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Seed Germination of *Arbutus unedo*, *A. andrachne* and Their Natural Hybrid *A. andrachnoides* in Relation to Temperature and Period of Storage

Konstantinos F. Bertsouklis and Maria Papafotiou[1]

Laboratory of Floriculture and Landscape Architecture, Department of Crop Sciences, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece

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***Abstract.* *Arbutus unedo* L., *A. andrachne* L., and their natural hybrid, *A.* ×*andrachnoides* Link, are the three *Arbutus* species of the Eastern Mediterranean Macchia. *A. unedo* is used as an ornamental plant and as cut foliage, whereas the other two species have the potential to be introduced to the floricultural industry. This study was carried out to clarify whether the seeds of these *Arbutus* species possess dormancy to determine the temperature range for their germination and the effects of storage period on germination. Seeds of the three species, which were stored dry at 25 °C for either three or 11 months, germinated at very high percentages (82% to 99%) and in a short period of time (24 to 46 days) when incubated at 15 or 10 °C without any pretreatment proving that they do not possess dormancy; germination was faster at 15 °C. At 20 °C only seeds of *A. unedo* and *A. andrachne* stored for three months germinated (29% to 34%), whereas at 25 °C, there was practically no germination. Seeds stored for three months germinated (61% to 75%) when incubated at 5 °C, but the germination was delayed and the seedlings did not grow further than the appearance of the radicle unless transferred at a higher temperature (10 to 25 °C). After 27 months of dry storage at 25 °C, seeds did not germinate when incubated at the range of 10 to 25 °C even after pretreatment with cold stratification.**

The evergreen woody shrubs *Arbutus unedo* (Strawberry tree), *A. andrachne* (Greek or Eastern strawberry tree) and their natural hybrid, *A.* ×*andrachnoides* (Ericaceae family), are the three *Arbutus* species found in the sclerophyllous Macchia zone of the Eastern Mediterranean (Torres et al., 2002). In certain countries they are listed as endangered plants (Garzuglia, 2006; Šatović, 2002). *A. unedo* is growing wild in all the Mediterranean and up to the coasts of south England (Villar, 1993) and is found as a nursery plant as north as the United Kingdom. It is used as a landscape plant and as a complement in the cut flower industry, mainly during Christmas time when it bears flowers and fruits (Metaxas et al., 2004). It could also be introduced as a pot plant bearing simultaneously white flowers and orange–red fruits. *A. andrachne* has potential use as a landscape plant because of its ornamental features, which include attractive plant architecture, winter fruiting, and smooth, cinnamon-red bark, peeling in long paper strips, revealing the inner gray–green bark (The European Garden Flora, 1997). *A.* ×*andrachnoides,* apart from its potential value as a landscape plant, is suitable for cut foliage production, because its growth speed is higher than *A. unedo,* the production of flowers and fruits is low, and has 90-d vase life (Cervelli et al., 2012). All three species could be used in Mediterranean parks and gardens contributing to high biodiversity and sustainability and emphasizing the traditional character of the area (Diekelmann and Schuster, 2002). They could also be used for reforestation in Mediterranean regions because of their potential for sprouting after fires, because they may have their aboveground burnt to ground level without major damage to the roots (Konstantinidis et al., 2006; Mesléard and Lepart, 1991).

Apart from ornamental value, *A. unedo* has edible fruits rich in vitamin C and there is increased demand for *A. unedo* and *A. andrachne* for medicinal purposes (Issa et al., 2008; Mostafa et al., 2010; Pavlovic et al., 2009).

Clonal propagation of these three species by cuttings is difficult (Al-Salem and Karam, 2001; Cervelli et al., 2012; Metaxas et al., 2004). There is a number of reports on in vitro propagation, most of them concerning *A. unedo* (El-Sayed El-Mahrouk et al., 2010; Gomes and Canhoto, 2009; Mereti et al., 2002) and a few *A. andrachne* and *A.* ×*andrachnoides* (Bertsouklis and Papafotiou, 2011, 2009; Mostafa et al., 2010).

In the horticultural and forestry practice, the plants are propagated mainly by seed. However, there is rather confusing information concerning the ecophysiology of *Arbutus* sp. seed germination. Mesléard and Lepart (1991) reported seed germination of *A. unedo* with alternating 15 and 20 °C without any pretreatment and concluded that seeds do not possess dormancy. In contrast, the recent years, Demirsoy et al. (2010), Ertekin and Kirdar (2010), Hammami et al. (2005), Kose (1998), and Tilki (2004) reported physiological dormancy in seeds stored for zero to five months, which was successfully overcome and germination occurred only after pretreatments with cold stratification or gibberellic acid (GA_3). Similarly, Karam and Al-Salem (2001), Kose (1998), Mostafa et al. (2010), Olmez et al. (2007), and Tilki and Guner (2007) reported dormancy in *A. andrachne* seeds stored for zero to five months, which was broken by cold stratification or GA_3 pretreatments; hot water scarification was also tested rather unsuccessfully (Karam and Al-Salem, 2001; Mostafa et al., 2010; Tilki and Guner, 2007). Concerning seed germination of other *Arbutus* species, cold stratification was also suggested for the pacific madrone *A. menziesii* (Harrington and Kraft, 2004). All these researchers, which reported seed dormancy of *Arbutus* species, placed seeds to germinate at high temperatures (20 to 30 °C), whereas cardinal temperatures used for germination of *Arbutus* are not determined. To the possession of dormancy in *A. andrachne* seed contrasts, preliminary work of ours showed germination at 15 °C without any pretreatment of seeds stored for either three or 11 months (Bertsouklis and Papafotiou, 2010). There are no reports in the literature for *A.* ×*andrachnoides* seed germination.

The objective of this study was to clarify whether seeds of *A. unedo*, *A. andrachne,* and *A.* ×*andrachnoides* possess dormancy and to determine cardinal temperatures for germination. In this way wrong practices in the nurseries that lead to low seed germination rates will be avoided (Olmez et al., 2007). The effect of storage period on seed germination of these three species was also investigated; there was no reference on this in the literature, except a preliminary work of ours on *A. andrachne* (Bertsouklis and Papafotiou, 2010).

Materials and Methods

Ripe fruits were collected late Dec. 2008 from one wild adult tree of each species, *A. unedo*, *A. andrachne*, and *A.* ×*andrachnoides,* located on the southeast side of Mount Parnitha (lat. 38°8′19″ N, long. 23°47′44″ E, 300 to 400 m altitude, Varympompi, Attiki). Fruits were collected when they became soft and had orange–red color (eight months after anthesis), at the stage of full maturity, but before they turned completely red.

Immediately after collection, the fruits were immersed in water and the seeds manually separated from the fruit flesh. Any floating seeds (≈10%) were discarded. Seeds were left to dry on tissue paper for 24 h at room temperature and then were placed in unsealed plastic petri dishes between filter paper. The

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[1]To whom reprint requests should be addressed; e-mail mpapaf@aua.gr.

petri dishes were placed in Styrofoam boxes (for darkness) and stored in a constant condition chamber at 25 °C and 30% relative humidity.

Germination of *Arbutus* seed was conducted after three, 11, and 27 months of storage. Storage periods were selected based on nursery practice, where seeds (collected in winter) are sown in spring or fall. Thus, seeds could be sown in the next spring (three months after collection), the next fall (11 months after collection), or in the spring two years after their collection (27 months after collection). Before initiating germination treatments, seeds were surface-sterilized with 20% (v/v) commercial bleach (4.6% w/v sodium hypochlorite) for 10 min, rinsed four times (3 min each) with sterile distilled water, and sown in 9-cm plastic petri dishes with hormone-free, half-strength Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) containing 2% (w/v) sucrose and solidified with 0.8% (w/v) agar. The pH of the medium was adjusted to 5.6 to 5.7 before autoclaving at 121 °C min for 20 min.

The following treatments were applied: 1) seeds directly after storage were surface-sterilized, sown on MS medium, and placed for germination in a constant condition growth chamber under a 16-h photoperiod of 37.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density of cool fluorescent light at 10, 15, 20, or 25 °C; 2) seeds directly after storage were surface-sterilized, sown on MS medium, and placed at 5 °C in the dark, in a refrigerator (cold stratification), for 40 d, and then transferred in the growth chamber (see previously) at 10, 15, 20, or 25 °C; and 3) seeds were treated like in (2) with the difference that cold stratification was applied for 60 d.

Germination was defined as the appearance of a radicle at least 2 mm long according to the rules of the International Seed Testing Association (1999). Germination was recorded every 2 d. One hundred twenty-five seeds were used per treatment (25 seeds per petri dish, five petri dishes per treatment). The significance of the results was tested by analysis of variance (JMP 6.0; SAS Institute, Cary, NC).

Results and Discussion

The present work investigated for the first time the temperature range for seed germination of *Arbutus* species and cardinal temperatures were defined for *A. unedo*, *A. andrachne,* and their natural hybrid, *A.* ×*andrachnoides*. Seeds of the current year (three months stored) of all three species germinated at high percentages (84% to 99%) and in a short period of time (24 to 30 d) when incubated at 15 and 10 °C. *A. unedo* and *A. andrachne* seeds germinated at low percentages at 20 °C and practically did not germinate at 25 °C (1% germination), whereas *A.* ×*andrachnoides* seeds did not germinate either at 20 or at 25 °C (Figs. 1A, 2A, and 3A). At 5 °C the seeds of all species germinated at high percentages (90% to 100%), but the germination was delayed (≈40 d in *A. andrachne* and 60 d in *A. unedo* and *A.* ×*andrachnoides*) and the seedlings did not grow further than the appearance of the radicle unless transferred at a higher temperature (Figs. 1B–C, 2B–C, and 3B–C). The optimum seed germination temperature for all three species was 15 °C at which the maximum germination percentage and germination rate were achieved without any pretreatment indicating that there was no dormancy either in current-year seeds (three months stored) or seeds stored for 11 months (Figs. 1A, 2A, and 3A). At 20 °C, temperature that is given as optimum for seed germination of sclerophyllous evergreen species in Baskin and Baskin (2001), germination percentage was low for *A. unedo* and *A. andrachne* and zero for *A.* ×*andrachnoides* (Figs. 1A, 2A, and 3A), which coincides with results of Hammami et al. (2005), Kose (1998), Tilki (2004), and Tilki and Guner (2007) for *A. unedo* and *A. andrachne* that led them to conclude that these species possess seed dormancy without testing lower temperatures for germination. To the contrary, higher than 20 °C temperatures (24, 26, even 30 °C) were tested by many other researchers (Demirsoy et al., 2010; Ertekin and Kirdar, 2010; Karam and Al-Salem, 2001; Olmez et al., 2007) that also led to failure of germination and led researchers to conclude seed dormancy. All these researchers suggested cold stratification, usually at 5 °C for 40 to 90 d, and/orGA_3 pretreatments for breaking the supposed dormancy. Like in these works, seeds of the current year were used (stored for zero to five months); cold stratification could have possibly acted as an appropriate temperature for germination, like the present work revealed (Figs. 1B–C, 2B–C, and 3B–C). This could explain why in Ertekin's and Kirdar (2010) work "cold stratification at 9 °C was more efficient than at 4 °C for *A. unedo* seed germination." Concerning the use of GA_3 for breaking supposed dormancy (Demirsoy et al., 2010; Karam and Al-Salem, 2001; Kose, 1998; Olmez et al., 2007; Tilki, 2004; Tilki and Guner, 2007), it may have promoted germination at supraoptimal high temperatures, as has been shown in lettuce (Dong et al., 2012). Our work, being in agreement with Mesléard's and Lepart (1991) who reported *A. unedo* seed germination with 12 h at 15 °C and 12 h at 20 °C, clearly indicated that *A. unedo*, *A. andrachne,* and *A.* ×*andrachnoides* do not possess any seed dormancy and germination percentages are very high if the appropriate range of temperatures (10 to 15 °C) are applied. Similar low temperatures (10 to 15 °C) were found optimum for seed germination of other drought-tolerant shrubs of the Mediterranean, too, such as *Lithodora zahnii* (Papafotiou and Kalantzis, 2009), *Dianthus fruticosus* (Papafotiou and Stragas, 2009), *Thymelaea hirsuta* (Shaltout and El-Shourbagy, 1989), and *Cistus incanus* spp. *creticus* (Thanos and Georgiou, 1988). Avoiding germination at high temperatures shows the adaptation of these species to the Mediterranean Macchia climate, where high temperatures coincide

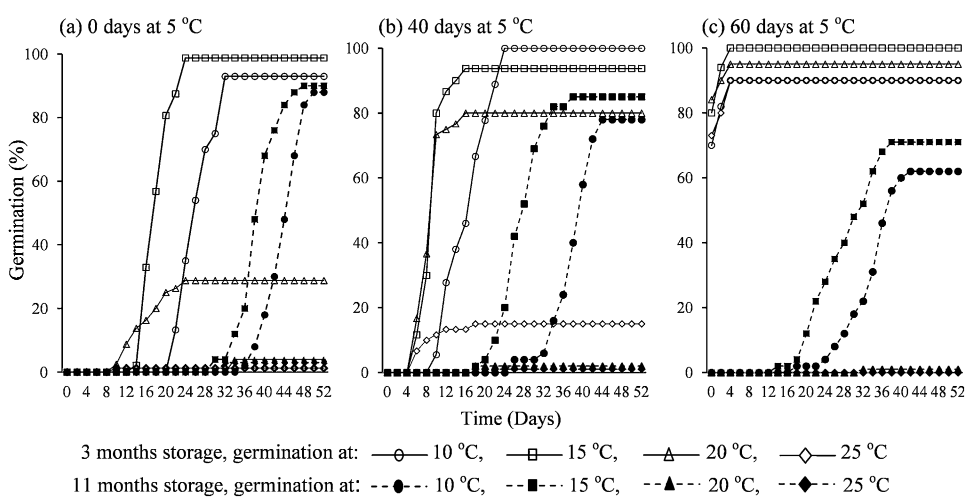

Fig. 1. (**A–C**) Germination time course curves of *A. andrachne* seeds as affected by storage period, cold stratification, and temperature at germination. Five replicates of 25 seeds were used for each treatment. SDs ranged from 0.00 to 8.00 and have been omitted for clarity.

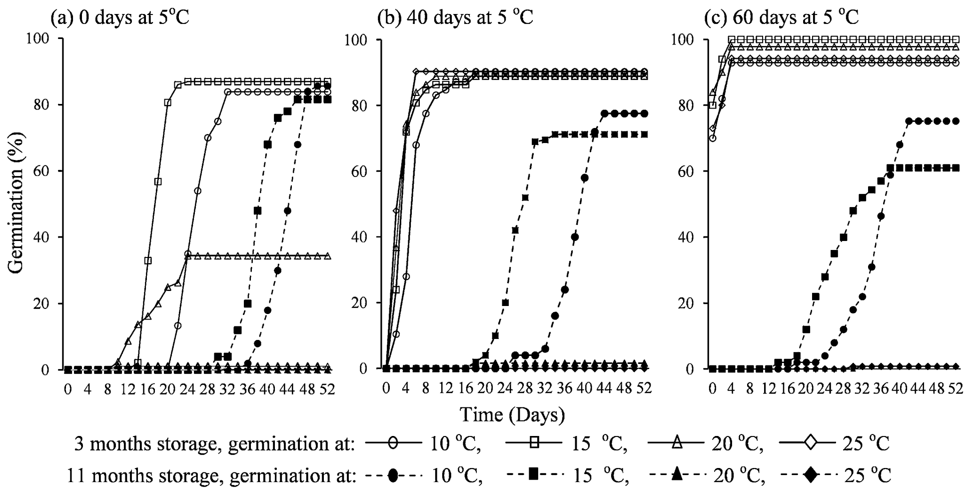

Fig. 2. (**A–C**) Germination time course curves of *Arbutus* ×*andrachnoides* seeds as affected by storage period, cold stratification, and temperature at germination. Five replicates of 25 seeds were used for each treatment. SDs ranged from 0.00 to 3.90 and have been omitted for clarity.

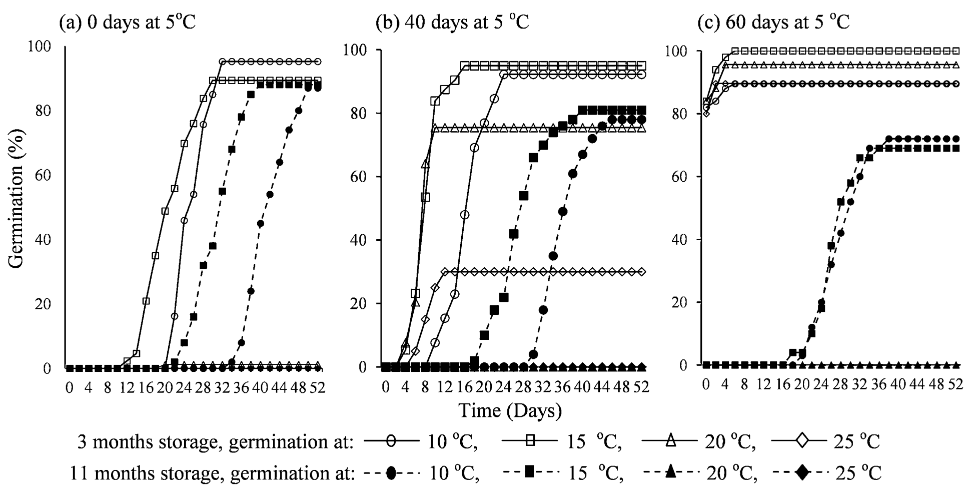

Fig. 3. (**A–C**) Germination time course curves of *Arbutus unedo* seeds as affected by storage period, cold stratification, and temperature at germination. Five replicates of 25 seeds were used for each treatment. SDs ranged from 0.00 to 5.95 and have been omitted for clarity.

or are shortly followed by the drought period that could be lethal for young seedlings. Germination response to temperature of *Watsonia* species (South African plants) was found to exhibit clear trends that are dependent on the season in which rainfall occurs at the geographic region from which the species originated; species from the winter rainfall region germinated optimally at 10 to 20 °C and those from the summer rainfall region at 15 to 25 °C (Ascough et al., 2007).

So, germination of *A. andrachne, A.* ×*andrachnoides,* and *A. unedo* seeds is a matter of the right temperature and unfavorable environmental conditions for germination should not be perceived as seed dormancy (Thompson and Ooi, 2010).

The upper threshold germination temperature for *A. unedo* and *A. andrachne* was indicated between 20 and 25 °C, whereas their hybrid, *A.* ×*andrachnoides,* was between 15 and 20 °C (Figs. 1A, 2A, and 3A). The narrower temperature range for seed germination of *A.* ×*andrachnoides* may explain its rarity in nature in comparison with its parental species. The lower threshold germination temperature for the three *Arbutus* species was not defined exactly, but we could put it at 5 °C, because at 10 °C, germination percentages were maximum and only the speed of germination was reduced compared with 15 °C, whereas at 5 °C, germination (as defined by the International Seed Testing Association, 1999) reached very high percentages (90% to 100%) in all three species (Figs. 1C, 2C, and 3C), but the seedlings did not grow further than the appearance of the radicle, even after 90 d (data not shown). Olmez et al. (2007) also observed germination in *A. andrachne* during the stratification treatment at 5 °C and they attributed this to the extremely low germination percentages that they got then under greenhouse or open field conditions in March. In our work, these seedlings grew in a very short period of time (1 to 2 d) when transferred at 10, 15, 20, or 25 °C (Figs. 1C, 2C, and 3C). In previous works on *A. unedo* (Demirsoy et al., 2010; Ertekin and Kirdar, 2010; Hammami et al., 2005; Kose, 1998; Tilki, 2004) and *A. andrachne* (Karam and Al-Salem, 2001; Kose, 1998; Olmez et al., 2007; Tilki and Guner 2007), this response of seeds that were put first at 5 °C and then at 20 to 25 °C was interpreted wrongly as dormancy breaking by cold stratification. At 5 °C, the maximum germination percentage was reached after ≈40 d in *A. andrachne* and 60 d in *A. unedo* and *A.* ×*andrachnoides* (Figs. 1B–C, 2B–C, 3B–C). Seeds transferred from 5 to 25 °C before the appearance of the radicle (mainly *A. unedo* and *A.* ×*andrachnoides* seeds incubated for 40 d at 5 °C) did not germinate (Figs. 1B, 2B, and 3B). At 5 °C, the seeds were kept in the dark, whereas in all the other temperatures, they were put in 16 h light/8 h dark, but these should not have affected their ability for germination, because both *A. unedo* and *A. andrachne* have been shown to germinate equally well in the light or dark (Bertsouklis et al., 2004; Karam and Al-Salem, 2001; Mesléard and Lepart, 1991; Tilki and Guner, 2007).

In nature these three *Arbutus* species disperse their seeds during the winter and because there is no dormancy, almost all of the seeds germinate in the spring. So, as Mesléard and Lepart (1991) showed for *A. unedo*, there are no seeds left in the soil to form a seed bank and after a summer fire, the survival of these species is based on their resprouting ability from the roots. Seed scarification by high temperature did not affect or even prevented germination of both *A. unedo* (Mesléard and Lepart, 1991) and *A. andrachne* (Karam and Al-Salem, 2001; Mostafa et al., 2010; Olmez et al., 2007).

The increase of storage period from three to 11 months decreased the range of germination temperatures in all three *Arbutus* species, restricting it between 10 and 15 °C, without affecting the total germination percentages, but reducing the speed of germination; seeds of the current year (three months' storage) germinated faster than seeds of the previous year (11 months' storage), as T_{50} (time to reach 50% germination) and germination curves show (Figs. 1A, 2A, and 3A). The delay of previous year seeds to reach total germination was higher in *A. unedo* (24 d) compared with *A. ×andrachnoides* (10 d) and *A. andrachne* (6 d). Seeds of all three species stored for 11 months and incubated at 5 °C (cold stratification) over 40 or 60 d did not germinate in contrast to current-year seeds that developed a radicle at this temperature. When the non-germinated seeds were transferred from 5 °C to 10 or 15 °C, germination was less than for seeds placed directly after the 11-month storage to germinate at 10 or 15 °C (Figs. 1A–C, 2A–C, and 3A–C). Two possible explanations could be given for this, either the aging of seeds for an extra 40 or 60 d (depending on the period of cold stratification) decreased their viability or cold stratification affected negatively germination; the latter, however, seems less possible because cold stratification did not have a negative effect on seeds that had been stored for three months.

Seeds stored for 27 months did not germinate in any of the treatments, even in the case where seeds received favorable germination temperatures after 60 d of cold stratification to counteract potential seed dormancy (data not shown). It seems rather impossible seeds that did not develop dormancy 11 months after their maturity to do so later on. So most probably seeds stored for 27 months lost their viability during storage. In previous reports on germination of *A. andrachne* and *A. unedo,* either storage period is not mentioned (Demirsoy et al., 2010; Hammami et al., 2005; Mesléard and Lepart, 1991), or seeds were put for germination immediately after harvest, or the next spring that is three to five months after harvest (Ertekin and Kirdar, 2010; Kose, 1998; Olmez et al., 2007; Tilki, 2004). The only reference found in the literature about storage capability of strawberry trees or madrones seeds is for *A. menziesii* (a native plant of California), the dried seeds of which could be stored for up to two years at room temperature (Immel, 2006). Seeds of *A. andrachne, A. ×andrachnoides,* and *A. unedo* seem to have decreased longevity at room temperature, because seeds stored for 27 months did not germinate in any treatment. Seed storage temperature has not been investigated for these *Arbutus* species and in some previous works on germination is not even reported (Kose, 1998; Tilki, 2004). Ertekin and Kirdar (2010) and Olmez et al. (2007) stored seeds of *A. andrachne* and *A. unedo*, respectively, at 4 to –6 °C until the next spring. The former had extremely low germination (maximum 16%), whereas the latter had 72% germination after 60 d cold stratification and 18 to 24 °C temperature at germination vs. 90% to 95% in a similar treatment at the present study (Figs. 1A and 3A). In preliminary experiments with *A. andrachne,* we used seeds supplied from the Forestry of Parnitha that were stored dry (inside cotton bags) in a refrigerator, and those seeds failed to germinate. So, for a short period of storage, dry-warm storage seems most appropriate for *Arbutus* seeds because dry-cold storage has rather negative effects on germination and wet-cold storage induces germination.

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