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1) Sterility mutants in soybeans.

In 1975 we selected green, partially sterile plants in farmers' fields when the normal plants had dropped their leaves and were ready for harvest. Seeds were harvested from the off-type plants and planted in the greenhouse (1975-76). The resulting plants which we called " F_1 's" were normal. Their progenies (" F_2 's") were grown in 1976 in the field and " F_3 's" were grown in 1977. Because of lack of greenhouse space, 'Calland' was not grown until a year later and so is one generation behind 'Wayne' and 'Woodworth'.

Segregation in F_2 and F_3 generations indicates that all three mutants are male steriles caused by single recessive genes (Table 1), i.e., \underline{Ms} is male fertile and $\underline{ms} \underline{ms}$ is male sterile. In Wayne, 38 F_3 rows segregated and 14 bred true, which fits a 2:1 ratio ($\chi^2 = 0.96$, $P = .33$). In Woodworth, the observed ratio was 50 to 24 which also fits a 2:1 ratio quite well ($\chi^2 = .03$, $P = .86$). All three male steriles have empty anthers but otherwise apparently normal flowers. Distributions of one-, two-, three- and four-seeded pods on male sterile segregates indicate rather high female fertility (Table 2). Mean number of seeds per pod was approximately two, with Woodworth being somewhat higher than Wayne. This cultivar may have a natural tendency for more four-seeded pods than Wayne has. It is interesting that two-seeded pods are lower in frequency than both three-seeded and one-seeded pods (with one exception). We have no explanation to offer at this time.

We do not yet know what loci are involved. Crosses of the type $\underline{ms}_1 \underline{ms}_1 \times \underline{Ms} \underline{ms}$ were made in the field this summer between Wayne, Woodworth, 'Northrup-King', 'Rampage', and $\underline{ms}_2 \underline{ms}_2$ stocks. Northrup-King and Rampage stocks were obtained from R. G. Palmer and $\underline{ms}_2 \underline{ms}_2$ from R. L. Bernard. One F_1 from $\underline{ms} \underline{ms}$ Rampage \times $\underline{Ms} \underline{ms}$ Woodworth and one F_1 from $\underline{ms} \underline{ms}$ Northrup-King \times $\underline{Ms} \underline{ms}$ Wayne were male sterile. Thus we probably have not discovered a new \underline{ms} locus. Our observations, however, suggest that if one desires a male sterile form in a particular variety he might seriously consider searching for a mutant rather than use a backcross program with known male steriles. Large populations can be screened effectively at harvest time. Male steriles can probably be separated from other sterile types by the presence of a high frequency of two-, three-, or even four-seeded pods. Sterile types with mostly one-seeded pods

Table 1
Distribution of male fertile and male sterile segregates in
"F₂" and "F₃" generations in three soybean cultivars

Cultivar	Generation	Male fertile	Male sterile	n	$\chi^2(3:1)$	P
Calland	F ₂	97	37	134	0.49	0.48
Wayne	F ₂	35	15	50	0.67	0.41
	F ₃	356	140	496 [†]	2.75	0.10
Woodworth	F ₂	66	26	92	2.09	0.15
	F ₃	459	162	621 [†]	0.39	0.53

[†]From 20 segregating progenies. Progenies were homogeneous, χ^2 's being 18.34 (P = .50) and 18.47 (P = .49) for Wayne and Woodworth respectively.

Table 2
Number of seeds per pod on male sterile segregates
in Wayne and Woodworth soybeans

Cultivar	Generation	Number of seeds per pod				Pod no.	\bar{X}/pod
		1	2	3	4		
Wayne	F ₂	36	41	43	--	120	1.94
Woodworth	F ₂	133	109	158	7	407	2.10
Wayne	F ₃	120	92	115	1	328	1.99
Woodworth	F ₃	101	90	129	16	336	2.18

are probably female sterile also.

In 1976, we crossed noduleless 'Clark' by a partial sterile (received several years ago from C. R. Weber at Ames, Iowa). This type tends to have multiple pistils but expressivity varies so that 1 to 5 pistils occur in different flowers on the same plant. Attempts to cross the multipistillate line as female were unsuccessful. One cross was obtained from the line used as a male. A small F₂ population of 63 plants was observed in the field in 1977,

giving 56 fertile to 7 partially sterile. Too few partial steriles occurred to fit a 3:1 ratio. This may have resulted from some genetically partial steriles having such a low percentage of multipistillate flowers that they were classified as normal. Flower color and nodulation behavior also segregate or should segregate in the same material. Nodulation data has not yet been obtained. Flower color showed no association with sterility: fertile, purple-42; fertile, white-14; partially sterile, purple-6; partially sterile, white-1. An F_3 progeny test will be conducted in 1978.

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2) Screening the USDA soybean germplasm collection for Kunitz trypsin inhibitor variants.*

The trypsin inhibitors as a group form one of the major anti-nutritional factors in soybean [*Glycine max* (L.) Merrill] seed. Several different trypsin inhibitors have been reported to be present in soybeans. However, much of the soybean trypsin inhibitor activity is thought to be due to the protein SBTI-A₂ which is generally known as the Kunitz trypsin inhibitor.

Seed from the USDA soybean germplasm collection have been screened using polyacrylamide gel electrophoresis for the presence or absence of electrophoretic forms of SBTI-A₂. Thus far, four electrophoretic forms have been discovered (Hymowitz and Hadley, 1972; Orf and Hymowitz, 1977a; Orf *et al.*, 1977; and Singh *et al.*, 1969). Three of the forms designated Ti^1 , Ti^2 and Ti^3 are electrophoretically distinguishable from one another by their different Rf values of 0.79, 0.75 and 0.83, respectively (Rf = mobility relative to the dye front in a 10% polyacrylamide gel anodic system using a pH 8.3 Tris-glycine

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buffer). The three forms are controlled by a codominant multiple allelic system at a single locus. The fourth form does not exhibit a SBTI-A₂ protein band in the gels. The lack of a protein band is inherited as a recessive allele. The gene for the lack of the SBTI-A₂ protein band has been designated ti.

The summary of the screening data is presented in Table 1. Of the 3038 soybean accessions tested, 2698 accessions, or 88.8%, had the Ti¹ allele. The Ti², Ti³ and ti alleles were found in 10.9, 0.3 and 0.06% of the population studied. Of the 359 accessions of Glycine soja tested, 337 accessions had Ti¹ and 24 accessions had Ti². Two accessions of Glycine soja, PI 378.694 and PI 407.258, were mixtures containing both Ti¹ and Ti² seed.

Sources for the Ti² allele within the Named Variety Collection are 'Aoda', 'Goku', 'Hakote', 'Jefferson', 'Jogun', 'Jogun (Ames)', 'Miller 67',

Table 1
Distribution of Kunitz trypsin inhibitor variants
in the USDA soybean germplasm collection*

Collection	<u>Ti</u> ¹	<u>Ti</u> ²	<u>Ti</u> ³	<u>ti</u>	Total
Asia: Japan	284	187	6		477
Korea	366	48	1	2	417
China	794	9			803
Remainder	345	37	1		383
Europe	405	29			434
Africa	56				56
Other: Named Varieties	320	15			335
Type Collection	89	5			94
' <u>G. gracilis</u> '	39				39
<u>G. soja</u> [†]	337	24			361

*Data taken in part from Clark et al., 1970; Hymowitz et al., 1971; Kaizuma and Hymowitz, 1978; Orf, 1976; and Skorupska and Hymowitz, 1978.

[†]Two accessions PI 378.694 and PI 407.258 were mixtures containing both Ti¹ and Ti² seed.

'Polysoy', 'Rokusun', 'Sato-3', 'Sousei', 'Toku', 'Tokyo', 'Tortoise Egg' and 'Wolverine'. Sources for the Ti^2 allele within the Type Collection are T69, T136, T141, T216 and T245.

Sources for the Ti^3 allele are PI 86.084, PI 196.172, PI 205.384, PI 227.557, PI 246.367, PI 304.217, PI 342.002 and PI 360.844. Sources for the ti allele are PI 157.440 and PI 196.168.

The screening and inheritance study phases of the project essentially are completed. However, the feeding trial phase of the project will increase in importance (Bajjalieh et al., 1977; Yen et al., 1971, 1973 and 1974). At present, feeding trials have been initiated to compare the nutritive value of raw defatted soybean meal from an accession without the Kunitz trypsin inhibitor with accessions containing the Kunitz trypsin inhibitor. In addition, linkage tests are being carried out to determine whether the Kunitz trypsin inhibitor is linked to certain chemical components of seed or certain morphological characters of plants (Orf and Hymowitz, 1977b).

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3) Soybean linkage tests between two seed proteins and other characters.*

F_2 linkage results between Ti and W_1 , Dt_1 and Ep are shown in Table 1. F_2 linkage results between Sp_1 and W_1 , Dt_1 , Ep and Le are shown in Table 2. In all cases the Chi-square values were calculated using contingency tables. Since all the probabilities are greater than .05, none of the gene pairs considered appear to be linked. Previously we have reported on the independent inheritance between Ti and Sp_1 (Orf and Hymowitz, 1977).

The Ti and Sp_1 F_2 genotypes were determined using previously described procedures (Hymowitz and Hadley, 1972; Orf and Hymowitz, 1976). The Ep phenotype was determined using the test described by Buttery and Buzzell (1968). The Le phenotype (Le controls a seed lectin; see Pull et al., pages 66-70 of this issue) was determined using polyacrylamide gel electrophoresis as described by Orf et al. (n.d.).

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Table 1

Observed numbers of individuals in the respective phenotypic classes
for F_2 linkage tests between \underline{Ti} and $\underline{W_1}$, $\underline{Dt_1}$ and \underline{Ep} from the cross
'Jefferson' ($\underline{Ti}^2 \underline{w_1} \underline{dt_1} \underline{ep}$) x 'Wilson' ($\underline{Ti}^1 \underline{W_1} \underline{Dt_1} \underline{Ep}$)

Phenotypes	$Ti^1 Ti^1$	$Ti^1 Ti^2$	$Ti^2 Ti^2$	$\chi^2 (2 \text{ df})$	P
W_1 —	42	82	53	1.97	0.37
$w_1 w_1$	9	25	21		
Dt_1 —	34	83	50	3.11	0.21
$dt_1 dt_1$	17	24	24		
Ep —	40	80	55	0.31	0.86
$ep ep$	11	27	19		

Table 2

Observed numbers of individuals in the respective phenotypic classes
for F_2 linkage tests between $\underline{Sp_1}$ and $\underline{W_1}$, $\underline{Dt_1}$, \underline{Ep} and \underline{Le} from
the crosses Jefferson ($\underline{Sp_1}^a \underline{w_1} \underline{dt_1} \underline{ep}$) x Wilson ($\underline{Sp_1}^b \underline{W_1} \underline{Dt_1} \underline{Ep}$)
and 'Amsoy' ($\underline{Sp_1}^a \underline{Le}$) x T102 ($\underline{Sp_1}^b \underline{le}$)

Phenotypes	$Sp_1^a Sp_1^a$	$Sp_1^a Sp_1^b$	$Sp_1^b Sp_1^b$	$\chi^2 (2 \text{ df})$	P
W_1 —	44	84	49	0.97	0.62
$w_1 w_1$	11	31	13		
Dt_1 —	42	83	42	1.06	0.59
$dt_1 dt_1$	13	32	20		
Ep —	40	91	44	1.77	0.41
$ep ep$	15	24	18		
Le —	20	49	23	1.68	0.43
$le le$	8	11	9		

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1) Velvetbean caterpillar resistance in soybean selections from crosses involving Mexican bean beetle resistant plants.

Segregating populations arising from crosses involving two sources of Mexican bean beetle (*Epilachna varivestis* Mulsant) resistance were screened and selected for resistance to velvetbean caterpillar (*Anticarsia gemmatilis* Hubner) in Guaiba, Rio Grande do Sul, Brazil and Isabela, Puerto Rico. The Mexican bean beetle resistant cultivars used in the crosses were PI 171.451 and PI 229.358 (Van Duyn *et al.*, 1971). The materials tested came from two sources: Dr. R. L. Bernard of the USDA Regional Soybean Laboratory, Urbana, IL and Dr. S. G. Turnipseed of Clemson University, Blackville, SC.

The Illinois material consisted of remnant F_2 seed of seven crosses of several Midwest varieties and PI 171.451 and PI 229.358. In late 1972, these F_2 populations were planted at Guaiba in single rows bordered on one side by the variety 'Clark 63' and on the other side by the variety 'Davis'. Plants which exhibited substantially less damage than either Clark 63 or Davis were tagged during the growing season and individually harvested. A total of