Cucurbit Genetics Cooperative

Report No. 7

July 1984

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Introduction

Resolution and Notes of Organization Meeting

Resolution and notes of organization meeting, October 28, 1976, Denver Hilton, Denver, Colorado, U.S.A. The following resolution was adopted by research workers interested in organizing a Cucurbit Genetics Cooperative: The Cucurbit Genetics Cooperative is organized to develop and advance the genetics of economically important cucurbits.

Membership to this Cooperative is voluntary and open to workers who have an interest in Cucurbit Genetics (an invitation to participate is extended to all Horticulturalists, Entomologists, Plant Pathologists, Geneticists, and others with an interest in Cucurbits.

Reports of the Cooperative will be issued on an annual basis. The reports will include articles submitted by members and for the use of the members of the Cucurbit Genetics Cooperative. None of the information in the annual report may be used in publications without the consent of the respective authors for a period of five years. After five years the information may be used in publications without the consent of the authors.

Dues for the Cucurbit Genetics Cooperative

Further, dues for the Cucurbit Genetics Cooperative dues for the Cucurbit Genetics Cooperative (CGC) will be $2.50* per year and will be used to defray cost of preparation and mailing of the annual report. Members from outside the U.S.A. are encouraged to pay dues in at least two-year increments because of bank charges incurred for clearing checks. Only postal money orders or checks drawn on U.S. banks are acceptable. The annual report will include four sections: Research Notes, Stocks and Germplasm desired or for Exchange, Membership Directory, and Financial Statement. Other sections will be added in future reports as desired, i.e. gene lists, linkage groups, etc.

In accordance with the above resolution, we requested that an invitation to join the CGC be published in the following:

- Agronomy News
- Euphytica
- HortScience
- Journal of Economic Entomology
- Journal of Heredity
- Phytopath News

We are most pleased to acknowledge the assistance of the editors of these publications.

*Dues structure and biennial membership, effective 1982 and 1983.

Subscriber	Dues (biennial membership)	Back issue fee
U.S.	$ 6.00	$ 3.50
Libraries	10.00	6.00
Foreign	10.00	6.00

Report of Seventh Annual Meeting

T. C. Wehner

The seventh annual meeting of the Cucurbit Genetics Cooperative was held in conjunction with the American Society for

Horticultural Science on October 19, 1983 in McAllen, Texas. There were 30 in attendance. The meeting was chaired by R. L. Lower. He reported on publication of CGC No. 6 and the financial status of CGC. The cost of publication and mailing for CGC Report No. 6 was $581.98 which left a balance of $1148.94. The membership was 157. CGC No. 6 was the largest report thus far in terms of number of contributions and pages.

Considerable discussion centered around the status of germplasm research and funding in the USA. Al Stoner (USDA) supplied general information on the status of present legislation in this area and indicated that USDA-ARS funding might be available for germplasm evaluation and improvement of cucurbits.

Todd Wehner moved that the chairman resubmit a request to the USDA to form a Commodity Advisory Committee (CAC) for cucurbits. The motion was seconded and passed.

The chair announced that a EUCARPIA meeting on vine crops would be held in Plovdiv, Bulgaria on July 4-7, 1984. There being no further business, the meeting was adjourned.

The **1984 Annual Meeting** of CGC will be held in Vancouver, Canada during the American Society for Horticultural Science meetings in August, 1984. Consult local program for exact time and place.

Note from the Chairman

Effective July 1, 1884 J. D. McCreight has assumed chairmanship of the Coordinating Committee of the CGC. It has been a pleasure to have served as Chair of the CGC in its formative years. I look forward with confidence to the continued growth of CGC. Thank you for this opportunity.

R. L. Lower

All future CGC correspondence should be sent to the attention of:

- Dr. James D. McCreight
- USDA, P.O. Box 5098
- Salinas, CA 93915
- U.S.A.

Comments From The Coordinating Committee

The call for papers for the 1985 report will go out in November, 1984, and they should be submitted to the Coordinating Committee by January 31, 1985. Hopefully, the eighth report will be published by June, 1985.

The Coordinating Committee acknowledges the service of the Nominating Committee: Paul Thompson-Chair, Ed Cox, and Claude Thomas for nominating Gary Elmstrom as replacement for J. D. McCreight representing muskmelons. Also, Thomas W. Whitaker has agreed to replace Chas A. John on the Gene List Committee.

We are eager to hear from the membership regarding the future direction of the CGC. It is a pleasure to acknowledge the assistance of Patricia Coan and Sue Higgins who were responsible for the typing, proofing, and duplicating of this report. We express our sincere appreciation.

Coordinating Committee

- W. R. Henderson (watermelon)
- J. A. Juvik (*Cucurbita* spp.)
- J. D. McCreight (muskmelon)
- R. W. Robinson (other genera)
- T. C. Wehner (cucumber)
- R. L. Lower, Chairman

Erratum

CGC Report No. 6:71-

- line 15 should read *possibility* not *possibly*
- line 18 should read *seeding* not *seedling*
- Literature Cited number 1
    - line 1 should read *yeraqot* not *yeragot*
    - line 3 should read *Hassadeh* not *Hassedeh*

Effects of the Determinate Locus on Number of Lateral Branches in Crosses between Four Cucumber Lines and *Cucumis sativus* var. *hardwickii* L.

D.E. Delaney and R.L. Lower

University of Wisconsin, Madison, WI 53706

The shorter vine and concentrated fruit set of determinate pickling cucumbers are well suited to once-over mechanical harvesting (3). However, determinate and short internode dwarf types have fewer and shorter lateral branches than the normal indeterminate vine type (3,4). A wild relative of the cucumber, *Cucumis sativus* var. *hardwickii*, has both multiple lateral branching and sequential fruiting characters, and has shown potential for increasing fruit yields (1,2). The objective of this study was to examine the problem of low lateral branch number in determinate cucumbers, and determine if this situation could be improved by the incorporation of exotic *hardwickii* germplasm.

Four determinate cucumber lines, 'Spacemaster', NCSU M21, NCSU M27 and USDA 1909 (P_2, P_3, P_4 and P_5, respectively) and a *Cucumis sativus* var. *hardwickii* line, LJ 90430 (P_1), were utilized in this study. The determinate parents varied in both vine length and fruit type, thus providing an opportunity to examine the effects of these characters on number of lateral branches. Four populations were generated in the fall and winter of 1982 and 1983. Each population included LJ 90430, and one of the four determinate parents, as well as the F_1, F_2 and backcrosses to each parent. All four populations were grown at the Hancock Experimental Station, Hancock, WI, in the summer of 1983. A split plot design with eight replications was used. Whole plots were populations and subplots were generations. Since the different generations were expected to contain different amounts of genetic variability, more plots were included of the segregating generations. Approximately 40 plants of each parent and F_1, 120 plants of the backcrosses and 240 plants of the F_2 were grown on 1.5 m centers.

Significant differences among generation means were observed for number of nodes and number of primary lateral branches (Table 1).

Table 1. Generation means for number of nodes, number of primary lateral branches, and ratio of nodes:laterals for two determinate *C. sativus* x *C. sativus* var. *hardwickii* crosses.

Generation	No. of Nodes > mean		No. of primary lateral branches > mean		Ratio of nodes:laterals	
	Number	Range	Number	Range	Number	Range
Cross 1						
P_1 (*C. sativus* var. *hardwickii*)	48.8a[1]	45-55[2]	12.6a	10-17	3.9e	2.9-5.0
P_3 (M21)	19.2h	15-22	3.8e	2-6	5.3b	2.9-6.2
F_1	41.9b	35-50	10.4b	7-15	4.1d	2.9-6.2
F_2	35.3d	18-52	8.8c	2-16	4.6c	2.1-15.0
*De_*	37.4c	25-52	9.6bc	2-16	4.3cd	2.1-15.0
dede	27.0f	18-32	5.2de	2-11	6.1a	2.7-15.0
BC_{11}	37.9c	32-48	10.5b	6-18	3.9d	2.0-7.2
BC_{13}	29.6e	15-49	6.87d	2-17	4.9c	2.3-10.0
De-	35.5d	27-49	9.1bc	4-17	4.2cd	2.3-8.5
dede	25.1g	15-32	5.1de	2-10	5.5b	3.0-10.0

Cross 2

P$_1$ (*C. sativus* var. *hardwickii*)	47.2a	42-58	12.3a	10-16	3.9e	2.9-5.0
P$_5$ (USDA 1909)	16.8h	14-21	2.6e	1-4	7.8a	4.2-16.0
F$_1$	45.1b	36-56	9.6b	5-13	5.0cd	3.1-9.0
F$_2$	34.7d	16-55	7.3c	0-15	5.6bc	2.7-25.0
*De_*	37.4c	25-55	8.1bc	0-15	5.1cd	2.7-16.0
dede	23.9f	16-32	4.0de	0-10	7.7a	3.4-25.0
BC$_{11}$	37.0c	28-50	9.3b	6-15	4.3de	2.0-8.5
BC$_{15}$	30.5e	13-51	5.6d	1-12	6.4b	2.5-20.0
De-	37.6c	27-51	6.9c	2-12	6.5b	3.8-13.2
dede	21.3g	13-30	3.2e	1-10	6.4b	2.5-20.0

[1] Mean separation in columns by LSD procedure, 0.05 level.

[2] Range

Data are shown for only two of the populations since trends were consistent across all populations. Among the determinate parents, 'Spacemaster' had the highest number of nodes, M21 was second highest, M27 had fewer nodes than M21, and 1909 had the lowest number of nodes. The same ranking was observed for number of primary lateral branches. This pattern continued for both traits in the F$_1$, F$_2$ and backcrosses, except for the 'Spacemaster' population. 'Spacemaster' is a slicing cucumber while the other lines are smaller fruited, Progeny from crosses between 'Spacemaster' and LJ 90430 had larger fruit but fewer lateral branches than the M21 population. Generally, however, determinate parents with a higher number of nodes yielded progeny with higher numbers of laterals in the F$_1$, F$_2$ and backcrosses. Determinate segregates in the F$_2$ and backcrosses to the determinate parent had fewer laterals than the indeterminate segregates. The linear relationship between number of nodes and number of primary lateral branches is illustrated in Fig. 1. There were very few individuals that were classified as determinate with lateral numbers in the range of LJ 90430.
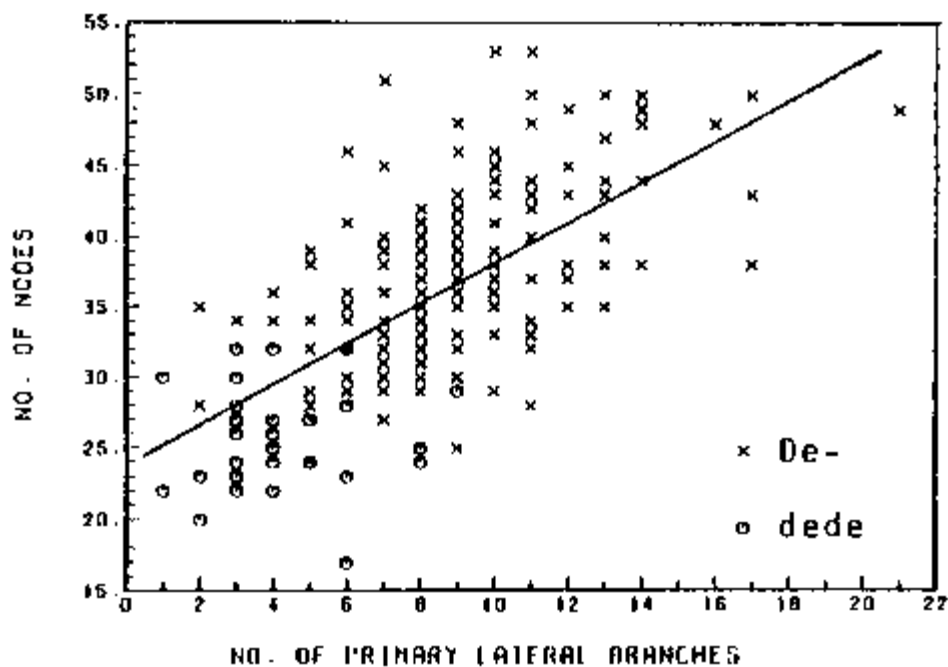
Significant differences were also observed among generations for the ratio of nodes to laterals. On the average there was a lateral for every 4 nodes on LJ 90430, while on the determinate parents and determinate segregates, there was a lateral for every 5 to 8 nodes. This indicates that determinate genotypes do not have fewer lateral branches merely because they have fewer nodes. Therefore, it should be possible to increase the number of lateral branches on determinate plant types.

Literature Cited

1. Horst, E.K. and R.L. Lower. 1978. *Cucumis hardwickii*: a source of germplasm for the cucumber breeder. Cucurbit Genetics Coop. Rept. 1:5.
2. Lower, R.L., J. Nienhuis and C.H. Miller. 1982. Gene action and heterosis for yield and vegetative characteristics in a cross between a gynoecious pickling cucumber inbred and a *Cucumis sativus* var. *hardwickii* line. J. Amer. Soc. Hort. Sci. 107:75-78.
3. Prend, J. and C.A. John. 1976. Improvement of pickling cucumber with the determinate (*de*) gene. HortScience 11:427-428.
4. Sandhu, M.S., H.C. Mohr and D.E. Knavel. 1972. Comparisons of a genetic dwarf and a normal vine cultivar of cucumber. HortScience 7:287.

NO. OF NODES

NO. OF PRIMARY LATERAL BRANCHES

× De-

o dede
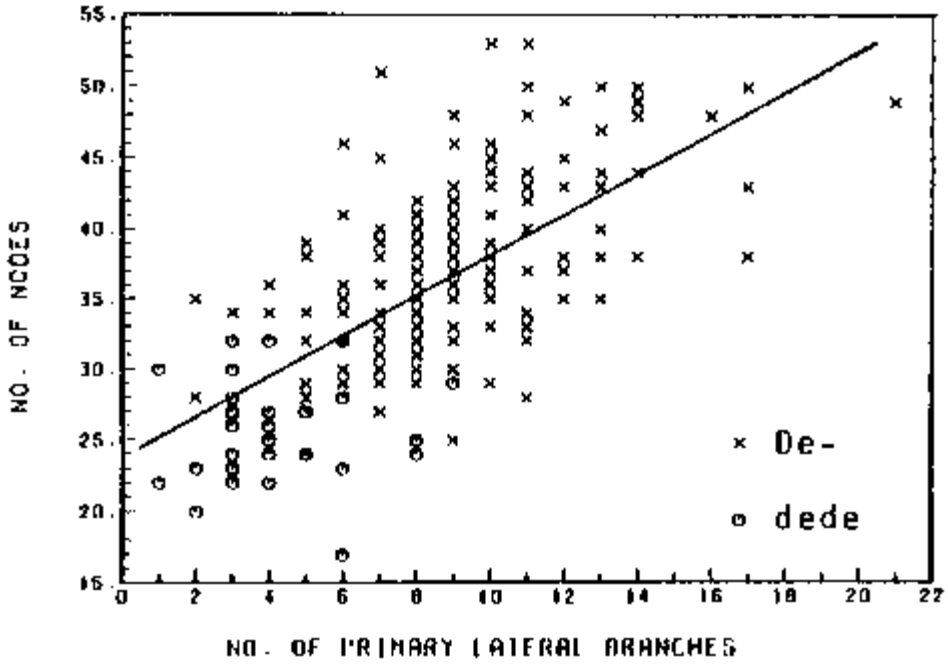
Facilitation of Self-pollination in Gynoecious Cucumber with Silver Nitrate Treatment of Cuttings

Soo-Nyeon Kwack and Kunimitsu Fujieda

University Farm, Faculty of Agriculture, Kyushu University, Kasuyamachi, Fukuoka, Japan

Obtaining self-pollinated seeds from gynoecious cucumber plants selected from segregating populations presents a serious problem for the plant breeder. In this study, we have investigated the application of gibberellic acid (GA) and silver nitrate (1,2,4) to induce staminate flowers in female lines of cucumber as a means of obtaining the necessary selfed seeds.

In order to determine the optimum method for the propagation of cucumber cuttings, cuttings were taken at the time of harvest from the tips of lateral branches of cucumber cultivar 'Sachimidori'. They were trimmed down to two expanded nodes from the tip, treated with indolebutyric acid, IBA, (0, 0.5, or 1.0%) on the cut face, and placed in baked rice chaff or pumice (less than 5 mm diameter). The cutting bed was covered with plastic film and black cheese cloth for 10 days. After 7 days, the percentage of rooted cuttings and the number of roots per cutting were recorded.

All cuttings in baked rice chaff produced roots regardless of IBA concentrations, but IBA treatment decreased the percentage of rooted cuttings in pumice. In both cases, IBA application increased the number of roots per cutting, but slightly suppressed root growth.

Induction of staminate flowers in gynoecious cuttings by GA_3 or $AgNO_3$ was investigated. Cuttings from gynoecious cultivars, 'Pandex', 'Noval' and 'Fertila', were rooted as above in baked rice chaff without IBA treatment. GA_3 (1000 ppm) or $AgNO_3$ (100 or 200 ppm) was applied as a foliar-spray, and after treatment, the cuttings were grown in water culture with OK-F-1 solution for five weeks.

All cultivars treated with GA_3 or $AgNO_3$ produced functional staminate flowers (Table 1). Plants treated with $AgNO_3$ formed staminate flowers at lower nodes than those treated with GA_3, and in addition, a larger number of nodes bore staminate flowers on plants treated with $AgNO_3$. It should be noted that, although it has been reported (4) that GA_3 lengthens internodes, we observed our GA_3 plants to have shorter internodes than controls.

When cuttings are taken from the tips of lateral branches of adult plants, placed in baked rice chaff, and treated with $AgNO_3$ after rooting, functional staminate flowers can be produced. This method makes possible the production of selfed seeds from gynoecious cucumber plants selected from a segregating population.

Table 1. Effects of GA_3 and $AgNO_3$ on induction of staminate flowers in gynoecious cucumber cuttings.

Cultivar	Treatment (ppm)	1st staminate node[z]	No. staminate nodes/plant[z]	Internode length (mm)[y]
'Pandex'	None	-	0	70
	GA_3 1000	14.3	4.0	59
	$AgNO_3$ 100	11.0	9.0	62
	$AgNO_3$ 200	8.7	13.7	71
'Noval'	None	-	0	73
	GA_3 1000	15.7	1.7	57
	$AgNO_3$ 100	10.7	9.7	74
	$AgNO_3$ 200	9.7	11.3	68

'Fertila'	None	-	0	73
	GA$_3$ 1000	11.3	2.3	62
	AgNO$_3$ 100	8.0	9.7	63
	AgNO$_3$ 200	7.7	14.7	67

[z]Based on the main stem.

[y]Mean length of 10 internodes from first to 11th node.

Literature Cited

1. Peterson, C.E. and L.D. Andher. 1960. Induction of staminate flowers on gynoecious cucumbers with gibberellin A$_3$. Science 131:1673-1674.
2. Pike, L.M. and C.E. Peterson. 1969. Gibberellin A4/7 for induction of staminate flowers on the gynoecious cucumber (*Cucumis sativus* L.). Euphytica 18:106-109.
3. Rodriquez, B.P. and V.N. Lambeth. 1972. Synergism and antagonism of GA and growth inhibitors on growth and sex expression in cucumber. J. Amer. Soc. Hort. Sci. 97:90-92.
4. Tolla, C.E. and C.E. Peterson. 1979. Comparison of gibberellin A4/7 and silver nitrate for induction of staminate flowers in gynoecious cucumber line. HortScience 14:542-544.

Pickling Cucumber Inbred Line Development by Full-sib Family Selection II

K. Lertrat and R. L. Lower

University of Wisconsin, Madison, WI 53706

One of the goals of our breeding program is the development of a commercial high-yielding mechanically harvestable hybrid cultivar. After the completion of three cycles of selection for improving fruit number per plant in two pickling cucumber populations, HSE-C_3 (*hardwickii* semi-exotic cycle 3) and GS-C_3 (gynoecious synthetic cycle 3), by S_1 recurrent selection (2). We have conducted an inbred line development program by using full-sib family selection (1).

The second generation of this full-sib family selection was completed in 1983. Average fruit yield of 107 S_1 x S_1 (HSE-S_1 x GS-S_1) crosses was 2.31 fruit per plant, ranging from 0.88 to 3.72 fruit per plant (Table 1). This fruit yield was not significantly higher than the average of six check hybrids (1.45 fruit per plant). However, mean yield was higher than that for a well-adapted hybrid, 'Calypso'. Twenty-one selfed lines from both populations (HSE-S_2, and GS-S_2) were selected on the basis of hybrid performance (selection intensity 20%) for further selection. Average fruit yield of the selected crosses was 3.20 fruit per plant.

Table 1. Summary of the second generation of selection, using full-sib family selection, for the inbred line development phase in HSE and GS populations.

	Number of crosses	S_1 x S_1	Range
All crosses	101	2.31	0.88-3.72
Selected crosses[a]	21	3.20	0.90-3.74
Hybrid checks[b]	6	1.45	0.86-1.97
Calypso		1.87	1.80-1.97

[a]Selection intensity 20%.

[b]Check hybrids included three monoecious cultivars (Clinton, Liberty and SMR 18) and three gynoecious hybrids (Calypso, Calico and Southern Belle).

Literature Cited

1. Lertrat, K. and R. L. Lower. 1983. Pickling cucumber inbred line development by full-sib family selection. Cucurbit Genetics Coop. Rpt. 6:16-17.
2. Nienhuis, J. 1982. Response to different selection procedures for increased fruit yield in two pickling cucumber populations. Ph.D. Thesis, University of Wisconsin-Madison.

Pickling Cucumber Population Improvement for Increased Fruit Yield II

K. Lertrat and R. L. Lower

University of Wisconsin, Madison, WI 53706

Direct selection to improve fruit number per plant has been used as one of the breeding strategies to improve pickling cucumber yield for a once-over mechanical harvesting system in our breeding program. The population improvement program has been conducted for several generations (1).

The second cycle of recurrent selection for specific combining ability using GY 14 as an inbred tester in two breeding populations, the *hardwickii* semiexotic (HSE) and the gynoecious synthetic population (GS), was completed in 1983.

In this cycle, the GY 2 tester was discarded due to the lack of scab resistance. Average fruit yield, at optimum harvest time, for GY 14 test crosses of HSE and GS were 1.75 and 1.63 fruit per plant, respectively (Table 1).

Table 1. Summary of the second cycle of recurrent selection for specific combining ability for increased fruit yield in pickling cucumber populations, HSE and GS, using Gy 14 as an inbred tester.

Population	Number of test crosses	Average fruit no. per plant	Range	Average fruit no. per plant of selected lines
HSE	123	1.75	1.14-3.67	2.16 (SI 20%)
GS	123	1.63	1.06-2.44	1.93 (SI 20%)
Hybrid checks[a]		1.29	0.90-1.88	

[a]Included three gynoecious F_1 hybrids (Calypso, Calico and Southern Belle) and three monoecious cultivars (SMR 18, Clinton and Liberty).

Test cross yields were higher than hybrid checks (1.29 fruit per plant). In summer 1983 the top 25 lines of HSE and GS (SI 20%), with their average fruit yield of 2.16 and 1.93, were selected for further population improvement. Yield was lower in 1983 than 1982 - presumably due to an unusually warm growing season.

Literature Cited

1. Lertrat, K. and R. L. Lower. 1983. Pickling cucumber population improvement for increased fruit yields. Cucurbit Genetics Coop. Rpt. 6:18-19.

Chlorflurenol-induced Seed Coat Development in Parthenocarpic Pickling Cucumbers

A.P.M. den Nijs and G. de Wolf

Institute for Horticultural Plant Breeding, P.O. Box 16, 6700 AA Wageningen, The Netherlands

Application of chlorflurenol to pickling cucumbers to accelerate and concentrate yield for once-over mechanical harvest by inducing parthenocarpic fruit set has met with difficulties related to unpredictable weather conditions. In the meantime, genetically parthenocarpic cultivars were developed which hold promise for reliable and increased field production if early fruit set due to the presence of staminate flowers can be prevented. For optimal harvest planning, a combination of genetically parthenocarpic cultivars and application of chlorflurenol has been advocated. The effect of the spraying is enhanced by the genetic parthenocarpy of the plants. However, the processing quality of the fruits must also be considered. Here we present some results on fruit quality, which were obtained during a separate study of the competition between pollinated and parthenocarpic fruits on the plant.

In two temperature-controlled glasshouses (23/17°C D/N and 20/23°C D/N), 18 plants of each of three parthenocarpic pickling cucumber cultivars (Aida, Andrea and Belia) were grown in pots which were randomly placed on trolleys. Chlorflurenol treatments started September 1 on half of the plants. Individual flowers were dipped in a 100 ppm solution (Curbiset, courtesy of Asepta N.V., Leiden, the Netherlands). Fruit length (L) and diameter (D) were measured and the inside of the fruits was observed at the end of the experiment.

Chlorflurenol hardly affected the mean relative growth rates of the fruits, regardless of cultivar. The ultimate volume of the fruits, however, was significantly increased by the treatment. The effect of chlorflurenol on the length/diameter (L/D) ratio for the three cultivars is presented in Table 1. The significantly lower ratio of treated fruits was exclusively due to increased diameter, whereas length remained the same. The reason for this becomes clear from Table 2, which shows the percentage of fruits with developed seed coats. Almost all chlorflurenol-induced fruits contained such seed coats in high numbers, whereas only few of the genetically parthenocarpic fruits did. Only a fair percentage of fruits of 'Belia' set at 23/17°C without treatment contained seed coats. All examined seed coats were completely empty.

Table 1. Effect of chlorflurenol application on the length/diameter ratio of parthenocarpic pickling cucumbers grown in 2 different temperature regimes (°C day/night).[z]

Cultivar	Untreated		Chlorflurenol	
	23/17	20/23	23/17	20/23
Alda	3.45	3.62	2.68	2.80
Andrea	3.30	3.10	2.57	2.83
Belia	2.94	2.92	2.65	2.71

[z]Data are means of 24 fruits per treatment.

Table 2. Percentage of parthenocarpic fruits with seed coats as affected by chlorflurenol treatment of cucumbers grown in 2 different temperature regimes (°C day/night).[z]

Cultivar	Untreated		Chlorflurenol	
	23/17	20/23	23/17	20/23
Alda	6	5	95	79
Andrea	16	5	89	84

Belia	60	14	86	60

[z]Data are means of 18 to 21 fruits per treatment.

Dutch slicing cucumbers contain barely-visible ovules in their parthenocarpic fruits. Dutch slicers were the source of parthenocarpy in the pickling cucumber cultivars used in this experiment (2), so the absence of seed coat development is to be expected. In breeding lines and exotic accessions, we have observed a wide range of seed coat development in parthenocarpic fruits set under insect-free glasshouse conditions. The number and size of such seed coats bears no relationship to the parthenocarpy of the genotypes.

Processing quality of parthenocarpic fruits has been disputed. Pasteurized fruits of Dutch genetically parthenocarpic cultivars proved to be too soft for consumer acceptance. Pollinating parthenocarpic cultivars resulted in even softer fruits, presumably caused by a larger seed cell. Chlorflurenol likewise increases fruit softness while decreasing the L/D ratio. Fruits often became egg-shaped (3). The decrease in L/D ratio was also observed in American non-parthenocarpic slicing and pickling cucumbers treated with chlorflurenol (1). The results presented here demonstrate that the induction of ovule development into seed coats by chlorflurenol may be a major factor in the loss of external and internal fruit quality of parthenocarpic pickling cultivars. Caution is therefore advised with harvest planning schemes involving genetically parthenocarpic cultivars and chlorflurenol application.

The effect of chlorflurenol on the ovules suggests that the mechanism of action of this chemical in inducing parthenocarpy differs from the genetically determined one.

Literature Cited

1. Cantliffe, D.J. 1977. The induction of fruit set in cucumbers by chlorflurenol with and without pollination. HortScience 12:58.
2. Ponti, O.M.B. de and F. Garretsen. 1976. Inheritance of parthenocarpy in pickling cucumbers (*Cucumis sativus* L.) and linkage with other characters. Euphytica 25:633-642.
3. Rol, W. 1981. Conservenkwaliteit van parthenocarpe augurkenrassen. Groente en Fruit 36(27):46-47.

Improving the Fertility of Tetraploid Cucumbers

A.P.M. den Nijs and D.L. Visser

Institute for Horticultural Plant Breeding (IVT), P.O.B. 16, 6700 AA Wageningen, The Netherlands

In our breeding program to introduce resistance genes from wild *Cucumis* species into *C. sativus*, we have attempted to overcome crossing barriers by using tetraploid cucumbers as the maternal parent in crosses with *C. melo* L. and *C. zeyheri* Sond. Interspecific pollinations, in all possible combinations, between diploid and tetraploid lines of *C. sativus* and *C. melo* yielded occasional fruits containing ovules that were sometimes enlarged but without embryos (1). Occasional embryo development up to the globular stage was obtained in diploid and tetraploid cucumbers pollinated by melon (2).

We assembled a small collection of tetraploid cucumber lines for a study of their crossability with other *Cucumis* species (Table 1). However, fruit set after pollinations by *C. melo* was disappointingly low, and self pollinations yielded only rarely fruits as well. Seed set upon selfing averaged 5 to 20 seeds per fruit (s/f) disclosing the low fertility of the tetraploids. This was also observed elsewhere (3). We decided to improve the fertility of the tetraploids before further crossability studies were carried out.

Table 1. Designation, creator and source of tetraploid breeding lines.

Code	Designation/derived from	Doubled by	Source
BDR	cv. Butchers Disease Resisting	Grimbly	GCRI, Littlehampton, UK
C16	4x/1976	Mackiewicz	Inst. of Gent., Reguly, Poland
Eng	English slicer	Grimbly	via Mackiewicz
RT	naturally occurring 4x	-	Arkansas Agr. Exp. Stn, USA
Addis	cv Addis	Lower	North Carolina S.U., USA
0918	PI 220860, from Korea	IVT	NCRPIS, Ames, Iowa, USA
1811	WJR 3147, from India	IVT	Vavilov Inst. P1. Ind., USSR

Crosses were made between the tetraploids listed in Table 1 in a half diallel to alleviate the inbreeding depression which could be responsible for the low fertility. Many crosses failed and average fruit set was under 25%. Seed set was also very low, but 'C16', 'Addis' and 'Gbn 1811' averaged about 20 seeds per fruit (s/f) after self pollination and outcrossing. Four out of six F_1 hybrids sown in 1982 germinated, and these were self pollinated and crossed with each other. Fruit set on the hybrid plants was over 90%, i.e. the same as on diploid material. Average seed set ranged from about 30 s/f for '1811 x Eng' and 'Addis x C16', to about 120 s/f for 'C16 x BDR' and 'RT x C16'. The outcrosses between the inbred tetraploid lines therefore significantly improved fertility.

One of the best hybrids, 'C16 x BDR', was studied in more detail in 1983, along with its parents, the F_2 and an outcross with 'RT x C16'. Some results are in Table 2. Poor germination resulted in only 2 plants of 'BDR', which like those of 'C16' lagged behind in plant development in comparison with the hybrid populations. Percentages of stainable pollen (means of at least 6 preparations per plant, at three dates) were uniformly high, which was also true for the fruit set. The very high fruit set on 'C16' and its reasonable seed set surprised us in view of earlier experience, although 'C16' is one of the more fertile lines. The environment must have been conducive to seed set in 1983. The very low seed set on 'BDR' and the dramatic increase to about 90 s/f on the F_1 hybrid agreed with earlier data. The average seed set on the F_2 plants was somewhat depressed which may reflect the again increased level of inbreeding. The combination with the unrelated tetraploid 'RT' appeared to have a positive effect on seed set.

Table 2. Fertility of inbred and hybrid tetraploid breeding lines.

Population	Number of plants	Percentage stain-pollen	Percentage fruit set after selfing	Total number of fruits	Seeds per fruit
P$_1$:C16	9	73	94	16	57
P$_2$:BDR	2	68	75	6	24
F$_1$:Cl6 x BDR	10	72	78	30	88
F$_2$:(Cl6 x BDR) x self	25	74	88	64	74
F$_1$:(Cl6 x BDR) x (RT x C16)	50	70	88	118	95

Although the seed set of these hybrid tetraploids is not yet up to the diploid level, we expect that the improved fertility will enable us to more accurately compare tetraploid and diploid cucumbers with respect to the crossability with melons and interesting wild *Cucumis* species.

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Effect of Length of Vegetative Phase on Total Dry Matter Production and Its Partitioning

D.R. Ramirez and T.C. Wehner

Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609

The onset of fruit development usually determines the extent of vegetative growth in crops. The length of both vegetative and reproductive phases in the growth of plants have been claimed to be important in determining yields in maize (*Zea mays* L.) (4) and cereals (1). Inhibition of vegetative growth by fruit development has been demonstrated in cucumbers (3). Assimilate supply from source leaves could also be limiting growth (2).

Dry matter production depends basically on the amount of photosynthetic leaf surface and the rate of carbon fixation (photosynthesis) per unit of leaf area. Most of the variation in yields of crop species was related to differences in rate of increase in leaf area rather than differences in net assimilation rate (5).

This experiment was aimed at determining the effect of an increase in the length of the vegetative phase on total dry weight production (a good estimate of net photosynthetic capacity). A second consideration was the effect on Partitioning of assimilates into source (leaves, stems) and sink (fruits) tissues.

Methods. Plants of the cultivar Calypso were grown in pots in the Horticultural Science greenhouses at the North Carolina State University in Raleigh, NC, from Jan. 27 through April 11, 1983. The experiment consisted of 4 flower removal treatments arranged in a randomized complete block design with 4 replications. Treatments consisted of the daily removal of female flowers for 0, 7, 14 and 21 days beginning when plants were 36 days old. The following parameters were measured at harvest: fresh and dry weights of leaves, stems and fruits; number of branches, leaves and fruits; stem length; and leaf area.

Results. Total fresh and dry weight production was not increased by increasing the length of the vegetative phase, but dry matter and fresh matter partitioning were greatly modified (Tables 1 and 3). Removal of flowers for 14 and 21 days significantly increased the percentage of dry and fresh weight that were devoted to leaf and stem growth and decreased the dry and fresh weight devoted to fruit growth. All flower removal treatments resulted in an increase in the number of branches and leaves, producing a larger plant (Table 2). Leaf area increased with increased flower removal but was only significant for the 21 day treatment. Flower removal for 7 days resulted in an increase in the number of fruits per plant, and fresh and dry weights of fruits (Tables 1 and 2), but the differences were not significant. This lack of significance could be due to the fact that fruit set was not uniform in the population of plants and treatment differences could have been masked by this variability. It seems that excessive vegetative development (as that obtained by the 14 and 21 day flower removal) is detrimental for fruit growth, as more assimilates are then diverted into unneeded leaf and stem growth.

Table 1. Fresh and dry weight production as affected by number of days of flower removal.[z]

Days of Flower Removal[y]	Fresh Weight Production (g)				Dry Weight Production (g)			
	Leaves	Stems	Fruits	Total	Leaves	Stems	Fruits	Total
0	65.7	65.3	726.7	857.7	8.2	4.0	29.3	41.4
7	69.6	72.0	761.4	903.0	8.6	4.4	30.6	43.7
14	84.3	90.8	665.0	840.1	10.4	6.4	25.5	42.1
21	115.4	113.6	635.3	864.2	13.9	8.7	19.4	42.0
LSD (5%)	10.3	18.2	101.1	118.1	1.0	1.3	3.5	4.3
CV (%)	8	13	9	9	6	14	8	6

Table 2. Branch number, leaf number, fruit number, stem length and leaf area as affected by number of days of flower removal.[z]

Days of Flower Removal[y]	Branch number	Leaf number	Fruit number	Stem length (cm)	Leaf area (cm^2)
0	0.0	16	2.5	110	3250
7	2.5	25	3.0	119	3473
14	4.0	26	2.3	134	4134
21	7.0	39	2.8	159	5190
LSD (5%)	2.0	4.6	0.7	36	897
CV (%)	37	11	17	18	14

[z]Data are means over four replications

[y]Treatments begun 36 days after planting

Table 3. Distribution of fresh or dry matter in plant parts as affected by the number of days of flower removal.[z]

Days of Flower Removal[y]	Distribution of Fresh Weight (%)			Distribution of Dry Weight (%)		
	Leaves	Stems	Fruits	Leaves	Stems	Fruits
0	19.8	9.5	70.7	7.7	7.6	84.7
7	19.7	10.2	70.1	7.7	8.0	84.3
14	24.7	14.9	60.5	10.1	10.9	79.1
21	33.2	20.8	46.0	13.4	13.1	73.4
LSD (5%)	1.9	3.0	4.5	0.9	1.6	2.0
CV (%)	5	14	5	6	10	2

[z]Data are means over four replications.

[y]Treatments begun 36 days after planting.

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Growth Analysis of Three Cucumber Lines Differing in Plant Habit and Yield

D.R. Ramirez and T.C. Wehner

Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609

It is known that the activity of a developing fruit in a cucumber plant inhibits the development of fruit that set later (1), as well as the development of other plant parts (2). This inhibitory effect ceases when the growing fruits are removed from the plant, allowing the production of several fruit per plant under multiple harvest conditions. The prevention of simultaneous development of several fruit per plant greatly reduces yield of fruit, especially in once-over harvest systems.

One explanation for this inhibitory effect could be that fruits of the commercial cultivars of *Cucumis sativus* L. constitute strong sinks for assimilates, drawing heavily on plant supplies and inhibiting in this way the development of other fruit. Inhibition or cessation of vegetative growth could indirectly limit the ability of a plant to support the growth of more fruit. This study was run to advance our understanding of the partitioning of assimilates in cucumber plants. The objective of this experiment was to determine relationships between vegetative and reproductive plant parts throughout the entire cycle of development of 3 lines that differ in growth habit and yield.

Methods. Growth analysis studies of 3 lines differing in growth habit and yield were conducted in the Horticultural Science greenhouses in Raleigh, NC during the Fall, 1982. The 3 lines studied were LJ 90430, an accession of *Cucumis sativus* var. *hardwickii*; 'Calypso', a widely-used indeterminate cultivar; and M 21, a dwarf, determinate breeding line for NCSU. After germination, plants of LJ 90430 were subjected to a 9.5 hour daylength for 17 days in order to induce fruit set.

The experiment design was a split plot in a randomized complete block with 4 replications. Whole plots were the 9 harvests and subplots were the 3 lines. Harvests were made 32, 39, 46, 53, 60, 67, 74, 81 and 88 days after planting beginning November 2 and ending December 28. At each harvest, one whole plot in each replication was removed and the following measurements made: fresh and dry weights of leaves, stems fruits, and roots; number of leaves, fruits and branches; stem length, and leaf area. Leaf area was determined with an electronic leaf area meter. After each harvest, the border plants were moved in to fill the space left.

Results. At the end of the growing period (88 days), both LJ 90430 and 'Calypso' produced the same amount of total dry weight and fruit dry weight. However, M 21 produced significantly less total dry weight. LJ 90430 incorporated a higher amount of dry weight into leaves, stems and roots than the other 2 lines (Table 1).

Table 1. Partition of dry weight production into leaves, stems, fruits and roots in three lines at final harvest (88 days)[z].

Line	Dry Weight (g)				
	Leaves	**Stems**	**Fruits**	**Roots**	**Total**
LJ 90430	10.2	9.9	40.9	2.6	63.1
Calypso	8.1	5.3	43.7	1.7	53.2
M 21	7.0	3.0	24.3	0.7	35.3
LSD (5%)	*1.3*	*0.8*	*12.6*	*0.9*	*15.1*
CV (%)	*9*	*8*	*20*	*31*	*17*

[z]Data are means over four replications on a per plant basis.

'Calypso' produced a significantly higher total fresh weight and fruit fresh weight than LJ 90430 and M 21 (Table 2). When dry and fresh weights were compared, it was evident that 'Calypso' fruits and stems had a higher water content. The number

of fruits per plant were significantly higher in LJ 90430 than in the other 2 lines.

Table 2. Fruit number,and fresh weight of leaves, stems and fruits in three lines at final harvest (88 days)[z].

Line	Fresh Weight (g)				
	Leaves	Stems	Fruits	Roots	Total
LJ 90430	26	79	120	504	703
Calypso	3	76	86	769	930
M 21	2	73	43	468	585
LSD (5%)	4	16	13	108	123
CV (%)	24	12	9	11	10

[z]Data are means over four replications on a per plant basis.

Leaf photosynthetic area was higher in 'Calypso' in the first 2 harvests (32 and 39 days), but LJ 90430 had a significantly higher leaf area than 'Calypso' and M 21 thereafter. This early advantage of 'Calypso' and to a lesser extent M 21 was related to earliness. Fruits started developing when plants were 39 days old in 'Calypso' and M 21, and when plants were 53 days in LJ 90430. At this time their leaf area had developed 63, 59 and 93%, respectively, of their total mean leaf area for the remainder of the period when it was fully developed (Table 3).

Table 3. Leaf area per plant for nine weekly harvests in three lines[z].

Line	Leaf area per plant (cm)								
	Days from planting to harvest								
	32	39	46	53	60	67	74	81	88
LJ 90430	74	1928	3734	5153	5715	5744	5601	5583	5395
Calypso	123	2489	3604	4025	3770	3832	3980	4082	4086
M 21	105	1928	3106	3547	3473	3254	2958	3151	3252
LSD (5%)	15	560	806	717	885	906	759	995	1075
CV (%)	9	15	13	10	12	12	11	13	15

[z]Data are means over four replications. Analysis were performed separately for each of the nine harvests.

These results indicate that 'Calypso' was capable of producing the same amount of total dry weight and a significantly higher total fresh weight and fruit fresh weight with a lower leaf photosynthetic area. However, the inhibitory effect of fruits in 'Calypso' and in M 21 was very high. It could be that early fruit set at the time when leaf area has not developed fully could enhance this inhibitory effect.

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Early Generation Testing in Cucumber

D.B. Rubino and T.C. Wehner

Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609

Early generation testing would benefit cucumber breeders by allowing poor lines to be discarded early in the inbreeding process. Often, lines are self pollinated six generations before testing. That method wastes time, space, and other resources on lines that will eventually be discarded. With early testing, lines are tested and evaluated after only one or two generations of self pollination.

Early testing was first suggested by Jenkins who found that maize (*Zea mays* L.) lines obtained their individuality in crosses in the S_1 or S_2 generation (1). Others have also found early testing to be successful (2,4). The efficiency of early testing for a given trait should depend greatly on the magnitude of the heritability for that trait. Heritabilities of 0.17 for fruit number, 0.25 for fruit color and 0.49 for carpel wall thickness were reported for cucumber (3). The objective of this experiment was to determine whether early testing was of value in cucumber.

Methods. Twenty-four S_0 (open-pollinated) plants from the North Carolina Medium Base Pickle (NCMBP) population were self-pollinated for six generations. Generations S_1 and S_6 of each family were crossed with 2 testers, NCMBP, the medium base pickle population from which the 24 families were derived (referred to as testcrosses), and Gy14A, a gynoecious inbred line (referred to as hybrids). The testcross and hybrid seeds along with remnant seeds from the 6 inbred generations were planted on July 13, 1983, in 1.5 m plots using a split-split plot design with 2 replications. Whole plots were the 3 testers, subplots were the 24 families, and sub-subplots were the 3 generations. A stand of 15 plants per plot was maintained with standard cultural practices. All plots were harvested when 'Calypso' check plots reached 10% oversize fruit (>51 mm in diameter).

In order to measure the value of early testing, the S_6 testcrosses were regressed on the S_1 testcrosses, the S_6 hybrids were regressed on the S_1 hybrids, and the S_6 inbreds were regressed on the S_1 through S_5 inbreds. The advantages of S_1 line testing (early testing) versus S_6 line testing were calculated using the equation for predicting gain from selection, assuming additive variance and phenotypic variance were the same for all generations. Thus, advantage = $(b_{6.1})(k_1/k_6)(2.5)$ where $b_{6.1}$ = the regression coefficient, and k_1 and k_6 the selection intensity in units of standard deviations for S_1 line testing and S_6 line testing, respectively. It was estimated that the number of lines that a cucumber breeder could handle were 300 for S_1 line testing and 100 for S_6 line testing. The equation was multiplied by 2.5, since 1 year is required for S_1 line testing versus 2.5 years required for S_6 line testing.

Results. The regression coefficients, and thus the prediction value for inbred yield, increased as the S_6 generation was approached (Table 1). This trend was not apparent for earliness and quality.

Table 1. Coefficients (b) for the regressions of S_6 on S_1 through S_5 inbred performance for yield, earliness, and quality.

	Yield (Fruit no./plot)			Fruit quality score[z]		
Regression	Total	Marketable	Earliness[y]	Shape	Seedcell	Color
$b_{6.1}$	0.52*	0.36*	0.76*	0.26*	0.17	0.21
$b_{6.2}$	0.26	0.21	0.44	0.45*	0.33*	0.22
$b_{6.3}$	0.69*	0.41*	0.60*	0.45*	0.00	0.09
$b_{6.4}$	0.70*	0.48*	0.62*	0.38*	0.12	0.14
$b_{6.5}$	0.82*	0.70*	0.74*	0.95*	0.00	0.26*

^{Z}Quality scored 1 to 9 (1 = poor, 5 = average, 9 = excellent).

^{y}Earliness is the number of oversize fruit per plot at harvest.

*b greater than standard error.

The calculated advantages indicated that early testing for specific combining ability (hybrid performance) and early testing for inbred performance should be used for yield and earliness but not for quality traits. However, early testing for general combining ability (testcross performance) was not as efficient for detecting superior lines.

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Effect of Inbreeding on Horticultural Performance of Cucumber Families Developed from a Variable Population

D.B. Rubino and T.C. Wehner

Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609

Species of the Cucurbitaceae have been reported to exhibit little inbreeding depression (1). However, Ghaderi and Lower (3) reported that in 5 of 6 crosses of cucumber, heterozygosis had significant positive linear effects for all yield characters measured. They also suggested that since hybrid vigor and inbreeding depressing are both aspects of the same phenomenon, inbreeding depression might also be expected to occur in cucumber (2). Therefore, self pollination should be expected to result in deterioration of cucumber lines as it does in maize (*Zea mays* L.), where a reduction in vigor accompanying inbreeding was reported as early as 1908 (4). The objective of this experiment was to determine if inbreeding depression occurs in cucumber families derived from an open-pollinated population.

Methods. Twenty-four cucumber families were developed by self-pollination of randomly-selected plants from the open-pollinated pickling cucumber population NCMBP, for 6 generations. Seeds from all generations were planted on July 13, 1983 using a split-plot in a randomized complete block design with 2 replications. Whole plots were the 24 families and subplots were the 6 generations. Check plots of 'Calypso' and NCMBP (S_0 plants) were included for reference points. A stand of 15 plants per 1.5m plot was maintained with standard cultural practices. All plots were harvested when 'Calypso' check plots reached 10% oversize fruit (>51mm in diameter).

At harvest, the total and marketable fruit number per plot were counted. Fruit shape and color, and the size of the seed cavity were rated visually on a scale of 1 (poor) to 9 (excellent). Earliness was measured as the number of oversize fruit per plot on the day of harvest. The regressions of yield, earliness, and quality on *F*, the coefficient of inbreeding, were used to measure the effect of the level of homozygosity on performance.

Results. The differences among generation means were small for all traits measured (Table 1). Inbreeding apparently had no deleterious effect on these traits. The regressions for all traits resulted in slopes that were not significantly different from 0 (Table 2). This indicated that no significant inbreeding depression occurred, since a reduction in vigor or quality with inbreeding would result in negative slopes.

Heterozygosis was reported to have a positive effect on yield in 5 of 6 cucumber crosses studied by Ghaderi and Lower (3). However, most of the genetic variance for yield in a population studied by Smith et al. (5) was found to be additive. Results from this study support previous observations (1) that inbreeding depression is not important in controlling the fruit yield, earliness, or quality of inbreds developed at random from the pickling cucumber population NCMBP.

Table 1. Yield, earliness, and fruit quality for 6 generations of inbreeding from the NCMBP population (generation 0).[z]

Generations of inbreeding	Yield (Fruit no./plot)		Earliness[y]	Fruit quality score[x]		
	Total	Marketable		Shape	Seedcell	Color
0	26.0	23.0	23.0	5.1	4.3	5.5

1	24.3	21.2	15.9	5.2	4.9	6.0
2	24.1	20.9	17.4	5.5	5.5	6.2
3	29.7	26.4	22.6	5.3	4.9	5.9
4	27.1	23.8	18.9	5.4	4.9	6.1
5	26.1	22.4	19.1	5.3	5.3	6.1
6	25.9	21.4	17.5	5.4	5.2	6.1
LSD (5%)	3.1	2.8	2.7	0.4	0.4	0.4
CV (%)	26.6	27.6	32.8	17.1	17.0	14.6

[z]Data are means over 24 families and 2 replications.

[y]Earliness is the number of oversize fruit per plot at harvest.

[x]Quality scored 1 to 9 (1=poor, 5=average, 9=excellent).

Table 2. Relationship of the level of inbreeding (F) to performance of 24 families for yield, earliness, and fruit quality.

Trait	Regression of trait on F (b)[z]
Yield	
Total (Fruit no.)	5.57
Marketable (Fruit no.)	3.90
Earliness[y]	6.35
Quality[x]	
Shape	0.36
Seedcell	0.32
Color	0.08

[z]None of the regression slopes were significant at the 5% level.

[y]Earliness is the number of oversize fruit per plot at harvest.

[x]Quality scored 1 to 9 (I=poor, 5=average, 9=excellent).

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Evaluation of Screening Methods and Sources of Resistance for *Rhizoctonia* Fruit Rot in Cucumber

Sloane, J.T., T.C. Wehner and S.F. Jenkins, Jr.

Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609

B. Graves

Southern Mississippi Experiment Station, Beaumont, MS 39423

Cucumber yield losses in the southern U.S. averaged 7 to 9% and reached as high as 40% in some areas (2). Cultural practices for control have not been economical to apply, and single gene resistance has not been found to date. Previous research (1) has identified sources of quantitative resistance, however. This study involved the evaluation of several methods for screening for *Rhizoctonia* fruit rot resistance, and the identification of resistant and susceptible lines for use in breeding programs.

Methods. The most resistant and most susceptible lines (totaling 149) from previous tests were evaluated using 2 screening methods. The lab test involved placing 50 mm diameter fruit harvested from field plots onto soil inoculated with *Rhizoctonia*. The soil was inoculated with oat grains (4800/m^2) that had been infested with 12 isolates of the fungus. The 12 isolates had been collected from North Carolina production areas. The fruit were scored after 10 days in a mist chamber where the soil was kept moist by applying 3 ml of water every 3 days for 1 min. Fruit were scored for percent of surface infected with *Rhizoctonia*.

The field test was run in a similar manner to the lab test, except that the fruit were not removed from the plants. Inoculation rates and procedures were similar except that the field plots were inoculated at vine tip-over stage. The first and second most infected fruit were flagged and scored twice weekly in each plot during the period 25 to 46 days after inoculation. Any plots having scores of 1% fruit infection or less were checked to determine if there were fruit in the plot that were more infected. This helped prevent us from confusing resistant plots with escapes. A second field test was run in Mississippi on naturally-infested soil using the same disease evaluation procedures as for the inoculated-field test.

Results. Data from the 1983 tests were combined with previous data to determine which lines of the initial 1063 screened were most resistant. The 4 most resistant lines were PI 163216, PI 197088, PI 280896 and PI 357852 (Table 1). Plants in some of the lines were segregating for plant type and fruit type, so selections were made within lines to stabilize resistance and other horticultural characteristics. The correlation between the lab and field tests was significant, but perhaps not high enough to use as a screening method. The field test was about as easy to run and was more closely correlated with results from a test run in Mississippi (data not shown). A more severe test is needed to distinguish among levels of resistance found in the best lines and in selections made from them.

Table 1. Belly rot resistance (percent of fruit surface infected) for the most resistant and most susceptible lines in 3 laboratory and 3 field tests.

Line-Selection	Origin	Laboratory test			Field test		
		1981	1982	1983	1982	1983	Miss.
PI 163216-4	India	0	0	0	0	0	0
PI 163216-6	India	0	0	0	0	0	0
PI 197088-5	India	0	0	0	0	0	0
PI 357852-1	Yugoslavia	0	0	-	0	-	0

PI 280096-2	USSR	0	0	-	0	-	0
PI 181752-3	Syria	22	3	-	18	0	6
PI 177360-5	Turkey	0	0	10	9	6	8
PI 419108-5	China	0	5	6	7	4	8
PI 419108-6	China	0	5	6	7	4	8
PI 344433-1	Iran	23	-	20	16	-	14
PI 267741-4	Japan	0	1	20	13	5	15
LSD (5%)		-	7	7	7	6	5
Mean		-	3	3	3	4	6
CV (%)		-	126	115	76	63	80
No. replications		1	2	3	2	2	1

Table 2. Correlation of *Rhizoctonia* fruit rot scores (percent of surface infected) among laboratory and field tests run in 1983.

Laboratory test Raleigh	Raleigh vs. Clinton	Field test Clinton	Clinton vs. Mississippi	Field test Mississippi
	0.50**		**0.40***	

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Heritability of Resistance to *Rhizoctonia* Fruit Rot in a Wide Base Cucumber Population

J.T. Sloane and T.C. Wehner

Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609

Rhizoctonia fruit rot is a major disease problem in the southern U.S., reducing yields by an average of 7 to 9% annually (3). No single-gene resistance to the disease has been found, however. Recently, screening methods have been developed and lines identified that have quantitatively-inherited resistance to the disease (2). In order to plan a breeding program to incorporate that resistance into commercially acceptable cultivars, a measurement of the heritability of the trait is needed. The objective of this study was to measure the heritability of Rhizoctonia fruit rot resistance in the wide-base cucumber population, NCWBP.

Methods. A wide-base population was developed by intercrossing all available cucumber Plant Introduction lines (from the Regional Plant Introduction Station at Ames, Iowa), breeding lines and cultivars, totaling 1063 lines. The lines were intercrossed in 1981 and the pickling cucumber types harvested and intercrossed in 1982 to form the North Carolina Wide Base Pickle (NCWBP) population. After intercrossing, 112 plants were selected at random and tested for *Rhizoctonia* fruit rot resistance. The plants were flagged and seed harvested from each plant at maturity for the progeny test. The progeny (half-sib families) were planted in 1983 and 3 plants in each family tested for fruit rot resistance. The test involved harvest of 1 fruit, approximately 50 mm in diameter, from each plant. Fruit were taken to the laboratory and placed on soil that had been inoculated with oat grains ($3200/m^2$) infested with a mixture of 12 *Rhizoctonia* isolates obtained from cucumber production areas in North Carolina. The soil was held in flats on a mist shelf system that applied 3 ml of water for 1 min every 3 days. After 10 days, the fruit were scored for percent of the surface infected with *Rhizoctonia*. Analysis of the data was by parent-progeny regression, which has been shown to estimate 1/2 the narrow-sense heritability, h^2_N (1).

Results. The population had a fairly high level of resistance and the detached fruit test was not as severe a test as has been developed more recently. However, there was a wide range of disease reaction, and the population mean of 2.1% fruit damage was considered to be an intermediate level between resistant and susceptible (Table 1). The heritability of resistance was estimated to be 0.24, considered low to moderate. Progress for incorporating resistance into cultivars should be steady. The formula for calculation of gain from selection was used to estimate that half-sib family selection (where the best 10% of 300 families tested were intercrossed in isolation each year) would result in a population mean of 0% fruit damage after 1 cycle of selection. However, more recent research has resulted in the development of a more severe screening procedure, so progress toward a higher level of resistance might not be so rapid. It appears that resistance can be transferred to commercially acceptable cultivars with slightly more effort than that being spent on transfer of high levels of anthracnose resistance.

Table 1. *Rhizoctonia* fruit rot resistance in the NCWBP population, and estimates of genetic variance and heritability from parent-progeny regression analysis of a sample of 112 plants.[z]

Statistic		Value
Mean		2.1%
Range	- Highest	12.0%
	- Lowest	0.0%
b(parent-offspring)		0.12
h^2_N		0.24

[z]Data were based on a single fruit per plant evaluated for percent of surface infected after 10 days of exposure to soil infested with

Rhizoctonia solani.

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Electrophoretic Comparison of Six Species of *Cucumis*

J.E. Staub and R.S. Kupper

Department of Horticulture, University of Wisconsin, Madison, WI 53706

T.C. Wehner

Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609

Evolutionary studies in the genus *Cucumis* have been conducted by Dane (2) and Esquinas-Alcazar (3) using isozymes as biochemical markers. In the study of Esquinas-Alcazar, electrophoretic variability was examined in 155 populations representing 21 species of *Cucumis* to determine intraspecific relationships within the species *C. melo* L. and interspecific relationships within the genus *Cucumis*. He used 6 enzymes [peroxidase (PRX), acid phosphatase (APR), glutamate oxalacetase transaminase (GOT), glucosephosphateisomerase (GPI), phosphoglucomutase (PGM), and 6- phosphoglucodehydrogenase (6-PDGH)] which were coded for a total of 16 enzyme-coding loci. The 21 species of *Cucumis* were classified, on the basis of genetic distances, into 4 groups. The *anguria* group included: *C. africanus*, *C. anguria*, *C. ficifolius*, *C. myriocarpus*, and *C. zeyheri*, while the *sativus* group included: *C. sativus*, *C. hardwickii*, and *C. trigonus*.

Two highly speculative hypotheses were proposed for the evolution of the *sativus* group. The hypotheses consisted of the following:

- The group could have been derived from an ancestral form of the *metuliferus* group through a primitive *C. hardwickii* following a reduction of chromosome number. This hypothesis is supported by the fact that the *metuliferus* group, which includes *C. membranifolium*, *C. metuliferus* and *C. sagittatus* is genetically close to the *anguria* and *sativus* groups. Moreover, Trivedi and Roy (8) concluded from cytogenetic studies that 12 might be the prime base chromosome number of *Cucumis*.
- The ancestral form of the *metuliferus* group and the *sativus* group could have had a common ancestor with 2n=14 chromosomes. This hypothesis implies that *Cucumis* species with a basic chromosome number x=12 evolved from those with 7 (1,4). Given that an unusual number of gene duplications have been observed between these groups (3), such duplication of genetic material could have taken place during the change in chromosome number.

In an earlier report (7), we documented the relative activity of 47 general metabolic enzymes and general protein in different tissues of selected botanical varieties of *Cucumis* [*Cucumis sativus* var. *sativus* L. and var. *hardwickii* (Royle) Kitamura]. In addition, we determined that enzyme polymorphisms existed in GPI, GR, isocitrate dehydrogenase (IDH), peptidase with phenyl-alanyl-proline (PEP- PAP), phosphogluconate dehydrogenase (PGD) and phosphoglucomutase (PGM). In this report we compare the electrophoretic phenotypes of several species of *Cucumis* for 16 enzymes [those mentioned above plus acid phosphatase (ACP), alkaline phosphatase (AKP), diaphorase (DIA), esterase (EST), fructose diphosphatase (FDP), glutamic pyruvic transaminase (GPT), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), shikimic dehydrogenase (SKDH), and triose phosphate isomerase (TPI)] coding for 19 loci. The objective of this study was to compare zymograms of each of the loci to determine the magnitude of potential genetic difference between several species of the *anguria* and *sativus* groups as classified by Esquinas-Alcazar.

Cotyledonary extracts of 5 *Cucumis* species of African origin, 3 *Cucumis sativus* var. *hardwickii* and 6 var. *sativus* were examined by horizontal starch gel electrophoresis. Isozyme banding patterns of the enzymes were recorded and comparisons were made among zymograms (Table 1).

Table 1. Electrophoretic variation in 19 enzyme loci of several species in the genus *Cucumis*.

Species or botanical variety	Cultivar name, inbred identification or PI no.[z]	Source[y]	Chromosome no.	Acp	Akp	Dia-1	Dia-2	Est	Fdp	Gpi	Gpt	Gr-1	Gr-2	Idh	Lap	Mdh	Pep-pap	Pgd-1	Pgd-2	Pgm	Skdh	Tpi
C. africanus	299570	S. Africa	24	4	11	2	22	24	11	33	11	--	12	22	2	2	11	22	33	34	12	3
C. anguria	147065	Brazil	24	3	11	3	--	33	11	33	11	--	12	22	3	3	22	22	33	33	22	2
C. ficifolius	196844	Ethiopia	48	2	11	2	--	--	--	23	11	--	12	22	3	4	--	22	33	33	22	1
C. myriocarpus	282447	S. Africa	24	3	12	3	22	33	11	33	22	11	12	22	4	3	22	22	33	33	23	2
C. zeyheri	282450	S. Africa	24	3	12	3	22	33	11	33	22	--	12	22	4	3	22	22	33	33	23	2
C. sativus var. *hardwickii*	183967	Umran, Kashia Hills, India	14	1	11	1	11	11	22	22	33	11	11	11	1	1	22	11	11	11	22	1
	215589	Dehra Dun, India	14	1	11	1	11	11	22	22	33	22	11	11	1	1	22	11	11	11	22	1

C. sativus var. sativus																						
	462369	India	14	1	11	1	11	11	22	22	33	11	11	33	1	1	11	11	11	11	22	1
	Marbel	Royal Sluis	14	1	11	1	11	11	22	22	33	11	11	33	1	1	11	11	11	11	22	1
	Riesenschall	Royal Sluis	14	1	11	1	11	11	22	22	33	11	12	33	1	1	11	11	22	22	22	1
	GY 2*	NCSU	14	1	11	1	11	11	22	22	33	11	11	33	1	1	11	11	11	22	22	1
	1397*	USDA/ARS	14	1	11	1	11	11	22	22	33	11	11	33	1	1	11	11	22	22	22	1
	200815	Burma	14	1	11	1	11	11	22	11	33	12	11	33	1	1	12	11	11	11	22	1
	188807	Philippines	14	1	11	1	11	11	22	22	33	12	12	33	1	1	11	11	22	11	22	1

[z]Inbreds are identified by "*".

[y]NCSU--North Carolina State University; USDA/ARS-- United States Department of Agriculture/Agricultural Research Service.

[x]Diagramatic representation of phenotypes which have been given a single digit type number are provided in Figure 1.

The allelic nomenclature follows a modified form described by Richmond (5) such that loci coding for ACP, AKP, DIA, EST, FDP, GPI, GPT, GR, IDH, LAP, MDH, PEP-PAP, PGD, PGM, SKDH, and TPI are designated as Acp, Akp, Dia, Est, Fdp, Gpi, Gpt, Gr, Idh, Lap, Mdh, Pep-pap, Pgd, Pgm, Skdh, and Tpi, respectively. Hyphenated numerals refer to multiple loci, numbered from most cathodal to most anodal. Alleles at a particular locus are designated by numerals numbered from most cathodal to most anodal. As an example, the combination of homomeric protein products of the locus GR-1, which has at least 2 alleles (1 and 2), produce a heteromeric product in a heterozygous individual which is designated GR-1 (12). Where no immediate genetic interpretation of the zymograms could be made, individual zymogram patterns were given type numbers (Fig. 1).
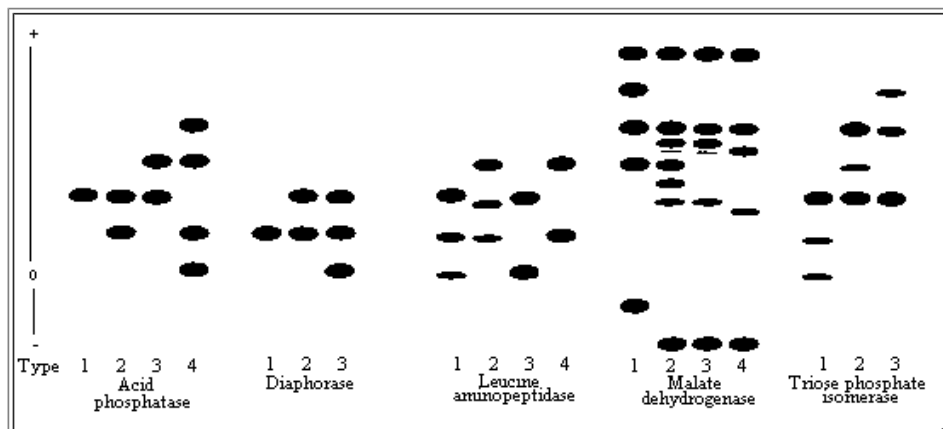

Figure 1. A diagramatic representation of electrophoretic variants observed in acid phosphatase, diaphorase, leucine aminopeptidase, malate dehydrogenase, and triose phosphate isomerase. Type numbers are given to identify each zymogram. The position of an isozyme does not reflect its relative mobility with regard to isozymes of other enzymes. Therefore, comparisons of relative isozyme mobilities should be made within and not between enzymes.

Electrophoretic phenotypes of all collections grouped as *sativus* were monomorphic for Acp, Dia, Est, Fdp, Gpt, Lap, Mdh, Pgd-1, Skah and Tpi, in which zymograms were single banded (Table 1). Phenotypic variation among botanical varieties was observed in Gpi, Gr-1, Gr-2, Idh, Pep-pap, Pgd-2 and Pgm. Those species grouped as *anguria* were monomorphic for Fdp, Gr-2, Idh, Pgd-1 and Pgd-2. If comparisons are made between groups, unique patterns (types) or alleles exist for Acp, Dia-1, Dia-2, Est,.Fdp, Gpi, Gpt, Idh, Lap, Mdh, Pgd-1, Pgd-2, Pqm and Tpi in the *anguria* group which do not appear in the *sativus* group.
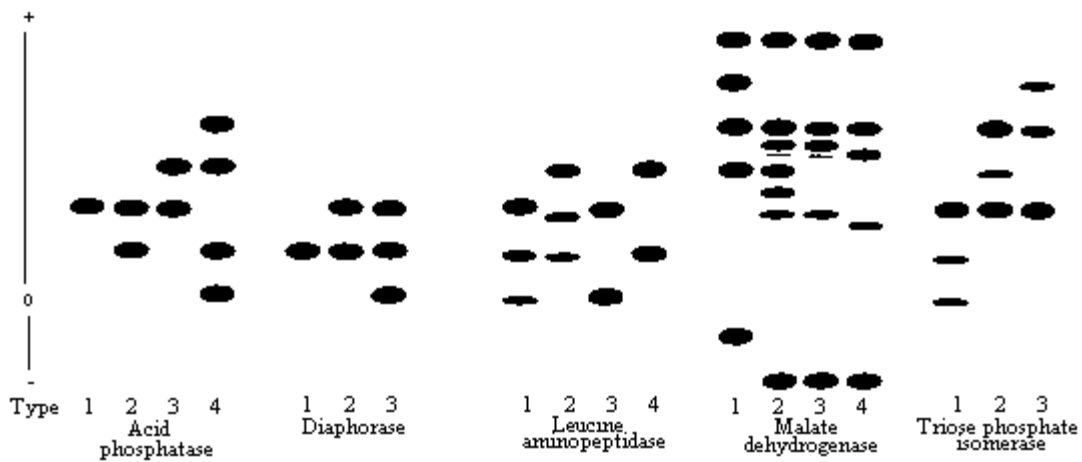
These data indicate that, although species within the groups share some common alleles, enough difference exists between them to suggest that their genetic distance is certainly as great as Esquinas-Alcazar states. Moreover, comparisons among collections in the sativus group suggest that the genetic relationship between the two botanical varieties is probably much closer than gross morphological differences (6) would lead one to believe. These findings support the hypothesis of Dane (2) and Esquinas- Alcazar (3) regarding the conspecific nature of var. *sativus* and var. *hardwickii*. It would be interesting, with regard to the evolution of the genus *Cucumis*, to examine *C. metuliferus* and *C. longipes* in more detail in order to determine their potential relationship to *C. sativus* var. *hardwickii* and *C. anguria*.

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Type 1 2 3 4   1 2 3   1 2 3 4   1 2 3 4   1 2 3
      Acid   Diaphorase   Leucine   Malate   Triose phosphate
      phosphatase   aminopeptidase   dehydrogenase   isomerase

Effect of End Borders on Plot Yield of Once-over Harvested Pickling and Fresh-market Cucumbers

T.C. Wehner

Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609

Yield of pickling and fresh-market cucumbers from plots harvested once-over is an efficient measure of yield in multiple-harvest trials (Wehner and Miller, unpublished data). Smith et al. (1) found the correlation between once-over and multiple-harvest yield to be less useful (r=0.31) in their test of 9 cultivars of pickling cucumbers. In the early stages of a breeding program where many families are being evaluated for yield, it would be efficient to use small plots harvested once-over as the selection method. Since seed supplies are usually limited, and because plots are small (1.5 x 1.5m was found to be optimal), trials are often run with plots separated by 1.5 m alleys to make it easy to identify the area to be harvested. It would, therefore, be important to know that end borders on plots do not affect the ranking of families for yield. The objective of this study was to determine whether yield of different genotypes of pickling and fresh-market cucumbers is affected by plot end borders.

Methods. Six cultivars and lines of pickling cucumbers ('Calypso', 'Castlepik', 'Clinton', 'NCSU M201, 'Pikmaster', and 'Tamor'), and 6 cultivars of fresh-market cucumbers ('Bush Champion', 'Dasher', 'Early Triumph', 'Poinsett 76', 'Sprint 440', and 'Verino') were chosen to represent the diversity of genotypes often used in breeding programs. The types represented included hybrid vs. inbred, gynoecious vs. monoecious, and tall vs. dwarf. The plots were planted on raised, shaped beds with 0.5 m tops and 1.5 m apart (center to center) on April 29 at the Horticultural Crops Research Station near Clinton, NC. Plots were 1.5 m long with either 1.5 m alleys or 1.5 m borders at each end. Harvest areas were marked with flags. Plots were harvested once-over when most of the plots reached 10% oversize (June 28 for the pickling cucumbers, and July 6 for the fresh-market cucumbers). Fruit were graded into marketable and cull and counted to determine total yield and yield per plant. The experiment design was a split-split-plot in a randomized complete block with 6 replications. Whole plots were the 2 crop types (pickling vs. fresh-market cucumbers), subplots were the 6 cultivars and lines, and sub-subplots were the 2 border treatments (ends vs. none).

Results. In the absence of end borders, the number of total and marketable fruit per plot was inflated 7 and 19%, respectively (Table 1). Plots with end borders had a higher percent of cull fruit as well (35% more). However, there was no significant interaction between cultivar and border treatment. Therefore, if end borders are left off plots, yield will be inflated, but the ranking of cultivars or lines will not be significantly affected. Thus, plant breeders can safely choose to plant small plots without end borders in order to make it easier to identify the harvest area, or to save seed where supplies are limited.

Table 1. Fruit yield of 12 cultivars and lines in 1.5m plots harvested once-over where plots had either 1.5 m of border or of alley at each end.[z]

Cultivar or line	Plot end treatment	Yield (fruit/plot)		Culls (%)	Fruit per plant	Plants in harvest area
		Total	Marketable			
Pickling Cucumbers						
Calypso	Ends	20	18	8	1.71	12
	None	25	24	4	1.91	13
Castlepik	Ends	24	22	10	1.70	14
	None	26	24	8	2.00	13
Clinton	Ends	18	18	0	1.52	12
	None	17	17	0	1.49	12

M21	Ends	22	21	3	1.65	13
	None	24	22	5	1.70	14
Pikmaster	Ends	20	18	7	1.54	13
	None	25	24	6	1.81	14
Tamor	Ends	19	17	11	1.73	12
	None	23	21	8	1.76	13
Fresh-market cucumbers						
Bush Champion	Ends	16	13	20	1.31	12
	None	17	15	12	1.41	12
Dasher	Ends	19	17	10	1.41	14
	None	23	21	7	1.79	13
Early Triumph	Ends	20	19	8	1.49	14
	None	27	26	3	1.92	14
Poinsett 76	Ends	17	14	17	1.28	13
	None	18	17	8	1.42	13
Sprint 440	Ends	20	18	9	1.58	11
	None	22	20	12	1.79	11
Verino	Ends	22	19	10	1.79	12
	None	27	24	11	2.11	13
F ratio (line x treatment)		1.06^{ns}	1.20^{ns}	1.52^{ns}	0.58^{ns}	0.69^{ns}
LSD (5%)		3	3	4	0.27	1
CV (%)		17	17	65	20	12
Mean	Ends	20	18	9	1.60	13
	None	21	21	7	1.76	11

[z]Data are means over 6 replications.

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Variation for Yield within Locations in Homogeneous and Heterogeneous Cucumber Populations

Todd C. Wehner

Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609

Environmental variation is an important source of error in evaluating yield of lines in small-plot trials. Smith et al. (2) found a large effect of environment for yield (fruit number per plot) in once-over harvested plots, and measured the narrow-sense heritability to be only 0.17. Environmental variation can be overcome by use of multiple years, locations and replications for yield trials. However, limited seed supplies and the large numbers of families to be evaluated in the early stages of a breeding program preclude the use of multiple test environments. The objective of this study was to determine the variability of unreplicated plots of genetically homogeneous or heterogeneous populations.

Methods. The pickling cucumber 'Calypso', without pollinator, and the diverse pickling cucumber population, NCMBP, were used as the homogeneous and heterogeneous populations, respectively. NCMBP is the North Carolina Medium Base Pickle population, which was developed by intercrossing most of the known pickling cucumber cultivars and lines in isolation for 3 years. The populations were planted on raised, shaped beds with 0.5 m tops and 1.5 m apart (center to center). The plots were 1.5 m long, and were planted on May 23, at the Horticultural Crops Research Station near Clinton, NC. The plots were overplanted and thinned to 15 plants at the first true leaf stage. Plots were harvested when most had 10% oversize (>50mm diameter) fruit in them, and the number of fruit per plot counted. Yield data were considered missing in plots where the plant stand was at all questionable.

Results. The homogeneous pickling cucumber population made up of the hybrid cultivar, 'Calypso', had yields that varied from 9 to 35 fruit per plot in 150 plots measured (Table 1). The variation for yield among plots of 'Calypso' is all caused by environmental effects. The heterogeneous population, NCMBP, had a greater range for yield than 'Calypso', with 10 to 56 fruit per plot in the 153 plots sampled (Table 2). However, using the data from the plots of 'Calypso', 56% of that range could be accounted for by environmental effects. Thus, it is important to recognize the strong effect the environment has on yield of small plots and to take steps to account for that variability. Replication, correction of plot yield using neighboring plots, or subdivision of the field into smaller units for selection as done by Gardner (1) are approaches that should be considered to help solve the problem of environmental variation within yield trials. while this information has been known for some time, it is more convincing when demonstrated directly as in Tables 1 and 2.

Table 1. Total fruit per plot in 25 tiers of 6 rows of 'Calypso'.[z]

Field tier	Field row					
	3	**4**	**12**	**13**	**21**	**22**
1	24	23	21	35	15	24
2	29	24	25	25	27	19
3	20	26	24	26	27	25
4	23	24	29	31	25	25
5	12	28	23	27	28	26
6	24	24	30	22	29	30
7	25	24	-	20	24	24
8	24	27	21	28	28	21
9	28	24	-	27	30	26
10	35	32	25	23	27	22

11	16	24	28	30	30	24
12	27	28	24	31	24	21
13	24	28	-	-	24	28
14	24	25	-	25	23	29
15	28	21	26	29	27	20
16	21	26	-	21	21	25
17	21	25	21	24	30	26
18	22	25	20	-	-	24
19	21	28	28	-	24	28
20	20	26	28	27	28	22
21	25	25	22	23	15	20
22	16	21	29	-	22	20
23	21	24	30	22	22	24
24	11	18	30	28	22	30
25	9	18	25	28	-	19
Mean	25					
Standard deviation	4					
Range	9-35					

[z] Plots (1.5 m long) harvested at 10% oversize.

Table 2. Total fruit per plot in 17 tiers of 9 rows of the North Carolina Medium Base Pickle Population.[z]

Field tier	Field row								
	1	2	3	4	5	6	7	8	9
1	33	37	26	48	27	35	40	43	28
2	36	32	36	41	27	28	25	10	23
3	43	40	38	32	32	33	44	36	35
4	25	47	40	56	38	27	40	35	34
5	28	43	33	40	39	33	51	32	28
6	45	37	31	40	39	12	41	23	39
7	44	44	41	42	42	37	49	35	41
8	38	45	46	53	33	29	38	42	37
9	38	29	40	43	48	16	38	33	42
10	39	44	52	46	40	27	30	50	43
11	31	34	35	21	40	39	33	45	39
12	45	31	34	44	35	26	33	43	45
13	41	34	45	46	34	24	27	45	38
14	39	22	31	48	37	21	40	37	36
15	35	37	26	56	52	25	46	38	45
16	25	47	36	46	38	23	56	38	37
17	36	34	27	43	32	28	39	43	27

Mean	37	
Standard deviation	8	
Range	10-56	
[z] Plots (1.5 m long) harvested at 10% oversize		

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Optimum Plot Size for Once-over Harvest of Pickling and Fresh- market Cucumbers

T.C. Wehner and W.H. Swallow

North Carolina State University, Raleigh, NC 27695

An efficient method for measuring yield in cucumbers is to count the fruit number in small plots harvested once-over. This can be done with both pickling and freshmarket cucumbers, and is a useful predictor of yield in multiple-harvest as well as once- over harvest conditions (Wehner and Miller, unpublished data). Optimum plot size for once-over harvest of pickling cucumbers has been estimated to be 1.5 x 2.4 m, based on data from cultivar yield trials (2). However, we wished to estimate optimum plot size for both pickling and fresh-market cucumbers using data from uniformity trials where only one cultivar of each type was tested, and to compare estimates from different fields and years. Furthermore, we wished to compare optimum plot sizes under conventional once-over harvesting methods with those under new labor-saving techniques for simulating once-over harvest using paraquat to defoliate the plots before evaluation. The objective of this study was then to estimate optimum plot size for pickling and fresh-market cucumbers grown in uniformity trials in 1982 and 1983 using labor costs for both conventional hand-pulled plots and herbicide-defoliated plots.

Methods. The experiment was planted at the Horticultural Crops Experiment Station near Clinton, North Carolina, on July 22, 1982, and May 23, 1983. Rows were seeded on raised, shaped beds 1.5 m apart center to center using 'Calypso' and 'Slicemaster' as the pickling and fresh-market cucumber cultivars, respectively. Plots were thinned to 15 plants at the first-leaf stage. Rows were harvested in 1.5 m increments and the total number of fruit counted in each plot on September 2 and 9, 1982, and on July 12 and 14, 1983, for pickling and fresh- market cucumber plots, respectively. For each year and crop, optimum plot size for judging yield as number of fruit per plot was determined by the method of Smith (1), using estimated costs (man-hours required) to conduct the trial with either conventional (hand-pulled plots) or herbicide-defoliation (paraquat) methods.

Results. The number of man-hours required to perform each operation in conventional and herbicide-defoliated yield trials is shown in Table 1; for both methods the greatest labor costs were in planting and harvesting. Estimates of optimum plot size when costs were taken into account are given in Table 2. The 1982 and 1983 estimates of optimum plot size for pickles, and the 1983 estimate for fresh-market cucumbers, were in close agreement and recommend small plots, about the size of the basic 1.5 x 1.5 m unit used in this experiment. The 1982 fresh-market estimate of optimum plot size is much larger; of the 4 plantings this one had the least variable yield data, but there was little correlation between yields of neighboring plots, so larger plots became worthwhile. The conventional (hand-pulled plots) method, being more labor-intensive, had smaller optimum plot size than the herbicide-defoliated (paraquat) method for all crops and years.

Table 1. Labor required (man-hours) for a once-over harvest trial measuring yield in cucumbers using 2 methods (conventional hand-pulled plots and paraquat-defoliated plots).

Operation	Conventional hand-pulled plots		Paraquat-defoliated plots	
	K_1	K_2	K_1	K_2
Field plan	.0032	0	.0032	0
Seed packeting	.0024	0	.0024	0
Planting	.0119	.0358	.0119	.0358
Thinning and stand counting	.0014	.0082	.0014	.0082
Harvesting	.0230	.1493	.0287	.0431
Data Analysis	.0096	0	.0096	0
Sub Total	.0515	.1933	.0572	.0871

Total	.2448	.1443

K_1 = cost per plot independent of plot size

K_2 = cost per plot based on 1.5 x 1.5 m plots; also the added cost for each 1.5 x 1.5 m increase in plot size

Table 2. Estimated optimum plot size for a once-over harvest trial measuring yields in pickling and fresh-market cucumbers for 2 methods (conventional hand-pulled plots and paraquat-defoliated plots).

Type	Year	Smith's b	Optimum Plot Size (in units of 1.5 x 1.5 m plots)	
			Conventional hand-pulled plots	Paraquat-defoliated plots
Pickle	1982	.656	0.51	1.25
	1983	.660	0.52	1.27
Fresh-market	1982	.950	5.06	12.48
	1983	.638	0.47	1.16

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Evaluating Downy Mildew Resistance in *Cucumis melo* L.

Cohen, Y., H. Eyal and A. Cohen

Department of Life Sciences, Bar-Ilan University, Israel

C.E. Thomas

U.S. Department of Agriculture, Agricultural Research Service, U.S. Vegetable Laboratory, Charleston, SC 29407

Downy mildew incited by *Pseudoperonospora cubensis* (Berk. & Curt.) Rostow. is an important disease on cantaloup (*C. melo*) in many production areas of both the U.S.A. and Israel. A major phase of our research has involved the evaluation of *C. melo* germplasm for resistance against this disease. As better sources of resistance have been identified, modifications have been made in our screening procedures to accommodate them. From 1977-82, the yellow vs. brown lesion reaction served as the basic criterion for evaluating young plants inoculated at the 2-leaf stage. This reaction was first described by Barnes and Epps (1) in cucumber, and was elaborated by Thomas in cantaloup (2). The following 1-9 scale has been used by Thomas and others to evaluate gradations of the yellow vs. brown lesion reaction observed:

1. entire leaf necrotic
2. coalesced yellow lesions, general sporulation
3. yellow lesions, moderate to abundant sporulation
4. brown lesions with yellow halo, moderate sporulation
5. large brown lesions, light to moderate sporulation
6. medium-sized brown lesions, light sporulation
7. small brown lesions, trace to very light sporulation
8. pin-prick brown lesions, no sporulation
9. entire leaf healthy, no evidence of infection

The main problem with this rating system was that leaf 1 and leaf 2 did not always react the same. Except in those cases where they did react the same, leaf 1 always exhibited a more susceptible reaction than did leaf 2. Therefore, the one digit rating assigned represented the most severe reaction present to avoid overestimating resistance.

In field plots a 1-9 scale was also used, but this scale rated gradations in amount of observed infection and subsequent disease damage as expressed by numbers of lesions and leaf loss. Since it did not rate lesion (reaction) type, correlations between artificial inoculations in the 2-leaf stage and reactions of older plants in field plots were difficult. Partly because of this difficulty, Thomas (3) used percent leaf loss to more critically describe the reaction of selected plant introductions and cultivars in field plots. This technique was very effective, but labor intensive.

More recently, due to the recognition of a different reaction (lesion) type as an expression of resistance and incorporation of this resistance into our breeding programs, a new protocol has been devised. This reaction type (RT) is characterized by 1 mm, circular, water-soaked, yellow lesions with no apparent sporulation. This protocol, which we refer to as a double rating technique, accommodates the newly recognized RT and better facilitates correlations between reactions of plants inoculated at the 2-leaf stage and older plants in the field.

Recognition of this resistant RT is difficult unless the following inoculation and post-inoculation procedures and conditions are followed. Plants are grown in the greenhouse (day:night temperature 20±2 C: 27±3 C) to the 2-true leaf stage, one plant per pot. The adaxial leaf surfaces are inoculated at about 1800 hr with a 1.0 x 10^4 sporangial suspension with a hand sprayer. Inoculated plants are placed overnight in a high humidity tent and then transferred to the greenhouse bench until seven days after inoculation, when they are returned to the high humidity tent for 20 hr to induce fungal sporulation. Disease

evaluations are made separately for leaf 1 and leaf 2 on each plant using the following index of RT's:

1. 10-15 mm, chlorotic, irregular, heavily-sporulating lesions
2. type '1' lesions, above, mixed with type '3' lesions, below
3. 3-4 mm, irregular to circular, water-soaked, chlorotic, sparsely sporulating lesions
4. 1 mm, circular, water-soaked, yellow lesions with no apparent sporulation

After this evaluation, plants are transplanted to the field (mid- April through mid-September) and inoculated with *P. cubensis* as described above when they reach the 10-leaf stage. Eight to 10 days after this field inoculation, separate evaluations are made for leaves 3-6 and leaves 7-10 using the reaction type index described above.

The above evaluation procedures result in a two digit number to describe the reaction of inoculated plants at either the 2-leaf or 10-leaf stage. The first digit (1-4) expresses the reaction of leaf 1 (or of leaves 3-6) and the second digit expresses that of leaf 2 (or of leaves 7-10) in the greenhouse (or in the field). Observed RT's range from 11, highly susceptible, to 44, highly resistant.

Using this protocol we have observed that on 2-leaf plants in the greenhouse, RT4 on leaf 1 was always associated with RT4 on leaf 2; whereas, RT4 on leaf 2 may have been associated with RT's 2 or 3 on leaf 1. Similarly in the field with 10-leaf plants, RT4 in lower leaves was always associated with RT4 in upper leaves; whereas RT4 in upper leaves may have been associated with RT's 2 or 3 in lower leaves.

F_2 populations of crosses made between a highly resistant breeding line (RT 44 in both greenhouse and field) and the cultivars 'Hemed', 'Ein-Dor' and 'Ananas-Yokneam' (RT's 11 and 12) segregated in greenhouse tests to RT's 11, 12, 13, 23, 24, 33, 34, and 44, with 3.2-12% of the populations showing RT 44. Most plants reacting with a RT 44 in the greenhouse (2-leaf) tests retained a RT 44 in the field until maturity, while none of the plants reacting with RT's 11-33 did so. Occasionally, plants reacting with RT 34 in the greenhouse exhibited a higher degree of resistance (RT 44) in the field.

The double rating technique described above facilitates the selection of highly resistant (RT's 34 and 44) plants both in the greenhouse and in the field. If RT4 is present in the germplasm and its selection is the objective of the evaluation, we recommend use of this technique to identify plants exhibiting RT 34 and 44. If RT4 is not present, we suggest use of the 1-9 rating scale to assess the yellow vs. brown lesion reaction.

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Climacteric and Nonclimacteric Ripening in *Cucumis melo*

Stephen Kendall and Timothy J. Ng

University of Maryland, College Park, MD 20742

Many diverse fruit types exist among cultivated genotypes of *Cucumis melo*. Netted muskmelons and honeydews have been reported to be climacteric, but nonclimacteric genotypes of *C. melo* have also been reported (1). In experiments spanning a 3 year period, we have found that 'Golden Beauty Casaba' (GBC) and C2, a casaba-type breeding line obtained from the Texas A&M Agricultural Experiment Station, are nonclimacteric in their ripening behavior. As the presence and degree of the climacteric may affect storage life, we initiated studies to investigate this phenomenon.

Crosses were made between several genotypes of *C. melo*. 'Perlita' (PER), a cultivar with good shipping quality, and MD63-53, a breeding line which lacks shipping quality, were the netted genotypes. Our experiments had previously shown that MD63-53 undergoes a climacteric prior to fruit abscission whereas PER experiences the climacteric rise after abscission. Table 1 presents data from an experiment dealing with field-grown melons harvested at physiological maturity (stem abscission for netted genotypes, softening of the blossom end for "non-slipping" genotypes) and stored at 10 C. Internal ethylene concentrations were determined by embedding hypodermic needles into the cavity of the fruit and sampling through septa at selected intervals. These results along with other experiments involving stored melons under a continuous air flow have confirmed that nonclimacteric genotypes of *C. melo* do exist and that hybrids between climacteric and nonclimacteric types experience a delayed climacteric when compared to the climacteric parent.

Table 1. Internal ethylene concentrations in genotypes of *Cucumis melo* after harvest when stored at 10 C.

Genotype[z]		Days from anthesis to maturity	Fruit weight (kg)	Internal C_2H_4 concentration (ppm)			
				Day 1	Day 5	Day 7	Day 20
PER	Mean (n=23)	37.2	1.47	20.3	32.4[y]	-	-
	SD	1.2	0.30	5.8	13.9	-	-
C2	Mean (n=7)	44.2	2.52	1.3	1.6	5.4	-
	SD	2.7	0.65	0.2	0.4	1.0	-
GBC	Mean (n=9)	42.4	3.33	nd[x]	0.1	0.3	4.1
	SD	2.9	1.10	-	0.1	0.6	2.5
PER x MD	Mean (n=14)	34.1	1.78	23.1	35.6	-	-
	SD	0.9	0.31	14.6	34.9	-	-
PER x C2	Mean (n=10)	39.9	2.71	15.5	12.0	16.0	-
	SD	2.0	0.58	6.5	6.0	8.3	-
PER x GBC	Mean (n=7)	48.3	3.65	-	3.7	4.0	42.2
	SD	2.3	1.11	-	3.0	4.3	12.4
MD x PER	Mean (n=9)	33.7	1.57	17.4	14.5	-	-
	SD	1.0	0.34	8.0	5.3	-	-
MD x C2	Mean (n=5)	35.2	2.04	9.3	7.7	-	-
	SD	0.8	0.32	6.4	2.7	-	-

[z]Genotype abbreviations are 'Perlita'(PER), 'Golden Beauty Casaba'(GBC), and MD63-53(MD).

[y]The last ethylene determination for each genotype was made after optimum horticultural maturity had been achieved.

[x]Ethylene was not present at a detectable level.

These differences in ripening patterns may be attributable to genetic differences in the fruit tissues, such as have been reported for ripening mutants of tomato (2). Oxygen availability to the fruit tissue may also be a factor since fruits of all nonclimacteric genotypes did not develop a net; the net in muskmelons is derived from the lenticels during fruit development and provides a channel for gaseous exchange with the surrounding atmosphere. Regardless of the physiological mode of action for this phenomenon, the implications remain that the use of nonclimacteric genotypes in breeding programs could be a valuable tool for genetically increasing the storage life of fruits of *C. melo*.

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Interaction of Zucchini Yellow Mosaic Virus Strains and Muskmelon Lines

Lecoq, H. and M. Pitrat

I.N.R.A., Centre de Recherches Agronomiques d'Avignon, B.P. 94, 84140 Montfavet, France

Zucchini yellow mosaic virus (ZYMV), a recently described member of the potyvirus group (3), is now commonly found infecting cucurbits in various parts of the world (2). It induces severe symptoms including mosaic, leaf deformations, plant stunting, necrosis and fruit alterations.

In muskmelon, two pathotypes were distinguished on 'Doublon' (1). One is pathotype F which causes a rapid wilting of the plant and subsequent general necrosis (WN). The second is pathotype NF which causes vein clearing, yellowing, mosaic and leaf deformations (VcY). Both pathotypes induce VcY symptoms in 'Vedrantais' muskmelon. The WN reaction of 'Doublon' has been attributed to the action of the semidominant *Flaccida necrosis* gene, symbolized (*Fn*) (5).

Muskmelon plant introduction (PI) 414723 is partially resistant to ZYMV (4). Most plants of PI 414723 remain symptomless when inoculated with strain E15 (pathotype NF) or with strain 1318 (pathotype F) and the virus does not become systemic. This resistance is controlled by the dominant gene *Zucchini Yellow Mosaic*, symbolized (*Zym*) (4). Some plants may, however, develop systemic, chloronecrotic lesions from which the virus may be recovered.

Some ZYMV strains, such as the ZYMV type strain from Italy (3) (pathotype NF) and strain E9 (pathotype F), induce systemic, chloronecrotic lesions on all inoculated PI 414723 plants. The virus can be recovered from the apex of plants infected with these strains. From such plants, we have selected in the laboratory ZYMV strains that completely overcome the resistance of PI 414723 and induce on this line typical VcY symptoms.

These results suggest another classification of ZYMV strains based on the symptoms of PI 414723 and 'Doublon' (Table 1).

Table 1. Classification of 6 ZYMV strains according to the reaction of PI 414723 and 'Doublon' muskmelon lines.

PI 414723	Doublon	
	Vein clearing yellowing (Pathotype NF)	Wilt necrosis (Pathotype F)
Symptomless (Pathotype 0)	E15	1318
Systemic, chloronecrotic lesions (Pathotype 1)	ZYMV type, Italy	E9
Vein clearing yellowing (Pathotype 2)	D41	Bo 21

It appears that the ability of a ZYMV isolate to induce the wilting reaction in 'Doublon' is independent of its virulence in PI 414723. The observed variability in the pathogenicity of ZYMV is important, and emphasizes the need for other sources of resistance to ZYMV even though pathotype 2 has not yet been found in the field.

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Evidence of a Recessive Powdery Mildew Resistance Gene in Muskmelon PI 414723

J. D. McCreight

U.S. Department of Agriculture, Agricultural Research Service, P.O. Box 5098, Salinas, CA

Plant Introduction (PI) 414723 (melon aphid, *Aphis gossypii* Glover, resistant inbred of PI 371795) is a source of aphid resistance (1) and zucchini yellow mosaic virus resistance in muskmelon. Observations of greenhouse plantings indicated that it was also resistant to powdery mildew caused by *Sphaerotheca fuliginea* (Schlecht.) Poll. PI 414723 was crossed with 'Topmark' which is susceptible to *S. fuliginea* races 1, 2, and 3. Twenty-five seedlings of PI 414723 and F_1 (PI 414723 x 'Topmark') were inoculated 3X over a 13-day period beginning at the cotyledon stage of growth. The inoculum, cultured on 'Hales Best Jumbo' and 'Topmark', was previously identified on a set differential hosts as *S. fuliginea* race 1 (unpublished data). The plants were rated for powdery mildew 3 and 4 weeks after the 1st inoculation.

PI 414723 was resistant. The F_1 (PI 414723 x 'Topmark') was susceptible, which indicates that the resistance in PI 414723 is conditioned by a recessive gene(s).

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Association of Fruit Quality with Seed Characters and Oil and Protein Content of Muskmelon Seeds

T. A. More and V. S. Seshadri

Division of Vegetable Crops, Indian Agricultural Research Institute, New Delhi 110012, India

Seeds of certain cucurbits are well known sources of oil and protein, although none have been used commercially (1). Madaan et al. (3) reported that muskmelon seeds are a potential source of edible oil. Our objective was to assess the variability of seed oil and protein content, in relation to seed and kernel weight, and total soluble solids (TSS) in muskmelon.

Oil and protein content of 78 collections from 10 countries were expressed as a percent of seed weight and kernel percentage. Oil content was estimated by the cold percolation method of Kartha and Sethi (2). Nitrogen content was determined by the micro- Kjeldhal method, and was expressed as total protein (N x 6.25). Kernel percentage was expressed as a percent of seed weight. Table 1 summarizes the data for each country.

Table 1. Average oil and protein content (% of seed weight), 100- seed weight (g), kernel percentage (of seed weight), and total soluble solids (%) of 78 muskmelons from 10 countries.

Country		Number of collections	Oil	Protein	Seed weight	Kernel percentage	Total soluble solids
Japan		7	26.8	15.5	1.3	54.1	7.0
Hungary		1	21.4	14.5	2.5	45.0	6.9
Israel		2	26.5	16.6	2.9	54.0	-
Bulgaria		1	30.5	19.0	4.0	60.8	9.2
France		3	27.7	15.9	2.4	56.5	6.5
USSR		7	28.4	18.5	2.7	58.9	5.7
USA and Australia	Cantaloupes	15	26.7	15.9	2.4	54.2	7.6
	Honey Dew	1	25.3	16.3	2.4	55.4	7.4
Pakistan		1	34.2	17.9	1.8	65.3	9.5
India	Dessert	31	31.2	16.7	3.2	62.6	8.4
	Non-dessert	9	24.1	14.8	1.5	48.1	4.7
SE (D) +/-			1.90	0.99	0.36	3.64	1.40

Highest oil content was in a collection from Pakistan. Highest protein content was in the Bulgarian collection. Seed weight of the Bulgarian collection was the heaviest, but the collection from Pakistan had the highest kernel percentage. Highest TSS was in the collection from Pakistan.

Non-dessert types of India were lower in oil and protein content, seed weight and kernel percentage, compared to the dessert types. There was not much difference between cantaloupes of USA and Australia, and noncantaloup dessert types of India, for oil and protein content. In that context, our results indicate that dessert types with higher TSS content were superior in seed characteristics.

Most of the correlations among oil and protein content, 100-seed weight, kernel percentage, and TSS were positive and significant (Table 2). The correlations of oil content with kernel percentage and protein with seed weight and kernel percentage were especially high.

Table 2. Correlation coefficients (r) for oil and protein content (% of seed weight), 100-seed weight (g), kernel percentage, and total soluble solids of 78 muskmelons from 10 countries.[z]

	Protein	Seed weight	Kernel percentage	Total soluble solids
Oil	0.52**	0.44**	0.78**	0.36**
Protein		0.89**	0.71**	0.54**
Seed weight			0.49**	0.42**
Kernel percentage				0.45**

[z]Significant at 0.01 (**) levels.

These results suggest that oil and protein content could be improved by selection for high 100-seed weight and high kernel percentage. These data also suggest that selection for dessert qualities resulted in simultaneous improvement in oil and protein content through higher seed weight and kernel percentage.

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Effect of Methodology on Expression of Intercultivar Differences in Response to NaCl Stress in Melons

H. Nerson, H.S. Paris, Z. Karchi, Y. Burger, and M. Edelstein

Department of Vegetable Crops, Agricultural Research Organization, Newe Ya'ar Experiment Station, P.O. Haifa, Israel

Most vegetable crops are sensitive or moderately sensitive to salinity (1). Melons are classified as moderately tolerant (3) and are a potential crop for irrigation with saline water (2).

Sources of salt tolerance within a crop must be identified as the first step in a breeding program whose goal is to develop cultivars having higher salt tolerance. Several methods of screening and selection for salt tolerance have been evaluated for many crops (4).

Our objective was to compare responses of four melon accessions from diverse genetic backgrounds to salinity stress induced by three screening methods, and to relate these results to field test results. Accessions compared were the Israeli muskmelon 'Galia', the Israeli casaba 'Noy 'Amid', the European casaba 'Rochet', and a muskmelon introduction from Iran designated "Persia 202". The three screening methods were: (a) Seedlings were grown in 250 ml pots filled with fine gravel which were put in slightly larger pots containing Hoagland solution. At the first leaf stage (about ten days after emergence), NaCl at the rate of 10,000 ppm was added to the nutrient solution, which was renewed every other day. The pots were placed in growth chambers at two temperature regimes (day/night), 31C/23C and 23C/15C, with a 12-hour photoperiod. (b) Similar to *a* except that the pots were filled with a 4:1 (by volume) mixture of local silty loam:vermiculite at two salinity levels, 10,000 and 15,000 ppm, and (c) Seedlings were grown in larger pots (600 ml), in the greenhouse, with the same mixture as in *b*. NaCl at 15,000 ppm was introduced at the same developmental stage as in *a* and *b* but by daily application of 50 cc/pot in the nutrient solution.

Fourteen days after the beginning of salinization, plants were analyzed for growth, the data being presented in Table 1. Comparison of control results among the various methods revealed three different levels of seedling growth. The most favorable conditions were in *c* (larger pot, fertile medium, and high radiation) while the poorest were in *a* (small pot, hydroponic, inert medium, and relatively low radiation). Differences among accessions in response to NaCl stress were inconsistent among the three methods. For a given method, the most clear-cut difference occurred in *c*, where 'Rochet' was far superior to the other accessions tested.

Table 1. Effect of three methods of NaCl application on absolute growth (mg dry weight) and relative growth (% of control) of four melon accessions, 14 days after salinity introduction.

Accession	Salinity level (ppm NaCl)	Method[z]					
		a		b		c	
		mg	%	mg	%	mg	%
Galia	0	203		437		1110	
	10,000	148	73 a	274	63ab		
	15,000			252	58 b	400	36 b
Noy 'Amid	0	201		555		1149	
	10,000	123	61 ab	380	68 ab		
	15,000			321	58 b	454	39 b
	0	231		469		991	

Persia 202	10,000	129	56 b	340	72 a			
	15,000			285	61 ab	368	37 b
Rochet	0	276		512		1109	
	10,000	162	59 ab	316	62 ab		
	15,000			304	59 b	695	63 a

ZAverage of temperature regimes (see text). Mean separation in relative growth (%) columns by Duncan's multiple range test, 5% level.

The two casabas, 'Rochet' and 'Noy Amid', were compared in a field experiment for sensitivity to salinity. Expressed in terms of percent yield reduction, 'Rochet' was less sensitive to salinity than 'Noy 'Amid', consistent with the performance of these two cultivars in method *c*. This suggests that screening for salt tolerance should be conducted under as near to optimal growing conditions as possible. Such conditions would reduce confounding effects of various other environmental stresses and enable each accession tested to express its full salt-tolerance potential.

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Linkage Studies in Muskmelon

Pitrat, M.

I.N.R.A., Centre de Recherches Agronomiques d'Avignon, B.P. 94, 84140 Montfavet, France

Until now only 4 gene linkages have been described in muskmelon: (1) *bush* (*b*) and *yellow virescent* (*yv*) - recombination 19.4% (5); (2) *virus aphid transmission* resistance (*Vat*) and *Flaccida necrosis* (*Fn*) - recombination 11.6%; (3) *red* stem (*r*) and *male sterile-1* (*ms-1*) - recombination 25.6% (1); and (4) *Zucchini yellow mosaic* virus resistance (*Zym*) and *andromonoecious* (*a*) - recombination 13.1% (3).

A program was begun to find linkage relationships between the following genes:

1. *Fusarium oxysporum* f. sp. *melonis* resistance: *Fom-1* (resistance to pathotypes 0 and 2) in 'Doublon' and *Fom-2* (resistance to pathotypes 0 and 1) in PI 414723 (supplied by G.W. Bohn).
2. *Watermelon mosaic virus*-1 resistance (*Wmv*) in PI 414723.
3. *Necrotic spot virus* resistance (*nsv*) to muskmelon necrotic spot virus in VA 435 supplied by Virginia Truck Expt. Sta.
4. *Zucchini yellow mosaic* virus resistance (*Zym*) in PI 414723.
5. *Virus aphid transmission* resistance (*Vat*) in PI 414723 or PI 161375.
6. *Powdery mildew* resistance-*X* (*Pm-X*) resistance to *Sphaerotheca fuliginea* race 2 in PI 414723 (exact powdery mildew resistance gene is under study).
7. *glabrous* (*gl*) in Arizona *gl B* supplied by R.E. Foster.
8. *yellow green* (*yg*) in Arizona *gl B*.
9. *red* stem (*r*) in 30569 supplied by J. D. McCreight.
10. *bush* (*b*) in 'Topmark Bush' supplied by F. W. Zink.

F_2 data from 4 of 19 gene combinations among these 10 genes studied to date strongly indicated linkage. The recombination fractions for these 4 gene pairs were calculated using the maximum likelihood method (Table 1). F_2 data indicated independent assortment between the other 15 gene pairs (Table 2).

Table 1. Linkage between 4 gene pairs in muskmelon.

Genes		Phase[z]	Number of plants observed in the expected dihybrid ratio				Chi square	Recombination	
			9	3	3	1		Value	Standard error
Fom-1	Wmv	R	104	52	44	0	19.876	0.0	7.07
Fom-2	yg	R	161	10	17	53	145.309	11.4	6.34
Zym	Pm-X	C	110	47	40	0	15.314	0.0	7.12
r	gl	R	367	106	128	8	27.745	30.9	3.61

[z]C=coupling; R=repulsion.

Table 2. Independent assortment between 15 gene pairs in muskmelon.

Genes		Phase[z]	Number of plants observed in the expected dihybrid ratio				Chi square
			9	3	3	1	
Fom-1	gl	R	159	61	51	19	1.163
Wmv	g	R	113	31	36	13	0.999
Vat	Zym	C	107	36	29	17	3.461

Wmv	nsv	R	111	32	45	12	2.347
gl	yg	C	1177	394	377	141	1.402
yg	b	R	138	41	49	19	1.594
yg	nsv	R	167	62	56	19	0.550
Pm-X	gl	R	151	54	44	7	6.486
Zym	nsv	R	96	40	35	18	4.834
Vat	b	R	100	38	32	11	0.638
Vat	nsv	R	105	48	32	14	4.444
b	gl	R	141	47	46	13	0.428
b	r	R	108	31	31	9	1.350
gl	nsv	R	178	50	50	24	3.330
Zym	b	R	83	25	23	12	1.796

[z]C=coupling; R=repulsion.

Vat was already found independent from *Fom-1*, *Fom-2* and *ms-1* (2), *Zym* independent from *Fom-1* and *Fom-2* (3) and *Fom-l* independent from *Fom-2* (4). The muskmelon linkage groups may tentatively be summarized from these and published data:

- Linkage group 1 : *b - yv*
- Linkage group 2 : *Vat - Fn*
- Linkage group 3 : *ms-1 - r - gl*
- Linkage group 4 : *Zym - a - Pm-X*
- Linkage group 5 : *Fom-l - Wmv*
- Linkage group 6 : *Fom-2 - yg*.

Gene *nsv* is independent from linkage groups 2 to 6. Its independence with group 1 is under study. The order in groups 3 and 4 is still unknown.

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Use of n-Pentane for Mixing Melon Pollen

Risser, Georgette and J.C. Rode

I.N.R.A., Centre de Recherches Agronomiques d'Avignon, 84140 Montfavet, France

Hand pollination of a melon flower with a mixture of pollen from several plants is difficult because melon pollen is sticky and aggregates. Our objective was to find a non-toxic solvent that could be used to wash and mix pollen from anthers of several plants for subsequent use in pollination.

In 1981, several solvents were tried without success, but in 1983 good results were obtained with n-pentane which had been reported to be useful for collecting *Berberis* pollen (1).

Pollen is washed from anthers and mixed with pollen from other plants by washing anthers in a watchglass filled with n-pentane. The pollen grains settle to the bottom of the glass, and the n- pentane evaporates very quickly.

We have used pollen collected in n-pentane up to 2.5 hr after collecting and had good fruit set and seed set. In 1984, we will determine how long muskmelon pollen is suitable for pollination after collecting in n-pentane.

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Inoculation Conditions to Evaluate Resistance to *Alternaria cucumerina* (Ellis & Everh.) Elliot in Muskmelon

Thomas, Claude E.

U.S. Department of Agriculture, Agricultural Research Service, U.S. Vegetable Laboratory, 2875 Savannah Highway, Charleston, SC 29407

Alternaria cucumerina incites leaf blight of various cucurbits throughout the world (2). This disease is particularly severe on muskmelon (*Cucumis melo* L.) in the southeastern and Midwestern production areas of the U.S.A. Studies by the author into disease control (4) and epidemiology of *Alternaria* leaf blight have developed information of use to those interested in the evaluation of muskmelon germplasm for resistance to this disease. The objectives of the studies reported herein were to develop practical inoculation procedures with *A. cucumerina* based on inoculum concentration, temperature, and duration of leaf wetness.

A. cucumerina was grown on V-8 juice agar under fluorescent illumination in an alternating regime of 8 hr light- 16 hr dark. Spores were harvested from 10-14-day-old cultures by flooding the surface of the media with sterile distilled water and scraping with a large glass coverslip to detach the conidia. The resultant suspension was then thoroughly mixed and clumps were dispersed by a 15-second treatment in the microcup of a Waring blender at high speed. The suspension was then diluted to the desired concentration. It was kept agitated to prevent settling of the conidia during this procedure.

Conidial suspensions were sprayed onto leaves using a DeVilbiss No. 15 atomizer or a Paasche Type H airbrush. The first two expanded leaves of ten 21-day-old 'Perlita' plants were sprayed to run-off and the plants were then placed in a high humidity chamber at the desired temperature for the duration of the leaf wetness period. At the end of the leaf wetness period, plants were removed and placed under fluorescent, supplemented by incandescent, illumination for 12-hr photoperiods at 20 C. The sixth night after inoculation, plants were placed back in the high humidity chamber for 16 hr at 20% to induce sporulation, which was observed but not evaluated in these studies. This high humidity treatment also makes severely diseased and desiccated leaves easier to handle during the evaluation process. Plants were removed from the chamber and determinations of lesion size, number, and type were made on the seventh day. All tests were repeated at least twice.

Inoculum concentration study. Inoculations were made with inoculum concentrations (conidia/ml) of: 0.5×10^3, 1.0×10^3, 2.0×10^3, 3.0×10^3, 4.0×10^3, 5.0×10^3, 6.0×10^3, 7.0×10^3, 8.0×10^3, 9.0×10^3, 1.0×10^4, 2.5×10^4, 5.0×10^4, 7.5×10^4 and zero (noninoculated check). Inoculated and check plants were then placed at high humidity for 16 hr at 20%. At inoculum concentrations of 2.5×10^4 and higher, many inoculated leaves were dead and desiccated by the sixth day. Lesions on those which were still alive were so numerous and coalesced that accurate determinations of number and size were extremely difficult. At 1.0×10^4 numerous coalesced lesions also precluded accurate determinations. As inoculum levels decreased, the number of coalesced lesions decreased, so that at 5.0×10^3 conidia/ml and lower, almost all lesions were discrete. At concentrations below 5.0×10^3, however, leaves without lesions (escapes) were encountered with increasing frequency as inoculum levels decreased. At 0.5×10^3 about 25% of the inoculated leaves escaped infection. Therefore, for subsequent studies, an inoculum concentration of 5.0×10^3 was used since it gave the most discrete lesions with minimal coalesced lesions without escapes. Carmody et al. (1) reports an optimum conidia concentration of 3.0×10^3 at 27C for leaf infection. Norton and Boyhan (3) used 2.0×10^4 at 25 C. Since these researchers were using the same *A. cucumerina* isolate (furnished by C.E. Thomas) in their work, the differences in inoculum concentrations are probably due to differences in temperature, duration of high humidity period, and plant age.

Temperature study. Ten plants were inoculated as above and were immediately placed under high humidity in dark

chambers at either 5, 10, 15, 20, 25, 30 or 35 C for 16 hr. Only a trace amount of infection occurred at either 5 C or 35 C. Infection was highest at 10 C and declined as temperature increased. A temperature of 20 C was chosen for use in subsequent studies. This temperature represents the lowest night temperature that one would reasonably expect to encounter with any frequency during a muskmelon growing season in areas where this disease occurs.

Leaf wetness study. Plants were inoculated as above and placed in the dark at high humidity at 20 C. Ten plants were removed from the high humidity chamber at 2 hr intervals from 2- 24 hr. The results of duration of leaf wetness studies (Figure 1) are given as totals for 20 leaves at each wetness treatment. There was no lesion development from the 2 hr treatment. Less than one lesion/leaf developed from the 4, 6, and 8 hr leaf wetness durations. Significant lesion development did not occur until 10 hr, and the number of successful infections increased sharply as the hours of leaf wetness increased. However, after 16 hr, the increased level of successful infections resulted in increased levels of coalesced lesions which made accurate comparisons difficult without careful examination of these lesions at magnifications of 25-50 x. Since this would not be conducive to a rapid evaluation technique, 16 hr of leaf wetness is recommended as the maximum duration.

Figure 1. Effect of duration of leaf wetness period on lesion development on *C. melo* by *A. cucumerina* at 20 C.
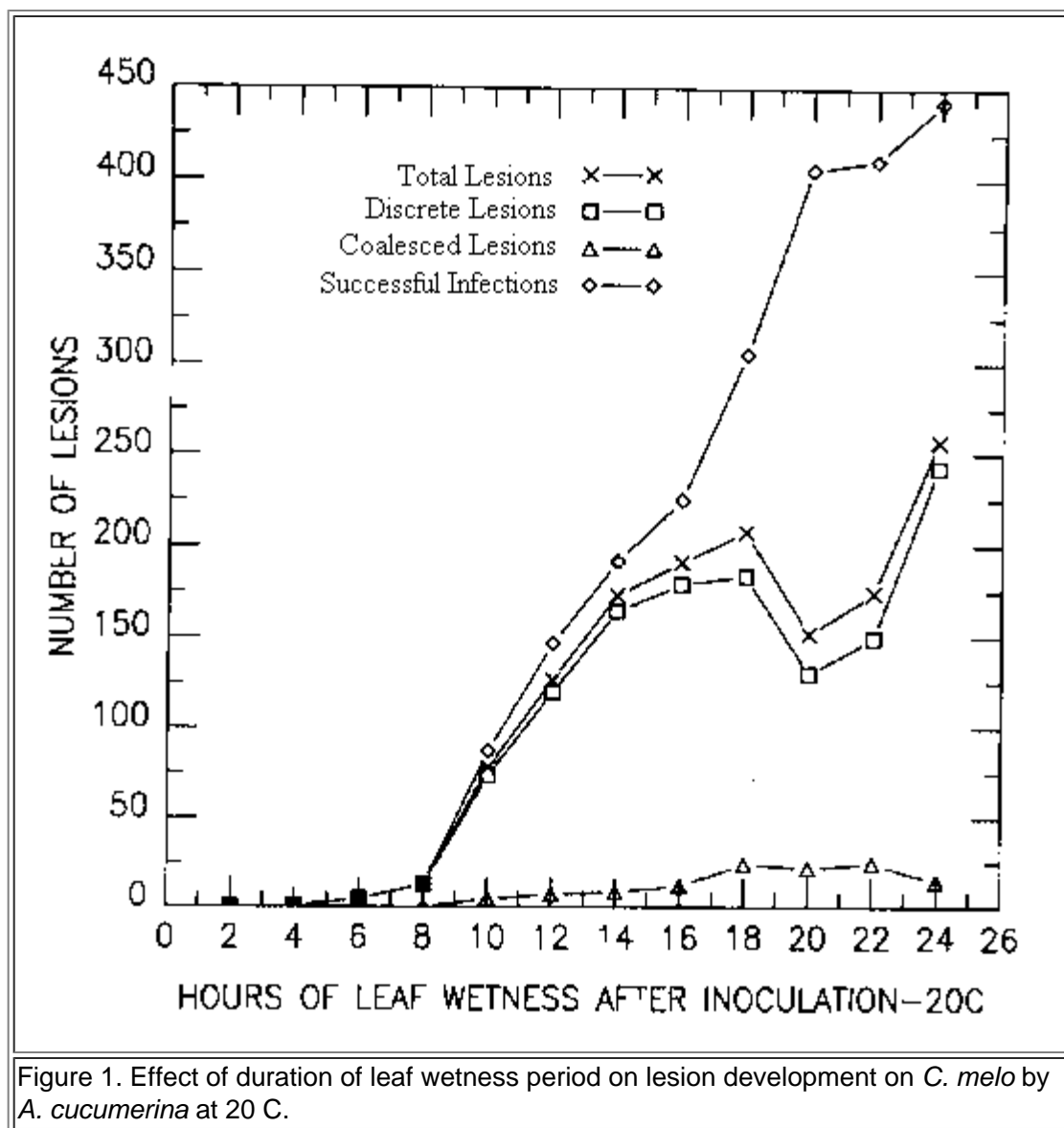
In summary, the recommended inoculation conditions for evaluation of *C. melo* against *A. cucumerina* at the two expanded leaf stage are: inoculum concentration of 5.0 x 10^3, 20 C, and 16 hr duration of leaf wetness in the dark. After this inoculation treatment, plants may be kept at constant temperatures or at usual greenhouse temperatures until they are evaluated. If sporulation is to be rated, a uniform 20 C, 16 hr leaf wetness, dark treatment should be provided on the sixth night after inoculation prior to evaluations on the seventh day.

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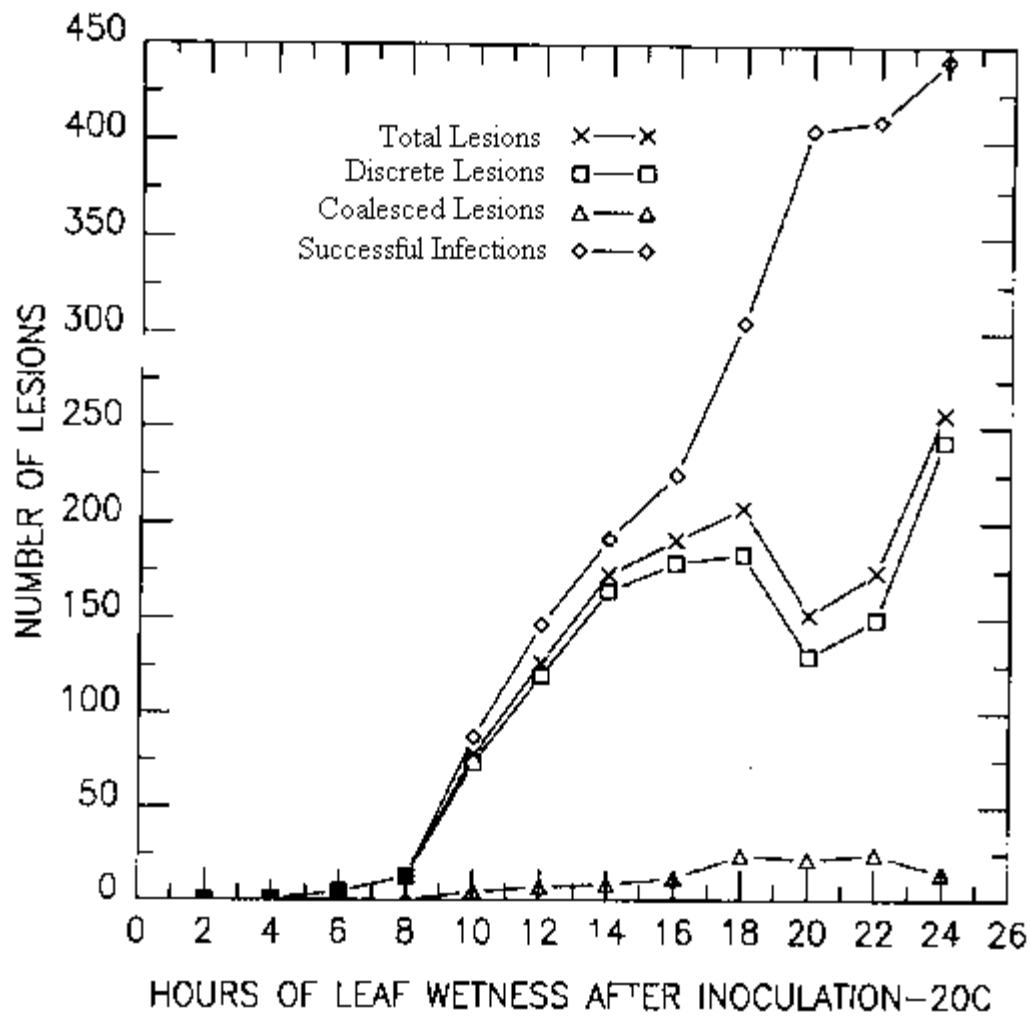
The Importance of Monitoring Races of Powdery Mildew on Muskmelon

Thomas, C.E., A.N. Kishaba, J.D. McCreight, and P.E. Nugent

USDA/ARS, U.S. Vegetable Laboratory, Charleston, SC 29407 (first and fourth authors), USDA/ARS, Boyden Entomological Laboratory, Riverside, CA 92521 (second author); and USDA/ARS, U.S. Agricultural Research Station, Salinas, CA 93915 (third author)

Powdery mildew is a widespread and often production limiting disease of muskmelon (*Cucumis melo* L.). Resistance against it is one object of many breeding programs. For many years the incitant fungus was considered to be *Erysiphe cichoracearum* DC ex Merat. All recent reports, however, indicate *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll. as the incitant. Sitterly (2) presents an excellent review of this situation. Three races of the powdery mildew pathogen have been demonstrated to occur in the U.S.A. (2, 4). The importance of monitoring these races, especially to breeding programs, is explained below.

In 1980 the authors began a cooperative research effort to determine which races of powdery mildew occurred on muskmelon in different geographic areas of the U.S.A. The differential cultivars listed by Thomas (4) and other cultivars with known reactions to individual races have been evaluated repeatedly for their reaction to powdery mildew in both the greenhouse and field at Brawley, Salinas, and Riverside, California; Charleston, South Carolina; and Weslaco, Texas.

Thus far we have confirmed a shift in the natural population of powdery mildew at Brawley, California, from a predominance of race 2 in 1982 to race 1 in 1983 and several shifts back and forth from race 3 to race 1 at Weslaco, Texas from 1980 through 1982. In 1983 we detected a shift in our greenhouse culture of powdery mildew at Charleston, South Carolina from race 3 to race 1. This shift occurred within a period of a few weeks and would have gone undetected for some time had we not maintained differential cultivars in the greenhouse.

Due to the population shifts that we and Sowell (3) have detected and the rapidity with which these shifts can occur, we recommend that races of the powdery mildew pathogen on muskmelon be monitored in research efforts, especially breeding programs. Table 1 lists the minimum recommended cultivars and their reactions to races 1, 2, and 3 of powdery mildew to accomplish this monitoring. The senior author will furnish small quantities of seed of these cultivars to interested researchers who are willing to share with us the reactions of these cultivars in their areas.

Table 1. Reactions of cantaloup cultivars to races of powdery mildew incited by *Sphaerotheca fuliginea*.

Cultivar	Reaction		
	Race 1	**Race 2**	**Race 3**
Hale's Best Jumbo	susceptible	susceptible	susceptible
PMR 45	resistant	susceptible	susceptible
PMR 6	resistant	resistant	susceptible

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A Preliminary Report on Screening Watermelons for Resistance to Watermelon Mosaic Viruses 1 and 2

Munger, H.M., T.A. More, and S. Awni

Cornell University, Ithaca, NY 14853.

About 150 watermelon seed lots planted in November, 1982, were inoculated as 3-week seedlings with WMV-1 and WMV-2. Except for about 10 entries, all were PI's supplied by the Regional Plant Introduction Station at Experiment, Georgia. For most entries 5 or 6 seedlings were inoculated with each virus.

Following inoculation mottling appeared on the leaves of nearly all the seedlings and differences were not readily apparent initially. In a few weeks certain entries showed much greater growth and diminished mottling. The best plant in each of the best entries was selected for transfer to a pot and carried through the winter. Most selected plants survived until May, 1983, when they were rated for resistance and several cuttings of each rooted. The surviving entries and their ratings are shown in Table 1.

Table 1. Watermelons planted Nov. 10, 1982, 6 plants inoculated with WMV1 and 6 with WMV2 on Dec. 2. Best plant saved from each entry below and held for field planting in 1983.

Cornell test no.	Entry	Origin	Resistance rating (May 1983)		O.P. seed from WMV2 isolation	Edibility
			WMV1	WMV2		
83	PI 179662	India	High	Med.	-	
80	PI 179878	India	High	-		
74	PI 179884	India	-	Med.	+	Red flesh[1]
63	PI 182934	India	-	Med.-High	+	Red flesh
51	PI 183398	India	-	Med.	+	Red flesh
133	PI 234603	New Zeal.	Med.	-		
20	PI 295848	S. Africa	Highest	High	++	Tasteless
123	PI 381703	India	Med.	-		
100	PI 381731	India	-	Med.	-	
94	PI 381740	India	-	High	++	Good[2]
86	PI 381751	India	-	Med.	+	
151	Wild citron	Egypt	High[3]	-		Bitter
152	WM5-4 (Webb's sel. from Egon)	Nigeria	High	High	+	Tasteless

[1]Rotted but flesh color suggested a commercial variety.

[2]12% soluble solids. Field assistant took it home to eat.

[3]Only seed produced in WMV1 isolation.

From 1 to 4 cuttings from each plant selected in the WMV-1 test were planted in one isolated field, and from the WMV-2 test in another, in single long rows. Plants were allowed to open- pollinate with the thought that natural crossing might lead to greater resistance through transgressive segregation. Unfortunately, the WMV-1 selections were in an unfavorable location and most did not survive transplanting in a hot, dry period. Most of the WMV-2 selections grew and set fruit normally, showing little evidence of virus disease. Part of the seed from each selection will go to Egypt for testing since virus diseases are limiting watermelon production there. There is adequate seed to share with other watermelon breeders interested in evaluating virus resistance.

These are highly preliminary results; larger samples of the more promising entries should be re-tested and more care given to getting seed, particularly from survivors of WMV-1 inoculation. In the collection tested, 75 entries came from India, 15 from Japan, 15 from South Africa, 10 from USSR, 10 from Central and South America, and 7 from tropical parts of Africa. The survivors from India had the best horticultural features but none had high resistance to both viruses. Entries with the best combined resistance to the 2 viruses came from Africa, but none was really edible.

Independence of Genes *Ses-B* and *M* in *Cucurbita pepo* L.

Bloksberg, L.N. and O. Shifriss

Department of Horticulture and Forestry, Cook College, Rutgers- The State University, New Brunswick, NJ 08903

Gene *B* conditions precocious depletion of chlorophyll in fruits leading to precocious fruit yellowing. In addition, *B* can deplete chlorophyll in leaves at early stages of plant development leading to leaf yellowing. This effect is particularly severe at low temperatures (3). However, gene *Ses-B* selectively suppresses the expression of *B* in leaves (6). Thus, plants of the *B*/*B* *Ses-B*/*Ses-B* genotype produce precociously yellow fruits, but their leaves appear persistently green as those of B^+/B^+ individuals.

Gene *M* conditions the mottled-leaf characteristic (2). The latter was also described as the silvery-leaf trait (5). This is a complex trait and some data suggest that it can impart a tendency to escape aphid-transmitted viruses (1, 4, 7).

'Jersey Golden Acorn' (JGA) is *B*/*B* *Ses-* B^+/*Ses-B^+ *m*/*m*. It produces precociously pigmented fruits, is susceptible to leaf yellowing, and bears non-silvery leaves. We attempted to substitute *Ses-B* for *Ses-B^+ and *M* for *m* in JGA by crossing it with one of our breeding lines, NJ4, *B*/*B* *Ses-B*/*Ses-B* *M*/*M*, followed by several backcrosses to JGA. This operation gave us an opportunity to study the relationship between *Ses-B* and *M*. The data in Table 1 clearly show that the two genes are independent. The incorporation of both *Ses-B* and *M* into JGA may increase the economic value of this cultivar.

Table 1. Independence of genes *Ses-B* and *M*.

	Number of plants[a] in respective classes				Total	Chi square	P
	Ses-B		*Ses-B^+				
	M	*m*	*M*	*m*			
P$_1$, 'Jersey Golden Acorn' *B*/*B* *Ses-B^+/*Ses-B^+ *m*/*m*	0	0	1(?)[b]	29	30	--	--
P$_2$, NJ4 *B*/*B* *Ses-B*/*Ses-B* *M*/*M*	30	0	0	0	30	--	--
F$_1$, P$_1$ x P$_2$	20	0	0	0	20	--	--
BC$_1$, F$_1$ x P$_1$	79	76	76	79	310	0.12	>0.98
F$_2$	89	28	28	12	157	0.63	>0.85

[a]All plants were grown under controlled conditions: 16 hr photoperiod, light 11 x 10^3 lu/m^2, 95% from fluorescent tubes and 5% from incandescent bulbs, 20 C day and 15 C night.

[b]The origin of this plant is not known, but it could reflect a low penetrance of the silvery-leaf trait among *m*/*m* individuals of some background.

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Potential of Restriction Endonuclease Analysis of Chloroplast DNA for the Determination of Phytogenetic Relationships among members of Cucurbitaceae

Juvik, J.A.

Department of Horticulture, University of Illinois, Urbana, IL 61801

J.D. Palmer

Department of Zoology, Duke University, Durham, NC 27706

A preliminary study was undertaken to determine the feasibility of using comparative restriction endonuclease analysis of chloroplast DNA as a method of accessing evolutionary relationships among members of the Cucurbitaceae. The size of the chloroplast genome of vascular plants [120-180 kilobase pairs (kb) (2)] is small enough to permit resolution of all the fragments produced after digestion of chloroplast DNA by many 6 base endonucleases and sufficiently large for rapid screening of a great many restriction sites using only a few enzymes (4). Previous studies (1,3) have indicated that changes in restriction patterns of the chloroplast genome are generally the result of base substitutions rather than major sequence rearrangements. These properties and the evolutionary conservatism of the chloroplast genome make it an excellent tool to study phylogenetic relationships among plant species, genera, and families (1,4,5).

Chloroplast DNA of 12 accessions from 11 species within 4 genera of Cucurbitaceae (Table 1) were purified according to the sucrose gradient method described by Palmer and Thompson (2). This method yielded relatively pure chloroplast DNA which was nearly free of both mitochondrial antinuclear DNA contamination. The chloroplast DNAs were digested with 9 different restriction endonucleases procured from Bethesda Research Laboratories and New England Biolabs using the instructions provided by the suppliers. The enzymes used were Sal I, Pvu II, Pat I, Sac I, Sac II, Kpn 1, Hind III, Ham HI, and Eco RI. Fragments were then separated on 0.7% - 1.0% horizontal agarose slab gels of 0.4 x 20 x 22 - 40 cm in 100 mM Tris-HCl, pH 8.1/12.5 mM NaOAc/0.25 mM EDTA.

Table 1. Numbers and size range of restriction endonuclease fragments fran the chloroplast DNA of 12 different accessions of Cucurbitaceae.

Genus/species Cultivar	Sal I		Pvu II		Sac II		Pst I		Kpn I		Sac I		Hind III		Bam HI		Eco RI	
	No.	Size range (kb)	No.	Size range (kb)	No.	Size range (kb)	No.	Size range (kb)	No.	Size range (kb)	No.	Size range (kb)	No.	Size range (kb)	No.	Size range (kb)	No.	Size range (kb)
Cucurbita pepo Fordhook zucchini	5	2.2-37	8	6.8-37	9	1.7-29	11	1.0-30	13	0.8-36	21	0.7-25	25	0.4-14	36	0.4-17	39	0.6-14
Cucurbita pepo Accession from Africa	5	2.2-37	8	6.8-37	9	1.7-29	11	1.0-30	12	0.8-36	19	0.7-25	24	0.4-14	35	0.4-17	37	0.6-14
Cucurbita maxima Hubbard	5	2.2-37	8	6.8-37	9	1.7-29	11	1.0-30	12	0.8-36	19	0.7-25	23	0.6-14	36	0.4-17	39	0.6-14

	#	size	#	size	#	size	#	size	#	size	#	size	#	size	#	size	#	size
squash																		
Cucurbita mixta Gold stripe cushaw	5	2.2-37	8	6.8-37	9	1.7-29	11	1.0-30	13	0.8-36	20	0.7-25	23	0.4-14	35	0.4-17	36	0.6-14
Cucurbita moschata Butternut squash	5	2.2-37	8	6.8-37	9	1.7-29	11	1.0-30	13	0.8-36	20	0.7-25	23	0.4-14	35	0.4-17	37	0.6-15
Cucurbita andreana Accession from Mexico	5	2.2-37	8	6.8-37	9	1.7-29	11	1.0-30	11	0.8-36	19	0.7-25	24	0.6-14	34	0.4-17	49	0.6-15
Cucurbita ficifolia Accession from Mexico	6	2.2-37	8	6.8-37	9	1.7-29	10	1.0-30	15	0.8-36	19	0.7-25	23	0.4-14	34	0.4-17	37	0.6-14
Cucurbita sororia wild accession	5	2.2-37	8	6.8-37	9	1.7-29	11	1.0-30	13	0.8-36	20	0.7-25	23	0.4-14	33	0.4-17	-	-
Cucumis sativus Beit Alpha Mt.	7	2.2-21	8	2.3-37	9	1.7-29	-	-	13	0.8-36	15	0.7-25	23	0.6-13	34	0.4-17	32	0.6-15
Cucumis melo Harvest queen muskmelon	10	2.1-37	10	1.9-37	8	1.7-29	15	1.6-37	17	0.8-36	15	0.9-33	22	0.4-14	34	0.4-17	35	0.6-15
Citrullus lanatus Charleston grey	8	2.2-37	10	1.9-37	9	1.7-31	12	1.0-33	12	0.8-36	14	0.9-25	19	0.5-14	35	0.4-17	32	0.6-15
Lagenaria siceraria Bottle gourd	8	2.2-37	10	1.9-37	8	4.3-33	12	1.0-37	16	0.8-36	16	0.9-25	19	0.6-14	38	0.4-17	34	0.6-15

Table 1 lists the plant accessions used in this study and the numbers and size ranges of fragments generated by restriction endonuclease digestion. Fragment sizes for each of the digests were estimated by comparison with a control consisting of a mixture of fragments of known kilobases. Summing fragment sizes for each digest provided an estimate of the size of the chloroplast genome of each accession. Estimates ranged from 150- 160 kb for all the accessions indicating an absence of significant variation in chloroplast DNA size. Variation in the number and individual fragment size was found to exist among the different chloroplast DNAs for each of the 9 restriction enzymes. Variation in fragment size and number is indicative of mutational events at restriction sites. For accessions within the genus *Cucurbita* no mutations were detected at the restriction sites specific to Pvu II and Sac II. For Sal I and Pst I only the *C. ficifolia* accession displayed mutations at cleavage sites specific to these endonucleases. Only *C. pepo*, *C. mixta* and *C. moschata* were identical in digestions using Kpn I. With Sac I, *C. maxima* chloroplast DNA was found to be homologous to *C. andreana* while *C. mixta*, *C. moschata*, and *C. sororia* also displayed identical cleavage patterns. All the Cucurbita species had distinct fragmentation patterns when digestions were conducted using Hind III, Ham HI, and Eco RI. A substantial number of restriction site mutations were found to exist between members of the four genera tested. Only the accessions *Citrullus lanatus* and *Lagenaria siceraria* had identical cleavage sites and fragment sizes when digested by the endonuclease Sal I.

The results of this study indicate: 1) that the method employed for chloroplast DNA purification is effective for members of the Cucurbitaceae; 2) that there appears to exist both substantial variation and sufficient homology within the family to map the chloroplast genome; and 3) it should be possible through the use of parsimony analysis of shared mutations to construct a

maternal phylogeny for plant species within the Cucurbitaceae.

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Embryo Culture of *Cucurbita andreana* and *C. martinezii*

Malter, A. B., R. J. Lebowitz, and J. A. Juvik

Department of Horticulture, University of Illinois, Urbana, IL 61801

Tissue culture may offer novel means of studying and improving Cucurbitaceae species. Cultures have already been used to analyze secondary metabolites (Yanagawa, et al., 1971), propagate gynoecious inbreds (Handley and Chambliss, 1979), regenerate plantlets from cotyledon-derived callus (Ding-Tai, et al., 1980; Jaleska, 1972 and 1974), and to study morphogenesis effects (Halder and Gadgil, 1981). The following text describes the preliminary results from embryo culture experiments using two species; *Cucurbita andreana* and *Cucurbita martinezii*.

Embryo culture of *C. andreana* and *C. martinezii* was achieved on a medium consisting of half strength Murashige and Skoog (1962) salts, 0.1 mg/liter thiamine HCl, 2.5 mg/liter niacin, 2.0 mg/liter pyridoxine HCl, 25 mg/liter ascorbic acid, 500 mg/liter malt extract, 40 mg/liter coconut milk, 300 mg/liter myo- inositol, 25 g/liter glucose, 0.1 mg/liter NAA, and 0.1 mg/liter kinetin. The medium was solidified by adding Bactoagar (8 gm/liter) after it was adjusted to pH = 5.65 using KOH. Eight ml aliquots of medium were dispensed into 25 x 150 mm glass culture tubes. The tubes, capped with Bellco Kaputs, were then sterilized by autoclaving for 15 minutes at 1.06 kg/cm2 at approximately 115 C.

Seeds from each species were surface disinfested by placing them in a solution consisting of 10% Chlorox and 0.1% Triton X-100 surfactant for 15 minutes. The disinfested seeds were then rinsed twice for 5 minutes in sterile distilled water and allowed to dry under a sterile transfer hood. The coats of each individual seed were removed and their embryos cultured on the medium described above. Cultured embryos were maintained at 23% under 2.2 Klux intensity light.

Radicals emerged from most of the *C. andreana* embryos within 9-12 days. Their cotyledons developed chlorophyll during this same period. After 12 days, 40% of the developing seedlings had produced a shoot and 4-17 lateral roots. After 16 days, 30% of the developing seedlings had produced one or more true leaves.

Embryo cultures of *C. martinezii* exhibited slightly less vigorous development than those of *C. andreana*. Radicals from most of these embryos required 12-16 days to emerge, however 56% of these seedlings had developed single leaves and lateral roots by the end of this 16 day period.

Seedlings from both species were subcultured at least 3-4 weeks after initial culturing when their shoots had developed at least two nodes and two or more true leaves. The seedlings were divided into segments consisting of individual shoot nodes, cotyledons, leaf blades with their petioles, leaves with basal buds, and short stem segments with the cotyledons still attached (see Table 1). These 5 types of tissue explants were placed on fresh medium and maintained under the same environmental conditions described earlier. Shoots and roots only differentiated from two of the different types of explants; leaves with basal buds, and short stem segments with the cotyledons still attached (see Table 1). Roots alone developed on some of the cotyledon explants.

Table 1. Differentiation seen in Cucurbita tissue explants.

Explant Type	No. of Cultures	Differentiation	
		Shoots	Roots
C. andreana			
Shoot node	15	-	-
Cotyledon	12	+	-
Leaf blade w/petiole	15	-	-

		13	+	+
	Leaf w/basal bud	13	+	+
	Stem segment w/cotyledon	10	+	+
C. martinezii				
	Shoot node	12	-	-
	Cotyledon	10	+	-
	Leaf blade w/petiole	12	-	-
	Leaf w/basal bud	13	+	+
	Stem segment w/cotyledon	10	+	+
+ = Present, - = Absent				

These results indicate that *in vitro* differentiation is controlled by endogenous factors as well as exogenous nutrients and hormones. Ding-Tai et al. (1980) and Jaleska (1972, 1974) reported similiar endogenous differentiation factors affecting *Cucumis melo* and *Cucurbita pepo* tissue cultures.

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Tolerance of *Cucurbita* spp. to Squash Leaf Curl

J.D. Mc Creight

U.S. Department of Agriculture, Agricultural Research Service, P.O. Box 5098, Salinas, CA 93915

Squash leaf curl (SLC) is a highly infectious virus disease that reached epidemic proportions in fall squash production in the desert southwest of the U.S. and the adjoining area of Mexico in 1981 (3). SLC virus is transmitted efficiently by the sweetpotato whitefly, *Bemesia tabaci* (Genn.), which is virtually impossible to control with chemicals (1,2). Resistance to SLC virus or its transmission are, therefore, the most promising means of controlling SLC. My objective was to test cultivars of *Cucurbita maxima* Duch., *C. mixta* Pang, *C. moschata* Duch., and *C. pepo* L. for resistance to SLC.

Seventy-four cultivars of these 4 *Cucurbita* spp. were rated in greenhouse tests using controlled inoculation and in a field test at Brawley, CA using natural infection. Plants were inoculated in the greenhouse by feeding SLC viruliferous whiteflies on 5 plants of each cultivar at the 2-3 leaf stage for 48-96 hr. Plants were rated 10-14 days after inoculation. The field test was planted in a randomized complete block design. There were 2, 2-plant hills per cultivar in each of 3 replications. The field was watered on July 27, 1983, and rated for SLC 50 and 91 days later. Plants in the greenhouse and field tests were rated on a scale from 1 (dead) to 9 (symptomless). The field test was rated on a plot basis. Enzyme linked immunosorbent assays (ELISA) were done for SLC virus on every surviving cultivar in one replication at the 91 day rating using the techniques of Cohen et al. (1).

Reactions (R and range) to SLC were more severe on all 4 species in the greenhouse tests than in the field test (Table 1). The *maxima* cultivars were in general more severely affected than those of the other 3 species. Reaction of the *mixta* cultivars were only slightly better than the *maxima* group in the greenhouse or field tests.

Table 1. Summary of ratings of 4 *Cucurbita* spp. to squash leaf curl virus in greenhouse and field tests[z].

Species	Number of cultivars	Greenhouse test		Field test			
				50 days		91 days	
		Mean	Range	Mean	Range	Mean	Range
maxima	15	2	1-3	3	1-9	2	1-8
mixta	3	3	2-3	4	2-9	4	1-9
moschata	6	3	2-6	8	5-9	7	5-9
pepo	50	2	1-5	5	1-9	5	1-9

[z]Rated on a 1 to 9 scale: 1 = dead; 3 = very severe curling and stunting; 5 = moderate symptoms; 7 = mild symptoms; 9 = symptomless.

Reaction of the *moschata* cultivars was not very good in the greenhouse tests except for 'Hercules' and 'Mediterranean' which showed relatively mild symptoms. In contrast, all the *moschata* cultivars performed well in the field test as indicated by a R severity rating of 8 at 50 days and 7 at 91 days after initial watering (Table 1).

Many *pepo* cultivars were severely affected by SLC in the greenhouse and field tests. Several *pepo* cultivars did, however, recover in greenhouse tests. For example, 'Black Magic' had severe SLC symptoms 14 days after inoculation, but by 31 days symptoms were mild, and the inoculated plants were almost indistinguishable from the control plants. The other 2 *maxima* and 2 *pepo* cultivars in the same test with 'Black Magic' did not recover. Eighteen of the 50 *pepo* cultivars showed tolerance to SLC in the field test, but there was large plot-to-plot variation.

ELISA readings from the field test were positive, and indicated that there was a high SLV virus titre even in those cultivars rated symptomless for SLC at time of sampling. This indicates that some cultivars of moschata and pepo are tolerant to SLC.

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Natural Hybridization of Wild *Cucurbita sororia* Group and Domesticated *C. mixta* in Southern Sonora, Mexico

Merrick, Laura C.

Cornell University, Ithaca, NY 14556 and University of California, Davis, CA 95616

Gary P. Nabhan

Native Seeds/SEARCH, and University of Arizona, Tucson, AZ 85721

Despite numerous experimental hybrids that have been made between *Cucurbita* species to determine taxonomic relationships among taxa in this genus, evidence of uncontrolled hybrids between taxa within their natural ranges has rarely been reported (1). The following observations and ethnobotanical interviews suggest that *C. mixta* land races long-cultivated by Sonoran Indians may continue to exchange genes with wild or weedy *Cucurbita* native to northwest Mexico.

In Sinaloa and adjacent Sonora and Chihuahua, botanists have collected numerous herbaria specimens of a wild *Cucurbita* belonging to the *C. sororia* group as it is defined by several authors (2,3). This group is supposedly represented by *C. palmeri* in the north, but their leaves are not as deeply lobed as *C. palmeri* described by Bailey (4). Sonoran specimens have sometimes been referred to as the more southerly *C. sororia*. Merrick (work in progress) is finding high seed set in hybrids made between various species in this group. If additional genetic and biogeographic work indicate that these taxa form one highly interfertile but variable, widespread compilospecies, the name *C. radicans* Naud. (4) has precedence. These taxa are closely clustered with *C. mixta* in numerical taxonomic studies and, as a group including *C. mixta*, are distinct from other *Cucurbita* species (3,5). Supporting evidence from crossing studies (Merrick, unpublished data) indicates fertile hybrids can be produced in controlled reciprocal crosses between *C. mixta* and *C. sororia* group taxa collected throughout the geographical range of these species in Mexico. More restricted crossability was found in experimental crosses between *C. mixta*/*C. sororia* group taxa and other *Cucurbita* wild and cultivated species. In native habitats, the flowering phenology, basic floral morphology, and pollinators of neighboring wild and cultivated *Cucurbita* are often the same, resulting in potentially suitable conditions for gene flow. Have *C. sororia* group taxa possibly contributed genes to *C. mixta* during its evolution under domestication?

Ethnobotanical evidence from areas of native agriculture in Northwest Mexico suggests occasional gene exchange. In August 1970, ethnobiologist Campbell Pennington collected seed of "calabasa caliente" in the Mountain Pima Indian locality of Yecora, Sonora. Dr. Thomas Whitaker gave them a preliminary i.d. of *C. mixta* "Taos type". Upon growout as Native Seeds/SEARCH #EO1-015, its seed produced round, nearly softball size fruit with extremely bitter flesh, and a peduncle less corky or enlarged than most *C. mixta*. Expression of the dominant *Bi* gene for cucurbitacin content, intermediate fruit size and relatively thin peduncle suggest that this may represent progeny of a *C. mixta* X *C. sororia* group hybrid from Sonora. Drs. Whitaker and Bemis have viewed the fruit and concurred with this hypothesis; progeny will continue to be evaluated.

While collecting plants in southern Sonora, each of us has encountered other possible evidence of such introgression. In September 1982, L.M. visited the garden of Indian Valentin Sasueta in the Mayo-Warihio community of San Bernardo. From his fence, she harvested fruits (#293) which matched the description given above for #EOI-015. Two other accessions (#294A and #294B) with relatively large fruit were obtained at a nearby airport landing strip not far from cultivated fields in the Alamos municipality. Grown out in Davis, #294B appeared to be an "escaped" *C. mixta*. It produced large fruits with non-bitter flesh, thickened peduncles, large seeds and *C. mixta*-type flowers. In Davis, plants of #293, #294A, and Sonoran accessions #EO2-001 and #EO2-003 from C.N. produced fruits of intermediate size (compared to over 90 accessions of *C. mixta* and over 40 accessions of wild *C. sororia* group taxa), bitter flesh, enlarged peduncles and relatively large *C. mixta*-like foliage and stem characteristics. The seeds of these accessions resembled those of the *C. mixta* "green striped cushaw

type" (6) land races cultivated in Northwestern Mexico (seed body shiny white, seed margin tan), but were distinctly smaller. The preceding characters match those found in experimentally produced *C. mixta*/*C. sororia* group taxa hybrids. The bitter Sonoran accessions exhibited variation between and within accessions for fruit size, shape, and coloration; floral characteristics; seed size and shape; and leaf coloration. These accessions have traits similar to those reported by Whitaker and Gentry (pers. comm.) as wild *C. mixta* from the Warihio Indian region around Alamos, and are often called "Chi Chi Coyote" or "Calabasa de Coyote" in Spanish, and "Ha'la'we Chipu" (Bitter Squash) in the native Warihio tongue (Eric Rowell, pers. comm.). Fruits of #293, #294A, and #294B were called "Chi Chi Coyote" by San Bernardo residents.

In August 1983, G.N. was accompanied by Mayo Indian José Valenzuela into the sierras between San Bernardo, Sonora and the Chihuahua border. At the edge of a slash-and-burn field, he pointed out a wild *Cucurbita* which he said was very bitter. He then volunteered in Spanish: "It is called Chi Chi Coyota. If Chi Chi Coyota is on the edge of your fields, and you plant squashes, you are going to lose them (for use) because the bad will enter the good (squashes)." The trouble, he added, is that it is difficult to tell this weed from the domesticated plant until the fruit begins to mature. He said that only the burros will try to eat its bitter gourds.

The folk term "Chi Chi Coyote" or its variants are used as well for the xerophytic wild *Cucurbita* of Northwest Mexico. In Baja California Norte, in the Sierra Juarez, a Mexican told G.N. that this was because they are sometimes used by mothers to discourage their children from further nursing. To wean a child, the cucurbitacin-rich, wet pulp of maturing fruit is rubbed on the woman's breast. After one "surprise", the child no longer wants to nurse. Perhaps this folk name is derived from "Coyote's Breasts" (breasts=chichis in Mexican vernacular), the Coyote being a supreme trickster in New World folklore. Sonoran and Arizona Indians associate Coyote with other wild relatives of their crops, including species of *Nicotiana*, *Proboscidea*, *Gossypium*, *Phaseolus*, and even *Cucumis* (*C. angularis*) (7). Their recognition of the close affinity of *C. sororia* group taxa with *C. mixta* predates that of Western scientists. Further studies will hopefully confirm this genetic introgression hypothesis and possibly establish that *C. mixta* evolution is non-centric, or diffuse in the geographical area from which it evolved and diverged from wild *Cucurbita* progenitors. Since southern Sonora borders on the true Sonoran Desert, this gene pool may be a source of genes conferring drought and heat resistance of use in the improvement of stress tolerance in *C. mixta* and *C. pepo*.

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Evidence of Gene Flow Between Cultivated *Cucurbita mixta* and a Field Edge Population of Wild *Cucurbita* at Onavas, Sonora

Nabhan, Gary P.

Office of Arid Lands Studies, University of Arizona, Tucson, AZ 85721

In an earlier-written note appearing in this newsletter, Merrick and Nabhan (1) suggest that natural hybridization occasionally occurs between wild *Cucurbita sororia* group populations and domesticated *C. mixta* in Mexico. Whitaker (2) has recently discussed an "undescribed" wild *Cucurbita* that climbs into trees and shrubs in the Rio Mayo watershed of Sonora, which is completely cross-compatible with and an apparent miniature of cultivated *C. mixta*. Because Whitaker (2) hypothesized that this taxon is either the possible ancestral type of *C. mixta*, or an impoverished feral form of it, further evaluation of wild *mixta*-like cucurbits in Sonora appears crucial to understanding *C. mixta* evolution.

The following is a progress report of NSF-funded population genetic and ethnobiological studies of cucurbits begun at Onavas, a Pima Indian village where native agriculture is of considerable antiquity (3). Fifteen miles east of Onavas, Dr. Amadeo Rea and I began observing and collecting wild *Cucurbita* cf. *palmeri* in thornscrub vegetation away from any farms. Upon reaching Onavas, we noticed an increase in density of cucurbit fruits within 150 m of floodplain field edges. Both wild and weedy cucurbits are called *chicoyote* in Spanish or *adavi* in Piman, and we enlisted local teenagers to collect fruit of this folk taxon of which 200 were scored for at least some of the traits discussed below.

Within the 200 fruits evaluated from Onavas, marginless seeds were found in 28. This trait is extremely rare in wild mesophytic gourds of the *C. sororia* complex, including *C. palmeri*, although it is found in domesticated *C. mixta* land races from Piman villages, such as Pennington's 1977 collection (NS/S E01-009). Upon observing this trait in Onavas gourds, Whitaker (pers. comm.) suggested that we check to see if their flesh was bitter due to the *Bi* wild type gene. Surprisingly, 26 of the 28 fruit with marginless seed had sweet, edible flesh, in contrast to the rest of the collection, many if not most of which were bitter. (Only four of dozens of the other fruits were found to be sweet before our tongues went on strike!)

To determine if the marginless seeded fruit could be considered a subpopulation distinct from the rest of the population, each fruit was scored for several characteristics, then chi square tests were run on the enumeration data from these two samples. Fruit with marginless seeds were not significantly different in mean fruit circumference, peduncle bristliness, nor skin coloration from the remainder of the fruit. However, the marginless-seeded fruit have peduncles which are significantly more corky and more persistent than those of the other fruit; (the null hypotheses were rejected at the .05 level of significance).

Peduncle corkiness, flesh sweetness, and marginless seeds are all traits known in *C. mixta* land races that are infrequent or unknown in wild mesophytic gourds from natural habitats in Sonora; this suggests gene exchange with the domesticates. The Onavas field edge population (GN 84-16) as a whole was extremely variable in its combinations of traits which I assume represent both wild-type and introgressed domesticate-type gene expression. It encompassed traits found in sample GN 84-15 collected 15 miles away from the fields, including yellow, oblong-shaped fruit averaging 25.8 cm in circumference, with bristly fluted peduncles and flat, tan seed 10 mm long. At the other end of the field edge population spectrum was a sweet, green and white striped fruit 35 cm in circumference, teardrop shaped, with white, inflated, etched seed. Though *mixta*-like, these latter seeds were still smaller than the 20 mm length of "wild *mixta*" seed from Alamos grown by Whitaker in 1970.

In Pima fields at Onavas where land races of *C. mixta* and *C. moschata* are grown, they are surrounded by these wild gourds. Pima elder Pedro Estrella believes that when wild *chicoyotes* grow near squashes, their bitterness "inoculates" the squash, making them unpalatable. This site will be intensively studied during the 1984 growing season.

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Epidemics of Zucchini Yellow Mosaic Virus and Other Cucurbit Viruses in Egypt in the Spring of 1983

R. Provvidenti

New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456

H.M. Munger

Cornell University, Ithaca, NY 14853

H.O. Paulus

University of California, Riverside, CA 92521

In Egypt, during the spring of 1983, viral diseases were responsible for devastating epidemics in cucumber, melon, squash and watermelon fields. Our surveys, which were conducted in May, included experimental and commercial fields in the Delta, along the Nile River, from Cairo to Sids, Ismailia (Suez Canal) and an agricultural development project in the Sinai.

Particularly affected were cultivars of summer squash *(Cucurbita pepo* L.), which reacted to viral infection with plant stunting, severe foliar symptoms and distorted fruits. These malformed fruits were frequently sold in local markets. In fields where viral infection had occurred in an early stage of plant growth, production was totally lost, since fruits either aborted or remained very small.

Because of the similarity in symptomatology, it was often difficult to differentiate plants infected with watermelon mosaic virus 1 (WMV-1) from those infected with zucchini yellow mosaic virus (ZYMV). Both of these viruses caused very severe foliage mosaic and knobbed fruits, and they appear to be the most prevalent and widespread. WMV-1 and ZYMV were followed in order of importance, by cucumber mosaic virus (CMV) and watermelon mosaic virus 2 (WMV-2). Squash mosaic virus (SqMV) was confined to isolated melon and squash plants, and its spread was impaired by low beetle populations. Conversely, CMV, WMV-1, WMV-2 and ZYMV were spread efficiently by several aphid species.

The presence of ZYMV in Egypt and of the other viruses was confirmed by the analysis of infected specimens. The identification was accomplished using electron microscopy, serology and diagnostic hosts. The Egyptian isolates of ZYMV incited symptoms closely resembling those caused by European isolates of this virus (1,3) and the American strain, ZYMV-CT (5).

In Egypt, a good control of these viral diseases was achieved when plants were grown initially under low plastic tunnels. When the plastic was removed to facilitate pollination, plants appeared to be healthy and produced a good crop. However, without the protective plastic shield, which had interfered with the activity of the vectors, these plants eventually succumbed to viral infections.

Plastic tunneling obviously adds to the production cost, but it offers an alternative to the total loss of the crop, particularly in years of devastating epidemics.

In addition to Egypt, ZYMV has been found in France, Germany, Israel, Italy, Morocco, Spain (2) and the USA (4,5).

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Further occurrence of Zucchini Yellow Mosaic Virus in the United States

Provvidenti, R. and D. Gonsalves

New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456

H.S. Humaydan

Joseph Harris Company, Inc., Rochester, NY 14624

During 1983 zucchini yellow mosaic virus (ZYMV) occurred in cucurbits grown in New York, Florida and California. In affected fields the virus caused severe losses in fruit production.

It appears that at least two strains of ZYMV are present in the United States. The first strain designated ZYMV-CT was found to infect squash in northern Connecticut in 1982 (3) and was subsequently isolated from squash specimens collected in the fall of the same year at Homestead, FL. This strain causes very severe yellow mosaic, knobbed fruits and plant stunting. These symptoms closely resemble those described for the European isolate of the virus (1,2). The second strain (ZYMV-FL) was originally isolated from squash growing in central Florida in the winter of 1982-83, and subsequently in 1983 from cucurbits grown in western New York and central California. ZYMV-FL incited symptoms that could be easily confused with those of watermelon mosaic virus 1 (WMV-1) infection. Foliage symptoms are also severe but they lack the intense yellowing caused by ZYMV-CT. However, plants are also stunted and fruits are knobbed. Both strains have the same host range and serologically are indistinguishable, but the incubation period of ZYMV-FL is 3-5 days longer. An antiserum was prepared to cytoplasmic inclusion proteins (CIP) of ZYMV-CT. This antiserum reacts with the American and foreign isolates of ZYMV, but not with the CIP of WMV-1 or WMV-2.

The destructive epidemics caused by ZYMV, here and abroad, illustrate the economic importance of this virus. Thus, our activity has been directed toward the search for sources of resistance or tolerance in cucurbit species. Utilizing hundreds of domestic and foreign cultivars, it has been established that, although rare, resistance or tolerance occurs in a few accessions of *Citrullus colocynthis. Cucumis melo*, *Cucumis sativus*, *Cucurbita ecuadorensis*, *C. maxima* and *Lagenaria siceraria.*

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Further Notes on the Silvery-Leaf Trait in *Cucurbita*

Shifriss, O.

Department of Horticulture and Forestry, Cook College, Rutgers University, New Brunswick, NJ 09803

The silvery-leaf trait (6) raises two issues: the biological control of its complex expression and its adaptive value. The biological control is poorly understood. But there is little doubt that, under field conditions in New Brunswick, inbreds of *C. pepo* L. differ greatly in (i) **time** during plant development at which trait is first manifested, (ii) **intensity** of its expression, and (iii) **extent** of its distribution over the upper leaf surface (5). The silvery expression can be confined to veins and/or vein axils (mottled- leaves) or can be extended over the entire leaf surface.

Five different forms, SL-1 to SL-5, have been described (6). Representatives of SL-1 are 'Early Prolific Straightneck' (EPS) and 'Jersey Golden Acorn'. These cultivars are assumed to carry *m/m* (*m* for non-mottled or non-silvery leaves) as well as some modifiers of *M* (*M* for mottled or silvery leaves) which have small silvery effects of their own. Representatives of SL-2 to SL-5 are assumed to be *M/M* lines which differ from one another in their modifiers or controllers. The most extreme form, SL-5, is represented by NJ260. This inbred can produce uniformly silvery leaves of intense expression throughout plant life.

Several new intermediate (between SL-2 and SL-5) inbreds were developed from crosses involving NJ260. In addition, two cultivars were found to differ from previously described forms: 'Bicolor Spoon' and 'Delicata'. Our inbred of 'Bicolor Spoon' is truly green or non-silvery. Hypothetically, it carries *M/m* but lacks the modifiers which have small silvery effects. As to 'Delicata', Tapley et al. (8) described its leaves as "silvery green". Under our field conditions, 'Delicata' produces green leaves during spring and midsummer, and uniformly silvery leaves in late summer and fall. The silvery expression in 'Delicata' often spreads from the tips to the entire surface of the leaves rather than from the veins or vein axils. If the present hypothesis is correct, 'Delicata' carries *M/M* and a unique combination of controllers. But I wish I were as confident in the hypothesis as I am in the description of the above lines.

Non-genetic factors profoundly affect the expression of the silvery-leaf trait. High expressivity is obtained predictably under the following controlled conditions: 16 hr photoperiod, 11×10^3 lu/m^2, 90% of which is from fluorescent tubes and 5% from incandescent bulbs, 20% at day and 15% at night. Expressivity is very low under greenhouse (shaded) conditions in midsummer when temperatures are often extremely high. However, extremely high temperatures under field conditions do not drastically reduce expressivity. These tentative observations suggest that expressivity is modified by a combination of non-genetic influences.

Unlike *C. pepo*, *C. moschata* (Duch.) Poir. is largely of tropical adaptation and most, if not all, its tropical cultivars have distinctly mottled leaves. Indeed, mottled-leaf is one of the distinguishing features of this species in several taxonomical treatises (e.g., in 9). An exception is a group of Butternut cultivars (2) which probably evolved in North America. The F_2 of an interspecific cross made between *C. pepo* 'Jersey Golden Acorn' (non-mottled), as seed parent, and *C. moschata* 'Burpee Butterbush' (non-mottled) consisted of an appreciable number of mottled as well as uniformly silvery segregates. These results indicate that the designation of a single locus for control of mottled-leaf in *Cucurbita* (4) is an over-simplification.

Is the silvery-leaf trait due to a neutral genotype that has been randomly fixed in our cultivars? Alternatively, does it have a selective advantage?

Initial observations suggested that the silvery-leaf trait provides an escape mechanism against aphids and aphid- transmitted virus diseases (5). Results of a subsequent field study supported this hypothesis, but showed that protection is not complete (3). Circumstantially, two other facts support this hypothesis. First, silvery leaves reflect more light than non- silvery leaves (7), and light reflectance is generally assumed to be involved in repelling aphids. Second, as pointed out above, most if not all tropical cultivars of *C. moschata* are mottled. Virus diseases are particularly destructive in the tropics. Therefore, leaf mottling may have a selective advantage if it provides some protection against aphids.

Results of a recent field experiment (1) showed that significantly more aphids are captured on trapping stakes placed near

silvery plants than near non-silvery plants. The difference is particularly striking in the upper trapping zone, the zone of highest aphid concentration. The same experiment showed also that significantly more aphids are captured on stakes placed in bare ground than in cultivated ground occupied by plants. Since the plants of the silvery cultivar (NJ260) used in this experiment were smaller than those of the non-silvery cultivar (EPS), each silvery plot consisted of larger areas of bare ground. According to our present interpretation, this factor together with increasing evidence for the repelling action of silvery plants (the light effect) could have contributed to the higher number of aphids captured near them.

It is difficult to design and interpret field experiments of this kind. The nature of the relationship between plants and aphid behavior is still obscure, aphid infestation is unpredictable, and present silvery and non-silvery lines are not isogenic. Nevertheless, the hypothesis that the silvery-leaf trait provides and escape mechanism against aphids is potentially amenable to critical testing.

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Isozyme Studies Indicate that the Genus *Cucurbita* is an Ancient Tetraploid

Weeden, N.F.

New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456

The high chromosome number (2n=40) of *Cucurbita* suggests that this genus may be of polyploid origin. Evidence for allotetraploidy in several *Cucurbita* species was provided in cytogenetic studies of Weiling (3). However, remarkably few examples of characters controlled by paired loci have been reported. Genetic analysis of isozyme phenotypes provides an excellent method for identifying gene duplications, for in many isozyme systems the number of loci expressed in diploid plants appears to be conserved (1). For example, 4 genes are normally involved in the expression of the aspartate aminotransferases in the plant cell, subunits of the cytosolic-, plastid-, mitochondrial- and microbody-specific isozymes each being coded by a separate locus (Weeden, unpublished). Twice this number of loci would be expected in tetraploid plants. The additive expression of diploid genomes in a tetraploid has been most clearly demonstrated in the recent allotetraploid *Tragopogon miscellus* (2).

Eight isozyme systems (aspartate aminotransferase, glucose phosphate isomerase, phosphoglucomutase, 6-phosphogluconate dehydrogenase, NAD-malate dehydrogenase, triose phosphate isomerase, NADP-isocitrate dehydrogenase and shikimic dehydrogenase) were selected for a study of gene expression in *Cucurbita*. In diploid plants each system is specified by a predictable number of genes (Table 1), usually one for each subcellular compartment in which the enzyme is found. The total number of gene products predicted in a diploid plant for the 8 systems is 16. Preliminary analysis of *Cucumis sativus* (2n=14) indicated that 17 loci were being expressed. In contrast, genetic studies on the isozyme phenotypes of *Cucurbita maxima*, *C. ecuadorensis*, *C. pepo*, *C. moschata* and *C. palmata* demonstrated that at least 28 loci were contributing isozymic forms. Similar studies on isozyme expression in *C. pepo* and *C. texana* have produced additional evidence for gene duplication in these species (T.C. Andrus and H.D. Wilson, unpublished).

Table 1. Comparison of isozyme loci expressed in known diploids and *Cucurbita*.

Isozyme system	# loci predicted in a diploid	# loci found in *Cucumis*	# loci found in *Cucurbita*
AAT	4	4	7
GPI	2	2	4
PGM	2	2	3
6PGD	2	2	3
MDH[*]	2	2	4
TPI	2	3	4
IDH	1	1	2
SKDH	1	1	1
Totals	16	17	28

[*]Cytosolic and mitochondrial forms only, the microbody-specific form was not resolved.

Although duplication of specific genes or chromosomal segments has been described in a considerable number of species (1), it is unlikely that a series of such events generated the extensive gene duplication observed in *Cucurbita*. Many of the loci involved in the isozyme systems investigated assort independently (Weeden, unpublished), thus indicating that a large portion of the genome would have had to have been duplicated. The results of the present study in conjunction with the high chromosome number in this genus and the cytogenetic findings of Weiling provide compelling evidence for the allotetraploid origin of *Cucurbita*.

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Linkage between an Isozyme Locus and One of the Genes Controlling Resistance to Watermelon Mosaic Virus 2 in *Cucurbita ecuadorensis*

Weeden, N.F., R.W. Robinson and F.Ignart[*]

Dept. Horticultural Sciences, New York Agricultural Experiment Station, Geneva NY 14456

Resistance to watermelon mosaic virus 2 (WMV-2) has been found in *Cucurbita ecuadorensis* (2). The resistant phenotype appears to be controlled by more than one locus (1), but little is known regarding the number of loci involved or the interactions between these loci.

As part of an on-going program to transfer multiple virus resistance from *Cucurbita ecuadorensis* to *C. maxima* two backcross populations [(*C. maxima* x *C. ecuadorensis*) x *C. maxima*] were grown in field plots at the New York State Agricultural Experiment Station, Geneva NY. Each plant was inoculated in the first true leaf stage with WMV- 2, and natural infection of WMV-2 also occurred in the field. Plants were phenotyped for 12 different isozyme systems (acid phosphatase, aldolase, aspartate aminotransferase, esterase, galactosidase, leucine aminopeptidase, malate dehydrogenase, phosphoglucomutase, peroxidase, shikimic dehydrogenase. superoxide dismutase and triose phosphate isomerase), permitting the observation of products from at least 21 segregating loci.

Correlation was found between resistance to WMV-2 and an allele of *Aldo-p*, the isozyme locus specifying the plastid-specific aldolase (3). A majority of the plants displaying a heterozygous *Aldo-p* phenotype at *Aldo-p* were resistant while all except one plant exhibiting the aldolase phenotype of *C. maxima* (homozygous slow) were susceptible (Table 1). We interpret these results to indicate that *C. ecuadorensis* possessed a WMV-2 resistance gene closely linked to *Aldo-p*. In the genetic background of the backcross population, the possession of this gene appeared to be a required but not a sufficient condition for expression of the resistant phenotype. The one individual exhibiting the slow aldolase allozyme in combination with the resistant phenotype could have resulted from a recombination event. The considerable number of WMV-2 susceptible plants heterozygous at *Aldo-p* may be due to an absence in these plants of other genes from *C. ecuadorensis* which contribute to the resistant phenotype. All other isozyme loci appeared to be assorting randomly with respect to WMV-2 resistance.

Table 1. Backcross segregation for watermelon mosaic virus 2 resistance and allozymes at the *Aldo-D* locus.

Population	n	WMV-2 susceptible		WMV-2 resistant		Chi-square	P
		aldolase		aldolase			
		slow	het.	slow	het.		
83-978	25	10	1	7	7	6.84	0.05<P<O.1
83-979	19	10	0	2	7	13.2**	P<0.01
Total	44	20	1	9	14	17.6**	P<0.01

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Present address: Institut de Recherches TEZIER, Domaine de Maninet, Route de Beaumont, B.P. 336, 26003 Valence- Cedex, Franc

A Nuclear Gene Codes for the Plastid-specific Aldolase in *Cucurbita* Species

Weeden, N.F.

Dept. Horticultural Sciences, New York State Agricultural Experiment Station, Geneva, NY 14456

At least two forms of fructose 1,6-diphosphate aldolase (EC 4.1.2.13) are present in leaves of *Cucurbita* species. One of these isozymes is localized in the plastid compartment while the other is cytosolic (1). Polymorphism in the plastid-specific aldolase has been observed using electrophoretic techniques. The genetic basis of this polymorphism was investigated in the backcross *C. maxima* x (*C. maxima* x *C. ecuadorensis*) and in backcross and F_2 populations of a cross involving the two *C. pepo* cultivars 'Black Jack' and 'Early Prolific Straightneck'. In all cases the aldolase phenotype segregated as if controlled by a single locus with codominant alleles (Table 1). The locus has been designated *Aldo-p*, the suffix reflecting the intracellular compartmentation of aldolase.

As is typical for allelic variants of isozymes, 'wild type' and 'mutant' terminology is not appropriate. Both allozymes perform the identical biochemical catalysis, and it would be very difficult to demonstrate which allozyme was derived from which. I have used the term "fast" and "slow" to discriminate between the two allozymes segregating in each population. It should not be assumed that the slow allozyme in the *C. maxima* x (*C. maxima* x *C. ecuadorensis*) backcross is the same as the slow allozyme in the *C. pepo* populations; instead, 4 *Aldo-p* alleles have probably been identified: 2 in *C. pepo*, a third in *C. maxima* and a fourth in *C. ecuadorensis.*

Table 1. Number of individuals with designated aldolase phenotypes in three segregating populations.

Population	Aldolase phenotype				
	n	slow	slow/fast	fast	x^2
C. maxima x (*C. maxima* x *C. ecuadorensis*)	45	21	24	-	0.20
Black Jack x Early Prolific Straightneck (F_2)	54	15	26	13	0.22
Black Jack x (Black Jack x EPSN)	20	10	10	-	0.00

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A Collection of Wild and Cultivated Cucurbitaceae from Zambia

Whitaker, T.W.

U.S. Department of Agriculture, Agricultural Research Service (collaborator) and University of California, San Diego, Department of Biology, P.O. Box 150, La Jolla, CA 92038

During April, May and June, 1981, the International Board for Plant Genetics Resources (IBPGR), in cooperation with the Department of Agriculture and Water Development of Zambia, sponsored a crop collecting expedition to explore the Southern, Western, Copperbelt and Laupula provinces of Zambia for crop germplasm (1). In addition to many other crops, 445 collections of cucurbits were made. Sampling areas included farmers' fields, threshing grounds, backyards, farm stores, village markets and natural vegetation along forest margins. In order to sample as wide a range of diversity as possible, most of the ecological zones and agricultural systems within each province were sampled.

The Team Leader was K.L. Mehra (IBPGR Consultant), National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi 110013, India. His efforts were supplemented by counterparts from the various provinces of Zambia and local agricultural specialists.

The seed samples of cucurbits were sent to me for identification by Dr. George A. White, Plant Introduction officer, USDA, Beltsville, MD. This is probably the most extensive and diversified collection of cucurbits ever assembled from Africa (see Table 1). This collection should furnish plant breeders working with the various cucurbit crops some new and much needed material for their research.

Table 1. Cucurbit genetic resources collected in Zambia (1981). The collection was made by an expedition sponsored by the International Board for Plant Genetic Resources, and the Zambia Department of Agriculture and Water Development; Leader - K. L. Mehra.

Genus	Species	No. of Collections
Cucurbita	*moschata*	113
	maxima	104
	pepo[Z]	3
Cucumis	*melo*	13
	sp.[Z]	80
Lagenaria	*siceraria*	134
Luffa	sp.	19
Citrullus	*lanatus*[Z]	55

[Z]There are 2 questionable samples tentatively identified as *Cucurbita maxima*; 1 *C. pepo*; 2 *Cucumis* sp., and 1 *Citrullus lanatus.*

This material will be processed and given Plant Introduction numbers and sent to the appropriate Regional Plant Introduction Station for increase, evaluation and subsequent distribution.

The determinations are reasonably accurate, but some items will have to be grown in the field or greenhouse to establish their identity.

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The Reliability of a Seedling Test for Resistance to Root-Knot Nematodes in Cucurbits

Boukema, I.W., G.T.M. Reuling and K. Hofman

Institute for Horticultural Plant Breeding (IVT), P.O.B. 16, 6700 AA Wageningen, The Netherlands

A seedling test is used to screen for resistance to root-knot nematodes *(Meloidogyne incognita* Chitw.) in cucurbits (1). The level of resistance is measured by determining the mean number of root-knot galls per plant. To verify whether this seedling test gives a reliable prediction of the resistance level, the reaction of four *Cucumis* species (Table 1), which showed different levels of resistance in former tests, was compared in a) the seedling test, and b) a test which approaches more the glasshouse situation (pot test).

The seedling test (1) was carried out in a growth cabinet at 24 C in five replications of eight plants per plot. Each seedling was inoculated with 50 larvae. After five weeks the number of galls per plant was counted and the pooled larvae production per plot was determined by hatching larvae from egg masses on the roots. The roots were therefore cut up and placed on nematode filters in tap water at room temperature.

The pot test was carried out in a glasshouse at a minimum temperature of 20 C, rising to 25-28 C on sunny days. *Cucumis* seedlings were transplanted to pots containing five liters of sieved glasshouse soil and inoculated one week after transplanting with 92 larvae per plant. The test was set up in six replications with six plants per plot. At 7, 10 and 17 weeks after inoculation two plants per plot were assessed. A gall index was assigned to the rootballs (scale 1-10, 1 = 0-10%, 10 = 90-100% of the roots covered with galls). Larvae production was determined by hatching larvae from egg masses on the roots per plot, as described for the seedling test.

Of the pot test, only results of the observation at 17 weeks are given, because they will approach most closely the value of the resistance of the studied genotypes in practice. At that date the gall indexes of the four *Cucumis* species differed significantly from each other (Table 1). The roots of the susceptible *C. sativus* were almost completely disintegrated at that time, while those of the moderately susceptible *C. anguria* var. *longipes* were partly disintegrated. The rootballs of the moderately resistant *C. metuliferus* and the resistant *C. zeyheri* 2x were still intact, but the former showed many more galls than the latter. Rather large numbers of larvae were produced on *C. metuliferus*, only very few on *C. zeyheri*. It should be noted that the *C. zeyheri* plants were initially growing very slowly, with almost no side roots. This may have influenced their high resistance level in this test.

Table 1. Means of the number of galls, of the number of larvae and of the gall index per plant in the seedling test and in the pot test, respectively 5 and 17 weeks after inoculation.

Cucumis species	Cv. or Accession No.	Seedling test		Pot test	
		galls	larvae[z]	gall-index	larvae[z]
C. sativus	G6	39.6 a[y]	5821 a	10.0 a	_[x]
C. anguria var. *longipes*	1751	28.2 b	4126 b	8.4	_[x]
C. metuliferus	1822	10.3 c	202 c	3.9 c	27.755 a
C. zeyheri 2x	0181	7.4 c	6 d	1.1 d	10 b

[z]For the analyses of variance a square root transformation was made.

[y]Means showing a common letter are not different at p=0.05.

[x]Number of larvae could not be determined because of disintegration of the roots.

At the first and the second observation date of the pot test, the ranking order of the gall index and the number of larvae agreed in most cases with that of the gall index after 17 weeks. However, the two resistant genotypes could not be distinguished.

In the seedling test the number of galls gave a good distinction between the susceptible, the moderately susceptible and the two resistant genotypes (Table 1). Both tests revealed that none of the genotypes are completely resistant. For the four genotypes studied, the level of resistance can be more precisely predicted if, besides the number of galls, the larvae production is measured in the seedling test.

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Embryo Size in *Cucumis sativus* x *C. melo* as Affected by Irradiation of the Pollen and Genotype of the Female Parent

Custers, J.B.M. and J.H.W. Bergervoet

Institute for Horticultural Plant Breeding, P.O. Box 16, 6700 AA Wageningen, The Netherlands

The cross *Cucumis sativus* x *C. melo* so far failed because the embryos cease their development at the globular- shaped stage (2). Embryo culture procedures suitable for non- hybrid proembryos and globular-shaped embryos of *Cucumis* spp. could not induce the hybrid globular-shaped embryos of *C. sativus* x *C. melo* to continue differentiation (3). The lack of capacity of progressive differentiation may in part be caused by the difference in basic chromosome number, i.e. x=7 in *C. sativus* and x=12 in *C. melo*. Davies (1), extrapolating from intervarietal crosses with irradiated pollen, has pointed out the possibility that irradiation of the pollen might also overcome embryo abortion caused by incompatibility of the two genomes involved. We surmise that pollen irradiation might induce selective elimination of the *C. melo* chromosomes from the hybrid cells of the young embryos of *C. sativus* x *C. melo*, which might improve the viability of these embryos. This contribution gives some preliminary data regarding this technique in this cross.

We used two accessions of *C. sativus* var. *hardwickii* (IVT Gene bank nos. 0777 and 1811 A) and one of *C. melo* ('Noy Yizreel', a cultivar from Israel with monogenic dominant powdery mildew resistance). The plants were grown in an insect-proof glasshouse (25 C D/18 C N) in the summer of 1983. The *C. melo* pollen was exposed to doses of 0, 10, 100 and 1000 Gy (1 Gy = 100 rad) of gamma radiation and used for pollination within two hours. The treatments were carried out on two dates. Fruits that developed were dissected three weeks after pollination. We assessed seed set (number of ovules > 6 mm per fruit), presence of endosperm and embryo (number of ovules with endosperm and with embryo respectively / 10 large ovules analyzed per fruit), endosperm size (measured from tip to haustorium), and embryo size (diameter or length).

Results of the experiment are in Table 1. In general the values of all the parameters decreased with increasing irradiation dose of the pollen. Mean seed sets, however, did not show significant differences at P = 0.05. Mean frequencies of ovules with endosperm and with embryo were significantly different at P = 0.05 only when the utmost irradiation doses were compared. Endosperm and embryo sizes were affected negatively by high doses of irradiation of the pollen, but clearly not by the low dose of 10 Gy. The difference between embryo sizes on plants of Gbn 0777 and Gbn 1811 A was most intriguing. Whereas the embryos on 0777 exhibited clearly tissue collapse, which was accompanied by yellow discolored dark areas in the endosperm, those on Gbn 1181 A appeared still firm and rather healthy and were surrounded by translucent endosperm. In a separate comparison of Gbn 1811 A with five other genotypes of *C. sativus* pollinated with non-irradiated pollen of *C. melo*, Gbn 1811 A also stood out because of the promising size of the hybrid embryos produced.

Table 1. Effects of pollen irradiation dose and female genotype on seed set, frequencies of ovules with endosperm and with embryo, and sizes of endosperm and embryo in the cross *Cucumis sativus* var. *hardwickii* x *C. melo*.

C. sativus (Gene bank no.)	*C. melo* pollen irradiation dose (Gy)	No. of fruits analyzed	Mean seed set	Mean frequency of ovules with		Mean size[*] (μ) of	
				Endosperm	Embryo	Endosperm	Embryo
0777	0	5	71a[**]	100a	100a	375a	85a
	10	7	52a	100a	88ab	361a	75a
	100	4	42a	76a	43be	228b	46b
	1000	4	40a	8b	0c	205b	-
	0	2	82a	100a	100a	519a	211a

1811 A	10	3	107a	90ab	86ab	539a	228a
	100	2	87a	85ab	40ab	310b	63b
	1000	2	80a	15b	0b	274b	-

[*]Calculated on the basis of the number of endosperms and embryos found in the analyzed ovules.

[**]Per genotype mean values designated by the same letter are not significantly different from each other at P = 0.05.

It seems worthy to study more thoroughly the effects of Gbn 1811 A and of low dose irradiated pollen as its beneficial influence was not excluded. We intend to apply *in vitro* embryo culture in order to put to use the above effects on *in vivo* embryo size.

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Trisomic Identification of Linkage Groups of *Cucurbita*

Graham, J.D., N.F. Weeden and R.W. Robinson

Department of Horticultural Sciences, New York State Agricultural Experiment Station, Geneva, NY 14456

Bemis (1) synthesized trisomic stocks of *Cucurbita*, having 40 chromosomes of *C. moschata* and one from *C. palmata*. The five different trisomics we obtained from him are morphologically very similar but not identical to 'Butternut', the *C. moschata* parent (2). Starch gel electrophoresis of young leaf extracts in combination with specific isozyme stains was used to develop an improved method of identifying *Cucurbita* trisomics.

Trisomic P2 is characterized by a hard, lignified fruit rind, similar to that of *C. palmata*, and a hybrid fumarase phenotype. The fumarase phenotype gave activity at positions corresponding to the bands observed in each parent plus the space between these two bands. Thus, the fumarase and hard rind genes are both on the *C. palmata* chromosome present in the P2 trisomic.

Trisomic P3 had unique phenotypes for aspartate aminotransferase (AAT) and glucose phosphate isomerase (GPI) isozymes. The trisomic had at least two AAT bands not present in *C. moschata*, one of them corresponding to one in the *C. palmata* zymogram and the other apparently a hybrid molecule consisting of one *moschata* and one *palmata* subunit. Trisomic P3 differed from each parental species for both the plastid specific form and cytosolic form of GPI. Two GPI and one AAT isozyme genes are therefore linked on the extra chromosome of the P3 trisomic.

No phenotype differences in isozymes from *C. moschata* were detected in P1, P4, and P6 trisomics. The parental species exhibited at least 20 allelic differences in the 12 enzyme systems investigated. Thus at least 15 of the loci, those for which a *C. palmata* allozyme was not observed in the trisomics, appear to be situated on chromosomes not represented in the selection of trisomics available to us.

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Nuclear DNA Variation in *Cucumis* Species

Ramachandran, C.

Department of Agricultural Botany, University College of Wales, Aberystwyth, U.K.

The genus *Cucumis* includes two sets of species with basic chromosome numbers x = 7 and x = 12, respectively. While the former group is indigenous to Asia, the latter group is believed to be native of Africa. The evolution and speciation within the genus and in particular the relationship between the species with different chromosome numbers is not clear.

Divergence and evolution of species of higher plants is accompanied by large variation in nuclear DNA amounts. A survey of 2C DNA amounts in 20 *Cucumis* species was done and the results are reported in this communication.

The author expresses his gratitude to the institute of Horticultural Plant Breeding, Wageningen, Netherlands for the supply of seeds of *Cucumis* species. The seeds were germinated in an incubator at 24 C and primary roots were fixed in 4 per cent formaldehyde for 2 hr at room temperature. The roots were washed in distilled water (several changes) for 24 hr and then fixed in acetoalcohol (1:3) for another 24 hr. Fixed roots can be stored in the same fixative in a refrigerator at 4 C until required. Roots were washed in distilled water and hydrolyzed in 5N HCl at room temperature for one hr. After hydrolysis the roots were stained in Feulgen stain (pH 2.2) for one hour and washed with three changes of SO_2 water, 10 minutes for each change. The root tips were transferred to distilled water and the meristems squashed in a drop of glycerol. The DNA measurements were made on a Vickers M85 microdensitometer. Fifteen 2C nuclei were measured in each of the three replicates in each species. The estimated DNA values were corrected to picograms using *Allium cepa* (2C = 33.05 pg) as a standard (2).

In Table 1, the total nuclear DNA amounts in 16 diploid and four tetraploid species of *Cucumis* are presented. The estimates vary from 1.373 to 3.886 pg. The African (2n = 24) group has species with both lower and higher DNA amounts than the Asiatic species. The botanical varieties within a particular species do not differ significantly for DNA content.

Table 1. Nuclear DNA content of *Cucumis* species.

	Species	Source	2n	Total DNA in picograms
Asiatic	*C. trigonus* (syn. *C. callosus*)	India	14	1.590
	C. sativus	India	14	1.777
	C. sativus var. *hardwickii*	IVT No. 1753	14	1.798
African	*C. melo* var. *agrestis*	IVT No. 1987	24	2.483
	C. melo var. *utilissimus*	India	24	2.358
	C. melo var. *momordica*	India	24	2.291
	C. metuliferus	IVT No. 1775	24	2.391
	C. anguria var. *longipes*	IVT No. 1735	24	1.587
	C. africanus	IVT No. 1984	24	1.782
	C. ficifolius	IVT No. 1801	24	1.373
	C. meeusi	IVT No. 1800	48	3.203
	C. dinteri	IVT No. 1794	24	2.167
	C. dipsaceus	IVT No. 1774	24	2.448
	C. figarei	IVT No. 1804	48	3.886
	C. zeyheri	IVT No. 0162	24	1.682

C. zeyheri	IVT No. 1053	48	2.846
C. sagitattus	IVT No. 2069	24	1.571
C. prophetarum	India	24	1.656
C. humifructus	South Africa	24	2.455
C. heptadactylus	IVT No. 1798	48	2.225

The two economically important vegetables in this genus, viz. cucumber (*C. sativus*) and muskmelon (*C. melo*), differ for their nuclear DNA contents. Hence, neither the fragmentation hypothesis of origin of muskmelon from cucumber (1) nor the fusion hypothesis of origin of cucumber from muskmelon (4) is supported. The detailed cytological studies at mitotic metaphase, pachytene and meiotic metaphase stages and also the Giemsa C-banding of somatic chromosomes in cucumber and muskmelon have revealed the untenability of the above two hypotheses (3). Another possibility of having any relationship between these two species is amplification or deletion of DNA segments within the chromosomes followed by species divergence. A more detailed study which would include quantitative estimation of different nuclear components (satellite sequence, middle repetitive DNA and nonrepetitive DNA) would be useful to assess the taxonomical relationship between these two species. The distribution of these sequences as revealed by *in situ* hybridization experiments would give valuable information regarding the chromosome evolution in these species.

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Monogenic Inheritance of Andromonoecy in Tetraploid *Cucumis ficifolius* A. Rich

Visser, D.L. and A.P.M. den Nijs

Institute for Horticultural Plant Breeding (IVT), P.O.B. 16, 6700 AA Wageningen, The Netherlands

Spontaneous fruit and seed set was observed in one out of ten plants of *Cucumis ficifolius A*. Rich (Genebanknumber 2061, IVT collection from Kenya), grown under isolation in an insect proof glasshouse in the summer of 1981. Under our conditions this monoecious wild species normally sets fruit only after artificial pollination. The single exceptional plant was selfed and used as pollen parent in crosses with several sibs of the same accession.

Thirty plants of a progeny obtained after selfing were examined in 1982, and all possessed perfect flowers in addition to staminate ones, so they were andromonoecious. Fruits set following inadvertent self pollinations contained on average 35 seeds. Not a single pistillate flower was observed on any of the 30 plants. All 27 plants of a progeny of a sib cross were monoecious, as was the selfed progeny of the monoecious female parent of this cross. The andromonoecious character thus appeared to be a recessive mutant character.

To investigate the inheritance of the trait, three monoecious plants of the sib cross were selfed and backcrossed with andromonoecious plants out of the self progeny of the putative mutant plant.

The resulting offspring was classified for sex expression, shown in Table 1. From the results it can be concluded that a single recessive gene governs andromonoecy in this species. We propose to designate the gene: *andromonoecy*, symbol *a*. This symbol is the same as in melon and watermelon, whereas in cucumber the symbol *m* has been given priority (2).

Table 1. Segregation of andromonoecious sextype in crosses in *C. ficifolius*.

Population	Number of plants		Probability (%)
	Monoecious	**Andromonoecious**	
F$_2$ (monoecious x andromonoecious)			x^2 (3:1)
1	85	32	50-70
2	36	6	10-20
3	98	23	95
Homogeneity			20-30
Total	219	70	70-90
(monoecious x andromonoecious) x andromonoecious			x^2 (1:1)
1	30	21	20-30
2	50	35	10-20
3	33	52	1-5
Homogeneity			1-5
Total	113	108	90-95

Counting of metaphase plates in root tips revealed 48 chromosomes in accession Gbn 2061, so it is a tetraploid like almost all accessions of *C. ficifolius* in our collection (Kroon, unpublished results). The apparently disomic inheritance of gene *a* testifies to the allotetraploid nature of the species. Meiotic chromosome studies of related tetraploid species revealed allotetraploidy (1), but no genetic segregation data have so far been presented to support this conclusion.

Monoecy is the prevalent sex expression in the genus *Cucumis*, *C. melo* excepted, and we have never before observed andromonoecy in any wild species of our collection. Rosa (3) considered monoecy as primitive in *C. melo*, from which the andromonoecious condition of the cultivated melons evolved. Our finding of one andromonoecious individual in one out of nine accessions of *C. ficifolius* presents another case of parallel evolution in the sex expression of the cucurbits.

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Special Request - Request for Germplasm of Cucurbits

A.P.M. den Nijs

Institute for Horticultural Plant Breeding (IVT), P.O.B. 16, 6700 AA Wageningen, The Netherlands

In our research to lower the energy requirements of glasshouse cucumbers for the heated winter culture in the Netherlands, we are studying rootstock/graft interactions in addition to selection of cucumber genotypes which grow well at lower temperatures.

The commonly used rootstock for cucumber is *Cucurbita ficifolia,* but a different wild cucurbit, *Sicyos angulatus* also holds promise for our winter culture at low temperatures. This rootstock has been in use in Japan for some time, especially because of its tolerance for low soil temperatures. We find our accession of this species (which is endemic in the americas) highly resistant to several soil diseases.

However, the plant is very slender and does not easily permit grafting. We therefore request seeds of other accessions of *Sicyos angulatus* or related cucurbit species to evaluate their potential as rootstock for the glasshouse cucumber.

Covenant and By-Laws of the Cucurbit Genetics Cooperative

ARTICLE I. Organization and Purposes

The Cucurbit Genetics Cooperative is an informal, unincorporated scientific society (hereinafter designated "CGC") organized without capital stock and intended not for business or profit but for the advancement of science and education in the field of genetics of cucurbits (Family: Cucurbitaceae). Its purposes include the following: to serve as a clearing house for scientists of the world interested in the genetics and breeding of cucurbits, to serve as a medium of exchange for information and materials of mutual interest, to assist in the publication of studies in the aforementioned field, and to accept and administer funds for the purposes indicated.

ARTICLE II. Membership and Dues

The membership of the CGC shall consist solely of active members; an active member is defined as any person who is actively interested in genetics and breeding of cucurbits and who pays biennial dues. Memberships are arranged by correspondence with the Chairman of the Coordinating Committee.

The amount of biennial dues shall be proposed by the Coordinating Committee and fixed, subject to approval at the Annual Meeting of the CGC. The amount of biennial dues shall remain constant until such time that the Coordinating Committee estimates that a change is necessary in order to compensate for a fund balance deemed excessive or inadequate to meet costs of the CGC.

Members who fail to pay their current biennial dues within the first six months of the biennium are dropped from active membership. Such members may be reinstated upon payment of the respective dues.

ARTICLE III. Committees

1. The Coordinating Committee shall govern policies and activities of the CGC. It shall consist of six members elected in order to represent areas of interest and importance in the field. The Coordinating Committee shall select its Chairman, who shall serve as a spokesman of the CGC, as well as its Secretary and Treasurer.
2. The Gene List Committee, consisting of five members, shall be responsible for formulating rules regulating the naming and symbolizing of genes, chromosomal alterations, or other hereditary modifications of the cucurbits. It shall record all newly reported mutations and periodically report lists of them in the Report of the CGC. It shall keep a record of all information pertaining to cucurbit linkages and periodically issue revised linkage maps in the Report of the CGC. Each committee member shall be responsible for genes and linkages of one of the following groups: cucumber, *Cucurbita* sp., muskmelon, watermelon, and other genera and species.
3. Other committees may be selected by the Coordinating Committee as the need for fulfilling other functions arises.

ARTICLE IV. Election and Appointment of Committees

1. The Chairman will serve an indefinite term while other members of the Coordinating Committee shall be elected for ten-year terms, replacement of a single retiring member taking place every other year. Election of a new member shall take place as follows: A Nominating Committee of three members shall be appointed by the Coordinating Committee. The aforesaid Nominating Committee shall nominate candidates for an anticipated opening on the Coordinating Committee, the number of nominees being at their discretion. The nominations shall be announced and election held by open-ballot at the Annual Meeting of the CGC. The nominee receiving the highest number of votes shall be declared elected. The newly elected member shall take office immediately.

In the event of death or retirement of a member of the Coordinating Committee before the expiration of his/her term, he/she shall be replaced by an appointee of the Coordinating Committee.

Members of other committees shall be appointed by the Coordinating Committee.

ARTICLE V. Publications

1. One of the primary functions of the CGC shall be to issue an Annual Report each year. The Annual Report shall contain sections in which research results and information concerning the exchange of stocks can be published. It shall also contain the annual financial statement. Revised membership lists and other useful information shall be issued periodically. The Editor shall be appointed by the Coordinating Committee and shall retain office for as many years as the Coordinating Committee deems appropriate.
2. Payment of biennial dues shall entitle each member to a copy of the Annual Report, newsletters, and any other duplicated information intended for distribution to the membership. The aforementioned publications shall not be sent to members who are in arrears in the payment of dues. Back numbers of the Annual Report, available indefinitely, shall be sold to active members at a rate determined by the Coordinating Committee.

ARTICLE VI. Meetings

An Annual Meeting shall be held at such time and place as determined by the Coordinating Committee. Members shall be notified of time and place of meetings by notices in the Annual Report or by notices mailed not less than one month prior to the meeting. A financial report and information on enrollment of members shall be presented at the Annual Meeting. Other business of the Annual Meeting may include topics of agenda selected by the Coordinating Committee or any items that members may wish to present.

ARTICLE VII. Fiscal Year

The fiscal year of the CGC shall end on December 31.

ARTICLE VIII. Amendments

These By-Laws may be amended by simple majority of members voting by mail ballot, provided a copy of the proposed amendments has been mailed to all the active members of the CGC at least one month previous to the balloting deadline.

ARTICLE IX. General Prohibitions

Notwithstanding any provision of the By-Laws or any other document that might be susceptible to a contrary interpretation:

1. The CGC shall be organized and operated exclusively for scientific and educational purposes.
2. No part of the net earnings of the CGC shall or may under any circumstances inure to the benefit of any individual.
3. No part of the activities of the CGC shall consist of carrying on propaganda or otherwise attempting to influence legislation of any political unit.
4. The CGC shall not participate in, or intervene in (including the publishing or distribution of statements), any political campaign on behalf of a candidate for public office.
5. The CGC shall not be organized or operated for profit.
6. The CGC shall not:
    a. lend any part of its income or corpus without the receipt of adequate security and a reasonable rate of interest to;
    b. pay any compensation in excess of a reasonable allowance for salaries or other compensation for personal services rendered to;
    c. make any part of its services available on a preferential basis to;
    d. make any purchase of securities or any other property, for more than adequate consideration in money's worth from;
    e. sell any securities or other property for less than adequate consideration in money or money's worth; or
    f. engage in any other transactions which result in a substantial diversion of income or corpus to any officer, member of the
       Coordinating Committee, or substantial contributor to the CGC.

The prohibitions contained in this subsection (6) do not mean to imply that the CGC may make such loans, payments, sales, or purchases to anyone else, unless authority be given or implied by other provisions of the By-laws.

ARTICLE X. Distribution on Dissolution

Upon dissolution of the CGC, the Coordinating Committee shall distribute the assets and accrued income to one or more scientific organizations as determined by the Committee, but which organization or organizations shall meet the limitations prescribed in sections 1-6 of Article IX.

Approvals:

W. Bemis

J. B. Norton

R. W. Robinson

W. R. Henderson

M. L. Robbins

R. L. Lower

CGC 6:103 (1983)