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Sensitivity to 2,4-D in Sunflower as an Indicator of Tolerance to the Sunflower Midge (Diptera: Cecidomyiidae)

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ABSTRACT Nine sunflower hybrids were evaluated for their sensitivity to 2,4-dichlorophenoxyacetic acid (2,4-D) as measured by the production of ethylene. Sensitivity to 2,4-D was then compared with the degree of field tolerance to the sunflower midge, *Contarinia schulzi* Gagné, exhibited by each hybrid. For the hybrids evaluated, ethylene production increased with 2,4-D concentration and sensitivity to 2,4-D was inversely proportional to midge tolerance. The procedure may be useful in identifying midge tolerant germplasm.

KEY WORDS Insecta, *Contarinia schulzi*, ethylene, host plant resistance

THE SUNFLOWER MIDGE, *Contarinia schulzi* Gagné, is an economic pest of cultivated sunflower (Schulz 1978). Midge larvae feed on tissues of the developing sunflower bud, which results in a distortion of the head and a loss of seed yield. The mechanism of distortion has not been determined. However, it has been shown that injection of 2,4-dichlorophenoxyacetic acid (2,4-D) into sunflower buds induces a distortion that is similar in appearance to that induced by sunflower midge larvae (Anderson 1989). Thus, distortion possibly arises in response to elevated auxin levels in the head tissue. In addition, sensitivity to 2,4-D was correlated negatively with midge tolerance among hybrids. It was proposed that sunflower hybrids tolerant to midge damage do not react as strongly to elevated auxin levels as susceptible hybrids. If true, exposure of sunflower hybrids to elevated levels of auxin should induce a response that is related to midge tolerance. This study was conducted to determine whether sensitivity to 2,4-D can screen sunflower hybrids for tolerance to the sunflower midge.

An objective measurement for sensitivity to 2,4-D was to use the increase in ethylene production in response to application of 2,4-D. Ethylene production by plants increases in quantities proportional to the degree of stress to which they are exposed (Tingey et al. 1976). Ethylene production has been used to test for phytotoxicity of various compounds (e.g., Simon et al. 1983) and to test different plant species for resistance to a phytotoxin (e.g., Hall et al. 1985). Ethylene production is also known to increase in response to elevated levels of auxin and auxin analogs such as 2,4-D (Abeles 1973). Auxin and stress influence the rate limiting step in the synthesis of ethylene (Yu & Yang 1979, 1980). Measurement of ethylene production was found to be sensitive within a broad response range, to yield objective data, to be relatively nonspecific over stress factors and plant taxa, to be reproducible, and to have biological significance (Tingey 1980).

Materials and Methods

Nine hybrids of known midge resistance (Anderson & Brewer 1991) were used: very susceptible 'Northrup King 212', susceptible 'Interstate 894', moderately resistant Agriculture Canada '85-346', and resistant 'Seedtec 315', 'Seedtec 316', Dahlgren 'DO643-7E', Dahlgren 'DO647-7E', Agriculture Canada '83-202', and Agriculture Canada '84-108'. Each hybrid was planted, six seedlings per 20.2 cm pot, using Sunshine Mix #1 (Fison's Horticulture Inc., Vancouver, B.C., Canada) potting media. Greenhouse conditions were a photoperiod of 15:9 (L:D) and 22°C. Lighting via 1,000-W HID sodium vapor lamps (rated at 135–140 lumens per watt) supplemented natural light. Plants were watered as needed and on the day before sampling to ensure leaf turgidity.

A stock solution of 10 mM 2,4-D in water was prepared for use in the experiment. A slight excess of diethanolamine was added with analytical grade 2,4-D (Sigma Chemical Company, St. Louis, Mo.) to improve water solubility. From the stock solution, dilutions were made to obtain seven concentrations of 2,4-D; 1 mM, 500, 250, 100, 50, 25, and 10 μM. Each dilution was buffered with 50 mM KH_2PO_4 and adjusted to pH 6.5 with 4N NaOH.

Leaf disks from seedlings were treated 19, 20, or 21 d after planting. Four of the six plants in each pot were selected for sampling based on

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uniformity of leaves. Two elliptic leaves (leaves 1 and 2) and two ovate leaves (leaves 3 and 4) were removed from each plant. Two disks, 2.0 cm in diameter, were taken from each leaf and placed on the surface of a 2,4-D solution or a 50 mM KH_2PO_4 buffer (control) in 250-ml jars (150-ml gas space above the liquid level). Each jar contained one disk from an elliptic leaf and one disk from an ovate leaf from each of the four plants of the same hybrid. Disks from the different leaf types were randomly assigned to the jars. Because pubescence was most consistent among hybrids on the underside of the leaf, disks were placed so the underside was in contact with the solution, minimizing differences in wettability among hybrids. Care was taken to avoid breaking the surface tension of the liquid so the leaf area available for gas exchange was approximately equal in all jars. The procedure was repeated for all hybrids in a randomized order. Two replications of each hybrid were exposed to 1 mM 2,4-D and a control for determination of ethylene production over time. Three replications of each hybrid were exposed to seven concentrations of 2,4-D and a control.

Because ethylene production is not affected by CO_2 concentration in the absence of light (Bassi & Spencer 1982), leaf disks were incubated in the dark. Because there may be a surge of ethylene production in response to the cutting of the disks (Suttle et al. 1983), the jars were left open for the initial 2 h and sealed with lids fitted with a septum. The gas-space of each jar was sampled 4, 6, 8, and 24 h after placing the leaf disks on solution for the time-course trials. Only the 8-h sampling interval was used for the dose trial.

Ethylene in the gas-space of each jar was quantified using a Shimadzu GC-9A (Shimadzu Corp., Kyoto, Japan) gas chromatograph. Ethylene was separated on a stainless steel column (61.0 cm by 0.3 cm) with an activated alumina support and a 60/80 mesh range. The carrier gas was helium at a flow rate of 32 ml/min. The column temperature was maintained at a constant temperature of 80°C. The injector temperature was 260°C, and the detector temperature was 260°C. Ethylene was detected with a flame-ionization detector. Under these conditions, the retention time of ethylene is ≈0.30–0.35 min, depending on the exact flow rate.

Gas samples (1 ml) were withdrawn from the gas-space of each jar and injected directly on the column. Each jar was sampled three times to obtain an average peak height, and this was compared with a standard curve to obtain the ethylene concentrations in each jar. A 1-ml ethylene standard of known concentration was injected periodically to correct for fluctuations in gas chromatograph performance.

Absolute ethylene production induced by a given dose of 2,4-D varied from day to day, largely because of light conditions in the greenhouse before testing. High light intensity before testing promotes ethylene production in the presence of CO_2 by increasing the activity of ACC oxidase in the last step of ethylene biosynthesis (Kao & Yang 1982, Bassi & Spencer 1982). Because the effect of 2,4-D (promotion of ACC synthase, Fig. 1) is established before this step in the pathway, light multiplies the effects of 2,4-D by a constant factor regardless of the concentration of 2,4-D. Division of treatment values by controls eliminates the effect of day-to-day variations in light intensity. Consequently, the sensitivity of each hybrid to 2,4-D was calculated as the percent increase in treatment ethylene production over the corresponding control.

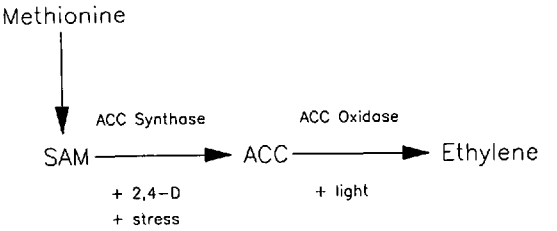

Fig. 1. Biosynthetic pathway of ethylene (for complete pathway, see Adams & Yang 1977). SAM, S-adenosylmethionine; ACC, 1-aminocyclopropane-1-carboxylic acid.

Mean 2,4-D sensitivity was regressed (SAS Institute 1985) on percent midge tolerance (Anderson & Brewer 1991) for each concentration of 2,4-D to determine if there was a relationship between midge tolerance and the hybrid response to 2,4-D. Percent midge tolerance is defined as the percent of resistance due to toler-

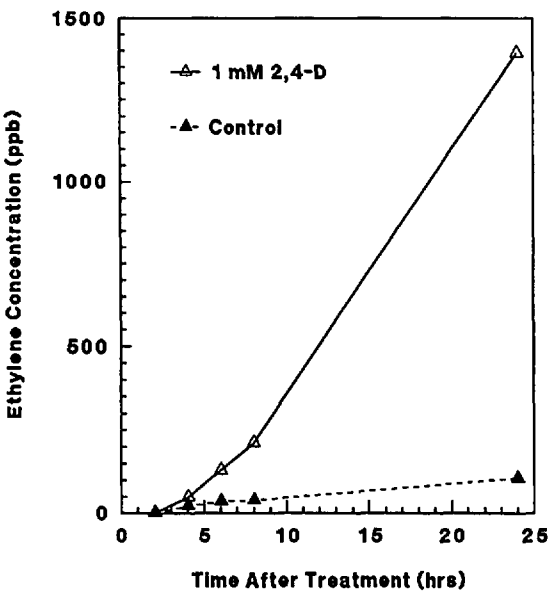

Fig. 2. Time-course plot of ethylene production in 'Interstate 894' in response to 1 mM 2,4-D treatment.

Table 1. Percent increase in ethylene production (sensitivity to 2,4-D) for nine sunflower hybrids at different doses of 2,4-D and percent increase regressed on tolerance to the sunflower midge, *C. schulzi*

Hybrid	Midge tolerance[a]	2,4-D concentration (μM)						
		10	25	50	100	250	500	1,000
DO643-7E	38.94A	89.48 ± 35.55	153.77 ± 46.05	176.86 ± 30.14	215.38 ± 20.68	300.06 ± 42.71	353.89 ± 94.03	390.99 ± 130.16
84-108	17.51B	110.20 ± 51.72	161.25 ± 47.11	240.38 ± 50.11	330.72 ± 123.13	478.58 ± 107.77	450.98 ± 213.84	646.12 ± 187.25
DO647-7E	14.41B	117.70 ± 32.53	195.07 ± 18.91	211.51 ± 47.02	323.07 ± 114.20	430.57 ± 81.62	514.58 ± 180.26	519.58 ± 170.95
83-202	9.04BC	103.37 ± 28.76	172.94 ± 55.39	259.30 ± 54.61	268.95 ± 95.31	485.14 ± 129.39	413.39 ± 166.42	495.24 ± 186.80
85-346	7.39C	144.96 ± 55.39	213.55 ± 53.98	289.09 ± 74.78	381.00 ± 154.89	586.96 ± 118.79	643.81 ± 304.91	837.10 ± 394.11
Seedtec 316	7.36C	123.52 ± 76.39	201.15 ± 49.98	246.94 ± 79.96	307.20 ± 47.59	479.46 ± 52.69	591.42 ± 165.39	635.41 ± 206.10
Interstate 894	6.93C	159.05 ± 22.54	298.56 ± 101.22	352.17 ± 61.79	444.61 ± 153.83	698.33 ± 139.08	836.93 ± 246.92	761.15 ± 411.97
Seedtec 315	6.92C	133.11 ± 59.48	197.32 ± 90.30	358.31 ± 127.08	488.17 ± 259.42	702.65 ± 265.18	671.36 ± 263.65	717.91 ± 247.45
Northrup King 212	0.00D	89.70 ± 19.19	160.93 ± 67.38	217.92 ± 59.63	271.92 ± 48.62	414.89 ± 84.11	475.63 ± 121.43	556.19 ± 188.98
F^b (Northrup King 212 included)		1.36	1.12	2.88	2.05	3.62	2.89	3.63
P		0.28	0.33	0.13	0.19	0.10	0.13	0.10
r^2		0.16	0.14	0.29	0.23	0.34	0.29	0.34
Slope ± SE		−0.86 ± 0.73	−1.46 ± 1.38	−2.97 ± 1.75	−3.71 ± 2.59	−6.89 ± 3.62	−7.25 ± 4.26	−7.35 ± 3.86
Intercept ± SE		129.34 ± 11.78	212.55 ± 22.14	297.22 ± 28.10	381.48 ± 41.51	591.57 ± 58.09	637.56 ± 68.38	706.36 ± 61.91
F^b (Northrup King 212 omitted)		6.65	2.69	6.91	4.86	9.47	5.28	6.45
P		0.04	0.15	0.04	0.07	0.02	0.06	0.04
r^2		0.53	0.31	0.54	0.45	0.61	0.47	0.52
Slope ± SE		−1.49 ± 0.58	−2.29 ± 1.39	−4.24 ± 1.61	−5.46 ± 2.48	−9.72 ± 3.16	−9.85 ± 4.28	−9.76 ± 3.84
Intercept ± SE		142.92 ± 9.85	230.23 ± 23.74	324.38 ± 27.48	419.00 ± 42.18	652.10 ± 53.78	693.03 ± 72.90	757.81 ± 65.39

Sensitivity to 2,4-D is expressed as the percent increase in ethylene production over the ethylene production of the controls (± SD).

[a] Percent relative tolerance (Anderson & Brewer 1991).

[b] Significance of the linear regression model for each concentration of 2,4-D. Statistics were calculated with 'Northrup King 212' included and again with 'Northrup King 212' omitted.

ance (with zero damage as 100% resistance) relative to susceptible 'Northrup King 212'.

Results and Discussion

The ethylene production of 'Interstate 894' in response to a test solution of 1 mM 2,4-D during a 24-h period (Fig. 2) illustrates the continued stimulation of ethylene production by 2,4-D. This is similar to that found by Kang et al. (1971). The remaining eight sunflower hybrids in the study showed similar responses. The slight increase in the slope of ethylene production by 2,4-D concentration indicated that factors other than 2,4-D had an influence on the rate of ethylene production. Sampling at eight hours (accumulation from the second to the eighth hour) was sufficient to distinguish differences in ethylene production among hybrids and minimized factors that influence ethylene accumulation in closed chambers at longer time intervals.

The linear regression model of 2,4-D sensitivity over midge tolerance was significant ($P \leq 0.1$) at the 250 and 1,000 μM concentrations of 2,4-D (Table 1). Sensitivity to 2,4-D in 'Northrup King 212' (midge tolerance value of 0.00, Anderson & Brewer 1991) was lower than expected and was partially responsible for the lack of significance at the other 2,4-D concentrations tested. The low 2,4-D sensitivity values for 'Northrup King 212' may have been due to stress factors other than 2,4-D. Although the plants used in this trial were generally healthy, plants of 'Northrup King 212' and, to a lesser extent, 'Seedtec 316' suffered some thrips damage that may have influenced the production of ethylene. Insect stress increases production of ethylene by increasing the activity of ACC synthase, the same site of influence as 2,4-D (Yu & Yang 1979). Thus, insect stress acts additively with 2,4-D in increasing ethylene production (Fig. 1). Because insect stress increases ethylene levels equally in both the 2,4-D treated leaf disks and the controls, it masks the effect of 2,4-D. Therefore, the low percent increase values for 'Northrup King 212' may be the result of thrips damage obscuring the effect of 2,4-D on ethylene production. When 'Northrup King 212' was omitted from the analysis, the linear regression model was significant for all but one 2,4-D concentration (Table 1).

It was initially proposed that midge tolerant hybrids do not react as strongly to elevated auxin levels as midge susceptible hybrids. The data support this theory. In addition to being more sensitive to 2,4-D at a given dose, susceptible hybrids are also more sensitive to changes in the dose of 2,4-D (illustrated in Table 1 by the decline in the regression line slopes with increasing dose). However, more work is needed to establish a physiological relationship between auxin levels and midge tolerance.

Regardless of the presence of physiological relationships, measurements of sensitivity to 2,4-D may be of value in the selection of midge tolerance in sunflower. Initial screening of sunflower lines may be conducted at times when midge populations are unavailable. Those lines showing a low sensitivity to 2,4-D would make good candidates for field confirmation of midge tolerance. Previous work (Anderson & Brewer 1991) revealed a high level of midge tolerance in 'DO643-7E' and a moderate level of tolerance in '84-108' and 'DO647-7E'. At 250 μM 2,4-D (with the highest F and r^2 values, Table 1) a 5-fold increase in ethylene production over control levels would distinguish these hybrids from the more susceptible hybrids.

There are several drawbacks to the use of this technique. The presence of water or nutrient stress, temperature stress, mechanical damage, insect and disease damage, and differences in plant maturity among hybrids may have a considerable influence on the expression of 2,4-D sensitivity in each hybrid and must be avoided when measuring ethylene production. In addition, this assay detects tolerance to auxin which indicates tolerance to the midge. Midge resistance based on antixenosis or antibiosis will not be detected. However, it may be desirable to select only those hybrids with midge tolerance which is difficult to detect in the field. The assay has the advantages of yielding objective data, and of detecting tolerance in sunflower to the sunflower midge without the need of an insect population.

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