the locular membrane permitted entire vesicles (sac plus stalk) to be individually obtained by plucking the stalk at the base with a sharp pointed forceps. The vesicles thus removed were soaked in a buffer solution (0.10*M* phosphate, *p*H 7.0) for 15 minutes and placed on liquid nutrient media in screw-cap vials. The tissues were supported by reagent grade sea sand (Merck) or Du Pont cellulose sponge (yellow color) topped with Whatman No. 42 ashless filter paper discs.

The nutrient medium was entirely inorganic in composition except for sucrose as a carbon source (Table 1) and a small quantity of organic material supplied as ferric citrate or iron chelate-138 (Geigy Chemical Corp.). The medium was buffered by phosphate salts ($K_2HPO_4 + KH_2PO_4$) which also served as the sole phosphate source. The buffer system used was that of Colowick and Kaplan (*3*).

The vesicles started proliferating approximately 2 to 3 weeks after they were planted (May 1957) and are still producing new cells (13 January, 1959) (Fig. 1, left). Subcultures also carry on mitotic activity for long periods (10 months) and have gained in fresh weight as much as 2600 percent. The vesicle stalk appears to be the primary seat of growth activity. This tissue is capable of proliferating when it is attached to or removed from the juice sac. Occasionally the juice sac will proliferate, but only when the stalk is attached. Growth is most vigorous in the *p*H range 7.0 to 7.7 and occurs to a lesser extent at *p*H 6.0. The growth is visibly the same at all the above *p*H levels. None occurs at *p*H levels below 6.0 under the conditions employed.

Nutrient media which had supported the growth of the excised tissue for 81 days yielded entirely negative results when they were tested for aerobic and anaerobic microorganisms. The results suggest that mature lemon juice vesicles possess the inherent capacity to carry on cell proliferation for indefinite periods under relatively simple *in vitro* conditions.

Table 1. Composition of nutrient solution. Phosphate (0.01*M* total) was supplied by the buffer salts.

Compound	Amt. (g/lit.)	Ion or element (ppm)
$CaCl_2 \cdot 2H_2O$	0.1470	Ca^{++}, 40.0 Cl^-, 71.0
KNO_3	0.5090	K^+, 200.0 NO_3^-, 312.0
$MgSO_4 \cdot 7H_2O$	0.1080	Mg^{++}, 10.0 SO_4^{--}, 42.3
$MnSO_4 \cdot 4H_2O$	0.0050	Mn^{++}, 1.25 SO_4^{--}, 2.16
$ZnSO_4 \cdot 7H_2O$	0.0022	Zn^{++}, 0.50 SO_4^{--}, 0.738
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.0037	Mo, 2.0 NH_4^+, 0.0003
H_3BO_3	0.0028	B, 0.50
Fe-138*	0.0467	Fe^{+++}, 5.0 chelate, 41.0
Fe-Citrate†	0.0273	Fe^{+++}, 5.0 citrate, 16.86
$CuSO_4 \cdot 5H_2O$	0.0012	Cu^{++}, 0.30 SO_4^{--}, 4.50
Sucrose	34.250	

* Chelate-138 was used in all nutrient solutions buffered above *p*H 6.0.
† Ferric citrate was used in all nutrient solutions buffered at *p*H 6.0 and lower.

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Requirements for Floral Initiation of Los Angeles Xanthium

Abstract. Cocklebur from the Los Angeles area was found to require more extensive short-day treatment for floral initiation than plants of the same species from the Chicago region. Data obtained by grafting the two regional types of cocklebur indicate that the leaves of the Los Angeles *Xanthium* produce a comparatively low amount of the flowering stimulus.

The cocklebur, *Xanthium strumarium* L. var. *canadense* (Mill.) T. and G. (*1*), which has been most commonly referred to in the past as *Xanthium pennsylvanicum* Wallr., is generally recognized as the most sensitive short-day plant now known. Although it can be maintained indefinitely in the vegetative state under long-day conditions, a single dark period of 8¾ hours or more will initiate floral development (*2*). The acknowledged esthetic deficiencies of the plant are more than compensated for by its usefulness as a test organism for assessing the effectiveness of a given treatment in terms of the short-day flowering response.

Most investigations employing *Xanthium* have used plant material derived from the fruit of wild plants collected in the vicinity of Chicago, Ill. The species range, however, extends in a wide belt from Florida through California, and from Quebec through North Dakota (*3*). Although it is taxonomically identical to the Chicago stock, *X. strumarium* from the Los Angeles area has been recognized (*4*) as being generally less sensitive to dark treatment than its Chicago counterpart.

The experiments described in this report constitute an evaluation of the critical day length for the Los Angeles *Xanthium* and, in addition, represent an attempt to determine a basis for the difference in response between the Los Angeles and the Chicago types.

Burs from wild Los Angeles stock were soaked in water for 12 hours before they were planted in flats of soil. Two weeks after emergence, 15 seedlings from each flat were selected for uniformity; all other plants were removed. All plants were maintained in cold frames throughout their growth period. Before and after the actual interval of photoinductive treatment the plants were maintained under continuous illumination, the natural day length being supplemented at night with incandescent light of intensity approximately 50 ft-ca at the leaf surface.

Two weeks after the last dark treatment the terminal buds were examined under a low-powered dissecting microscope and assessed with respect to the relative stage of floral development that had been attained. Results were evaluated in accordance with a numerical scale based on the diameter and morphological stage of development of the terminal staminate inflorescence. Vegetative plants were rated as zero on the scale. The first morphological change in the stem apex that could be clearly recognized as flowering was assigned a value of 1.0. A flowering apex measuring 0.25 mm in diameter was evaluated as 2.0. An additional increment of 1.0 was allowed for each 0.25-mm increase in the diameter of the developing inflorescence (*5*).

The treatments employed in the determination of the critical day length are listed in Table 1. From these data it is apparent that no flowering occurs in Los Angeles plants subjected to dark periods of 11 hours' duration or less. A single dark treatment of 20 hours was ineffective. The critical day length of the Los Angeles stock is, therefore, between 11 and 12 hours of darkness, and more than one photoinductive treatment is required for minimal floral initiation. Three photoinductive cycles with dark periods of 12-hour duration resulted in a low flowering response. An increase in

Table 1. Effect of dark treatment on the flowering response of Los Angeles *Xanthium*.

Darkness (hr)	Av. flowering response, with short-day treatments				
	No. of treatments				Continuous treatment
	1	3	5	5*	
9		0.0	0.0	0.0	0.0
10			0.0	0.0	0.0
11		0.0	0.0	0.0	0.0
12		0.2	1.9	0.7	10.9
13		0.9	4.0	2.9	
16	0.0	3.2			
20	0.0				

* Noninductive day length alternated with short-day treatment.

Table 2. A comparison of relative responsiveness to photoperiodic treatments of leaf and bud combinations from Los Angeles and Chicago *Xanthium*.

Treatment	Av. flowering response
A. Chicago bud grafted to Los Angeles leaf	0.0
B. Los Angeles bud grafted to Chicago leaf	2.0
C. Los Angeles bud grafted to Los Angeles leaf	0.0
D. Chicago bud grafted to Chicago leaf	4.4
E. Los Angeles bud defoliated	0.0
F. Chicago bud defoliated	0.0
G. Chicago plant (not grafted)	13.4
H. Los Angeles plant (not grafted)	2.8

either the length or the frequency of the dark period resulted in a corresponding increase in floral development. It is of interest to note that alternation of noninductive day length and short-day treatment (Table 1) effectively lowered the flowering response. This is in accord with the results of Lincoln *et al.* (*6*).

In explanation of the low level of flowering response of the Los Angeles *Xanthium*, as compared with the floral development that would be expected in the Chicago stock under comparable conditions of illumination, two possibilities were considered: (i) The buds of the Los Angeles plants may require a greater amount of stimulus to initiate flowering, and (ii) the leaves of the Los Angeles plants may produce less stimulus than the leaves of the Chicago type produce under comparable photoinductive conditions.

The following experiment was designed to test the validity of these postulates. The plants were germinated in flats and 1 week after emergence were transplanted singly to 4-in. pots. The individual plants of the Los Angeles and Chicago types were trimmed and debudded, a single leaf being retained on the donor plant and a single terminal bud on the receptor plant. The plants were grafted 4 weeks after transplanting, and the first short-day illumination was provided 3 days after grafting. All other conditions were the same as those described above.

The data listed in Table 2 indicate that five inductive cycles involving dark periods of 16 hours' duration do not initiate flowering in either a Chicago or a Los Angeles bud in association with a Los Angeles leaf. A Chicago leaf, however, under the same photoinductive treatment will cause floral initiation in either bud type. The intact control plants of both the Chicago (treatment G) and the Los Angeles (treatment H) types flowered in response to the photoinductive treatment. As would be expected from the data given in Table 1, the Los Angeles plants were less responsive to short-day treatment than the Chicago plants. The graft apparently restricted to some extent the amount of stimulus moving from the leaf to the scion bud. It is apparent that the low response of the Los Angeles plants can be attributed in some measure to the small amount of stimulus supplied by the leaf. The response of the Los Angeles bud to a Chicago leaf was less than that of the Chicago bud to a leaf of the same type. The Los Angeles bud therefore may have a somewhat higher requirement for comparable floral initiation.

Variations in natural day-length conditions may exert a selective influence on the photoperiodic responses of a plant. A single plant species distributed over a wide longitudinal range may exhibit differences in photoperiodic sensitivity corresponding to the gradation in longitudinal distribution (*7*). The observed difference in the response of Los Angeles *Xanthium* to photoperiodic induction may represent a similar pattern of natural selection.

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Hetero Blood Types and Breeding Performance

Abstract. The phenomenon of hybrid vigor apparently depends to some extent upon the degree of diversity between the genes of the two parents. Results are presented which suggest that diversity of cellular antigens might be used as an indicator of general genetic diversity between mates.

In livestock breeding, the phenomenon of hybrid vigor is often manifested by vigorous offspring with a relatively high rate of survival. Generally, the more different the parents are genetically, the more pronounced is the phenomenon of hybrid vigor (*1*). The absence of means to measure the genetic diversity between animals to be used in a breeding program aimed at utilizing hybrid vigor is a deterrent to a widespread exploitation of this phenomenon. The cellular blood antigens of cattle are controlled by genes located on at least 11 different chromosomes, and it has been demonstrated that the frequencies of the blood types vary between different breeds and strains (*2*). A study was made of breeding results in dairy cattle in which the cellular antigens of the mates were known.

Table 1. Data on matings between 310 females and 32 males of the Holstein-Friesian breed.

No. of antigens in which mates differed	No. of matings	No. of offspring	No. of offspring that survived (%)
1– 5	305	140	46
6– 7	433	212	49
8– 9	357	176	49
10–15	169	101	60
Total	1264	629	50

In order to study whether similarity or dissimilarity of mates influences the probability of survival of the young, a tabulation was made of 1264 matings between 310 females and 32 males of the Holstein-Friesian breed, in which the presence or absence of 20 different cellular antigens had been determined (*3*). Only matings between supposedly fertile animals were included in the tabulation shown here.

The matings were classified on the basis of the number of cellular antigens in which the two mates differed. The results are presented in Table 1.

The rate of survival increased as the difference in antigens increased. The chi-square of 8.72 is significant at the 0.05 level.

No claims are made that cellular antigens are responsible for fertility and successful embryonic development. However, dissimilarity of antigens may be used as an indicator not only of dissimilarity of genes affecting blood antigens but of dissimilarity of other genes affecting productive traits as well. Further studies on other productive traits seem justified in order to determine whether differences manifested with respect to blood antigens might be used as a guide in mating for "hybrid vigor" (*4*).

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