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EVALUATION OF HELIANTHUS FOR RESISTANCE TO INSECT PESTS[1]

By

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Abstract

Resistance to the larva of the sunflower beetle (Zygogramma exclamationis (L.)) was found in Helianthus annus, H. petiolaris, H. paradoxus, H. ciliaris, H. salicifolius, and H. tuberosus. Resistance was expressed as lengthened stadia, reduced leaf consumption, and increased mortality for larvae reared on these species. Adults reared on these Helianthus species had reduced longevity and did not lay any eggs. Hybrid 896 was used as the susceptible check.

Adults of the carrot beetle (Bothynus gibbosus (De Geer)) did significantly more damage to roots of Hybrid 896 than to roots of H. tuberosus, H. maximiliani, H. niveus, H. x laetiflorus, H. salicifolius, H. mollis, H. grosseserratus, H. argophyllus, or H. ciliaris.

Females of an aphid (Masonphis masoni (Knowlton)) produced as many progeny on 4 annual species of Helianthus as they produced on Hybrid 896. However, they produced significantly fewer progeny on H. ciliaris, H. grosseserratus, H. maximiliani, H. salicifolius, H. mollis, H. x laetiflorus, and H. tuberosus than they produced on Hybrid 896.

Preparatory to evaluation of Helianthus species for resistance to the sunflower moth, a behavioral study of feeding larvae was conducted. The study showed that larvae of the sunflower moth fed primarily on pollen as 1st instars, corollas as 2nd instars, and began doing significant damage to floral ovaries as 3rd instars.

Introduction

The emergence of sunflower as a major source of premier vegetable oil resulted largely because varieties and hybrids have been developed that are resistant to pestilences such as broomrape, Orobanche cumana Wallr., downy mildew, Plasmopara halstedii (Farl.) Berl. & de Toni, rust, Puccinia helianthi Schw., and verticullium wilt, Verticillium albo-atrum Reinke & Berth. (Pustovoit 1966, and Cobia and Zimmer 1975). Despite the excellent progress that has been made in the development of disease-resistant varieties and hybrids of sunflowers, very little attention has been given to the development of insect-resistant sunflower. Exceptions include the report by Pustovoit (1966) of the Soviet Union of apparent resistance to the aphid Brachycaudus helichrysi (Kaltenbach), in Helianthus mollis Lambert, H. tomentosus Michx., and H. macrophyllus Willd., and resistance to the larva of the "European sunflower moth", Homoeosoma nebulellum (Schiffermuller) in H. tuberosus var. purpurens Ckll. (Heiser 1969

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considers H. tomentosus Michx. to be a synonym of H. tuberosus L., and H. macrophyllus to be a synonym of H. strumosus L.). Also, in the United States, Kinman (1966) and Teetes et al (1971) reported tentative resistance and degrees of susceptibility to the larva of the sunflower moth, H. electellum (Hulst), in H. petiolaris Nutt., and among varieties. These latter studies and those by Carlson and Witt (1974), and Johnson and Beard (1977) have emphasized resistance conferred by the armored layer (phytomelanin) characteristic of achenes. More recently, Waiss et al. (1977) extracted the florets of sunflower lines having the phytomelanin characteristic and obtained two diterpenoid acids that appeared to be toxic to early-instar larvae of the sunflower moth.

Reported herein are results from initial evaluations of Helianthus species for resistance to the sunflower moth, H. electellum; sunflower beetle, Zygogramma exclamationis (F.); carrot beetle, Bothynus gibbosus (De Geer); and an aphid, Masonaphis masoni (Knowlton).

General Methods

All plants were seeded in 15-cm pots and maintained in the greenhouse until screening for resistance began. All evaluations were done in the laboratory under a photophase of 14 h and a temperature of 27°±2°C. Tests were conducted on plants 30-45 cm tall in ventilated pyroxylin cages. "Hybrid 896" served as susceptible check in all tests and as a host for maintenance of parent colonies of the aphid and the sunflower beetle. The carrot beetle was maintained on sweet potato before being used for a test. Helianthus species being evaluated were thinned to one plant/pot for the aphid and sunflower beetle tests, and to 2 plants/pot in the carrot beetle test. Species of Helianthus evaluated for resistance were agrophyllus Torrey and Gray, annuus L., ciliaris DC., debilis Nutt., grosseserratus Martens, H. x laetiflorus Pers., maximiliani Schrader, mollis, neglectus Heiser, niveus subspecies canescens (Gray) Heiser, paradoxus Heiser, petiolaris, salicifolius A. Dietr., and tuberosus.

Zygogramma exclamationis

The sunflower beetle is a North American chrysomelid defoliator of sunflower, both as a larva and as an adult. In a few instances, insecticidal application has been necessary to control larval populations of the sunflower beetle in seedling sunflower.

Methods - In evaluating Helianthus species for resistance to the sunflower beetle, two approaches were used: (1) Ten newly hatched 1st-instar larvae were placed with a moist sable-hair brush on each of 10 plants of each Helianthus sp. and left until pupation. Larval mortality, leaf consumption, pupal weight, and total mortality were recorded. (2) A newly emerged pair of adults was placed on each of 5 plants of each Helianthus sp. and observed for longevity, fecundity, and fertility.

Results - Stadia for larvae of the sunflower beetle were significantly longer (P = 0.05) when the insects were reared on H. petiolaris and H. annuus than when they were reared on Hybrid 896 (Table 1). Larval stadia on H. debilis, H. paradoxus, and H. tuberosus approximated those for larvae on Hybrid 896. Larvae refused to eat on H. ciliaris and H. salicifolius and lived for less than one day. Mean leaf consumption of larvae reared on H. petiolaris, H. paradoxus, and H. tuberosus was significantly less than it was for larvae reared on Hybrid 896. Although mortality was very low for larvae reared on H. debilis, mortality of pupae from these larvae was very high (Table 1). Total mortality was significantly higher for larvae reared on all species except H. petiolaris than it was for those reared on Hybrid 896.

Longevity of males was significantly shorter (P = 0.05) on the four Helianthus species evaluated for resistance than it was on Hybrid 896 (Table 2). The longevity of females was also significantly shorter except for those on H. tuberosus. Females did not lay any eggs on the four Helianthus test species, though an average of 1020 eggs/female was laid on Hybrid 896 (Rogers 1977).

The results of these preliminary tests indicate that perennial species of Helianthus may be excellent sources of germplasm for resistance to the sunflower beetle.

TABLE 1. Mean stadia, leaf consumption, and mortality of larvae of Zygogramma exclamationis reared on Helianthus species.[1]

Helianthus species	$\bar{x}$ Stadia (days)[2]	$\bar{x}$ Leaf consumption/ larva (cm^2)	% Mortality	
			Larvae	Total
petiolaris	42 a	15 c	48 a	52 b
annuus	39 ab	29 ab	84 a	94 a
debilis	29 c	30 a	16 b	84 a
paradoxus	29 c	21 b	86 a	90 a
ciliaris	< 1 d[3]	0 c	100 a	100 a
salicifolius	< 1 d[3]	0 c	100 a	100 a
tuberosus	29[4]	5 c	96 a	100 a
Hybrid 896	31 c	37 a	22 b	35 b

1 $\bar{x}$'s followed by same letter are not significantly different @ P = 0.05.

2 $\bar{x}$ days from hatching of egg to emergence of adult

3 all larvae died on day of infestation.

4 all died as pupae

TABLE 2. Mean longevity and fecundity of adult Zygogramma exclamationis on Helianthus species.[1]

Helianthus Species	$\bar{x}$ Longevity (Days)		$\bar{x}$ No. eggs/ Female
	Female	Male	
paradoxus	11 a	13 a	0 b
salicifolius	22 a	21 a	0 a
ciliaris	35 a	59 a	0 a
tuberosus	68 b	18 a	0 a
Hybrid 896	84 b	109 b	1020 b[2]

1 $\bar{x}$'s followed by the same letter are not significantly different @ P = 0.05.

2 From fecundity of females determined in an accompanying bionomics study (Rogers 1977)

Bothynus gibbosus

The carrot beetle is a noctural scarab that damages sunflower by adult-feeding on the roots (Rogers 1974). Although small plots have frequently been destroyed by the carrot beetle, there has been only one report during the last three years of this beetle severely damaging sunflower in commerical fields.

Methods - Resistance to the carrot beetle was determined by placing three pairs of adults (collected from a light trap) in each of five pots of each Helianthus species. At the end of 2 weeks, the soil containing the plants and beetles was carefully removed from the pots. The soil was then removed from the roots by gentle agitation in water. The amount of damage done to both the primary and the secondary root systems was determined.

Results - Carrot beetles damaged significantly more (P = 0.05) primary roots of Hybrid 896 and H. tuberosus than of any other species (Table 3). There were no signs of feeding on the primary roots of H. salicifolius, H. mollis, H. grosseserratus, H. argophyllus, or H. ciliaris. The mean percentage of tap roots damaged was significantly greater for Hybrid 896 than for any test species, a significantly greater (P = 0.05) volume of secondary root destruction occurred on Hybrid 896. Carrot beetles fed more readily on the secondary roots than on the primary roots. Only H. argophyllus showed no evidence of any root damage. These results indicate that there may be resistance to the carrot beetle among native Helianthus species.

TABLE 3. Injury by Bothynus gibbosus adults to the roots of Helianthus species in the laboratory.[1, 2]

Helianthus Species	No. tap roots damaged	% tap roots damaged	Volume roots damaged
Hybrid 896	1.4 a	47 a	65 a
tuberosus	1.6 a	16 b	16 b
maximiliani	0.6 b	2 b	8 b
niveus canescens	0.2 b	5 b	9 b
H. x laetiflorus	0 b	4 b	6 b
salicifolius	0 b	0 b	2 b
mollis	0 b	0 b	4 b
grosseserratus	0 b	0 b	7 b
argophyllus	0 b	0 b	0 b
ciliaris	0 b	0 b	5 b

[1] $\bar{x}$'s followed by the same letter are not significantly different @ P = 0.05.
[2] Injury expressed as $\bar{x}$ per replication.

Masonaphis masoni

Aphids have not yet become an economic threat to sunflower production in the United States. However, large populations are sometimes found on sunflower.

Methods - In this test, apterous (wingless) females were transferred from parent colonies to plants of the *Helianthus* species with a moist sable-hair brush. Resistance was determined by two methods: (1) Ten apterous females were placed on each of 10 plants of each *Helianthus* species; then after 7 days, the nymphs and adults were counted. (2) Ten apterous females were installed on each of five plant of each *Helianthus* species and left for 30 days (or until the plants died). The number of progeny produced and the effects on host plants were recorded.

Results - Table 4 shows the response of *M. masoni* to several species of *Helianthus* evaluated for resistance. After 7 days, there were significantly more (P = 0.05) aphids on *H. paradoxus* and significantly fewer aphids on *H. niveus* than on the hybrid check. More aphids were present on the annual species after 30 days than on the check. Thus, the four annual species of *Helianthus* were as suitable as a host for *M. masoni* as Hybrid 896.

TABLE 4. Progeny developing from ten apterous females of *Masonaphis masoni* held on *Helianthus* species in the laboratory.

Helianthus species	$\bar{x}$ No. aphids per plant After 7 days	After 30 days
Annual species		
paradoxus	128 a	413 b
neglectus	89 b	425 b
argophyllus	84 b	552 a
niveus canescens	56 c	359 c
Hybrid 896	91 b	294 c
Perennial species		
Hybrid 896	91 a	294 c
ciliaris	59 b	4 c
grosseserratus	43 c	99 b
maximiliani	37 cd	107 b
salicifolius	33 cd	68 bc
mollis	30 d	24 bc
H. x laetiflorus	10 e	0 c
tuberosus	2 e	0 c

[1] $\bar{x}$'s followed by the same letters are not significantly different @ P = 0.05.

The response of *M. masoni* to seven perennial species of *Helianthus* was drastically different (Table 4). In both the 7-day and 30-day tests, significantly fewer (P = 0.05) aphids were found on these perennial species than on the hybrid check. All the aphids died on *H. tuberosus* and *H. x laetiflorus*, and most died on *H. ciliaris*. These results indicate that antibiosis may have have been involved as a resistance mechanism. *Helianthus x laetiflorus* is a natural hybrid resulting from crossing by *H. tuberosus* and *H. rigidus*, a species that we have not yet evaluated. As with sunflower beetle, resistance to aphids appeared to be present in perennial species of *Helianthus*. Thus, such species may possess valuable germplasm for incorporation into commerical hybrids to give resistance to foliage-feeding insects.

Homoeosoma electellum

The sunflower moth is the key pest of sunflower in the United States. This pest is important not only for the seed that the larva destroys but also as a precursor to infection of sunlfower by Rhizopus oryzae Went and Prinsen-Geerligs, the cause of head rot of sunlfower (Rogers et al, 1978). Preparatory to evaluating Helianthus species for resistance to the sunflower moth, the feeding behavior of the larva was studied.

Method - Seedlings of Hybrid 896 were transplanted from the field to 30.5 cm pots and maintained in the greenhouse until flowering began. When ca. 20% of the florets on a head were shedding pollen, 10 newly hatched lst-instar larvae were placed on inflorescence of 35 plants. Five of the 35 plants were then taken to the laboratory and examined by a dissecting microscope (15X) for four 20-min. periods on each of four days. Notes were taken of feeding and non-feeding behavior. After 4 days, the heads were dissected and examined microscopically and the extent of injury done by the larvae was recorded.

The other 30 plants were held in the greenhouse until the receptacles were dissected and examined microscopically (5 plants on 3,6,9,12,15, and 18 days after infestation). During dissections, larval maturity and extent of feeding damage were recorded.

Results - About 65% of the larvae established silken retreats in the inter-corolla spaces. These larvae fed primarily on pllen during the first 4 days (Table 5). The larvae that became established inside opened florets fed about equally on pollen and anthers. However, the anthers support pollen, so they may have been damaged incidentally as the larva attempted to remove pollen.

TABLE 5. Feeding behavior of 1- to 4-day-old larvae of Homoeosoma electellum florets of Hybrid 896 in laboratory.

Feeding Site	No. observed feedings		% feeding at specific sites
	Total	$\bar{x}$/day[1]	
Between florets			
Pollen	106	26.5 a	82.8
Corolla	18	4.5 b	14.0
Ovary[2]	4	1.0 b	3.2
Inside florets			
Pollen	19	4.7 b	38.7
Anther[3]	21	5.3 b	42.8
Style[4]	9	2.3	18.5

[1] $\bar{x}$'s followed by same letter are not significantly different @ P = 0.05.

[2] Immature seed

[3] Including filaments

[4] Including stigmas

The sequential feeding damage to floral parts by larvae as they matured is summarized in Table 6. Floral damage was limited to corollas during the first three days. By the sixth day, significantly more (P = 0.05) corollas were being damaged by larvae (2nd instars) than had been damaged by 3 days (1st instars) after infestation. By the ninth day after infestation (early 3rd-instar stage), whole florets were being damaged by feeding larvae; also significant feeding was noted on the ovaries. Larvae that remained on the heads until maturity destroyed an average 22.8 seeds and prevented fertilization of an average 95.5 seeds by destroying the florets prior to fertilization of floral ovaries. It appears that larva may cause more indirect seed loss via floral destruction than by direct feeding on the developing seeds.

Therefore, in developing sunflower hybrids resistant to the sunflower moth, the most productive approach would apparently be mechanisms that adversely affect early-instar larvae before they begin feeding extensively on florets or immature seeds. On the basis of the preliminary findings of Waiss et al (1977), we are optimistic that such a mechanism may be found in the form of chemical toxins in either the pollen or florets of some species of Helianthus.

TABLE 6. Sequential feeding damage to disk florets by larvae of Homoeosoma electellum on Hybrid 896 in the laboratory.

Age of larvae (Days)	$\bar{x}$ No. floral parts damaged/larva[1]		
	Corolla	Whole floret	Ovary
3	0.7 a	0 a	0 a
6	5.5 b	0 a	0.1 a
9	5.3 b	10.4 ab	8.2 b
12[2]		24.0 abc	12.3 b
15		54.8 bcd	19.6 b
18		95.5 d	22.8 b

[1] $\bar{x}$'s followed by same letter are not significantly different @ P = 0.05.

[2] Included among "whole-floret" data for age 12-18 days.

Future Plans

We are presently bioassaying floral extracts of Heliatnhus species for toxicity to larvae of the sunflower moth by feeding them in a standard wheat germ diet. Dr. R.D. Stipanovic, Research Chemist, FR, SEA, USDA, College Station, Texas, is a cooperator in this project. Dr. Stipanovic will determine the chemical nature of resistance mechanisms in the florets. Also, we are cooperating with Dr. D.H. O'Brien, Department of Chemistry, Texas A&M University, who will seek to determine the chemical nature of resistance to foliage-feeding insects. A major effort henceforth will be to develop insect-resistant parental lines for hybrid sunflower production.

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