

## Environmental effects on genetic variation of chilling resistance in cucumber

Lambertus Smeets & Todd C. Wehner

*Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609, U.S.A.*

Received 4 February 1997; accepted 25 May 1997

*Key words:* *Cucumis sativus*, chilling injury, cold shock damage, low-temperature tolerance, vegetable breeding

### Summary

Environmental effects on genetic variation for chilling resistance were studied in nine cultivars and breeding lines (referred to as cultigens hereafter) of cucumber (*Cucumis sativus* L.). Five experiments were carried out in controlled-environment chambers to measure the effects of growth temperature, photoperiod, duration of chilling, light level during chilling, and watering frequency on chilling resistance of seedlings at the cotyledon and first true leaf growth stages. Significant interactions were found between cultigen and all environmental factors studied except for the photoperiod and watering frequency. Cultigen rank was affected by growth temperature before chilling, chilling duration, and light level during chilling, but shifts in rank were not consistent. Genetic variation was largest when the plants were grown at 22/18 °C, most pronounced after a chilling duration of 5 to 9 hours and a light level during chilling of 270  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Variation was larger at the first true leaf stage than at the cotyledon stage. Differences among cultigens in chilling damage were largest 5 days after chilling. Therefore, it seems that testing for genetic variation in chilling damage can be restricted to one set of environmental conditions. We recommend the following conditions for screening cucumber for genetic variation in chilling resistance: grow the plants at 22/18 °C, under a 9-hour photoperiod with a 3-hour night interruption, water them once daily, subject them at the first true leaf stage to a chilling treatment of 7 hours at 4 °C at a light level of 270  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , and evaluate damage 5 days after treatment.

*Abbreviations:* SAS – Statistical Analysis System from SAS Institute (Cary, NC) for data analysis; GLM – General Linear Models procedure of SAS

### Introduction

North Carolina is a leading producer of field-grown cucumbers (*Cucumis sativus* L.) in the United States. The state ranks second in the production of pickling cucumbers, and fourth in slicing cucumbers (United States Department of Agriculture, 1993). A spring and a summer crop are grown, with the spring crop in the coastal plain (the primary production area) being planted about 15 April (Hughes et al., 1983). The crop is planted one month later in the mountains (the secondary production area). Earlier planting often results in poor germination and emergence. Moreover, the plants may be injured by chilling (low temperatures above freezing) in their early stage of growth. After chilling, the cucumber leaves become yellow or necrot-

ic and the seedlings may be killed. In order to extend the production season for growers and processors, cultivars must be developed that have both cold germination ability and chilling resistance.

There is genetic variation among cucumber cultigens (used hereafter to refer collectively to cultivars and breeding lines as described by Soule, 1985) for germination at low temperature (Lower, 1974; Nienhuis et al., 1983; Wehner, 1981). Cold germination has a heritability of 0.15 to 0.61, depending on test temperature and population used (Wehner, 1982, 1984), and can be improved without correlated changes in other important horticultural traits (Staub et al., 1988). Genetic variation for chilling resistance in cucumber appears to exist (Aoki et al., 1988, 1989; Cabrera et al., 1992; Liu et al., 1984; Saczynska et al., 1993). How-

ever, absence of variation for that trait in cucumber has also been reported (Rietze, 1988; Staub, 1988).

Chilling damage in cucumber, as in other thermophilic plants, depends mainly on chilling temperature, duration of chilling, and on the light conditions during chilling (Minchin & Simon, 1973; Rietze, 1988; Rietze & Wiebe, 1987, 1989; Van Hasselt, 1972; Wang, 1986; Wright & Simon, 1973). The environment before and after chilling also is important. Chilling damage is affected by the temperature, light conditions and water status of the plants prior to chilling (Lafuente et al., 1990; Pomeroy & Mudd, 1987; Rietze & Wiebe, 1989; Rikin et al., 1976; Saltveit, 1991; Wilson & Crawford, 1974) and the light conditions after chilling (Lasley et al., 1979; Rietze & Wiebe, 1989).

We could find no information on whether genetic variation in chilling sensitivity in cucumber is affected by the environment. As a result, the environmental conditions for screening for genetic variation in chilling resistance in cucumber are also not known. Therefore, we evaluated a diverse set of cultigens for the effect of environment on genetic variation in chilling resistance to determine the most effective environment for screening.

## Materials and methods

### *Plant material*

Nine cultigens were chosen on the basis of genetic diversity in important horticultural traits including resistance to chilling and low temperature germination. The cultigens were AR 79-75; 'Chipper', Gy 14, 'Marketmore 76', NCSU M 28, NCSU M 29, 'Pixie', 'Poinsett 76', and 'Wisconsin SMR 18'. AR 79-75, 'Chipper', 'Pixie' and 'Wisconsin SMR 18' had chilling resistance (Wehner et al., 1997), and 'Pixie' and 'Wisconsin SMR 18' had cold germination ability (Lower, 1974).

Experiments were conducted at the Southeastern Plant Environment Laboratory (Phytotron) at North Carolina State University (Downs & Thomas, 1991). Seeds were sown in peat pots (57 mm square) in a medium of gravel and peat-lite (2:1 ratio) and placed in flats. Three seeds were sown per pot, and thinned to one plant just after emergence. Each flat was divided in half. In one half plants were grown to the cotyledon stage, and in the other half to the first true leaf stage. Each half of a flat contained nine rows of three pots each, one row per cultigen. In order to have both groups of plants at

the proper growth stage at the same time, plants for the first true leaf stage were seeded 4 to 7 days earlier (depending on the growth temperature) than for the cotyledon stage. The flats were placed in controlled-environment chambers set at different temperatures at a 9-hour photoperiod (8 am – 5 pm) with a 3 hour night interruption (11 pm – 2 am). Photosynthetically active radiation (400–700 nm) was  $639 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  during the day, and  $39 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  during the night interruption. Photomorphogenic radiation (700–800 nm) was  $73 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  during the day and  $58 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  during the night interruption. Plants were watered once daily with the standard Phytotron nutrient solution (Downs & Thomas, 1991).

### *Chilling treatments*

After the plants in both halves of the flats had reached the proper stage of development (cotyledon and first true leaf stage respectively), they were chilled at  $4^\circ\text{C}$  in the light (light level  $500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). Plants were moved from the chamber in which they had been grown to the chamber where they were chilled at 8 am. After chilling they were returned to the growth chamber from where they were taken prior to chilling and placed at the same temperature and light regime as before.

### *Assessment of injury*

Leaf damage was always rated 1 and 7 days after the chilling treatment. The scale was 0 = no damage, 1–2 = trace, 3–4 = slight, 5–6 = moderate, 7–8 = advanced, 9 = dead (Figure 1). Data were collected as means over all leaves on the three plants that constituted the plot (one treatment combination).

### *Experiment design and data analysis*

Environmental factors studied were growth temperature, photoperiod, watering frequency, chilling duration and light level during chilling. Five experiments were run using a split-plot treatment arrangement in a randomized complete block design with 2 replications. The nine cultigens described above were included in all experiments. Plants were grown, chilled and rated as described above.

Data analysis was by the procedure GLM in SAS (SAS, 1988). Means were tested using Fisher's LSD when the F ratio for that treatment indicated significance at the 5% level. For all significant interactions involving cultigen, we checked to see if cultigen rank



Figure 1. Chilling damage in cucumber seedlings, with ratings of 1 through 9 (for the 1st true leaf stage) from top left to bottom right (0 damage not shown).

was affected, and whether the interactions were consistent over experiments.

In Experiment 1, the effect of a chilling treatment of 0, 3, 5, 7 or 9 hours was studied for plants grown at 26/22 °C. Whole plots were the five chilling durations,

subplots were the two growth stages, and sub-subplots were the nine cultivars.

In Experiment 2, the effect of watering once or twice daily was studied for plants grown at 22/18 °C or 30/26 °C which were subjected to a chilling treatment of 0 or 9 hours. Whole plots were the two growth

temperatures, subplots were the two waterings, sub-subplots were the two chilling durations, sub-sub-subplots were the two growth stages, and sub-sub-sub-subplots were the nine cultigens.

In Experiment 3, the effect of 0, 3, 5, 7 or 9 hours of chilling was studied in plants grown at 22/18 °C or 26/22 °C. Whole plots were the two growth temperatures, subplots were the five chilling durations, sub-subplots were the two growth stages, and sub-sub-subplots were the nine cultigens.

In Experiment 4, the effect of a chilling treatment of 0, 5, 7 or 9 hours was studied for plants grown at 30/26 °C and chilled at light levels of 0, 150, 270 or 500  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Whole plots were the four light levels, subplots were the four chilling durations, sub-subplots the two growth stages and sub-sub-subplots the nine cultigens.

In Experiment 5, the effect of a 9-hour photoperiod with or without a 3-hour night interruption was studied for plants grown at 22/18 °C and chilled for 7 hours. Whole plots were the two photoperiods and subplots were the nine cultigens.

## Results

In each of the five experiments, damage ratings on days 1 and 7 after the chilling treatment were always highly correlated ( $r = 0.89, 0.88, 0.89, 0.93$  and  $0.86$  for Experiments 1, 2, 3, 4 and 5, respectively). Since on day 1, damage had not developed fully, damage on day 7 gave a better impression of the performance of the cultigens. Therefore, only the data for day 7 were presented in the tables.

### Experiment 1

Analysis of variance showed that cultigen had a significant effect on damage rating. There was significant interaction between growth stage and cultigen.

Growth stage had an effect on cultigen rank for chilling damage (Table 1). For AR 79-75 and 'Poinsett 76' the rank for chilling damage shifted from high at the cotyledon stage to low at the first true leaf stage. For M 29, the reverse was the case. The differences among cultigens were slightly higher when chilled at the first true leaf stage than at the cotyledon stage.

The interaction between the duration of chilling and cultigen was not significant. Cultigen rank for chilling damage (mean of 4 chilling durations) was: 'Pixie' (4.0), 'Chipper' (5.3), 'Wisconsin SMR 18' (5.3), AR

Table 1. Interaction between growth stage and cultigen for chilling damage (rank) in cucumber seedlings in experiment 1\*

Cultigen	Growth stage at chilling	
	Cotyledon	First true leaf
Resistant		
AR 79-75	6.5 (5)	5.0 (2)
Chipper	5.5 (3)	5.0 (2)
Pixie	5.0 (1)	4.8 (1)
Wis. SMR 18	5.4 (2)	5.3 (4)
Susceptible		
Gy 14	7.1 (9)	6.3 (7)
M 28	6.5 (5)	6.0 (6)
M 29	6.4 (4)	7.1 (9)
Marketmore 76	7.0 (8)	6.6 (8)
Poinsett 76	6.8 (7)	5.5 (5)
Control	0.0	0.0
Mean (w/o control)	6.2	5.7
Range (w/o control)	2.1	2.3
LSD (5%)		
for row and column comparisons		1.1
CV (%)		13

\* Damage rating was 0–9 (0 = none, 1–2 = trace, 3–4 = slight, 5–6 = moderate, 7–8 = advanced, 9 = dead). Data are means of 4 chilling durations and 2 replications.

79-75 (5.8), 'Poinsett 76' (6.1), M 28 (6.3), Gy 14 (6.7), M 29 (6.8), and 'Marketmore 76' (6.9). The range for the cultigens in chilling damage was largest after 7 hours of chilling (1.5, 2.3, 3.5 and 1.8 for a chilling duration of 3, 5, 7 and 9 hours, respectively). Mean chilling damage increased with an increase in chilling duration (3.4, 5.3, 7.1 and 8.1 for 3, 5, 7 and 9 hours, respectively).

### Experiment 2

Analysis of variance showed that watering frequency did not affect chilling injury. Cultigen had significant effects on damage rating. There was a significant interaction for growth stage and cultigen, and for growth temperature, growth stage and cultigen.

At both growth temperatures, there were shifts in cultigen rank for chilling damage when the plants were chilled at the first true leaf stage instead of at the cotyledon stage (Table 2). Moreover the effect of growth stage on the cultigen rank was affected by the growth temperature. When grown at 22/18 °C, 'Chipper' had a higher rank when chilled at the first true leaf stage than at the cotyledon stage. At 30/26 °C the rank for 'Chipper' was similar for both growth stages. For AR

Table 2. Interaction between growth temperature, cultigen and growth stage for chilling damage (rank) in cucumber seedlings in experiment 2\*

Cultigen	Growth temperature			
	22/18 °C		30/26 °C	
	Stage at chilling		Stage at chilling	
	Cotyledon	First true leaf	Cotyledon	First true leaf
Resistant				
AR 79-75	6.0 (3)	4.5 (3)	6.3 (4)	5.5 (2)
Chipper	5.3 (1)	5.3 (4)	4.3 (2)	6.5 (3)
Pixie	5.3 (1)	2.8 (1)	4.0 (1)	5.3 (1)
Wis. SMR 18	6.3 (4)	3.8 (2)	4.8 (3)	7.3 (4)
Susceptible				
Gy 14	7.8 (8)	7.0 (7)	8.0 (6)	8.5 (6)
M 28	6.8 (5)	7.0 (7)	8.3 (9)	8.5 (6)
M 29	7.5 (7)	7.5 (9)	8.0 (6)	9.0 (8)
Marketmore 76	7.8 (8)	6.8 (6)	7.8 (5)	9.0 (8)
Poinsett 76	7.0 (6)	6.5 (5)	8.0 (6)	8.0 (5)
Control	0.0	0.0	0.0	0.0
Mean (w/o control)	6.6	5.7	6.6	7.5
Range (w/o control)	2.5	4.8	4.3	3.8
LSD (5%) for row and column comparisons			0.8	
CV (%)			12	

\* Damage rating was 0–9 (0 = none, 1–2 = trace, 3–4 = slight, 5–6 = moderate, 7–8 = advanced, 9 = dead). Data are means of 2 watering regimes and 2 replications.

Table 3. Interaction between growth temperature and cultigen for chilling damage (rank) in cucumber seedlings in experiment 3\*

Cultigen	Growth temperature	
	22/18 °C	26/22 °C
Resistant		
AR 79-75	1.7 (1)	3.8 (4)
Chipper	2.1 (3)	3.5 (2)
Pixie	1.9 (2)	3.4 (1)
Wis. SMR 18	2.3 (4)	3.9 (6)
Susceptible		
Gy 14	3.2 (6)	4.1 (8)
M 28	2.9 (5)	3.8 (4)
M 29	3.8 (8)	4.2 (9)
Marketmore 76	3.5 (7)	3.9 (6)
Poinsett 76	4.1 (9)	3.6 (3)
Control	0.0	0.0
Mean (w/o control)	2.8	3.8
Range (w/o control)	2.4	0.8
LSD (5%)		
for row and column comparisons		0.7
CV (%)		32

\* Damage rating was 0–9 (0 = none, 1–2 = trace, 3–4 = slight, 5–6 = moderate, 7–8 = advanced, 9 = dead). Data are means of 4 chilling durations, 2 growth stages and 2 replications.

79-75 the reverse was the case. There was no effect of growth stage on cultigen rank when the plants were grown at 22/18 °C, but there were differences when they were grown at 30/26 °C. The rank was higher at the cotyledon than at the first true leaf stage. Nevertheless, chilling damage at both growth temperatures was always lowest for ‘Chipper’, AR 79-75, ‘Pixie’ and ‘Wisconsin SMR 18’ and highest for the other cultigens irrespective of the growth temperature and growth stage. The differences among cultigens were largest when the plants were grown at 22/18 °C and were chilled at the first true leaf stage.

### Experiment 3

Analysis of variance showed that cultigen had a significant effect on damage rating. Significant interactions occurred for growth temperature and cultigen.

Cultigen rank was affected by growth temperature (Table 3). At 22/18 °C, chilling damage for AR 79-75, ‘Chipper’, and ‘Pixie’ was significantly lower than for Gy 14, M 28, M 29, ‘Marketmore 76’, and ‘Poinsett 76’. At 26/22 °C there were no significant differences in chilling damage among cultigens. As those shifts did not occur in Experiment 2 (no significant interac-

Table 4. Range and correlation for chilling damage in cucumber seedlings 1, 3, 5 and 7 days after chilling in experiment 3\*

Cultigen	Days after chilling			
	1 day	3 days	5 days	7 days
Resistant				
AR 79-75	0.5	1.5	1.8	2.0
Chipper	0.8	1.3	2.0	2.3
Pixie	1.5	3.0	3.5	2.8
Wis. SMR 18	1.8	2.5	2.8	2.8
Susceptible				
Gy 14	2.0	3.5	3.8	3.3
M 28	2.3	4.0	4.5	4.0
M 29	2.0	3.8	3.8	3.0
Marketmore 76	2.3	4.3	4.5	4.3
Poinsett 76	2.5	4.0	5.0	4.8
Mean (w/o control)	1.7	3.1	3.5	3.3
Range (w/o control)	2.0	3.0	3.2	2.8
Correlation with day 7	0.90**	0.87**	0.94**	1.00
LSD (5%) for row and column comparisons			1.7	
CV (%)			32	

\* Damage rating was 0–9 (0 = none, 1–2 = trace, 3–4 = slight, 5–6 = moderate, 7–8 = advanced, 9 = dead). Data are means of 3 chilling durations, 2 growth stages and 2 replications.

tion between growth temperature and cultigen), that response was inconsistent.

There were no significant interactions between growth stage and cultigen, and among growth temperature, growth stage and cultigen in Experiment 3. Thus, the shifts in rank observed in Experiment 1 at 26/22 °C for AR 79-75 and 'Poinsett 76' from a high rank for chilling damage at the cotyledon stage to a low rank at the first true leaf stage, and in Experiment 2 at 22/18 °C for 'Chipper' from a low rank for chilling damage at the cotyledon stage to a high rank at the first true leaf stage, were not consistent either. Differences among cultigens in Experiment 3 were largest when the plants were grown at 22/18 °C.

Genetic differences in chilling resistance could be assessed easily after only 1 day (Table 4). However, differences among cultigens in chilling damage were largest 5 days after the chilling treatments. Thus, when screening for genetic variation in chilling resistance, rating should be done 5 days after chilling.

#### Experiment 4

Analysis of variance showed that cultigen had a significant effect on damage rating. Moreover, there were significant interactions between duration of chilling

and cultigen, light level during chilling and cultigen, and growth stage and cultigen.

There were no large shifts in cultigen rank for chilling damage as the duration of chilling was increased (Table 5). Cultigens with low chilling damage were the same at all chilling durations. The differences among cultigens in chilling damage were largest at the 7-hour duration of chilling.

An increase in light level during chilling resulted in only one large shift in cultigen rank for chilling damage (Table 6). 'Marketmore 76' shifted from an intermediate position at the lower light levels to the highest position at the highest light levels. That shift was unimportant, since 'Marketmore 76' would be considered susceptible at all light levels. The differences among cultigens in chilling damage were largest at a light level of 270  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ .

The growth stage at which the cultigens were subjected to the chilling treatment had an effect on the rank of cultigens for chilling damage (Table 7). For 'Marketmore 76' the rank shifted from intermediate damage at the cotyledon stage to high damage at the first true leaf stage. However, that shift did not occur in Experiment 2. There was no shift of AR 79-75 from a higher rank for chilling damage at the cotyledon stage to a lower rank at the first true leaf stage, as was found

Table 5. Interaction between duration of chilling and cultigen for chilling damage (rank) in cucumber seedlings in experiment 4\*

Cultigen	Duration of chilling		
	5 hours	7 hours	9 hours
Resistant			
AR 79-75	1.0 (1)	3.2 (1)	4.9 (2)
Chipper	1.3 (2)	3.2 (1)	4.7 (1)
Pixie	1.4 (3)	4.3 (3)	5.7 (3)
Wis. SMR 18	1.7 (5)	4.6 (5)	6.0 (4)
Susceptible			
Gy 14	1.9 (6)	6.3 (8)	7.3 (9)
M 28	2.0 (7)	5.4 (6)	6.7 (7)
M 29	2.1 (8)	6.3 (8)	6.9 (8)
Marketmore 76	2.1 (8)	5.4 (6)	6.6 (6)
Poinsett 76	1.6 (4)	4.3 (3)	6.0 (4)
Control	0.0	0.0	0.0
Mean (w/o control)	1.7	4.8	6.1
Range (w/o control)	1.1	3.1	2.6
LSD (5%)	1.7		
for row and column comparisons	26		
CV (%)	26		

\* Damage rating was 0–9 (0 = none, 1–2 = trace, 3–4 = slight, 5–6 = moderate, 7–8 = advanced, 9 = dead). Data are means of 3 light levels, 2 growth stages and 2 replications.

in Experiment 2. Thus, none of the shifts were consistent. The differences among cultigens for chilling damage were larger at the first true leaf stage than at the cotyledon stage.

#### Experiment 5

Analysis of variance showed that cultigen had a significant effect on chilling damage. Chilling damage for the plants grown at a photoperiod of 9 hours or 9 hours with a 3 hour night interruption was moderate to advanced (Table 8). The difference in damage between the two photoperiods was not significant. The rank of cultigens for chilling damage was not affected by the photoperiod at which the plants were grown. The differences in damage among cultigens were largest for the plants grown at a photoperiod of 9 hours with a 3 hour night interruption.

#### Discussion

At a chilling temperature, damage depends on the duration of chilling (Rietze, 1988; Rietze & Wiebe, 1987; Van Hasselt, 1972) and the light level during chilling

Table 6. Interaction between light level during chilling and cultigen for chilling damage (rank) in cucumber seedlings in experiment 4\*

Cultigen	Light level during chilling ( $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ )		
	150	270	500
Resistant			
AR 79-75	1.3 (1)	3.0 (1)	4.8 (2)
Chipper	2.1 (2)	3.0 (1)	4.0 (1)
Pixie	2.2 (3)	3.7 (3)	5.5 (5)
Wis. SMR 18	2.6 (4)	4.9 (6)	5.0 (3)
Susceptible			
Gy 14	3.7 (9)	6.0 (9)	5.8 (7)
M 28	3.3 (7)	5.2 (7)	5.5 (5)
M 29	3.3 (7)	5.7 (8)	6.3 (8)
Marketmore 76	2.6 (4)	4.6 (5)	6.8 (9)
Poinsett 76	2.6 (4)	4.0 (4)	5.3 (4)
Control	0.0	0.0	0.0
Mean (w/o control)	2.6	4.5	5.4
Range (w/o control)	2.4	3.0	2.8
LSD (5%)	0.9		
for row and column comparisons	26		
CV (%)	26		

\* Damage rating was 0–9 (0 = none, 1–2 = trace, 3–4 = slight, 5–6 = moderate, 7–8 = advanced, 9 = dead). Data are means of 3 chilling durations, 2 growth stages and 2 replications.

(Rietze, 1988; Rietze & Wiebe, 1987, 1989; Táncoz, 1974; Van Hasselt, 1972; Wang, 1986; Wright & Simon, 1973). Both effects were confirmed in our experiments. Mean chilling damage increased with an increase in the duration of chilling and with an increase in light level during chilling. The magnitude of the effects seems to depend on cultigen.

Growth temperature had an effect on chilling damage. Mean chilling damage was lower when the plants were grown at 22/18 °C than at 26/22 °C. Similar effects have been described in the literature. Wilson & Crawford (1973) found that cucumber plants grown at 25 °C and subjected to 12 °C for 4 days prior to chilling were less damaged than plants grown at a constant 25 °C. Saltveit (1991) observed that the rate of ion leakage from cucumber mesocarp discs after 4 days of chilling at 2.5 °C increased significantly when the tissue was subjected to 32 °C for 6 hours instead of being exposed continuously to 12 °C. However, lower chilling damage induced by high temperature prior to chilling has also been reported. Chilling damage was reduced greatly when cucumber protoplasts were placed at 27 °C for 6 hours prior to chilling at 4 °C

Table 7. Interaction between growth stage and cultigen for chilling damage (rank) in cucumber seedlings in experiment 4\*

Cultigen	Growth stage at chilling	
	Cotyledon	First true leaf
Resistant		
AR 79-75	2.3 (1)	3.7 (1)
Chipper	2.3 (1)	3.7 (1)
Pixie	2.6 (3)	4.9 (5)
Wis. SMR 18	3.5 (7)	4.8 (4)
Susceptible		
Gy 14	4.1 (9)	6.2 (7)
M 28	3.4 (6)	5.9 (6)
M 29	3.6 (8)	6.5 (9)
Marketmore 76	3.1 (4)	6.2 (7)
Poinsett 76	3.2 (5)	4.7 (3)
Control	0.0	0.0
Mean (w/o control)	3.1	5.2
Range (w/o control)	1.8	2.8
LSD (5%)	0.7	
for row and column comparisons	0.7	
CV (%)	26	

\* Damage rating was 0–9 (0 = none, 1–2 = trace, 3–4 = slight, 5–6 = moderate, 7–8 = advanced, 9 = dead). Data are means of 3 chilling durations, 3 light levels and 2 replications.

(Pomeroy & Mudd, 1987). Exposure to 37 °C for 6 hours before chilling reduced significantly the rate of ion leakage from leaf discs of fully-expanded cotyledons of cucumber (Lafuente et al., 1991).

We found no effect of watering frequency on chilling damage (Experiment 2). However, Rikin et al. (1971) found, when chilling cucumber seedlings in the dark, that withholding water increased resistance to chilling. In our experiment, in which the seedlings were chilled in the light, watering once daily resulted in optimal conditions for chilling damage; watering twice daily did not increase the amount of damage.

An interruption of the dark period with 3 hours of light followed by 6 hours of darkness before chilling in the light did not decrease chilling sensitivity (Experiment 5). Rietze & Wiebe (1989) demonstrated that cucumber seedlings were most sensitive to chilling in the light at the beginning of the day. This rhythm disappears when the plants are grown in continuous light but could be induced with 6 hours of darkness prior to chilling in the light. Thus, a dark period prior to chilling in the light was essential to obtain optimal sensitivity to chilling. Six hours of darkness seems to be sufficient.

Table 8. Effect of photoperiod on chilling damage (rank) in nine cucumber cultigens grown at 22/18 °C in experiment 5\*

Cultigen	Photoperiod	
	9 hours	9 + 3 hours
Resistant		
AR 79-75	7.0 (4)	6.9 (4)
Chipper	5.7 (1)	5.4 (1)
Pixie	6.9 (3)	6.3 (3)
Wis. SMR 18	6.8 (2)	6.2 (2)
Susceptible		
Gy 14	7.7 (8)	7.3 (6)
M 28	7.4 (5)	7.1 (5)
M 29	7.6 (7)	7.8 (9)
Marketmore 76	7.4 (5)	7.6 (8)
Poinsett 76	7.6 (7)	7.3 (6)
Control	0.0	0.0
Mean (w/o control)	7.1	6.8
Range (w/o control)	2.0	2.4
F ratio (9 vs. 9 + 3)	1.4 <sup>ns</sup>	
CV (%)	11	

\* Damage rating was 0–9 (0 = none, 1–2 = trace, 3–4 = slight, 5–6 = moderate, 7–8 = advanced, 9 = dead). Data are means for true leaf ratings in 4 replications.

There was significant interaction between cultigen and four factors: growth temperature, duration of chilling, light level during chilling, and growth stage at chilling (Experiments 1, 2, 3 and 4). For those interactions, there were shifts in cultigen rank for chilling damage. However, none of those shifts appeared to be consistent.

## Conclusions

In developing chilling resistant cultivars, screening for genetic variation in chilling resistance at the whole-plant level can be restricted to one set of environmental conditions. The following conditions are recommended for screening for chilling resistance: grow the plants at 22/18 °C at a 9-hour photoperiod (with a 3-hour night interruption), water them once daily, subject them at the first true leaf stage to a chilling treatment of 7 hours at 4 °C at a light level of 270  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , and evaluate damage 5 days after treatment.

## Acknowledgements

We gratefully acknowledge the technical assistance of Mr. Rufus R. Horton, Jr. (Dept. Hort. Sci.), Dr. R. Jack Downs, Dr. Judith F. Thomas, and the technicians of the Southeastern Plant Environment Laboratory (Phytotron), N.C. State Univ.

## References

- Aoki, S., M. Oda & K. Hosino, 1989. Varietal differences in chilling-induced depression of photosynthesis and leaf growth in cucumber seedlings. *J Japan Soc Hort Sci* 58: 173–179.
- Aoki, S., M. Oda & M. Nagaoka, 1988. Chilling and heat sensitivities in cucumber seedlings measured by chlorophyll fluorescence. *Bull Nat Res Inst Veg, Ornament Plants & Tea Japan. Ser. A, No. 2*: 81–92.
- Cabrera, R.M., M.E. Saltveit & K. Owens, 1992. Cucumber cultivars differ in their response to chilling temperatures. *J Amer Soc Hort Sci* 117: 802–807.
- Downs, R.J. & J.F. Thomas, 1991. *Phytotron Procedural Manual for Controlled-Environment Research at the Southeastern Plant Environment Laboratory*. North Carolina Agricultural Research Service Tech Bul 244 (revised), 43 pp.
- Hughes, G.R., C.W. Averre & K.A. Sorensen, 1983. Growing pickling cucumbers in North Carolina. *N.C. Agric. Ext Serv Bulletin No. AG-315*, 15 pp.
- Lafuente, M.T., A. Belver, M.G. Guye & M.E. Saltveit, 1991. Effect of temperature conditioning on chilling injury of cucumber cotyledons. Possible role of abscisic acid and heat shock proteins. *Plant Physiol* 95: 443–449.
- Lasley, S.E., M.P. Garber & C.F. Hodges, 1979. Aftereffects of light and chilling temperatures on photosynthesis in excised cucumber cotyledons. *J Amer Soc Hort Sci* 104: 477–480.
- Liu, H.X., Y.R. Wang, S.X. Zeng, P. Li, Y.Z. Chen, D.F. Chen & J.Y. Guo (C.Y. Kuo), 1984. The effect of chilling stress on respiratory metabolism in cucumber seedlings with different degrees of tolerance. *Acta Phytophysiol Sin* 10: 191–199.
- Lower, R.L., 1974. Measurement and selection for cold tolerance in cucumber. *Pickle Pak Sci* 4: 8–11.
- Minchin, A. & E.W. Simon, 1973. Chilling injury in cucumber leaves in relation to temperature. *J Exp Bot* 24: 1231–1235.
- Nienhuis, J., R.L. Lower & J.E. Staub, 1983. Selection for improved low temperature germination in cucumber. *J Amer Soc Hort Sci* 108: 1040–1043.
- Pomeroy, M.K. & J.B. Mudd, 1987. Chilling sensitivity of cucumber cotyledon protoplasts and seedlings. *Plant Physiol* 84: 677–681.
- Rietze, E., 1988. Wirkungen kurzfristiger Kälteperioden auf Gurken (*Cucumis sativus* L.). Ph.D. thesis, University of Hannover, Germany, p. 90.
- Rietze, E. & H.J. Wiebe, 1987. Kälte im Licht mögen Pflanzen nicht. *Deutscher Gartenbau* 41: 2436–2437.
- Rietze, E. & H.J. Wiebe, 1989. Diurnal rhythm of chilling sensitivity of cucumbers in light. *Sci Hortic* 38: 231–237.
- Rikin, A., A. Blumenfeld & A.E. Richmond, 1976. Chilling resistance as affected by stressing environments and abscisic acid. *Bot Gaz* 137: 307–312.
- Saczynska, V., J. Gemel & Z. Kaniuga, 1993. Chilling susceptibility of *Cucumis* species. *Phytochemistry* 33: 61–67.
- Saltveit, M.E., 1991. Prior temperature exposure affects subsequent chilling sensitivity. *Physiol Plant* 82: 529–536.
- SAS/STAT User's Guide, Release 6.03 edition, 1988. SAS Institute, Cary, NC, USA.
- Soule, J., 1985. *Glossary for horticultural crops*. John Wiley & Sons, New York, 308 pp.
- Staub, J.E., 1988. Lack of chilling resistance in *Cucumis sativus* var. *hardwickii* (R.) Alef. *Cucurbit Genet Coop Rpt* 11: 29–32.
- Staub, J.E., R.L. Lower & J. Nienhuis, 1988. Correlated responses to selection for low temperature germination in cucumber. *HortScience* 23: 745–746.
- Tánczos, O.G., 1974. Invloed van lage temperatuur op de bladeren van *Cucumis sativus* L. Ph.D. thesis, University of Groningen, The Netherlands, 89 pp.
- United States Department of Agriculture. *Agricultural Statistics*. 1993, p 146.
- Van Hasselt, P.R., 1972. Photo-oxidation of leaf pigments in *Cucumis* leaf discs during chilling. *Acta Bot Neerl* 21: 539–548.
- Wang, C.Y., 1986. Effects of temperature and light on ACC and MACC in chilled cucumber seedlings. *Sci Hortic* 30: 47–52.
- Wehner, T.C., 1981. Screening for low-temperature germination ability in cucumber. *HortScience* 16: 399 (Abstr.).
- Wehner, T.C., 1982. Genetic variation for low-temperature germination ability in cucumber. *Cucurbit Genet Coop Rpt* 5: 16–17.
- Wehner, T.C., 1984. Estimates of heritabilities and variance components for low-temperature germination ability in cucumber. *J Amer Soc Hort Sci* 109: 664–667.
- Wehner, T.C., J.E. Staub & J.P. Palta, 1997. Tolerance to low temperatures in cucumber. *HortScience* (in press).
- Wilson, J.M. & R.M.M. Crawford, 1974. Leaf fatty-acid content in relation to hardening and chilling injury. *J Exp Bot* 25: 121–131.
- Wright, M. & E.W. Wilson, 1973. Chilling injury in cucumber leaves. *J Exp Bot* 24: 400–411.