J. AMER. SOC. HORT. SCI. 114(5):819–823. 1989.

Light-mediated Inhibition of in Vitro Late Embryogeny of *Ilex*

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Additional index words. Embryo culture, *Ilex aquifolium, Ilex opaca*, dormancy

Abstract. **An inhibitory effect of light on in vitro late embyogeny of *I. aquifolium* and *I. opaca* cv. Farage was observed. A negative log-linear correlation between the final embryo size and hours of continuous light during pre-incubation, at an intensity of 64 W·m^{-2}, was established with *I. aquifolium*. It took ≈15 hr of pre-incubation light to result in 50% growth inhibition. Negative log-linear correlations between the final embryo lengths and light intensities were also observed for *I. aquifolium* and *I. opaca*. The rudimentary embryos of *I. opaca* were more sensitive to light inhibition than those of *I. aquifolium*. During incubation for 11 and 14 days with a 16-hr photoperiod, light intensities for 50% growth inhibition were ≈5 and 11 W·m^{-2} for *I. opaca* and *I. aquifolium*, respectively. Since growth inhibition by light could not be reversed by the presence of GA_3 concentrations up to 1000 μM, some factor(s) other than the accumulation of abscisic acid or related compounds is probably responsible for such a phenomenon.**

The major handicap in holly breeding is the delayed germination of seeds of many holly species. This is caused by the rudimentary nature of holly embryos because they remain in the immature heart stage for a long period of time after the fruits reach maturity (6, 10, 17). An in vitro embryo culture technique has been previously developed to overcome holly seed dormancy. This technique induces the resumption of late embryogeny from the heart-shaped stage to mature embryo and causes rapid germination (10, 16).

Light inhibition of in vitro late embryogeny of excised rudimentary embryos of several *Ilex* spp. has been observed previously (9, 11). We found no work on the inhibitory effect of light on embryonic development of other genera. The ultrastructure of the quiescent rudimentary *Ilex* embryos and the changes accompanying the activation of these embryos in late in vitro embryogeny closely resemble those of quiescent mature embryos and their initial germination changes in other genera (17). Thus, the inhibitory effect of light on *Ilex* embryos should be equivalent to the inhibitory effects of light on seed germination of other genera.

The occurrence of negative photoblastic seed germination (light-induced germination inhibition) has been documented in *Amaranthus caudatus* (18), *Bromus sterilis* (7), *Laportea bulbifera* (26), *Nemophila insignis* (2), and is common among cucurbits [e.g., *Citrullus lanatus* (3) and *Cucumis anguria* (24)]. The negative photoblastic nature of seed germination is expressed only under conditions of water stress in certain species; e.g., *Avena fatua* (8), *Sinapis alba* (21), and some other cultivated species [e.g., cucumber, radish, and sunflower (23)].

Detailed studies have been previously made with *I. opaca* Ait. cv. Farage (13) on the effects of photoperiod on late embryogeny, but no study has been made to reveal the relationship between light intensity and growth inhibition of *Ilex* embryos. The studies we now report were designed to investigate these relationships.

Received for publication 13 Oct. 1988. This work was supported in part by the Fulbright Commission of the United States and CAPES (the Brazilian Fulbright agent) for a sabbatical grant to A.G. and by the continuous A.R.T. funds from Wm. Paterson College. We are grateful to John Hewitt for statistical analysis and to Robert F. Callahan for the critical review of this manuscript. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

Materials and Methods

General. Open-pollinated ripe drupes of *I. aquifolium* (English holly) and *I. opaca* Ait. (American holly) cv. Farage were collected at Rutgers Univ., New Brunswick, N.J. They were stored under refrigeration (0C) from several days to 2 months until use. Linsmaier and Skoog medium (19) with 4% sucrose and 0.7% Difco Bacto-agar was used as culture medium. Embryo dissection and culture medium preparation procedures were described earlier (10, 14).

Twenty four-well, 13.5 × 7.5-cm sterile plastic tissue culture plates (Tissue Culture Cluster, Costar, Cambridge, Mass.) were used as the culture containers. About 3 ml of sterilized culture medium was added to each well, each of which was 18 mm deep and 16 mm in diameter. One excised heart-stage embryo was placed on the medium surface of each well. The plate was covered with a tight-fitting plastic lid.

Cultures were incubated at 25C in a Sherar plant growth chamber with a light intensity of 64 W·m^{-2} (measured with a radiometer; Lambda Instruments) at the culture level, achieved with a mixture of cool-white fluorescent and incandescent lamps. Their red to far-red radiations were 3.1 and 3.9 μW·cm^{-2}, respectively (measured with Plant Growth Photometer IL150, International Light Inc., Newburyport, Mass.). Lower light intensities were achieved by covering the culture plates with layers of cheesecloth. Dark incubation was achieved by wrapping the plates in aluminum foil.

One culture plate, containing 24 excised embryos, was used for each treatment. Each experiment was repeated one to three times. Data of the same treatment were combined and resulted in 48 to 96 readings per treatment. The initial and the final embryo lengths were measured with an ocular micrometer under a stereomicroscope. Since some of the embryos had already germinated (with radicle elongation) at the end of the incubation period, the length of these embryos were converted, during data computation, to the average lengths of full-grown mature embryos, which were 3.4 mm for *I. aquifolium* and 2.5 mm for *I. opaca*.

Experimental design. To test effects of light duration on in vitro late embryogeny of *I. aquifolium*, excised embryos were pre-incubated under 64 W·m^{-2} for 6, 12, 24, or 48 hr. They were subsequently incubated in darkness for 14 days.

The effects of light intensity on in vitro late embryogeny were

tested on excised embryos of *I. aquifolium* that were incubated under darkness or a 16-hr photoperiod of 4, 8, 16, 32, or 64 W·m^{-2} for 14 days. *I. opaca* was also incubated under 2 W·m^{-2}. The incubation period for the latter species was 11 to 12 days.

Excised embryos of *I. opaca* were used to study the nature of light-induced dormancy. GA_3 at 0, 1, 10, 100, or 1000 μM was added to the autoclaved medium by Millipore filtration. One culture plate of each GA_3 concentration was incubated under darkness and one under a 16-hr photoperiod of ≈11 W·m^{-2} from cool-white fluorescent tubes for 12 days.

Results

The initial embryo lengths at time of excision were 530 ± 50 μm (n = 38) for *I. aquifolium* and 345 ± 33 μm (n = 50) for *I. opaca*. Most of them were at the heart stage, with a small percentage at early and late heart stages.

Effects of light duration on in vitro late embryogeny of I. aquifolium. A negative log-linear correlation between the final embryo size and hours at light pre-incubation was observed on a semilog scale (Fig. 1). The growth rate of individual embryos varied widely in each treatment.

Effects of light intensity on in vitro late embryogeny. The regression lanes, means, and SEs of final embryo lengths following incubation at each light intensity for both species are presented in Fig. 2. Since a light intensity of 32 W·m^{-2} already reached the maximum growth inhibition for *I. opaca*, treatment incubation under higher light intensity, i.e., 64 W·m^{-2}, was excluded during the calculation of regression line for this species. Negative log-linear correlations between the final embryo lengths and light intensities were observed on this semilog graph for both species. The embryo size distribution at each light intensity for *I. aquifolium* is shown in Table 1 and Fig. 3. It was evident that the higher the incubating light intensity, the greater the amount of growth inhibition that resulted. More than 60% of the embryos remained at various heart stages, and none reached intermediate or mature stages when the incubating light intensities reached 32 W·m^{-2} or higher. But, when the incubating light intensities were 8 W·m^{-2} or lower, >64% of the embryos reached intermediate and mature stages. Under these low light intensities, <7% of the embryos remained at various heart stages.

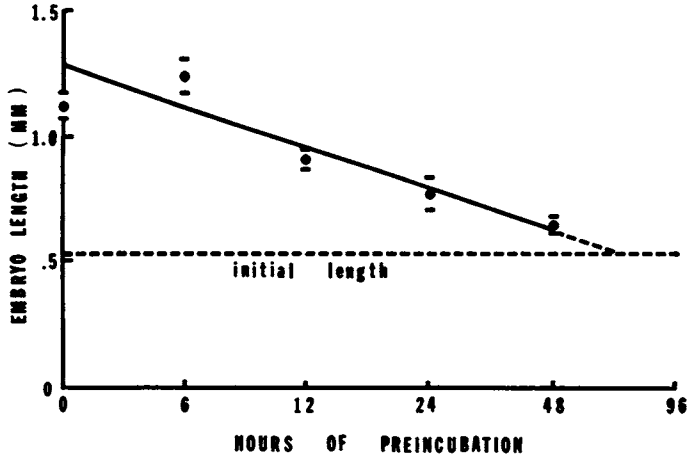

Fig. 1. Effect of duration of pre-incubation in light on in vitro late embryogeny. The rudimentary embryos of *I. aquifolium* were incubated at 25C in darkness for 14 days after light pre-incubation. Dot and bars = mean and range for SE. Regression line is based on the equation: Y = 1.2708 − 0.1626X, when the points on the X-axis are transformed from 0, 6, 12, 24, and 48 hr to 0, 1, 2, 3, and 4, respectively. N = 207, r = 0.3314, P < 0.0001.

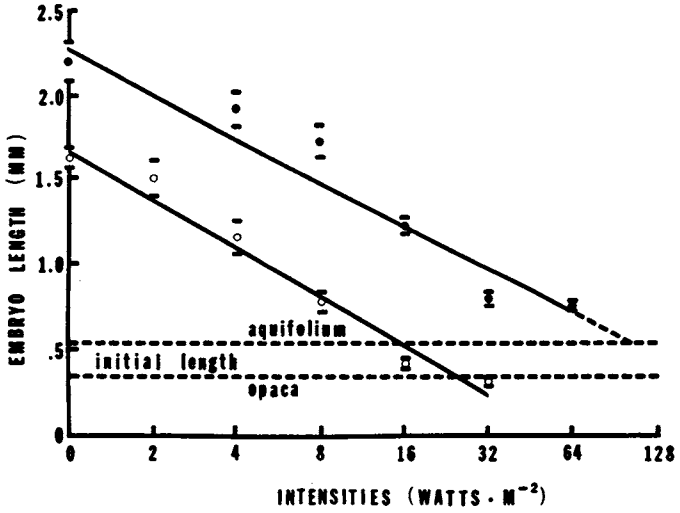

Fig. 2. Effect of light intensity on in vitro *Ilex* late embryogeny. The rudimentary embryos of *I. aquifolium* and *I. opaca* cv. Farage were incubated in darkness or in a 16-hr photoperiod of appropriate intensity for 14 and 11 days, respectively. Dot (for *I. aquifolium*) and circle (for *I. opaca*) = mean. Bars = range for SE. Regression lines for *I. aquifolium* (upper line) and *I. opaca* (lower line) are based on the equations: Y = 2.2763 − 0.2605X (N = 353; r = 0.5916) and Y = 1.6644 − 0.2884X (n = 375; r = 4455), respectively, when the points on X-axis are transformed from 0, 2, 4, 8, 16, 32, and 64 W·m^{-2} to 0, 1, 2, 3, 4, 5, and 6, respectively. r values significant at P < 0.0001.

Table 1. Distribution of embryonic stages after the rudimentary, heart-stage embryos of *I. aquifolium* were incubated in a 16-hr photoperiod for 14 days under various light intensities.

Embryoic stage[z]	Light intensities (W·m^{-2})					
	0	4	8	16	32	64
	No. embryos[y]					
1	3	0	3	7	29	44
2	12	12	16	32	18	0
3	34	36	35	11	0	0
Total	49	48	54	50	47	44

[z]1 = little or no growth (early heart, heart, and late heart stages); 2 = slight growth (torpedo stage); 3 = much growth (intermediate, mature, and germinated stages).

[y]Number of embryos at the given developmental stage after incubation under the specified light intensity; χ^2 independence test on embryo size distribution at various light intensities, P < 0.0001.

The nature of light-induced dormancy. Although high GA_3 concentrations under dark conditions were able to stimulate additional embryo elongation in comparison with the control and resulted in higher fresh weights, it could not counterbalance the inhibitory effect of light (Table 2). Instead, a progressive enhancement of the inhibitory effect of light was observed as the concentration of GA_3 increased.

Discussion

Negative photoblastism. Our data suggest an active role of light in growth inhibition. Once the maximum level of light inhibition was reached, it could not be reversed by subsequent

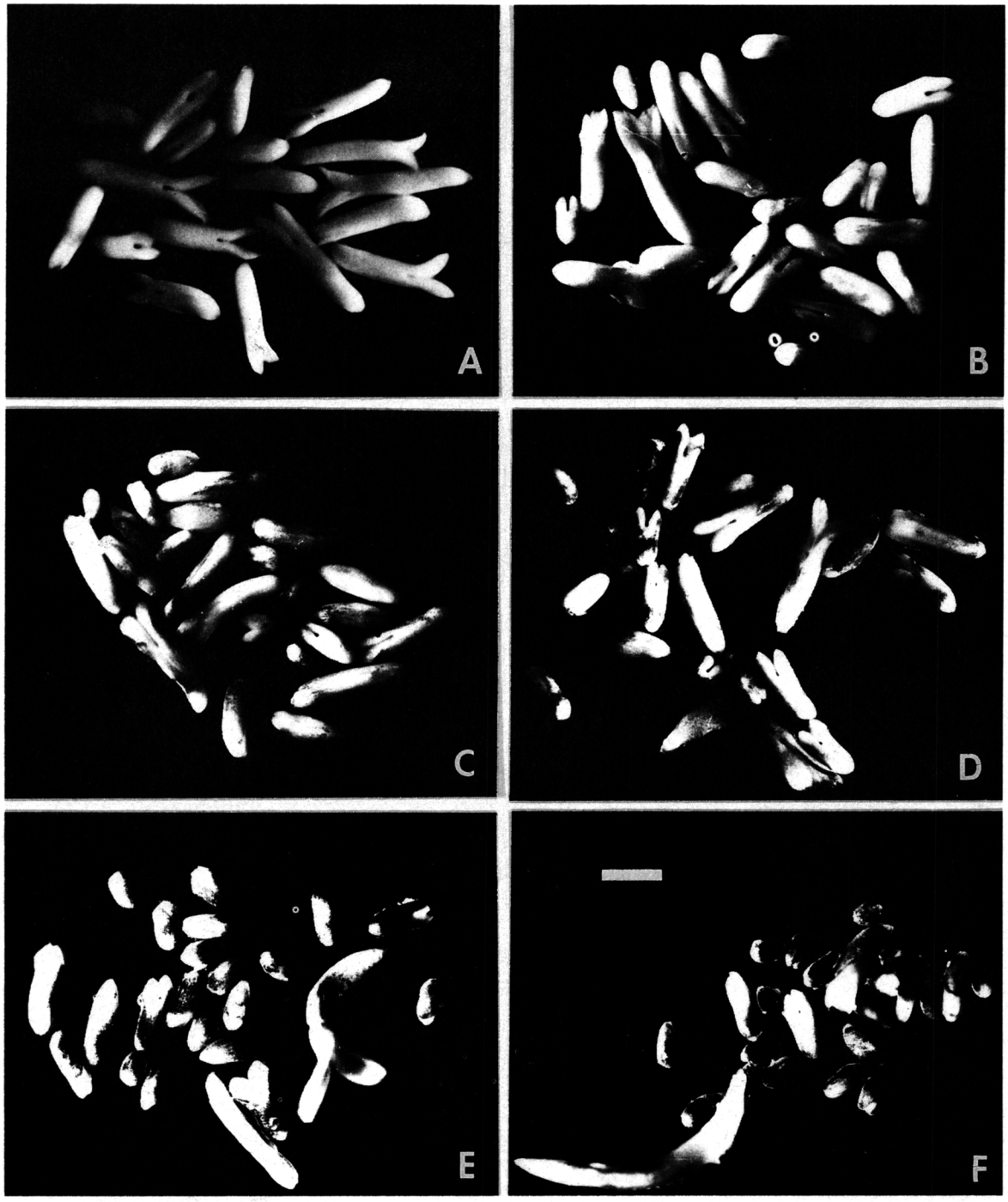

Fig. 3. Effect of light intensity on in vitro *Ilex* late embryogeny. The rudimenary embryos of *I. aquifolium* were incubated in a 16-hr photoperiod at 0 (**A**), 4 (**B**), 8 (**C**), 16 (**D**), 32 (**E**), or 64 (**F**) $W \cdot m^{-2}$ for 14 days. Bar = 1 mm.

dark incubation. Some type of secondary dormancy must induced in the excised rudimentary embryos by light.

The negative photoblastic response of seed germination, as with light-induced growth inhibition of excised heart-stage *Ilex* embryos reported here, generally requires a long period of light exposure (1) of high energy levels. Thus, the so called high irradiance response (HIR; ref. 22) is involved. Light-induced germination inhibition was reportedly mediated through the photochrome system (4, 7, 20). Since blue and far-red light are known to inhibit seed germination of negative photoblastic species (27), as well as the in vitro embryonic development of *Ilex* (12), the poorly understood cryptochrome, a blue/UV-A light-absorbing pigment (26), may also participate in such HIR phenomena. One of the difficulties in evaluating the involvement of cryptochrome and phytochrome with the HIR of the negative photoblastism is that blue wavelengths are absorbed much more than red or far-red by seed coats and fruit walls. The use of naked excised *Ilex* embryos in culture is an ideal system to study pigment involvement without the complication of light absorption by seed coat or fruit wall.

Botha et al. (5) provided evidence to support the possibility of light as an inhibitory factor for germination of *Citrullus lanatus* seeds within the fruits. Hu (15) proposed that sunlight, which penetrates the semitransparent fruit and seed tissue during

Table 2. Effect of GA_3 concentrations on in vitro late embryogenesis of *I. opaca* cv. Farage. The rudimentary embryos were incubated either under 16-hr photoperiod (≈4000 lux) or in darkness for 12 days.[z]

GA_3 (μM)	Dark incubation	Light incubation		
		Embryo		
	Fresh wt. (mg)	Fresh wt. (mg)	Length (mm)	Remained in heart stages (%)
0	788 ± 124	140 ± 49	0.81 ± 0.15	77
1	472 ± 86	72.9 ± 40	0.70 ± 0.15	83
10	651 ± 92	17.8 ± 3.7	0.40 ± 0.03	96
100	837 ± 187	17.2 ± 1.6	0.36 ± 0.01	100
1000	1010 ± 156	15.8 ± 1.5	0.38 ± 0.01	100

[z]Mean ± SE.

fruit development, initiated in situ growth cessation of *Ilex* embryos at the heart stage. These embryos were found to be insensitive to the inhibitory effect of light before (the early heart stage) and after (the end of late heart stage) this heart stage (13, 15; unpublished data).

The effects of light duration on in vitro late embryogeny. Figure 1 shows that the longer the light pre-incubation the stronger the resulting growth inhibition. It would take ≈15 hr of pre-incubation light to result in a 50% growth inhibition. If this negative log-linear relationship holds true when the regression line is extended, a total growth inhibition would take place at ≈75 hr of light pre-incubation. At this point, the inhibitory effect could no longer be reversed by subsequent dark incubation (Fig. 1; ref. 13). This fact agrees with the observation on the negative photoblastic seed germination of *Nemophila* (2) and *Amaranthus* (18), but disagrees with that of *Citrullus lanatus* (3) and *Bromus sterilis* (7), which resumed maximum germination when transferred back to darkness from light. Nevertheless, a total growth inhibition may never be reached in *I. aquifolium* because the *Ilex* embryo is physiologically sensitive to the inhibitory effect of light only at a brief developmental period, namely the heart stage, but not before or after (13, 15; unpublished data). Apparently, in the mature fruits of this species, a fraction of the embryos had already reached the late-heart stage. Those late-heart stage embryos already had passed their light-sensitive period and could grow even in the presence of continuous high-intensity light (Fig. 3). Similar results have been obtained in *I. opaca* (13).

The effects of light intensity on in vitro late embryogeny. It is evident from Figs. 2 and 3 and Table 1 that the higher the incubation light intensity, the greater is the amount of growth inhibition. The rudimentary embryos of *I. opaca* were more sensitive to light inhibition than those of *I. aquifolium*. Based on the regression lines in Fig. 2, light intensities for a 50% growth inhibition were ≈5 and 11 $W \cdot m^{-2}$ for *I. opaca* and *I. aquifolium*, respectively. The minimum light intensities for total growth inhibition were ≈27 $W \cdot m^{-2}$ for *I. opaca* and 100 $W \cdot m^{-2}$ for *I. aquifoium*, if the regression line holds true when being extended. Again, a total growth inhibition by light may not be possible in *I. aquifolium*, as discussed in the previous section. The slopes of the regression lines in Fig. 2 would become steeper (more negative) as the duration of the incubation period increased.

The nature of light-induced dormancy. Since the light-induced growth inhibition could not be nullified by the presence of GA_3, some factor(s) other than the accumulation of abscisic acid related compounds is probably involved. This point was further supported by the following observations (unpublished data): 23 rudimentary embryos of *I. pernyi* were incubated under light for 13 days. These light-inhibited embryos were then stored in a refrigerator (4C) for 117 days, followed by dark incubation at 25C. Although most embryos retained a healthy appearance, no growth was detected even after 25 days of incubation. The nature of such light-induced secondary dormancy [primary dormancy describes maintenance of embryos at a rudimentary stage by an endosperm inhibitor (17)] is still unclear.

Although it is well-known that gibberellin breaks dormancy of seeds and buds of numerous species, it may also induce dormancy in certain plants. Okagami (25) reviewed the dormancy-inducing phenomenon of gibberellin in bulbils of *Begonia* and *Dioscorea*, as well as in buds of some woody plants. He presented data on the modification of seed germination by GA_3 in *Dioscorea*. Resembling the in vitro late embryogeny of *I. opaca* reported here, GA_3 promoted seed germination of *D. tenuipes* in darkness and as a germination inhibitor under white light. Inhibition reached a peak at a GA_3 concentration of 3 μM, then decreased as the concentration increased further. With *I. opaca*, inhibition increased sharply with GA_3 concentrations up to 10 μM, although higher concentrations were less effective. The mechanism of such enhancement of light inhibition by gibberellin is still unknown.

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J. Amer. Soc. Hort. Sci. 114(5):823–827. 1989.

Genetic Parameters Estimated for an Advanced-cycle Strawberry Breeding Population at Two Locations

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Additional index words. *Fragaria* ×*ananassa*, genotype × environment interaction, heritability

Abstract. **Strawberry (*Fragaria* ×*ananassa*) seedlings were evaluated for yield, fruit weight, and commercial appearance in two field trials established in 1985 and 1986. Genetic analyses for unbalanced diallels were performed to quantify genetic, environmental, and interaction variance for each trial separately, and for crosses common to two locations in a single year. When data from crosses common to two test locations were analyzed simultaneously, narrow-sense heritabilities (h^2) averaged 0.35 (±0.11), 0.21 (±0.07), and 0.08 (±0.06) for yield, fruit weight, and appearance score. Broad-sense heritabilities (H^2) were 0.35 (±0.11), 0.27 (±0.12), and 0.21 (±0.11) for the same traits, respectively. These estimates do not differ significantly from heritabilities estimated from the ancestral breeding population 20 years ago. Estimates of H^2 for single-location analyses were biased upwards by dominance × location interactions for all traits. Additive × location interactions were detected for appearance score and contributed a small bias to single-location estimates of h^2. Use of biased estimates in predicting genetic gain could lead to errors in choice of appropriate selection strategy.**

Improved cultivars and cultural practices have contributed to the substantial improvements in production traits of strawberries in California over the past four decades (1). The objective of the Univ. of California breeding program is to develop new cultivars with improved preformance, and adaptation to the superior environments created by improved cultural practices. Over time, successful selection is expected to alter the amount and distribution of genetic variation within the breeding population. Periodic assessments and information regarding directional change in genetic parameters are important, because the effectiveness of different breeding, testing, and selection strategies depends on the availability and distribution of genetic variation.

Hansche et al. (7) estimated narrow-sense heritabilities for strawberry fruit yield, fruit weight, and a commercial appearance score as 0.48, 0.20, and −0.02, respectively, using data from a large sample of offspring-parent pairs collected in California between 1960 and 1966. They also compared estimates of genotypic and additive genetic variance, concluding that dominance effects were unimportant for yield, detectable for fruit weight, and large for appearance score. Conversely, Comstock et al. (3) and Spangelo et al. (11) have detected different patterns of inheritance for yield and fruit weight in cultivated strawberries, concluding that these traits are conditioned largely

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