

# Genotypic Differentiation in Temperature Requirements for Stratification in Subtropical Peach Seeds

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**Abstract.** The variability of species under local ecosystems, particularly in response to temperatures during endodormancy, permits adaptation of temperate fruit trees to subtropical climates. Information about the behavior of endodormant fruit trees and seeds is based on a narrow genetic base from higher latitudes. This work was conducted to generate information about responses of endodormant seeds from several subtropical peach (*Prunus persica* L.) genotypes, as a basis for breeding and selection in these regions. Samples of peach seeds were collected from genotypes originating at a range of altitudes in tropical-subtropical regions to evaluate their responses to different temperatures and lengths of stratification periods. When seeds were stratified at 7 °C, some genotypes with very low-chilling requirement registered a high percent germination in <40 days, and all accessions studied reached 95% germination before day 80. When seeds were stratified at warmer temperatures (10 and 14 °C), germination started earlier and was high at 10 °C for most accessions. Although seeds of some late-blooming accessions germinated earlier at 10 or 14 °C than at 7 °C, percent germination was lower and time-response curves were flatter. This contrasts with previous reports on genotypes with high-chilling requirement, where no germination was registered at 14 °C. These observations provide a background for screening seedlings for adaptation to local conditions, and suggest that endodormancy models should be based on information generated from local genotypes when applied in subtropical regions.

The dormancy period of seeds and buds of temperate fruit trees is strongly and positively influenced by chilling temperatures ranging from 4 to 8 °C (Chandler, 1957; Dennis, 1987; Erez and Lavee, 1971; Seeley and Damavandy, 1985; Weinberger, 1967). Higher temperatures (above 20 °C) registered during this period will negate the chilling effect (Couvillon and Erez, 1985; Erez et al., 1979a, 1979b). Once a certain amount of chilling is accumulated, warmer temperatures (13 to 15 °C), alternating with chilling, enhance budbreak (Erez and Couvillon, 1987).

Even though seeds and buds are obviously different, their response to chilling temperatures during dormancy tends to be similar (Dennis, 1996; Frisby and Seeley, 1993b). They have both been studied to understand dormancy phenomena of temperate fruit trees (Chandler 1957; Couvillon and Erez, 1985; Erez et al., 1979a, 1979b; Frisby and Seeley, 1993b). However, the information generated to date is based almost exclusively on a narrow genetic pool with high-chilling requirements (Dennis, 1987; Erez and Couvillon, 1987; Erez and Lavee, 1971; Fishman et al., 1987; Frisby and Seeley, 1993a, 1993b; Seeley and Damavandy, 1985). Only a few studies have

emphasized genetic diversity at the species level (Westwood and Bjornstad, 1968), particularly on subtropical germplasm distributed in meridional regions.

Genetic analysis of the inheritance of the chilling requirement has suggested that it is multigenic, with only a few genes playing major roles (Thompson et al., 1985). Some genes have been associated with hormone synthesis (Koorneef et al., 1982).

The genetic mechanisms determining chilling requirement in temperate fruit trees are probably very similar within a species, but more information on the range of diversity for temperature requirement during dormancy will be necessary to understand the evolution of temperate fruit trees in the subtropics.

Peaches were introduced into Mexico early in the 16th century (Hedrick, 1917). They were spread rapidly into a wide range of ecosystems, from the dry, cool regions in the high Mexican plateau to lower elevations in the subtropical and tropical northeastern coast, where they grow side by side with vanilla and bananas (Pérez-González, 1989).

The southern range of distribution of temperate fruit trees is determined by the chilling requirement and the amount of chilling accumulated during rest (Weinberger, 1956). Seeds of temperate fruit species enter dormancy and require cool temperatures and a moist environment to induce germination and normal seedling growth. However, intra- and interspecific variability in seed chilling requirements during stratification has been reported for several species and genotypes of pear (*Pyrus commu-*

*nis*) (Westwood and Bjornstad, 1968), almond (*Prunus dulcis*) (Kester et al., 1977), apple (*Malus domestica*) (Pasternak and Powell, 1980), and peach (Pérez-González, 1990).

A close relationship exists between the chilling required to induce flower budbreak and that required to promote germination of seeds of these genotypes. When peach seeds from lower latitudes are stratified at 5 to 7 °C, some genotypes, like the evergreen 'Tetela', reach 90% germination in <55 d, while seeds of late-blooming selections such as I8-16 remain dormant (Pérez-González, 1990). Frisby and Seeley (1993a, 1993b) reported similar observations for genotypes that require >80 d for high germination.

Monitoring seed germination for endodormancy studies is easier than manipulating whole trees (Seeley and Damavandy, 1985). Optimum stratification temperatures for pears originating from a wide range of latitudes require between 2 and 10 °C for good germination (Westwood and Bjornstad, 1968), vs. 4 to 6 °C for several temperate-zone species from northern regions (Seeley and Damavandy, 1985). For peach seed, optimum stratification temperature was recently reported to be 4 °C in a series of papers by Frisby and Seeley (1993a, 1993b). These and other observations provided the basis for a model for peach seed stratification (Seeley and Damavandy, 1985). The more efficient temperatures, as evidenced by earlier and higher percentages of germination, ranged between 2 and 6 °C. But the same authors noticed some genotypic differences in seed chilling requirements, confirming previous findings (Chang and Werner, 1984; Pérez-González, 1990).

Models designed to estimate chilling accumulation are based on genotypes from higher latitudes (Ashcroft et al., 1977; Weinberger, 1967), but such models rarely apply when used in southern regions (Pérez-González, 1993). Some adjustments to these models have been proposed (del Real-Laborde, 1987; Erez and Couvillon, 1987; Fishman et al., 1987), but none has been applied to responses of locally adapted genotypes.

The main objective of this work was to generate information on temperature requirements for breaking the dormancy of peach seeds from subtropical populations and, thereby, provide an additional criterion when screening genotypes for adaptation to local ecosystems.

## Materials and Methods

Seed samples from the INIFAP peach germplasm collection in Central Mexico (north-eastern Guanajuato) were collected in the summers of 1991 and 1995. A wide range of genotypes from early to late blooming was used in this study (Table 1).

Ripe fruits were harvested and pits were removed and thoroughly washed, sun-dried for 2-3 h, placed in paper bags, tagged, and stored at 17 to 23 °C in a dry place until November.

Pits were cracked and the extracted seeds were washed in a soap solution, surface steril-

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Table 1. Description of accessions studied.

Name	Origin	Blossom season <sup>a</sup>	Harvest season <sup>2</sup>
Okinawa	South Pacific	November	July
Magno	Brazil	January	June
Perú	Perú	January–February	August
Tetela	Mexico–Morelos	February	August
Guatemala	Guatemala–Quetzalt.	Late February	August
Kakamas	South Africa	Early March	July
I8-16	Mexico–Zacatecas	Mid-March	September

<sup>1</sup>In Guanajuato, Mexico.

ized for 5 min in a 3.5% sodium hypochlorite solution, and then rinsed twice in distilled water. A minimum of 240 seeds from each accession was used for the studies, with 10 seeds per replication and four replications per treatment (genotype–temperature combination). Seeds for each replication were spread over a paper towel moistened with a 2% captan solution and placed in a transparent plastic bag (10 × 15 cm).

*Effect of length of the stratification period at 7 °C.* Twelve bags per accession were randomly selected and deposited in cardboard boxes (15 × 20 × 25 cm) that were placed at 7 °C; thermostats controlled the temperatures within 1.5 °C of these settings. After 35, 45, and 55 d, four bags from each accession were removed and placed at ambient temperatures (23 to 25 °C day/16 to 18 °C night) for 20 d. Germination was recorded daily and seeds were considered germinated when radicals were 1–2 mm long.

*Influence of temperature during stratification.* Ten bags per genotype were placed in cardboard boxes and transferred to rooms set at 7, 10, and 14 °C (temperatures fluctuated 1.5 °C within settings). Germination was recorded at 3-d intervals for the next 100 d, and each experiment was repeated at least twice in 1991 and 1995.

*Effects of stratification at ambient soil temperatures.* To determine seed responses to contrasting winters in terms of chilling accumulation, two experiments (1991 and 1994) were performed. Chilling hours were estimated with the Weinberger (1967) regression equation, adjusted for altitude (Pérez-González, 1993), based on mean November–

February ambient temperatures. Estimated chilling hours were 530 in 1991, and 310 in 1994. On 1 Dec., seed samples were put in cardboard boxes along with a maximum-minimum thermometer. A 50-cm hole was dug in the ground, the boxes were put at the bottom and covered with moist sand and straw. Temperatures inside the cardboard boxes were monitored daily; minimum temperatures ranged from 8 to 11 °C in 1991 and from 9 to 12.5 °C in 1994, while maximums varied from 14.9 to 18.2 °C in 1991 and from 16.5 to 19 °C in 1995. The number of germinated seeds per replication was recorded twice a week.

## Results

*Effect of length of the stratification period at 7 °C.* There were marked differences among genotypes in rate of germination as a function of duration of stratification (Table 2). For the subtropical genotypes 'Okinawa' and 'Magno', 47% and 9%, respectively, of their seeds had germinated at 7 °C after 35 d. On transfer to room temperature, the remaining seeds germinated within the next 4 d (Table 2). Germination was high for most of the other accessions following transfer to 7 °C, with the exception of 'Kakamas', in which germination was <50% over a period of 7 d. I8-16, a late-blooming genotype, also showed very high germination.

After 45 d at 7 °C, 'Okinawa' had already reached 100% germination, followed by the evergreen 'Tetela' with 92%, 'Magno' (76%), and 'Kakamas' (32%). Subsequent germination was very high for all treatments and was more rapid than in seeds held for 35 d at 7 °C. After transfer to ambient temperatures, all

remaining seeds of 'Tetela' germinated in <24 h. 'Magno' seeds required 3 d, while 'Kakamas' and I8-16 required 4 to 5 d (Table 2).

I8-16 seeds initiated germination at 7 °C only after 55 d, while 'Kakamas' had 35% germination, and I8-16 had only 2%. By that time, 93% to 100% of 'Okinawa', 'Magno', and 'Tetela' seeds had germinated. Once seed samples were placed at ambient temperatures, all genotypes reached maximum germination in <3 d.

*Influence of temperatures during stratification.* At all temperatures, accessions differed in the time required for germination (Figs. 1 and 2). When stratified at 7 °C, 'Okinawa' and 'Tetela' reached 80% germination in <40 d, at the time when 'Perú' and 'Magno' were just beginning to germinate, and 100% 15 d later. I8-16 started germinating only after 50 to 60 d, when all but 'Kakamas' and 'Guatemala' had reached 100%.

All accessions germinated earlier at 10 °C than at 7 °C. Total germination was slightly lower at 10 °C than at 7 °C for all but 'Tetela' and I8-16. For some accessions, such as 'Kakamas', germination also started earlier, but was more prolonged.

However, the greatest differences among genotypes were observed when seed samples were stratified at 14 °C. While germination started earlier than at 7 and 10 °C for most genotypes, the final percentage was generally lower, ranging from nearly 100% in 'Tetela' to <40% in 'Kakamas' and I8-16. Within each of the latter accessions, the last part of the germination curve tended to be more horizontal and was spread over a wider range, from 30 to 80 d depending upon genotype.

'Okinawa' and 'Tetela' showed the earliest germination peaks, at ≈35 and 42 d, respectively (Fig. 2), with minor differences between 7 and 10 °C. As stratification temperatures were increased to 14 °C, the germination curves became flatter and clearly separated from that at 7 °C. This response was more obvious in 'Magno' and 'Kakamas', and even in Okinawa, the lowest chilling requirement genotype within this group.

*Effects of stratification at ambient soil temperatures.* The differences in germination per-

Table 2. Percent germination registered in five peach accessions stratified at 7 °C during 35, 45, and 55 d, then transferred to 18 to 24 °C.

Genotype	Days at 7 °C	Germination at 7 °C (%) <sup>a</sup>	% Germination after day <sup>b</sup>							Sum (%)
			1	2	3	4	5	6	7	
Okinawa	35	47	34	19						100
	45	100								100
	55	100								100
Magno	35	9	77	5	4	4				99
	45	76	14	5	5					100
	55	95	5							100
Tetela	35	0	4	48	40	8				100
	45	92	8							100
	55	93	7							100
Kakamas	35	0	3	18	6	15			3	48
	45	32	14	22	7	7	3			85
	55	39	34	10	7					90
I8-16	35	0	6	28	16	18	16	10	6	100
	45	0	5	85	8	1				99
	55	2	42	56						100

<sup>a</sup>Percent germinated at time of removal from 7 °C.

<sup>b</sup>Days at ambient temperatures (18 to 24 °C) after seeds were removed from 7 °C.

SEED TECHNOLOGY

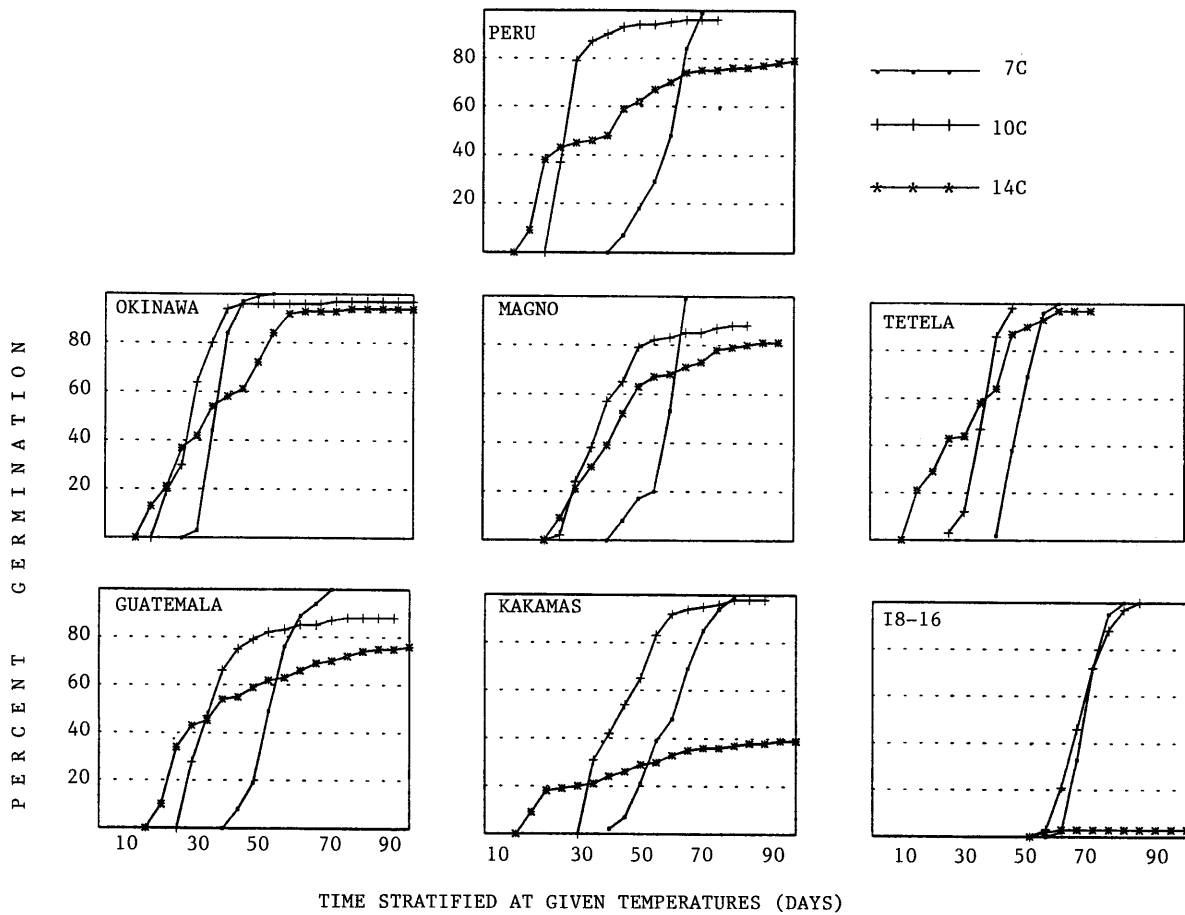


Fig. 1. Individual responses of seven peach genotypes stratified at 7, 10, and 14 °C (cumulative).

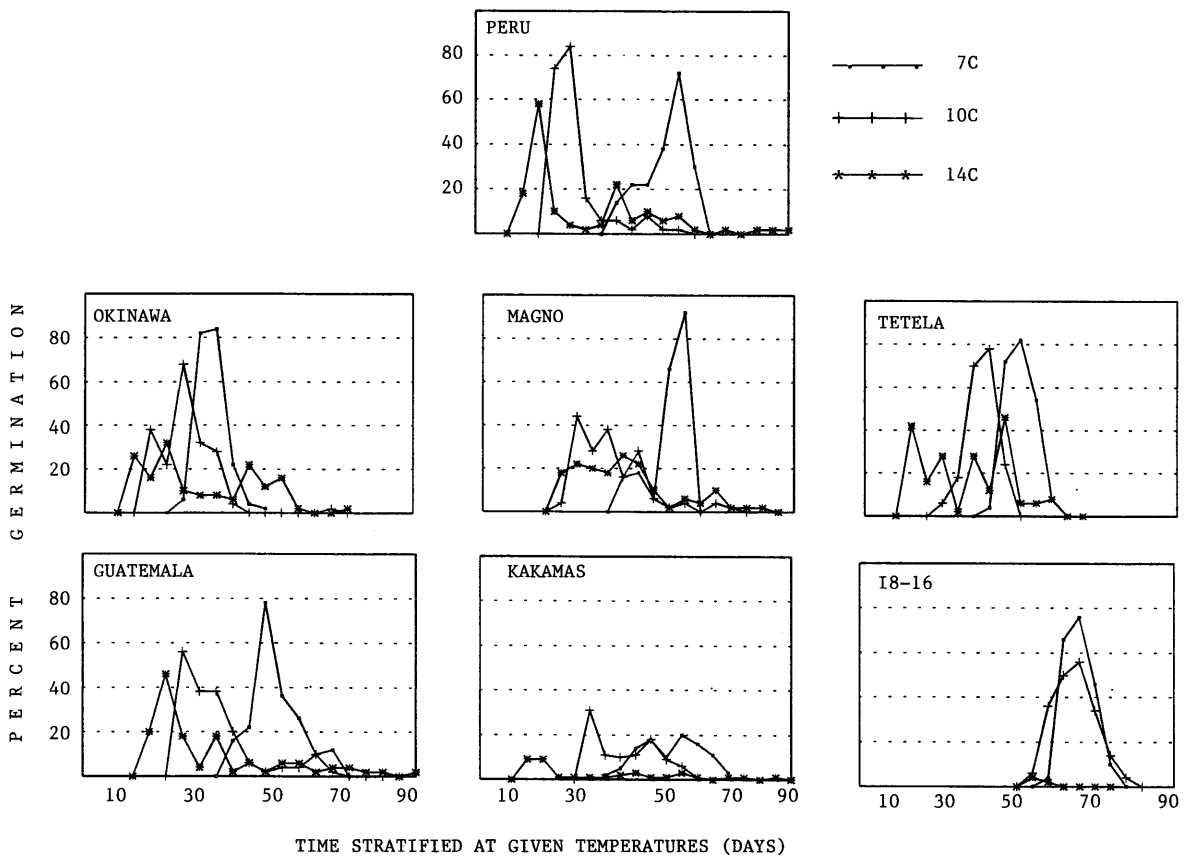


Fig. 2. Sequence of germination registered in seven peach genotypes stratified at 7, 10, and 14 °C.

centages and rates observed at ambient temperatures in the 2 years (Fig. 3) reflect the differences in chilling accumulation between the two winters. Germination was higher and occurred in a shorter period in 1991 (520 chilling hours), whereas in 1994 (310 chilling hours) high-chilling genotypes germinated poorly and showed an extended germination period. Differences between years were much less for 'Tetela' and 'Okinawa', because of their lower chilling requirement (or higher temperature threshold). In these genotypes, differences between 7, 10, and 14 °C (Fig. 1), and between years (Fig. 2), were smaller, and in some cases, germination was even better at 10 °C than at 7 °C.

### Discussion

For the first experiment, the effects of stratification and germination temperature are clearly separated, but in the second, these two factors were confounded. However, if seeds of 'Tetela' germinate as well or better at 10 °C than at 7 °C, and if the optimum stratification has been defined as that with high germination over a short period of time, the genetic basis for adaptation to warmer climates is demonstrated.

Germination was high for all accessions in all stratification treatments, except for 'Kakamas', stratified 35 d at 7 °C. The germination period was more extended after stratifi-

cation for 35 d, than when seeds were held for 55 d. The response of 'Kakamas' is more typical of that described in genotypes requiring more chilling (Frisby and Seeley, 1993a and b; Seeley and Damavandy, 1985). This could be explained in terms of the two-step dynamic model proposed by Erez and Couvillon (1987) and Fishman et al. (1987). Seeds that did not germinate at 7 °C could have been at stage A-B, as in 'Kakamas', while other genotypes from the subtropics were at stage B-C and germinated well, although their germination period was still wider than after 55 d (Fig. 1). This response is similar to the delayed foliation observed in trees with medium to high-chilling requirements during warm winters (Chandler, 1957; Weinberger, 1956).

Although no formal data were collected on growth, some seedlings derived from 'Kakamas', 'Perú', and, to a lesser extent, 'Okinawa' showed different degrees of leaf russetting after short stratification periods and after germination at 14 °C, typical of insufficient chilling. However, the growth of 'Tetela' seedlings was normal. This suggests that different genetic systems operate during the stratification stage than during growth and seedling development. Therefore, selection criteria should be independent and additive to provide a solid background for adaptation to subtropical environments. If these variables are linked with some traits expressed at later stages of development, such as blossom density, fruit set, and fruit shape at warmer temperatures,

the possibilities for developing a temperate fruit industry in the subtropics could be improved.

Germination rates (Fig. 2) are similar to results expressed as cumulative germination (Fig. 1), but peaks reveal clear-cut differences between some accessions. Temperatures higher than 7 °C tend to widen the distances between germination peaks. This response could be explained in terms of their lower chilling requirements for stratification, which might be closer to 7 °C in 'Kakamas', between 7 and 10 °C for I8-16, and ≈10 °C for 'Tetela'.

Germination in 'Tetela' was 100% at 14 °C, as compared with no germination reported for 'Johnson Elberta' at this temperature (Frisby and Seeley, 1993a). Differences in germination behavior observed among genotypes and between years have caused serious problems for the nursery industry, as observed during Winter 1995-96 in California when 'Lovell' seeds stratified in the field showed very poor germination as compared to 'Nemaguard' (L. Vargas and R. Martella, personal communication). Differences in percent germination and length of the stratification period during stratification could be used to select genotypes for warm climates at very early stages. This would provide the background for further seedling evaluation at different elevations (with strong contrasts in chilling accumulation) and avoid frost problems associated with earlier blooming, as genotypes with lower chilling requirement exhibit earlier blooming and faster germination (Pérez-González, 1990).

Almost all genotypes tended to germinate more rapidly at warmer stratification temperatures. However, at higher temperatures total germination was reduced and marked differences among genotypes were observed, with 'Tetela' and 'Okinawa' being more stable, and 'Kakamas' and I8-16 exhibiting decreased and more extended germination.

Peach seed germination shows a normal distribution, with rapidly germinating seeds at the extreme left (Fig. 2). These differences in the speed of seed germination, among and within accessions, could represent the basis for adaptation to subtropical climates. Warmer temperatures are common in frost-free regions where some of the "evergreen" peaches are grown. Under these conditions, rapid germination is not detrimental.

Explanations for sigmoid germination curves were offered by Seeley and Damavandy (1985). In this study, the germination curve was wider for hybrid genotypes such as 'Magno' and 'Kakamas', suggesting progenitors with contrasting chilling requirements. Germination curves were narrower in relatively homozygous genotypes, like 'Tetela' and I8-16, expressing little variability in terms of chilling requirements. However, in both groups, it may be possible to separate early vs. late-germinating genotypes. This diversity could provide the genetic background to reduce the problem of variability in chilling accumulation between years and sites at different elevations, common to most subtropical regions.

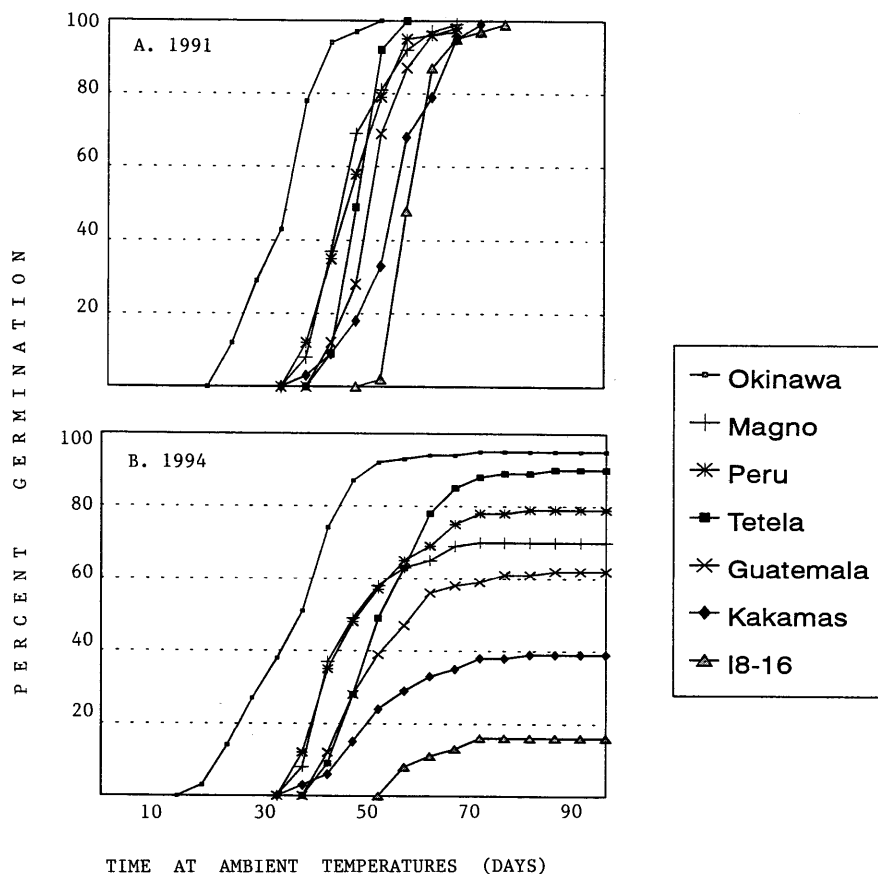


Fig. 3. Cumulative germination observed in seven peach seed accessions stratified in 1991 (A) and 1994 (B) at ambient temperatures in the soil at Guanajuato, Mexico.

## Literature Cited

- If an adequate stratification period is associated with a high percentage of germination over a short period of time, then 10 °C is a more suitable stratification temperature for genotypes such as 'Tetela' and 7 to 10 °C for I8-16. If we consider percent germination as the main criterion, both accessions exhibit similar behavior at lower temperatures. However, germination declines slightly from 10 to 14 °C in 'Tetela', but stops completely in I8-16. These results support the concept that genotypes differ in temperature thresholds required for germination, and perhaps for the breaking of endodormancy. Therefore, the models used to describe and predict germination behavior and endodormancy should be based on genotypes with similar temperature requirements grown in specific ecosystems, instead of applying information generated from a single genotype in one environment to all situations.
- Budbreak and seed germination exhibit normal distributions that can be either leptokurtic (narrow) with a high budbreak or seed germination when chilling requirements during rest are satisfied, or platikurtic (flatter) under warmer conditions. These differences reflect the genetic constitution of each accession. Heterozygous genotypes produce heterogeneous families; low-chilling genotypes germinate earlier, while those with higher chilling requirement germinate later or not at all. This variability favors survival in variable ecosystems. Seedlings from early germinating seeds (<45 d of stratification) could have greater potential in subtropical climates, while those germinating after 80 d might have greater possibilities at higher altitudes or in northern locations, where lower temperatures during rest are common. Seeds of several well-known genotypes with well-known chilling requirement could be stratified at ambient temperatures to evaluate the amount of chilling accumulated over a given period. If only the low-chilling genotypes germinate well, while medium to high ones germinate poorly, chemical sprays could be applied to the latter.
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