Effect of Short Photoperiods, Cycocel, and Gibberellic Acid upon Flower Bud Initiation and Development in Azalea 'Hexe'[1]

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Abstract. A technique using 2-branched rooted cuttings of azalea 'Hexe' was devised to provide uniform plant material for studying the effect of photoperiod and growth regulators on early stages of flower bud initiation and development. Flower initiation was most rapid under 8-hr daylengths, as a definite change of shape of apex was observed the 4th to 5th weeks. No change was observed on plants subjected to interrupted nights with 50 ft-c incandescent light supplied between 10 PM and 2 AM.

Short days plus a drench of 0.4 g Cycocel, (2-chloroethyl) trimethylammonium chloride, accelerated initiation over short days alone. Gibberellic acid applied to Cycocel-treated plants at 3 and 4 weeks of short days delayed flower initiation. Gibberellic acid applied to short day plants during the 4th to 6th weeks was effective in preventing flower initiation in this cultivar.

Introduction

The evergreen florist azalea has usually been considered as insensitive to length of day in induction of flowers. Since 1956 (12), it has been generally accepted that a period of approximately 8 weeks at a minimum temperature of 65°F is necessary for uniform bud set.

Skinner (14) reported that some azaleas produced more flower buds on long days but some rhododendron species produced more flower buds on short days. No promotive effect of short days on budding azalea plants was observed by Post (11). Using the relatively easy-to-bud cultivar 'Coral Bells', Kiplinger (5, 6) reported that shortening the daylength was an aid to flower development, without specific reference to initiation. Borthwick et al. (1) found no marked effect of photoperiods of 9 to 18 hr on time of flower bud initiation of several azalea cultivars.

More recently, using 8-hr photoperiods as adjuncts to his work with retardants in promoting flower bud initiation, Stuart (17) found the cultivars 'Alaska' and 'Hexe' to initiate flower buds while plants on long days continued vegetative growth. He has also reported (18) that reduction of light intensity or daylength after initiation resulted in more rapid development of the initiated buds.

While recognizing a large error variation in comparison of shoots for different treatments, McDowell and Larson (9) found no effect of short (8-hr) or natural (14 hr 41 min) daylengths on the date of flower bud initiation in 3 cultivars 'Alaska', 'Redwing', and 'Hershey Red'.

In view of the diverse genetic background of the florist azalea, it is not surprising that some cultivars might be found which are responsive to short days for promotion of flower initiation. The cultivar 'Hexe' is one which appears to have such a response.

The effects of the retardants, Cycocel and B-nine, in promoting flower bud initiation in the azalea have been noted by many researchers, among them Stuart (16, 17, 18), Gill (3), Crittendon et al. (2), Wilkins and Gartner (19), Shanks (13), and McDowell and Larson (9). Only the last-named have sought to relate the actual initiation process to environmental and chemical conditions. In their experiments with 'Redwing', the retardants Cycocel and B-nine did cause earlier bud initiation and a greater rate of development than did no-chemical treatments, while imposition of daylength treatments was not effective in altering time of initiation.

The main objective of the study was to determine the effects of photoperiod and growth regulating chemicals on flower initiation and early development. Another objective was to study the morphological development of the flower bud. The study of flower initiation and development requires uniform stock with which to work. The usual size and growth habit of azalea tend to increase the diversity of morphological conditions found on the plant. Therefore, experiments were set up with the objective of evaluating the use of rooted cuttings as experimental plants for studying flowering in azalea.

Materials and Methods

In the initial experiments, rooted cuttings of 'Hexe' provided by California Camellia Gardens, were potted in peat moss and grown at 70°F under natural daylength conditions for 4 weeks. At this time, they were pinched below any flower or elongating vegetative bud and placed under natural daylengths with a supplemental illumination of 50 ft-c from 10 PM to 2 AM from an incandescent source. When new shoots were large enough to distinguish (about 2 weeks) the plants were pruned to the 2 largest shoots. At this time 2 comparable groups of 20 plants each were selected and one placed under an 8-hr daylength (SD) while the other continued under the interrupted night (IN) regime. Because these shoots tended to develop unevenly, a later experiment included a 3 week period of 45° after pinching. This cold period conditioned the buds so that more even breaking and uniform subsequent growth resulted. At the end of the treatment period, a node count was taken on each of the breaks to provide evidence for the time and uniformity of flower initiation.

For identification of the stages of floral initiation and development plants were subjected to short days and interrupted nights as previously described. At weekly intervals beginning 3 weeks after the start of SD and until the 8th week, shoot tips from plants in both treatments were collected and preserved for microscopic study according to standard paraffin techniques. Serial sections were stained with safranin and tannic acid/iron chloride. A rating system for development paralleling that of Kohl and Sciaroni (7) was used.

Gibberellic acid (as KGA_3) was applied as an aqueous solution containing 0.05% Tween 20 in a 10 µl droplet placed in the leafy tip of each stem. Cycocel was applied as a soil drench of 0.4 g in 100 ml water per 4-inch pan.

Results

Macroscopic flower buds could be observed on short day plants after 8 weeks, while buds were not observable until several weeks later, if at all, on those plants given

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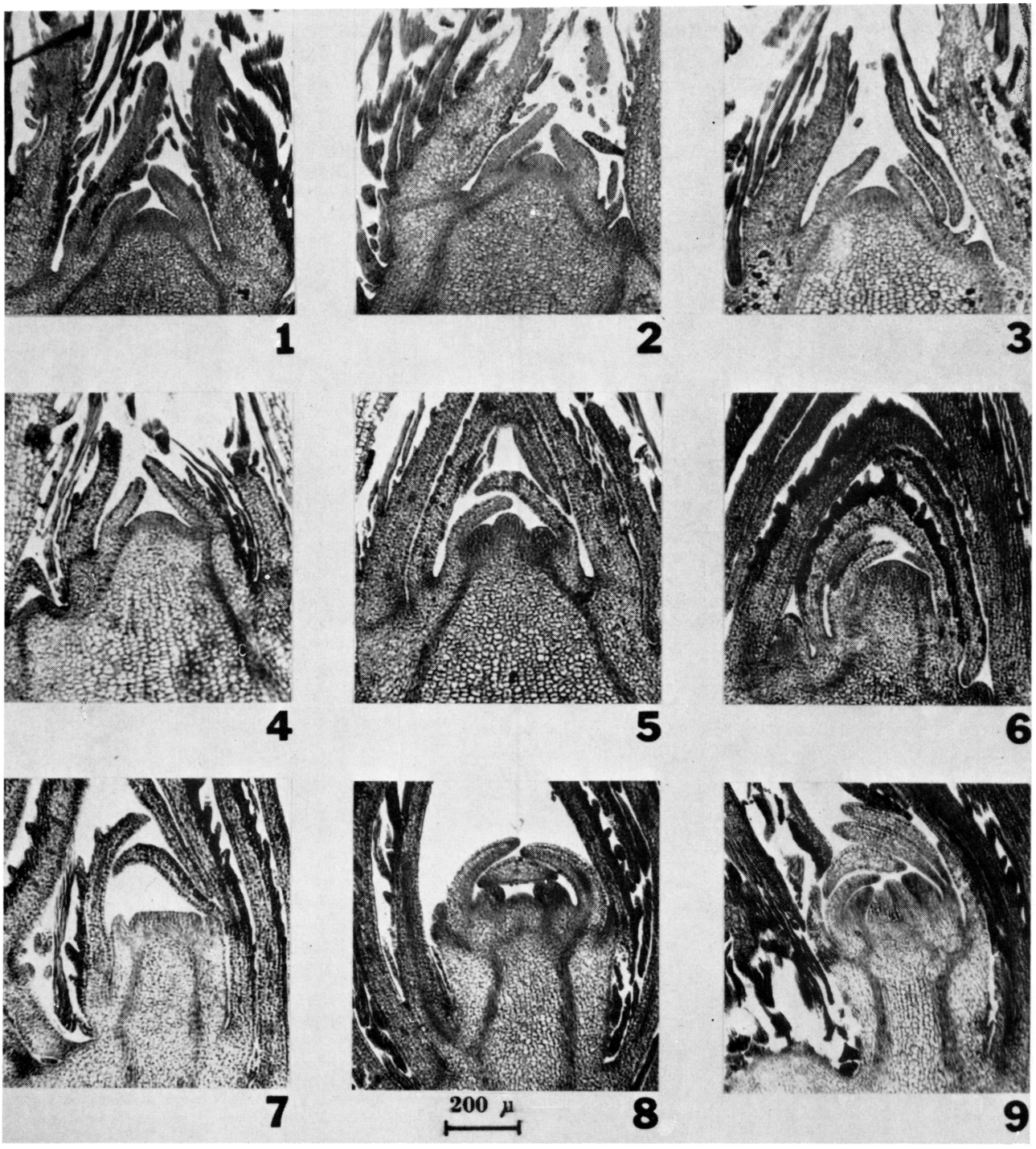

Fig. 1. Stages in the initiation and early development of an azalea flower. 1–1: 4th week of interrupted nights (IN), vegetative. 1–2: 3rd week of short days (SD), vegetative. 1–3: 4th week, SD, transition stage with apex beginning to broaden. 1–4: 5th week, SD, transition stage with apex broadening. 1–5: 5th week, SD, development of inflorescence of 3 flowers; no floral organs apparent. 1–6: 6th week, SD, development of sepal primordia. 1–7: 7th week, SD, sepal and petal primordia, apparent. 1–8: 8th week, SD, sepal, petal, anther, and carpel primordia present. 1–9: 8th week, SD, more advanced development with sepals, petals, anthers, and open style differentiated. All photomicrographs at same magnification. Bar represents 200 microns.

interrupted nights. After a 3 to 4 month period to allow for initiation under both conditions, a notable difference existed in number of nodes to flower initiation between those groups exposed to SD and IN (Table 1). The uniformity of response was not out of line with usual biological variation in controlled plants. Blind apices appeared in the third trial which may have been associated with high temperatures under the black cloth.

Following 3 weeks of treatment with SD and IN, apices in both systems were apparently vegetative (Fig. 1). After 4 weeks of SD, the SD apex had begun to broaden out and, at this time, corresponded approximately to stage 1 of Kohl and Sciaroni (7). The IN apex remained vegetative. Development progressed steadily in the short day apices during the period studied to the equivalent of stage 5 of Kohl and Sciaroni (7) with the style differentiated and open while the IN plants remained vegetative.

Table 1. Nodes to flower initiation and per cent shoots of cv. 'Hexe' budded under 8-hr daylengths (Short days) and natural daylengths + 4 hr, 50 ft-c, interruption from 10 PM to 2 AM (Interrupted night) and 70° night temperatures.

Expt. no.	Date commenced	No. days	Short days		Interrupted night	
			No. nodes	% budded	No. nodes	% budded
I.....	3/4/65	137	24.4 ± 4.1	100	54.9 ± 14.9	55
II.....	4/6/65	104	24.8 ± 2.9	100	52.4 ± 12.3	75
III.....	4/15/66	100	18.5 ± 2.3	54	25.4 ± 5.0	0
			14.5 ± 1.8	46[a]		

[a]Blind apices.

A promotive effect upon bud initiation by SD and Cycocel and the delay resulting from IN or applications of gibberellic acid were evident (Fig. 2). Seven weekly gibberellic acid applications of 1 μg/tip overcame the promotive effects of SD and the Cycocel drench. Cycocel accelerated initiation under IN conditions and appeared to act additively with SD.

In studies in which 2 μg gibberellic acid was applied to each shoot tip following 3, 4, 5, 6, or 7 weeks of SD, initiation was apparently delayed by GA during the first 4 weeks of short days in both Cycocel-treated and non-treated plants (Fig. 3). Cycocel accelerated flower initiation over short days alone.

Gibberellic acid had no effect upon development of the flower once it was initiated (Fig. 4). Cycocel-treated plants were approximately a week ahead of the control plants when compared on the basis of data presented in Fig. 1. In this experiment the first signs of initiation, an increase in height and width of the apex, were apparent at the 4th week for Cycocel-treated plants.

In cases where a flower bud did not develop, bud scales had formed, but the terminal apex had failed to continue its development. These apices at 100 days commonly appeared as a vegetative apex with leaf primordia forcing their way out of the bud scales (Fig. 5). In other cases the apex persisted and developed little if any while a new vegetative apex arose elsewhere within the bud scales. Such structures were termed remnant apices by Naskali (10). This type of non-reproductive, yet not characteristically vegetative, apex occurred largely in the treatments receiving gibberellic acid at the 5th and 6th weeks of SD (Fig. 3). The 5th and 6th weeks of SD were brighter and warmer than the preceding periods which may have affected some critical aspect of flower development. With Cycocel treatments, however, few remnant apices developed.

Discussion

The short day response of 'Hexe' and the development of techniques for producing uniform plant material enabled studies of the interaction of the growth retardant, Cycocel, and gibberellic acid on flower initiation. No greater variance was noted for a population of 10 2-stemmed plants than for a larger number of plants; thus, an increase in the number of treatments could be effected without burdening the data-taking operation with matters of uniformity and selection.

The apical meristem appeared to consist of a 3–4 lay-

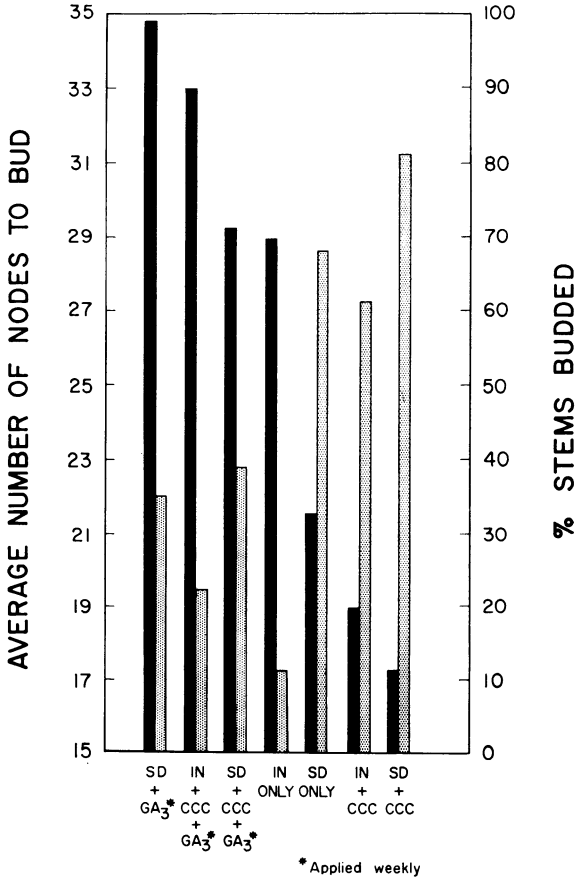

Fig. 2. Average number of nodes from break to bud (solid bar) and per cent stems budded out of 20 (stippled bar) in azalea plants given interrupted nights (IN) or short days (SD). GA_3 applied weekly at rate of 1 μg/tip. CCC (Cycocel) applied as a drench at rate of 0.4 gm/plant at beginning of experiment.

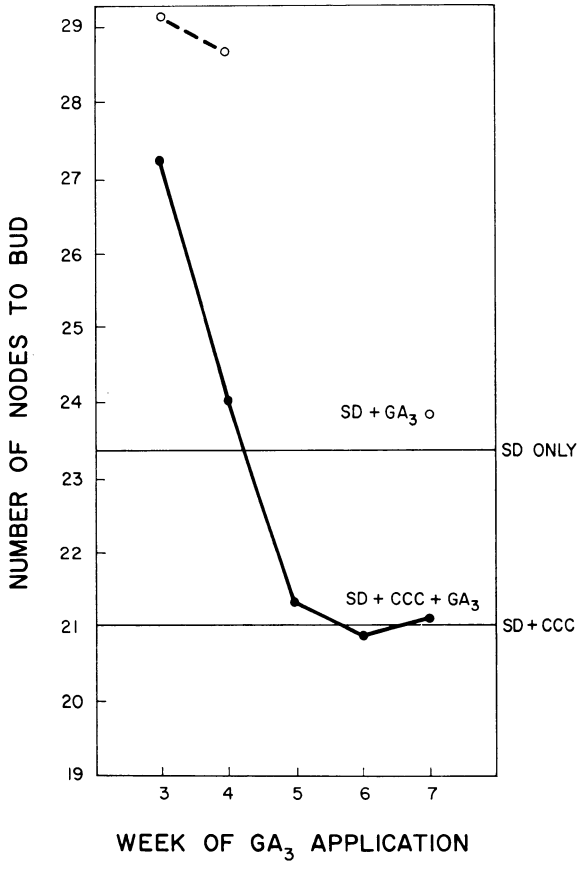

Fig. 3. Effect of CCC (Cycocel) drench and 2 μg gibberellic acid applied to tip of plant on number of nodes to flower bud following varying periods of short days. No flower buds were found on non-CCC-treated plants receiving GA_3 (open circles) at the 5th or 6th weeks of short days (SD).

ered tunica and corpus. In the vegetative meristem the distance from the topmost node to the dome apex ranged from 20 to 50 microns, averaging about 30. The width varied between 60 and 120 microns. Such periclinal divisions as signal the onset of a reproductive status occurred deep on the flanks of the apex in the second and third layers. In several apices there appeared to be a central zone of activity.

During the 4th week of SD, the apex broadened while retaining or increasing its characteristic vegetative height. By the 5th week, active divisions in the third layer had raised humps on the flanks of the dome. It was at this time that the multi-flowered apex could arise (Fig. 1–5). The apex may initiate either a single flower or an inflorescence of 2 to 5 flowers, 3 being most common.

In the 2 sets of experiments in which microscopic examination of short day apices was carried out, the average stages of development were parallel for given periods of exposure. In the second instance, differences in the rate of development were traceable to the use of Cycocel rather than gibberellic acid in that the Cycocel treatments were responsible for more rapid development of the flowers once initiated. The influence of Cycocel was sufficiently great that an application of gibberellic acid after 5 weeks of SD failed to disrupt normal development, and few remnant apices were found. Early application of gibberellic acid resulted in a delay of flower bud initiation under the favorable conditions created by SD and Cycocel. In plants with a longer exposure to SD, gibberellic acid had no effect upon development but may have stimulated activity in latent meristems to give rise to remnant apices. In this respect, a close resemblance is observed to the lateral meristems activated by the low temperature treatments of Struckmeyer and Roberts (15). Alternatively, a delay in development ultimately culminating in a blind or remnant apex may have resulted from warm temperatures at a critical time. The failure of microscopic examination to reveal the early effect of the gibberellic acid application may be due to collecting the sample too soon.

The analogy to the observations of Jorgensen (4) is pronounced. The bud scales do develop first and the floral parts later. These data support his proposal for a two-stage process in azalea flower bud initiation and suggest that further development can be modified by Cycocel or gibberellic acid. Additional studies can be envisioned which would attempt to promote and/or modify flower development through the manipulation of environment and the use of chemicals.

In a practical sense, if a flower bud can be initiated earlier and its development completed sooner, the plant may be ready for cold storage and forcing sooner. Larson and McIntyre (8) have already advocated the use of a short day period in producing uniformly budded azaleas for "year-round flowering".

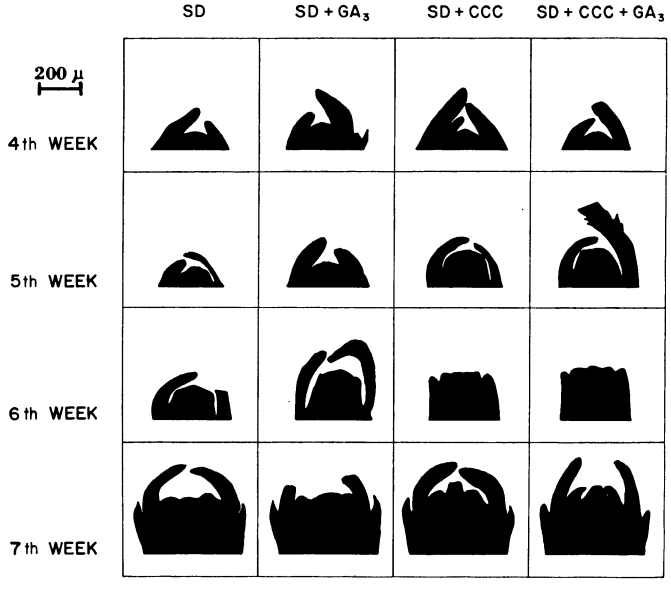

Fig. 4. Stages of development of apices following varying periods of short days (SD) and treatment with GA_3, CCC (Cycocel) and combination of GA_3 and CCC. Bar represents 200 microns.

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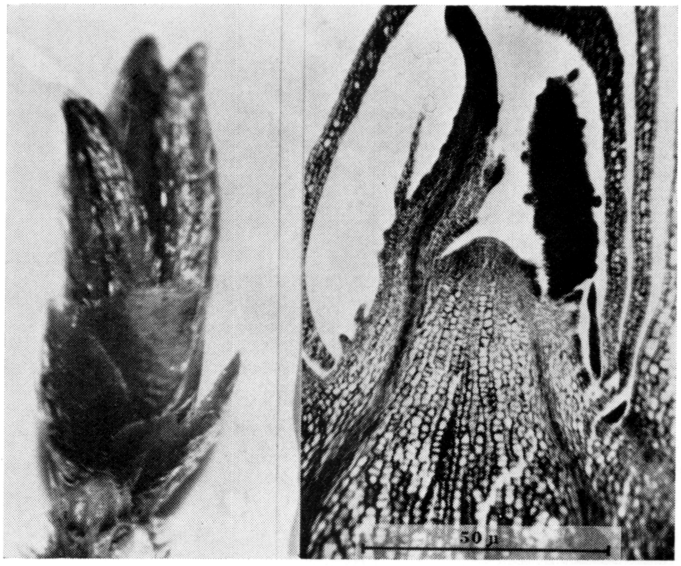

Fig. 5. Left — appearance of typical remnant apex. Note bud scales, new leaves. Right — photomicrograph of remnant apex in which no activity leading to foliar primordia was occurring. Both photographs taken from material receiving 100 short days. Bar represents 50 microns.

Methods of Selecting Tomatoes for Drosophila Resistance[1]

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Abstract. Tomatoes can be screened for resistance to *Drosophila* by using a slit-fruit technique, or by trapping adult flies in jars containing sections of fruit of different varieties. These techniques can be used on a turntable to eliminate light effects, or by randomization in a greenhouse or an enclosed room with uniform light. Significant differences were found, both when *Drosophila* could choose between varieties and when they were restricted to cages containing fruit of only one variety. Due to the large amount of variation encountered when screening tomatoes for *Drosophila* resistance, large numbers of replications are necessary. The variation can be partially accounted for by differences in fruit maturity and differences between plants of a variety or line.

Introduction

Drosophila, or the vinegar fruit fly, remains one of the most serious problems of the tomato-processing industry because of the contamination problem it creates. Insecticides, crack-resistant tomato varieties, cultural practices, prompt processing after harvest, and sanitation practices in field and processing plant all help to reduce the problem, but still more effective control measures are needed.

Mason et al. (1) and Stoner and Mason (3) demonstrated that fruit of tomato varieties differ in their attractiveness to *D. melanogaster*. They used a turntable technique in their studies to eliminate the effect of light differences, but the number of fruit that could be screened at one time was limited by the size of the turntable. Their studies suggested the possibility of breeding *Drosophila*-resistant tomatoes. The resistance they demonstrated is not complete; however, any reduction in the natural field population may be important in reducing the insect contamination problem.

In the following studies we attempted: 1) to develop other techniques that could be easily incorporated into tomato breeding programs, and 2) to define some of the problems encountered in screening for *Drosophila* resistance. The data illustrate the validity of the techniques and the amount of variation that can be expected when testing for *Drosophila* resistance.

Materials and Methods

Since greenhouses are essential for tomato-breeding programs and are usually not fully utilized during the outdoor growing season, we believed that they could perhaps be utilized for *Drosophila* resistance studies. We compared varieties with known levels of resistance in a 10 × 12 ft glass greenhouse that was shaded on the outside with a shading compound, and equipped with automatically controlled heating, cooling, and ventilation equipment. The 2 benches in the greenhouse were covered with 3-ft high cheesecloth cages to confine the insects (Fig. 1). The 4 layers of cheesecloth also reduced the light intensity in the cages, thereby making conditions more conducive to *Drosophila* activity during the bright daylight hours.

Fig. 1. Greenhouse bench covered by cheesecloth cage used in screening tomato varieties for resistance to *Drosophila melanogaster*.

Sound, red-ripe fruit of the varieties 'Campbell 16', 'Chico Grande', 'Manalucie', 'H1350', 'Bouncer', and 'VF-145B' were harvested from field plots in 1967 for the tests. The fruits were washed and labeled with a felt-tip marking pen. A slit 1½ inches in length was made in the outer pericarp of each fruit with a razor blade as previously described (1, 3). Care was taken not to expose any of the locular material. We placed the slit fruit directly on the pea gravel covering the greenhouse bench. We spaced 40 fruits approximately 9 × 12 inches apart on each of the 11 × 3 foot benches. Fruits were grouped together in replications to facilitate statistical analysis.

In each cage we released approximately 1000 unsexed *Drosophila* of varying ages with the freshly slit tomatoes at 4 PM and allowed them to remain overnight. At 8 AM the following day we removed the fruit, and counted the number of eggs laid in the slits with the aid of binocular microscopes. Temperature was maintained between 70° and 80° F during the tests.

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