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Effect of gibberellic acid and ethephon on the germination of European beech dormant and chilled beechnuts

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ABSTRACT: The effect of ethephon (80, 100 and 120 mg·l^{-1}) and gibberellic acid (GA_3) (40, 300 and 1,000 mg·l^{-1}) on the germination capacity (GC) and mean germination time (MGT) of European beech (*Fagus sylvatica* [L.]) dormant beechnuts or beechnuts pre-chilled for four weeks was determined. Compared to the control (dormant untreated seeds) or beechnuts treated with tap water no significant increase in mean GC was detected after the application of ethephon or GA_3 to dormant seeds. Conversely, both ethephon and GA_3 treatments reduced (ethephon significantly) GC when applied to beechnuts chilled for four weeks prior to treatment. The effect of the treatments on germination speed (MGT) and dormancy release significantly improved when beechnuts were chilled for four weeks prior to the application of ethephon or GA_3. However, the effect of GA_3 on MGT of chilled beechnuts was not so distinct compared to dormant untreated seeds. Reduction in MGT was most obvious in seeds hydrated with 1,000 mg·l^{-1} GA_3 prior to germination. Their cold requirement time was reduced by three weeks compared to beechnuts hydrated in tap water.

Keywords: ethephon; *Fagus sylvatica;* germination; gibberellic acid

European beech (*Fagus sylvatica* [L.]) is one of the most widely-grown, deciduous forest trees in Czech forests. It takes up 7.0% (182 thousand ha) of the total Czech forest land and this area is slowly increasing. Although natural beech regeneration is successful, artificial reforestation is still needed. In 2008 beech was planted on 3 865 ha, which represents 19% of the reforested area in that year (Report on the state of forests and forestry in the Czech Republic in 2008). As such, it is necessary to ensure that sufficient beechnuts are available for nursery sowing and increased seedling production.

Beechnuts, seeds of European beech, are deeply dormant orthodox seeds. Beechnut lots vary dramatically in dormancy which is released by moist chilling of beechnuts to 28–30% moisture content (mc) for 4–12 weeks, sometimes 20 weeks (Gosling 1991; Suszka et al. 1994; Procházková et al. 2002). This long chilling can drastically decrease germination or even result in a complete loss of germination in less vigorous seedlots. Faster dormancy release should result in reducing the chilling period, improve germination and increase seedling stands in nurseries.

The effect of low temperature on dormancy release of seeds of various tree species can be replaced by applying chemicals such as gibberellic acid or ethylene. These chemicals stimulate the germination of non-dormant seeds (Bewley, Black 1982; Procházka, Šebánek 1997; Baskin, Baskin 2001). Ethylene (ethene) is a colourless gas produced by plants and microorganisms, including fungi (Gloser 1998). It can be applied to plants in a solid, water-soluble form – ethephon (acid 2-chlorethylphosphate). In plant tissues, ethephon degrades to ethylene, chlorides and phosphates (Procházka, Šebánek 1997).

The physiological effects of ethylene are highly variable. The most common effect is slowing down the elongation of stems and roots with simultaneous thickening while other effects include loss of geotropism, production of adventitious roots, shedding of leaves, flowers and fruits, and stimulation of fruit maturation and seed germination (Gloser 1998).

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The molecular mechanism of ethylene impact on dormancy release is not well known (Cervantes et al. 1994; Corbineau, Côme 1995 ex Borghetti et al. 2002). Eastwell and Spencer (1982 ex Procházka, Šebánek 1997) assumed that ethylene increases the production of xylanase forming channels through the walls of the aleuron cells of seed, supporting the release of α-amylase, an enzyme that stimulates germination by degrading starch. Though ethylene overcomes dormancy and increases seed germination of many species (Bewley, Black 1982; Baskin, Baskin 2001), other seeds are unaffected or their germination is even inhibited (Baskin, Baskin 2001). The effective ethylene concentration that stimulates germination is 0.1–200 $\mu l \cdot l^{-1}$ (Bewley, Black 1982), i.e. 0.1–200 $cm^3 \cdot m^{-3}$.

Gibberellic acid (GA_3) is one of the most frequently used gibberellins (Hudson 2005). Gibberellins accumulate in developing embryos and by the time of seed maturation they exist in a fixed form. After seed imbibition, gibberellins are released and the embryo starts synthesizing gibberellins *de novo*. In barley grain, where this process has been studied in detail, free gibberellins were found to be transported to the aleuron layer of the seed, where they induced the production of α-amylase and subsequently hydrolytic enzymes (Jones, Jacobson 1991 ex Procházka, Šebánek 1997).

Hydrolytic enzymes then move to the endosperm, where they degrade reserve sugars and proteins, and provide enough energy and building materials for the growing embryos. The induction of α-amylase is very effectively inhibited by abscise acid (ABA). Inhibitors such as ABA gradually degrade and the gibberellin level increases during cold stratification. By exogenous application of gibberellins it is possible to intensify the effect of cold stratification, thus the stratification of seeds is partly replaced with gibberellins (Procházka, Šebánek 1997). This procedure works especially well in species with weak or medium-deep seed physiological dormancy, but less so in seeds with deep physiological dormancy (Nikolaeva et al. 1973; Nikolaeva 1977 ex Baskin, Baskin 2001). Better effects can be obtained in such seeds by gentle scarification or puncturing or scratching the seed coats (Bewley, Black 1982). However, the application of gibberellins can be lethal for seeds of some species or can induce significant elongation and etiolation of the seedlings or cause seedling mortality. The lethal concentration of GA_3 for seeds of some species is 1,000 ppm, while 500 ppm has no effect and the 750 ppm concentration has a positive influence (Hudson 2005). According to Bewley and Black (1982) the effective concentrations of gibberellins for releasing seed dormancy are 10^{-5} to 10^{-3}M.

The purpose of the present paper was to determine the effect of gibberellic acid and ethephon on overcoming beechnut dormancy and stimulation of beechnut germination.

MATERIALS AND METHODS

Seeds

Three beechnut seedlots with a moisture content of 8.6–9.0% and stored in sealed plastic bags at –7°C for two to four years at the Tree Seed Centre in Tyniste n. Orlici were used in our experiments. The beechnuts originated from two natural forest regions and two altitudinal zones (Table 1). In the laboratory the beechnuts were kept in sealed

Table 1. Beechnut (*Fagus sylvatica*) seedlots and their initial quality before applying ethephon (May 2005) or GA_3 (October 2005)

Seedlot No.	Year of ripening	Natural forest region, Czech Republic	Altitude zone (m a.s.l.)	Viability (%)*		1,000 seed weight (g)**	Moisture content (%)***
				May 2005	October 2005		
229	2001	Stredomoravske Karpaty (36)	400–550	58	64	241.4	9.0
241	2003	Luzicka piskovcova vrchovina (19)	600–700	66	66	277.4	8.6
243	2003	Luzicka piskovcova vrchovina (19)	400–550	77	71	267.8	9.0

*Based on four replicates of 100 seeds each; **Based on two replicates of 10 g of seeds each; ***Based on eight replicates of 100 seeds each

plastic bags at –5°C until used. The initial viability of the beechnuts varied from 58 to 77% and the 1,000 seed weight from 241.4 to 277.4 g (Table 1).

Treatments (control, application of ethephon, GA_3 or tap water)

Ethephon (2-chlorethylphosphonic acid, $C_2H_6ClO_3P$) or gibberellic acid (GA_3, $C_{19}H_{22}O_6$) were applied either to dormant beechnuts (8–9% mc, hereafter "9%") or beechnuts (28–30% mc, hereafter "30%") chilled for 4 weeks (Table 2). Each treatment consisted of eight replications of 50 seeds per seedlot.

In treatment 1 (control) seeds with 9% mc were incorporated (without previous soaking) into a moist peat-sand substrate (28–30% mc) and incubated in a closed plastic boxes at 4±1°C (hereafter "4°C") in the dark (see germination determination).

In treatments 2 to 5 the moisture content of dormant beechnuts (9%) was slowly increased to a target 30% mc by sprinkling the beechnuts with tap water, ethephon (80, 100 and 120 mg·l^{-1}) or GA_3 (40, 300 and 1,000 mg·l^{-1}) for five to seven days. Tap water, ethephon or GA_3 were used to reach the target mc of the beechnuts. Then the beechnuts were mixed with the moist peat-sand substrate and incubated as described above (control).

In treatments 6 to 9 the mc of beechnuts was increased by sprinkling them with tap water to obtain the target mc (30%). Then the beechnuts were chilled (without medium) at 4°C in the dark and after four weeks chilling they were imbibed either with tap water, ethephon (80, 100 and 120 mg·l^{-1}) or GA_3 (40, 300 and 1,000 mg·l^{-1}) for 20 hours. The imbibition resulted in 31–35% mc of beechnuts. Then the beechnuts were mixed with the moist peat-sand substrate and incubated as described above.

Moisture content, 1,000 seed weight, viability, germination capacity and mean germination time

The moisture content (fresh weight basis) was determined on two replications of cut beechnuts (10 g each) dried at 103 ± 2°C for 1 hour in a Brabender apparatus (Brabender OHG, Duisburg, Germany) (CSN 48 1211 1997). The thousand seed weight (8 × 100 seeds) and viability (tetrazolium test) (4 × 100) were determined according the ISTA Rules (2005).

Germination tests were done using a peat-sand substrate (1:1 by volume) (CSN 48 1211 2006) with 400 seeds of each seedlot being mixed with a peat-sand substrate (one volume of seed to two volumes of substrate, 28–30% mc) for germination in 17 × 12 cm boxes at 4°C in the dark. The boxes were fitted with translucent lids and were opened weekly to check the germinants. Beechnuts with 5–10 mm long radicles were considered as germinated and discarded after counting. Germination counts were done weekly from the first week after sowing until when no germinants were observed in two consec-

Table 2. Treatments applied to the beechnuts. In treatments 1–5 dormant beechnuts were hydrated by sprinkling them with tap water, ethephon or GA_3 for 5 to 7 days before mixing them into the germination substrate. In treatments 6–9 dormant beechnuts were hydrated by sprinkling them with tap water to increase their moisture content to 30%, where upon they were chilled (without substrate) at 4°C for 4 weeks, then soaked for 20 h in tap water, ethephon or GA_3 before mixing them into the germination substrate

Treatments	Material	Tap water	Ethephon (mg·l^{-1})	GA_3 (mg·l^{-1})
1 (control)	dormant seeds (9.0% mc)	no	–	–
2			80	40
3			100	300
4			120	1,000
5		yes	–	–
6	seeds chilled for 4 weeks (30% mc)		80	40
7			100	300
8			120	1,000
9		yes	–	–

Table 3. Analysis of variance results showing the effects of pre-stratification treatments with ethephon and GA_3 on beechnuts (*Fagus sylvatica*) on their germination capacity (GC) and mean germination time (MGT)

Effect	df	GC				MGT			
		SS	MS	*F*	*P*	SS	MS	*F*	*P*
Treatment (ethephon)	8	8,626.1	1,078.3	14.9	0.000	270.7	33.8	90.8	0.000
Seedlots	2	23,464.0	11,732.0	161.5	0.000	315.8	157.9	423.6	0.000
Treatment × Seedlot	16	2,702.5	168.9	2.3	0.003	24.5	1.5	4.1	0.000
Treatment (GA_3)	8	4,294.7	536.8	7.0	0.000	696.8	87.1	384.9	0.000
Seedlots	2	21,758.9	10,879.5	141.5	0.000	219.6	109.8	485.3	0.000
Treatment*Seedlot	16	2,401.1	150.1	2.0	0.018	33.3	2.1	9.2	0.000

df – degrees of freedom, SS – sum of squares, MS – mean squares, *F* – *F*-distribution, *P* – probability

utive weeks. Then, all the remaining (non-germinated) seeds were cut and the dead (rotten), empty and 'fresh' seeds were counted. The fresh seeds (if any) were included in germinated seeds. The germination tests terminated after ca five months when germination ceased. Germination capacity (GC) and MGT were calculated as the mean of eight replications plus or minus the standard error.

Mean germination time (MGT) was used to determine the speed of germination. It was calculated according to the modified formula: $MGT = \Sigma(n_i \times t_i) \times n^{-1}_{total}$ where n_i is the number of seeds germinated in a specific week (t) and n_{total} is the total number of germinated seeds (Younsheng, Szikaie 1985 ex Falleri et al. 1997).

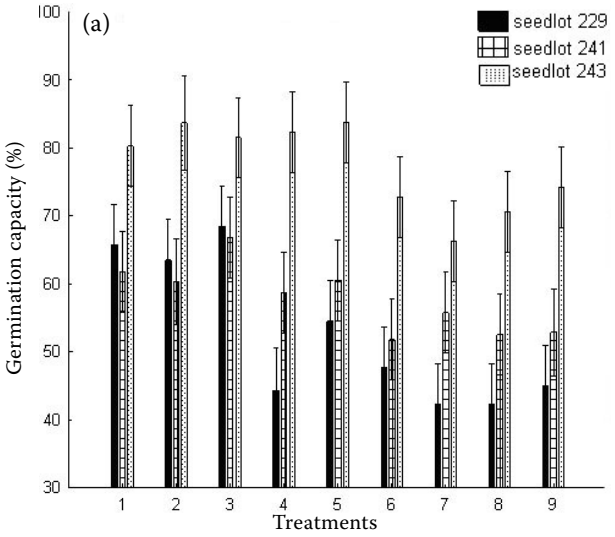
Data analyses

Seedlot and treatment effects, and their interactions, were determined by two-way ANOVA and the significance of mean differences was determined using the Scheffe's test (StatSoft Inc. 2005).

Table 4. Germination capacity (GC) and mean germination time (MGT) of dormant or chilled beechnuts (*Fagus sylvatica*) treated with tap water, ethephon or GA_3

	Treatments	Ethephon		GA_3	
		GC (%)	MGT (weeks)	GC (%)	MGT (weeks)
Dormant seeds (9.0% mc)	control (no soaking)	69.3^{b}	13.0^{d}	61.7^{ab}	14.3^{g}
	80 mg·l^{-1} ethephon or 40 mg·l^{-1} GA_3	68.2^{b}	12.1^{ac}	65.3^{b}	9.5^{de}
	100 mg·l^{-1} ethephon or 300 mg·l^{-1} GA_3	72.3^{b}	12.0^{ac}	64.3^{b}	8.7^{abc}
	120 mg·l^{-1} ethephon or 1,000 mg·l^{-1} GA_3	62.5^{ab}	12.5^{ad}	63.5^{ab}	8.2^{a}
	tap water	66.3^{bc}	12.6^{ad}	57.6^{ab}	11.4^{f}
Seeds chilled for 4 weeks (30% mc)	80 mg·l^{-1}ethephon or 40 mg·l^{-1} GA_3	57.4^{ac}	10.0^{b}	55.4^{ab}	9.2^{cd}
	100 mg·l^{-1} ethephon or 300 mg·l^{-1} GA_3	54.8^{a}	10.1^{b}	55.3^{ab}	9.0^{bc}
	120 mg·l^{-1} ethephon or 1,000 mg·l^{-1} GA_3	55.1^{a}	10.0^{b}	53.9^{a}	8.7^{ab}
	tap water	57.6^{ac}	11.7^{c}	53.6^{a}	9.8^{e}

Data are the means of eight replicates of 50 seeds each. Values in the same column followed by the same letter are not significantly different (Scheffe test, $\alpha = 0.05$).



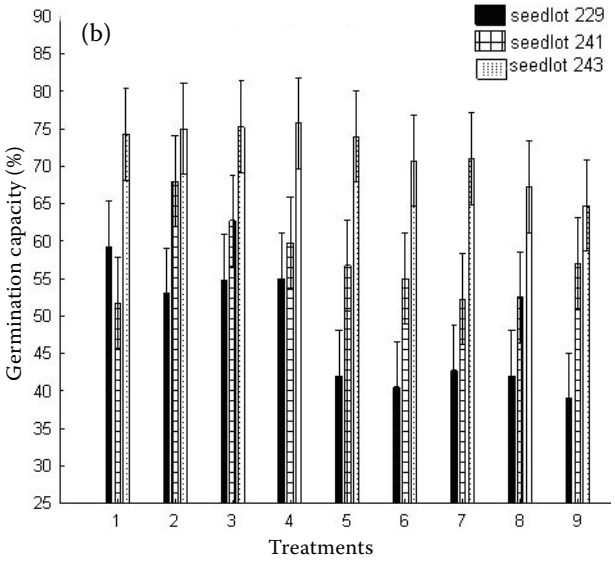


Fig. 1. Germination capacity of beechnuts (*Fagus sylvatica*) treated with ethephon (a) or GA_3 (b)

1 – control, 2 – dormant seeds treated with 80 $mg \cdot l^{-1}$ of ethephon or 40 $mg \cdot l^{-1}$ of GA_3, 3 – dormant seeds treated with 100 $mg \cdot l^{-1}$ of ethephon or 300 $mg \cdot l^{-1}$ of GA_3, 4 – dormant seeds treated with 120 $mg \cdot l^{-1}$ of ethephon or 1,000 $mg \cdot l^{-1}$ of GA_3, 5 – dormant seeds treated with tap water, 6 – seeds chilled for 4 weeks and then treated with 80 $mg \cdot l^{-1}$ of ethephon or 40 $mg \cdot l^{-1}$ of G_{A3}, 7 – seeds chilled for 4 weeks and then treated with 100 $mg \cdot l^{-1}$ of ethephon or 300 $mg \cdot l^{-1}$ of GA_3, 8 – seeds chilled for 4 weeks and then treated with 120 $mg \cdot l^{-1}$ of ethephon or 1,000 $mg \cdot l^{-1}$ of GA_3, 9 – seeds chilled for 4 weeks and then treated with tap water. Vertical bars show means and SE

RESULTS

The ANOVA detected a significant effect ($\alpha = 0.05$) of seedlots and ethephon or GA_3 treatments on germination capacity and MGT. The two-way interaction (seedlot × treatment) effect on germination was significant only for ethephon but not for GA_3 while a highly significant interaction effect on MGT was detected (Table 3).

Compared to control (dormant non-soaked) seeds or beechnuts treated with tap water no significant increase in mean germination capacity was detected after applying ethephon or GA_3 to dormant seeds. Conversely, the ethephon and GA_3 treatments reduced (ethephon significantly) germination capacity when applied to beechnuts chilled for four weeks prior to treatment (Table 4; Figs. 1 and 2).

The highest, but insignificant, mean germination capacity (72.3%) occurred when dormant beechnuts were imbibed in 100 $mg \cdot l^{-1}$ of ethephon and then chilled (Table 4).

The effect of the treatments on germination speed (MGT) and dormancy release significantly improved when beechnuts were chilled for four weeks prior to applying ethephon or GA_3 (Fig. 2). However, the effect of GA_3 on MGT of chilled beechnuts was not so distinct compared to dormant seeds (Fig. 2b).

DISCUSSION

Our results show that neither ethephon (80, 100 or 120 $mg \cdot l^{-1}$) nor GA_3 (40, 300 or 1,000 $mg \cdot l^{-1}$) increases the germination capacity of beechnuts. This contradicts the results of Fernandez et al. (1997), who also increased the mc of dormant beechnuts to 30% by soaking them in GA_3 (100 or 300 $mg \cdot l^{-1}$) or ethephon (100 $mg \cdot l^{-1}$) or tap water. After 3 weeks

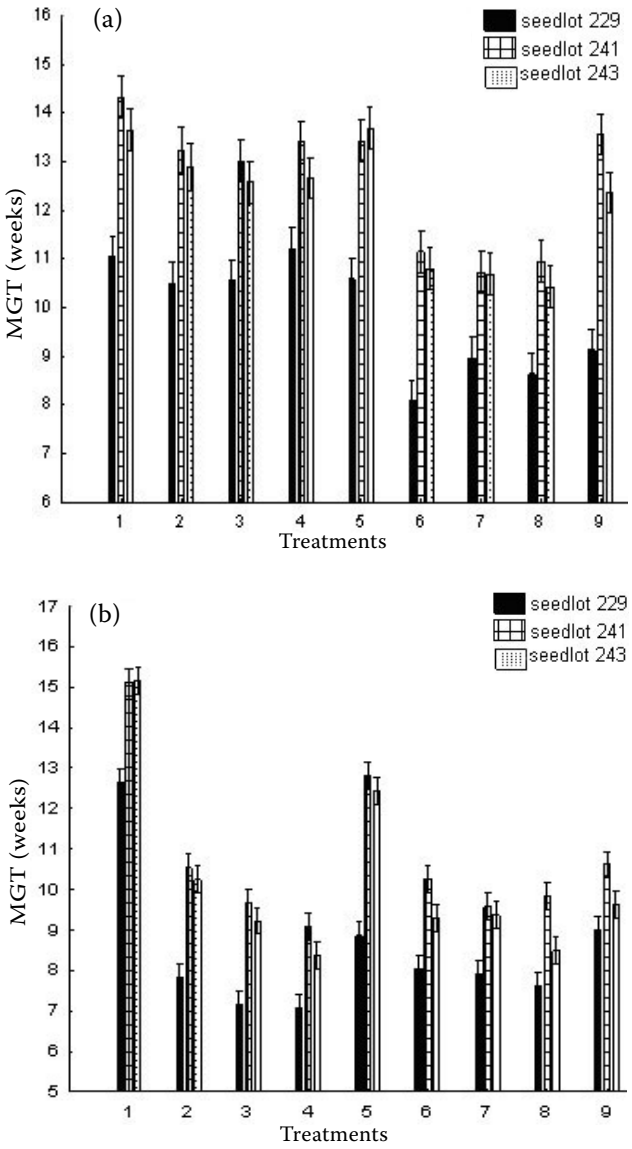


Fig. 2. Mean germination time of beechnuts (*Fagus sylvatica*) treated with ethephon (a) or GA_3 (b)

1 – control, 2 – dormant seeds treated with 80 $mg \cdot l^{-1}$ of ethephon or 40 $mg \cdot l^{-1}$ of GA_3, 3 – dormant seeds treated with 100 $mg \cdot l^{-1}$ of ethephon or 300 $mg \cdot l^{-1}$ of GA_3, 4 – dormant seeds treated with 120 $mg \cdot l^{-1}$ of ethephon or 1,000 $mg \cdot l^{-1}$ of GA_3, 5 – dormant seeds treated with tap water, 6 – seeds chilled for 4 weeks and then treated with 80 $mg \cdot l^{-1}$ of ethephon or 40 $mg \cdot l^{-1}$ of GA_3, 7 – seeds chilled for 4 weeks and then treated with 100 $mg \cdot l^{-1}$ of ethephon or 300 $mg \cdot l^{-1}$ of GA_3, 8 – seeds chilled for 4 weeks and then treated with 120 $mg \cdot l^{-1}$ of ethephon or 1,000 $mg \cdot l^{-1}$ of GA_3, 9 – seeds chilled for 4 weeks and then treated with tap water. Vertical bars show means and SE

of chilling beechnuts treated with GA_3 or ethephon germinated ca 20% better than beechnuts imbibed with tap water. Even prolonging the chilling period for tap water treated beechnuts did not increase germination. Clearly chilling duration was sufficient to break dormancy and the effect of GA_3 and ethephon only stimulated the germination of less vigorous beechnuts.

Similarly, GA_3 (200 $mg \cdot l^{-1}$) improves the germination of stored beechnuts by 15–18% compared to control beechnuts (Muller, Bonnet-Masimbert 1983; Muller 1983 ex Suszka 1990). In earlier experiments Frankland, Wareing (1966 ex Suszka 1990) found that the application of gibberellic acids was effective only for beechnuts where the pericarp had been removed, while seeds with the intact pericarp were not affected.

Mortensen and Eriksen (2004) also observed a positive effect of gibberellic acid only on one of two seedlots, while the treatment of dormant seeds with GA_3 (35 $mg \cdot l^{-1}$) resulted in no change in germination capacity compared to beechnuts chilled for six weeks. However, the germination capacity of 10-week chilled beechnuts was the same (over 90%) as for seeds treated with GA_3. Thus, GA_3 only reduced dormancy release without rise in germination. They observed the same effect for ethephon (144 $mg \cdot l^{-1}$), which reduced the chilling requirement by about three weeks without any increase in germination capacity.

In our experiments the ethephon treatment was similar to that of Falleri et al. (1997) and Muller and Larope (2003). Dormant beechnuts with the intact pericarp were hydrated in ethephon (100 $g \cdot l^{-1}$) or tap water (control) to reach 30% mc and then chilled for various periods. After seven weeks of chilling, beechnuts imbibed in ethephon reached 83% germination, while only 74% of seeds treated in water (control) germinated and the longer chilling did not increase germination capacity

either (Falleri et al. 1997). Muller and Larope (2003) also found that germination capacity and emergence of stored beechnuts treated with ethephon after three weeks of subsequent chilling were nearly the same (74%) as in the control (71%).

It is evident that the treatment of beechnuts with gibberellic acid or ethephon only results in a slight increase in the germination capacity of some, less vigorous seedlots. More frequently the application of these two chemicals speeds up dormancy release without affecting germination capacity. Our results showed similar germination capacity of beechnuts treated with these chemicals compared to untreated seeds. However, the germination rate (MGT) after ethephon and GA_3 application increased compared to control beechnuts.

In our studies higher germination occurred in dormant, not chilled beechnuts hydrated to 30% mc prior to the germination test while soaking seeds in tap water or ethephon or GA_3 after 4-weeks chilling resulted in lower germination (Table 4). Thus, short chilling prior to applying ethephon or GA_3 did not improve the germination capacity, but instead beechnut germination was poorer. The reason might be the mixing of chilled beechnuts with higher mc (31 to 35%) with peat-sand substrate of ca 30% mc. The optimum mc of beechnuts for dormancy release reported by Suszka et al. (1994) was 30–32% while Hlavová (1999) or Martincová et al. (1999) did not recommend mc above 30% due to the increasing risk of moulding.

Our germination test of beechnuts was done at the same temperature (3–5°C) as the pre-sowing (dormancy release) treatment. While according to the ISTA Rules (ISTA 2010) the germination test must be carried out on top of the germination paper, Suszka et al. (1994) recommended the mixing of beechnuts with moist substrate. However, no recommendation has been made regarding the precise mc of beechnuts prior to the germination test. We have found that beechnuts germinated faster when their mc was increased to 28% at least before germinating them. The MGT of beechnuts hydrated with tap water was slightly reduced as compared to control (no soaking) beechnuts, but GA_3 significantly speeded up germination compared to beechnuts which were allowed to gradually absorb water from the germination substrate (Table 4; Fig. 2).

We found no apparent effect of different concentrations of ethephon or GA_3 on germination capacity. The only exception was the application of 1,000 $mg \cdot l^{-1}$ GA_3 to dormant seeds that reduced the MGT to six weeks compared to the control (Table 4). In previous studies, the application of rather low concentrations of GA_3 affected the germination rate (MGT), e.g. 200 $mg \cdot l^{-1}$ on intact beechnuts or 35 $mg \cdot l^{-1}$ on beechnuts without pericarp (Bonnet-Masimbert and Muller 1976 ex Suszka 1990; Nicolás et al. 1996; Mortensen and Eriksen 2004). Fernandez et al. (1997) found no difference in the germination of pre-chilled, intact beechnuts treated either with 100 or 300 $mg \cdot l^{-1}$ GA_3 while dormant seeds germinated better when treated with 300 or 1,000 $mg \cdot l^{-1}$ GA_3 compared to 10-100 $mg \cdot l^{-1}$ GA_3. Evidently, a lower dose of GA_3 did not compensate for pre-chilling. In the case of ethephon the recommended concentrations are 100 $mg \cdot l^{-1}$ (Falleri et al. 1997; Fernandez et al. 1997; Muller and Larope 2003) or 144 $mg \cdot l^{-1}$ (Mortensen and Eriksen 2004). The effect of higher concentrations of ethephon on beechnut germination is not known.

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