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PLANT RESISTANCE

Screening Sunflower for Tolerance to Sunflower Midge Using the Synthetic Auxin 2,4-Dichlorophenoxyacetic Acid

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ABSTRACT The sunflower midge, *Contarinia schulzi* Gagné, causes economic damage by inducing abnormal growth in infested heads (capitula) of sunflower, *Helianthus annuus* L. The primary objective of this study was to determine whether sunflower midge damage could be simulated and whether that simulated damage could be used to select midge-tolerant sunflower germplasm. An additional objective was to develop a quantitative alternative to the scoring systems used to visually estimate damage. Sunflower plants were treated by injecting buds with the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D), which resulted in a distortion of the head morphology that was similar in appearance to the damage caused by the sunflower midge. The extent of distortion depended not only on the dose of 2,4-D but also on the size and growth stage of the head when injected. Among sunflower hybrids tested, resistance to the sunflower midge was significantly, negatively correlated with 2,4-D damage. Therefore, injection of sunflower heads with 2,4-D appears to be an effective method of screening sunflower germplasm for tolerance to the sunflower midge. Two distortion indices, based on measurements of head shape, were compared with the visual damage system. Although the visual rating system is faster, distortion index 2 gave similar results and is preferred when it is necessary to avoid individual differences in scoring techniques.

KEY WORDS *Contarinia schulzi, Helianthus,* tolerance

FEEDING ACTIVITY BY LARVAE of the sunflower midge, *Contarinia schulzi* Gagné, (Diptera: Cecidomyiidae) damages plants by inducing abnormal growth of the head (capitulum) of sunflower, *Helianthus annuus* L. A sunflower field with a high infestation of the sunflower midge may have sufficient damage to reduce yield (Schulz 1973). Sunflower midge control is difficult because insecticides are not effective (McMullen 1985) and breeding for resistance has been impeded by inadequate naturally occurring, insect populations for screening.

As an alternative to screening for resistance with the sunflower midge, an attempt was made to simulate this insect's damage. In preliminary testing (G.J.B., unpublished data), we were able to induce a distortion in sunflower heads that appeared similar to slight sunflower midge damage by injecting healthy heads with crude plant extracts from either sunflower midge infested or uninfested sunflower heads. Because distortion occurred when extracts were made from both infested and uninfested heads, we suspected that a natural phytohormone was in the extracts, and when healthy heads were injected with the extracts, the phytohormone level was elevated above normal levels and caused the abnormal growth response.

Because natural phytohormones are rapidly metabolized by sunflower (Mani 1964), they do not give prolonged stimulation when injected into sunflower tissue. Thus, natural phytohormones are not good candidates for artificially simulating sunflower midge damage. However, 2,4-dichlorophenoxyacetic acid (2,4-D), a synthetic auxin, is not metabolized effectively by sunflower tissue (Mani 1964). Thus, the use of 2,4-D may influence long-term biological activity in the sunflower and simulate the continual influence of feeding by sunflower midge larvae. Anderson & Brewer (1992) used leaf disk assays to show that sunflower sensitivity to 2,4-D, as measured by ethylene production, is correlated positively with resistance to the sunflower midge. Tolerant hybrids were less sensitive to 2,4-D than sunflower midge intolerant hybrids. The three objectives of the current study were: (1) to simulate sunflower midge damage by injecting 2,4-D into sunflower hybrids of differing susceptibility to the sunflower midge, (2) to evaluate sunflower plants for sunflower midge resis-

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tance by injecting with 2,4-D in the absence of natural infestation, and (3) to develop a quantitative method to assess sunflower head distortion caused by either natural sunflower midge infestation or simulated distortion (induced by 2,4-D injection).

Materials and Methods

Eleven sunflower hybrids were used in greenhouse and field tests. They ranged in sunflower midge resistance from very susceptible ('Northrup King 212'), to susceptible ('Interstate 894'), to moderately resistant ('Agriculture Canada 85-346'), and to resistant ('Seedtec 315', 'Seedtec 316', 'Dahlgren DO643-7E', 'Dahlgren DO647-7E', 'Agriculture Canada 83-202', and 'Agriculture Canada 84-108') hybrids (Anderson & Brewer 1991). The resistance of two other sunflower hybrids, 'Cargil 207' and 'Dahlgren DO855', was not known.

A stock solution of 10 mM 2,4-D in water was prepared using analytical grade 2,4-D (Sigma Chemical, St. Louis, MO). To improve the solubility of 2,4-D, diethanolamine was added to the stock solution at the rate of 0.5 ml/1 liter of solution. From this stock solution, dilutions were made to obtain two concentrations.

The 2,4-D solution was applied by positioning a hypodermic needle at a right angle to the petiole and along the outside margin of the bud (unopened capitulum). The needle was inserted into the bud to a depth of ≈0.5 cm, withdrawn slightly (≈1 mm) to create a small reservoir, and the test solution injected. A total of 0.5 ml of the test solution was distributed equally among three sites around the periphery of the head, ≈120 degrees apart. Thus, each injection site received ≈0.167 ml of the test solution. Preliminary testing with a diethanolamine-water solution did not affect growth of sunflower heads; therefore, for simplicity, controls were injected with water only.

Bud Diameter. Sunflower hybrids, 'Northrup King 212', 'Interstate 894', 'Agriculture Canada 85-346', 'Agriculture Canada 83-202', 'Agriculture Canada 84-108', 'Seedtec 315', 'Seedtec 316', 'Dahlgren DO643-7E', and 'Dahlgren DO647-7E', were used to study the effect of bud size on response to 2,4-D in the greenhouse at Fargo, ND. Conditions during evaluation were at a photoperiod of 16:8 (L:D) h and an approximate temperature of 24°C. Buds of each hybrid were injected with 2,4-D (10 mM or 1 mM concentration) when the buds were 4.5–5.0 cm diameter.

Plants of hybrids 'Northrup King 212', 'Interstate 894', 'Seedtec 315', 'Seedtec 316', and 'Dahlgren DO643-7E' were injected with a 10-mM concentration of 2,4-D when their buds were in three additional size classes. The second group of plants had buds of 3.0–3.5 cm in diameter and the third group had buds of 6.0–6.5 cm diameter.

The fourth treatment class, termed best-fit, was determined using data from Anderson & Brewer (1991 and M.D.A., unpublished data). In that field trial, sunflower hybrids were planted at the same time but the bud diameters of the hybrids tested differed when sunflower midge were present and ovipositing. For the best-fit class, hybrids were tested at bud diameters approximating the bud size of the hybrids when they were infested in the field by sunflower midge (Table 1). Thus, for the best-fit class, hybrid 'Seedtec 315' was treated at bud diameter 3.0–3.5 cm; hybrids 'Seedtec 316', 'Interstate 894', and 'Northrup King 212' were treated at bud diameter 4.5–5.0 cm; hybrid 'Dahlgren DO643-7E' was treated at bud diameter 6.0–6.5 cm. Replicates per treatment and hybrid ranged from two to six, depending on availability of plants in the appropriate stage.

Plants were rated for tissue distortion 11 d after injection (when head growth stopped) using a sunflower midge damage rating scale (modified from Anderson & Brewer 1991) as follows: 0, none; 2, light; 3, moderate; and 4, severe distortion.

Anderson & Brewer (1991) calculated the relative proportions of larval antibiosis, antixenosis (given as resistance to infestation), and tolerance (resistance to damage not attributable to antixenosis or antibiosis) in sunflower hybrids tested in the field. Because the relationship between the field tolerance scores to sunflower midge, from Anderson & Brewer (1991), and 2,4-D damage ratings was nonlinear, the tolerance scores were log-transformed. Within each size class, the transformed tolerance scores were regressed on the mean 2,4-D damage rating reported in the current study using PROC REG (SAS Institute 1985).

Plant Growth Stage. We injected plants of hybrid 'Interstate 894' under greenhouse culture with a 5 mM concentration of 2,4-D at growth stages early bud (R2), middle bud (R3), and late bud (R4) (based on stage ratings of Schneiter & Miller 1981) rather than at certain bud diameters. This was done to provide a uniform phenological stage when comparing plants grown in different environments. Because we thought that a 10 mM dose of 2,4-D could result in too many scores at the upper end of the damage rating scale, a 5 mM concentration of 2,4-D was used in this and all subsequent tests. Six or seven replications per growth stage were used. For this study, two plants injected with 0.5 ml of distilled water at stage R4 served as controls.

The effect of treatment at different plant growth stages was also determined for six hybrids ('Northrup King 212', 'Interstate 894', 'Seedtec 316', 'Dahlgren DO643-7E', 'Cargil 207', and 'Dahlgren DO855') in a completely randomized design. Two sets of three or four plants of each hybrid were injected with 0.5 ml of 5 mM concentration of 2,4-D. Set one was treated at growth stage R2. Set two was treated at growth stage R4. Controls ($n = 2$) for each hybrid were injected with distilled water. Plants of each hybrid were planted on the same date and were treated as they reached the proper growth stage. A damage rating scale developed by Bracken (1991) was used in this and subsequent tests: 0, no distortion; 1, creases in surface of the head; 2, curling of bracts inward and slight cupping toward center of the head; 3, pronounced cupping to center of the head; 4, severe cupping to center of the head; and 5, head closed, no seeds present.

Damage ratings were taken on plants that completed flowering (stage R6). In the first test, differences among plants of 'Interstate 894' treated at different growth stages were determined by analysis of variance (PROC GLM, SAS Institute 1985). In the second test, hybrid, plant growth stage, and interaction effects were determined by analysis of variance (PROC GLM, SAS Institute 1985).

Greenhouse Hybrid Trial. Hybrids 'Northrup King 212', 'Interstate 894', 'Seedtec 316', 'Dahlgren DO643-7E', 'Cargil 207', and 'Dahlgren DO855' were tested for response to 2,4-D treatment in greenhouses at Crookston, MN, and Fargo, ND. Plants were treated with 0.5 ml of a 5 mM concentration of 2,4-D injected into stage R4 plants. Control plants were injected with 0.5 ml of distilled water. A completely randomized design with three replications was used.

Damage ratings were taken on stage R6 plants. Hybrid response to treatment at the two locations was determined with analysis of variance using PROC GLM (SAS Institute 1985). For the four hybrids with field tolerance scores ('Northrup King 212', 'Interstate 894', 'Seedtec 316', and 'Dahlgren DO643-7E'), the mean log-transformed scores were regressed on mean damage rating using PROC REG (SAS Institute 1985).

Field Hybrid Trials. Hybrids 'Northrup King 212', 'Interstate 894', 'Seedtec 316', 'Dahlgren DO643-7E', 'Cargil 207', and 'Dahlgren DO855' were tested in field trials using a randomized complete block design with four replicates. This study was conducted in 1990 and 1991 at Crookston, MN, and Mapleton, ND. At the Mapleton site, sunflower midge adults, eggs, and larvae were observed both study years. However, sunflower midge was not detected at the Crookston site in either of the study years.

Treatments were natural infestation by the sunflower midge (Mapleton site only), the three-injection procedure, and a single-injection procedure. Because treating many plants with three injections is slow, we compared the three-injection technique with a single-injection procedure. In the single injection treatment the entire 0.5 ml dose of 2,4-D was injected into the center of the petiole just below the bud. Controls were injection of buds of plants in stage R4 with 0.5 ml of distilled water. Two plants per replicate were treated.

At the Mapleton site, plants to be treated with 2,4-D were protected from natural sunflower midge infestation. This was done by either spraying periodically with an insecticide (malathion) or covering the buds with Delnet pollination bags to exclude insects (Applied Extrusion Technologies, Middletown, DE). This was started at stage R2 and continued until the heads were in full bloom, stage R5.2.

In the second year, damage assessments in addition to visual scoring were evaluated. The distances across the face of heads were measured in two directions at right angles to each other (d_1 and d_2). Measurement d_1 was made across the widest part of the head. We converted the measurements to two indices that measure deviation of the heads from a round shape, which is the normal shape for uninfested plants. The two indices are: round index-1, $d_1 - d_2$; and round index-2, (round index-1)/($d_1 + d_2$). Round index-2 removes bias attributable to inherent differences in the sizes of heads of different hybrids.

Damage ratings resulting from natural midge infestation were correlated with damage ratings from plants injected with 2,4-D at one or three locations. Analysis of variance of the effects of injection treatment or hybrid, block within location, location, and treatment by location interaction on damage rating and distortion indices was made. Plants of each hybrid treated by the three-injection method were scored using the damage rating system and the distortion indices, and the results were correlated. The log tolerance scores were regressed on the damage rating and the distortion indices scores. Analyses were done using PROC CORR, REG, and GLM (SAS Institute 1985).

Total weight of seeds per head ($n = 2$–6 plants per block) was measured for hybrids tested at Crookston in the second year. Mean weight per block was used in correlation analysis of the yield data on damage rating, round index-1, and round index-2 (PROC CORR, SAS Institute 1985).

Results

Treatments with water controls were made in the various trials. In no case was damage apparent in water treated plants.

Bud Diameter. After 11 d, heads were distorted to varying degrees depending on the hybrid. Distortion was characterized by an asymmetrical growth of the front and back of the head in a way that was similar in appearance to damage induced by the sunflower midge. The back

Table 1. Damage ratings in nine sunflower hybrids treated with different doses of 2,4-D at various floral bud diameters

Hybrid	2,4-D				Sunflower midge	
	Concentration, mM	Treatment diameter, cm	n	Damage rating, mean ± SEM[a]	Field tolerance, mean ± SEM[b]	Diameter head receiving oviposition, cm mean ± SEM[c]
DO643-7E	10	3.0–3.5	3	2.0 ± 0.00	38.94 ± 3.22	6.12 ± 0.28
	10	4.5–5.0	5	2.3 ± 0.13		
	10	6.0–6.5	3	2.2 ± 0.17		
	1	4.5–5.0	4	1.9 ± 0.15		
84-108	10	4.5–5.0	5	2.4 ± 0.09	17.51 ± 1.21	4.57 ± 0.36
	1	4.5–5.0	4	1.9 ± 0.15		
DO647-7E	10	4.5–5.0	6	2.3 ± 0.12	14.41 ± 0.68	4.73 ± 0.27
	1	4.5–5.0	2	1.5 ± 0.14		
83-202	10	4.5–5.0	5	2.9 ± 0.09	9.04 ± 0.87	4.85 ± 0.30
	1	4.5–5.0	4	1.6 ± 0.15		
85-346	10	4.5–5.0	4	2.5 ± 0.00	7.39 ± 1.08	4.68 ± 0.22
	1	4.5–5.0	3	1.8 ± 0.17		
ST 316	10	3.0–3.5	4	2.1 ± 0.15	7.36 ± 0.49	4.53 ± 0.37
	10	4.5–5.0	4	2.4 ± 0.15		
	10	6.0–6.5	3	2.2 ± 0.17		
	1	4.5–5.0	3	1.7 ± 0.17		
IS 894	10	3.0–3.5	3	3.7 ± 0.35	6.93 ± 0.84	4.32 ± 0.32
	10	4.5–5.0	4	3.0 ± 0.35		
	10	6.0–6.5	4	2.3 ± 0.15		
	1	4.5–5.0	3	2.2 ± 0.17		
ST 315	10	3.0–3.5	5	2.6 ± 0.31	6.92 ± 0.41	3.22 ± 0.25
	10	4.5–5.0	4	2.3 ± 0.15		
	10	6.0–6.5	3	2.0 ± 0.00		
	1	4.5–5.0	3	1.5 ± 0.00		
NK 212	10	3.0–3.5	4	2.6 ± 0.15	0.00 ± 0.00	5.32 ± 0.35
	10	4.5–5.0	3	3.7 ± 0.35		
	10	6.0–6.5	2	3.3 ± 0.28		
	1	4.5–5.0	4	2.5 ± 0.00		

[a] 2,4-D damage based on 0–4 rating scale; 0, no distortion to 4, severe distortion.
[b] Percentage tolerance relative to very susceptible hybrid 'NK 212'; data from Anderson & Brewer (1991) except for '85-346' and 'ST316', which were previously unpublished.
[c] Diameter of sunflower buds when midge adults were present and ovipositing in the field; data from Anderson & Brewer (1991) except for '85-346' and 'ST316', which were previously unpublished.

of the head overgrew the front so that the head was concave. In severe cases, the head was *clamshell-like* in appearance and lacked seeds. Occasionally there were three regions of overgrowth. The damage ratings resulting from injection of 2,4-D into sunflower buds are given in Table 1. For all nine hybrids treated, distortion in heads of the 4.5–5.0 cm size class was significantly greater when the 10 mM concentration was used than when the 1 mM concentration was used.

Regression of field tolerance levels on damage ratings resulting from treatment of hybrids with 10 mM 2,4-D was used to test the relationship between hybrid susceptibility to sunflower midge and to 2,4-D treatment. Regressions of sunflower midge tolerance on 2,4-D damage rating for the 4.5–5.0 cm size class and the best-fit size class were significant (Table 2). However, regression of damage occurring in heads of the smaller (3.0–3.5 cm) and larger (6.0–6.5 cm) size classes with tolerance were not significant.

Plant Growth Stage. Damage in plants of hybrid 'Interstate 894' was significantly lower in the oldest growth stage (R4) treated ($F = 37.6$; df = 2, 19; $P = 0.0001$). The damage rating (mean ± SEM) for treatment at stage R2 was 4.3 ± 0.2 ($n = 8$); at stage R3 it was 4.9 ± 0.1 ($n = 4$); and at stage R4 it was 2.5 ± 0.2 ($n = 7$).

In the second test, we observed both hybrid and plant growth stage effects. Hybrids, stage, and hybrid by stage interaction effects were significant (Table 3). The damage rating (mean ± SEM) for stage R2 was 3.65 ± 0.1 ($n = 23$); for stage R4 it was 1.76 ± 0.3 ($n = 21$).

Table 2. Relationship between tolerance scores of sunflower hybrids for resistance to sunflower midge with damage ratings when treated with 10 mM 2,4-D at various classes of bud diameters in the greenhouse

Bud diameter class, cm	n	Intercept, mean ± SEM	Slope, mean ± SEM	P
3.0–3.5	5	2.89 ± 0.6	−0.33 ± 0.6	>0.05
4.5–5.0	9	3.53 ± 0.3	−0.92 ± 0.3	<0.01
6.0–6.5	5	3.04 ± 0.3	−0.74 ± 0.3	>0.05
Best fit[a]	9	3.61 ± 0.2	−0.97 ± 0.2	<0.01

Model, log(tolerance score) = a + b (damage rating), fit to data in Table 1.
[a] Composite distribution of the three size classes for each hybrid to match the distribution of head diameters observed to be attacked in the field by the sunflower midge (Table 1).

Table 3. Analysis of variance table for the effect of plant growth stage on damage ratings of sunflower hybrids treated with 0.5 ml of 5 mM 2,4-D

Source	*F*	df (source, error)	*P*
Hybrid	3.8	5, 32	0.0111
Stage	58.6	1, 32	0.0001
Hybrid X Stage	12.9	5, 32	0.0050

Greenhouse Hybrid Trial. In the test of hybrids at two greenhouses, the hybrid, location, and hybrid by location interaction were significant (Table 4). Damage to the hybrids was higher at the Fargo location than at Crookston. The most highly damaged hybrid at Fargo was 'Northrup King 212'; at Crookston it was 'Dahlgren DO855'. Damage to 'Seedtec 316' was lowest at both locations (Table 5). Log tolerance scores had a significant, negative regression with damage ratings (Table 6).

Field Hybrid Trials. In the first year, sunflower midge damage at the Mapleton location was too low to rate the hybrids visually for damage. However, in the second year sunflower midge damage was higher, and natural damage was compared with damage attributable to 2,4-D treatment. Midge damage was significantly correlated with damage from the three-injection treatment (r = 0.75, n = 24, P = 0.0001) but not with the one-injection treatment (r = 0.29, n = 24, P = 0.1703).

In the second year of the field study, single-injection and three-injection treatments were compared over both locations. Injection treatments and locations were significant. Injection procedure by location interaction was not significant (Table 4). Damage ratings for both the one-injection and the three-injection treatments were higher at the Mapleton location than Crookston location. The mean ± SEM for the one-injection treatment (3.5 ± 0.2) was significantly higher than that for the three-injection treatment (2.6 ± 0.2) (Table 4). Sunflower heads treated with a single injection of 2,4-D often had a large hole in the center and were trumpet shaped in appearance. While sunflower midge-infested plants sometimes develop a central hole, it is usually not as deep as the 2,4-D-induced hole. Heads treated with a single injection of 2,4-D bore less resemblance to sunflower midge damaged heads than did heads treated at three sites. Data from the three-injection treatment were used in all subsequent analyses.

Hybrid and location effects were significant during both years of the hybrid field trials. Hybrid by location interaction was not significant (Table 4). The hybrid and location means are presented in Table 5. 'Northrup King 212' was the most damaged hybrid both years, although not significantly so in the second year. No hybrid was consistently least damaged. For both years, damage ratings at Mapleton exceeded those at Crookston.

Damage ratings from the three-injection treatment were correlated significantly with round index-1 (n = 48, r = 0.67, P = 0.0001) and round index-2 (n = 48, r = 0.68, P = 0.0001) scores. Log-transformed tolerance scores of hybrids 'Northrup King 212', 'Seedtec 316', 'Interstate 894', and 'Dahlgren DO643-7E' were regressed significantly on damage ratings and the round indices (Table 6).

Damage ratings for hybrids at the Crookston site in the second year were correlated with yield loss (n = 95, r = −0.45, P = 0.0001) (Fig. 1). Yield loss also was correlated significantly with round index-1 (n = 95, r = −0.31, P = 0.0026) and round index-2 (n = 95, r = −0.37, P = 0.0002).

Table 4. Analysis of variance table for the effect of hybrids and location, and injection procedure and location, on damage ratings of plants treated in the greenhouse and field with 0.5 ml of 5 mM 2,4-D

Source	*F*	df (source, error)	*P*
Greenhouse			
Hybrid	12.4	5, 25	0.0001
Location	5.2	1, 25	0.0317
Hybrid X location	5.6	5, 25	0.0014
Field, year-1			
Hybrid	27.2	5, 30	0.0001
Location	6.3	1, 30	0.0177
Hybrid X location	1.8	5, 30	0.1405
Field, year-2			
Hybrid	10.9	5, 30	0.0001
Location	151.0	1, 30	0.0001
Hybrid X location	1.8	5, 30	0.1520
Field, year-2			
Injection	42.9	1, 92	0.0001
Location	163.4	1, 92	0.0001
Injection X location	0.6	1, 92	0.4466

Discussion

The sunflower midge reduces yield when infestations are high enough to distort the growth of developing sunflower heads. In a series of greenhouse and field trials, we were able to simulate sunflower midge growth distortion by injecting sunflower heads with a synthetic auxin, 2,4-D.

Our results also suggest that injection of 2,4-D into sunflower buds can be used to identify sunflower midge tolerant germplasm. Tolerance to the sunflower midge was significantly, negatively correlated with 2,4-D damage rating. Furthermore, in the second year of the field test (when natural sunflower midge infestation was high enough to rate), hybrids highly damaged by the 2,4-D treatment also had high sunflower midge damage ratings, and hybrids with low 2,4-D damage had low sunflower midge damage ratings. Thus, treatment of sunflower hybrids

Table 5. Three studies of damage ratings for six sunflower hybrids treated with a 5 mM dose of 2,4-D

Hybrid	Greenhouse study			Field study, year 1			Field study, year 2		
	Crookston	Fargo	Pooled	Crookston	Mapleton	Pooled	Crookston	Mapleton	Pooled
'NK 212'	3.00 ± 0.6	4.33 ± 0.2	3.67 ± 0.4a	3.34 ± 0.2	4.28 ± 0.1	3.83 ± 0.2a	2.71 ± 0.3	4.09 ± 0.2	3.40 ± 0.3a
'DO855'	3.33 ± 0.3	1.83 ± 0.2	2.58 ± 0.4ab	1.98 ± 0.4	2.55 ± 0.4	2.26 ± 0.3b	1.54 ± 0.3	3.41 ± 0.4	2.48 ± 0.4bcd
'IS 894'	2.00 ± 0.0	2.88 ± 0.3	2.50 ± 0.2b	1.73 ± 0.3	1.38 ± 0.1	1.55 ± 0.1bc	2.04 ± 0.3	3.83 ± 0.1	2.94 ± 0.4ab
'CAR 207'	2.33 ± 0.3	2.17 ± 0.3	2.25 ± 0.2b	1.53 ± 0.2	1.38 ± 0.2	1.45 ± 0.1bc	2.21 ± 0.4	3.25 ± 0.1	2.73 ± 0.3abc
'DO643-7E'	1.00 ± 0.6	3.00 ± 0.6	2.00 ± 0.6b	1.20 ± 0.2	1.88 ± 0.4	1.54 ± 0.3bc	0.63 ± 0.2	2.99 ± 0.1	1.81 ± 0.5d
'ST 316'	0.67 ± 0.3	1.00 ± 0.0	0.83 ± 0.2c	0.80 ± 0.2	1.48 ± 0.2	1.14 ± 0.2c	1.25 ± 0.1	3.11 ± 0.1	2.18 ± 0.4cd
Pooled means	2.06 ± 0.3b	2.55 ± 0.3a		1.77 ± 0.2b	2.15 ± 0.2a		1.73 ± 0.2b	3.45 ± 0.1a	

Mean ± SEM for damage ratings based on the 0–5 rating scale: 0, no distortion to 5, head closed, no seeds. For hybrids at locations; $n = 3$ for greenhouse study and $n = 4$ for field studies. Pooled means within a column not followed by a common lowercase letter or within a row and study not followed by a common lowercase letter are significantly different ($P < 0.05$); by Tukey's studentized range test (SAS Institute 1985).

with 2,4-D can be used to predict tolerance to sunflower midge.

Although this study did not directly test the role of auxin in sunflower midge–sunflower interaction, the results suggest that sunflower midge damage is caused by increased levels of auxins. Future tests will analyze auxin levels in sunflower midge infested, uninfested, tolerant and susceptible hybrids.

The 2,4-D injection technique was accurate in predicting sunflower midge tolerance only when three injections, rather than a single injection, were used. Damage resulting from the single-injection procedure was more severe, and the morphology of damaged heads bore less resemblance to sunflower midge damaged heads than did the three-injection treatment. Because most sunflower midge larvae feed on the periphery of the sunflower head, the three-injection procedure more closely mimics sunflower midge damage than does a single injection to the center of the bud.

Also important to accurate simulation of sunflower midge damage was the bud size, or stage, and 2,4-D dose. As the plant develops through successive plant growth stages, bud, and later, head size increases. Growth continues through stage R6 when head growth stops, although seed filling is still occurring. If buds are treated while small (at an early growth stage, R2), damage is too severe to differentiate hybrid responses to treatment. The effects of treatment at growth stage R4 was recognized easily and gave consistent results. A 0.5-ml dose of either 5 or 10 mM 2,4-D is adequate to produce a consistent response.

Use of damage ratings must be consistent. Often, different observers will give different scores to the same plants. While this is minimized by the use of the Bracken (1991) rating scale, inaccuracies are still possible. To eliminate error caused by individual differences in scoring techniques, it is advisable to use one of the distortion indices. Round index-2 is preferred because it takes into account innate differences in average head size.

Table 6. Relationship between tolerance scores of sunflower hybrids for resistance to sunflower midge with damage ratings and round indices when treated with 5 mM 2,4-D in different studies

Study	n	Intercept, mean ± SEM	Slope, mean ± SEM	P
Damage rating				
Greenhouse	24	3.39 ± 0.4	−1.48 ± 0.4	0.0005
Field, year-1	24	3.83 ± 0.2	−2.73 ± 0.2	0.0001
Field, year-2	47	3.61 ± 0.3	−0.86 ± 0.3	0.0019
Round index-1				
Field, year-2	17	5.32 ± 1.1	−2.49 ± 1.0	0.0208
Round index-2				
Field, year-2	47	0.20 ± 0.0	−0.10 ± 0.0	0.0150

Model, log (tolerance score) = a + b (damage rating, round index-1, or round index-2), fit using PROC REG (SAS Institute 1985).

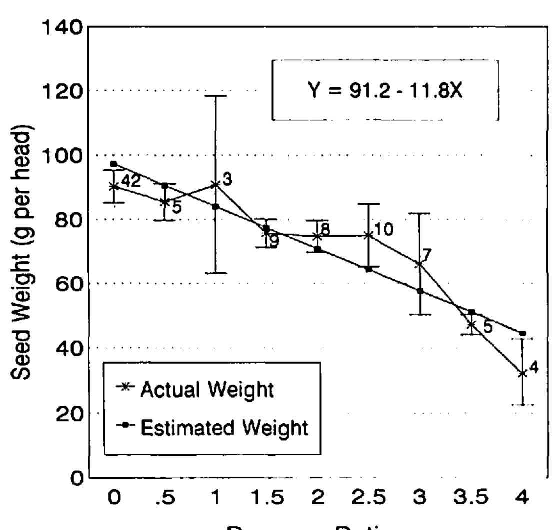

Fig. 1. Weight of seed produced per head by sunflower hybrids treated with a 5-ml dose of 0.5 mM 2,4-D at growth stage R4. Data pooled into 0.5 damage rating score units. Data expressed as mean ± SEM. n Shown next to data points.

The technique of using a synthetic auxin is a novel method of identifying insect tolerant germplasm in the absence of a natural infestation. This method will not identify antibiotic or antixenotic germplasm, but field screening for resistance to the sunflower midge is impractical. Thus, the auxin method is a promising procedure for identifying sunflower midge tolerant germplasm.

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