upland cotton plants in jars of soil two months after planting were subjected to low daylight intensity of around 50-foot candles by keeping the plants in a laboratory room for a single 4-day period. Five weeks later, these plants had on the average only 5.4 good-sized bolls per plant and each plant had shed an average of 30 squares and young bolls. At the same time, the control plants had 21.2 bolls per plant with only 17.5 squares and bolls shed. This represents a reduction of 75 per cent. in the number of mature bolls, associated with the low light intensity treatment. Most of those fruiting forms were shed during the third and fourth days while the plants were indoors. Similar increases in rates of shedding have been obtained by shading cotton plants in the greenhouse and in the field with black cloth. Such shading has reduced the direct sunlight intensity at midday from around 12,000 foot candles to 300–1,000 foot candles. In connection with these studies at College Station, light intensities of less than 1,000 foot candles have been measured at noon on cloudy days during the growing season for cotton.

In addition to the abscission of young bolls just after flowering, at which stage most of the shedding in the field takes place, these low light intensities for as short a period as two days caused the shedding of many cotton squares. These effects are equal to results obtained by prolonged severe wilting, which is recognized as providing a strong stimulus for shedding. Presumably, the accelerated rates of shedding following shading were associated with interrupted photosynthetic activity and low carbohydrate nutrition. Increased vegetative growth followed the shedding of buds and bolls.

Evidence of varietal differences in the response of cotton to conditions of low light intensity has been obtained.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

USE OF ENZYMES TO IMPROVE CYTOLOGICAL TECHNIQUES

DURING the last few years cytological studies on a tetraploid form of *Lilium longiflorum* Thunb. have been in progress at the Plant Industry Station, Beltsville, Md. It was found very difficult to analyze chromosome associations during the first meiotic metaphase because they tended to form clumps. To overcome this difficulty a number of treatments designed to digest the cell wall and destroy the gel properties of the cytoplasm were used. If this could be accomplished without injury to the paired chromosomes, well-spread figures could be secured in which analysis of polyvalents would be possible. Among the treatments herein described, several with enzymes gave definitely beneficial results.

The plants used for these experiments were a tetraploid clon of *Lilium longiflorum,* several diploid forms of the same species and a triploid clon of *L. tigrinum* Ker Gawler. Buds were collected in which most of the pollen mother cells were at the first meiotic metaphase. The perianth segments were removed and the buds then fixed in a solution composed of 3 parts absolute alcohol to 1 part glacial acetic acid. After overnight immersion the buds were run through 95 per cent. alcohol to 70 per cent., where they were held until wanted.

The anthers, as needed, were cut into small pieces, about 1 to 2 mm long, thus making it possible to give a series of different treatments to portions of the same anther. All slides were stained with aceto-carmine.

In the first series of tests only inorganic chemical treatments were used. These were: a cellulose solvent, copper oxide ammonia; a pectin solvent, 1 per cent. ammonium oxalate; and a suberin solvent, 10 per cent. alcoholic potassium hydroxide. All the slides from the treatments were poor in comparison with the untreated.

These unsatisfactory results led us to try a biological approach, and 1 per cent. solutions of three enzyme preparations were tried: Malt diastase; Polyzime "P," which is used in desizing fabrics; and Clarase,[1] a proprietary enzyme complex. For these treatments the fixed anthers were first run through lower strengths of alcohol to water and thoroughly washed. Use of the first two preparations produced little if any benefit in comparison with the check slide which in this instance was made following the washing in water. The Clarase treatment, however, brought about a partial softening of the cell walls and digestion of the cytoplasm so that in most cells the paired chromosomes were well spread out, thus making it possible to determine the types of chromosome associations present. Several periods of exposure were tried and as little as 15 to 20 minutes was about as effective as several hours.

Since the diastatic enzymes were ineffective it was thought that possibly the beneficial effects of Clarase

[1] Manufactured by the Takamine Laboratory, Clifton, N. J.

might be caused by a proteolytic enzyme. Accordingly, 1 per cent. solutions of pancreatin (supplied by the makers of Clarase), and of papain were used. Both preparations were ineffective, and actually caused chromosome clumping. The results were very poor when compared with the checks.

Recent studies by Greathouse, Klemme and Barker[2] on the deterioration of cellulose by fungi, suggested that certain of these organisms might be of value in the present problem. Fortunately we were able to secure cultures of *Aspergillus niger* Van Tieghem, *Chaetomium globosum* Kunze, and a species of *Metarrhizium* through the courtesy of G. A. Greathouse of this Bureau. Single flask cultures were extracted 10 to 15 days after inoculation by grinding the contents of the flask with quartz sand in a mortar containing 10 ml of a sodium acetate buffer, pH 5.0. The supernatant liquid was tested on anther sections as previously described for the enzymes. This series of treatments also included a 5 per cent. solution of Clarase in sodium acetate at pH 5.0. All treatments gave beneficial results and produced slides superior to the checks. The cell walls of most pollen mother cells were softened and the cytoplasm partially digested. As a result of these changes the cells were easily flattened and the chromosomes spread. Tests were also made in which the freshly prepared Clarase solution and fungus extracts were heated to boiling prior to use. The slides resulting from material treated in the boiled solutions were definitely inferior to those from the unheated solutions, which indicates that active enzymes were necessary to produce the beneficial effect.

The results herein briefly reported are preliminary to further work under way with other treatments and modes of application. There are many aspects of the problem needing further investigation. For instance, it is not possible as yet to measure the concentration of the fungus extracts used, or to identify the enzyme or enzyme complex that is effective. So far the method has been used on only one species other than Lilium. Dr. A. E. Clarke of this division has used Clarase on the pollen mother cells of an amphidiploid Allium with beneficial results. Some observations have also been made on smears of treated Lilium root tips. These have shown that fungus extracts affect the middle lamella so that the cells separate readily. Further investigations of pre-treated root-tip smears are under way.

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2 G. A. Greathouse, D. E. Klemme and H. D. Barker, *Ind. and Eng. Chem. Anal. Ed.*, 14: 614–620, 1942.

A NYLON BLOOD AND PLASMA FILTER

FOR the last nine months we ran laboratory tests on Nylon blood and plasma filters. Our observations and tests fully coincided with those made by Dr. S. Brandt Rose, *et al.*, as reported in SCIENCE (Vol. 98, No. 2534).

However, we found the sewing of these tiny filters rather cumbersome, and noted that small, but objectionable quantities of Nylon fibers would be entrained in the filtrate.

We took advantage of the thermo-plastic qualities of Nylon and welded the seams rather than sewing them. This method is much faster than sewing and eliminates the shedding of Nylon fibers. Furthermore, the filters can be fabricated with a cone point, and thereby can be utilized for making the drip count.

The following method was found to be satisfactory in making the filters: A double layer of finely textured Nylon cloth was placed on a flat metallic surface. A sheet metal template was made for the filters, and this was placed firmly over the Nylon cloth. The outline of the template was then traced with an electrically heated metal stylus. The stylus of an electric wood-burning set was used for this with excellent results. Flat, colorless, flexible seams were obtained after only a small amount of practice. These seams were tested carefully and were found to be safer for use in transfusion filters than sewn ones.

Prior to using the electric cutting method, experiments with flame cutting were conducted, but the results were found to be too unreliable.

The filters were welded directly to the glass delivery tube of the transfusion assembly.

Some chemical tests were made on the Nylon material. It was found to be soluble in mineral acids and was destroyed by alkalies. It withstood treatment with hydrogen peroxide and solutions of sodium citrate and sodium citrate-dextrose.

The apparatus was developed in the laboratory only, and was not used in giving transfusions to patients.

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