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dissertation entitled

EFFECTS OF STRATIFICATION TEMPERATURE ON BREAKING
DORMANCY AND ON PROTEIN CHANGES
IN PEACH SEEDS

presented by

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has been accepted towards fulfillment
of the requirements for

Ph.D. degree in HORTICULTURE

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Date April 1, 1991

EFFECTS OF STRATIFICATION TEMPERATURE
ON BREAKING DORMANCY
AND ON PROTEIN CHANGES IN PEACH SEEDS

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Horticulture

1991

ABSTRACT

EFFECTS OF STRATIFICATION TEMPERATURE ON BREAKING DORMANCY AND ON PROTEIN CHANGES IN PEACH SEEDS

By

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Constant vs alternating temperatures were compared as to their effectiveness in breaking the dormancy (endodormancy) of peach seeds. Temperature alternation between 5° and 10°C (5°/10°) for 16 and 8 hr, respectively, hastened germination, whereas a similar regime 5°/15° delayed germination, in comparison with constant 5° for 1008 hr (6 wk). Seeds were also held at constant temperatures (5°, 10°, or 15°) for 3 wk intervals, all receiving 6 wk at 5°. Response varied with time of exposure to the higher temperature. Exposure to 10° or 15° during the first 3 wk inhibited subsequent germination, whereas exposure during the last 3 wk stimulated it. Exposure to 10° during the second 3-week period promoted, whereas exposure to 15° inhibited germination. The inhibitory effect of high temperatures was dependent upon cycle length. This inhibition decreased or disappeared as the cycle ratio (days at 5°/days at high temperature) increased. Protein changes in peach seeds were evaluated during stratification at 5° and incubation at 20°. Soluble protein content remained constant in both axes and cotyledons regardless of stratification temperature and duration. However, changes were observed in the protein profile, but only at 5°C and only in the cotyledons. These changes occurred prior to changes in size of the axis. Similar changes occurred in cotyledons when the seeds were partially imbibed and even when the cotyledons were stratified separately from the axes. These changes therefore appear to occur prior to, and to be independent of, germination.

DEDICATION

This work is dedicated to:

- My deceased father for his sacrifices, love and support.
- My mother for her love.
- My brothers and sisters for their love and help.
- My wife, Saida, for her support, patience, encouragement, and numerous sacrifices.
- My wonderful daughter, Meryem, and fine son Adel.
- My in-laws for their support.
- My friends for their support and encouragement.

ACKNOWLEDGMENTS

I wish to express my sincere appreciation and deepest gratitude to Dr. Frank G. Dennis for his precious guidance, counsel, help, constant support, understanding, friendship, generosity, hospitality and encouragement throughout this study and thesis preparation.

I am grateful to Drs. Martin J. Bukovac, James A. Flore and Charles E. Cress, for their help and continuous support and for accepting to serve as members of the examining committee; to Dr. Clifford J. Pollard for serving in the advisory committee; and to Dr. Kenneth L. Poff for reading the thesis and substituting for Dr. Pollard in the final examination.

I wish also to express my warmest thanks and sincere appreciation to my friend, Dr. Barbara S. Peterson, for her continuous help and encouragement.

My sincere thanks are also extended to the Director and the General Secretary of IAV Hassan II and its personnel, USAID, Dr. Johnson, the University of Minnesota, and the faculty members of the Department of Horticulture at Michigan State University for their help of various kinds at various times, moral and financial support.

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PART ONE

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LITERATURE REVIEW

INTRODUCTION

Seeds and buds of most deciduous fruit trees go through a dormant phase. Dormancy, an adaptation which allows survival under adverse environmental conditions, is of considerable economic importance. Dormant organs are more resistant to unfavorable conditions, such as low temperatures and drought, than are growing organs.

A number of treatments, including light and chilling, are effective in breaking dormancy. However, the mechanisms of action of these treatments are not well understood. Most deciduous fruit trees require a period of cold treatment prior to resumption of growth. The duration of chilling required varies greatly among species and cultivars.

The lack of adequate chilling in zones with warm climates constitutes a serious limitation to the culture of temperate fruit trees. Symptoms include: 1) irregularity and delay in bud opening; 2) deformed leaves; 3) multiple pistils; 4) poor style and pollen development; 5) abscission of buds. Thus, a better understanding of the onset, maintenance, and removal of dormancy would help to increase the range of conditions under which temperate zone fruits can be grown.

DORMANCY: DEFINITIONS AND DISCUSSION

Lang et al. (57) reviewed the terminology used to describe dormancy. The authors considered the terms inadequate and confusing because: 1) they are easily misused; 2) they are imprecise and lack physiological meaning; 3) semantic differences between these terms are often minute; 4) they are not easily translated from one language to another; 5) they tend to be a mixture of physiological and seasonal terms; 6) they have not been universally applied to all plant parts exibiting similar dormancy phenomenon. There are almost as many definitions for dormancy as there are scientists working on this phenomenon. Evenari (36) defined dormancy as the absence of visible growth of viable seeds (or buds) under favorable conditions of moisture, temperature, and oxygen. Samish (72) defined dormancy as a temporary suspension of visible growth, regardless of its cause. He differentiated between 1) correlative inhibition when conditions causing rest are due to factors arising outside the organ, and 2) rest, when the factors responsible for growth cessation arise within the organ itself. He further distinguished between different phases of rest. <u>Quiescence</u>, which usually precedes the entrance into rest, results from unfavorable growing conditions (temperature, water). <u>Preliminary-rest</u> results when the dormant bud will no longer grow in response to favorable conditions, but can easily be forced by subjection to heat, wounding or defoliation. During <u>Mid-rest</u> only special treatments, such as light or cold, will allow growth resumption. <u>After-rest</u> is a quiescent period which follows chilling if the growing conditions are not favorable.

Doorenbos (29) distinguished between: 1) summer-dormancy, when growth of buds is inhibited by physiological processes inside the plant but outside the bud; 2) winter-dormancy, when growth is prevented by a physiological process inside the bud itself ; and 3) imposed dormancy, when growth cessation results from unfavorable environmental conditions. Saure (74) critized Doorenbos's terminology because "winter-dormancy" begins during mid-summer, reaches its maximum by leaf fall, and can be satisfied by mid-winter or even earlier. In addition, this term does not apply to tropical and subtropical regions where dormancy can exist despite the absence of temperatures characteristic of "winter". Saure (74) also noticed that "imposed dormancy" implies that this condition should be removed as soon as favorable environmental conditions prevail. In general this indeed occurs in temperate zones, but bud opening is often delayed in regions with warm climates. Saure (74) proposed a modification of Doorenbos's terminology to separate the cause from the season of inhibition. He defines: 1) predormancy as the stage during which the lateral buds are directly prevented from breaking by the growing shoot tip or adjacent leaves; 2) true dormancy or rest as the phase when the source of inhibition is located within the buds; 3) imposed dormancy as the period in which inhibition is due to external factors and thus may be considered as the expression of an exogenous inhibition. The intensity of true dormancy varies with time rather than being constant. The low intensity stage of this phase was termed after-rest by Samish (72), post-dormancy by Vegis (91), and late dormancy by Saure (74). During this stage buds and/or seeds are able to start growing in a narrow range of moderate temperatures. This range widens progressively until growth activity reaches its maximum.

Thus an extensive terminology has been used to describe the phenomenon of dormancy. Lang et al. (57) proposed a new terminology using the base word dormancy, which they define as a temporary suspension of visible growth of any structure containing a meristem. They differentiate between 1) ecodormancy, which is regulated by environmental factors such as temperature, water, light, and nutrients necessary for growth; 2) paradormancy, which is due to physiological factors originating in a part of the plant other than the affected structure itself (e.g. apical dominance) ; and 3) endodormancy, which is regulated by physiological factors originating within the affected structure. Lang et al. (57) summarized the extensive list of terms found in the literature. They attempted to classify all of them under one of the three categories of dormancy they advanced in their proposed terminology (Table 1).

TYPES OF DORMANCY

Amen (3) attributed seed dormancy to the inability of the embryo to resume growth as a result of an internal metabolic block or the production of an inhibitor. Prolonged periods of chilling required to break dormancy are usually attributed to a requirement for physical as well as chemical changes within the embryo, i.e. metabolism of the inhibitors and/or the accumulation of growth promoting substances (6). The causes of dormancy are numerous and varied (3). These causes include: 1) rudimentary embryos (orchids); 2) physiologically immature embryos (lettuce, barley); 3) seed coats which are mechanically resistant to embryo growth (Alisma plantago, Eucalyptus pauciflora); 4) low or no permeability of seed coats to water and/or gases (legumes); 5) the presence of germination inhibitors (apple, peach); 6) combinations of two or more of the above; 7) secondary dormancy. The different

Table 1. Terms used to describe dormancy and the possibility of replacing them by simplified terminology (after Lang et al. 1987).

Endodormancy: After-ripening, autogenic dormancy, autonomic dormancy, constitutional dormancy, constitutive dormancy, deep dormancy, deep physiological dormancy, deep rest, dormancy, dormancy I, dormancy II, early dormancy, endogenous dormancy, induced dormancy, innate dormancy, intrinsic dormancy, late dormancy, main rest, middle rest, organic dormancy, permanent dormancy, physiodormancy, physiological dormancy, primary dormancy, real dormancy, rest, secondary dormancy, spontaneous dormancy, true dormancy, true winter dormancy, winter dormancy, winter rest.

Paradormancy: Correlative dormancy, correlative inhibition, early rest, predormancy, preliminary rest, relative dormancy, shallow dormancy, summer dormancy, temporary dormancy.

Ecodormancy: After-rest, aitogenic dormancy, aitonomic dormancy, conditional dormancy, environmental dormancy, exogenous dormancy, external dormancy, imposed dormancy, post-dormancy, post-rest, quiescence, relative dormancy.

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types of dormancy may have common control mechanisms. Dormancy can be removed by exposing seeds to moisture and light, dry storage or moist chilling. The latter is commonly used in breaking peach seed (and bud) dormancy.

BREAKING DORMANCY BY CHILLING

1. Effects of constant temperatures on seed dormancy

Stokes (84) stated that after-ripening of seeds of most deciduous fruit trees can be achieved by subjecting them in an imbibed state to low temperature (1° to 10°C). The most effective temperatures are between 2° and 5°C. She listed optimum temperatures, effective ranges, and time required at optimum temperature for removal of dormancy in over 80 species. After-ripening does not take place at temperatures below freezing, freezing causes injury in some species (84). The required chilling period varies between 4 and 20 wk depending on species and cultivar. Environmental factors, seed source and pollen source influence chilling requirement (84). The author summarized the characteristics of low temperature in after-ripening of rosaceous seeds as follows: 1) effectiveness of low temperatures is enhanced (i.e., increased percentage and/or rate of germination) by prolonging the exposure period; 2) the temperature/response curve is of the optimum curve type (as exposure time is increased the optimum temperature declines); 3) any temperature within the effective temperature range induces optimal germination if allowed to act long enough; 4) high temperatures reduce or negate the effect of chilling; 5) the after-ripening of partially stratified seeds can be completed at higher temperatures (10-15°C), which have little or no effect if applied from the beginning. Seeley and Damavandy (76) conducted an extensive study to determine the effect of a series of temperatures (-2°, 0°, 2°, 4°, 6°, 10°, 12°, 14°, and 16°C) on the removal of seed dormancy in seven rosaceous species (apple, quince, peach, pear, apricot, and cherry (*Prunus avium* and *Prunus mahaleb*)). The seeds were held at these temperatures for periods varying from 0 to 2880 hr (120 hr intervals), then their germination was tested at 25°C for 10 days. Optimum temperatures for all species fell between 4° and 6°C. All factors tested (seed source, temperature, time) and their interactions were significant in every species. The temperature response-curves for peach seeds (76) and buds (69) were parallel. However, the optimum temperature for seeds was slightly lower (4°C) than that for buds.

The symmetry of seed chilling response for different species was evaluated by measuring the curve area below and above the optimum temperature (Table 2). The curves varied from highly asymmetric (peach) to very symmetric (quince and Mazzard cherry). The symmetry response in pear was apparently influenced by environmental conditions prevailing during seed maturation, and/or pollen source. 'Bartlett' pear seeds from trees in a cold location had a lower optimum temperature and required less chilling than those from a warm location. Seed germination increased with chilling in a sigmoid curve. The authors suggested that the sigmoidal shape of the curve could be attributed to: 1) genetic variations among seeds within a population which could be normally distributed in regard to chilling; 2) constant change in chill unit value during the chilling period; 3) biochemical reactions in the first (lag) phase which are required to synthesize or liberate dormancy-breaking chemicals, leading to a linear active phase, then a saturation phase (maximum response).

Table 2. Symmetry of seed chilling response (after Seeley and Damavandy 1985).

Species	Optimum Temp.(oC)	Relative Area Below Opt.	Relative Area Above Opt.	Ratio Below/Above
Peach	4	30	70	0.4
Mahaleb Cherry	4	30	70	0.4
Apple	4	33	67	0.5
Pear (Cold climate)	4	34	66	0.5
Apricot	6	44	56	0.8
Quince	6	50	50	1.0
Mazzard Cherry	6	50	50	1.0
Pear (Warm climate)	6	52	48	1.1

Carlson and Tukey (18) studied the after-ripening requirements of several sources and varieties of peach seeds. Each variety had specific after-ripening requirements. The period required at 3°C to attain maximum germination varied from 504 hr for 'McAllister' to 2016 hr for 'Lovell' and even longer for 'Muir'. Sharma and Singh (79), evaluated the effect of constant temperatures (0°, 7°, 10°, and 24°C) on breaking dormancy of 'Sharbati' peach seeds. Ten degrees gave the highest percentage germination regardless of stratification period up to 10 wk. In contrast, seeds stratified at 0° failed to germinate, and few of those held at 24°C germinated. The beneficial effect of 10°C on stratification extended to seedling growth. The seedlings raised from seeds stratified at 10° were taller than those from seeds kept at 7°, while those from seeds held at 24°C were dwarfed.

2. Effects of constant temperatures on bud dormancy

Westwood and Bjornstad (99), studying the chilling requirements of fourteen pear species from different locations, found that the effective temperatures ranged between 2° and 10°C. Species originating from warmer climates had lower chilling requirements and a higher optimum range (7° to 10°C) than those from colder climates. Weinberger (95) determined chilling requirements for over sixty peach varieties. The chilling requirement varied between 700 hr for 'Sunhigh' and 1300 hr below 7.2°C for 'Mayflower'. The chilling requirement of leaf buds was higher than that of flower buds. Freeman and Martin (41) found that mist, low light, and low temperature treatment hastened dormancy removal and subsequent growth of peach floral buds. High temperature and high light intensity had the opposite effect. Six degrees appeared to be the optimum temperature for peach buds (32). However, the temperatures below 6°C were just as effective. Chilling efficiency fell as the temperature was increased to 10° which was 50% as effective as 6°C. The bud temperature responses depended on their location on shoots. Thus, maximum bud break was obtained at 8°, 6°, and 3°C for terminal and lateral buds on primary shoots, and lateral buds on lateral shoots, respectively. In rabbiteye blueberry cv, flower buds have a higher chilling requirement than vegetative buds (81), while the opposite was reported for peach buds (95). Mechanical defoliation enhanced the breaking of vegetative buds in blueberry, but had no effect on flower buds.

Gurdian and Biggs (46) compared the effect of 7.2° vs 12.8°C on potted trees of four peach cv with different chilling requirements. They reported that low chilling cv 'Flordasun', 'Flordahome', and 'Okinawa' responded well to both temperatures. In fact, the percentage bud break of nonchilled trees of these cv reached 70 to 80% after 48 days of forcing. However, the shoots remained rosetted in the absence of chilling. In contrast to low chilling cv, 'Nemaguard' (a high chilling cv) responded differently, 7.2° was more effective than 12.8°C. The effect of both temperatures on low chilling cv appeared to be quantitative since the buds resumed growth in the absence of chilling. Percentage bud break in all cv was enhanced by both temperatures. Except for 'Nemaguard' the length of time at either temperature had little effect on the final percentage of buds released from dormancy. Exposure to both temperatures promoted shoot growth of the four cv.

3. Relationship between chilling requirements of seeds and buds

Kester (53), studied the relationship between chilling requirement of seeds and buds in almond. The author reported that: 1) Plants with a chilling requirement for continued growth generally produce seeds with a chilling requirement for germination. 2) The breaking of dormancy is promoted by low temperatures and inhibited by higher ones. 3) The equilibrium point between promotion-inhibition is slightly higher than 15.6°C. 4) 10°C appeared to be the optimum temperature for after-ripening. 5) There was a correlation between the chilling requirement of seeds and date of bloom of the mother plants; early blooming varieties, whether used as male or female parents, produced seeds with short chilling requirements. Kester concluded that embryo genotype controls the chilling requirement in almond. The author suggested that almond embryos can be used to determine chilling requirement of the resultant tree. The chilling requirements of pear seeds were highly correlated with those of the mother plants (99). Crosses between species with high and low chilling requirements yielded seeds with intermediate chilling requirements.

4. Effects of high temperatures on breaking dormancy

Weinberger (96) reported that high temperatures counteracted the effects of chilling. High temperatures caused a delay in bloom and foliation and reduced fruit set of 'Sullivan Elberta' peach. In contrast, the breaking of rest was enhanced by shading which acts by reducing bud temperature. Bennett (7) showed that exposure of pear buds to high temperatures (23°C) during chilling delayed their development. Continuous moderate temperatures (13-15°C) delayed peach flower bud break more than did higher temperature for brief periods. However, the latter had a more pronounced effect on leaf buds. The negative effect of high temperatures was more pronounced in December than in November or January. In another study, Weinberger (97) related monthly mean temperatures to prolonged dormancy. January and December temperatures were the most closely associated with breaking of rest in peach buds. Average temperatures for these months were highly correlated (0.93) with prolonged dormancy. Immersion of pear twigs in a water bath (45°C) resulted in dormancy removal (19). A period of hot weather in August-September, with temperature fluctuating between 36° and 45°C, had a striking effect on breaking apple bud dormancy (19). Many of the trees bloomed heavily and produced some new leaves 4 wk later. The effect was more pronounced on low chilling cv. Chandler (19) was able to break dormancy of potted trees by holding them for 6 hr at 44-46°C. He concluded that 6 hr at 45° was just as effective in removing dormancy as 100 hr at 4.5°C. Chaudhry et al. (20) found that 3.5 hr at 45° was as effective as 12 wk at 3°C in breaking rest of pear buds. However, the bud activity and the vigor of sprouting were higher in cold-treated plants. Thevenot et al. (87) reported that apple embryo dormancy can be broken by subjecting seeds to 30° or 35°C in a moist atmosphere. They attributed the efficacy of high temperatures in removing dormancy to the limitation of oxygen to the embryo. Immersion in hot water (50-52°C) for short periods induced sprouting in grapevine cuttings (74). Temperatures above 20°C did not inhibit bud break of peach cv of South China parentage (62). A wide range of temperatures can reduce dormancy intensity. However, temperatures in the effective range do not have the same effectiveness (74). Erez and Lavee (32) proposed using weighted chilling hr to determine the chilling requirement of different species or cv. In this model, 1 hr at the optimum temperature is given the value of 1 chill unit, and the values of other temperatures are assigned relative to the optimum depending on their effectiveness in removing rest. This model has been adopted by many researchers involved in modeling chilling requirements. The weighted chilling hr method and the summation of chilling hr below 7.2°C assume that the effect of a temperature is constant through the entire chilling period. Kobayashi et al. (55) contended that the effect of a chilling temperature is not constant; rather, it changes with the stage of development.

ALTERNATING TEMPERATURES

Under natural conditions, temperature is not constant but varies diurnally and seasonally. Vegis (91) suggested that a given temperature could exert its maximum effect on growth even if intermittent. Several hr a day could be satisfactory provided the temperature during the rest of the day did not act in an opposite direction.

1. Vernalization vs after-ripening

Purvis and Gregory (67) observed that vernalization of 'Petkus' winter rye at 10°C could be reversed by high temperatures (20° to 25°C). The degree of devernalization depended upon the duration of the previous vernalization period. Some devernalization occurred at 15° and 17°C. They postulated that a neutral temperature may exist between 10° and 15°C at which no effect on subsequent flowering occurs. Vernalization and the breaking of rest have some similar effective temperature ranges. However, the two phenomena are different in that vernalization is the acquisition or acceleration of the ability to flower by a chilling treatment, whereas the breaking of rest allows active growth when favorable conditions return but does not direcly cause the formation of new kinds of organs (21).

2. Effects of alternating temperatures on peach bud dormancy

Overcash and Campbell (66) compared the effect of continuous 4° vs 4/21°C in a diurnal cycle (16 and 8 hr respectively) on breaking rest of 'Sunhigh' and 'Redhaven' peaches. Continuous 4°C was more effective in breaking rest. Interruption with high temperature resulted in a partial negation of chilling. Peach leaf bud opening was reduced by 33% when the average temperature was raised from 10-12° 18° for 15 days while 80% reduction resulted from raising the average temperature to 22.2°C for the same period (96). Erez and Lavee (32) compared the effect of constant vs alternating temperatures on potted 'Redhaven' peach trees. No negation by a high temperature occurred up to 18° However, 21°or 8 hr in a diurnal cycle completely negated chilling accumulation. When chilling was interrupted by 2 long periods (11-12 days) of high temperatures (20° lateral bud opening was enhanced in comparison with the continuous chilling. The cycle length appeared to be the major cause of response inversion. Intermittent high temperature antagonizes chilling on a 24 hr cycle but not on longer cycles. However, the authors did not mention the importance of the amount of chilling accumulated prior to warm temperature interruption. In fact, the first long period of interruption was applied only after the buds had accumulated 912 hr at 6°C. A high temperature at later stages of the chilling period could affect bud growth, thereby resulting in increased bud opening. In addition these buds were held longer at the forcing conditions. The degree of reversal of chilling depends on the temperature, duration of interruption, and the chilling previously accumulated (67). Erez et al. (33) studied the effect of high temperature on chilling accumulation in 'Redhaven' and 'Redskin' peaches. They used a range of constant temperatures between 4° and 24°C and a diurnal cycle of 16 hr at 6° and 8 hr at higher temperatures (15°, 18°, 21°, or 24°C). Six degrees used in cycling treatments was not included as a constant temperature. In addition, trees were illuminated during alternating temperature treatments while those held at continuous 4°C were not. Twenty-four degrees did not break rest. A high percentage of bud break was obtained at 4° and at 6/15°C. The latter treatment was significantly more effective per hr at low temperature than the former; however, the total time was much longer in alternating treatment, and 15°C may have acted for a longer time on growth rather than on rest *per se*. When alternated with low temperatures, temperatures below or equal to 18°C did not prevent chilling accumulation. However, temperatures above 18°C had an inhibitory effect. The authors postulated that an hr at these higher temperatures should be given -2 as a chill unit rather than -1 as was suggested by Richardson et al. (69). They based their postulation on the inhibition of 16 hr at 6° by 8 hr at 21° or 24°C. Erez and Couvillon (35) reported 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° it was just as efficient as 8°C. Moderate temperatures (10°-15°C) enhanced rest completion when they were applied subsequent to chilling temperatures. The most effective moderate temperature was 13°C. The response of leaf buds to intermediate temperatures was much higher than that of flower buds. In one experiment 17/6°C (8/16 hr) inhibited chilling accumulation in 'Redhaven' buds while 15/6°C was without effect. However, in another experiment when 15° was alternated with 6° bud break was enhanced. Eighteen degrees was neutral and 21° completely inhibited the effect of 6°. 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 hr, respectively) enhanced flower bud opening only when applied in the last stage. However, cycling was ineffective in promoting leaf bud break in this experiment; in fact, it inhibited leaf bud opening when applied during the first third of the chilling period.

3. Effects of alternating temperatures in relation to cycle length

Erez et al. (34) evaluated the effect of cycle length on chilling negation by high temperatures in leaf buds of rooted cuttings of 'Redhaven' and 'Redskin' peach. Cycle lengths were 1, 3, 6, and 9 days. For 2/3 of the cycle the buds were held at low temperature (4° for 1 day cycle and 6° for other cycles) for the other 1/3 they were kept at 24°C. Little or no bud break occurred in 'Redskin' plants kept under 1 and 3 day cycles; 6 and 9 day cycles were just as effective as continuous 4°C. However, the cuttings were not uniformly treated. The 1 day cycle had 6° as a low temperature, while 4°C was used for other cycles, and had much higher light intensity (4800 lux vs 40 lux). For 'Redhaven' bud break was significantly lower than that of the control (4°C) but only in 1 day cycle. The authors concluded that chilling negation by high temperatures is dependent on cycle length. Purvis and Gregory (67) working with Petkus winter rye drew the same conclusion.

4. Effects of alternating temperatures on nectarine bud dormancy

Gilreath and Buchanan (42) used 1-year-old cuttings of 'Sungold' nectarine to study the effect of constant (0.6°, 3.3°, 7°, 10°, and 15°C) and alternating temperatures in a diurnal cycle of 14 hr (low temperature) and 10 hr (high temperature) 7/30°, 7/15°, 0/7°C. The effect of chilling interruption for 14 days at 30°C was also evaluated. The plants were removed at 100 hr increments from 350 to 750 chilling hr. Bud break was evaluated, at a temperature regime of 27° during the day and 13°C at night, as number of days required to reach an arbitrary stage. In plants held at constant temperatures floral bud break was faster at 10° than at 7°C for most chilling periods. This trend was also evident in plants subjected to 14 days of interruption halfway through the chilling period. There was no visible bud activity in plants held at 15°C for less than 750 hr. Fifteen degrees, 3.3°, and 0.6° were less effective than 10° and 7°C after 60 days of forcing. Chilling interruption increased the initial bud break but reduced the final number of buds that developed. No bud activity was apparent in plants given 350 hr chilling with 14 days at 30°C. The authors concluded that high temperature must have negated 175 hr accumulated prior to high temperature exposure. Gilreath and Buchanan (42) postulated that this constitutes a confirmation of a "temperature-dependent fixation process". The effect of high temperature on chilling negation depends on the duration of low temperature exposure. The chilling effect becomes more stable and irreversible as chilling proceeds and more "product" is generated. In regard to alternating temperatures they reported that floral bud break was faster following 7/0° than at 15/7°, 30/7°, and continuous 0.6° but not as fast as continuous 7°C. Bud break in plants held at 15/7° was intermediate between continuous 7° and 15°C. No bud break occurred in plants held at 30/7° for up to 750 hr at 7°C, even after 67 days of forcing. The authors concluded that a single prolonged exposure to high temperature is not as antagonistic to chilling accumulation as frequent periods of high temperature following short periods of chilling. Gilreath and Buchanan (42) reported that high temperature negation was less pronounced in low than high chilling peach cvs. They further stated that low chilling peaches and nectarines reach cold base at a higher temperature, and that the same trend is likely for the chilling requirement and such cultivars could be more tolerant of high temperatures. No vegetative bud break occurred in plants held continuously at either 0.6° or 15°C. There were no significant differences among other treatments. Gilreath and Buchanan (42) compared the temperature-chill unit model derived from the combined floral bud break data to the Utah model (69). They reported that the low chilling model is broader and has a higher optimum temperature. Both models reach maximum chilling negation (-1 chill unit) at the same temperature. There is no difference in response at the extreme low end of the curve, approaching -1°C (0 chill unit). Cultivar differences are most apparent at the optimum temperature and in the positive chill unit region at temperatures above the optimum. They observed a shift in optimum chilling temperature of 2° (from 6° to 8°C). This shift is significant but the portion of the curve where the greatest divergence is noted between 8° and 18°C may be even more important in total chilling accumulation. The authors stated that winter temperatures in warm climates often fall within this range, and therefore their possible contribution to chilling should not be ignored. They postulated that assigning fewer chill units to these temperatures may be the reason for failure of high chilling models to predict rest completion under mild conditions.

5. Effects of alternating temperatures on blueberry bud dormancy

Spiers (81) found no inhibitory effect of 18° (for 10 hr) in alternation with 7°C (14 hr) on chilling accumulation in rabitteye blueberry "Tifblue". 23°C for 35 hr per week (7 hr a day, 5 days a wk) partially reduced low temperature effect. The effect of insufficient chilling was more pronounced on flower than on vegetative buds. Gilreath and Buchanan (43) evaluated the effect of the same temperature regimes used for "Sungold" nectarine (see above) on breaking rest of high ("Tifblue") and low chilling ("Woodard" and "Bluegem") blueberry cv. The cuttings were removed at 100 hr increments ranging from 250 to 650 hr for "Woodard" and "Bluegem" and from 250 to 850 hr for "Tifblue". Terminal floral bud break was similar for all cv at constant temperatures of 0.6°, 3.3°, 7°, and 10°C. "Tifblue" did not respond as well as low chilling cv to 15°, no visible bud activity being apparent following 550 hr exposure to 15°C. The same trend was evident in alternating temperature treatments. Differences among cv were more pronounced in lateral than in terminal buds. "Tifblue" did not respond at all to 15° or 7/15° and responded to 10°C only when exposed to the maximum chilling duration (850 hr). The authors concluded that these findings indicate a narrower range of effective chilling temperatures for "Tifblue" and suggest that "Woodard" and "Bluegem" are better adapted to mild climatic conditions due to a widening of the temperature range over which chilling is effective. Vegetative bud break following diurnal alternation was similar to that obtained under constant temperatures. 7/15° was more effective in satisfying rest than regimes of 0/7° and 7/30°C following 250, 350, and 450 hr of chilling, but not at 550 hr or longer chilling hr duration. 0/7°C gave bud break intermediate between 0.6° and 7°C only when at least 450 hr total time was accumulated. In fact this regime slowed bud break under shorter exposure periods. High chilling cv showed a narrower range of effective temperatures than low chilling ones (43). Continuous 15° or 7/15° for 14/10 hr, respectively, were as effective as constant 7°C on low chilling cv but were ineffective in breaking rest of high chilling cv. Interrupting the chilling period halfway with 2 wk at 30°C enhanced the rate but did not affect total bud break. However, when 30° was applied in a diurnal cycle (for 10 hr) with 7°C, a partial negation of bud break resulted.

6. Effects of alternating temperatures on cherry bud dormancy

Fifteen degrees was not effective in breaking rest of 'Montmorency' sour cherry flower buds, but a 5/15° (16/8 hr) cycle was more effective than constant 5°C (37). Felker and Robitaille (37) postulated that this cycle provides sufficient chilling accumulation, insufficient continuous hr at 15°C to nullify chilling, and enough concomitant heat unit accumulation to increase the rate of bud break.

MECHANISMS CONTROLLING SEED DORMANCY REMOVAL

Scientists have devoted considerable research effort attempting to understand the physiological basis of rest and the mechanisms of action of low temperatures. The most popular hypotheses involve hormones; one of these postulates that growth inhibiting substances accumulate in seeds as they mature, leading to the onset of dormancy. During rest-breaking treatments (chilling, light, dry storage, etc.) the inhibitors are metabolized and/or growth promoting substances (cytokinins, gibberellins) are synthesized and the seeds attain the potential to germinate and yield normal seedlings. I shall examine the available evidence regarding this hypothesis.

Abscisic acid (ABA)

The presence of ABA in seeds and other plant parts is well established, and many attempts have been made to determine its involvement in dormancy. This problem has been approached by: 1) comparing ABA content of dormant and nondormant seeds of the same or related species; and 2) effect of dormancy-breaking treatments on ABA content, such as leaching of inhibitors from the structures covering embryos to allow germination (94). In the latter it is not clear whether leaching per se or imbibition alone is sufficient to initiate other metabolic processes leading to a decrease in inhibitor content.

Rudnicki (70) reported that levels of ABA in apple seeds increased with their maturation. ABA levels were higher in seed coats than in embryos. He postulated that translocation of endogenous ABA of seed coat to embryo during stratification may play a regulatory role in blocking some metabolic activities of embryo growth. Rudnicki and Czapski (71) investigated the distribution and degradation of ^{14}C-ABA in apple seeds during stratification at 4°C. Total activity decreased in seed coats and endosperm but increased in cotyledons and embryonic axes. Seeley and Powell (75) found that levels of ABA were high in vegetative apple buds during mid-summer before and after onset of dormancy. ABA content increased to a maximum just prior to leaf fall and decreased to a minimum just prior to bloom. Leaf removal reduced rest intensity. The authors concluded that ABA peak and rest intensity are well correlated. Balboa-Zavala and Dennis (4) investigated the content of ABA in apple seeds as they mature. ABA levels were fairly high prior to the onset of dormancy. As the latter developed both, free and bound ABA decreased and as the embryos became fully dormant the ABA levels rose sharply. ABA levels in seed coats and cotyledons did not appear to be related to the intensity of dormancy. Patterns of ABA changes in apple buds ('Antonovka') were similar at 4° and 24°C (13). High chilling peach seeds contained higher levels of free and bound ABA-like inhibitor(s) than low chilling ones (27). Diaz and Martin (27) suggested that this difference in initial ABA content could be the cause of the difference in chilling requirements. ABA-like activity in peach flower buds increased during autumn until leaf fall, then decreased throughout winter until rest termination; levels of ABA were correlated with chilling requirement of flower buds of three peach cv (15). During maturation of peach seeds the ABA levels increased in embryonic axes but remained low in the seed coats (11). The pattern of ABA in maturing peach seeds varied from year to year but germination properties did not vary accordingly. In most early studies on ABA levels vs intensity of rest no attempt was made to determine the effect of stratification temperature on ABA content. When this precaution was taken, ABA content in apple (4) and peach seeds (12) declined in a similar fashion at 5^{o} and $20^{o}C$. But only the former treatment was effective in breaking dormancy. The authors concluded that ABA alone does not control dormancy. The decline in ABA content may be a prerequisite for dormancy removal but chilling must have one or more additional effects. Ramsay and Martin (68) investigated the seasonal changes of growth promoters and inhibitors in apricot buds. The onset of rest was correlated with a decrease in inhibitor and an increase in GA-like activity. The end of rest was correlated with a decrease in the level of both ABA and GA-like activity. Inhibitor content decreased to undetected levels as chilling proceeded. Dennis et al. (26) found no consistent correlation between ABA content and seed dormancy in several species of Pyrus. Using seeds with a wide range of chilling requirements from 2 wk ('Bartlett') to 13 wk ('Russian') the authors could not relate either ABA or DPA levels to the depth of dormancy. Mielke and Dennis (64) reported that both free and bound ABA levels increased during autumn leaf abscission in sour cherry flower buds. The increase in ABA was prevented by mechanical defoliation. However, early manual defoliation did not affect rest intensity. Levels of endogenous ABA decreased in buds of several *Vitis vinifera* cv during cold treatment (31). However, ABA content was higher in lateral buds destined to grow the year of formation than in dormant axillary buds which required a chilling treatment. The authors concluded that the interaction (if any) between ABA and chilling would be of secondary importance in bud burst of grapes.

Karssen et al. (52) used ABA-deficient mutants of *Arabidopsis thaliana* to determine: 1) the origin of ABA in seeds; 2) the role of ABA in onset of dormancy; 3) the regulatory site of seed dormancy induction. Reciprocal crosses of wild type and ABA-deficient mutants showed a dual origin in developing seeds: seed coat ABA, which peaks mid-way through seed development, and embryonic ABA, which reached much lower levels, but persisted for sometime after the maximum in maternal (seed coat) ABA. The onset of dormancy correlated well with the presence of the embryonic ABA fraction but not with seed coat fraction. Effect of maternal ABA was similar to that of exogenously applied ABA which did not induce dormancy in ABA-deficient mutants. Dormancy was induced only when the genome of the embryo contained the dominant allele and thus the embryonic ABA fraction. Maternal ABA was not related to dormancy induction; dormancy developed despite its absence. The pattern of dormancy induction was dependent upon the genotypes of embryo and endosperm. The authors stated that the probability that ABA and dormancy are not causally related is very low since genetic analysis showed that a single gene is involved in the mutation. In other species such as tobacco, in which the female parent has a greater influence on dormancy induction than the pollen parent, the maternal genotype may be more actively involved in onset of dormancy. A dual origin of ABA has not been established in seeds of other species.

SUMMARY

Considerable effort has been made to determine the relationship between ABA content and dormancy in seeds and buds. ABA declines during chilling in almost all species investigated (10). However, the decline in ABA content is not limited to conditions that break dormancy. ABA can decline at the same rate and to the same level when imbibed seeds are held at warm temperatures which are inefficient in breaking rest. Black (10) postulated that a decrease in ABA content is not sufficient for dormancy removal. The latter must require changes, in addition to, or other than, a decrease in ABA. In fact, ABA may decline or disappear several weeks prior to the satisfaction of seed chilling requirement (5). Other changes required for dormancy removal could involve other growth regulators.

MECHANISMS OF ALTERNATING TEMPERATURES

Low temperature is required for breaking dormancy, while high temperatures generally have a negative effect (84, 53, 96, 41). High temperatures inhibit chilling accumulation when interspersed during stratification. Purvis and Gregory (67) observed that high temperatures (25^oC) negate the effect of low temperatures (10^oC) on vernalization of Petkus winter rye. Devernalization was temperature and cycle length dependent. They postulated that the first step in vernalization is reversible. When high and low temperatures are alternated the advance towards the vernalized condition occurs during low temperature and during the subsequent high temperature period the effect is partially or completely annulled. The net effect of a single cycle would depend upon the temperature coefficients of the forward and backward reactions. The total effect of a series of cycles will depend upon the mode of summation of the effect of the individual cycle as well as on the change in sensitivity as vernalization proceeds. When relative durations of exposure to low and high temperatures were maintained at a fixed ratio the degree of reversal was inversely proportional to the absolute cycle length. The vernalization is further fixed if a period of moderate temperature (15°C) precedes the exposure to high temperature.

Purvis and Gregory (67) proposed a scheme to rationalize the effect of low and high temperatures and their interaction:

$$A \underset{a'}{\overset{a}{\rightleftharpoons}} A' \xrightarrow{b} B \underset{c'}{\overset{c}{\rightleftharpoons}} C \xrightarrow{d} D$$

a : occurs at low temperatures

a': occurs at high temperatures

b : occurs either at low or moderate temperatures (15°C)

c : occurs in the dark

c': occurs in the light

d : occurs in the light.

A is a precursor converted to A' at low temperatures, A'is an intermediary of a specific substance B, C is produced by reversible reaction proceeding forward in darkness (SD induction) and backward in light, and C to D proceeds only in light. C and D are substances responsible for flower initiation and development. B could also be converted to a substance E which could be a vegetative hormone favoring leaf production.

Erez and Couvillon (33, 34, 35), evaluating the effects of constant and alternating temperatures reported that a high temperature (> 20^{o}C) for 8 hr a day negated chilling accumulation. In contrast a moderate temperature (15^{o}C) promoted peach bud break when applied for 8 hr on a daily cycle in the later stages of dormancy. The authors proposed a scheme (a modification of Purvis and Gregory's) to explain the promotive effect of moderate temperatures on breaking dormancy of peach buds.

$$A \underset{a'}{\overset{a}{\rightleftharpoons}} B \xrightarrow{b} C$$

a : occurs at low temperature

a': occurs at high temperatures

b : occurs at low temperature but occurs at a faster rate at intermediate temperatures.

Intermediate temperatures permit more rapid conversion of B to C, thus increasing the efficiency of low temperature on breaking rest. In contrast high temperatures (> 20^{o}C), when interspersed with chilling temperatures, negate chilling accumulation and favor the conversion of B back to A (Figure 1). The chemical natures of A, B, and C are not known and no attempt has been made by the authors to explain what these precursors might be. In contrast to the two-step reactions proposed by Purvis and Gregory (67) and Erez and Couvillon (35), Saure (74) suggested that there could be two distinct but overlapping temperature reactions. The first reaction has a low optimum temperature, but as chilling accumulates this optimum widens. During early stages of chilling (true dormancy) the inhibition potential is much higher and chilling could be counteracted even by relatively moderate temperature

(Figure 2). As chilling proceeds (late dormancy) the buds would accumulate enough chilling so that the second reaction can proceed and the range of temperatures capable of negating subsequent chilling becomes narrower (> 23°C) (Figure 3).

CONCLUSIONS

Dormancy is a general phenomenon experienced by almost all plant organs during their life cycles. A considerable amount of work has been devoted to attempting to understand the physiological basis of rest. A number of models and concepts have been developed to explain the mechanisms of rest, but none is capable of explaining all phenomena. Some good correlations exist between physiological processes in seeds and buds and natural inhibitor or promotor levels. However, there are no cases known in which the role of an endogenous plant hormone has been proven beyond doubt.

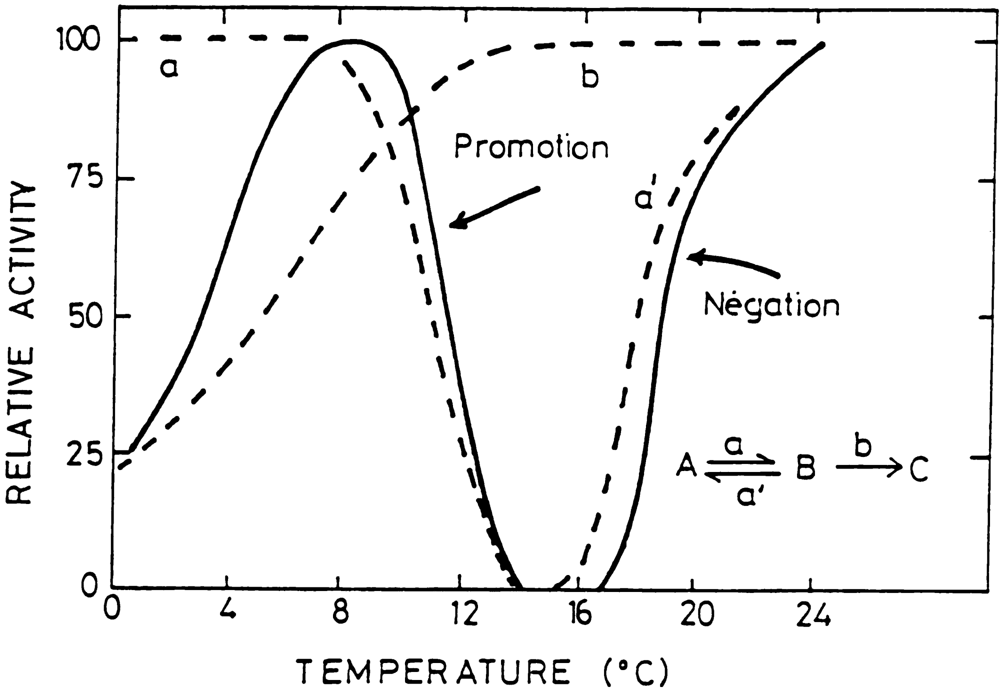

Figure 1. Temperature response curve of proposed reactions occurring during chilling of resting peach buds(Erez and Couvillon 1987).

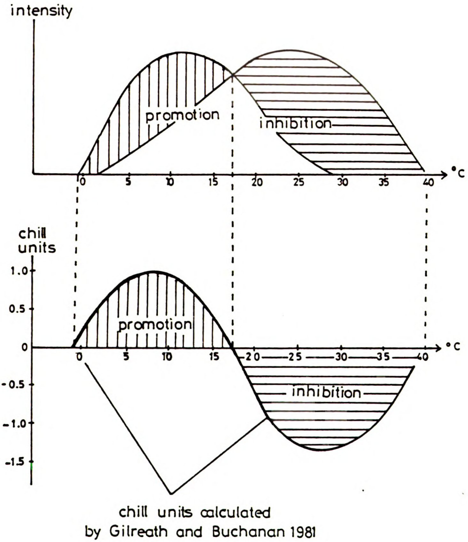

Figure 2. Hypothetical model of a dual temperature action in dormancy release as derived from calculated chill units. (Saure 1985).

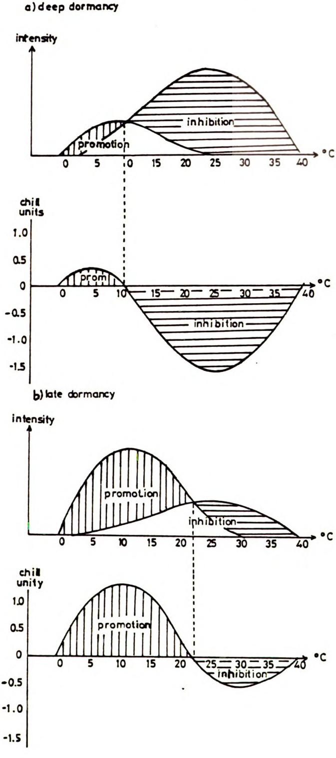

Figure 3. The widening temperature range for the promotion of budbreak and the reduced range for an inhibiting effect of higher temperatures as deduced from a hypothetical model of dual temperature action in dormancy release. (Saure 1985).

PART TWO

EFFECTS OF CONSTANT AND ALTERNATING TEMPERATURES ON BREAKING DORMANCY OF PEACH SEEDS

INTRODUCTION

Peach seeds (and buds), like those of other temperate fruit trees, go through a dormant period after maturity. Removal of such dormancy is usually accomplished by a moist cold treatment. Effective temperature range for removal of dormancy in rosaceous seeds and buds is between 0^o and 10^o, with an optimum between 2^o and 7^oC (76, 84). However, the optimum temperature does not appear to be constant throughout the chilling period but rather changes with time. Saure (74) speculated that as seeds or buds accumulate chilling the optimum is shifted to a higher temperature. Any temperature within the effective range can remove dormancy if allowed to act long enough. As a consequence chilling requirement is sometimes expressed as the number of weighted chilling hours (32). The chilling requirements of buds and seeds of the same species are very similar (53). Seeds are easy to manipulate and work with, and are therefore better suited for such studies.

PURPOSE OF MY RESEARCH

Since chilling requirement varies greatly among peach cultivars, my objectives were to determine: (a) the temperature response of peach seeds from several sources which differ in chilling requirements, (b) whether the effect of temperature changes as stratification proceeds, and (c) whether the promotive effect of moderate temperature observed in peach buds also occurs with seeds.

MATERIAL AND METHODS

Seeds of 5 different types were used: Siberian C (SibC), Lovell, a Chilean seedling, a Brazilean seedling (Pessigo), and FL 9-4 which have 800, 1000, 450, 450, and 150 hr chilling requirements, respectively. The seeds were obtained from the following sources: Siberian C - Hilltop Nurseries, Hartford, Michigan; Lovell - a commercial orchard in California; Chilean - Chile; Pessigo - Brazil; and FL 9-4 - Dr. Wayne Sherman, University of Florida, Gainesville, FL. In all experiments the seeds were treated in a similar fashion: dry peach pits 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 hr. Seeds were 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 various periods of stratification, germination capacity was evaluated by holding seeds for 10 days at 20°C in the dark. The final germination (%) represents total germination during stratification plus 10 days at 20°C. For alternating temperature treatments seeds were held in styrofoam boxes placed in a 5°C room. The temperatures within the boxes were raised for specified periods of time each day by using a time-clock to illuminate an incandescent light bulb. A thermostat prevented overheating and a fan mixed the air inside the box to equalize temperature. Controls were kept at constant temperatures (5° for alternating and 20°C for continuous temperature treatments).

RESULTS

Section I. Effects of constant temperatures throughout stratifcation period

A. Effect of stratification temperature on germination of peach seeds with varying chilling requirements

The purposes of this experiment were to determine the temperature response curves of peach seeds exhibiting different chilling requirements, evaluate the nature of these differences and assess whether they are only quantitative (number of chilling hr) or qualitative as well. The results were analyzed factorially (temperature x time of stratification), after arcsin transformation of percentage germination for each cultivar (Figures 4-7).

Germination of SibC seeds increased steadily with time at both 0° and 5° and 0° was as effective as 5°C (Figure 4). Temperatures of 15° and 20° were totally ineffective in stimulating germination, whereas 10°C resulted in about 20% germination after 1344 hr. In Chilean seeds, 0° was most effective at 336 and 672 hr followed by 5°C (Figure 5). However, 10° was equally effective after 1008 hr, while 15° and 20°C were ineffective regardless of stratification time. The chilling requirement was completed after 672 hr at 0°, after 1008 hr at 5° or 10°C. Although chilling Pessigo seeds for 336 hr increased germination, response was essentially saturated within 672 hr at 0°, 5°, or 10°C (Figure 6). At 15°C some increase in germination was apparent following an additional 336 hr of chilling. Five degrees was somewhat more effective than 0° or 10°, 15° was much less so, and 20°C had little effect. In FL 9-4 seeds held at 5°, little response was obtained by extending chilling beyond 336 hr; however, at 0°C 672 hr of chilling was much more effective than 336 hr (Figure 7). Response to 10° paralleled that of Pessigo seeds at 15°, whereas neither 15° nor 20°C were effective.

Maximum germination at 20° was reached after exposure to 0°, 5°, and 10°C for 1008 hr for low chilling peach cv (Chilean, Pessigo, and FL 9-4) and and 1344 hr for SibC. (Figure 8). Chilean and Pessigo exhibited a broader temperature response curve than did SibC or FL 9-4. This suggests that some low chilling cv may have a wider range of effective temperatures that break dormancy. Gilreath and Buchanan (43) reported that low chilling cv of blueberry and peach have higher optimum temperature and broader effective temperature range.

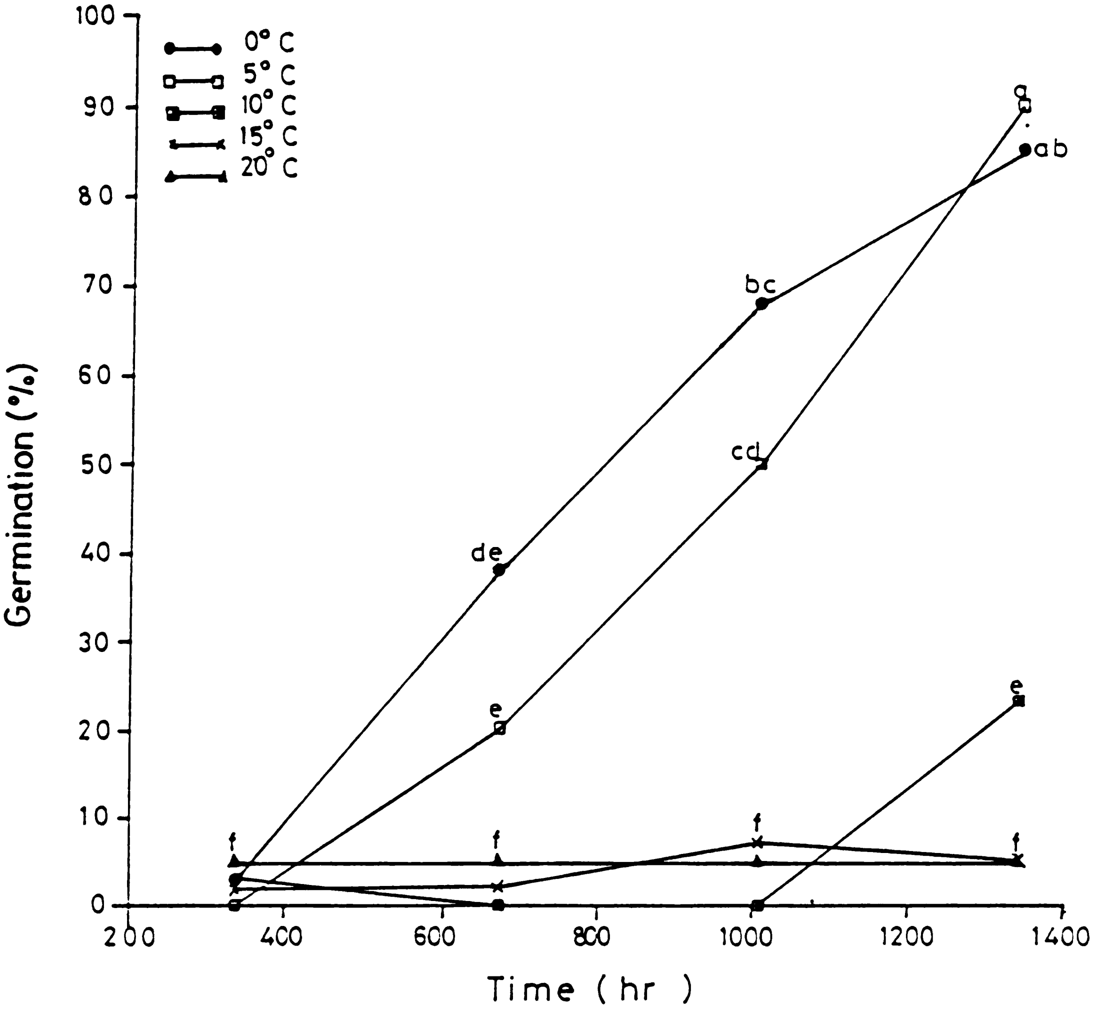

Figure 4. Effect of stratification temperature andduration ongermination(%) (during stratification plus 10 days at 20° C) of Siberian C peach seeds.

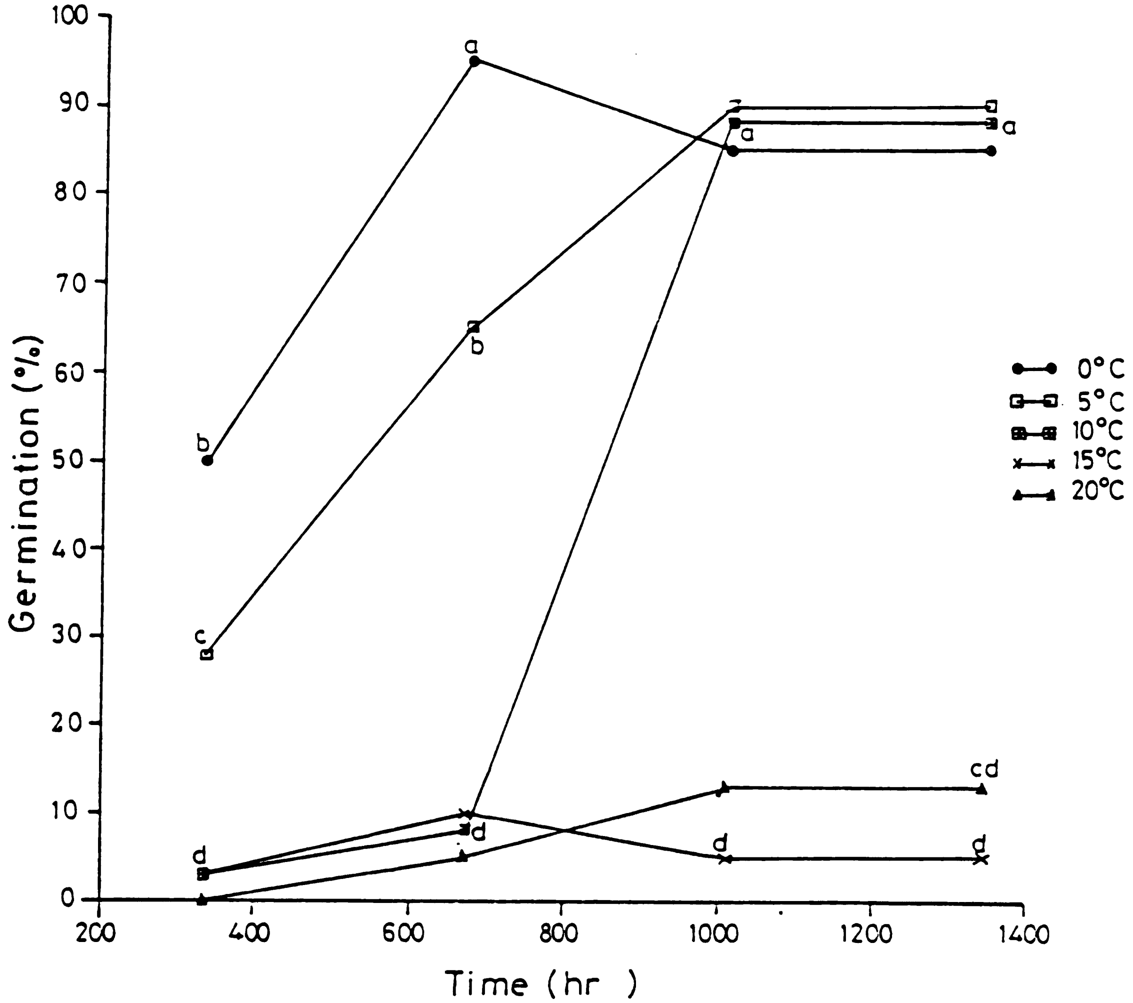

Figure 5. Effect of stratification temperature and duration on germination (%) (during stratification plus 10 days at 20°C) of Chilean peach seeds.

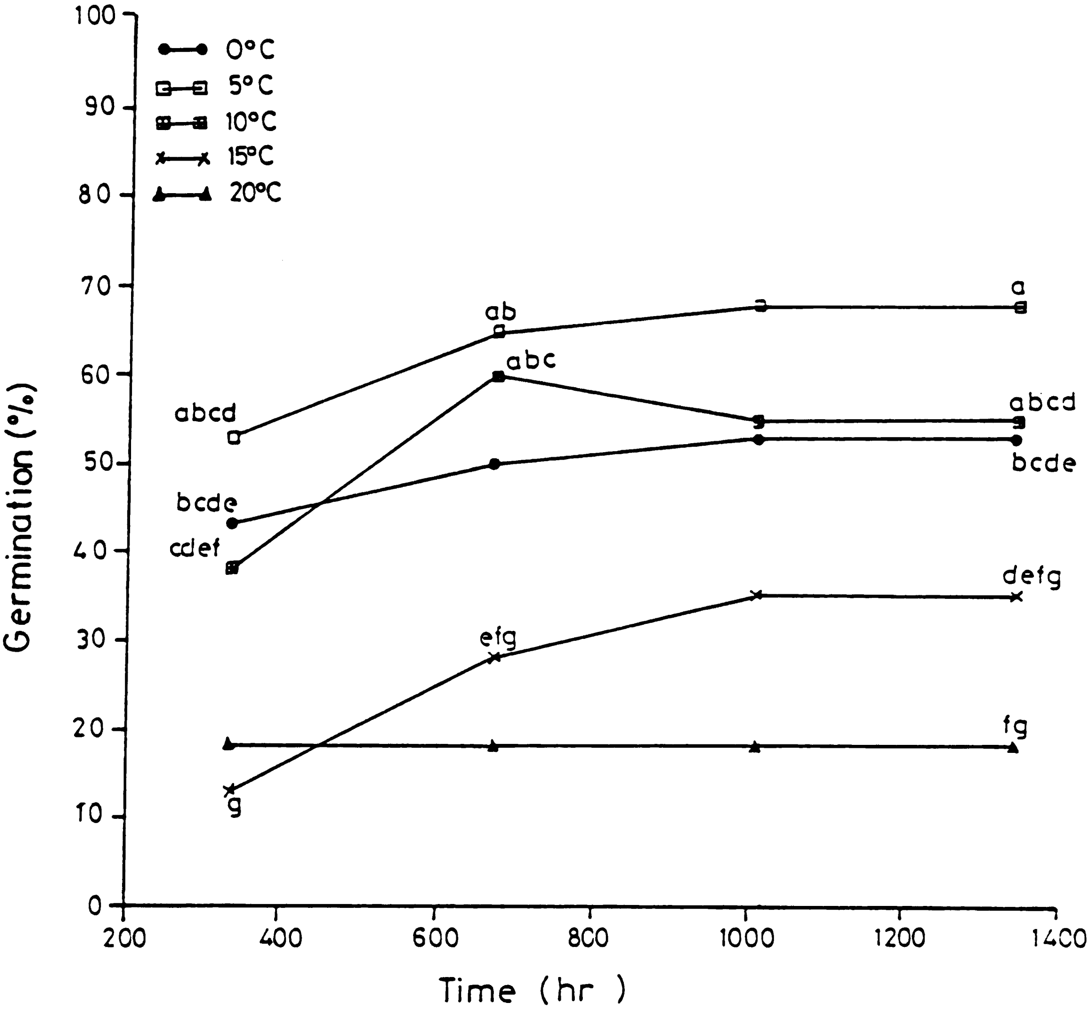

Figure 6. Effect of stratification temperature and duration on germination (%) (during stratification plus 10 days at 20°C) of Pessigo peach seeds.

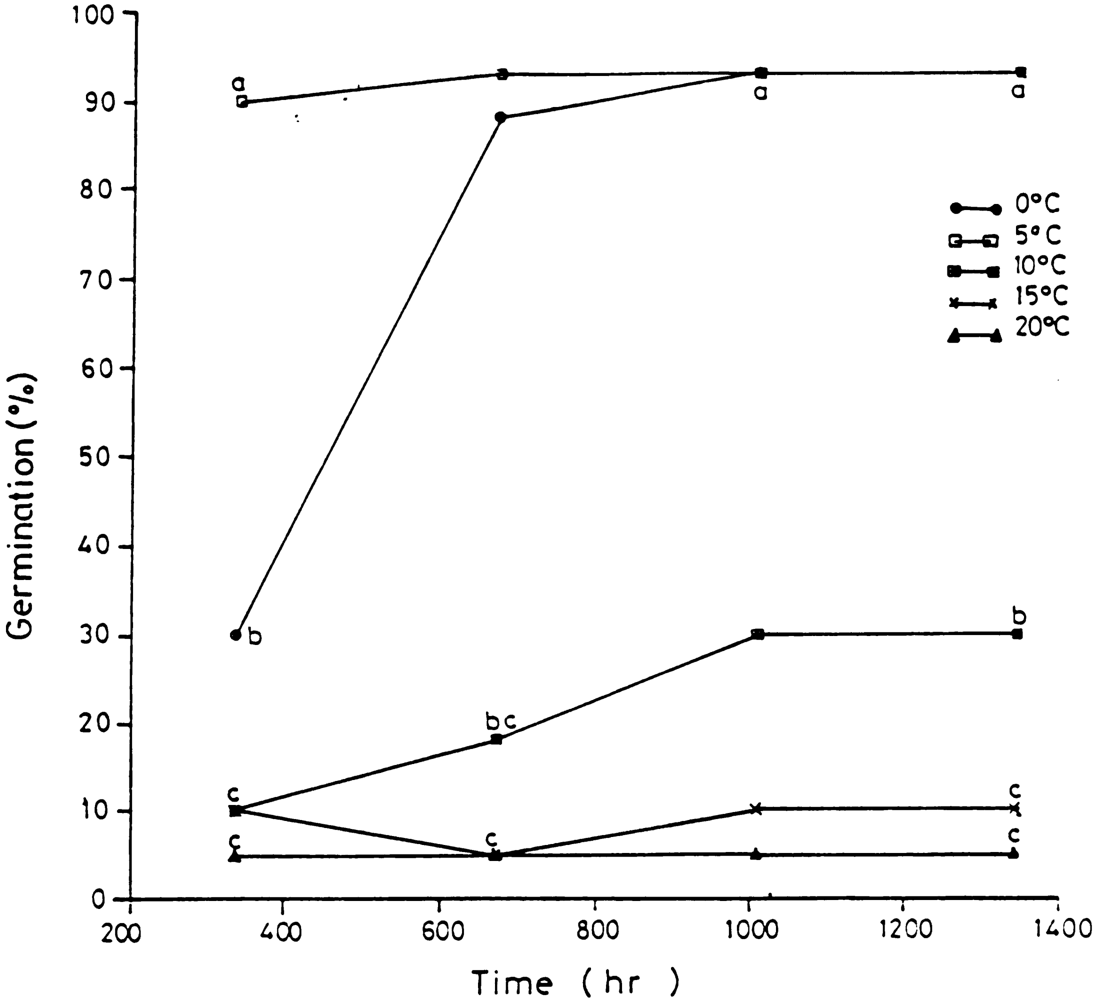

Figure 7. Effect of stratification temperature and duration on germination (%) (during stratificaction plus 10 days at 20°C) of FL 9-4 peach seeds.

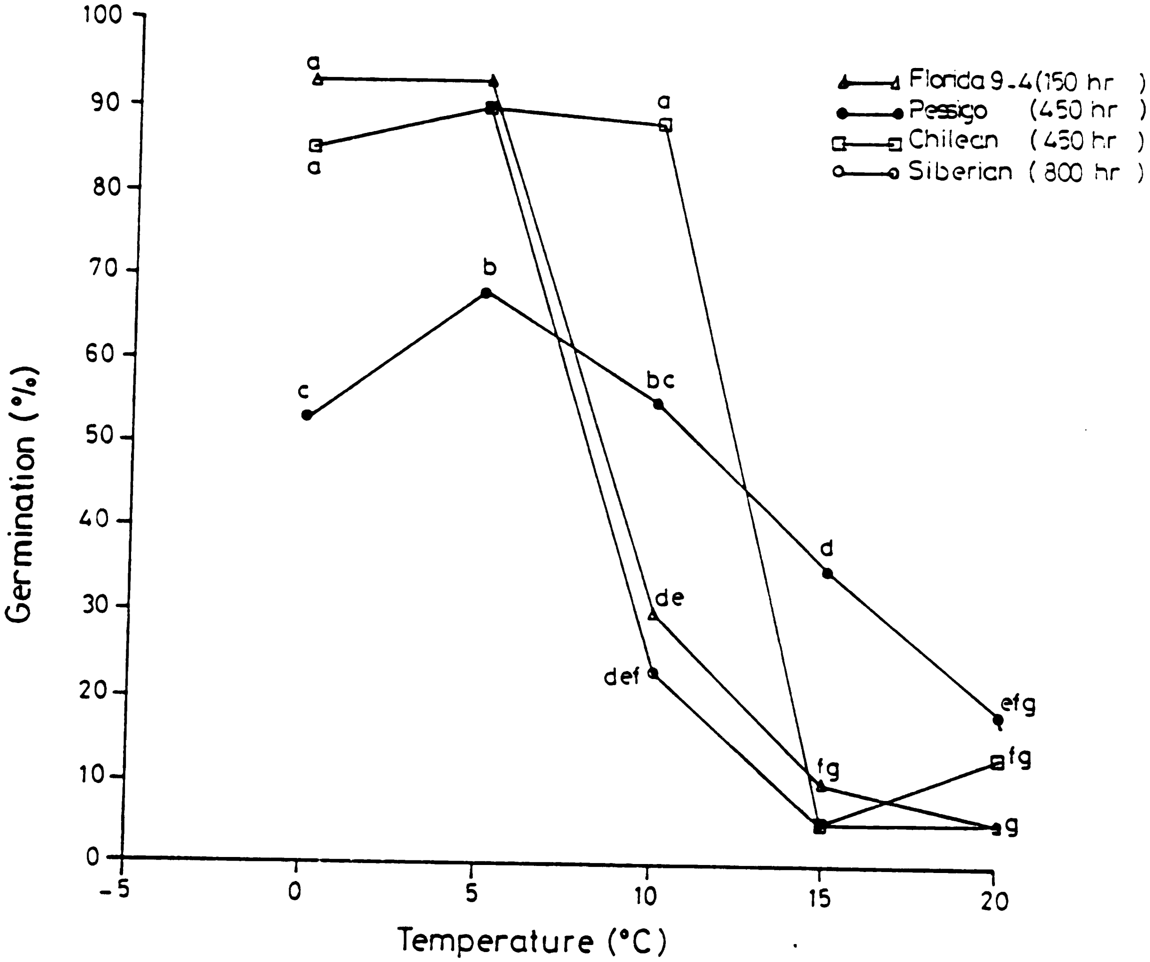

Figure 8. Effect of stratification temperature on germination (%) of peach seeds with varying chilling requirements (Siberian C 1344, Chilean, Pessigo and FL 9-4 1008 hr).

B. Effect of constant temperatures on peach seeds with high chilling requirements

The purpose of this experiment was to determine the optimum temperature and relative efficiency of temperature so that weighted chilling hr can be calculated. SibC and Lovell peach seeds were held at -5°, 0°, 5°, 10°, 15° or 20°C for periods up to 2016 hr (12 wk). The germination was determined after 10 days at 20°C following each stratification treatment. The results were analyzed statistically after transforming percentage data to arcsin square root values. Mean separation was performed by the Duncan's multiple range test at the 5% level. In the first experiment with SibC seeds, 5° appeared to be the optimum temperature, followed by 0°, 10° was less effective, although 60% of the seeds germinated after 2016 hr of stratification, and 15°C had very little effect (Figure 9). None of the seeds kept at -5° (freezing) or 20° had germinated after 12 wk. Apparently exposure to -5° irreversibly injured the seeds since no germination was observed even after the seeds were restratified at 5°C for 2184 hr. The experiment was repeated to determine whether the effect of the temperatures used was consistent. Again in this experiment 5° appeared to be the optimum temperature, while -5°C had a detrimental effect on seed viability (Figure 10). When seeds of 'Lovell', another peach cultivar with a high chilling requirement, were used the results were similar to those obtained with SibC. Five degrees again was optimum, followed by 0°, whereas 10° and 15°C had little and no chilling effect, respectively (Figure 11).

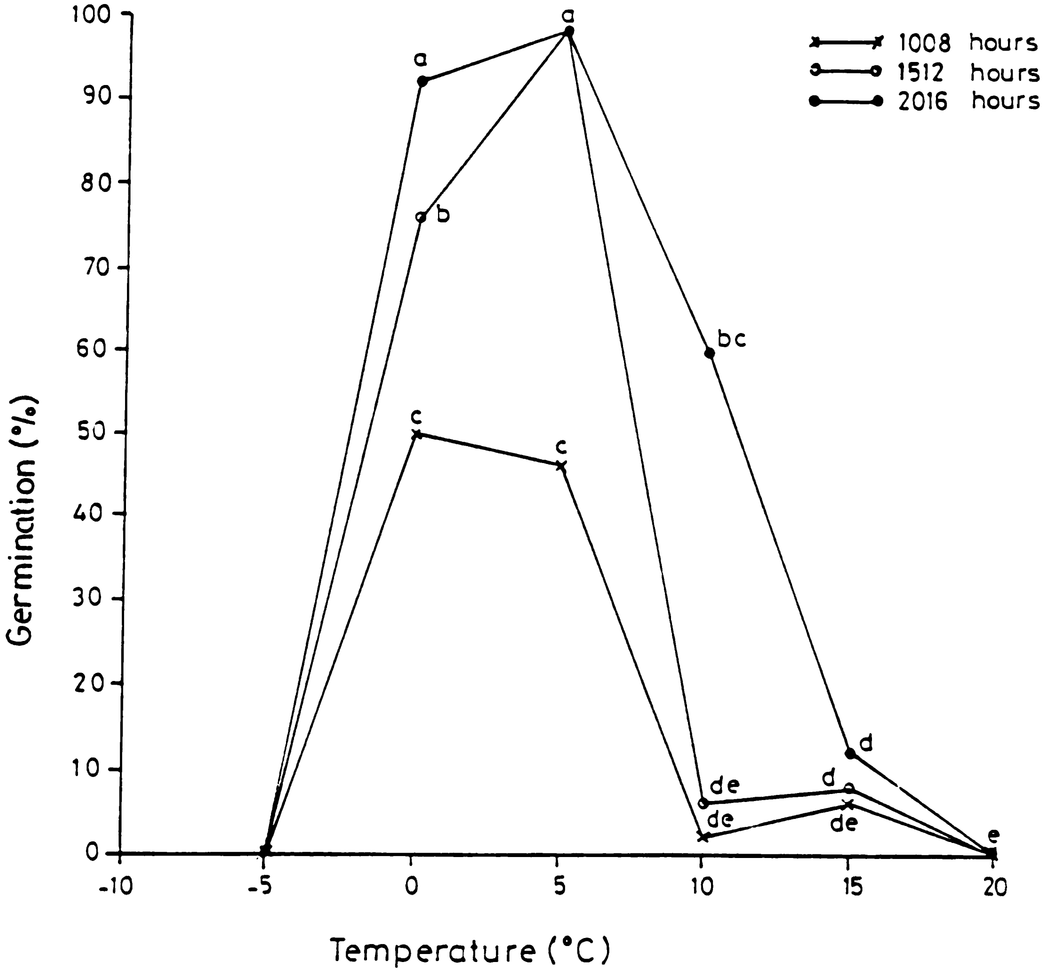

Figure 9. Effect of stratification temperature and duration on germination (%) (during stratification plus 10 days at 20°C) of Siberian C peach seeds.

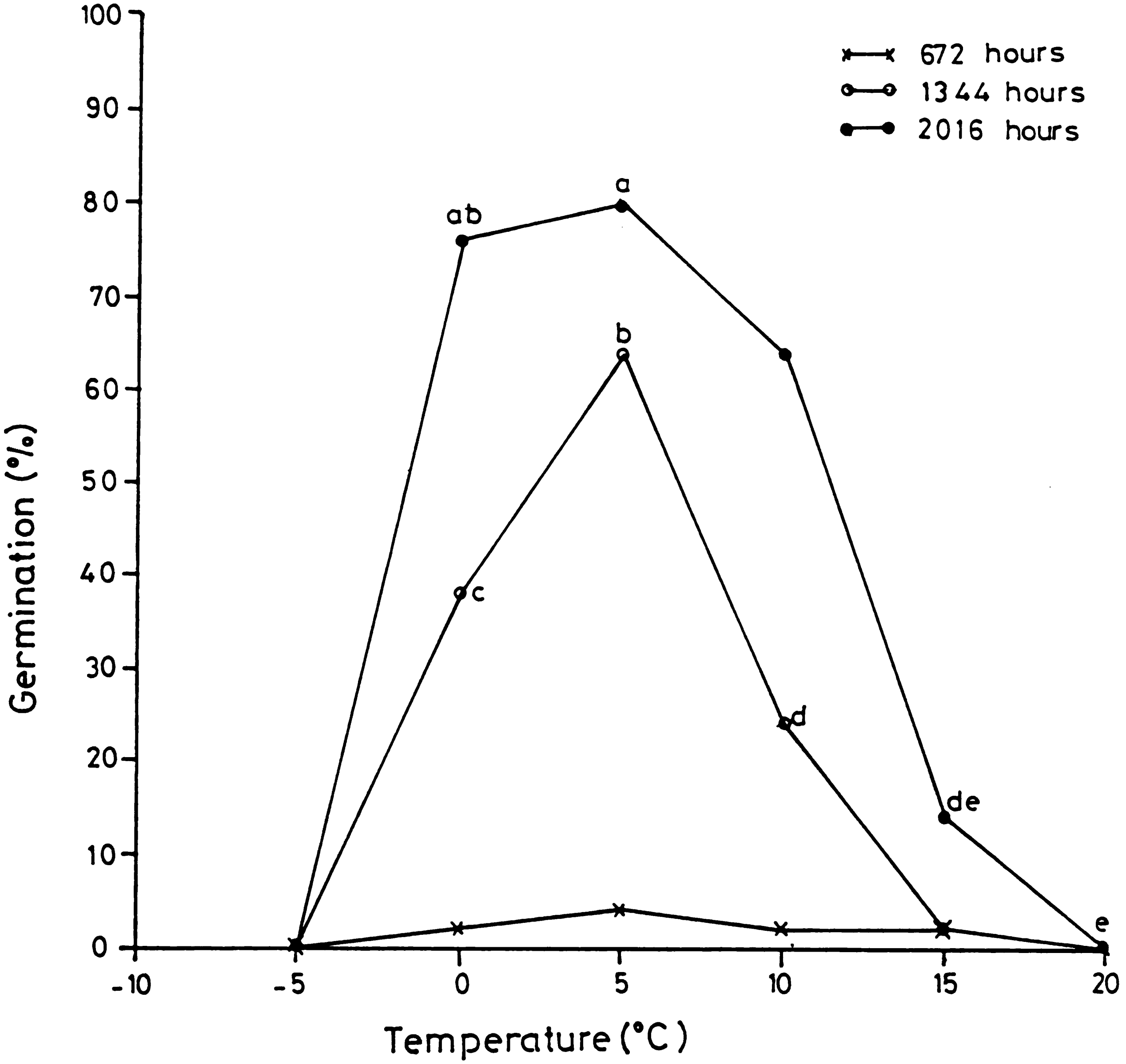

Figure 10. Effect of stratification temperature and duration on germination (%) (during stratification plus 10 days at 20°C) of Siberian C peach seeds.

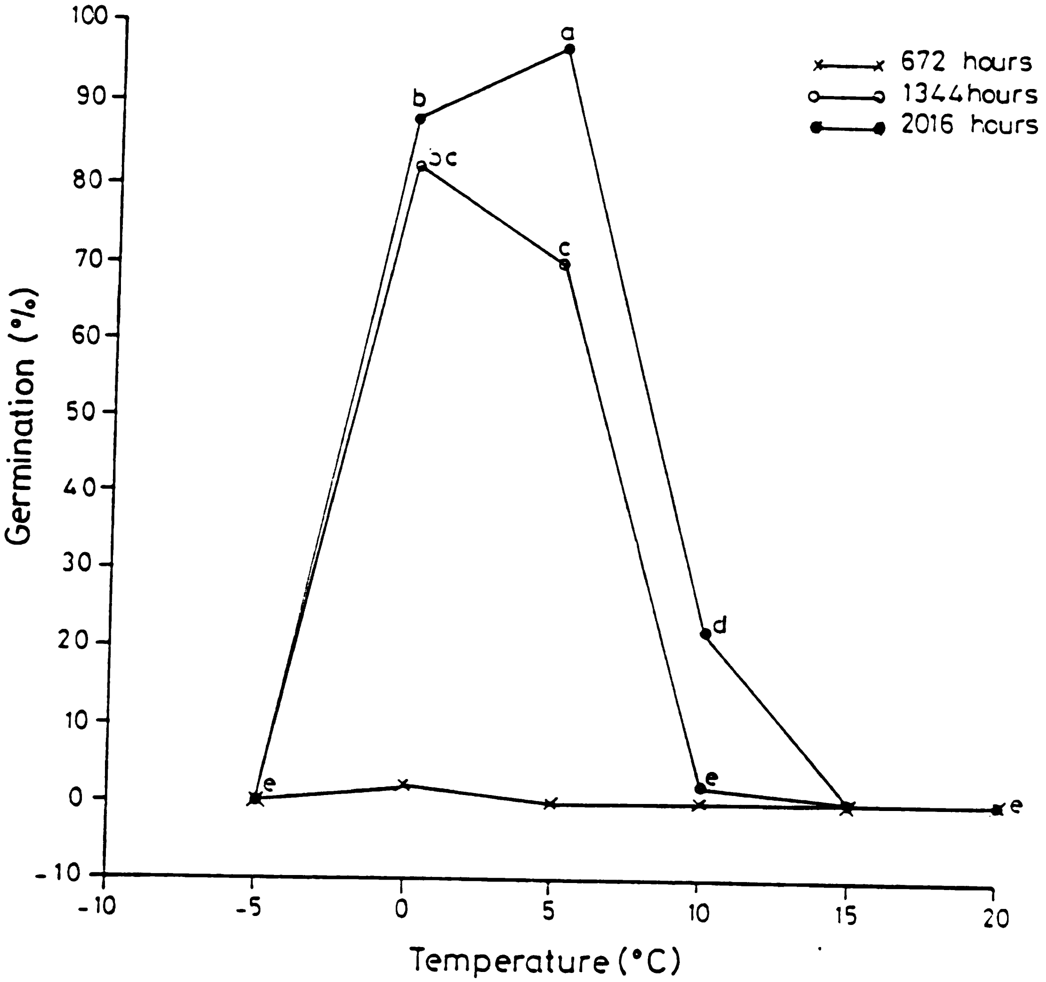

Figure 11. Effect of stratification temperature and duration on germination (%) (during stratification plus 10 days at 20°C) of Lovell peach seeds.

Fifteen degrees by itself had little chilling effect. The efficiency of 10° varied with stratification time and experiments as well as seed source. The effect of 10°C does not appear to be consistent; it was partially effective in some instances but ineffective in others. One hr at 10°C can be given a relative chill value of 0.4 in experiment 1 and 0.6 in experiment 2 for SibC and only 0.2 for Lovell. This variability appeared also with low chilling peach seeds.

Section II. Effects of alternating temperatures

The purpose of the following experiments was to 1) assess the effectiveness of moderate temperatures, on breaking dormancy when alternated with low temperature, 2) assess whether or not the promotive effect of such moderate temperatures observed with peach buds occurs also with seeds, and 3) evaluate the inhibitory effect of high temperatures in relation to cyle length and stage of stratification.

A. Effects of alternating temperatures in a diurnal cycle

1. Effects of continuously alternating temperatures in a diurnal cycle

SibC peach seeds were exposed to diurnal cycles (16 hr at 5° and 8 hr at 10°, 15°, 20°, 25°C) to determine if the promotive effect reported for peach buds occurs with seeds. The results were statistically analyzed as a factorial (temperature x time) after arcsin transformation of square root of percentage germination (Figure 12). Little difference was evident in percentage germination between treatments up to 672 hr at 5°, only constant 5° vs 5/20° differed significantly from one another. However, the 5/10° regime significantly promoted germination, once 896 hr at 5° were accumulated, in comparison with 1008 hr at continuous 5°C, but differences thereafter were non-significant. Alternating 5° with 15°, 20° or 25° significantly reduced chilling accumulation and even after the seeds held at these temperature regimes accumulated 1344 hr at 5°C the germination percentage remained very low. When the same alternating temperature regimes were applied to 'Lovell' 20° and 25° counteracted the effect of 5° regardless of period of stratification, and 15°C partially negated chilling accumulation. In contrast, 10°C had a promotive effect in early phases of chilling and was just as effective as the control thereafter (Figure 13).

The same cycle was used for the two low chilling peach cv Chilean and FL 9-4. Chilean seeds held continuously at 5°C gave the highest percentage germination on the first sampling date. However, after 672 hr at 5°, 5/10°, 5/13°, and 5/15°C all promoted germination, while 5/20° inhibited it. Both promotive and inhibitory effects disappeared when the cycle was repeated until the seeds accumulated 1008 hr at 5°C (Figure 14).

With FL 9-4 seeds moderate temperatures (10°, 13°, and 15°) when alternated with 5° were no more effective than continuous 5°C regardless of the chilling period. 5/20° caused partial negation when seeds were stratified for short periods, but not after 672 hr at 5°C (Figure 15).

Ten degrees (8 hr) when alternated with 5° (16 hr) during stratification promoted germination at 20° of seeds with high chilling requirements, however, a temperature of 15°C or higher inhibited it. Similar observations were reported by Aduib and Seeley (1) where 10° promoted while 15°C inhibited the germination of "Halford" peach seeds when these temperatures were applied in a diurnal cycle of 16/8 hr at 5°C and higher temperatures, respectively. Thus 15°C, does not appear to be as effective in removing seed dormancy as was reported for peach buds, suggesting that seeds may have a temperature threshold for enhancing chilling effect on 16/8 hr cycle. When moderate temperatures were applied to peach seeds with low chilling requirements they did not inhibit subsequent germination at 20°C. In fact both 13° and 15° promoted germination of Chilean peach seeds held for 672 hr at 5°C. However, 10° ~ 5° for Chilean, although 15°C is much less so.

2. Diurnal alternation at different stages of stratification

To determine if the effect of temperature alternation depends upon the chilling accumulated or stage of stratification, the chilling was divided into 3 equal subperiods (I, II, III) of 504 hr each. The seeds were held at continuous 5°, or at 5/10°, 5/13°, 5/15°, or 5/20°C (16 hr at 5° and 8 hr at higher temperature). Temperatures were alternated either throughout the chilling period or only during one or two of the 3 periods.

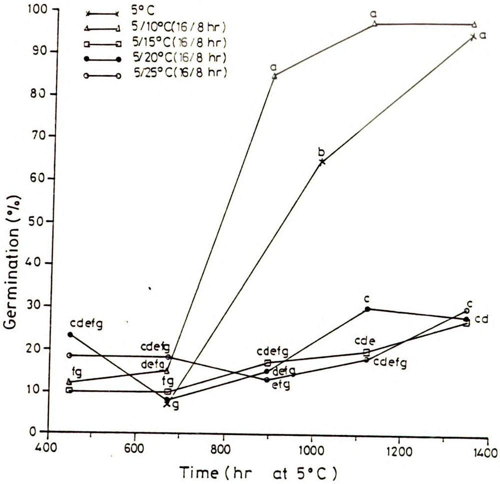

Figure 12. Effect of continuous vs alternating temperatures (5°C for 16 hr alternated with higher temperatures for 8 hr) during stratification on germination(%) (during stratification plus 10 days at 20°C) of Siberian C peach seeds.

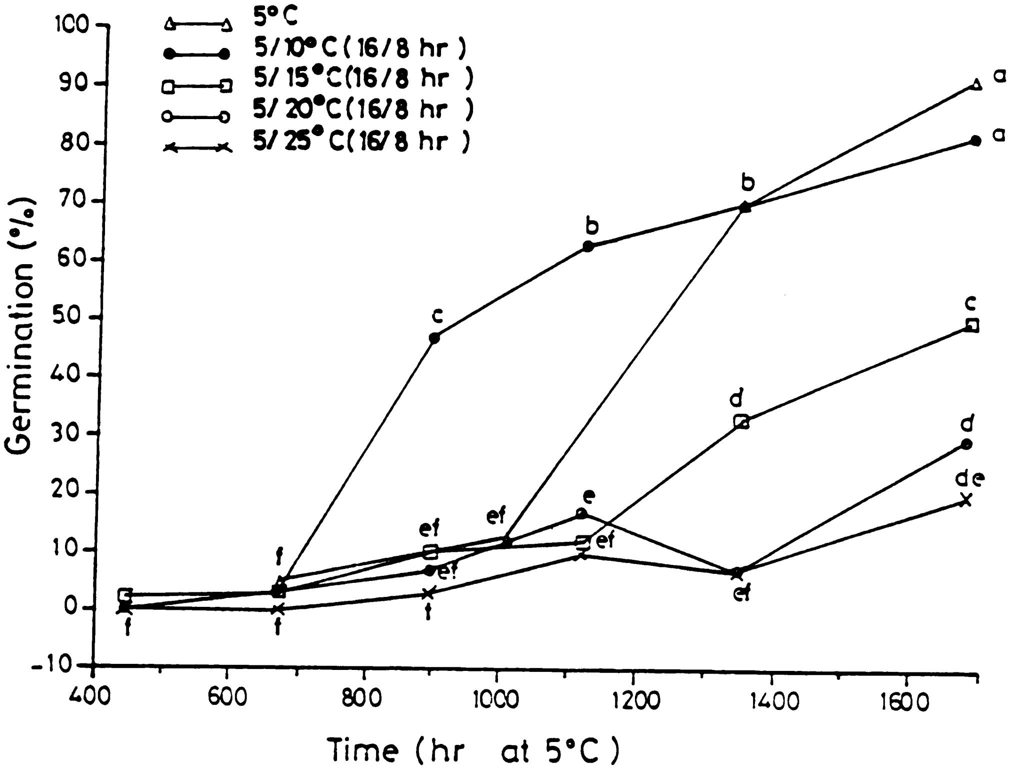

Figure 13. Effect of continuous vs alternating temperatures (5°C for 16 hr alernated with higher temperatures for 8 hr) during stratification on germination(%) (during stratification plus 10 days at 20°C) of Lovell peach seeds.

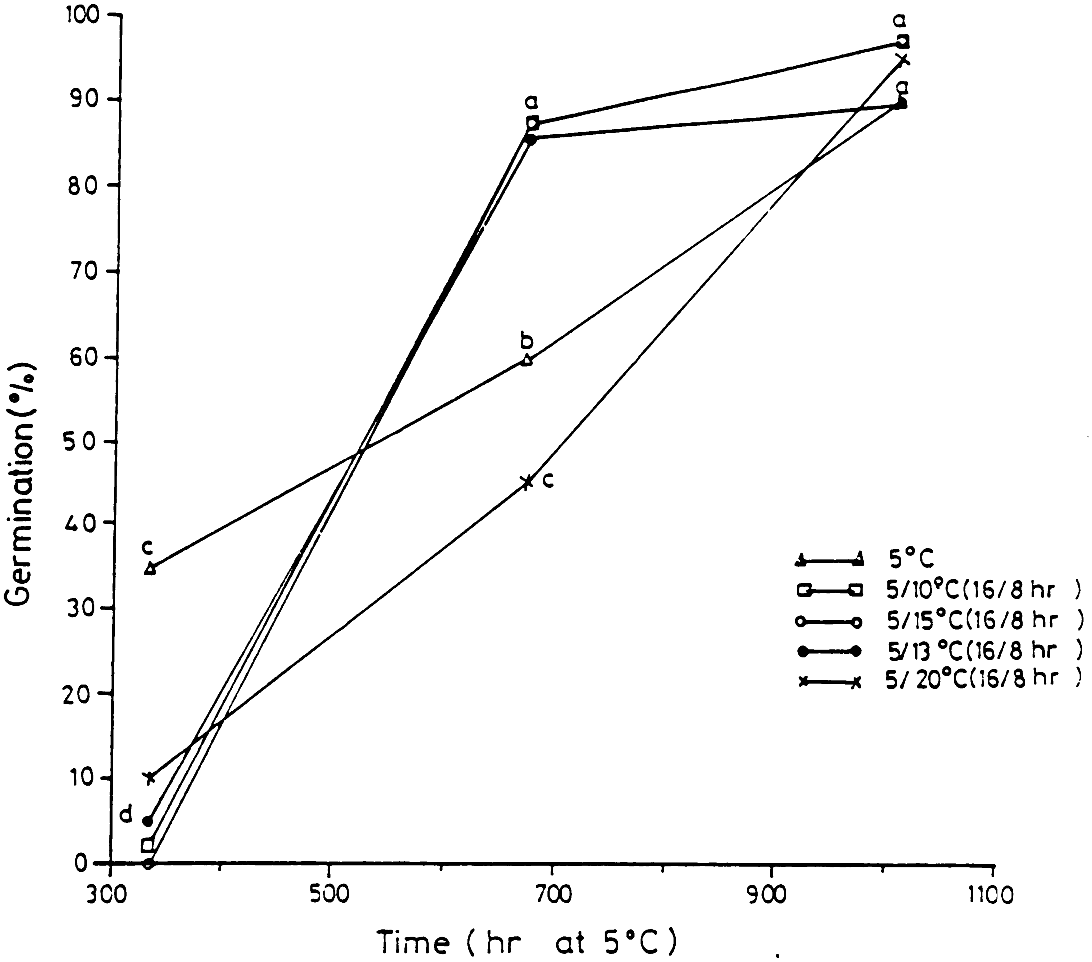

Figure 14. Effect of continuous vs alternating temperatures (5°C for 16 hr alternated with higher temperatures for 8 hr) during stratification on germination (% during stratification plus 10 days at 20°C) of Chilean peach seeds.

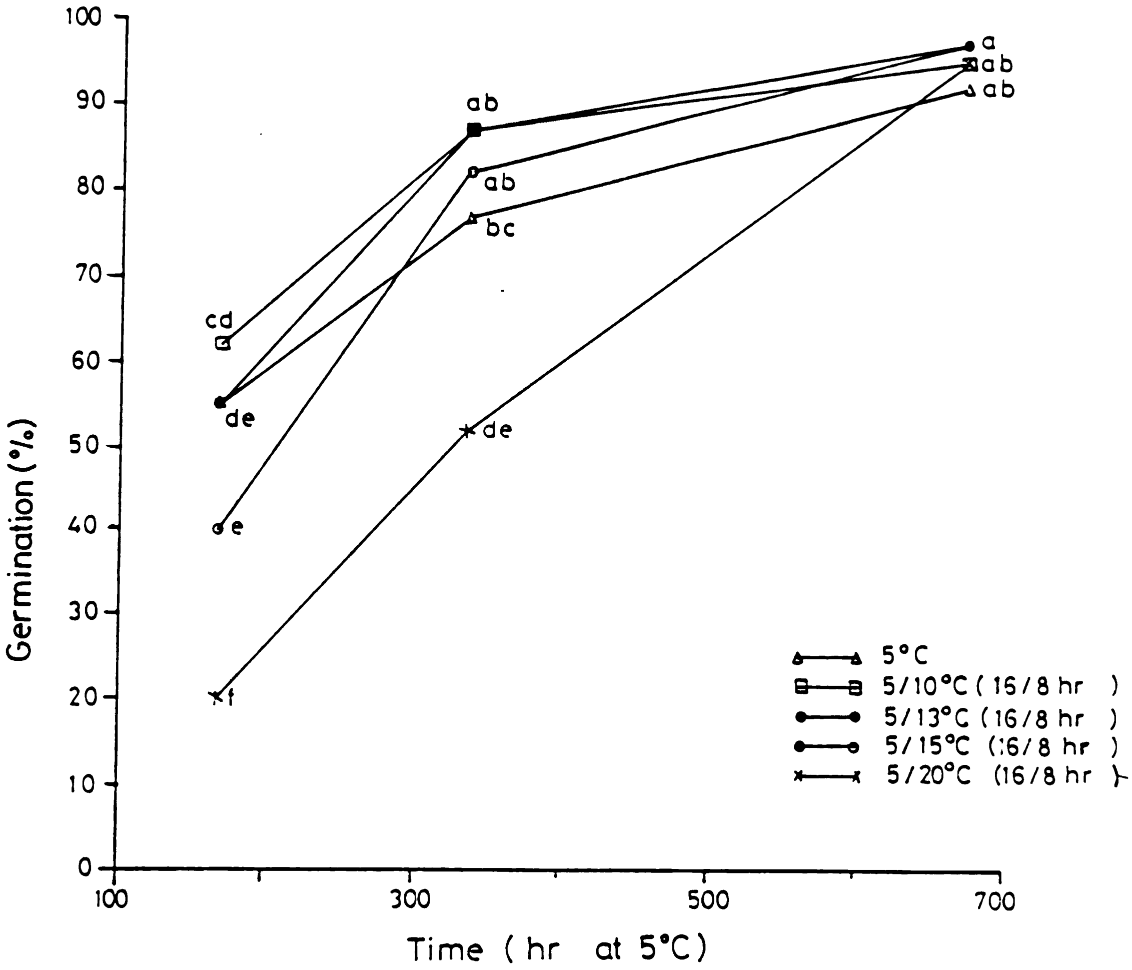

Figure 15. Effect of continuous vs alternating temperatures (5°C for 16 hr alternated with higher temperatures for 8 hr) during stratification on germination (%) (during stratification plus 10 days at 20°C) of FL 9-4 peach seeds.

a. Effect of continuously alternating temperatures

All treatments were continued until 1008 hours at 5°C were accumulated (Figure 16). Alternating temperature of 5/10° again promoted germination while 5/15° and 5/20° inhibited it; 5/13°C reduced germination, but not significantly.

b. Diurnal alternation during 1 of the 3 stages

In this experiment the seeds were held at alternating temperatures (5/10°, 5/13°, 5/15°, or 5/20°) for one of the three stages (I, II, or III) and at constant 5° for the two other stages until all the treatments had accumulated 1344 hr at constant 5°C. The final percentage germination after 10 days at 20°C was transformed to arcsin square root (germination %) and analyzed as a two factor (temperature x stage) factorial.

Temperatures of 13°, 15°, and 20° all inhibited germination significantly when alternated with 5°C during the first period (I) of stratification (Figure 17). However, they had no significant effect in subsequent two stages. A promotive effect of 10° was not evident, probably because the control germinated well after 1344 hr at 5°C; promotion would have required a shorter chilling period. It is difficult to explain the inhibitory effect in early stages, of a temperature regime such as 5/13° or 5/15°C. The same effect was apparent in peach buds (35) although, the authors did not attempt to explain the inhibition.

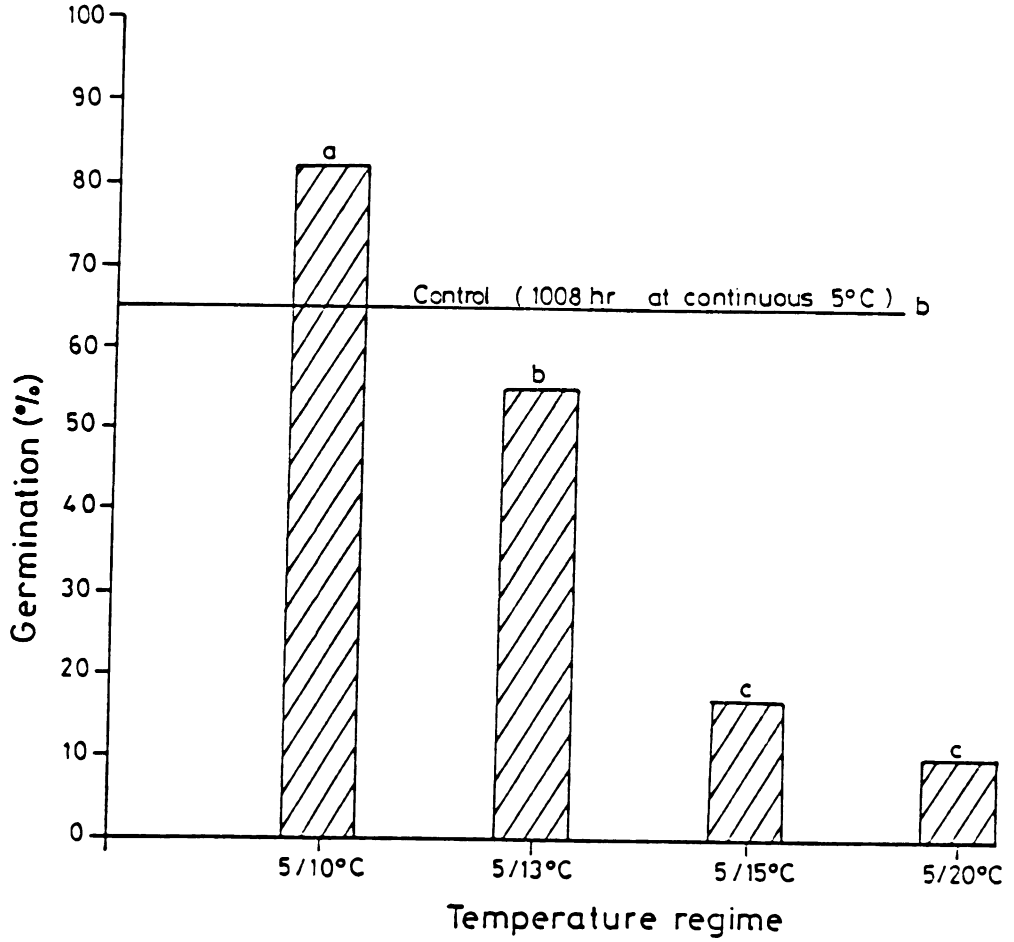

Figure 16. Effect of stratification temperature 5°C vs 5° for 16 hr cycled with higher temperatures for 8 hr, for 1008 hr at 5°C, on germination (%) (during stratification plus 10 days at 20°C) of Siberian C peach seeds.

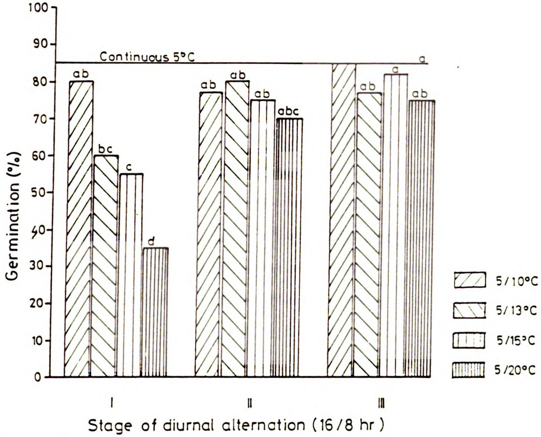

Figure 17. Effect of stratification temperature 5°C vs 5° for 16 hr alternated with higher temperatures for 8 hr, for 1344hr at 5°C, on germination (%) (during stratification plus 10 days at 20°C) of Siberian C peach seeds.

c. Diurnal alternation at any 2 of the 3 stages

All seeds were held for 1176 hr at 5°C. The results are summarized in Figure 18. 5/13° and 5/15° regimes did not inhibit germination significantly in this experiment as they did when applied in the first stage alone (Figure 17). However, in the first experiment seeds accumulated more chilling (1344 vs 1176 hr) and more total time. Only the 5/20°C temperature regime significantly negated germination when applied during the first and second or first and third stages. None of the temperatures significantly inhibited germination in the later stages. This suggests that either the chilling unit changes with time, or that there are two distinct but overlapping reactions with two different optimum temperatures, as Saure (74) suggested. Therefore the inhibitory effect of a high temperature in early stages could be compensated for by its promotive effect as chilling accumulates.

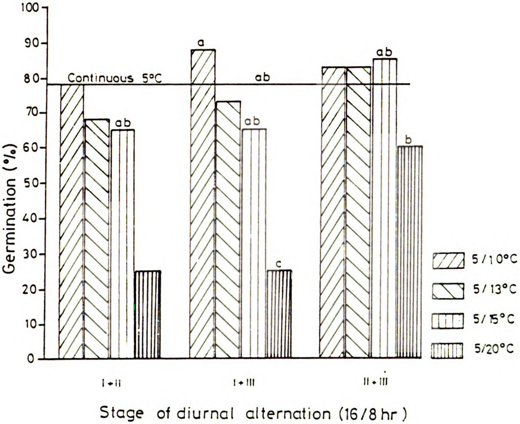

Figure 18. Effect of stratification temperature 5°C vs 5° for 16 hr alternated with higher temperatures for 8 hr, for 1176 hr at 5°C, on germination (%) (during stratification plus 10 days at 20°C) of SiberianC peach seeds.

B. Alternating temperatures in long cycles

1. First experiment

In addition to diurnal alternation, the effect of a series of long cycles was examined in order to determine whether or not the promotion or negation of a certain temperature is dependent on cycle length. In a first experiment, the ratios of days at 5°C to days at higher temperatures (10°, 15°, 20°, and 25° used were 1/1, 2/1, 3/1, 2/2, 4/1, 5/1, 3/3, 4/2, 6/1, 4/4, 6/3, and 5/5. All cycles were repeated until the seeds accumulated 1344 hr at 5°C. The percentage germination data were transformed to arcsin of square root of percentage germination, then analyzed as a 2 factor factorial.

a. Cycles of days at 5°C/days at higher temperature-1/1

Temperature significantly affected response, but cycle length did not, and interaction was non-significant (Figure 19). 5/20° and 5/25°C significantly reduced germination regardless of cycle length, whereas 5/10° and 5/15° had neither a promotive nor an inhibitory effect. Probably germination response was saturated following chilling for 1344 hr. Promotion or inhibition by a moderate temperature would have required shorter stratification periods.

b. Cycles of days at 5°C/days at higher temperature-2/1

At this ratio the main effects of both temperature and cycle length, as well as their interaction, were significant (Figure 20). Only 20° and 25°C significantly inhibited germination. The degree of inhibition by 20°, but not 25°, decreased as the cycle length increased from 6 days to 9 days. No promotion was apparent at any temperature or cycle length, probably because the seeds held at continuous 5°C were approaching maximum percentage germination after 1344 hr.

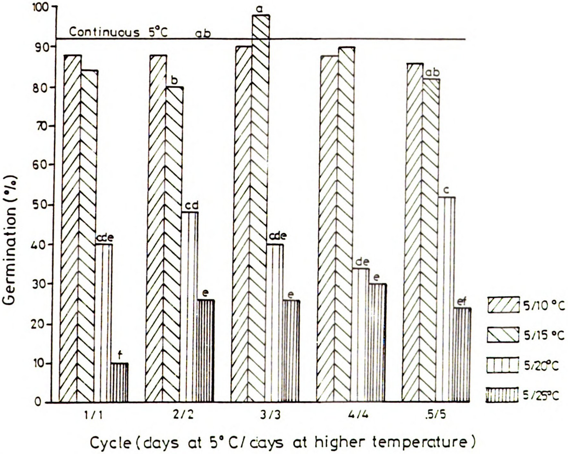

Figure 19. Effect of stratification temperature 5°C vs 5° alternated with higher temperature, for 1344 hr at 5°C, on germination (%) (during stratification plus 10 days at 20°) of SiberianC peach seeds.

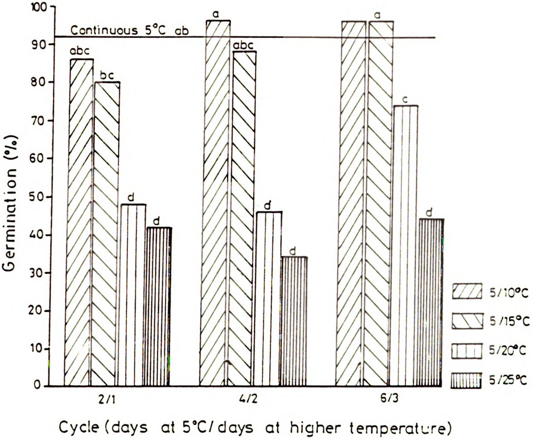

Figure 20. Effect of stratification temperature 5°C vs 5°alternated with higher temperatures, for 1344 hr at 5°C, on germination (%) (during stratification plus 10 days at 20°C) of Siberian C peach seeds.

c. Cycles of a single day at high temperatures and increasing time at 5°C

Main effects of both temperature, cycle length and their interaction were significant (Figure 21). Moderate temperatures (10° and 15°C) did not significantly reduce germination at any cycle length. However, 20° and 25°C partially negated chilling on short cycles but not on those longer than 4 days. Thus, the inhibitory effect of high temperature declines as the proportion of time at low temperature increases. Four days at 5°C are required to prevent the inhibitory effect of high temperatures. The threshold cycle for both 20° and 25°C appeared to be 4 days. Any cycle shorter than this threshold significantly inhibited chilling accumulation. However, no beneficial effect of moderate temperatures (10° or 15°C) was evident since the stratification period was too long (1344 hr at 5°C) and germination percentage was saturated.

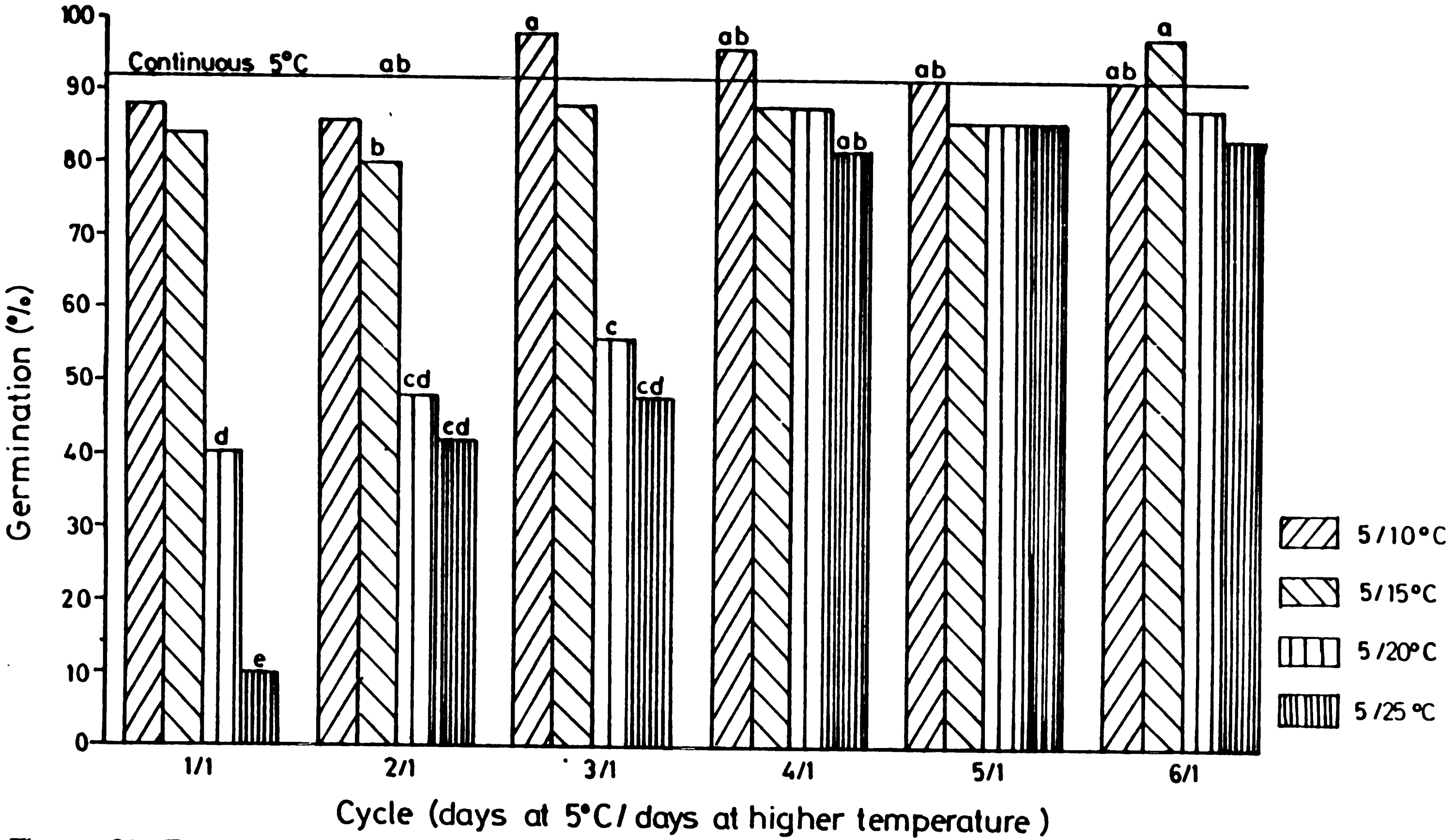

Figure 21. Effect of stratification temperature 5°C vs 5° alternated with higher temperatures, for 1344 hr at 5°C, on germination (%) (during stratification plus 10 days at 20°C) of Siberian C peach seeds.

2. Second experiment

Siberian C peach seeds were subjected to the same treatments as in experiment 1, but the time of exposure to 5°C was limited to 1008 hr. In addition to long cycles a diurnal cycle (16/8 hr) was included.

a. Cycles of days at 5°C/days at higher temperature-1/1

Ten degrees, 13°, 15° and 20° were alternated with 5°C for 16/8 hr, 1/1, 2/2, and 3/3 days respectively. The results are summarized in Figure 22. The alternating 5/10°C promoted germination in comparison with constant 5°, but the difference was significant only on the 6 day (3/3) cycle. The effects of 13° were non-significant, but 15°C significantly inhibited chilling accumulation in the diurnal and the 2 day cycles. In contrast 20°C had an inhibitory effect regardless of cycle length. However, when main effects for temperature were compared 10° and 13° significantly promoted chilling accumulation while 15° and 20°C inhibited it. The main effects for cycle length and interaction were not significant.

b. Cycles of days at 5°C/days at higher temperature-2/1

The cycles used were 16/8 hr, 2/1, 4/2, and 6/3 days. 10° and 13° slightly promoted germination (not significant at 5% level) in comparison with continuous 5°, while 15°C inhibited it on diurnal and 3 day cycles but not on longer ones (Figure 23). This confirms the fact that the inhibitory effect of 15° is cycle length dependent. On the other hand, 20°C was inhibitory, regardless of cycle length.

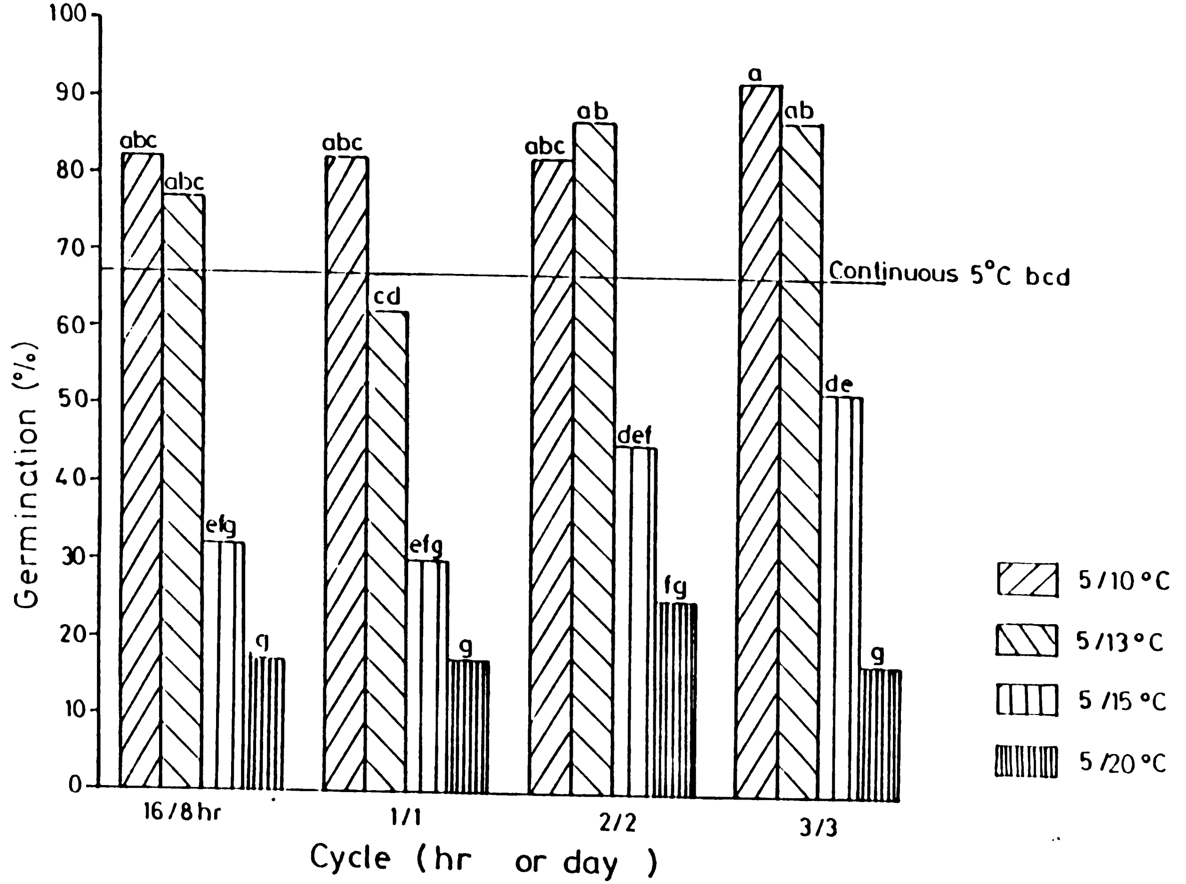

Figure 22. Effect of stratification temperature 5°C vs 5° alternated with higher temperatures, for 1008 hr at 5°C, on germination (%) (during stratification plus 10 days at 20°C) of Siberian C peach seeds.

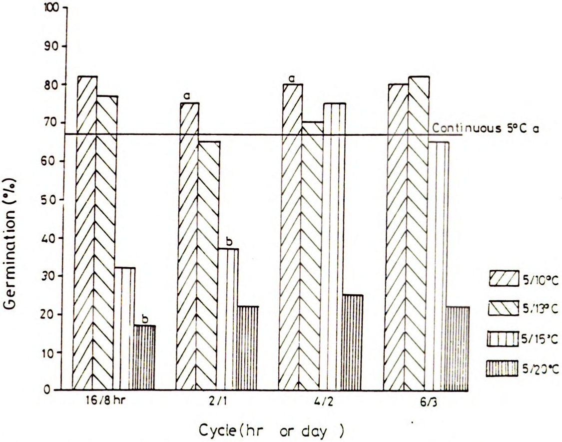

Figure 23. Effect of stratification temperature 5° C vs 5° alternated with higher temperatures, for 1008 hr at 5°C, on germination (%) (during stratification plus 10 days at 20°C) of SiberianC peach seeds.

c. Cycles with a single day at high temperatures and increasing time at 5°C

Increasing cycle length again showed that the inhibition of germination by 15° and 20°C depends on relative time of exposure to high vs low temperature (Figure 24). 15°C inhibited germination at 16/8 hr, 1/1, and 2/1 days but not as the cycle length was increased to 4 and 5 days. Comparison of temperature main effects showed that 10° promoted germination while 15° and 20°C inhibited it relative to constant 5°. On the other hand 13° was without effect. Main effects of both temperature and cycle length and their interaction were significant. The inhibitory effect of 20°C also declined as cycle length increased, but was still evident on the 5 day cycle. The threshold cycle was similar to that in experiment 1. Inhibition by 20°C was much greater in seeds chilled for 1008 hr than in those chilled for 1344 hr.

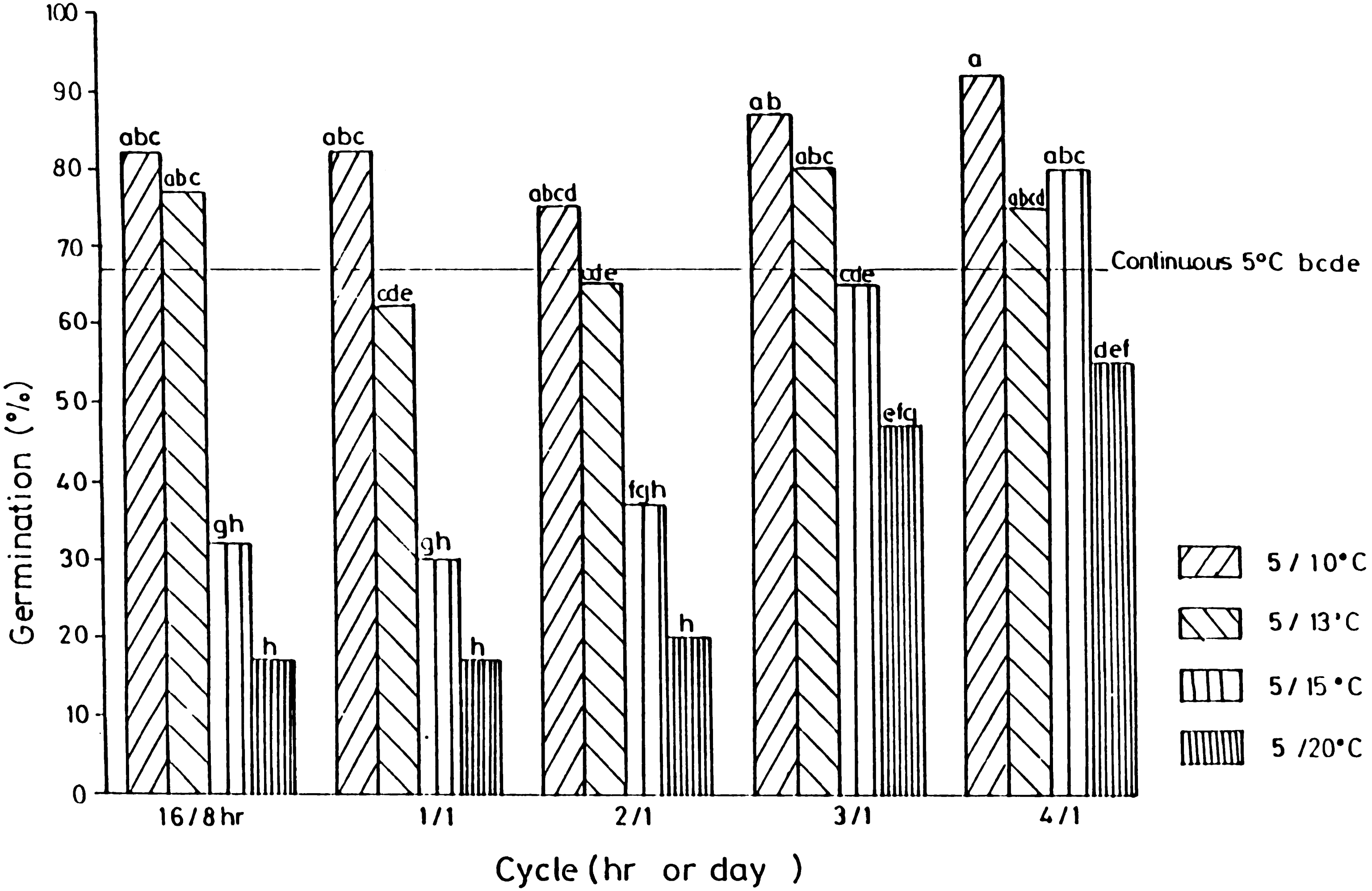

Figure 24. Effect of stratification temperature, 5°C vs 5° alternated with higher temperatures, for 1008 hr at 5°C, on germination (%) (during stratification plus 10 days at 20°C) of Siberian C peach seeds.

Section III. Effects of constant temperatures at various times during stratification

A total chilling period of 1512 hr (9 wk) was divided into three equal periods of 504 hours (3 weeks) each and continuous temperatures of 5°, 10°, and 15°C were used in to assess: 1) the effect of moderate temperatures at different stages of chilling; 2) whether or not the chill unit value changes with time.

Experiment 1: Ten degrees was just as effective when given in the third stage alone as when used continuously in a diurnal cycle, but did not stimulate germination when applied during the first stage. 15°C had a similar promotive effect when it was applied subsequent to 1008 hr of chilling, but reduced response to subsequent chilling when given during the first stage (Figure 25).

Experiment 2: Again both 10° and 15°C had a promotive effect when applied in the last stage, but inhibited germination in stage 1. Ten degrees had a positive effect when applied in the second stage of stratification while 15°C had an opposite effect (Figure 26).

Experiment 3: In a third experiment, the control (1008 hr at 5°C) germinated better than in the two first experiments. Seeds exposed to moderate temperatures in later stages appeared to respond, differences were not significant when given in the early stages, 15° inhibited response to chilling, but 10° had no effect (Figure 27).

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 could 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 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.

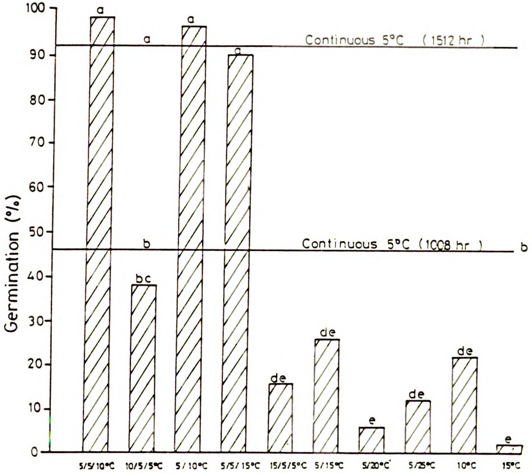

Figure 25. Effect of stratification temperature 5° continuous or alternated with higher temperatures, for 1008 hr at 5°C, on germination (%) (during stratification plus 10 days at 20°C) of Siberian C peach seeds.

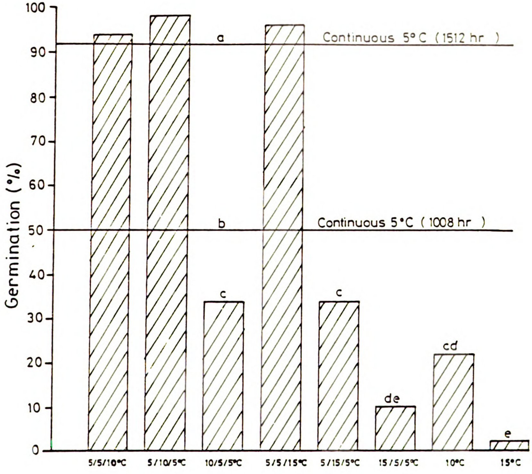

Figure 26. Effect of stratification temperature 5° continuous or alternated with higher temperatures during different stages for 1008 hr on germination (%) (during stratification plus 10 days at 20°C) of Siberian C peach seeds.

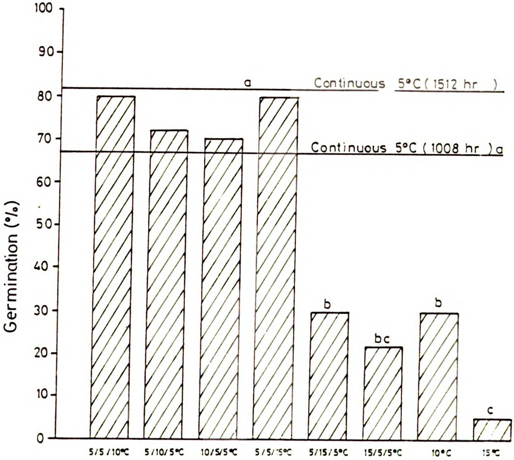

Figure 27. Effect of stratification temperature 5°C continuous or alternated with higher temperatures, for 1008 hr at 5°C, on germination (%) (during stratification plus 10 days at 20°C) of Siberian C peach seeds.

Section IV. Attempt to elucidate the mechanism of temperature effect

It would be helpful if one could separate between the effect of a temperature on chilling and its effect on germination *per se*. One approach to this problem is to determine if partially chilled seeds germinate better at an intermediate temperature such as 10° or 15° than at 20°C or higher. A second approach is to use polyethylene glycol (PEG) to prevent germination during stratification. PEG, a viscous liquid miscible with water, lowers the osmotic potential of the medium, limiting water uptake and thereby preventing radicle elongation.

1. Effect of temperature on germination of partially and completely stratified seeds

Siberian C peach seeds were stratified for various periods at 5°, then their ability to germinate was tested at 10°, 15°, 20° and 25°C (Figure 28). Temperatures did not affect germination significantly except that 25° inhibited germination after 1344 hr at 5°C.

2. Effect of PEG and stratification temperature on germination of Siberian C peach seeds

Experiment 1. Temperature (5°, 5/10°, and 5/15°C), and PEG concentration (0, -1, -3, and -5 bars) were tested in a completely randomized factorial design. The seeds were held at different temperatures until 896 hr at 5° had been accumulated. The PEG was then washed out and the seeds were germinated at 20°C (Figure 29). Neither PEG nor germination temperature affected the results; 10° on a diurnal cycle was promotive while 15°C had an inhibitory effect relative to the control (896 hr at constant 5°C). Some seeds held at 5° for 1344 hr germinated during stratification despite the presence of PEG at -1 or -3 bars.

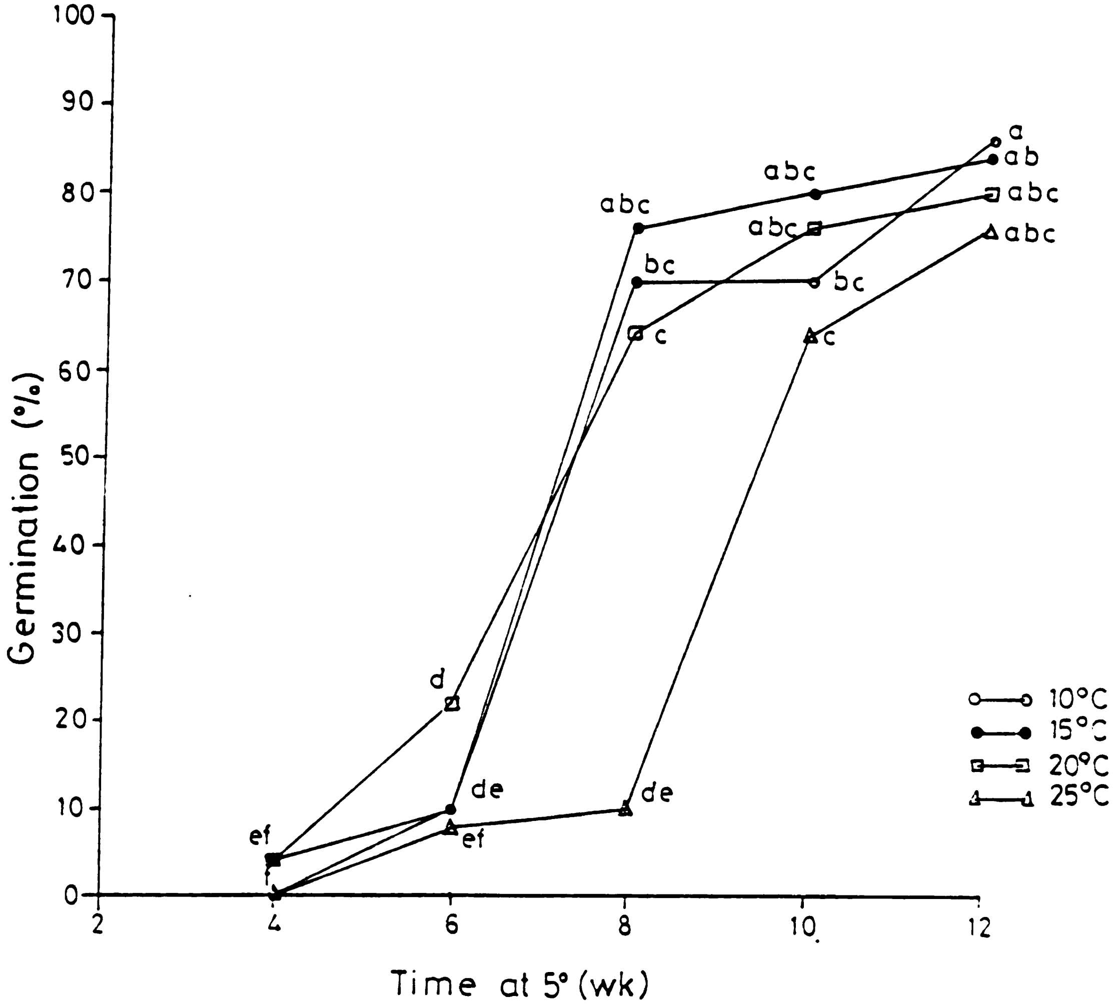

Figure 28. Effect of temperature on germination of Siberian C peach seeds following stratification at 5°C.

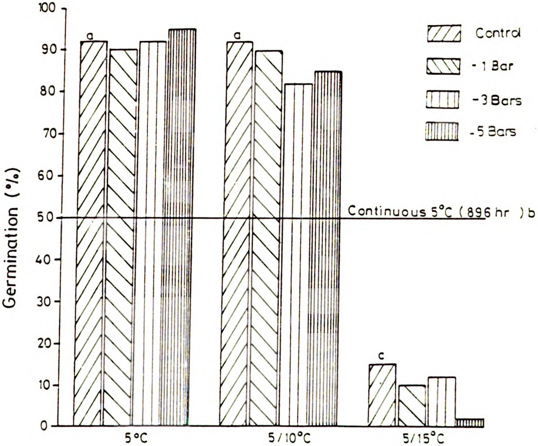

Figure 29. Effect of stratification temperature 5° continuous (1344 hr) or alternated wiht higher temperatures for 16/8 hr , for 896 hr at 5°C, with or without PEG on germination (%) (during stratification plus 10 days at 20°C) of SiberianC peach seeds.

Experiment 2. The chilling period was divided into 3 equal periods of 3 wk each. 5°, 10°, and 15°C were alternated at different stages with or without PEG. The PEG was washed off following stratification and seeds were held for 10 days at 20°C for germination.

In the absence of PEG, 10° and 15° had the expected effects: both promoted germination in stage 3, and 10° was also effective in stage 2; both inhibited in stage 1 and 15° inhibited in stage 2 as well (Figure 30). Response of PEG-treated seeds was much more difficult to rationalize. Ten degrees promoted in all stages at the high PEG concentration, but only in stages 2 and 3 at the low concentration. Fifteen degrees inhibited 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. Because PEG limits radicle emergence, rather than the early phases of germination, per se, this experiment does not provide conclusive evidence as to the mechanism of temperature effect. 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 has 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 inhibited. The chill unit value might also change with time, so that in early stages low temperature is required for removal of an inhibitor (ABA metabolism) or synthesis of a promoter (GA) and that a moderate temperature at a later stage is beneficial for enzymatic or metabolic reactions.

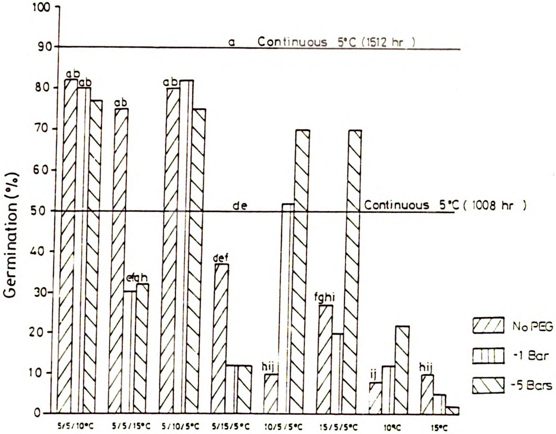

Figure 30. Effect of stratification temperature 5°C continuous or alternated with higher temperatures, for 1008 hr at 5°C, on germination (%) (during stratification plus 10 days at 20°C) of Siberian C peach seeds.

Section V. Effect of chilling interruption

Siberian C peach seeds were chilled at 5°C for a total of 1344 hr (8 wk). Chilling was interrupted for 1 wk interval starting with the second wk. The interruption consisted of exposing seeds to -5°, 0°, 10°, 15°, 20°, 25°, or 35°C. A temperature of -5°C was consistently inhibitory, especially at later stages of stratification (Figure 31). After 1 wk of chilling only 20° and 25°C significantly reduced the percentage germination. The effect of 20°C was not significant thereafter. However, 25° remained inhibitory until the seeds had accumulated 5 wk of chilling. These data suggest that the effect of chilling is "fixed" and that its negation by high temperature depends on the quantity of chilling previously accumulated. Surprisingly, 35°C was not inhibitory in the early stages of chilling (up to 4 wk), but partially negated chilling accumulation thereafter. Such high temperatures may have no negative effect unless the seeds have accumulated a critical number of chilling hr. The partial negation induced by both -5° and 35° seemed to be associated with injury since none of the non-germinating seeds from these treatments germinated despite restratification at 5°C for 13 wk.

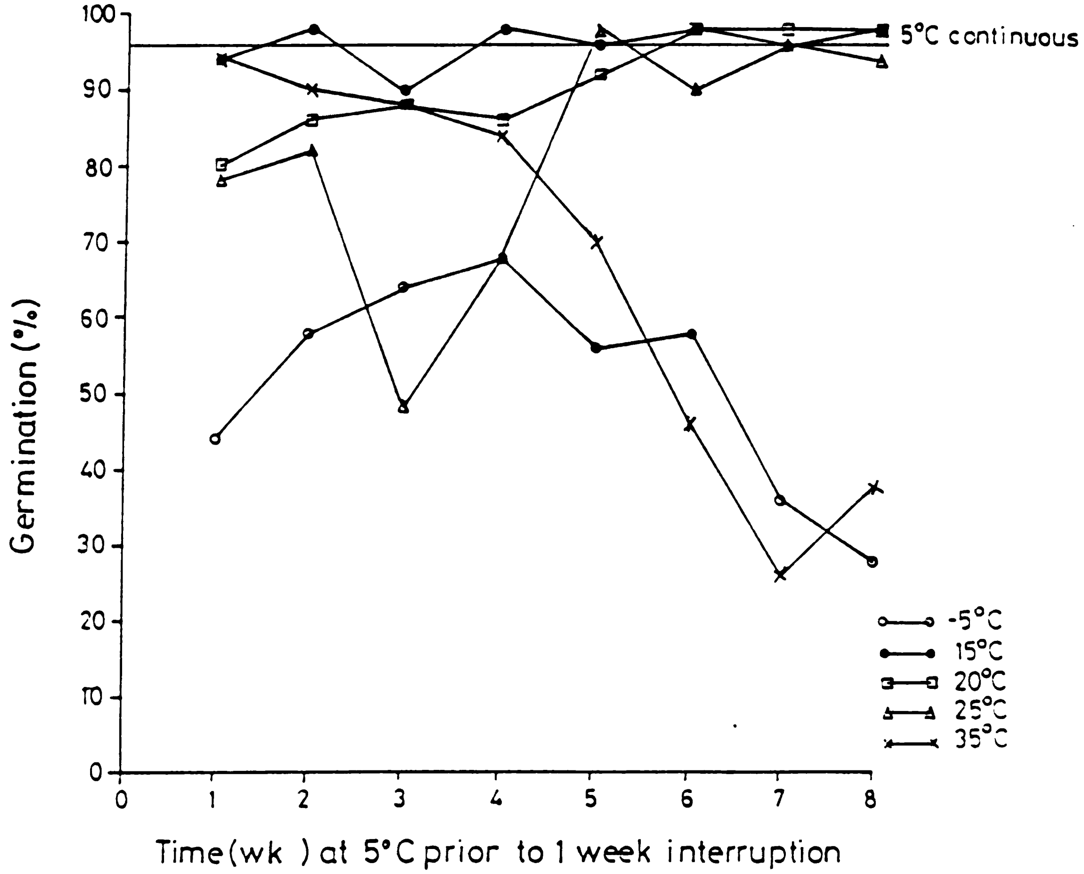

Figure 31. Effect of 1 wk chilling interruption at different stages of stratification (1344 hr /5°C) on germination (%) (during stratification plus 10 days at 20°C) of Siberian C peach seeds.

CONCLUSIONS

From a series of experiments on the effects of different temperature regimes on breaking peach seed dormancy we can conclude that:

(i) Peach seeds with varying chilling requirements have the same optimum temperature, but some low chilling cvs showed a rather broader optimum suggesting that differences in response to chilling among cultivars may be qualitative as well as quantitative. Similar observations have been reported by Gilreath and Buchanan (42,43).

(ii) Among the range of constant temperatures tested 5° appeared to be the optimum, closely followed by 0°C. Fifteen degrees had very little or no effect, and the effect of 10°C was not consistent from one experiment to another. The relative efficiency of 10 varied from 20 to 60% depending on stratification period and experiment.

(iii) Experiments with continuously alternating diurnal temperatures between 5° (16 hr) and 10° to 25° (8 hr) indicated that 10° had a promotive effect while temperatures of 15° or higher inhibited germination relative to constant 5°C. This confirms the observations of Aduib and Seeley (1).

(iv) However, 15° (continuously or in a diurnal cycle with 5° 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 is composed of two reactions of different nature and having different optimum temperatures. If the latter is true the value of a chill unit is not constant through the chilling period, but rather changes both with time and with the quantity of chilling previously accumulated.

(v) The inhibitory effect of high temperature is dependent upon cycle length and the amount of chilling previously accumulated, suggesting that chilling goes through a fixation state.

(vi) When chilling of seeds at 5° was interrupted by 1 wk at higher or lower temperature, 35° was inhibitory only after 5 wk or more of chilling had been experienced; 20° and 25°C, on the other hand, were only inhibitory early in the stratification period. In contrast a freezing temperature (-5°C) inhibited germination regardless of stratification time; the inhibition appeared to be associated with injury.

DISCUSSION

After-ripening of seeds of most deciduous fruit trees can be achieved by subjecting them in an imbibed state to low temperature (0 to 10C) (19). The most effective temperatures are between 2 and 8C. The characteristics of low temperature in after-ripening of rosaceous seeds can be summarized as follows: i) effectiveness of low temperatures is enhanced by prolonging the exposure period; ii) the temperature/response curve is of the optimum curve type; iii) any temperature within the effective range for breaking dormancy induces optimal germination if allowed to act long enough; iv) high temperatures reduce or negate the effect of chilling; v) the after-ripening of partially stratified seeds can be completed at moderate temperatures (10-15C), which have little or no effect if applied from the beginning. This suggests that only initial phases of after-ripening process require low temperature.

Optimum temperature range for breaking of dormancy

Moderate temperatures alone can remove dormancy in some cases. Ten degrees compared with 0, 7, and 24C gave the highest percentage germination of 'Sharbati' peach seeds regardless of stratification period (17). The beneficial effect of 10C on stratification extended to seedling growth. The seedlings raised from seeds stratified at 10C were taller than those from seeds kept at 7C, while those from seeds held at 24C were dwarfed. Ten degrees was also the optimum temperature for after-ripening of almond seeds (13). 'Sungold' nectarine cuttings held at constant temperatures showed a faster floral bud break at 10C than at 7C regardless of chilling period (10).

Low chilling peach cv 'Flordasun', 'Flordahome', and 'Okinawa' responded well to both 7.2 and 12.8C (12). In fact, the percentage bud break of non-chilled trees of these cv reached 70 to 80% after 48 days of forcing, but the shoots remained rosetted. However, 7.2C was more effective than 12.8C in removing rest of 'Nemaguard' (high chilling cv). There was no visible bud activity in 'Sungold' plants held at 15C for less than 750 hr (10). Fifteen degrees, 3.3, and 0.6C were less effective than 10 and 7C after 60 days of forcing.

Terminal floral bud break was similar for 2 low chilling ('Woodard' and 'Bluegem') and 1 high chilling ('Tifblue') blueberry cv, at constant temperatures of 0.6, 3.3, 7, and 10C (11). 'Tifblue' did not respond as well as low chilling cv to 15C, no visible bud activity being apparent following 550 hr exposure to 15C. 'Tifblue' did not respond at all to 15 and responded to 10C only when exposed to the maximum chilling duration (850 hr). Gilreath and Buchanan (11) found a narrower range of effective chilling temperatures for 'Tifblue' and suggested that 'Woodard' and 'Bluegem' are better adapted to mild climatic conditions due to widening of the temperature range over which chilling is effective. High chilling cv showed a narrower range of effective temperatures than low chilling ones (11).

Pear seeds, cultivar 'Bartlett', from a warmer climate had a higher optimum temperature than those from a cold location and displayed a broader temperature response curve (16). The evaluation of chilling requirements of fourteen pear species from different locations revealed that the effective temperature range lies between 2 and 10C (21). Species originating from warmer climates had lower chilling requirements and a higher optimum range (7 to 10C) than those from colder climates.

In my experiments, maximum germination at 20C was reached after exposure of seeds to 0, 5, and 10C for 1008 hr for low chilling peach cv (Chilean, Pessigo, and FL 9-4) and 1344 hr for Sib C. Chilean and Pessigo exhibited a broader temperature response curve than did Sib C or FL 9-4. This supports the view that low chilling cultivars and seed populations may have a wider range of effective temperatures that break dormancy. Fifteen degrees by itself had little chilling effect. The efficiency of 10C varied with stratification time and experiment as well as seed source.

Negation of chilling by high temperature

Exposure to temperatures of 18C or higher can counteract the effects of previous chilling. Peach leaf bud opening was reduced by 33% when the average temperature was raised from 10-12C to 18C for 15 days while 80% reduction resulted from raising the average temperature to 22.2C for the same period (20). High temperatures counteracted the effects of chilling, causing a delay in bloom and foliation and reducing fruit set of 'Sullivan Elberta' peach (20). In contrast, the breaking of rest was enhanced by shading, which reduces bud temperature.

Bennett (2) showed that exposure of pear buds to high temperatures (23C) during chilling delayed their development. Continuous moderate temperatures (13-15C) delayed peach flower bud break more than did higher temperature for brief periods.

Chilling interruption increased the initial bud break (10) in 'Sungold' nectarine, but reduced the final number of buds that developed. No bud activity was apparent in plants given 350 hr chilling with 14 days at 30C. The authors concluded that high temperature must have negated the 175 hr accumulated prior to high temperature exposure. Gilreath and Buchanan (10) postulated that this constitutes a confirmation of a "temperature-dependent fixation process".

Interruption of chilling by 2 long periods (11-12 days) of high temperatures (20C) enhanced lateral bud opening in 'Redhaven' peach trees in comparison with the continuous chilling (5).

A 2-week period at 30C midway in the chilling period enhanced the rate but did not affect total bud break of blueberry cv (11). High temperature negation was less pronounced in low than high chilling peach cv (10). Low chilling peaches and nectarines have a higher optimum chilling temperature, and such cultivars could be more tolerant of high temperatures.

Effects of diurnal temperature alternation

Both promotive and inhibitory effects of temperature alternation have been reported (Table 1). Spiers and Draper (18) found no inhibitory effect of 18C (for 10 hr) in alternation with 7C (14 hr) on chilling accumulation in rabbiteye blueberry "Tifblue", but 20C (7 hr a day, 5 days a week) partially reduced the low temperature effect. The effect of insufficient chilling was more pronounced on flower than on vegetative buds. Temperatures of 7/15C for 14/10 hr, or continuous 15C, were as effective as constant 7C on low chilling cv of blueberry but were ineffective in breaking rest of a high chilling cv (11). However, when 30C was applied in a diurnal cycle (10 hr) with 7C, a partial negation of bud break resulted (11).

Fifteen degrees was not effective in breaking rest of 'Montmorency' sour cherry flower buds, but a 5/15C (16/8 hr) cycle was more effective than constant 5C (8).

Erez et al. (5) exposed potted peach trees to diurnal temperature cycles (16 hr at 4C, 8 hr at higher temperatures). Twenty degrees for 8 hr completely negated chilling accumulation, whereas temperatures below

18C were not inhibitory. Intermittent exposure to 10 to 15C enhanced chilling efficiency (response per hour of exposure to 4C) relative to constant 4C.

In one experiment 6/17C (16/8 hr) inhibited chilling accumulation in 'Redhaven' buds while 6/15C was without effect (7). However, in another experiment when 15C was alternated with 6C bud break was enhanced. Eighteen degrees was neutral and 21C completely inhibited the effect of 6C.

In section II of this thesis, 5/10C (16/8 hr) during stratification promoted germination at 20C of peach seeds with high chilling requirements in comparison with constant 5C; however, 5/15C, 5/20, and 5/25 inhibited it. Similar observations were reported by Aduib and Seeley (1); 5/10C promoted, while 5/15C inhibited, the germination of 'Halford' peach seeds. Thus, 15C hastens peach bud break, while inhibiting the breaking of dormancy. This suggests that seeds may have a lower temperature threshold for enhancing the chilling effect on a 16/8 hr cycle. In contrast 5/15C cycles did not inhibit subsequent germination at 20C of peach seeds with low chilling requirements. In fact both 13C and 15 promoted germination of Chilean peach seeds held for 672 hr at 5C.

Table 3. Effects of alternating vs constant temperatures on breaking of rest. All effects are statistically significant.

	Temperature (°C) alternating with 4 to 6				
Cultivar & organs	10	13	15	20	Ref.
Peach leaf buds					
Redhaven		+	+	-	32, 35
Redskin			+	-	32
Montmorency sour cherry flower bud			+		37
Peach seeds					
Halford	+		-		1
Siberian C	+		-	-	Mahhou
Chilean	+	+	+	-	Mahhou

Effect of timing of treatment

When the total chilling period was divided into 3 equal periods and cycling was applied in different stages, 4/15C (16/8 hr respectively) enhanced peach flower bud opening only when applied in the last stages. However, cycling was ineffective in promoting leaf bud break in this experiment; in fact, it inhibited leaf bud opening when applied during the first third of the chilling period (7).

When I applied similar treatments to seeds (Section II) temperatures of 13, 15, and 20C inhibited germination significantly when alternated with 5C during the first period of stratification, but not during either of the last 2 periods. 5/13 and 5/15C did not inhibit subsequent germination when applied during any 2 of the periods, and 20C inhibited germination only when applied during the first and second or first and third periods. None of the temperature regimes significantly inhibited germination when applied throughout the second and third stages.

Effects of constant temperatures for varying periods of time

To determine whether cycling was necessary to stimulate germination, constant temperature treatments were compared with alternating temperatures (Section III). The efficiency of 10C varied between 25 and 37% relative to continuous 5C depending on the experiment. However, 15C had no effect; no more than 5% of the seeds germinated in any of the three experiments following 9 wk at this temperature. Constant 10C during the third subperiod was just as effective as when alternated with 5C in a diurnal cycle. It did not, however, stimulate germination when applied in the first subperiod of chilling. Fifteen degrees had a similar promotive effect when applied subsequent to 1008 hr of chilling, but reduced response to chilling when given during the first or second subperiod.

Although the 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.

Effects of interruption of chilling by high temperatures at various times

When stratification period of 10 wk of apple seeds at 4C was interrupted for 1 wk at either 0, 2, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24C del Real Laborde (4) found no significant differences during the first 6 wk. However, the interruption of chilling during the 7th or 8th wk reduced subsequent germination. Interruption of chilling the 3rd 1/4 of the chilling period (1680 hr) by 2, 6, 18, 20, or 24C also reduced seed germination. Maximum inhibition was observed at 20 and 24C. Temperatures of 18, 20, and 24C enhanced seed germination when applied during the first 1/4 of the chilling period.

In this thesis chilling of Siberian C peach seeds was interrupted for 1 week after various periods at 5C. After 1 week of chilling only 20 and 25C reduced chilling accumulation. The effect of 20C was not significant thereafter. However, 25C remained inhibitory until the seeds had accumulated 5 wk of chilling. This suggests that the effect of chilling is "fixed" and that its negation depends on the amount of chilling previously accumulated. Surprisingly, 35C was not inhibitory in early stages (up to 4 wk), but negated chilling thereafter. A temperature of -5C was consistently inhibitory particularly at later stages of stratification. The negative effect of -5 and 35C was associated with injury; none of the non-germinating seeds from these treatments germinated despite restratification at 5C for 13 wk.

Effect of cycle length on negation of chilling

The inhibitory effect of high temperatures is cycle length dependent. The degree of reversal of vernalization by high temperature in Petkus rye depends on the temperature, duration of interruption, and the amount of chilling previously accumulated (14).

Exposure to 20C for 4 hr or less daily enhanced peach bud break in comparison with constant 4C (3). Increasing the high temperature exposure period to 6 hr resulted in chilling negation.

The effect of cycle length on chilling negation by high temperatures in leaf buds of rooted cuttings of 'Redhaven' and 'Redskin' peach has been evaluated (6). Cycles of 1, 3, 6, and 9 days were used with 2/3 of the cycle length at low temperature (4C for 1 day and 6C for other cycles) and the remaining 1/3 at 24C. Little or no bud break occurred in 'Redskin' plants exposed to 1 and 3 day cycles, whereas 6 and 9 day cycles were just as effective, per hour at 4C, as continuous 4C. The authors concluded that chilling negation by high temperatures is dependent on cycle length.

In this thesis the inhibitory effect of a given exposure to high temperature on subsequent germination of peach seeds decreased as the cycle length increased. Moderate temperatures were not effective in stimulating germination because germination of the controls was too high. Cycles of a single day at high temperatures and increasing time at 5C showed that the inhibitory effect of 20 and 25C decreased with cycle length and disappeared on cycles longer than 4 days. Thus the threshold cycle appears to be 4 days.

Effects of moderate temperatures on dormancy vs germination

The ability to distinguish between the effects of temperature on after-ripening *per se* vs germination is crucial. 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 (Section IV), therefore better germination at moderate temperature does not explain the promotive effects of such temperatures.

In several experiments polyethylene glycol (PEG) was used to prevent germination. 5/10C promoted and 5/15C inhibited the breaking of dormancy relative to seeds held continuously at 5C, even when germination was prevented by PEG.

When continuous moderate temperatures were alternated with 5C during the subperiods of chilling, 10C and 15C had the expected effects; both promoted germination in the last subperiod and 10 was also effective in subperiod II. Both inhibited in stage I, and 15C inhibited in stage II as well. Response of PEG-treated seeds was much more difficult to rationalize. Ten degrees promoted in all stages at the high PEG concentration, but only in stages 2 and 3 at the low concentration. Fifteen degrees inhibited at all stages at the low concentration and at both stages 2 and 3 at the high concentration, but promoted germination in stage I at high concentration. Stress (high temperature or osmoticum) might favor the breaking of dormancy in the early stages of after-ripening. PEG limits radicle emergence, and therefore probably restricts growth rather than the early processes of germination.

Possible explanations of temperature effects

To explain the promotive effect of moderate temperatures on breaking dormancy of peach buds Erez and Couvillon (7) proposed a scheme (a modification of Purvis and Gregory's (14)). The scheme involves a two-step reaction the first of which is reversible.

$$A \underset{a'}{\overset{a}{\rightleftharpoons}} B \xrightarrow{b} C$$

a : occurs at low temperature

a': occurs at high temperature

b : occurs at low temperature but occurs at a faster rate at intermediate temperatures. Thus, moderate temperatures (10-15C) hasten the breaking of dormancy by stimulating conversion of B to C.

This scheme is, however, simplistic and, more importantly, does not explain the inhibitory effect of moderate temperatures during early stages of after-ripening. This inhibitory effect was observed in our experiments with peach seeds. The same effect was apparent in peach buds (7), even though the authors did not attempt to explain it.

Fishman et al. (9) proposed what they called a phenomenological theory to explain the action of temperature on the breaking of dormancy. In their scheme they assume the occurrence of a thermally unstable precursor (PDBF), which is converted by chilling to a factor(s) (DBF) responsible for breaking dormancy. The conversion of the precursor to the dormancy breaking factor is assumed to be irreversible. Once the amount of PDBF reaches a critical level (threshold) all of it undergoes an irreversible transition (fixation) to the DBF.

k_0, and k_1 are rate coefficients for formation (low temperature) and destruction (high temperature) of PDBF.

The rates of formation and destruction of PDBF are proportional to its concentration and change with temperature. When the concentration of PDBF is low, its formation is rapid. As its concentration increases, the rate of its destruction increases. At this point, temperature becomes crucial. As long as the temperature remains at or below a critical point (ca. 12C), the reaction continues until the critical concentration (-1) is reached, and all of PDBF is converted to DBF. If the temperature is above 10C, the initial rate formation of PDBF is higher, but the increased rate of destruction prevents it from ever reaching the critical level and dormancy is maintained. Temperatures above 18C can reduce the levels of PDBF already formed because $k_0 < k_1$. The basis for the promotive effects of alternating temperatures between 4C and 15C is that initial exposure to 15C causes PDBF to accumulate faster initially. When the temperature is shifted to 4C, less time is needed to reach the critical level. Therefore, more DBF accumulates over a given time period. Cycle time is crucial in this scheme if dormancy is to be broken more rapidly.

When low temperature is alternated with moderate temperature the critical level of PDBF is reached faster and therefore the efficiency of chilling is increased. The total time required to accumulate the amount of chilling units is shorter at cycling temperatures than at constant low temperature. This scheme is clearly an oversimplification of a very complex process. However, it is of interest as a working hypothesis. When applied to my data it does not provide an explanation for the inhibitory effect of exposure to moderate temperatures on chilling accumulation during stage 1, or of the promotive effect of constant 10C or 15C during stages 2 and/or 3.

In contrast to the two-step reaction scheme, Saure (15) suggested that there could be two distinct but overlapping temperature reactions, one producing a factor which breaks dormancy (low temperature) the other a factor which maintains or enforces dormancy (high temperature). The first reaction has a low temperature range at first, but as chilling accumulates this range widens. During early stages of chilling (true dormancy) the inhibitory potential is much higher and chilling could be counteracted even by relatively moderate temperature. As chilling proceeds (late dormancy) the buds would accumulate enough chilling so that the second reaction can proceed and the range of temperatures capable of negating subsequent chilling becomes narrower (>23°C). This hypothesis is partially supported by my data, for both 10 and 15C were inhibitory in stage 1, 10 was promotive but 15C inhibitory in stage 2, and both were promotive in stage 3. Thus there appeared to be a widening of the temperature range for the breaking of dormancy as after-ripening proceeded.

Conceivably the process(es) occurring during after-ripening are set in motion or triggered by low temperatures. Once the initial steps are underway subsequent reactions take place at a faster rate at moderate temperatures. High or moderate temperatures at early stages may trigger antagonistic processes. The cascade of events leading to dormancy removal may also involve a number of intermediates or precursors. The initial steps or phases of the series, but not later steps, require low temperature. These could take place under low temperature as well as moderate temperatures and their rate may even be enhanced at the latter. However, if moderate temperatures are applied at early stages several processes may be initiated or turned on at the same time, but the rate of occurrence may be slowed down. The system becomes less efficient.

Felker and Robitaille (8) postulated that 5/15C (16/8 hr) cycles provide sufficient chilling accumulation, insufficient continuous hr at 15C to nullify chilling, and enough concomitant heat unit accumulation to increase the rate of bud break. This concept is supported by my data, for moderate temperatures promoted germination only when applied during later stages of after-ripening, when the seeds were more capable of germination. However, it does not explain the negative effect of high temperatures. These should also favor bud development and seed germination.

Physiological processes are generally complex and involve a series or cascade of events and steps. These may not have the same optimum temperatures and requirements. Thus seed's receptivity to an external stimulus (temperature) may change with time depending on its physiological state. During early phases of stratification certain receptors may be turned on or activated by low temperature. With longer exposure such receptors become acclimated and may respond to a relatively wider range of temperatures. Other receptors or processes could also come into play.

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PART THREE

EFFECTS OF STRATIFICATION TEMPERATURE ON METABOLIC CHANGES

LITERATURE REVIEW

INTRODUCTION

Dormant seeds require an environmental stimulus, e.g., light or cool temperatures before germination can occur. Alternatively, the stimulus may be replaced by imbibing the seeds in a solution of phytohormone (usually a gibberellin). Many studies to elucidate the mechanisms whereby such stimuli break dormancy have emphasized nucleic acid and protein synthesis. Dormancy could result from the blockage of one or both of these processes. Dormancy should not be equated with metabolic quiescence, since dormant seeds are capable of a variety of metabolic reactions. Dormant lettuce are capable of appreciable RNA (40) and protein (38, 39) synthesis, as are dormant seeds of *Agrostemma githago* (48), *Melandrium noctiflorum* (49), *Paulownia tomentosa* (45), *Xanthium pensylvanicum* (73) and *Fraxinus excelsior* (89). Any promotive action of hormones or environmental stimuli is likely to entail some modification or enhancement of this "basal" metabolism. A considerable body of work has been carried out to determine the effects of RNA and protein synthesis inhibitors on GA_3-induced germination. The results obtained have, however, been very variable, the effects of the inhibitors ranging from completely inhibitory to completely ineffective. Bewley (8) pointed out that conclusions drawn from inhibitor studies should be treated with caution, since (a) proof is often lacking that inhibitors are taken up by the seed and that they do indeed inhibit protein or RNA synthesis; (b) very high concentrations of some inhibitors are needed for them to be effective, perhaps because of their limited ability to penetrate the seeds. In the tissues that they do penetrate there is a possibility of side - effects due to their high concentration; (c) the specifity of RNA and protein synthesis inhibitors is not as narrow as was once assumed, e.g., actinomycin D is not a specific mRNA synthesis inhibitor; (d) to date, no evidence exists that an effective inhibitor preferentially eliminates hormone-induced RNA or protein synthesis. Such inhibitors could affect "basal" metabolism as well as, or instead of, specifically hormone-induced processes. Reduction of basal metabolism, presumably essential for the metabolic well-being of the seed, could result indirectly in reduced germination.

Gibberellin appears to promote RNA and/or protein synthesis in some seeds. ABA is inhibitory but its effects are counteracted by cytokinin (54). However, there is no compelling evidence that any of these hormones act directly at the level of transcription and translation or regulate the synthesis of proteins essential for germination if, indeed, such proteins exist. The same conclusions can be drawn from experiments on the effects of light, mediated via the phytochrome system, upon protein and RNA synthesis during the breaking of dormancy (8).

Proteolysis plays an important role in seed germination (61). Proteases convert seed proteins to amino acids which are required for the developing plant. Protein degradation is a continuous process during protein turnover and appears to be selective with regard to individual species of protein. In many seeds storage proteins are deposited in a distinct organelle or protein body. Its boundary is formed by a single membrane homologous to a vacuolar membrane. The breakdown of storage proteins probably takes place within these bodies. The disappearance of storage proteins in germinating seeds is generally associated with an increase in proteolytic activities. In germinating cow peas (*Vigna unguiculata*) protein mobilization is closely related not only to enhanced protease activity but also to the location and timing of protein breakdown within the cotyledons (47). The storage proteins present in both the protein bodies and the cytoplasm are hydrolyzed.

For dormancy to be overcome in many species such as peach, the seed must undergo a period of exposure to low temperature (stratification). Much effort has been devoted to understanding the mechanisms of action of low temperature release from dormancy. This research generally falls into two categories:

1. the effects of temperature on hormonal changes in relation to dormancy;

2. the effects of temperature on changes in protein synthesis and/or enzyme activities. In this review only the second area of research will be discussed as it relates to the metabolism of proteins, including changes in isozymes.

ISOZYME CHANGES

1. Peroxidase changes

Peroxidase activity in apple embryos changed very little with the breaking of dormancy, decreasing only slightly at the end of the second month of after-ripening (58).

No cathodic and only 4 anodic isoperoxidases occurred in extracts of dormant pear embryos, wheareas seven anodic and two cathodic isoperoxidases were detected in extracts of non-dormant embryos (stratified for 30 days) (85). During stratification four different types of changes occurred in anodic isoperoxidases: (a) the activity of isoperoxidase 1 remained unchanged; (b) an increase in activity of isoperoxidase 3 occurred during the first 8 days; (c) increases in activities of isoperoxidases 4, 5, 6 and 7 occurred at a later stage; and (d) the activity of isoperoxidase 2 increased progressively with the breaking of dormancy. The presence of GA_3 or kinetin at 20 uM enhanced the activity of certain isoperoxidases. These enhancements were blocked in the presence of ABA, which by itself had an inhibitory effect on both germination and isoperoxidase synthesis. Cycloheximide and 6-methylpurine inhibited both the synthesis of isoperoxidases and the breaking of dormancy.

Thevenot et al. (86) reported that peroxidase activity in dormant apple embryos was low, but increased significantly in isolated embryos held at 5° and even more rapidly in those held at 20°C. A number of embryos were able to germinate at 20° and to grow more rapidly than those held at 5°C. The increase in peroxidase activity therefore could have been associated with germination. The peroxidase activity in embryos confined within the seed remained very low at 5° and 20°C and unable to germinate. The authors concluded that peroxidase activity was not involved in the removal of embryonal dormancy but rather in the germination processes.

Evaluation of the spectrum of isoperoxidases showed that embryos excised form freshly imbibed seeds contained a very low number of isoperoxidases: four anodic ones (A_2, A_3, A_4 and A_6) and a cathodic one (C_2) of low activity. When the isolated embryos were placed at 5° or 20°C the activities of the isoenzymes initially present greatly increased and new isoenzymes (A_1, A_5, C_1, C_3, C_5, C_6, and C_7) appeared progressively. Evolution of the isoperoxidases was more pronounced at

20^{o} than at $5^{o}C$ and the isoenzyme C_4 was only found in isolated embryos held at $20^{o}C$. Thevenot et al. (86) concluded that quantitative and qualitative variations in the isoperoxidase spectrum were related, not to the release of dormancy, but rather to the processes of germination and growth which take place even at low temperature.

2. Lipase changes

Smolenska and Lewak (80) reported the presence of two lipolytic enzymes, or groups of enzymes, differing in optimum pH (7.5 and 5) in germinating apple embryos from non-stratified seeds. Light stimulated germination, and the activity of alkaline lipase (optimum pH 7.5) was higher, and that of acid lipase lower, in light-cultured embryos. In dark-grown embryos the main lipolytic activity was attributed to the acidic lipase. They suggested that light stimulates germination by inducing the conversion of phytochrome into its active P_{fr} form. This activates GA biosynthesis (GA_4) which induces mobilization of storage lipids by activating or stimulating biosynthesis of alkaline lipase.

During stratification at $4^{o}C$ of apple seeds the activity of acid lipase appears about 20 days earlier (on the 50th day) than that of the alkaline lipase (100). However, the authors did not include a control at a warm temperature. The optimum temperature for acid lipase activity (5^{o}) was close to that for the seed after-ripening process (4^{o}), whereas the optimum was about $30^{o}C$ for alkaline lipase. Activities of both enzymes were determined at both 4^{o} and $30^{o}C$ in extracts from embryos isolated from seed after different periods of stratification. The activity of alkaline lipase at $30^{o}C$ was negligible during the first 35 day of stratification, increased between the 40th and the 70th day, then decreased sharply. The rise in acid lipase activity at $4^{o}C$ began at about 10 day and reached its maximum at 50 day. The decrease in acid lipase activity during the second half of the after-ripening period was less pronounced than that for the alkaline enzyme. The authors suggested that hydrolysis of storage lipids during apple seed stratification is catalyzed primarily by acid lipase. Alkaline and acid lipase activities were assayed in *in vitro*-cultured embryos isolated from (i) dormant seeds; (ii) seeds at the end of the first phase of after-ripening (20 day); (iii) seeds at the middle of the catabolic phase (50 day); (iv) seeds at initiation of germination. In all cases germination was stimulated by light. Light stimulated alkaline lipase activity only in embryos isolated from seeds in the phases (i), (ii) and (iii) but had no effect on acid lipase activity. The authors concluded that hydrolysis of storage fats, during stratification of seeds or germination of excised embryos was catalyzed by two different enzyme systems, each controlled by different environmental factors. Acid lipase activity was stimulated by low temperature and thus was more closely related to the removal of dormancy. Alkaline lipase activity was stimulated by light and associated with germination of fully or partly dormant embryos. The acidic lipase activity appears when protease is most active (30 to 50 day) (58). Zarska and Lewak (100) suggested that both proteins and fats are hydrolyzed during the same phase of after-ripening, and that both processes are at least partly dependent upon exposure to low temperature.

3. Dehydrogenase changes

The specific activities of glucose-6-phosphate and 6-phosphogluconic acid dehydrogenases increased in hazel seeds during stratification at 5° but remained relatively constant at 20°C (44). The major increase in activity of these enzymes coincided with a marked change in germination potential of the chilled seed. The authors suggested a probable connection between enzyme activity and dormancy removal via the pentose phosphate pathway.

RESERVE FOOD CHANGES

Reserve food materials were transferred from the endosperm to the embryo of *Heracleum sphondylium* during chilling at 2°C (83). The embryo grew to twice the volume and twelve times its original dry weight during 9 wk of chilling, with a concomitant loss of dry matter from the endosperm. However, these changes did not occur and the seeds remained dormant when they were kept moist at 15°C. Warm temperature interruption did not reverse the progress of dormancy-breaking at any stage, and the total chilling periods were additive. Stokes (83) concluded that chilling did not remove an inhibitor, but merely caused a change in the mobilization of food reserves and their transfer to the embryo, possibly by inducing a factor in the embryo which diffused to the endosperm and there stimulated proteolysis.

The reserve food materials within the embryo itself can also change during the chilling treatment. The freshly imbibed embryos of *Fraxinus excelsior* store mainly lipids, and starch is almost undetectable. During dormancy the lipid content decreases from 20% to less than 4% of the dry weight; much starch appears in the tissues and at the same time the protein content increases from 14% to 24% of the dry weight (82). However, the mobilization of reserve food materials does not appear to be an essential stage in the breaking of dormancy, since lipid breakdown, starch formation and increase of sugar can all take place even if the seed is held at temperatures which do not break dormancy (90).

A correlation was found between the accumulation of starch grains during chilling and an increasing ability of apple embryos to germinate (22). Starch content in the axis of apple embryos increased when seeds were held on a moist medium at 5°, but decreased when seeds were held at either 5° or 20°C inside the fruits (14). Both treatments, however, broke embryo dormancy. This suggests that variations in starch content are not linked to dormancy release.

NUCLEIC ACID CHANGES

Little RNA synthesis was evident in dormant potato buds, but RNA synthesis was stimulated by breaking dormancy with ethylene chlorhydrin (88). DNA extracted from dormant buds did not support RNA synthesis, but DNA from non-dormant buds did so. Actinomycin D, an inhibitor of messenger RNA production, inhibited RNA production in non-dormant buds. Breaking of dormancy in the buds of potato therefore appeared to be associated with gene derepression and the synthesis of mRNA.

Chilling increases the capacity for nucleic acid synthesis in pear embryos (54). All nucleic acid fractions increased, but especially transfer RNA and a fraction considered to be a hybrid RNA-DNA molecule. This hybrid fraction may be important in dormancy-breaking.

During chilling of Corylus avellana embryos RNA synthesis increased, as measured by the incorporation of ^{32}P into RNA in the tissues (50). The earliest change was in DNA template availability, followed by an increase in RNA polymerase activity. Thus, the breaking of dormancy is apparently accompanied by an increased capacity of the DNA to support RNA synthesis.

In non-dormant seeds such as wheat, the mRNA necessary for at least the initial stages of germination may already be present in the dry seed. Actinomycin D does not prevent protein synthesis, or the initial stages of germination, in imbibing seeds, even though it inhibits RNA synthesis (60). In dormant species, however, the absence of mRNA could provide a convenient method for the control of germination at the level of transcription of the genome (92).

In non-dormant seed RNA and protein synthesis are concomitant events commencing at the earliest times of imbibition (8). mRNA's are almost surely conserved in mature dry seeds. They are in a protected form, perhaps in the nucleus or in the cytoplasm associated with proteins as mRNP particles. Some of the conserved mRNA is utilized at the earliest times of imbibition and germination to direct protein synthesis. There is an excess of mRNA in relation to the early requirements of the cells, but the messages that are not utilized may not be qualitatively different from those that are. Within a few hours synthesis of new mRNA's begins, and continued protein synthesis probably becomes increasingly dependent upon them. _De novo_ synthesized mRNA's appear to be similar to those conserved within the dry seed, although subtle but important changes in protein synthesis could occur as a consequence of changes to minor mRNA species that are undetectable using currently available techniques.

Mature dry embryos contain ribosomes that are active in synthesizing proteins _in vitro_, and yet rRNA synthesis is an early event during germination. Ribosomes containing _de novo_ synthesized rRNA may become associated with the protein-synthesizing complex early during germination, but whether or not new rRNA's in new ribosomes are essential for protein synthesis at any stage during germination is unknown.

Transfer RNA's and their aminoacylating enzymes are present within dry seeds and systems for protein synthesis _in vitro_ have been developed from wheat (59) and rye embryos (17) without any requirement for addition of these components. Synthesis of tRNAs, however, begins within 20 min in imbibed rye embryos (77) and within 1 hr in dissected _Phaseolus vulgaris_ axes (93), but their involvement in protein synthesis remains to be determined. Protein and RNA synthesis precedes and accompanies cell elongation, division and differentiation in embryonic axes, but occurs in the absence of such cellular changes within storage organs.

The induction and termination of dormancy in hazel nut seeds may be related to the balance of natural growth regulators (GAs and ABA) (78). Treatments which broke dormancy stimulated synthesis of RNA within the embryonic axis prior to growth. Synthesis of poly (A)-rich RNA (poly (A) + RNA) declined during induction and increased during the breaking of dormancy. GA_3 treatment and cotyledon excision, which break dormancy, changed the size distribution of RNA-containing polyadenylic acid (poly (A) + RNA) synthesized in the embryonic axes. This change preceded germination by at least 5 days. The changes in size and encoded peptides of poly (A) + RNA suggest that dormancy breaking may be associated with the transcription of novel mRNAs specific for germination. The poly (A) in the newly synthesized poly (A) + RNA from axes of dormant and non-dormant seeds were of similar size. Greater messenger activity was detectable in poly (A) alone (poly (A) - RNA) than in poly (A) + RNA in imbibed dormant and non-dormant axes. Both poly (A) + mRNA and poly (A) - mRNA were present in axes of dry seeds, the former declining markedly during storage. However, whether a causal relationship exists between mRNA metabolism and dormancy or whether mRNA metabolism merely reflects metabolic activity is not yet clear.

PROTEIN METABOLISM

Weisman (98) observed an increase in free amino acid level in apple embryos, but the rise occurred at both 5° and 20°C. Thus the increase in free amino acid content was not associated with the breaking of dormancy. In apple embryos kept within the fruit at 0°C, the pool of free amino acids was relatively stable during 4 months; only arginine, aspartic and glutamic acids increased slightly. No visible change occurred in the morphology of storage protein bodies to indicate their degradation. Weisman (98) concluded that proteolysis observed during stratification was not related to the release of dormancy but could be an essential process in germination or early growth of the embryo.

Dawidowicz-Grzegorzewska et al. (23) found that the content of free amino acids in apple embryos rose dramatically during cold treatment, a four-fold increase being noted after 30 days at 5°C. The amount of aspartic and glutamic acids increased up to the 17th day of cold treatment, after wich the level of aspartic acid decreased. The levels of arginine and asparagine plus threonine were constant until the 12th day of cold treatment, then increased. However, few of these changes were observed in embryos kept inside the fruit at 0°C for 4 months. The authors confirmed the findings and the conclusion of Weisman (98).

Protease activity in apple embryos increased 5-fold during the first 50 day of stratification, decreased sharply during the next 20 day then remained at a level similar to that in dormant seeds (58). The amount of soluble protein in apple embryos increases during the first two months of stratification, then decreases slightly. No change occurred during the last 3 wk. Biosynthesis of new protein, which is relatively low during the first 6 wk of after-ripening, sharply increases in the course of the last month (58).

Protein bodies were observed in almost all cells of dormant apple embryos (22). During stratification degradation was confined to those bodies in the embryo axis. Considerable swelling of the bodies and their inclusions occurred soon after exposure to chilling temperatures. They disappeared from the embryo axis and were gradually replaced by vacuoles. Removal of storage proteins was followed successively by a general increase in intensity of cytoplasmic staining, and proliferation of the new cell organelles, among which amyloplasts were clearly recognizable. Degradation of protein bodies in the cotyledons started after those in the embryo axis had disappeared.

Protein degradation in apple embryos during cold stratification was associated with a significant increase of nucleolar volume. The size and structure of the nucleolus served as a sensitive parameter of cellular activity (22). Simultaneous increase in size and development of nucleolar vacuoles was interpreted as an indication of metabolic activity and this was associated with greater ability of seeds to synthesize RNA. None of these changes was observed during warm (25°C) stratification.

Degradation of protein bodies in apple embryos was initiated after 21 day of cold treatment. After 42 day all radicle cells and part of the lower hypocotyl cells were devoid of the storage proteins. Increase in some free amino acids and the disappearance of storage protein bodies in apple embryos during cold treatment led Dawidowicz et al. (23) to postulate that dormancy release of apple embryos is related to proteolysis.

Hydrolysis of reserve protein occurs at both 5° and 20°C when apple embryos are placed on a moist medium. However, proteolysis does not occur in seeds left inside the fruits at 0°C (14). These data, combined with those of Weisman (98) on free amino acid suggest that proteolysis is not required for dormancy removal.

Eichholtz et al. (30) found that total soluble proteins in axes and cotyledons of apple seeds changed very little during stratification at 5° or 15°C. No changes occurred in the profiles of cotyledon polypeptides. However, notable profile changes occurred in axes at 5° but not at 15°. Four polypeptides with apparent subunit molecular weights of 70, 50, 32 and 28.5 kd increased in axes held at 5°. The 70 kd polypeptide tripled during stratification at 5°. The 48.5 kd, occurring only in axes at 5°, was first visible on gels after 3 wk at 5° and reached a maximum after 7 wk. Major polypeptides (47, 38, 36, 29.5 and 24.5 kd) decreased much more at 5° than at 15°. These results led the authors to conclude that during stratification the protein reserves are specifically mobilized in the axes of seeds.

In contrast, Tao and Khan (85) found no significant changes in soluble protein content of pear embryos during chilling. An increase in sample size did not alter the results.

Alscher-Herman and Khan (2) reported that stratification increased the *in vitro* translational capacities of polyribosomes isolated from pear seeds, with a maximum occurring at 13 day. Peak activity occurred simultaneously with the ability of excised embryos to grow, at the end of the period of stratification required for normal development of excised embryos, i.e., expansion of both cotyledons, rather than only the cotyledon in contact with a moist medium. The authors suggested that this might reflect synthesis of particular proteins whose activity is crucial to the dormancy breaking process. The increase was greatly diminished in seeds which were held continously at 25°C.

Michalski (63) submitted Mazzard cherry pits to the following treatments: (i) constant 3° for 32 wk; (ii) 20° for 3 wk plus 3° for 32 wk; (iii) 3° for 5 wk plus 25° for 2 wk plus 3° for 32 wk; (iv) 20° for 3 wk plus 3° for 2 wk plus 25° for 2 wk, plus 3° for 32 wk. The author evaluated periodically pit cracking, seed germination and protein synthesis (by incorporation ^{14}C-leucine) in embryo axes at both 3° and 20°C. The results of this study are summarized in Table 4.

Table 4. Effects of stratification temperature on pit cracking, germination, and protein synthesis in Mazzard cherry seeds (Michalski 1982).

Treatment	Pit cracking (%)	Seed germ. (%)	Peaks of protein synthesis at 20°C (cpm)		
			1	2	3
continuous 3° 32 wk	18	15	15000(0)	16000(5)	-
20° 5 wk 3° 32 wk	89	88	15000(0)	29000 (24 hr)	12500(6)
3° 5 wk 25° 2 wk 3° 32 wk	89	85	15000(0)	16000(5)	12000(6)
20° 3 wk 3° 2 wk 25° 2 wk 3° 32 wk	89	88	15000(0)	29000(0)	17000(0)

() time in wk at 3°C at which the peak of protein synthesis occurred.

Michalski (63) concluded that the rate of leucine incorporation was relatively high (15000 cpm) at the beginning suggesting that protein synthesis started during the first hr of water absorption by the embryo axes. A rise in temperature (from 3° to 20° or 25°C) reduced protein synthesis during the first 2 wk of the warm period, while reduction of temperature induced a protein synthesis increase 24 hr later. In the first wk of cold stratification with one warm phase two consecutive peaks of protein synthesis were observed before the start of stone cracking. In the continuous cold only one peak occurred and germinative capacity remained very low. The author suggested that two consecutive peaks of protein synthesis intensity could be required for preparation of seeds to germinate.

Protein synthesis (measured by the incorporation of radioactive leucine) during the first 9 hr of imbibition was higher in the dormant cocklebur axes and cotyledons than in non-dormant ones (73). However, when imbibed for more than 12 h the reverse was observed. Cycloheximide blocked protein synthesis in the axes regardless of their dormant status, and prevented germination of non-dormant seeds. However, cycloheximide at 3 mM slightly stimulated germination of dormant seed. Satoh and Esashi (73) suggested that proteins may be involved in the maintenance of dormancy in cocklebur seeds.

Following the breaking of seed dormancy by 3 months of dry storage at room temperature protein synthesis in embryos of _Agrostema githago_ L. increased in 3 successive stages (25): (a) concurrent with swelling; (b) during the lag phase between the completion of water uptake and the onset of growth; (c) immediately after protrusion of the radicle through the seed coat. The increase during the lag phase occurred only in non-dormant seeds indicating that after-ripening affected protein synthesis prior to the onset of growth.

Seeds of sugar pine (_Pinus lambertiana_ Dougl.) require moist chilling to break their dormancy. The capacity for protein synthesis increased rapidly during the first 7 day at 25°C in embryos and megagametophytes and then decreased gradually through the rest of the 90 day treatment period (65). These seeds remained dormant. Protein synthesis capacity of embryos held at 5° surpassed that of those kept at 25° after 60 day but remained significantly lower in megagametophytes at 5° than at 25°C throughout the 90 day period. The pattern of increase in the capacity for protein synthesis in embryos held at 5° generally paralleled increases in germinability (50% after 30 day and

84% at the end of the treatment period).

Protein banding patterns of embryos were virtually identical regardless of temperature or stratification time. However, 4 bands were consistently darker at 25° than at 5°C after 30 and 60 day of stratification. The patterns in megagametophytes also remained virtually unchanged over the 90 day treatment period, as did their staining intensity. The megagametophytes had only 9 distinguishable protein bands compared to the 16 bands observed in embryos. The megagametophyte profile contained two rather prominent bands which may represent major storage proteins. Although the rate of protein synthesis in the embryos and percent germinability during stratification were highly associated, warm incubation (25°C) also resulted in a high protein synthesis rate but failed to break dormancy. Noland and Murphy (65) speculated that the synthesis of germination-inhibiting proteins may control seed dormancy. After-ripening treatment could affect the rate of production and/or degradation of such protein(s) thereby stimulating germination (24).

The higher leucine-tyrosine aminopeptidase (LTA) activity in sugar pine embryos at 5° vs 25°C could be an indication of the destruction or modification of dormancy-maintaining protein(s) (65). A slight stimulation of dormant cocklebur seed germination by cycloheximide (73) also supports the concept of a germination inhibiting protein. Furthermore, actinomycin D and chloramphenicol significantly stimulated germination of dormant lettuce (Lactuca sativa, L. cv Grand Rapids) seeds (9). These results imply that synthesis of protein is involved in maintaining dormancy and that germination may be promoted by hydrolysis or modification of germination-inhibiting proteins.

Leucine-tyrosine aminopeptidase (LTA) activity in sugar pine embryos kept at 25° increased to a peak at 14 day and then dropped to its lowest level at 30 day, whereas the LTA activity of 5°C embryos reached a maximum at 60 day (70% germination) and then began decreasing (65). The LTA activity in embryos held at 5° was significantly higher ($P=0.05$), than that in those held at 25°C at 30 and 60 day. The LTA activity of the megagametophytes changed little at either temperature; the LTA activity at 5° was significantly higher ($P=0.05$) than at 25° only at 30 day. Noland and Murphy (65) speculated that the higher LTA activity in 5° treated-embryos could be an indication of the destruction or modification of dormancy-maintaining proteins.

Protein hydrolysis in the endosperm was accompanied by an increase in protein in the embryo in ash (*Fraxinus excelsior*) during warm moist storage (90). When the seed was stored at chilling temperatures immediately after imbibition, the mobilization of nitrogenous substances did not occur. This may explain the requirement for a period of warm temperature storage before chilling treatment in this species.

Jarvis and Hunter (51) reported that treatment with GA_3 stimulated both germination and protein synthesis in embryonic axes of hazel seeds. Associated with this increased protein synthesis were increases in tRNA and aminoacyl-tRNA synthetase activity.

Protein synthesis does not occur within the mature dry seed, and polysomes are usually absent (8). Upon imbibition the ribosomes, together with mRNA, initiation and elongation factors, tRNA, amino acids, aminoacyl tRNA synthetases, ATP and GTP, commence protein synthesis. These components are presumably conserved within mature dry seeds and appear to be present in sufficient quantities for protein synthesis.

When water is added to a dry, non-dormant seed, a variety of metabolic processes quickly resume, including RNA and protein synthesis (8) and protein synthesis is probably a pre-requisite for germination. Germination-related protein synthesis probably occurs within the embryonic axis itself. One should distinguish between studies on protein synthesis in the axis and those on protein synthesis in storage organs, for protein synthesis in the latter is probably not related to germination *per se*, but to the mobilization of stored reserves, a post-germination phenomenon.

Bewley (8) stated that at the present time it is impossible to identify any proteins that are essential for completion of the germination process, or to determine if such proteins (if they exist) require the synthesis of mRNA's at any time during germination. Changes of a few proteins within a few cells of the radicle could be responsible for the initiation of radicle elongation.

Protein and RNA metabolism are complex processes, for synthesis and/or activation of hydrolytic enzymes are accompanied by catabolism of both proteins and RNAs (8). Changes in metabolism within storage organs are generally unrelated to germination; they are almost exclusively related to the mobilization of stored reserves, a post-germination phenomenon associated with seedling growth. The mobilization of stored reserves is an event that requires the participation of many enzymes, a substantial number of which must be synthesized *de novo*.

CONCLUSIONS

Changes in metabolism during dormancy have been studied by a number of workers. These studies led to variable results ranging from high correlation to the absence of any relationship between after-ripening and metabolic changes. Most workers agree on the occurrence of various metabolic changes but disagree on their role and on the nature of a relationship existing between these changes and dormancy removal. Variation could be explained by: (i) researchers have used a wide variety of species and plant materials; (ii) the absence of an appropriate control; (iii) the lack of powerful analytical techniques capable of detecting minor changes; (iv) problems related to terminology and the need for a mean to clearly separate between physiological stages especially, after-ripening *per se* and germination.

At this point we can summarize the findings of previous studies as follows: (i) reserve food changes do not appear to play a role in the breaking of dormancy since lipid breakdown, starch accumulation and increase in sugar can take place even at higher temperatures having no effects on dormancy removal; (ii) the breaking of dormancy is apparently accompanied by an increased capacity of nucleic acids synthesis; (iii) free amino acids do not appear to be related to dormancy removal but could play an important role in germination and/or growth of the embryo. Free amino acid content increases as much as or more at 20^o than at 5^o in apple embryos. None of these changes occurred in embryos inside the fruits held at 0^oC. However, hydrolysis of the proteins to their constituent amino acids is an advanced stage of breakdown and could well be due to germination and probably to growth which is higher at 20^o but could take place even at low but non freezing temperatures. It is clear that most if not all previous studies on protein metabolism in relation to dormancy focused on quantitative rather than qualitative changes. The quantitative changes must be important in order to be detected using current quantitation procedures. Electrophoresis constitutes a powerful tool for evaluation of protein profile during stratification and warm incubation. In this respect we decided to evaluate the qualitative and quantitative changes in peach seed proteins in response to stratification temperature.

PART FOUR

EFFECTS OF STRATIFICATION TEMPERATURE AND TIME ON PROTEIN CHANGES IN PEACH SEEDS

INTRODUCTION

The objectives of this study were to assess the effects of stratification temperature and duration on qualitative and quantitative changes in the protein content of peach seed cotyledons and embryonic axes. Several treatments were used to determine the effects of preventing embryo development during stratification, or hastening the breaking of dormancy with gibberellic acid, on the observed changes in protein profile.

MATERIAL AND METHODS

1. Plant Material

Two lots of Siberian C (SibC) peach pits were obtained from Hilltop Nurseries, Hartford, MI, USA, and one lot each of 'Farouki' and 'Maloussi' (local seedlings used as rootstocks) were purchased from seed collectors in the Missour region of Morocco. Following removal of the pericarp, the pits were washed, dried at room temperature, and stored at 5°C until used.

Three experiments were conducted concurrently, using one set of intact, fully imbibed seeds as controls for all three.

a. Effects of degree of imbibition

To determine whether the observed changes in protein profile occurred during the breaking of dormancy of seeds which were unable to germinate, 'Farouki' and 'Maloussi' peach seeds were soaked in water for 5, 15, or 72 hr (25, 50, and 100% of full imbibition, as determined by water content). The seeds were then wrapped in a piece of cheesecloth which was attached to the top of a 250 ml jar containing 25 ml of Captan solution (0.03%). The jar was tightly sealed with a cap and Parafilm, then held at 5°C for 2 to 10 wk. At 2 wk intervals the following data were recorded: i) fresh and dry weights; ii) germination both with and without additional imbibition; iii) soluble protein contents and protein profiles for both axes and cotyledons.

b. <u>Effects of stratification of cotyledons separately from embryonic axes</u>

The embryonic axis might be responsible for changes observed in protein content of the cotyledons, even though similar changes did not occur in the axis itself. To test this possibility, seeds were imbibed for 72 hr, then cut transversely with a razor. The chalazal and micropylar ends of the seeds were then stratified independently at 5°C. The cotyledons remaining in the chalazal end represented 94-97% and 89-95% fresh weight of the intact cotyledons, respectively, for 'Farouki' and 'Maloussi'. After 0 to 8 wk, the embryonic axis was dissected out of the micropylar end, and the cotyledons from the chalazal end. Fresh and dry weights of the cotyledons in chalazal and micropylar ends and of the axis were recorded, but germination capacity of the axis was not tested. Protein content was determined as described above.

c. <u>Effects of gibberellic acid</u>

GA_3 stimulated germination in partially chilled peach seeds (28). To determine if protein hydrolysis was also hastened by GA treatment, seeds were soaked in distilled water for 48 hr, then some of the seeds were transferred to a solution of GA_3 (500 ppm) for the remaining 24 hr of a 72 hr imbibition period. Control seeds were held for 72 hr in water (same controls as used in Parts 1 and 2 above). Thirty seeds from each treatment (time of stratification at 5°C x GA_3 for 24 hr) were used to evaluate germination at 20°C. Protein content was determined as described above.

2. Stratification, germination, fresh and dry weights

Seeds were removed from the pits with a mechanical cracker, washed in 'Captan' solution (0.03%) and imbibed for 72 hr in the same solution. The seeds were then stratified on sterile filter paper moistened with Captan solution in petri dishes held in the dark at either 5° or 20°C for 1 to 10 wk.

For evaluation of germination capacity, three replications of 10 seeds each were held in petri dishes at 20°C in the dark. The seeds were considered to have germinated if the radicle had elongated 3 mm or more after 14 day.

Thirty seeds from each treatment were dissected into cotyledons and embryonic axes for determination of both fresh and dry weights. Fresh weights were recorded immediately after dissection, and dry weights after 72 hr at 90°C.

3. Analysis of protein content

For determination of soluble protein content, 10 embryonic axes were extracted in 0.5 ml of 62.5 mM Tris-HCl (pH 6.8) in a small mortar. The homogenate, along with two 0.25 ml rinses, was left overnight at room temperature (about 20°C) and cleared by centrifugation at 14,000 rpm for 20 min. The supernatant was filtered through Whatman n°1 filter paper and stored in a freezer until use. The equivalent of one pair of cotyledons from eight seeds were homogenized in 2 ml of the same buffer in a small mortar. The homogenate, together with two 1 ml rinses, was treated as above.

Protein content was determined according to Bradford's (16) method. This assay is based on the fact that the absorbance for an acid solution of Coomassie Brillant Blue G-250 shifts from 465 nm to 595 nm when binding to protein occurs.

For SDS polyacrylamide gel electrophoresis (PAGE) samples were extracted as described above with 62.5 mM Tris-HCl containing SDS (2%), glycerol (10%), 2-B-mercaptoethanol (5%) and bromophenol blue (0.002%, as a tracking dye). Electrophoresis was performed according to Laemmli (56), with 12% (w/v) polyacrylamide on 1.5 mm slab gels, for the separating and 4% (w/v) for the stacking gels at constant temperature (20°C) and constant current of 20 mA. Fifty ug of proteins were loaded per well. Gels were stained with Coomassie Brilliant Blue R-250 in 40% methanol plus 10% glacial acetic acid for 4 hr, then destained for 12 hr. Three replicate gels were run for each sampling date.

The relative mobilities (Rf) of the proteins were determined from photographs of the gels. Molecular weight (MW) is a log function of the relative mobility. The relative mobilities of proteins of known MW were used to establish a standard curve. This curve was used to estimate the MW of proteins in the extracts. Low range molecular weight standards (purchased from Bio-Rad, 32nd & Griffin Ave., Richmond CA 94804) were used in each electrophoretic run. The 6 standards and their MW in kilodaltons were: rabbit muscle phosphorylase b (RMPb), 97.4 kd; bovine serum albumin (BSA), 66.20 kd; hen egg white ovalbumin (HEWO), 42.699 kd; bovine carbonic anhydrase (BCA), 31.0 kd; soybean trypsin inhibitor (STI), 21.5 kd; hen egg white lysozyme (HEWL), 14.4 kd.

RESULTS

1. Effects of stratification time and temperature

In the first experiment, none of the Siberian C seeds germinated regardless of stratification duration or temperature (data not shown). Fresh and dry weights of cotyledons and embryonic axes did not change appreciably during stratification at either 5° or 20°C (data not shown). Soluble protein content of cotyledons and embryonic axes ranged from 26 to 30%, and 15 to 18% DW, respectively, but was affected by neither temperature nor time of stratification (data not shown). Protein patterns on PAGE gels also remained unchanged (Figures 32 and 33).

In the second experiment, seeds held at 20°C again did not germinate. Those held at 5° began to germinate after 5 wk, and percentage germination increased with time at 5°. Germination percentages for 5 through 8 wk were 20, 45, 75, and 94%, respectively (all significantly different from one another at the 5% level). Fresh weights and water content of both cotyledons and embryonic axes increased with time at both 5° and 20°, with a greater rate of increase at the lower temperature (Figures 34-35). Dry weights remained constant at approximately 0.65 and 260 mg, for embryonic axes and cotyledons, respectively (data not shown). Soluble protein content again remained unchanged at 26 to 28% DW (cotyledons) and 14-16% (embryonic axes), regardless of temperature or time of stratification (data not shown).

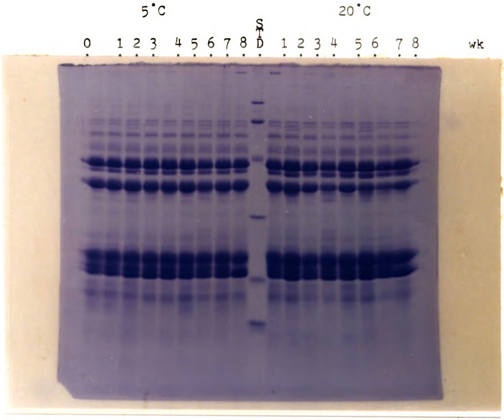

Figure 32. Effects of stratification temperature and duration on protein profile of Siberian C peach cotyledons.

0 : Control (imbibed for 72 hr, no chilling)
STD : low Range Molecular Weight Standards :

1. RMPb(97.4); 2. BSA(66.2); 3. HEWO(42.7);
4. BCA(31.0); 5. STI(21.5); 6. HEWL(14.4Kd)

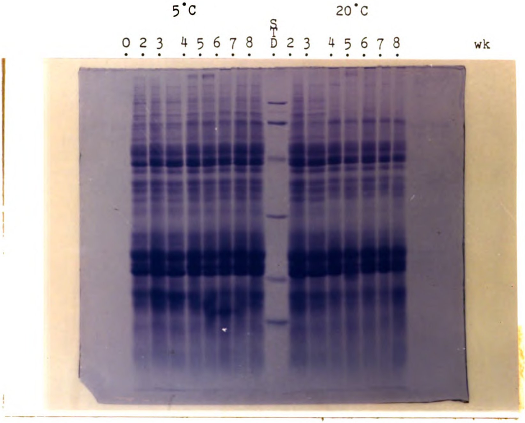

Figure 33. Effects of stratification temperature and duration on protein profile of Siberian C peach embryonic axes.

0 : Control (imbibed for 72 hr, no chilling)
STD : Low Range Molecular Weight Standards :

1. RMPb(97.4); 2. BSA(66.2); 3. HEWO(42.7); 4. BCA(31.0); 5. STI(21.5); 6. HEWL(14.4Kd)

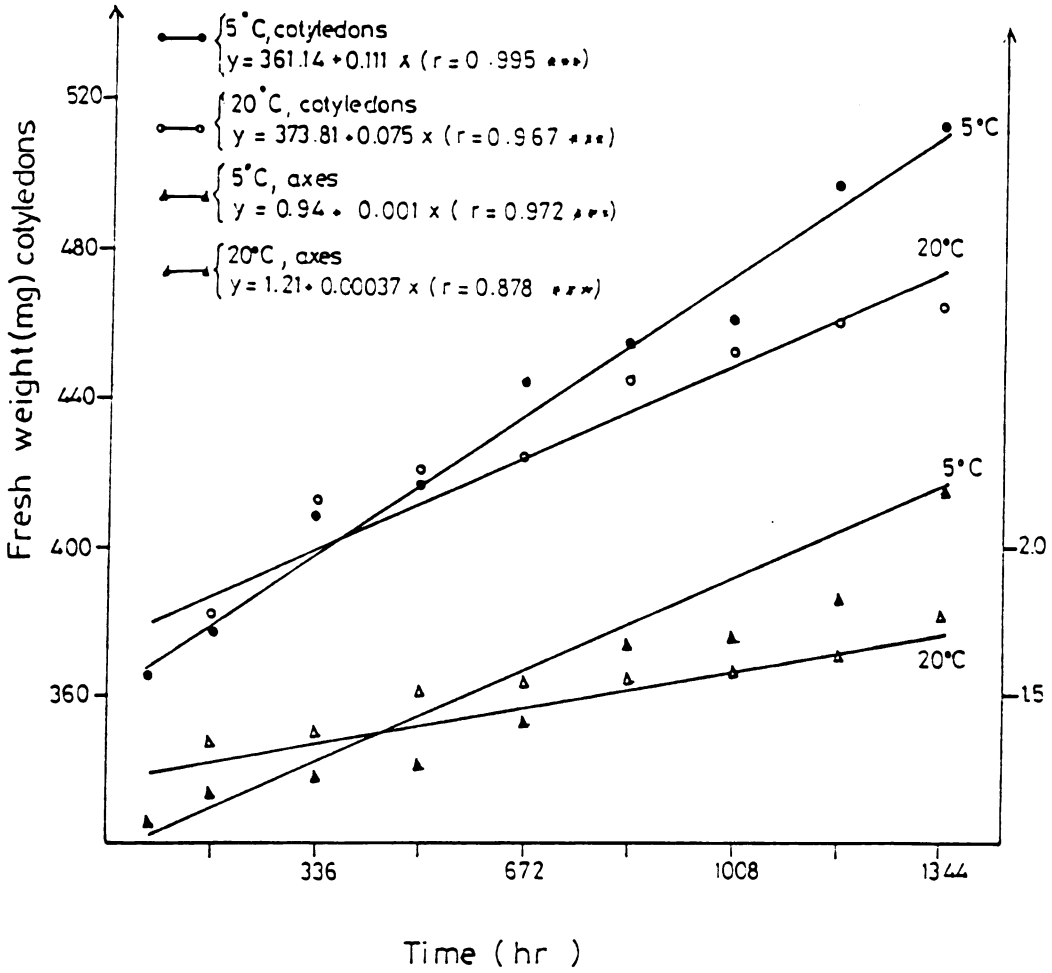

Figure 34. Effects of stratification temperature, and duration on fresh weight (mg) of Siberian C peach embryonic axes and cotyledons.

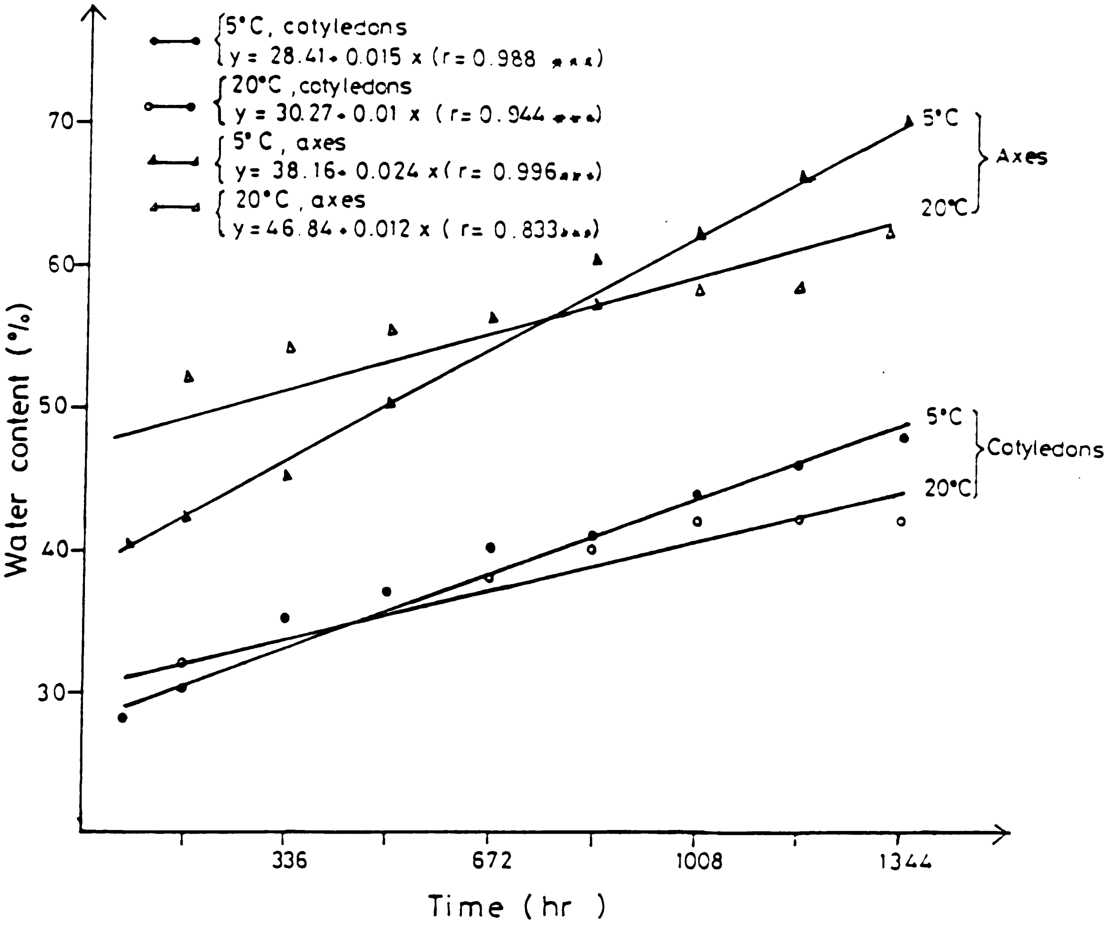

Figure 35. Effects of stratification temperature and duration on water content (%) of Siberian C peach embryonic axes and cotyledons.

PAGE revealed a decrease in the concentrations of 9 polypeptides in the cotyledons of seeds held at 5°C during the 5th to 8th wk (Figure 36), coinciding with an increase in germination capacity. These decreases were accompanied by the appearance of 2 or 3 new polypeptides of low molecular weights (10 - 14 kd). None of these changes occurred in the embryonic axes of these seeds (Figure 37), or in either cotyledons or axes of seeds held at 20°C. Although the concentration of polypeptide n°13 (36 kd) decreased in axes of seeds held at both temperatures, levels of the same polypeptide, or one of similar molecular weight, in the cotyledons declined only at 5°C.

That major changes occurred in cotyledons only at 5°C, and only when the seeds were capable of germination, suggested that the changes were associated with after-ripening, germination, or both.

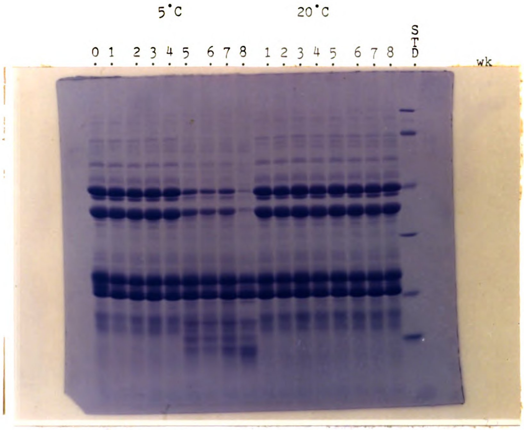

Figure 36. Effects of stratification temperature and duration on protein profile of Siberian C peach cotyledons.

O : Control (imbibed for 72 hr, no chilling)
STD : Low Range Molecular Weight Standards :

1. RMPb(97.4); 2. BSA(66.2); 3. HEWO(42.7);
4. BCA(31.0); 5. STI(21.5); 6. HEWL(14.4Kd)

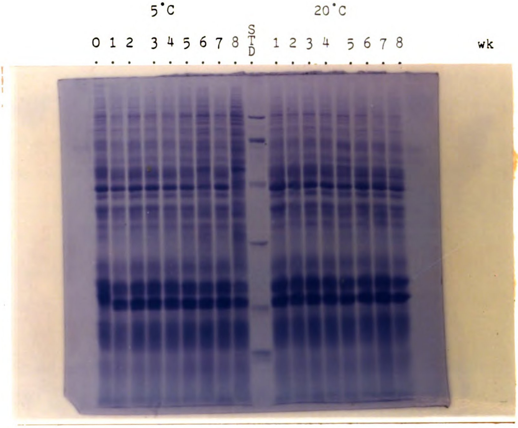

Figure 37. Effects of stratification temperature and duration on protein profile of Siberian C peach embryonic axes.

O : Control (imbibed for 72 hr, no chilling)
STD : Low Range Molecular Weight Standards :

1. RMPb(97.4); 2. BSA(66.2); 3. HEWO(42.7); 4. BCA(31.0); 5. STI(21.5); 6. HEWL(14.4Kd)

2. Effects of degree of imbibition

Germination of fully imbibed seeds at 20° increased steadily as time of stratification at 5° increased. Partially imbibed seeds did not germinate regardless of time of stratification (data not shown), but when soaked in water, their germination paralleled that of fully imbibed seeds (Figures 38-39). Thus after-ripening occurred even though the seeds were incapable of germination.

Both fresh weight and water content of cotyledons and embryonic axes increased with time of stratification of fully imbibed seeds, but the rate of increase was greater at 5° than at 20°C. Data for partially imbibed seeds held at 5° paralleled those for fully imbibed seeds, but all values were lower, as might be expected. Thus the water content of seed parts of partially imbibed seeds following 8 weeks of stratification was approximately equal or less than that of seed parts of fully imbibed but non-stratified seeds. None of the treatments affected dry weights of either axes or cotyledons (data not shown).

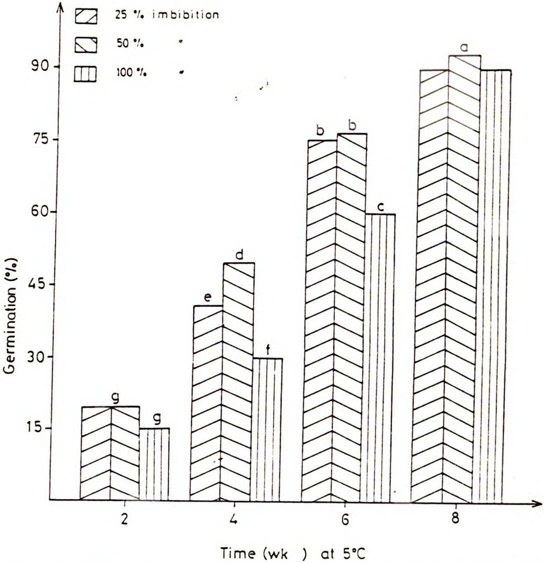

Figure 38. Effect of degree of imbibition and duration of stratification at 5°C on germination (%) of 'Farouki' peach seeds.

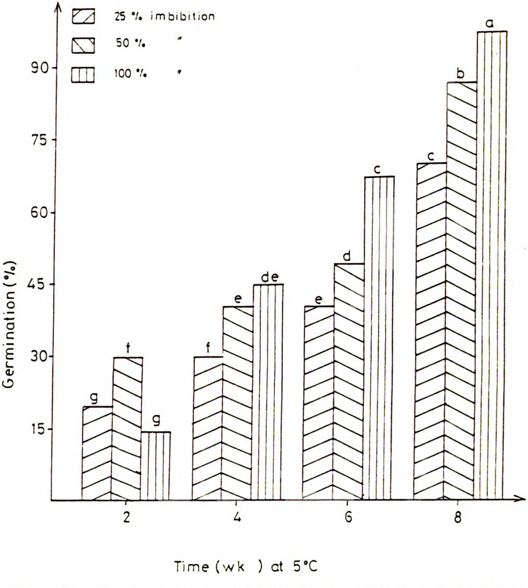

Figure 39. Effects of degree of imbibition and duration of stratification at 5°C on germination (%) of 'Malcussi' peach seeds.

Soluble protein content varied from 19 to 24% (cotyledons) or 9 to 12% (embryonic axes), and was not affected by cultivar or treatment (data not shown). Changes in protein profiles in fully imbibed seeds confirmed those previously observed in Siberian C seeds. Decreases in intensity of 9 bands were evident in extracts of the cotyledons after 2 wk of stratification and the decreases continued through the end of the experiment (8 wk). Again, two bands of lower molecular weight appeared at the same time. However, none of these changes occurred in axes of these seeds or in either axes or cotyledons of fully imbibed seeds kept continuously at 20°C (incapable of germination) (Figures 40-43). Although the concentrations of polypeptides 11 and 13, 41 and 36 kd respectively decreased in axes of seeds held at both temperatures, levels of the same polypeptides, or ones of similar molecular weights, in the cotyledons decreased only at 5°.

Similar changes occurred in cotyledons of partially imbibed seeds during stratification at 5°C, but they occurred more slowly (Figures 44-49). Thus the intensities of bands 11 and 13 in partially imbibed seeds stratified for 10 wk were approximately equivalent to those of the same bands in fully imbibed seeds stratified for only 3 wk. In 'Maloussi' seeds, low molecular weight bands also were slower to appear, especially in seeds imbibed for only 5 hr.

Data from this experiment confirm the effects of stratification temperature observed in 'Siberian C' seeds, and indicate that such changes occur during the breaking of dormancy even in seeds which are incapable of germination because of limited water content. However, the changes are delayed by partial imbibition whereas germination capacity (ability to germinate when fully imbibed) is not.

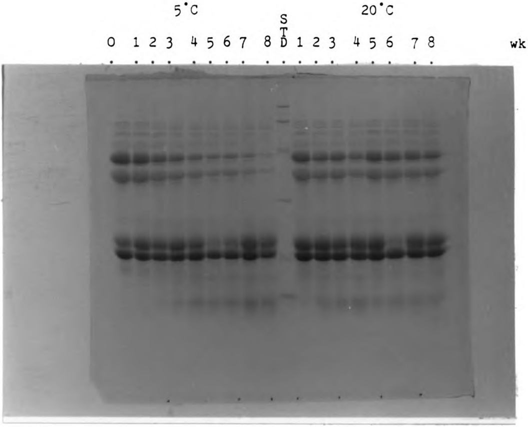

Figure 40. Effects of stratification temperature and duration on protein profile of "Farouki" peach cotyledons.

0 : Control (imbibed for 72 hr, no chilling)
STD : Low Range Molecular Weight Standards :

1. RMPb(97.4); 2. BSA(66.2); 3. HEWO(42.7); 4. BCA(31.0); 5. STI(21.5); 6. HEWL(14.4Kd)

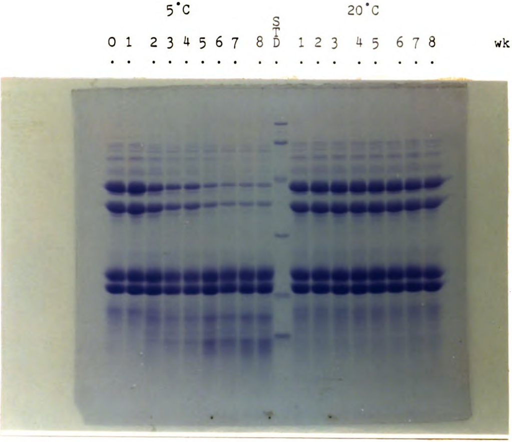

Figure 41. Effects of stratification temperature and duration on protein profile of "Maloussi" peach cotyledons.

O : Control (imbibed for 72 hr, no chilling)
STD : Low Range Molecular Weight Standards :

1. RMPb(97.4); 2. BSA(66.2); 3. HEWO(42.7);
4. BCA(31.0); 5. STI(21.5); 6. HEWL(14.4Kd)

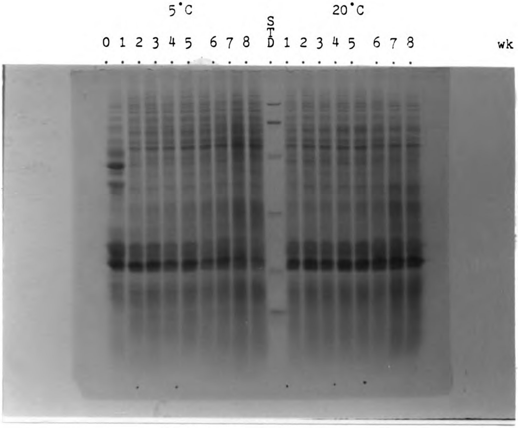

Figure 42 . Effects of stratification temperature and duration on protein profile of "Farouki" peach embryonic axes.

O : Control (imbibed for 72 hr, no chilling)
STD : Low Range Molecular Weight Standards :

1. RMPb(97.4); 2. BSA(66.2); 3. HEWO(42.7);
4. BCA(31.0); 5. STI(21.5); 6. HEWL(14.4Kd)

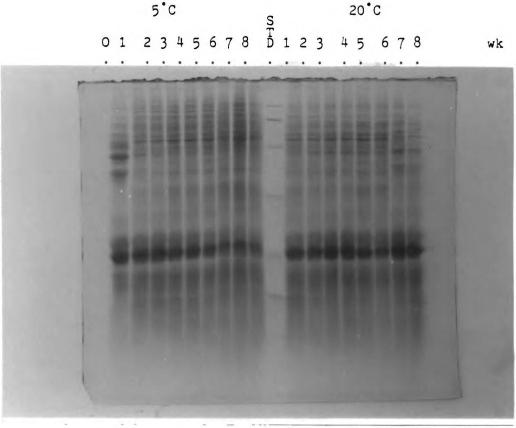

Figure 43. Effects of stratification temperature and duration on protein profile of "Maloussi" peach embryonic axes.

O : Control (imbibed for 72 hr, no chilling)
STD : Low Range Molecular Weight Standards :

1. RMPb(97.4); 2. BSA(66.2); 3. HEWO(42.7);
4. BCA(31.0); 5. STI(21.5); 6. HEWL(14.4Kd)

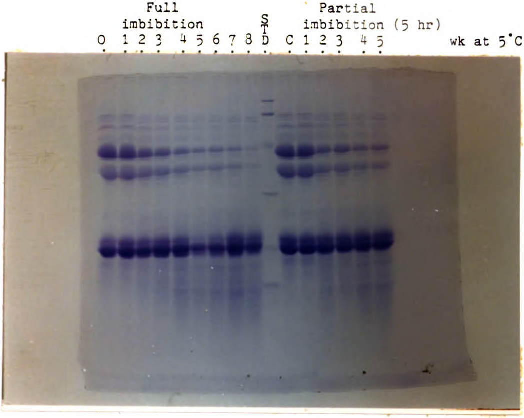

Figure 44. Effects of stratification temperature and duration on protein profile of "Farouki" peach cotyledons from fully and partially imbibed (5 hr) seeds.

O : Control (imbibed for 72 hr, no chilling)
C : Control (imbibed for 5 hr, no chilling)
STD : Low Range Molecular Weight Standards :

1. RMPb(97.4); 2. BSA(66.2); 3. HEWO(42.7); 4. BCA(31.0); 5. STI(21.5); 6. HEWL(14.4Kd)

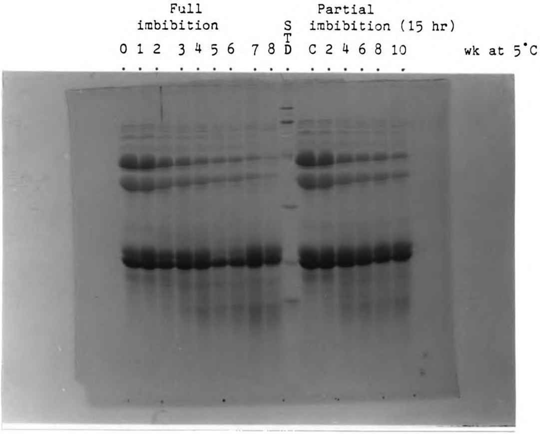

Figure 45. Effects of stratification temperature and duration on protein profile of "Farouki" peach cotyledons from fully and partially imbibed (15 hr) seeds.

O : Control (imbibed for 72 hr, no chilling)
C : Control (imbibed for 15 hr, no chilling)
STD : Low Range Molecular Weight Standards :

1. RMPb(97.4); 2. BSA(66.2); 3. HEWO(42.7);
4. BCA(31.0); 5. STI(21.5); 6. HEWL(14.4Kd)

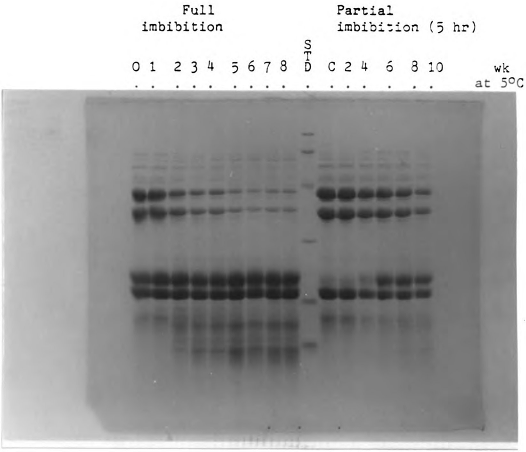

Figure 46. Effects of stratification temperature and duration on protein profile of "Maloussi" peach cotyledons from partially (5 hr) and fully imbibed seeds.

0 : Control (imbibed for 72 hr, no chilling)
C : Control (imbibed for 5 hr, no chilling)
STD : Low Range Molecular Weight Standards :

1. RMPb(97.4); 2. BSA(66.2); 3. HEWO(42.7); 4. BCA(31.0); 5. STI(21.5); 6. HEWL(14.4Kd)

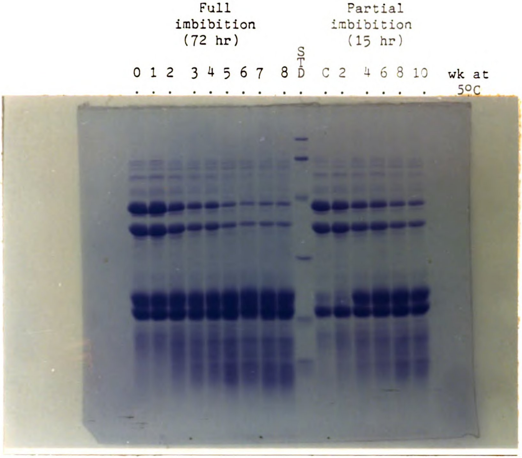

Figure 47. Effects of stratification temperature and duration on protein profile of "Maloussi" peach cotyledons from partially (15 hr) and fully imbibed seeds.

0 : Control (imbibed for 72 hr, no chilling)
C : Control (imbibed for 15 hr, ni chilling)
STD : Low Range Molecular Weight Standards :

1. RMPb(97.4); 2. BSA(66.2); 3. HEWO(42.7);
4. BCA(31.0); 5. STI(21.5); 6. HEWL(14.4Kd)

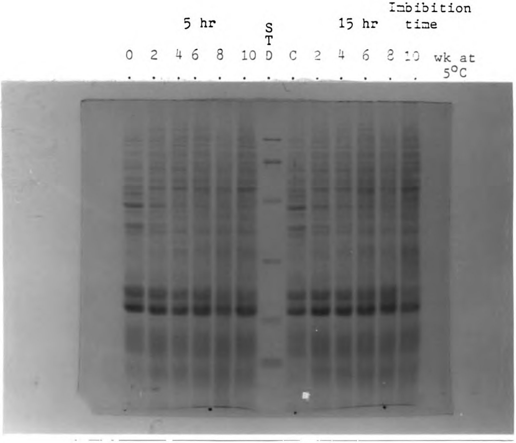

Figure 48. Effects of stratification temperature and duration on protein profile of "Farouki" peach embryonic axes from partially imbibed seeds.

O : Control (imbibed for 5 hr, no chilling)
C : Control (imbibed for 15 hr, no chilling)
STD : Low Range Molecular Weight Standards :

1. RMPb(97.4); 2. BSA(66.2); 3. HEWO(42.7);
4. BCA(31.0); 5. STI(21.5); 6. HEWL(14.4Kd)

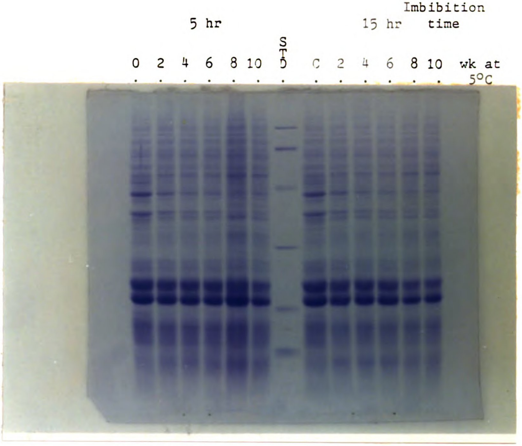

Figure 49. Effects of stratification temperature and duration on protein profile of "Maloussi" peach embryonic axes from partially imbibed seeds.

O : Control (imbibed for 5 hr, no chilling)
C : Control (imbibed for 15 hr, no chilling)
STD : Low Range Molecular Weight Standards :

1. RMFb(97.4); 2. BSA(66.2); 3. HEWO(42.7);
4. BCA(31.0); 5. STI(21.5); 6. HEWL(14.4Kd)

3. Effects of stratification of cotyledons separately from embryonic axes

Fresh weights and water contents of both embryonic axes and cotyledons increased with time at 5°C. Dry weights of cotyledons were approximately 78 and 88%, respectively for 'Farouki' and 'Maloussi', of those of intact seeds because of removal of portions in cutting the seeds. Dry weights and total soluble protein content were again not affected by time at 5°C (data not shown). Protein profiles in the cotyledons were unaffected by removal of the embryonic axis prior to stratification; the two bands at 41 and 36 kd decreased in intensity at the same rates, and new bands with high mobility appeared as the larger polypeptides disappeared (Figures 50-51). Thus the embryonic axis is not required for changes to occur in the cotyledons. Protein profile of axes showed no visible changes except the disappearance of bands at 41 and 36 kd observed earlier at both 5° and 20°C (Figures 52-53).

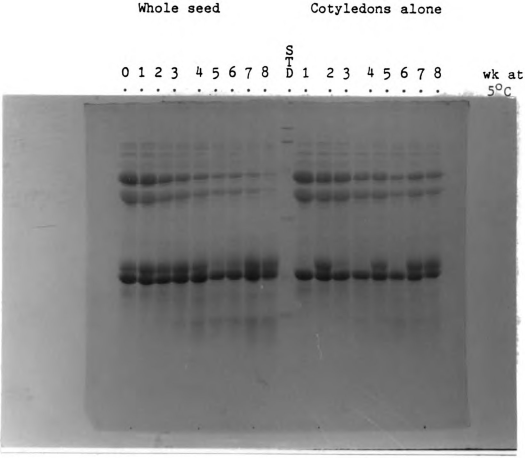

Figure 50. Effects of stratification temperature and duration on protein profile of "Farouki" peach cotyledons in the presence and the absence of embryonic axes.

0 : Control (imbibed for 72 hr, no chilling)
STD : Low Range Molecular Weight Standards :

1. RMPb(97.4); 2. BSA(66.2); 3. HEWO(42.7); 4. BCA(31.0); 5. STI(21.5); 6. HEWL(14.4Kd)

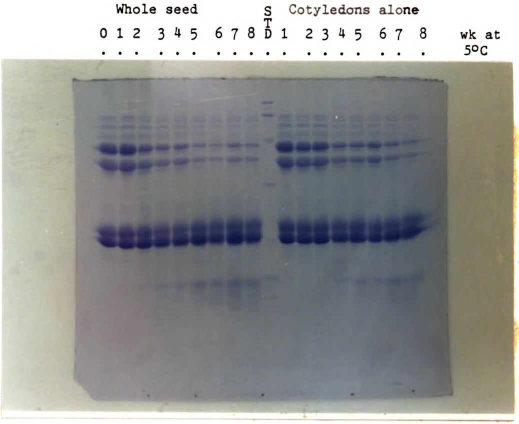

Figure 51. Effects of stratification temperature and duration on protein profile of "Maloussi" peach cotyledons in the presence and the absence of embryonic axes.

O : Control (imbibed for 72 hr, no chilling)

STD : Low Range Molecular Weight Standards :

1. RMPb(97.4); 2. BSA(66.2); 3. HEWO(42.7); 4. BCA(31.0); 5. STI(21.5); 6. HEWL(14.4Kd)

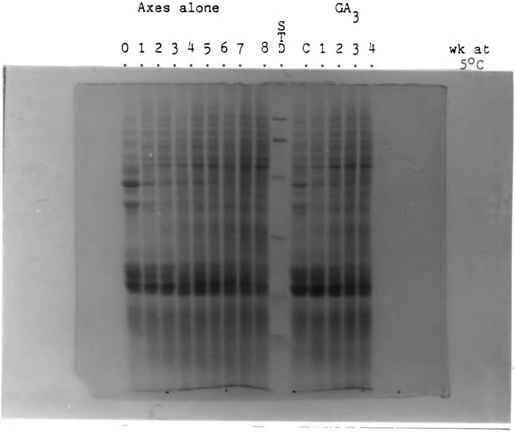

Figure 52. Effects of gibberellic acid (GA$_3$ at 500 ppm for 24 hr) and stratification temperature and duration on protein profile of partially and separately stratified "Farouki" peach embryonic axes.

O : Control (imbibed for 72 hr, no chilling)
C : Control (imbibed for 48 hr, then soaked in GA$_3$ solution for 24 hr, no chilling)
STD : Low Range Molecular Weight Standards :

1. RMPb(97.4); 2. BSA(66.2); 3. HEWO(42.7);
4. BCA(31.0); 5. STI(21.5); 6. HEWL(14.4Kd)

GA$_3$ treated seeds were dissected 48 hr following the treatment.

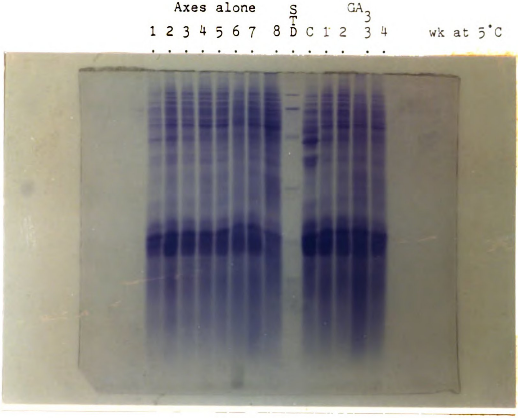

Figure 53. Effects of gibberellic acid (GA_3 at 500 ppm for 24 hr) and the absence of cotyledons on protein profile of "Maloussi" peach embryonic axes from seeds partially and fully stratified.

O : Control (imbibed for 48 hr then soaked in GA_3 solution for 24 hr, no chilling)

STD : Low Range Molecular Weight Standards :

1. RMPb(97.4); 2. BSA(66.2); 3. HEWO(42.7); 4. BCA(31.0); 5. STI(21.5); 6. HEWL(14.4Kd)

GA_3-treated seeds were dissected 48 hr following the treatment.

4. Effects of gibberellic acid

Following GA treatment, 35 to 40% of the seeds germinated without chilling whereas none of the control seeds germinated. Chilling further stimulated germination, which reached 90 to 95% after 4 wk (Table 5), whereas 8 wk of chilling were required to attain similar germination percentages in the absence of GA treatment.

Table 5. Effects of gibberellic acid (GA_3 at 500 ppm for 24 hr) on germination (%) of partially stratified 'Farouki' and 'Maloussi' peach seeds.

	Time (wk) at 5°C				
Cultivar	0	1	2	3	4
'Farouki'	40 d	51 c	83 b	90 a	95 a
'Maloussi'	35 D	50 C	65 B	70 B	90 A

GA did not affect total soluble protein content (data not shown). It hastened the loss of intensity of protein bands in the cotyledons in 'Farouki' (Figure 54), but not in 'Maloussi' (Figure 55). However, germination of GA-treated seeds occurred before any major changes occurred in proteins. The two prominent bands at 36 and 41 kd remained essentially unchanged in both cultivars after 1 wk of chilling, yet germination capacity was 50% at this time (Table 5).

No consistent changes in protein profile were evident in embryonic axes as a result of GA treatment.

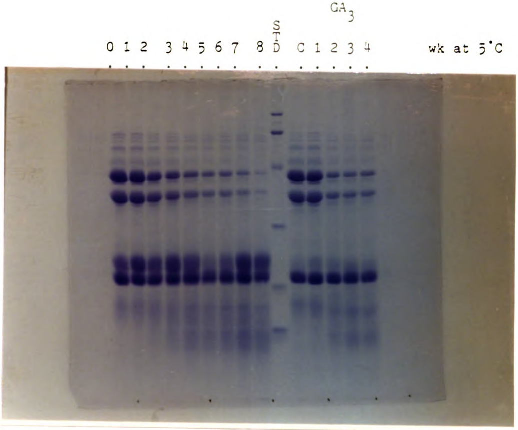

Figure 54. Effects of gibberellic acid (GA$_3$ at 500 ppm for 24 hr) on protein profile of "Farouki" peach cotyledons from partially stratified seeds.

0 : Control (imbibed for 72 hr, no chilling)
C : Control (imbibed for 48 hr, then soaked in GA$_3$ solution for 24 hr, no chilling).
STD : Low Range Molecular Weight Standards :

1. RMPb(97.4); 2. BSA(66.2); 3. HEWO(42.7);
4. BCA(31.0); 5. STI(21.5); 6. HEWL(14.4Kd)

GA$_3$ treated-seeds were dissected and extracted 48 hr following the treatment.

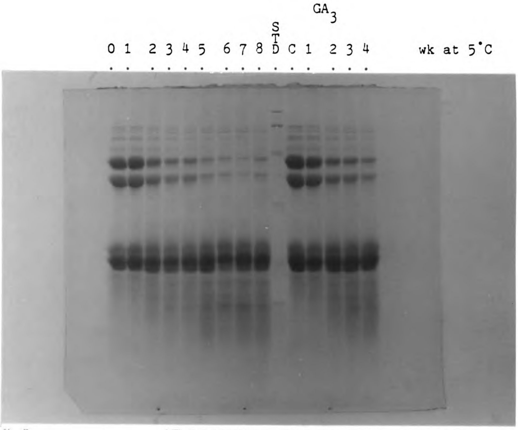

Figure 55. Effects of gibberellic acid (GA$_3$ at 500 ppm for 24 hr) on protein profile of "Maloussi" peach cotyledons from partially stratified seeds.

0 : Control (imbibed for 72 hr, no chilling)
C : Control (imbibed for 48 hr, then soaked in GA$_3$ solution for 24 hr, no chilling).
STD : Low Range Molecular Weight Standards :

1. RMPb(97.4); 2. BSA(66.2); 3. HEWO(42.7); 4. BCA(31.0); 5. STI(21.5); 6. HEWL(14.4Kd)

GA$_3$-treated seeds were dissected and extracted 48 hr following the treatment.

CONCLUSIONS

Satoh and Esashi (73) and Noland and Murphy (65) postulated that protein synthesis is involved in the maintenance of dormancy. Dormancy-breaking treatments could induce (or promote) hydrolysis or modification of dormancy-maintaining proteins, leading to germination. Others (58) have associated the breaking of dormancy with the appearance of new proteins.

In this study, hydrolysis of certain poplypeptides occurred in the cotyledons of peach seeds stratified at 5°C only when the seeds were capable of germination. As these polypeptides were hydrolyzed, additional proteins of lower molecular weights appeared. None of these changes occurred at 20°C, which does not break dormancy, or in embryonic axes at either temperature. Partial imbibition prior to stratification at 5°C prevented germination unless the seeds were soaked after chilling; however, on full hydration, germination was equivalent to that of seeds which were fully imbibed prior to stratification. Protein changes in cotyledons of partially imbibed seeds paralleled those in fully imbibed seeds, but occurred later or at a slower rate. Thus changes in germination capacity and protein profile were out of phase. The observed changes occurred in cotyledons held at 5° even when the embryonic axis was removed prior to stratification. Therefore they are not induced by hormonal or other changes in the axis. Treatment of seeds with GA_3 prior to stratification induced immediate germination (35-40%) and partially substituted for chilling in stimulating germination. However, significant changes in protein content did not occur until the seeds had been held for 2 wk at 5°C, these changes closely paralleled those observed during chilling of seeds not treated with GA. Thus the protein changes appear to be associated with chilling, rather than germination, and are favored by hydration.

The polypeptides whose concentrations decline during chilling all have apparent molecular weights greater than 31 kd and therefore are probably storage proteins. These proteins are probably hydrolyzed to smaller ones rather than to their constituant amino acids. In fact, in this study no changes were observed in soluble protein content regardless of stratification temperature or time. The hydrolysis of the prominent polypeptides 36 and 41 kd occurs in both axes and cotyledons. However, imbibition is sufficient in axes, whereas stratification is necessary in the cotyledons.

DISCUSSION

Dormant seeds require an environmental stimulus, e.g. light or cool temperatures, before germination can occur. Much effort has been devoted to understanding the metabolic basis of these effects.

Proteolysis plays an important role in seed germination (7). Proteases convert seed proteins to amino acids, which are required for the developing plant. The disappearance of storage proteins in germinating seeds is generally associated with an increase in proteolytic activities.

Reserve proteins were hydrolyzed in the endosperm and transferred to the embryo of Heracleum sphondylium during chilling at 2C (10). These changes did not occur and the seeds remained dormant when they were kept moist at 15C. Warm temperature interruption did not reverse the progress of dormancy-breaking at any stage, and the total chilling periods were additive. Chilling induced mobilization of protein reserves and their transfer to the embryo, possibly by inducing a factor in the embryo which diffused to the endosperm and there stimulated proteolysis. It is assumed that the embryo plays a major role in the proteolysis taking place in the endosperm. Low temperature allows the activation or synthesis of enzymes that are diffused to the reserve organ where they hydrolyze protein reserves. It is suggested that the embryo plays a major role or is responsible for the changes taking place in the organs surrounding it.

Free amino acid content in apple embryos increased at both 5 and 20C (12), Thus, the increase was not associated with the breaking of dormancy. Proteolysis observed during stratification was not related to the release of dormancy but could be an essential process in germination or early growth of the embryo. Hydrolysis of reserve protein occurs at both 5 and 20C when apple embryos are placed on a moist medium. However, proteolysis does not occur in seeds left inside the fruits at 0C (4).

Total soluble protein content in axes and cotyledons of apple seeds changed very little during stratification at 5C or 15C (6). No changes occurred in the profiles of cotyledon polypeptides. However, notable profile changes occurred in axes at 5C but not at 15C. Four polypeptides with apparent subunit molecular weights of 70, 50, 32, and 28.5 kd increased in axes held at 5C. The 70 kd polypeptide tripled during stratification at 5C. The 48.5 kd, occurring only in axes at 5C, was first visible on gels after 3 wk at 5C and reached a maximum after 7 wk. Major polypeptides (47, 38, 36, 29.5, and 24.5 kd) decreased much more at 5C than at 15C. The authors speculated that during stratification the protein reserves are specifically mobilized in the axes of the seeds (6). No significant changes were observed in total soluble protein content of pear embryos during chilling (11).

Protein synthesis was higher in the dormant cocklebur axes and cotyledons than in non-dormant ones during the first 9 hr of imbibition (9), but the reverse was observed thereafter. Cycloheximide blocked protein synthesis in the axes regardless of their dormant status, and prevented germination of non-dormant seeds. However, cycloheximide stimulated germination of dormant seed. Proteins may be involved in the maintenance of dormancy in cocklebur seeds (9). Protein synthesis capacity of embryos of sugar pine held at 5C surpassed that of those kept at 25C after 60 day, but remained significantly lower in megagametophytes at 5C than at 25C throughout the 90 day period (8).

The increase in the capacity for protein synthesis in embryos held at 5C paralleled increases in germinability. Protein banding patterns of embryos were virtually identical regardless of temperature or stratification time. However, 4 bands were consistently darker at 25C than at 5C after 30 and 60 day of stratification. Although the rate of protein synthesis in the embryos and percent germinability during stratification were highly associated, warm incubation (25C) also resulted in a high protein synthesis rate but failed to break dormancy (8). Noland and Murphy (8) speculated that the synthesis of germination-inhibiting proteins may control seed dormancy. After-ripening treatment could affect the rate of production and/or degradation of such proteins thereby allowing germination to occur (5). A higher leucine-tyrosine amino peptidase (LTA) activity in sugar pine embryos at 5 vs 25C could be an indication of the degradation or modification of dormancy-maintaining proteins (8). Stimulation of dormant cocklebur seed germination by cycloheximide (9) also supports the concept of a germination inhibiting protein. Furthermore, actinomycin D and chloramphenicol significantly stimulated germination of dormant lettuce seeds (2). These findings imply that synthesis of protein may be involved in maintaining dormancy and that germination may be allowed or promoted by hydrolysis or modification of germination-inhibiting proteins.

Protein synthesis is probably a pre-requisite for germination (1). Germination-related protein synthesis probably occurs within the embryonic axis itself. Distinction must be made between protein synthesis in the axis and the storage organs (cotyledons). Protein synthesis in the latter is probably not related to germination *per se* but to the mobilization of stored reserve proteins a post-germination phenomenon. Protein and RNA metabolism are complex processes for synthesis and/or activation of hydrolytic enzymes are accompanied by catabolism of both proteins and RNAs (1). Changes in metabolism within storage organs are generally unrelated to germination; they are almost exclusively related to the mobilization of stored reserves, a post-germination phenomenon associated with seedling growth. The mobilization of stored reserves is an event that requires the participation of many enzymes, a substantial number of which must be synthesized _de novo_ (1).

Callaway et al. (3) evaluated protein changes in peach buds and seeds. They found that after 336 hr of chilling no germination of bud break occurred. After 24 hr, concentrations of 2 polypeptides were greater at low than at high temperature. One polypeptide was present only at 4C, while another polypeptide was present only at 25C. After 168 hr a new polypeptide appeared at 4C, while one polypeptide increased at 25C. Following 336 hr 4 polypeptides increased in chilled seeds.

In peach buds they observed the following changes: after 24 hr 2 polypeptides were present at higher concentration at 25C than at 4C. One polypeptide appeared only at 4C. After 168 hr 4 polypeptides appeared only at low temperature, and 2 polypeptides were present in higher concentrations at 25C than 4C. After 336 hr 5 polypeptides were present in greater concentrations at 4C while 4 polypeptides were more abundant at 25C.

Table 6. Protein changes observed in peach seeds and buds during exposure to 4C and 25C. (Callaway, 1988)

Time (hr)	pI	kd	4C	25C
Seeds				
24	6.2	67	++	+
	5.5	48	++	+
	6.5	51	+	
	6.5	12	+	
168	4.8	70	+	
	4.7	20	+	++
336	5.2	44	++	+
	4.8	68	++	+
	5.2	84	++	+
	4.7	89	++	+
Buds				
24	7.8	67	+	++
	7.1	38	+	++
	5.9	60	+	
168	7.1	83	+	
	7.3	77	+	
	7.1	59	+	
	7.1	49	+	
	7.6	56	+	++
	7.6	62	+	++
336	5.3	58	++	+
	5.0	85	++	+
	5.1	65	++	+
	5.6	115	++	+
	5.4	109	++	+
	6.1	30	+	++
	6.2	21	+	++
	5.8	21	+	++
	5.5	21	+	++

When radiolabelling was used, protein synthesis was detected after 24 hr. Three polypeptides were observed only at high temperature. Synthesis of 1 polypeptide was reduced at 4C, while synthesis of 3 others increased. Quantitative increases in the synthesis of several low molecular weight polypeptides was noted at 4C after 1 week. Five polypeptides were detectable only at low temperature. However, one was present only at 4C and the synthesis of 2 was higher at 25C. After 336 hr less of one was synthesized at 25C and another was synthesized only at 4C. Callaway et al. (3) speculated that polypeptides present at higher concentration at 25C could represent inhibitors of rest removal. Their role would be to maintain rest until the proper amount of chilling has accumulated. Those of greater relative concentration at 4C, could represent proteins that are either synthesized during after-ripening or resist chilling specific degradation. They speculated that changes seen at this early stages (2 wk) may reflect the primary events in release from rest. The polypeptide differences could represent some of the initial and potentially controlling changes in gene expression leading to rest release.

Various metabolic changes occur during after-ripening. However, researchers disagree on the role of such changes in breaking dormancy. This variation could be explained by:

* the use of a wide variety of species and plant materials

* the absence of an appropriate control

* the lack of powerful analytical techniques capable of detecting minor changes

* problems related to terminology

* the absence of adequate means of clearly separating between physiological stages, particularly after-ripening vs. germination

* it is not certain that all the active tissue is extracted

* interaction is generally ignored

* what is measured is assumed to be the only factor involved and changes in this factor are assumed to be related to the breaking of dormancy

* tissue being extracted serves several functions and some or most of them may not be involved in dormancy.

In my experiments with Siberian C peach seeds that were incapable of germination, all the parameters measured (fresh and dry weights, water content, protein content, protein profile) remained stable regardless of stratification temperature and duration. However, in viable seeds fresh weights and water content of both cotyledons and embryonic axes increased with time at both 5C and 20C, with a greater rate of increase at the lower temperature. Dry weights remained constant, suggesting that the increase in fresh weight was due solely to water uptake. Soluble protein content remained unchanged in both cotyledons and embryonic axes regardless of temperature or time of exposure. SDS-PAGE revealed a decrease in the concentration of 9 polypeptides in the cotyledons of seeds held at 5C, coinciding with the increase in germination capacity of the seeds. These decreases were accompanied by the appearance of 2 or 3 new polypeptides of lower molecular weights. These major changes occurred only at 5C and only when the seeds were capable of germination. These changes could be associated with either after-ripening or germination.

__After-ripening__. The changes in proteins observed in cotyledons of peach seeds held at 5C were induced by chilling and occurred prior to the beginning of germination. Presumably the critical metabolic changes important in dormancy are those taking place in the embryonic axis itself or controlled by the axis. The embryonic axis could send a chemical messenger to the surrounding tissues where it induces synthesis of hydrolytic enzymes. This speculation finds support in the better understood system of germination in cereal seeds during dry storage. During germination gibberellins synthesized in the embryo are transferred to the aleurone layer where α-amylase (among other hydrolytic enzymes) is synthesized __de__ __novo__. The enzyme diffuses to the endosperm where it degrades starch to provide energy for the growing embryo.

__Germination__. The most common criticism of studies where metabolic changes have been reported during stratification is that these changes are associated with germination rather than after-ripening. The metabolic changes usually occur only after germination has begun. Therefore such changes could be induced by or related to early phases of germination. This speculation is supported by the fact that similar changes take place even at higher temperature.

It was important therefore, to establish whether i) changes in protein profile of peach seed cotyledons was associated with after-ripening __per__ __se__ or with germination, and ii) whether the presence of the embryonic axis was required for such changes to take place.

To separate between after-ripening and germination __per__ __se__ we used i) partial imbibition; ii) separation of embryonic axes from cotyledons before stratification; iii) application of exogenous gibberellic acid (GA_3).

Partially imbibed seeds were unable to germinate regardless of the stratification period. However, after additional imbibition the germination of these seeds paralleled that of fully imbibed seeds. This suggests that after-ripening occurred even in partially imbibed seeds. However, we define germination as the expansion of the radicle or beginning of growth. The initial phases of germination may not require full imbibition and could take place even though water supply is limited. However, further growth would require absorption of additional water.

Changes in protein profiles in fully imbibed 'Farouki' and 'Maloussi' peach seeds were similar to those observed in Siberian C. Similar changes occurred in cotyledons of partially imbibed seeds during stratification at 5°C, although more slowly. This indicates that after-ripening takes place even in partially imbibed seeds and that the changes in protein profiles occur during stratification even in seeds incapable of germination because of limited water content. However, changes are delayed by partial imbibition whereas germination capacity (ability to germinate when fully imbibed) is not.

To test the possibility that the embryonic axis might be responsible for changes in protein content of the cotyledons, embryonic axes were separated from cotyledons before stratification at 5C. Dry weights and soluble protein content remained stable in both axes and cotyledons regardless of stratification duration. Changes in protein profiles of the cotyledons in the absence of embryonic axes were similar to those of cotyledons from whole seeds. The embryonic axis therefore, is not required for protein changes to occur in the cotyledons.

Exogenous application of gibberellic acid hastened germination in non-chilled and chilled peach seeds, but did not affect soluble protein content. It hastened the loss of intensity of protein bands in the cotyledons in 'Farouki' but not in 'Maloussi' peach seeds. However, germination of GA-treated seeds occurred before any major changes occurred in proteins. This suggests that protein changes in cotyledons are mediated by GA and are related to chilling, rather than to germination.

Many other changes in protein content probably occur during stratification. There may be other changes that are of small magnitude but of great significance to dormancy or other physiological phenomena. Only one dimensional PAGE was used. This allowed the separation of over 40 protein bands in the cotyledons. Plant tissues contain thousands of proteins, hence many small differences could have been overlooked. Furthermore, radiolabelling was not employed to detect newly-synthesized proteins. This method, too, is capable of detecting small amounts of proteins, particularly those that are metabolically active.

The changes in protein could be associated with either after-ripening or germination or both. Both phenomena may induce the same changes. After-ripening may not require full imbibition to trigger the process, but germination would require full imbibition. In studying physiological processes plant physiologists have a tendency to dissect and break down these processes into small and distinct steps, leading to oversimplification. There may be no clear separation between after-ripening and germination. One single process involving several steps could lead to radicle expansion and resumption of growth.

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