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Correlations of Chilling Requirements for Germination, Blooming and Leafing within and among Seedling Populations of Almond[1]

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Abstract. **Relationships among blooming and leafing dates of parents, offspring, and the chilling requirements of the intervening seed were determined among a group of families of almond (*Prunus amygdalus* Batsch). Parent-offspring heritabilities for blooming and leafing dates were high. Leafing and blooming dates of individuals and families, although significantly correlated, were more or less independent traits. Seed chilling was a function of both seed and pollen parents and was correlated with mean bloom and leafing of parents. The correlation coefficient between chilling of a seed and the blooming date of the corresponding offspring plant was significant and large when calculated for families and significant, but low, when calculated for individuals. The seed-leafing correlation was significant when the *early* and *late* leafing progeny were considered separately.**

Most woody plant species of the temperate zone require chilling for flowering and emergence of new shoots in the spring. Their seeds also require moist-chilling (stratification), the amount of which is usually associated with the amount of chilling required by the buds (17). In addition, bud exposure to warm temperatures subsequent to completion of their chilling requirement appears to be necessary (15, 16). Presumably similar biological systems are involved in the processes that control germination, bud emergence and flowering.

This chilling requirement is expressed as a time-temp relationship where the minimum temp for buds is near freezing or slightly below and the upper limit near to 7.2°C (45°F). For seeds the range has been considered to be between freezing or slightly below and an upper limit around 15.5°C (60°F) (10). The adaptation of many species and cultivars to a particular location may be determined by these chilling requirements.

Recently, the seed chilling requirement was shown to be a function of the genotype of the embryo as determined by both the seed and pollen parents in almond (6, 11) and some other species (1). It can thus be considered to be an expression of xenia (3). Since the embryo is the first phase of the seedling generation, selection for chilling requirement for flowering and leafing might logically be possible at the time of seed germination.

The purpose of this study was to establish the relationships between the chilling requirement of the seed and that of the parents and offspring for blooming and leafing. To be useful for selection, the chilling requirement of the individual seed must be highly correlated with the blooming date of the plant which develops from that seed.

Materials and Methods

Materials. Seeds were produced by hand-pollination of emasculated flowers. The 21 parental clones used represented a range of blooming and leafing times and included a group of cultivars from species whose leafing time was much later than blooming. The crosses (Table 1) produced approximately 1000 seedlings involving 27 plant families. In addition, data from 13 open pollinated families was used in some calculations.

Procedure. The *seed chilling index* was defined as the number of days required for the radicle of an individual seed to emerge after being stratified at 10°C (50°F). Seeds were harvested from the mature fruits, dried and stored at 2.2°C (36°F). In preparation for stratification, shells were removed, seeds were soaked overnight in a slurry of fungicide in a covered Erlenmyer flask, excess liquid removed and the unit placed at 10°C (50°F) for stratification. At triweekly inspections, seeds that germinated were removed, date recorded, and then were transferred to another flask for storage at 2.2°C (36°F) until all were ready for planting. The propagation sequence was to plant in the greenhouse, transfer after a month to the nursery and transplant to the orchard in the following dormant season. Orchard spacing was 1.8 × 4.3 m. For each seedling tree, the record of the *seed chilling index* of the individual seed from which it developed was maintained.

The times of bloom and leafing were determined by observing trees 3 times per week. The *bloom index* was designated as

Table 1. Bloom and bloom:leaf indices for parental genotypes, the crosses made and the mean bloom:leaf indices of the offspring families.

			Mean bloom:leaf index offspring families			
			Seed parent			
Pollen parent	Bloom index	Bloom: leaf index	Non-pareil	Mission	Tardy Non-pareil	Sel. 5:33
Hybrid A	+0	+8	+1.4	+4.9	+0.6	ND
P. argentea 5-37	+4	+7	ND	+4.2	ND	ND
Hybrid B 9-3	+4	+9	+4.5	+6.4	ND	ND
P. argentea 15-33	+5	+8	ND	+5.7		
P. argentea c1-2	+4	+10	ND	+5.9	ND	ND
P. argentea 15-38	+6	+9	ND	+3.2		
P. bucharica	+6	+7	+2.6	−0.6	+0.5	ND
P. tangutica	+7	+6	+4.0	+5.3	ND	ND
Iranian 4-24	E	E			+4.5	
P. amygdalus						
Sel. 25-26	+6	−1	ND	ND	−2.0	ND
Nonpareil	+8	−1	ND	ND	ND	ND
Merced	+9	−4	ND	−1.1	ND	ND
Mission	+10	0	ND	ND	ND	ND
Davey	+12	−1	−3.2	ND	ND	ND
Sel. 1-100	+12	−1	ND	ND	−3.6	−3.6
Sel. 5-33	+12	−1	ND	ND	−4.6	ND
Sel. 1-85	+17	E	−2.3			
Tardy Nonpareil	+17	−4	ND	ND	−1.6	−4.5
Nemaguard	+17	?	ND	ND	ND	−0.3
P. persica						
Ventura	+	+	−1.8	ND	ND	−1.7
Altair	E	E	−1.4	ND	ND	ND

E = early.
ND = no data.

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Table 2. Correlations involving germination, blooming and leafing indices in plant and seed families of almond.

Comparison	No.	Calculated r	Required r for significance	
			5%	1%
1. Mean bloom index for parents vs. Mean seed chilling index	26	0.686	0.381	0.487
2. Mean seed chilling index (seed family) vs. Mean bloom index (seedling family)	26	0.854	0.381	0.487
3. Individual seed chilling index vs. Individual bloom index	1000	0.129	0.062	0.081
4. Mean leafing index for parents vs. Mean seed chilling index	15	0.441 (a) 0.525 (b)	0.482	0.606
5. Mean seed chilling index (seed family) vs. Mean leafing index (seedling family)				
a. late leafing	23	0.529	0.398	0.507
b. early leafing	16	0.496	0.482	0.606
6. Individual seed chilling index (seed family) vs. Individual leaf index	1068	0.154	0.062	0.081
7. Individual bloom index (seedling family) vs. Individual leaf index	1000	0.340	0.062	0.081

z) $Y = -1.709 + 1.0135\ X$
b) $Y = 0.41X^{1.30}$

the number of days after February 20 when "first bloom" occurred, the date when 5–10% of the blossoms were open (12). The *leafing index* was designated as the number of days after Feb. 20 when shoots emerged. The emergence date was the day when approximately 50% of the shoots had emerged sufficiently for leaves to expand. The *bloom-leaf index* was defined as the number of days from bloom to leafing. It could be a positive or negative value.

Observations on bloom and leafing have been made for 3 years, but only the 2nd year's data were used for the calculations when the plants were 4 years old. Statistical correlations were calculated by a programmed 9805-A Hewlett-Packard calculator. Heritability was estimated by calculating the regression of offspring to mean of the parents (7).

Results

Blooming and germination. The parental means for *bloom index* were highly correlated (0.686) to mean *seed chilling index* values (Table 2) among the families studied. Germination curves (Fig. 1) for 3 representative progenies produced on one seed tree by 3 different pollen parents were significantly different from each other, but that of reciprocal crosses of 1 parental combination were identical.

The linear correlation of *seed chilling index* values to *bloom index* values of the resulting seedling plants was highly significant and large in magnitude (0.854), when family means were compared (Table 2). When the *chilling index* value of an individual seed was compared to the *bloom index* value of the same plant, the correlation coefficient was significant, but low in magnitude (0.129).

Heritability of *bloom index* was 0.804 ± 0.087 from calculations involving 13 parents, 20 families and 490 offspring. This high value shows that the genetic relationship between parent and offspring progenies was high and evidently controlled by additive genes.

Leafing and germination. Two groups of genotypes were identified among the parental clones with regard to leafing (Table 1). In one, referred to as the *early* leafing group, trees leaf out at the time of blooming and have either negative or low *bloom-leaf index* values. This group includes certain almond cultivars (*Prunus amygdalus*) and some peaches (*P. persica*). In the second, referred to as the *late* leafing group, the trees leaf out after blooming and have large positive *bloom-leaf index* values. The second includes selections from other almond species or from hybridization between them and *P. amygdalus*. Table 1 data show that the *late* leafing trait is inherited and suggests that a bimodal distribution is produced among the plant families

The correlation (0.441) between *leafing index* values of parents with *seed chilling index* values was not significant at the 5% level when compared on a linear basis ($Y = A + BX$). A better fit of the regression curve (Fig. 2) was produced with the relationship $Y = AX^B$ and the correlation was significant (0.525). The later leafing parents of this group did not necessarily produce seeds with a correspondingly large *chilling index* value.

The correlation between mean *seed chilling index* and mean *leafing index* was not significant when all offspring families were considered together. However, when the family means for the early group and late group were considered separately the two linear correlations were significant and relatively large in magnitude (0.529 and 0.496).

When the *seed chilling index* values for individual seeds and the *leafing index* value of the corresponding plant were considered, the correlation was significant but low in magnitude (0.154), about the same as for blooming. The correlation between *bloom index* and the *leaf index* for the same individual was somewhat higher in magnitude than the seed-plant indices and highly significant (0.340).

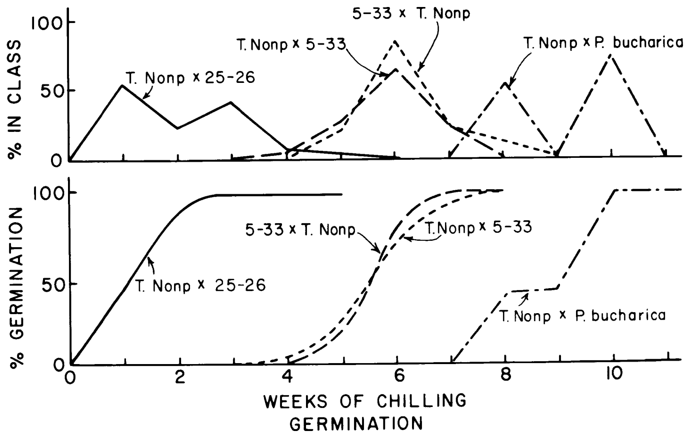

Fig. 1. Germination of 4 progenies produced by using 3 pollen parents on a single 'Tardy Nonpareil' tree and one reciprocal cross. Lower: Accumulative germination. Upper: No. of seeds germinating on any single date.

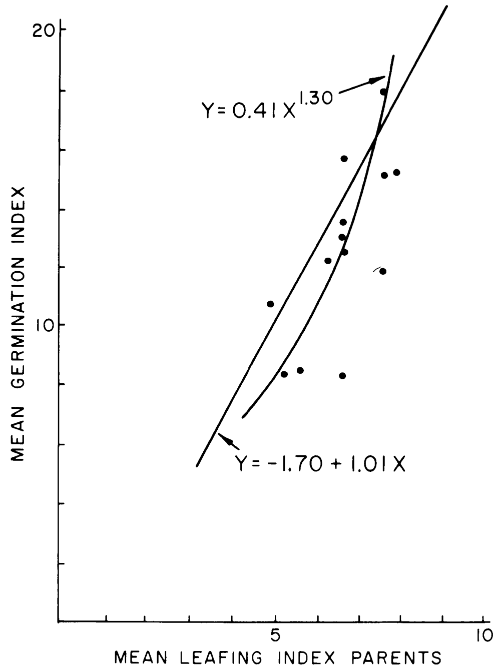

Fig. 2. Relationship between mean leafing index of parents and the mean germination index of seed families produced.

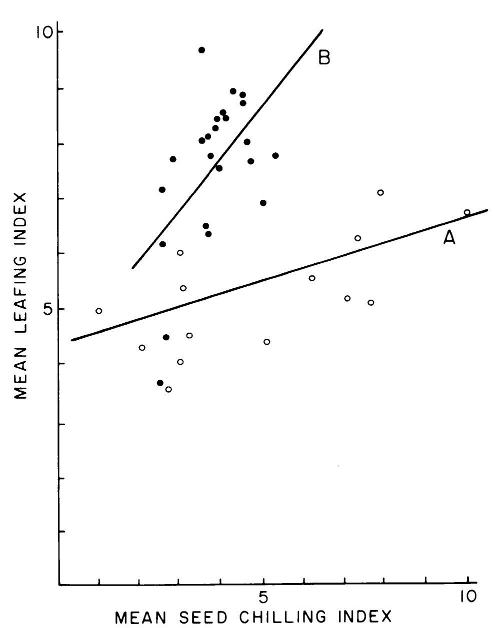

Fig. 3. Relationship between mean seed chilling index and the mean leafing indices for the 2 groups of seedling families produced from those seeds.

Heritability of *leafing index* was high for the 20 seedling families of 478 individuals produced from the 13 parents (0.829 ± 0.088).

Discussion

In perennial plant species, relationship among genetic traits affecting physiological processes can be investigated among families, within families or within individuals propagated vegetatively as clones. Selection of individual genotypes from genetic populations is the basis of cultivar improvement programs. In this study we have analyzed the relationship among the 3 traits (leafing, blooming and germination) from the standpoint of separate genetic families and from the standpoint of individuals.

The high heritability of leafing and blooming time in the group used in this study suggests that additive quantitative genes are probably involved, similar to that shown in other fruit and nut species (7, 8, 9). These results were obtained in interspecific hybrid populations and such traits can evidently be transferred readily from one to the other of the species involved.

The control of time of leafing and blooming within individual plants, however, appears to be independent although some degree of relationship exists as indicated by the significant but low correlation value. Shoot emergence responds differently than flower opening to light (5) and to chemical treatment (4). This independence is not surprising in view of the different morphological sequencing of vegetative and flower bud development.

The independence of leafing and blooming is reflected in the widely differing values for *bloom-leaf index* among the plants used in this study. The possibility that shoot emergence is correlated to other physiological characteristics such as cold resistance has been suggested elsewhere (2) and justifies further consideration.

However, our initial interest in this study was to relate seed chilling requirement to plant chilling requirement. The seed is the bridge between the two generations and includes two kinds of tissue: a. that of the offspring genotype, i.e., the embryo, and endosperm (a function of both the male and female parents), and b. tissue of the maternal parent genotype, i.e., nucellus, integuments. Our results indicate a separation of the control of seed chilling into at least 2 components. One is controlled by the embryo genotype. This is indicated mostly by the differences in mean germination between seed progeny produced by different pollen parents on the same seed tree and the lack of difference between reciprocals but also indicated by the high correlation between the progeny means of seeds and the corresponding plants. The second component is indicated by the low, but highly significant, correlation value between the germination of an individual seed and the blooming and leafing dates of the resulting plant. Although variability among individual seeds within a single progeny can result from genetic variation inherent in the embryo, it could also result from an assortment of developmental and environmental factors associated with the maternal parent.

Seed chilling has been thought to be controlled by interactions among various endogenous hormonal systems (12). One can infer that the 2 components described could represent separate genetically controlled hormonal systems. Physiological investigations of seed dormancy should utilize seed populations of uniform genetic origin for both seed and pollen parent.

The *seed chilling index* is seriously limited as a selection tool for predicting the blooming and leafing characteristics of the tree derived from it, because of the relatively low seed to plant correlation. However, the seed could be effectively utilized as a tool to investigate dormancy problems and to characterize genotypes if seed populations were studied in conjunction with the individuals they come from. For example, we have identified low chilling hybrid rogue plants that resulted from pollen contamination involving nearby plants of different species than those under study (unpublished).

Early selection for time of bloom on the basis of leafing time of the plant appears to be more useful for individual plants than the *seed chilling index,* but there also are limitations imposed by the relative independence of leafing and blooming in some genotypes.

Grigorian (6) reported similar results for the pollen effect of almond cultivars of different blooming dates on seed chilling requirements. However, he found that exceptions to a strictly additive relationship existed for certain cultivars and suggested that some dominance of late bloom might be involved. He also found that certain late blooming cultivars produced seeds with low chilling requirements and attributed this fact to difference in the heat requirement of those cultivars for bloom. We have also found that late blooming mutation of 'Nonpareil' ('Tardy Nonpareil') does not produce a corresponding change in the chilling requirement of seeds from the new clone (11). Various genetic systems for control of bloom, leafing and germination may likely exist that could be identified by investigating a wider range of plant materials.

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Fertility and Irrigation Effects on 'Temple' Orange. I. Yield and Leaf Analyses[1]

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Abstract. **Six year's data with so-called 'Temple' orange (*Citrus* hybrid) on 'Cleopatra' mandarin rootstock (*Citrus reticulata* Blanco), show that increased rates of N and K increased fruit yield. P also increased yield. Spring vs. fall irrigation resulted in a significant increase in fruit production in 1 of 6 years, although slightly higher fruit production was observed every year with spring irrigation. Increased N rates resulted in higher leaf N and Mg but lower leaf P, K, and Ca. Increased K rates raised leaf K but lowered leaf Mg. The addition of P in the fertilizer resulted in higher leaf P and Ca but lower K and Mg contents. Spring irrigation induced lower K but higher Mg in certain years.**

Considerable research has been done to determine the fertilizer and irrigation requirements of many citrus cultivars (1, 2, 4, 6, 8, 10, 11, 13, 15, 16); however, very little research has been reported for 'Temple'. 'Temple' is presumed to be a natural mandarin hybrid of either *C. reticulata* and *C. sinensis* (L.) Osbeck or possibly *C. reticulata* and *C. grandis* (L.) Osbeck. This would make it a tangor or tangelo (5). It is primarily a fresh fruit cultivar that normally does not provide satisfactory returns to the grower when used for processing; therefore, in addition to adequate yield, it must have good external and internal quality. The majority of the plantings are found in Florida although some 130 ha are being grown in California (14). Since 1970, the recorded Florida plantings have been relatively stable at approx 8,900 ha with an annual on-the-tree value between 6 and 10 million dollars (14).

Calvert (3) reported the mineral element content of 'Temple' leaves treated with various mixed analyses of N, K, and Mg. This research was done on poorly drained, organic-hardpan soils in Florida's Indian River flatwoods area. Calvert reported that increased N rates increased yield, but decreased individual fruit size, % soluble solids, and fruit juice acidity. The leaf N increased as the N rates increased; however, leaf P and K decreased as N was increased. Increased K rates showed higher leaf K but lower Ca and had little effect on leaf N and P.

The primary purposes of this research were to examine the effects of fertilizer and irrigation practices on yield, leaf analysis, and internal and external fruit quality on a deep central Florida sand. The fruit quality data are found in another paper (7).

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