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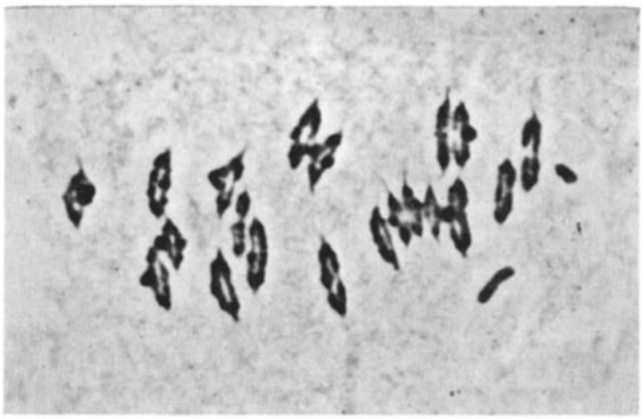

Fig. 3. Metaphase I of meiosis in Line 1 × ditelocentric 7D showing 20 bivalents, 1 normal univalent (below), and 1 telocentric univalent (above). (× ca. 1100).

7D. If the *Agropyron* chromosome was substituted for the marked chromosome, then the telocentric chromosome(s) would be unpaired in the F_1. If it were not substituted for that chromosome, then in the F_1 the telocentric(s) would pair with a normal wheat chromosome, and two entire univalents would be present. In the F_1's of the cross to ditelo 7A and double ditelo 7B the telocentric(s) was paired with an entire chromosome, but the F_1 of the cross to ditelo 7D possessed 20 bivalents, 1 entire univalent and 1 telocentric univalent ($20'' + 1' + 1^t$) as in Fig. 3.

The F_1 of Line 2 × ditelo 7D was also observed to possess $20'' + 1' + 1^t$. The *Agropyron* chromosome is, therefore, substituted for chromosome 7D in each line. A close examination of the entire univalent in these F_1 plants, that is, the *Agropyron* chromosome, revealed the same subterminal constrictions noted earlier.

Since both lines possess the same *Agropyron* chromosome in place of the same wheat chromosome, a cross between the two should yield an F_1 with $21''$. This was observed in all except one of six F_1 plants examined. The one exception, which possessed $20'' + 2'$, must clearly have resulted from an atypical gamete, viz., a normal wheat gamete.

Both substitution lines are vigorous and fully fertile, reflecting genetic balance. Thus the *Agropyron elongatum* chromosome bearing this wheat leaf rust resistance may be considered a member of homoeologus group No. 7 as described in wheat. This relationship was recently suggested by Sharma & Knott (2) from transmission studies of a translocation chromosome involving chromosome 7D and this *Agropyron* chromosome.

Although spontaneous wheat-alien substitution lines are very valuable for purposes of determining wheat-alien chromosome homoeologies, unfortunately the known number of such lines is quite small. However, production of substitution lines using wheat monosomics should enable these homoeologies to be determined.

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[4] Sharma, D., and D. R. Knott. 1966. The transfer of leaf-rust resistance from *Agropyron* to *Triticum* by irradiation. Can. J. Gen. Cytol., 8:137-143.

GREENHOUSE TECHNIQUE FOR STUDYING FUSARIUM WILT IN COTTON[1]

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THE FUSARIUM WILT, *Fusarium oxyporum* f. *vasinfectum,* is one of the more important disease problems in cotton, *Gossypium hirsutum,* for the southeastern area of the United States. One would be greatly aided if a screening technique could be developed to study the inheritance of resistance to fusarium wilt in the greenhouse. To date no adequately sensitive greenhouse technique has been developed for critically studying the inheritance of this disease.

Most of the prior work for studying the inheritance of fusarium wilt has been conducted under field conditions. Reasonably effective techniques are in use for field ratings of replicated progeny at Tallassee, Alabama (2). Such field areas, however, are very difficult to locate and develop, and even at best, the breeder must work with progeny units. Field studies probably give a fair estimate of entry means. However, it is doubtful that they indicate differences between plants within entries. A previous greenhouse method of seedling testing was described in some detail (4). This technique required some length of time to record resistance following the seeding, plus it only gave entry means. During the summer of 1962 it was proposed that a greenhouse technique be developed at North Carolina State University to critically screen for the resistance of fusarium wilt in cotton. The objectives of this study were to develop a simple technique and one reliable enough to make individual phenotypic selections for resistance in a segregating population. Since 1962 further greenhouse techniques, called the washed root method, were described by Perry (1) and Wickens (3). These techniques involved the pouring of an aliquot of inoculum around the roots of 7-day-old cotton seedlings. With these techniques, no attempt was made to control the concentration of conidia beyond a measured amount of inoculum per plant. Thus, it is difficult to repeat similar reactions even within the same variety or line of cotton.

The greenhouse procedure used at North Carolina State University was a very simple and reliable technique for screening fusarium wilt in cotton. The procedure was as follows: Cotton was seeded in a medium of 2 parts of sterilized soil to 1 part of sand. The flats were placed in a well lighted greenhouse, with an average temperature of 75 F. Fusarium inoculum was started the same day as the cotton was seeded in a modified Richards' solution. The ingredients were mixed in the following order: distilled water, 1,000 ml; potassium nitrate, 10 g; potassium monobasic phosphate, 5 g; magnesium sulfate, 2.5 g, ferric chloride, 0.02 g, and sucrose, 20 g. It was then plugged and autoclaved at 1.05 kg/cm^2 (15 psi) for 20 minutes.

One week later the cotton seedlings were pulled from the planting medium and inoculated in the

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Table 1. Percentages of fusarium wilt infection in two lines of cotton with different conidia concentrations 4 weeks after inoculation.

	Conidia concentration per ml			
	"Washed root technique"			
Line	No control	500,000	1,000,000	2,000,000
Auburn 56-1	94.0	33.8	84.0	95.6
M8-4	98.2	95.0	99.8	100.0

following manner. First a concentration of 500,000 conidia per ml was made. After the seedlings were pulled from the planting medium, they were suspended in the inoculum for 5 minutes and then transplanted into 5.7-cm (2¼-inch) peat moss pots. The transplanting medium was 3 parts of sterilized soil to 1 part of sand. Fertilizer was blended into the transplanting medium at the prescribed field rates. The inoculated transplants were then placed in a greenhouse with temperatures of 85 to 95 F. The pots were then lightly watered. A paper shade was placed over them for 36 to 48 hours to prevent wilting and dying. After being placed in greenhouses at temperatures of 85 to 95 F., the fusarium wilt symptoms can be scored from 4 to 5 weeks later. They were scored as 0 = healthy, 25 = stunted growth and internal symptoms, 50 = outward wilt symptoms, and 100 = dead plants. Selected resistant seedlings can be transplanted and grown to maturity at which time one may thus make internal observations for wilt. If wilt occurs in the selected plant, the progeny from such a plant may be discarded or put into a new classified group of resistance. This allows one to conduct a breeding program as plants can be crossed or selfed.

Two lines of cotton were used in developing a greenhouse technique for resistance to fusarium wilt. Auburn 56-1 was considered a fairly resistant line of cotton, while M8-4 was considered a fairly susceptible line. Several techniques were attempted prior to the reports of Perry (1) and Wickens (3) and one was quite similar to their washed root method. Data were collected comparing no control of conidia concentration with the control of conidia concentration (Table 1). Using the washed root technique one could not control the fusarium concentration; therefore, one could not differentiate resistance between different lines of cotton.

In summary, this technique is simple and allows one to score individual plants in a segregating population. Selected resistant seedlings can be transplanted and grown to maturity. It allows one to conduct a breeding program as plants can be crossed or selfed. The time involved from seeding date to scoring date only involves six weeks. Thus, one can screen large populations in short periods of time in the greenhouse.

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SELF- AND CROSS-INCOMPATIBILITY AND GENERAL SEED SETTING STUDIES WITH ZIGZAG CLOVER, *Trifolium medium* L.[1]

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ABSTRACT

Forty-two noninbred zigzag clover plants were evaluated for self-compatibility and all were highly self-incompatible. Variation for cross-compatibility of F_1 plants from self-incompatible parents was continuous. The results indicate that the inheritance of self- and cross-incompatibility in zigzag clover is complex. A highly significant difference was found among 64 plants under field conditions for open-pollination seed set. Damage due to the clover seed chalcid reduced seed set considerably.

ZIGZAG clover is highly self-incompatible, a high polyploid, and a poor seed producer. Chromosome numbers, summarized for the genus *Trifolium* by Britten (1), indicate that the somatic number for zigzag clover ranges from 78 to 84. Robertson and Armstrong (4) found that the chromosomes tended to pair as bivalents at meiosis. Seed failure was reported by Keim (2) to be due to genetic factors, to clover seed chalcid damage, and to preference of pollinators for other plants. Robertson and Armstrong (4) also found seed yield to be genetically controlled.

In a self-incompatible species it is desirable to learn something about the genetics of self- and cross-incompatibility prior to the initiation of a systematic improvement program. Therefore, the purpose of this study was to obtain such information plus information concerning the general seed-setting ability of the species under open-pollination conditions.

Materials and Methods

The vegetative materials used in this study came from the nurseries of Dr. E. A. Hollowell (retired), U. S. Department of Agriculture, Beltsville, Md., and Dr. A. Gershoy (retired), University of Vermont. Several seed lots were provided by the New Crops Research Branch, USDA. Open-pollination progenies from the above plants were produced in Colorado and were included in this study.

Self-incompatibility studies were made in the field. Small cloth bags were placed over the heads prior to floret opening to exclude pollinating insects. At the appropriate time, the bag was removed, florets were manipulated with a small piece of folded paper trimmed to a point on one end, and the bag was replaced. It was generally necessary to repeat this procedure several times because of the time required for maturation of all florets of an individual head. Old florets on the first day of manipulation and immature florets remaining on the last day of manipulation were removed. Generally, florets from 4 heads or an average of 150 florets per plant were manipulated. The hands of the operator were washed with 95% ethyl alcohol between manipulations.

Five noninbred plants, selected on the basis of superior vigor, were used in the cross-compatibility study. However, only the F_1 progenies of the 85 × 9-23 and 85 × 9 HF crosses were used for further study. Plants 85 and 9 HF came from Dr. Gershoy's nursery and 9-23 is a plant introduction. There were 10 F_1 plants per progeny. Each F_1 plant was self-pollinated and crossed reciprocally with its nine F_1 sibs and with both parents. Some of the crosses were made in a screened greenhouse but most of them were made in the field because of relatively poor flowering in the greenhouse. There was considerable variation

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