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Use of synthetic plant auxin to select for insect tolerance in sunflower

Gary J. Brewer and N. V. Rama Raje Urs*
Dept. of Entomology, North Dakota State University, Fargo, North Dakota 58105 USA
*Dahlgren and Company, Inc., 1220 Sunflower Street, Crookston, Minnesota 56716 USA

SUMMARY

The development of sunflower midge, Contarinia schulzi Gagnè, resistant sunflower, Helianthus annuus L., has been impeded due to lack of adequate insect populations in selection nurseries. The impetus for this study was to explore if midge damage could be simulated. And secondly, to learn if midge tolerant germplasm could be identified by simulating midge damage. Commercially available synthetic auxin was injected into sunflower heads (capitula) to induce an abnormal growth in the heads. The head distortion resulting from 2,4-dichlorophenoxyacetic acid (2,4-D) treatment was similar in appearance to the damage caused by the sunflower midge. Damage was evaluated using a visual rating system and two quantitative distortion indexes. Among hybrids tested, tolerance to the sunflower midge was significantly regressed on 2,4-D damage rating score and both distortion indexes. Correlation of natural midge damage with 2,4-D induced damage was significant. Damage ratings and the distortion indexes from plants treated with 2,4-D were correlated. All three estimates of damage were negatively correlated with yield. Midge populations in selection nurseries are often too small to allow resistant germplasm to be identified. Therefore, injection of sunflower heads with 2,4-D is the most effective method of screening sunflower germplasm for tolerance to the sunflower midge.

INTRODUCTION

Feeding by larvae of the sunflower midge, Contarinia schulzi Gagnè, induces an abnormal growth of the head (capitulum) of sunflower. The atypical growth of a sunflower head infested with the midge meets the definition of a gall as defined by Mani

(1992). In heavily infested heads, a severe yield reduction results (Schulz 1973). Resistance to the midge is the only effective control. However, breeding for resistance is delayed by lack of reliable populations of the midge. Therefore, a method of identifying midge resistant germplasm without the midge is needed.

In preliminary testing we were able to induce a midge-like distortion in sunflower heads. This was accomplished by injecting healthy flower buds with extracts from either midge infested or uninfested sunflower heads. This led us to postulate that the abnormal growth of midge infested sunflower heads may be due to elevated auxin levels. The objectives of this study were to determine:

1. Whether injecting synthetic auxin, 2,4-dichlorophenoxyacetic acid (2,4-D), into a sunflower head could induce a growth abnormality similar to that of a midge infestation.
2. If 2,4-D could be used to identify tolerance to the sunflower midge in sunflower germplasm.
3. Whether a reliable and simple quantitative method to assess sunflower head distortion, either due to 2,4-D or natural midge infestation, could be developed.

MATERIALS AND METHODS

Nine hybrids of known midge resistance (Anderson & Brewer 1991) were tested for two years in greenhouse and field trials to evaluate their reaction to 2,4-D injection. The hybrids tested ranged from very susceptible to resistant.

A stock solution of 5 mM 2,4-D in water was prepared using analytical grade 2,4-D. The addition of diethanolamine (0.5 ml per 1 L of solution) to the stock solution enhanced the solubility of 2,4-D. The 2,4-D solution was applied by inserting a hypodermic needle horizontally into the sunflower bud to a depth of approximately 0.5 cm, withdrawing slightly to create a small reservoir, and injecting the 2,4-D solution. A total of 0.5 ml of the test solution was distributed equally among three sites around the periphery of the head, approximately 120 degrees apart. Thus, each injection site received about 0.167 ml of the

2,4-D solution. Plants were observed for head abnormalities following treatment and were rated for tissue distortion using a sunflower midge damage rating scale (Bracken 1991).

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

5 - extreme cupping, head closed, no seeds present

Damage ratings were taken on physiologically mature plants. The midge tolerance score of each hybrid (determined by Anderson and Brewer 1991) was converted to its logarithm and regressed on the mean 2,4-D damage rating. In the second year of the field trials, the midge population was high enough to significantly damage the test hybrids. Damage ratings from midge infested plants were correlated with damage ratings of other plants from the same location that were protected from midge infestation but were treated with 2,4-D.

Two assessment methods, other than visual scoring, were also evaluated. The distance across the face of treated heads was measured in two directions at right angles to each other. We converted the measurements to two indexes that gauge head deviation from a round shape, the normal shape for uninfested plants. The two indexes are given below:

D_1 = measurement 1.

D_2 = measurement 2.

Round-1 index = $d_1 - d_2$ or $d_2 - d_1$ (whichever gave a positive value).

Round-2 index = Round-1 index / $(d_1 + d_2)$

The round-2 index eliminates bias due to inherent disparities in the sizes of heads of different hybrids. Correlation was used to compare the results of the two indexes with damage rating scores for the same plants.

Seed weight (grams of seed per head) of treated heads was taken of the hybrids treated with 2,4-D. The results from two to six heads per treatment per block were averaged for statistical purposes. Correlation and regression of the yield data on damage rating, round-1 index, and round-2 index were computed.

RESULTS

At day eleven following treatment, heads were distorted to varying degrees depending on the hybrid. In susceptible hybrids, the distortion closely resembled midge induced growth abnormality. Resistant hybrids were only slightly distorted. There was no distortion in heads of plants injected with distilled water.

The tolerance scores of the hybrids tested were regressed on 2,4-D damage ratings and the two distortion indexes (Table 1). For all three measures of damage, the regression was significant. Midge damage was significantly correlated with damage from the three injection treatment.

Table 1. Regression and correlation matrix for logarithms of midge tolerance score and midge damage rating with 2,4-D damage rating and distortion indexes.

Variable	Statistic	2,4-D Damage Rating	Round-1 Index	Round-2 Index
Midge	r	-0.44	-0.34	-0.35
tolerance	n	47	47	47
	slope	-0.86	-2.49	-0.10
	intercept	3.61	5.32	0.20
Midge	r	0.75		
damage	n	24		
rating	slope	1.57		
	intercept	-4.73		

Damage resulting from 2,4-D treatment was significantly correlated with the round-1 index (r=0.66, n=71) and the round-2 index (r=0.68, n=71). Yield was negatively correlated with 2,4-D damage rating (r=-0.45, n=95), the round-1 index (r=-0.31, n=95), and the round-2 index (r=-0.37, n=95).

DISCUSSION

We used both greenhouse and field tests to confirm the feasibility of using 2,4-D to simulate sunflower midge damage. The sunflower midge reduces yield when infestations are high enough to distort the growth of developing sunflower heads. We postulate that the distortion occurring in infested heads is a result of increased levels of endogenous auxins. To test our hypothesis and to simulate midge damage, we used the synthetic auxin, 2,4-D. 2,4-D was used because it altered the growth of developing sunflower heads in a way that very closely resembled the abnormal growth resulting from natural midge infestation.

The technique was to be considered effective in simulating midge damage only if repeated testing confirmed a relationship between hybrid tolerance to the midge and tolerance to 2,4-D treatment. Also important, was to evaluate the technique for a good correlation between midge induced damage and 2,4-D induced damage. Our results suggest that these criteria were met. Greenhouse studies and two years of field trials at two locations indicate that the regression of midge tolerance on 2,4-D damage rating was significant. Furthermore in the second year of the field test (when midge infestation was high enough to rate), 2,4-D damage correlated well with midge damage. Thus, treatment of sunflower hybrids with 2,4-D can be used to predict midge tolerance.

The effective use of damage ratings is dependent on the consistent use of the rating scale. Often different observers will give different scores to the same plants. While this is minimized by using the Bracken (1991) rating scale, which includes precise illustrated descriptions of the damage, inaccuracies still are possible. Because midge damage causes changes in sunflower head shape, it is possible to measure those changes. Both of the distortion indexes, which measure changes in head shape, can be used to replace the damage rating system.

An advantage of the rating scale developed by Bracken (1991) is that besides estimating damage, the rating classes are correlated with yield loss. The distortion indexes share that advantage, in that they also are correlated with yield loss.

CONCLUSION

We did these assays under two assumptions. First, that midge induced head distortion is ultimately due to an increase in endogenous auxin levels. Second, that tolerance to the midge depends on how the plant responds to elevated auxin levels. Because of the correlation between tolerance to 2,4-D distortion and to the midge, our assumption appears justified. The correlation also shows that this technique can be used to screen sunflower hybrids for sunflower midge tolerance.

The technique of using a synthetic auxin is, as far as is known to the authors, a novel method of identifying insect tolerant germplasm in the absence of a natural infestation. But this method will not identify germplasm with antibiotic or antixenotic resistance mechanisms. However, because of the extreme difficulty of establishing sunflower midge nurseries, screening for resistance with the midge is impractical. This method is currently the best procedure for identifying midge resistant germplasm.

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