleaned of pulp, and cleaned bird-passed seeds, regardless if seeds were regurgitated or defecated. Fruits of *V. tinus* are consumed and dispersed by birds (Thebaud and Debussche, 1992; Herrera, 1998), and the present results suggest that removal of pulp from seeds by frugivores is an important factor for germination.

The present study suggests that the first complete seedlings of *V. tinus* would start to emerge, in the Mediterranean region, 1.5 years after fruit maturation in October. Seed dispersal would occur during winter, embryo growth during the first summer, root protrusion and establishment during the second autumn and winter, and cotyledon emergence

during the second spring. Seedlings would probably emerge at the same occasion regardless of when dispersal occurred during a winter season (autumn to spring).

When collecting the seeds used in the present study, it was noteworthy that the entire seed crop was still on the plants in late spring. The timing of germination has been an aspect evaluated in the past to test hypotheses concerning the adaptive reproduction strategy of plants that produce 'persistent fruits' (cf. Jones and Wheelwright, 1987). Jones and Wheelwright (1987) suggested that their germination results for *V. opulus* supported theoretically expected predictions for fruit retention. They found no difference in the timing or success of germination of seeds eaten in November vs. April, even though the length of time between dispersal and germination differed (9 vs. 4 months). However, seeds dispersed in November and April would have been exposed to equal amounts of warm (summer) temperatures. Seeds of *V. opulus*, reported to have epicotyl dormancy (Giersbach, 1937; Zolobova, 1970; Nikolaeva *et al.*, 1985), are likely to have similar phenology of emergence as those of *V. tinus* (Fig. 1). It is predicted that if fruits of *V. opulus* were available and eaten at a time of the year (e.g. late summer) when they would not have received a sufficient temperature treatment for embryo growth and overcoming dormancy, germination would have been delayed. The timing of dispersal events, particularly when investigating plants with persistent fruits like some *Viburnum* species, needs to be placed into a framework of knowledge about the phenology of embryo growth, dormancy alleviation and germination.

Seeds and stones of *V. tinus* treated in annual cycles managed to germinate quickly at 15/5 and also at 5 °C (Fig. 1), while they did not germinate at continuous 5 °C, and at continuous 15/5 °C they germinated more slowly and to a lesser degree than in annual cycles (Fig. 2). Consequently, *V. tinus* seems to have a physiological mechanism, in addition to the morphological one, that prevents germination when directly subjected to some environmental conditions. However, after exposure to a factor that overcomes dormancy, these conditions are suitable for germination. Hence, it is concluded that *V. tinus* exhibits morphophysiological dormancy. Considering the dormancy of the 16 *Viburnum* taxa investigated so far, 14 apparently have morphophysiological dormancy (Adams, 1927; Giersbach, 1937; Gould, 1966; Zolobova, 1970; Fedec and Knowles, 1973; Gill and Pogge, 1974; Nikolaeva *et al.*, 1985; Chien *et al.*, 2002; Hidayati *et al.*, in press), so this classification is not unexpected.

Functionally, *V. tinus* has the same root protrusion and shoot emergence pattern as previously described for most other *Viburnum* species (Giersbach, 1937; Gould, 1966; Zolobova, 1970; Fedec and Knowles, 1973; Nikolaeva *et al.*, 1985; Hidayati *et al.*, in press). When subjected to an annual cycle, root protrusion occurs after the first summer (or warm) period and shoot emergence after or during the following winter (or cold) period (Fig. 1). This type of germination pattern for *Viburnum* has been reported as epicotyl dormancy. Nevertheless, the present results are not completely supportive for inferring epicotyl dormancy in *V. tinus*.

First, there was no strict requirement of a warm period for germination in *V. tinus*, but warm summer temperature alleviated a weak physiological dormancy. The fact that substantial germination (45–75 %) was recorded at continuous 15/5 °C (Fig. 2) suggests that high temperatures are not necessary to initiate embryo growth, nor a prerequisite for root protrusion. In contrast, radicles protruded from 72 and 50 % of *V. acerifolium* seeds held for 48 weeks at 25/15 and 20/10 °C, respectively; no seeds germinated at 15/6 °C (Hidayati *et al.*, in press). At 15/5 °C, the absence of increased mean length of embryos in seeds and stones after 60 and 80 weeks (Fig. 2) can be explained by a possible difference in dormancy level between individuals. In the seeds and stones with the weakest dormancy, embryo growth and subsequent root protrusion occurred, whereas the embryos in seeds and stones with stronger dormancy remained at their initial length. Such a differentiation in responses among seeds in a batch could be expected at suboptimal conditions for the species, and especially so as near-unsuitable conditions are approached. At continuous 25/15 °C embryo growth was considerable during the first 12 weeks but root protrusion was prevented for another 30 weeks (Fig. 2). This indicates that the high summer temperature is not suitable for root protrusion, but that it eventually can happen at such temperatures after embryo growth has occurred.

Secondly, there was no requirement for a cold period for shoot emergence in *V. tinus*. A substantial time lag occurred between root protrusion and shoot emergence (Table 1), but the development of a shoot continued regardless of continuous temperature regime or changes between temperature regimes in the annual cycles (Figs 1 and 2). It appears that, after planting, roots grew to a substantial size, whereupon shoot development took place. The development was actually quicker at high than at low continuous temperatures (Table 1), which should not have been the case if low temperatures (<10 °C) were essential to weaken dormancy of the epicotyl. During 5 °C in annual cycles, plant development slowed down, but when transferred to 15/5 °C quick and simultaneous shoot emergence occurred (Fig. 1). At continuous 25/15 °C approx. 50 % of germs failed to develop shoots (Table 1), indicating that this temperature was not suitable for shoot emergence.

The interpretation of the present *V. tinus* results is that dormancy alleviation and germination is a slow process: the first phase (alleviation of dormancy, embryo growth and root protrusion) requires a minimum of 10 weeks (Fig. 2) and the second (epicotyl growth and shoot emergence) a minimum of 6 weeks (Table 1). This slow process is then combined with different temperature regimes suitable for embryo growth (15/5 °C to 25/15 °C), and root protrusion and shoot emergence (5 °C to 20/10 °C) (Figs 1 and 2 and Table 1). The substantial germination at continuous temperatures (Fig. 2) can be explained by the overlap between temperature regimes suitable for the different stages of seedling development. For example, continuous 20/10 °C was suitable for all parts of the germination process; dormancy alleviation, embryo growth, root protrusion and shoot emergence.

Epicotyl dormancy is commonly reported in *Viburnum* species, in members of several sections of the genus growing in different vegetation types. Standardized laboratory experiments have not been performed in most previous germination studies on members of this genus. In fact, several of the published dormancy classifications involving epicotyl dormancy are based on observations of seedling emergence patterns, rather than on experiments with controlled temperature sequences. It is preferable that seed dormancy is defined as a strict seed characteristic, not affected by ambient environmental conditions (Vleeshouwers *et al.*, 1995; Baskin and Baskin, 1998). The concept of epicotyl dormancy becomes unclear if based on functional properties, partly affected by germination conditions, rather than on innate physiological mechanisms in the seed. Hence, data among the various species of *Viburnum* are not comparable to the degree necessary for a meaningful phylogenetic analysis. The present results also emphasize the importance of adequate controls in a move-along experiment or exposing seeds to an annual temperature cycle (*sensu* Baskin and Baskin, 2003). Without seeds kept at the continuous temperature regimes for the entire length of the experiment, the interpretation of data could have been incorrect in the present study.

It is concluded that *V. tinus* does not have deep simple epicotyl morphophysiological dormancy according to the strict definition (Nikolaeva, 1977; Baskin and Baskin, 1998). Instead, after a weak physiological dormancy is alleviated, continuous development of the seedling occurs, even though the phenological phenomenon in response to annual changing temperature conditions is easily mistaken for epicotyl dormancy.

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