Effects of Soaking, Washing, and Warm Pretreatment on the Germination of Russian-Olive and Autumn-Olive Seeds

Richard L. Jinks and Lorenzo Ciccarese

Forestry Commission Research Agency, Wrecclesham, Farnham, Surrey, United Kingdom, and Centro di Sperimentazione Agricola e Forestale, Rome, Italy

There is evidence that water-soluble inhibitors in seed coats are in part responsible for seed dormancy in some Elaeagnus *species. The effects of washing, soaking, and moist warm pretreatment on germination of Russian-olive* (E. angustifolia *L.) and autumn-olive* (E. umbellata *Thunb.) seeds are reported in this paper. Autumn-olive seeds were not particularly dormant and germinated without pretreatment. However, germination of the more dormant Russian-olive seeds was improved by washing the seeds in tap water for 6 days prior to prechilling. A similar proportion of seeds also germinated during warm pretreatment in peat and sand without prechilling.* Tree Planters' Notes 48(1 /2):18-23; 1997.

Russian-olive *(Elaeagnus angustifolia* L.) and autumn-olive *(E. umbellata* Thunb.) were introduced into Europe and North America from western and eastern Asia, respectively (Bean 1973). Both species are capable of fixing nitrogen and are tolerant of salt, a wide range of soil pH, and drought (Dirr 1983). As well as being of ornamental value, both species make good windbreaks and are very useful for planting on reclamation sites and on roadsides (Gambi 1972; Vogel 1987). They also have significant conservation value, providing cover for animals and nectar for bees. Their berries are an important source of food for wildlife in winter, and they are suitable for processing into jams and syrups (Bounous 1990). However, Russian-olive can be invasive and is considered a noxious weed by a number of states in the United States (Tesky 1992).

Low and unpredictable germination of *Elaeagnus* seeds can be a problem in nurseries, possibly limiting more widespread use of these and other members of the genus in Europe (Bounous and others 1992). Seeds of *Elaeagnus* species are considered dormant and require pretreatment before sowing, usually by prechilling seeds at 1 to 5 °C (34 to 41 °F) for 10 to 90 days (Olson 1974). The need for prechilling suggests that embryo dormancy is a block to germination. The minimum effective stratification period for autumn-olive is 16 weeks (Fowler and Fowler 1987), and 9 to 12 weeks for Russian-olive (Hogue and LaCroix 1970). Fruit and seed coats are also involved in dormancy regulation because removal of the endocarp and seed coat results in rapid germination of Russian-olive (Hogue and LaCroix 1970; Zaborovskij and Varasova 1961). The effectiveness of sulfuric acid as a scarification treatment suggests that these structures may physically restrict emergence of the embryo (Heit 1967). However, Hamilton and Carpenter (1975, 1976) have shown that coumarin-like inhibitors are present in the endocarp and testa, as well as in the embryo, of both Russian-olive and autumn-olive and these compounds may be responsible for inhibition of germination. Fung (1984) found that soaking silver-olive *(E. commutata* Bernh.) seeds in water at 50 °C (122 °F) improved germination, and he suggested that inhibition was caused by a water-soluble substance that is readily leached. However, Morgenson (1990) reported that soaking silver-olive seeds at room temperature was not as effective at overcoming dormancy as moist prechilling at 4 °C (39 °F) for 30 to 90 days.

The objective of this study was to determine to what extent soaking or washing seeds of Russian-olive and autumn-olive could replace cold pretreatment as a practical method for breaking dormancy. In addition the effects of a warm moist incubation at 20 °C (68 °F) applied before chilling on dormancy release was also investigated. Moist warm pretreatment of seed before prechilling can improve germination of some species such as members of the genus *Prunus* (Gordon and Rowe 1982), and there are apparently no reports on its effects on the germination of *Elaeagnus* seeds.

Materials and Methods

Samples of seeds of both species were collected in 1992 from sites near Reggio Emilia in the Po Valley in northern Italy (44.42° N 10.37° E). The samples were combined, cleaned, and then dried to 11.5% moisture content (fresh weight basis) and stored in polythene bags at 4 °C (39 °F). Cut tests showed that 96% of Russian-olive seed and 99% of autumn-olive seed were firm and considered alive. In addition a tetrazolium test carried out on autumn-olive seed indicated that 89% of the seeds were viable.

Seed samples of each species were given 1 of 7 pretreatments, comprising 5 types of treatment, 2 of which were applied for 2 durations. These were followed by a 4-week period of chilling at 4 °C (39 °F). Treatments were replicated 4 times. The 7 pretreatments were

Control:	Seeds received no treatment
Soaking:	Samples of seed were soaked in equal volumes of distilled water at 15 °C (59 °F) for 3 or 6 days
Washing:	Seeds were washed in running tap water at about 15 °C (59 °F) for 3 or 6 days
Hot soaking:	Seeds were immersed in hot distilled water at 50 °C (122 °F) and allowed to cool in the same water for 24 hours
Warm pretreatment:	Dry seeds were mixed with moist peat and sand medium (1:1, v/v) and incubated at 20 °C (68 °F) for 4 weeks

All seeds were mixed with moist peat and sand (1:1, v/v) for the subsequent prechilling. Samples of seeds were tested for germination both before and after prechilling.

For each germination test, 100 seeds from each replicate were sown on moist filter paper in individual plastic germination boxes (Gosling 1988); the boxes of seed were then incubated at constant 15 °C (59 °F). Germination was assessed 3 times a week for 8 weeks. A seed was considered to have germinated when its radicle was at least 3 times the length of the seed coat. During the warm pretreatment, seeds of both species began germinating after 10 days, and the numbers of germinated seeds were counted at regular intervals during the remainder of this pretreatment. At the end of the warm stratification period, only ungerminated seeds were either tested or transferred to the cold prechill treatment. At the end of the germination tests, the remaining seeds were cut and categorized as being either dead, abnormal, or fresh; the latter category refers to imbibed seeds that failed to germinate under test conditions but remained clean and firm and had the potential to develop into normal seedlings (ISTA 1996). Mean germination time (MGT) (Jones and Gosling 1994) was calculated for autumn-olive only, because germination in several treatments applied to Russian-olive seed was too low. Effects of treatments on germination and MGT were tested by 2-way analysis of variance using procedures in Genstat (Payne and others 1993). An angular transformation was applied to all percentage data before analysis; MGT values were transformed to log(MGT). Reported differences between treatment means are considered significant at $P<0.05$.

Results and Discussion

More than 65% of autumn-olive seeds and 38% of Russian-olive seeds germinated during the course of the warm stratification period (figures la and 2a). A further 15% of autumn-olive, and 1% of Russian-olive germinated in a subsequent laboratory test without chilling, and there was no additional germination of warm pretreated seed after the 4-week chilling treatment.

Maximum germination of the untreated autumn-olive seed was nearly 78%, and this together with the extensive germination occurring during the 4-week warm pretreatment, suggests that this seedlot was not particularly dormant. There was no significant difference in percentage germination among the soaking, washing or hot soaking treatments before seeds were chilled. However, the average germination of these treatment groups (69%) was about 9% lower than that for untreated seed (figure la). After chilling, germination did not differ significantly among treatments (figure la). Chilling increased germination of untreated seed by about 8%, and of the washed or soaked seed by 18% to give an overall average of 86%. Only about 6% of seeds were found to be dead at the end of the germination tests (figure 1c), slightly more of the prechilled seed (8%) died compared with non-chilled seed (4%). The majority of seeds that did not germinate remained fresh and could be considered dormant (figure lb).

Prechilling significantly reduced germination time from an average of 24 days to 17 days (figure 3). Washing for either 3 or 6 days, however, caused a slight but significant reduction of about 3 days in the MGT of non-chilled seed. Generally, washing and soaking did not improve germination of autumn-olive seed and actually reduced germination of unchilled seed slightly. Chilling was the only treatment that produced significant gains in both the amount and rate of germination for this species, although the response to chilling was much less than reported by Fowler and Fowler (1987).

Seeds of Russian-olive were much more dormant than in autumn-olive. Germination of unchilled untreated and soaked seed reached only about 5% (figure 2a). Washing in running water for 3 or 6 days, or soaking in hot water, significantly increased the germination of unchilled seed to about 15%, whereas warm pretreatment increased germination to nearly 40%. Chilling did not increase germination of untreated, soaked, and hot soaked seed, nor was there any further increase in germination of warm pretreated seed. However, chilling significantly increased germination of the washed seed

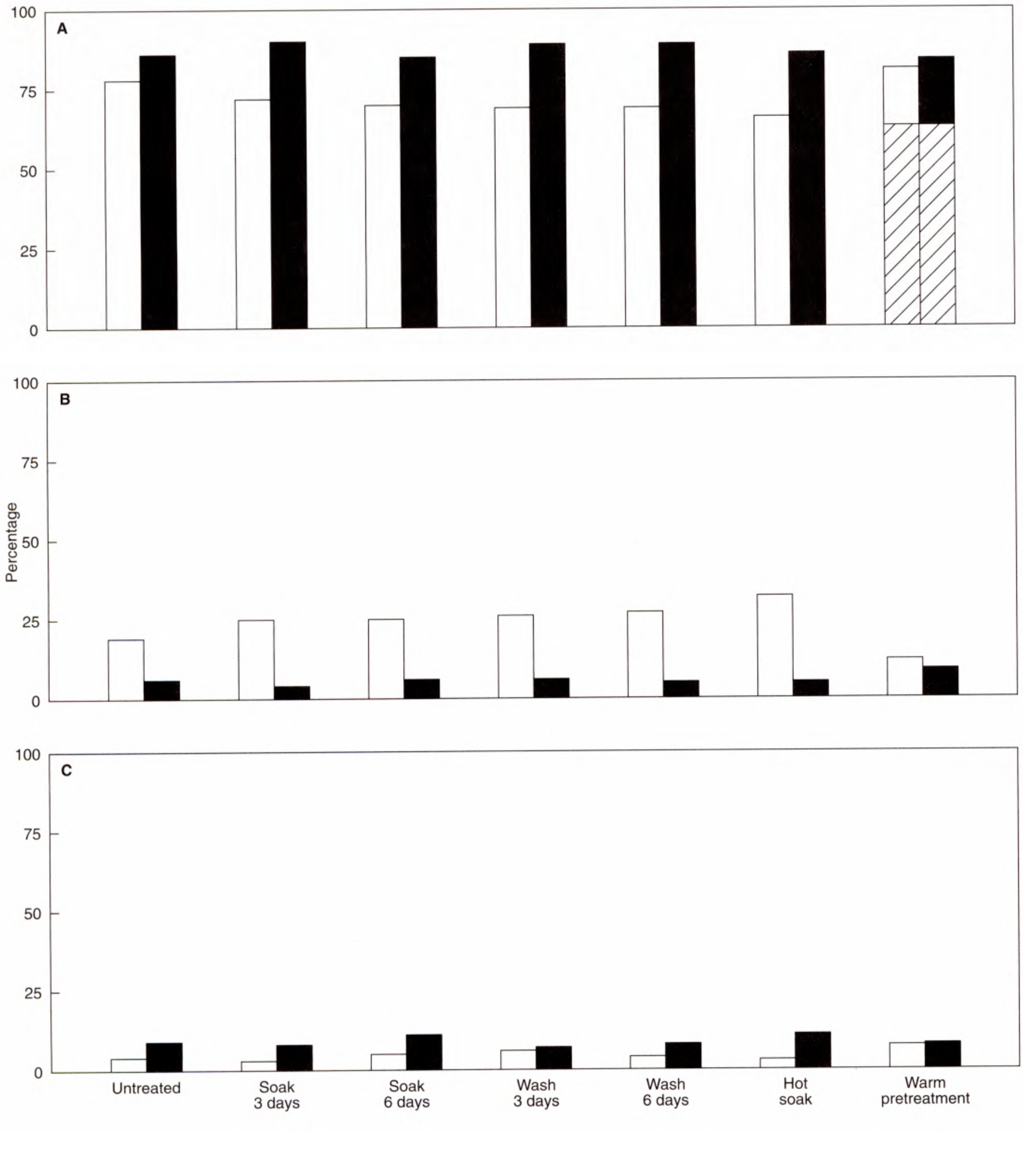

Figure *1—Effects of soaking, washing, and warm pretreatment on (A) percentage germination, (B) percentage of ungerminated but fresh seeds, and (C) percentage of dead or abnormal autumn-olive* (Elaeagnus umbellata) *seeds. Key: germination test results determined before chilling (open columns); after chilling (solid columns) at 4 °C (39 °F) for 4 weeks; the percentage of seeds that germinated during warm pretreatment (hatched columns).*

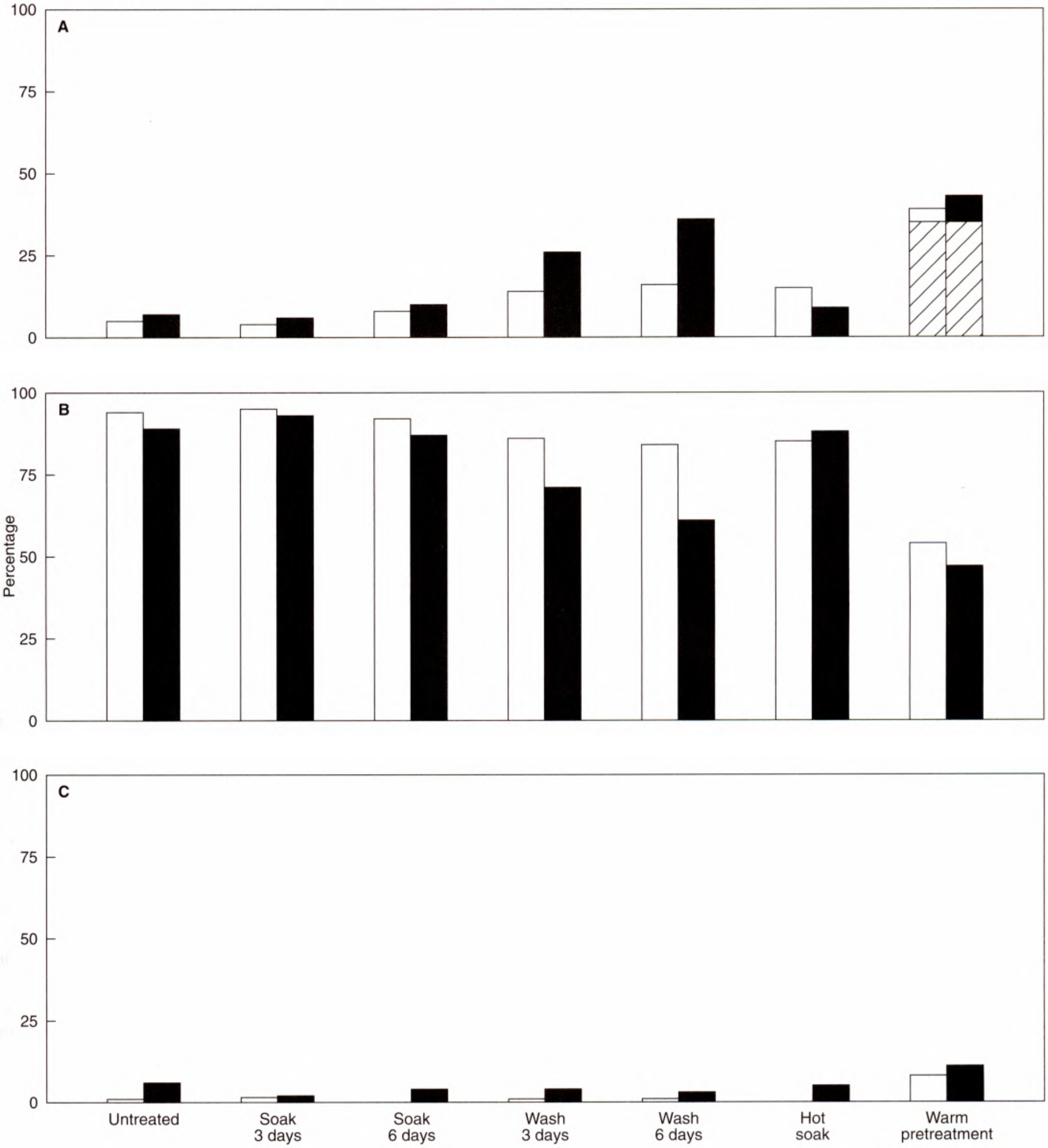

Figure 2— *Effects of soaking, washing, and warm pretreatment on (A) percentage germination, (B) percentage of ungerminated but fresh seeds, and (C) percentage of dead or abnormal Russian-olive* (Elaeagnus angustifolia) *seeds. Key: germination test results before (open columns) and after chilling (solid columns) at 4 °C (39 °F) for* 4 *weeks; the percentage of seeds that germinated during warm pretreatment (hatched columns).*

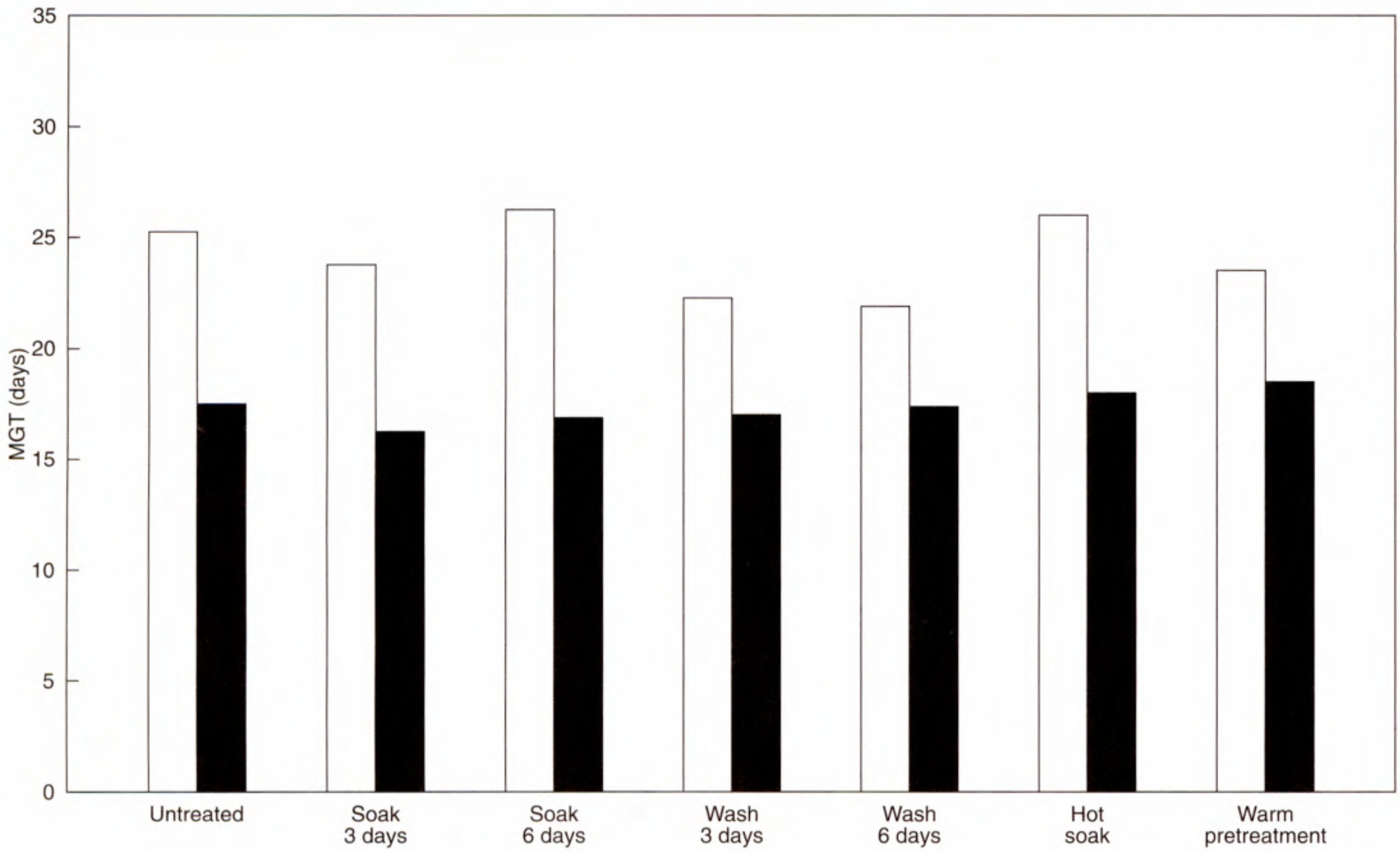

Figure 3—*Effects of soaking, washing, and warm pretreatment on the mean germination time (MGT) of autumn-olive* (Elaeagnus umbellata) *seeds. Key: MGT for seeds tested before (open columns) and after (solid columns) chilling at 4 °C (39 °F) for 4 weeks.*

from an average of 15% to 31%, and washing for 6 days produced significantly more germination (37%) than for 3 days (26%). Most of the ungerminated seeds in each treatment were still fresh and were considered to be dormant (figure 2b). The proportion of dead seeds following incubation was low (figure 2c); generally less than 1% of the unchilled seed died, but 8% died during warm pretreatment in peat and sand. Prechilling significantly increased seed death to an average of nearly 5% across all treatments (figure 2c). The results for Russian-olive show that washing seed in running water before cold pretreatment can improve germination but that moist warm pretreatment alone was just as effective. Extending the length of the chilling period to 12 weeks (Hamilton and Carpenter 1975) could increase germination further.

Overall, these 2 species of *Elaeagnus* had very different levels of seed dormancy. Autumn-olive seed was least dormant, for more than 70% of the seeds germinated without pretreatment. This particular seedlot was less dormant than previously reported for autumn-olive (Fowler and Fowler 1987). In contrast, the seedlot of Russian-olive was dormant and would probably require the recommended 9 to 12 weeks of chilling to obtain maximum germination (Hogue and LaCroix 1970). The present results suggest that this period could be significantly shortened by washing seed in running water for 6 days before incubation at chilling temperatures. A proportion of untreated seed was also capable of germinating at warm temperatures (20 °C = 68 °F) in a peat and sand mix, and this method could be used to obtain some seedlings in a shorter time than by conventional pretreatment. The medium was probably more effective at reducing the levels of inhibitory substances in seed coats than incubating seed on moist filter paper.

Conclusions

The results for autumn-olive suggest that seeds of this species may not always be particularly dormant. However, the Russian-olive seedlot was dormant, and improvements in germination by either washing seeds for 6 days and then prechilling, or by giving a warm stratification for at least 4 weeks, suggest that a water-soluble inhibitor may be an important feature of the dormancy mechanism of this species as shown by (Hamilton and Carpenter 1975).

Address correspondence to: Dr. Richard L. Jinks, Forestry Commission Research Division, Alice Holt Lodge, Wrecclesham, Farnham, Surrey GU10 4LH UK; e-mail: jinks@fcrd.gov.uk

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