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Effects of temperatures and polyethylene glycol on breaking dormancy and on germination of peach (*Prunus persica L.*) seeds

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مفعول درجات الحرارة و بولي إيثيلان كليكول على إيزال غفوة و إنبات بذور الخوخ

قارنا مفعول درجات حرارة قارة و متغيرة، خلال مراحل مختلفة من التطبق، على إيزال غفوة بذور الخوخ. قسمنا المدة الزمنية للتطبق و هي 9 أسابيع إلى ثلاث فترات متساوية. حسنت 15 درجة الإنبات في الفترة الثالثة و كانت بدون مفعول في الفترتين الأولى و الثانية . لم تتغير هذه النتائج لما أضفنا Polythyleneglycol من أجل منع الانبات خلال التطبق. تدل هذه النتائج على أن مفعول درجات الحرارة يتغير مع تطور التطبق و نناقش كذلك في هذه المقال آثار اخضاع البذور لدرجات حرارة مرتفعة في مراحل مختلفة من التطبق.

الكلمات المفتاحية : بذور الخوخ – الحرارة – غفوة – تطبق.

Effets de la temperature et du polyethylene glycol sur la levée de dormance et la germination des graines de pêcher (*Prunus persica L.*)

Les effets des températures constantes et alternantes sur la levée de dormance des graines de pêcher ont été évalués à différents stades de la stratification. La durée totale de stratification de 9 semaines a été divisée en 3 périodes de 3 semaines chacune. La température de dix degré a amélioré la germination pendant la 2ème et la 3ème période alors qu'elle n'a pas eu d'effet pendant la 1ère période. Quinze degré a amélioré la germination pendant la 3ème période mais l'a inhibée pendant les deux premières phases (1ère et 2ème). Les mêmes effets ont été observés en présence de polyethylène glycol. Ces résultats indiquent que l'unité de froid change avec la stratification. L'effet de l'interruption du froid par des températures élevées est également discuté.

Mots Clés : Graines de pêcher - Température - PEG - Dormance - Stratification

Effects of temperatures and polyethylene glycol on breaking dormancy and on germination of peach (*Prunus persica L.*) seeds

The effects of constant and alternating temperatures at various times during stratification on peach seed dormancy were evaluated. Stratification for a total of 9 weeks was divided into 3 equal periods of 3 weeks each. Both 10 and 15°C promoted subsequent germination when applied only during the last period. A temperature of 10°C promoted germination when applied during the 2nd period, but had no effect when applied during the 1st period, whereas 15°C was inhibitory in both first and second period. The same effects were observed even when polyethylene glycol was used to prevent germination. These results suggest that the chill unit changes as chilling accumulates. However, the inhibitory effect of moderate temperatures applied in the early stages of stratification is difficult to explain. The effect of chilling interruption by high temperatures at various times is also discussed.

Key Words : Peach seeds - Temperature - PEG - Dormancy - Stratification

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INTRODUCTION

Pe. ı seeds require exposure to moist chilling before germination can occur. The effective temperatures for breaking dormancy of rosaceous seeds range between 0° and 10°C . Six degrees appeared to be the optimum temperature for peach buds (Erez & Lavee 1971). Chilling efficiency fell as the temperature was increased to 10°C which was 50% as effective as 6°C .

Under natural conditions, temperature is not constant but varies diurnally and seasonally. A given temperature could exert its maximum effect on a physiological process (germination, growth...) even if intermittent. Several hours a day could be satisfactory provided the temperature during the rest of the day does not act in an opposite direction.

Erez & Lavee (1971) reported that 21°C for 8 h in a diurnal cycle completely negated chilling accumulation. When chilling was interrupted by 2 long periods (12 days) of high temperature (20°C), lateral bud opening was enhanced in comparison with the continuous chilling.

Erez & Couvillon (1987) found that chilling efficiency rises with temperature between 0° and 8°C if no moderate temperatures are interspersed. Zero degrees by itself was without effect, but when applied in a diurnal cycle with 15°C , it was just as effective as 8°C . Temperatures above 18°C for 8 h a day inhibited chilling accumulation while those below or equal to 18°C either promoted or were without effect on subsequent seed germination (Erez *et al.*, 1979). Erez & Couvillon (1987) reported that in one experiment 17°/6°C (8/16 h) inhibited chilling accumulation in 'Redhaven' peach buds while 15°/6°C was without effect.

However, in an other experiment when 15° was alternated with 6° bud break was enhanced, 18°C was neutral and 21°C completely negated the effect of 6°C .

When the total chilling period was divided into 3 equal periods and cycling was applied in different stages, 15° in combination with 4°C in a diurnal cycle (8/16 h respectively) enhanced flower bud opening only the last stage (Erez & Couvillon, 1987). Aduib & Seeley (1985) found that thermoperiodically treated 'Halford' peach seeds had lower germination than those given constant 5°C. However, 5°/10°C (16/8 h) enhanced germination percentage. Both seed germination and number of nodes per plant were lower under 5°/15°C than continuous 5°C .

The objectives of the present study were to :

- 1. Compare the effects of alternating temperatures with transfer from one constant temperature to another at different stages of chilling;
- 2. Determine whether or not the chill unit value changes with time;
- 3. Separate between the effect of a temperature on chilling and its effect on germination *per se*. For this, polyethylene glycol 6000 (PEG) was used. PEG, a viscous liquid miscible with water, lowers the osmotic potential of the medium, limiting water uptake and thereby preventing radicle elongation;
- 4. Determine the effect of temperatures on germination ability of partially stratified seeds;
- 5. Determine the effects of chilling interruption.

MATERIALS AND METHODS

Siberian C peach seeds were used. Dry pits were obtained from Hilltop Nurseries, Hartford, Michigan, and were held at 5°C until used. Seeds were removed from the pits and soaked in a fungicide solution (0.03% "Captan" = N-[(trichloromethyl) thio]-4-cyclohexene 1,2 - dicarboximide) for 24 h, then placed in petri dishes containing 2 layers of filter paper moistened with Captan solution. Four dishes (10 seeds per dish) were used per treatment. After stratification, germination capacity was evaluated by holding seeds for 10 days at 20°C in the dark. The final germination percentage represents total germination during stratification plus 10 days at 20°C .

1. Effects of constant and alternating temperature at various periods during stratification

A total chilling period of 1512 h (9 weeks) was divided into three equal periods of 504 hours (3 wk) each and continuous temperatures of 5, 10, and 15°C were applied. Constant and alternating temperature (16 h at 5°C plus 8 h at 10, 15, 20 or 25°C) were included for comparison. There were 3 experiments 1, 2, and 3 and the temperature regimes applied in each experiment were as follows.

• **Experiment 1**

1^{st}-3^{rd} week	4^{th}-6^{th} week	7^{th}-9^{th} week
5/10°C	5/10°C	5/10°C
5/15°C	5/15°C	5/15°C
5/20°C	5/20°C	5/20°C
5/25°C	5/25°C	5/25°C
5°C	5°C	-
5°C	5°C	5°C
5°C	5°C	10°C
5°C	5°C	15°C
10°C	5°C	5°C
15°C	5°C	5°C
10°C	10°C	10°C
15°C	15°C	15°C

• **Experiments 2 et 3**

They had same temperature regimes. They differ from experiment 1 by the absence of the first 4 treatments regarding diurnal alternation and by the addition of two treatments where moderate temperature was given during the second period (5 - 10 - 5°C and 5 - 15 - 5°C).

2. Effect of temperature on germination

Siberian C peach seeds were stratified for various periods at 5°C , then their ability to germinate was evaluated at 10°, 15°, 20° and 25°C .

3. Effects of stratification temperature in the presence of PEG

• **Experiment 1**

Temperatures of 5° continuous, and 5° (16 h) alternated with 10 or 15°C (8 h) and PEG at concentrations of 0, -1, -3, or -5 bars were used. The seeds were held at different temperatures until 896 h at 5°C had been accumulated. The PEG was then washed off and the seeds were germinated at 20°C.

• **Experiment 2**

The chilling period was divided into 3 equal periods of 3 weeks each. Temperatures of 5°, 10°, and 15°C were alternated at different stages with or without PEG (0, -1, -5 bars) by transferring from one constant temperature to another. The osmotic potential of polyethylene glycol was determined and related to concentration according to Michel and Kaufmann (1973). PEG was washed off following stratification.

4. Effects of chilling interruption

Siberian C peach seeds were chilled at 5°C for a total of 1344 h (8 wk). Chilling was interrupted for 1 wk interval starting with the 2^{nd} wk. The interruption consisted of exposing seeds to -5°, 0°, 10°, 15°, 20°, 25°, or 35°C .

RESULTS AND DISCUSSION

1. Effects of alternating temperatures at various periods during stratification

• **Experiment 1**

The treatment of 5/10°C for 16 and 8 h, respectively, significantly promoted subsequent germination. However, 5/15°C inhibited chilling accumulation and so did 20° and 25°C . Ten degrees was just as effective when given in the third period alone as when used continuously in a diurnal cycle, but did not stimulate germination when applied during the first period. Fifteen degrees had a similar promotive effect when it was applied subsequent to 1008 h of chilling, but reduced response to subsequent chilling when given during the first period (Table 1).

Table 1. Effects of constant and alternating temperatures at various periods during stratification on the germination (%) (during stratification plus 10 days at 20°C) of Siberian C peach seeds

Temperature (°C) duringweeks			Total time	..........Germination (%)........		
				..........Experiment...........		
1-3	4-6	7-9	at 5°C (h)	1	2	3
5/10	5/10	5/10	1008	96a	-	-
5/15	5/15	5/15	"	26cd	-	-
5/20	5/20	5/20	"	6e	-	
5/25	5/25	5/25	"	12de	-	-
5	5	-	1008	46b	50b	65a
5	5	5	1512	92a	92a	82a
5	5	10	1008	98a	94a	80a
5	5	15	"	90a	96a	80a
5	10	5	"	-	98a	72a
5	15	5	"	-	34c	30b
10	5	5	"	38bc	34c	70a
15	5	5	"	16de	10de	22bc
10	10	10	0	22de	22cd	30b
15	15	15	0	2e	2e	5c

Mean separation within each experiment by Duncan's Multiple Range Test at the 5% level

• **Experiment 2**

Again, both 10° and 15°C promoted subsequent germination when applied in the last period, but inhibited germination in the first period. Ten degrees promoted germination when applied in the second period of stratification while 15°C inhibited it (Table 1).

• **Experiment 3**

In this experiment, the control (1008 h at 5°C) germinated better than in the two first experiments and none of the treatments promoted germination significantly. However, 15°C inhibited response to chilling, when applied in either stage I or II, whereas 10°C did not (Table 1).

2. Effect of temperature on germination

Germination of Siberian C peach seeds did not appear to be affected by temperature. The effects of 10°, 15°, and 20°C on germination following stratification at 5°C did not differ significantly. Twenty five degrees was inhibitory only after 8 wk (1344 h) of exposure to 5°C (Table 2).

Table 2. Effect of temperature on germination (%) of Siberian C peach seeds following stratification at 5°C

Time (h) at 5°C	Germination temperature (°C)			
	10	15	20	25
672	0f	4ef	4ef	0f
1008	10de	10de	22d	8ef
1344	70bc	76abc	64c	10de
1680	70bc	80abc	76abc	64c
2016	86a	84ab	80abc	76abc

Mean separation by Duncan's Multiple Range Test at the 5% level

3. Effect of PEG and stratification temperature

• Experiment 1

PEG did not affect subsequent germination at 20°C . Ten degrees on a diurnal cycle promoted while 15°C inhibited germination in comparison with the control (896 h at 5°C continuous) (Table 3).

Table 3. Effect of stratification temperature at 5°C continuous or at 5°C for 16 h alternated with higher temperature for 8 h, and PEG on germination (%) of Siberian C peach seeds following 896 h of chilling at 5°C

Temperature (°C)	Germination (%) PEG osmotic potential (bars)			
	0	-1	-3	-5
5	50b	50b	55b	50b
5/10	92a	90a	82a	95a
5/15	15c	10c	12c	2c

Mean separation by Duncan's Multiple Range Test at the 5% level

• Experiment 2

In the absence of PEG, 10° and 15°C had the expected effects : they both promoted germination in stage 3, and 10°C was also effective in stage 2. Both temperatures were inhibitory when applied in stage 1, and 15°C inhibited germination in stage 2 as well (Table 4).

Response of PEG-treated seeds was much more difficult to rationalize. Ten degrees promoted germination in all stages at the high PEG concentration, but only in stages 2 and 3 at the low concentration. Fifteen degrees was inhibitory at all stages at the low concentration and at both stages 2 and 3 at the high concentration; but promoted germination in stage 1 at high concentration.

Table 4. Effect of constant and alternating temperatures at various times during stratification, and polyethylene glycol on germination (%) (during stratification plus 10 days at 20°C) of Siberian C peach seeds

Temperature during weeks			Total time at 5°C (h)	............Germination (%)........... PEG osmotic potential (bar)		
1-3	4-6	7-9		0	-1	-5
5	5	-	1008	50de	50de	50de
5	5	5	1512	92a	90ab	86ab
5	5	10	1008	82ab	80ab	77ab
5	5	15	1008	75ab	30efgh	30defgh
5	10	5	1008	80ab	82ab	75ab
5	15	5	1008	37def	12ghij	12ghij
10	5	5	1008	10hij	52cd	70bc
15	5	5	1008	25fghi	20fghij	70bc
10	10	10	0	8ij	12ghij	22fghij
15	15	15	0	10hij	5j	2j

Mean separation by Duncan's Multiple Range Test at the 5% level

4. Effect of chilling interruption

A temperature of -5°C was consistently inhibitory, especially at later stages of stratification (Table 5).

After 1 week of chilling only 20°C and 25°C significantly reduced subsequent germination. The effect of 20°C was not significant thereafter. However, 25°C remained inhibitory until the seeds had accumulated 5 weeks of chilling.

Table 5. Effects of temperature and time of interruption of chilling at 5°C for 1 wk on subsequent germination (%) of Siberian C peach seeds after a total time of 1344 h at 5°C

Time*	Germination (%)						
	Temperature of interruption (°C)						
	-5	0	10	15	20	25	35
1	44jk	94abc	96ab	94abc	80cdef	78def	94abc
2	58ghi	98a	98a	98a	86abcd	82bcde	90abcd
3	64gh	98a	98a	90abcd	88abcd	48ijk	88abcd
4	68fgh	96ab	96ab	98a	86abcd	68fgh	84abcd
5	56hij	96ab	96ab	98a	92abc	98a	70efg
6	58ghi	98a	98a	96ab	98a	90abcd	46ijk
7	36kl	96ab	96ab	98a	98a	96ab	26l
8	28l	96ab	98a	98a	98a	94abc	38kl
Control**	96a						

Mean separation by Duncan's Multiple Range Test at the 5% level
* Time (weeks)at 5°C prior to interruption
** Control (continuous 5°C

DISCUSSION

The fact that 15° was generally inhibitory in the diurnal cycle and during early stages of stratification, but promoted germination in stage 3, suggests that the beneficial effect of a moderate temperature during late stages may be attributed to promotion of germination. However, response was not consistent. The promotive effect of moderate temperature in late stages could also be attributed to a change in chill unit as stratification proceeds. It is more difficult to explain the inhibitory effect of moderate temperatures in early stages, since one would expect that such temperatures would be without effect on subsequent chilling.

To determine whether cycling was necessary to stimulate germination, constant temperature treatments were compared with alternating temperatures. The efficiency of 10°C varied between 25 and 36% relative to continuous 5°C depending on the experiment. However, 15°C had no effect; no more than 5% of the seeds germinated in any of the three experiments following 9 weeks at this temperature. Constant 10°C during the third period was just as effective as when alternated with 5°C in a diurnal cycle. It did not, however, stimulate germination when applied in the first period of chilling. Fifteen degrees had a similar promotive effect when applied subsequent to 1008 h of chilling, but reduced response to chilling when given during the first or second period. Similar results were reported by a number of authors. Thus, Erez *et al.* (1979) found that moderate temperature (10-13°C) promoted chilling accumulation when applied in diurnal cycles. A temperature of 15°C had was promotive only when given in late stages of chilling. On the other hand Aduib & Seeley (1985) reported that 10°C for 8 hours a day alternated with 5°C promoted subsequent germination of 'Halford' peach seeds. However, 15°C inhibited chilling accumulation when applied in a similar regime. It appears from our results and those of other researchers that the beneficial effect of moderate temperature, on subsequent seed germination or bud break, is more pronounced when applied in late stages of chilling. Although this beneficial effect of a moderate temperature during late stages may be attributed to promotion of germination or to a change in optimum temperature as stratification proceeds, the inhibitory effect of moderate temperatures in early stages is difficult to explain, since one would expect such temperatures to be without effect on subsequent chilling.

The use of PEG in order to prevent germination did not provide conclusive evidence as to the mechanisms of temperature effect. PEG limits radicle emergence, and therefore, probably, restricts growth rather than the early processes of germination. It is possible that a moderate temperature such as 15°C affects germination rather than chilling. This suggestion is supported by the fact that 15°C by itself had no chilling effect and that its beneficial effect was observed in all but one experiment when it was given in the late stages while during the early ones it was inhibitory. The chill unit value might also change with time, so that in early stages low temperature is required for removal of an inhibitor or synthesis of a promoter and that a moderate temperature at a later stage is beneficial for enzymatic or metabolic reactions.

The ability to distinguish between the effects of temperature on after-ripening *per se vs* germination is crucial. As mentioned above, the promotive effect of moderate temperatures in the last stages of after-ripening could be associated with germination rather than with chilling accumulation. However, germination of partially stratified peach seeds did not vary appreciably with temperature, therefore better germination at moderate temperature does not explain the promotive effects of such temperatures.

When stratification period of 10 wk of apple seeds at 4°C was interrupted for 1 week at either 0°, 2°, 6°, 8°, 10°, 12°, 14°, 16°, 18°, 20°, 22°, or 24°C Del Re Laborde (1987) found no significant differences during the first 6 weeks. However, the interruption of chilling during the 7th or 8th week reduce subsequent germination. Interruption of chilling the 3rd quarter of the stratification period (1680 h) by 2°, 6°, 18°, 20°, or 24°C also reduced seed germination. Maximum inhibition was observed at 20° and 24°C . Temperatures of 18°, 20°, and 24°C enhanced seed germination when applied during the first quarter of the chilling period. Interruption of chilling of Siberian C peach seeds for 1 week after various periods at 5°C showed that the effect of chilling is "fixed". The negation of chilling depends on the amount of chilling previously accumulated. Surprisingly, 35°C did not inhibit subsequent germination in early stages (up to 4 weeks), but negate chilling thereafter. A temperature of -5°C was consistently inhibitory particularly at later stages of stratification. The negative effect of -5° and 35°C was associated with injury; none of the non-germinating seeds from these treatments germinated despite restratification at 5°C for 13 weeks.

CONCLUSION

Experiments with continuously alternating diurnal temperatures, between 5°C (16 h) and 10° to 25°C (8 h), revealed that 10°C promoted while temperatures of 15°C or higher inhibited subsequent germination relative to constant 5°C . However, 15°C (continuously or in a diurnal cycle with 5°C) generally promoted germination when given during the latter part of the chilling period. This suggests that either moderate temperature affects germination rather than chilling *per se*, and/ or the chilling mechanism involves two reactions of different nature having different optimum temperatures. If the latter is true then the value of a chill unit will not be constant through the chilling period but will rather change with time and with the quantity of chilling previously accumulated. The chilling interruption experiment indicated that 35°C was inhibitory only after 5 weeks or more of chilling had been accumulated; 20° and 25°C on the other hand were inhibitory only in the early stages of the stratification period. In contrast a freezing temperature (-5°C) inhibited subsequent germination regardless of stratification time; the inhibition appeared to be associated with injury.

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