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RESISTANCE TO THE BANDED SUNFLOWER MOTH (*Lepidoptera: Cochylidae*) FROM COMPONENTS IN SUNFLOWER SEED, FLORETS, RAY FLOWERS AND LEAVES*

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SUMMARY

Hybrid sunflower florets have been reported to contain two acetone soluble kauranoid compounds that conferred resistance, in the form of "larvicidal" effects, to the sunflower moth, *Homeosoma electellum* (Hulst). The objective of this study was to determine if the seed, florets, ray flowers or leaves from a hybrid sunflower contained acetone soluble kauranoid or other components that conferred resistance to larvae of the banded sunflower moth *Cochylis hospes* Walsingham. The mortality and development time of larvae reared on diets prepared with unextracted seed (rearing diet), floret, ray flower or leaf material were compared with mortality and development of larvae fed complementary diets that contained acetone extracted seed, floret, ray flower or leaf material as the test ingredient. Important findings of the study were as follows: 1) larval mortality decreased significantly when larvae were fed diet prepared with acetone-extracted seed compared with diet with unextracted seed 2) substitution of florets into the diet was not larvicidal although substitution of florets into the diet resulted in a significant increase in larval development time, 3) the development time on diet with ray flowers significantly increased, 4) mortality on the leaf diet was as high as on the rearing diet, and 5) the effects of florets, ray flowers, or leaves on mortality or development time of *C. hospes* were not related to acetone soluble components in sunflower because the results in each case were similar when either acetone extracted or intact floret, ray flower or leaf material were substituted in the diet. The difference in response reported here for *C. hospes* and those reported for the sunflower moth *H. electellum* may reflect species differences in adaptation to the host plant or there may be significant differences in the chemical composition of different sunflower hybrids. Selection of sunflower for constituents that conferred resistance in the form of increased mortality and development time of *C. hospes* could be useful for greater control of this pest insect.

Key words: ***Cochylis hospes, Helianthus annuus*, insect, larval development, mortality, resistance.**

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INTRODUCTION

In the North American Great Plains, larvae of the banded sunflower moth, *Cochylis hospes* Walsingham, infest the heads of cultivated sunflower. The eggs are oviposited on the bracts of the sunflower head and the larvae feed on florets, ray flowers, pollen and seed as the sunflower head matures (Westdal 1949; Charlet and Gross, 1990). The sunflower moth *Homeosoma electellum* is a pest of sunflower and has a similar life cycle and ecological niche to *C. hospes*. *H. electellum* prefers to oviposit on the open inflorescences of the sunflower (DePew, 1983). Few reports are available that demonstrate sunflower resistance to these insect pests such as increased mortality due to chemical components or developmental delays that expose larval stages to the hazards of weather, predation, parasitism or disease for extended periods. One such study, Waiss *et al.*, (1977), reported that hybrid sunflower florets were a particularly rich source, containing up to 5% by weight, of two hexane (or acetone, Metzger and Hazebroek, 1989) soluble kauranoid compounds identified as trachyloban-19-oic and (-)kaur-16-en-19-oic acid which had "larvicidal" effects on larvae of the sunflower moth. *H. electellum* larvae that were fed on synthetic diets that contained 5% of the hexane floret extract died while normal larval development occurred when they were fed diets containing 20% of the extraction residue (Waiss *et al.*, 1977) The objective of this study was to document host plant resistance to *C. hospes* by studying mortality and development time of larvae that were reared on insect diet that contained a finely ground meal of intact or acetone extracted seeds, florets, ray flowers, or leaves from a hybrid sunflower and to determine if the larvicidal effects of kauranoids in florets could be extended to *C. hospes*.

MATERIALS AND METHODS

Insects and Rearing Diet. Banded sunflower moth eggs were obtained from a colony that has been maintained in the laboratory since 1988. The diet used to rear the banded sunflower moth is based on an artificial diet developed by Vanderzant *et al.*, (1962) to rear the bollworm *Helicoverpa zea* (Boddie). Mortality of *C. hospes* on the Vanderzant diet averaged 59.0 ± 5.6 % but substitution of 100 g of ground sunflower seed for casein (100 g) (Barker, 1988) to reduced mortality to 44%. The modified Vanderzant diet was satisfactory as a rearing diet because the fecundity of the banded sunflower moth was such that the 56% that survived amounted to thousands of individuals per week and sunflower seed, in contrast to casein, is a natural food source for this moth. In addition, sunflower seed is cheap, commercially available as hulled seed and can be obtained as "organically grown" free of pesticide residues. The colony was maintained at 28 °C, light : dark cycle of 15:9 h and 50% relative humidity. Experimental insects were held under the same light regimen, temperature and humidity as the rearing colony. To reduce loss of wild traits that is common in laboratory reared insects, larvae are collected annually from field populations in the Fargo, ND area and added to the colony when they emerge as adults.

Sunflower. Leaf, petal, and floret material was obtained from sunflower hybrid 894, a standard research sunflower in the northern Great Plains. Leaves 15, 16, and 17 were taken from the midsection of the plant. Hybrid 894 plants were grown without the use of insecticide. The hybrid source of organically grown seed that was obtained from commercial sources was unidentified.

Preparation of sunflower seed, florets, ray flowers and leaves. Preparation of the four types of sunflower material was similar except for minor differences as follows: leaves, florets, and ray flowers were air dried, ground and then pulverized in a ball mill to reduce them to a fine powder that would be evenly distributed in the diet. Seeds were homogenized to a fine meal in a Waring® blender. In each case, half of the material was set aside and used for the preparation of diet with unextracted material and the other half was extracted with acetone to remove the putative larvicidal agents trachyloban-19-oic and (-) kaur-16-en-19-oic acids. Acetone extractions were made by adding one liter of acetone to 500 g of pulverized material which was then stirred on a magnetic stirrer for 2 - 3 hours. The slurry was then poured into a Buchner funnel lined with Whatman #4 filter paper and the acetone was removed with suction. Acetone extraction of each 500 g batch of sunflower material was repeated three times with 1 liter of fresh acetone. The extracted material was spread over the bottom of a 40 x 24 cm pan and air dried in a fume hood for 72 hours and then stored at -20°C until needed.

Bioassay of seed, floret, ray flower and leaf diets. In each of three replicates, a set of four diets was prepared that contained one of the following as an ingredient: unextracted seed (rearing diet), floret, ray flower or leaf material. A complementary set of four diets were prepared that contained acetone extracted seed, floret, ray flowers or leaf material as a test ingredient. The diet was pressed through a 0.5 cm^2 mesh hardware cloth to form pieces that were 0.5 cm^2 x 2 cm long. The average weight of each piece of diet was 0.81 ± 0.02 gr. Neonates were divided into 8 groups of 100 and individually placed in vented 1.5 ml micro test tubes that contained a piece of one of the test diets. Larvae of each group were observed daily for mortality and development time from the beginning of the first instar to the pupal stage. The rearing diet was used as a control to compare mortality and development time of larvae fed the other seven diets because the value of these parameters with respect to the rearing diet had become well known to the author from efforts to maintain the laboratory colony.

Statistics. Analysis of variance and Student-Newman-Keuls (SNK) for separation of significant differences between means were performed using Jandel Scientific Sigmastat® software.

RESULTS AND DISCUSSION

Seed diets. Larval mortality on the rearing diet prepared with intact seed was about 44% but extraction of the seed with acetone for diet preparation resulted in a significant reduction of mortality to about 19% (F=12.6; df=7,16; P=0.001, Table 1). This result suggested that acetone removed material from sunflower seeds that reduced the survival of banded sunflower moth larvae. The development time on diet prepared with extracted seed decreased but it was not statistically significant. Survival of the colony has not been threatened by the presence of acetone soluble seed component(s) that had deleterious effects on larval survival, but 44% larval mortality suggested plant resistance. Selection of the sunflower plants to enhance the trait has potential for greater control of this pest.

Floret diet. Substitution of 100 g of either acetone extracted or unextracted florets to the diet significantly reduced mortality of banded sunflower moth larvae compared with larvae that were fed the rearing diet (F=15.9; df=7,16; P≤0.001, Table 1). The decrease in mortality of banded sunflower moth larvae contrasted with the reported larvicidal effects of hybrid florets on sunflower moth larvae. At least two interpretations of these results are possible: (1) there was an insect species difference, with banded sunflower moth larvae showing immunity or resistance to floret components that were larvicidal for head moth larvae, or (2) there are qualitative or quantitative differences in floret components of sunflower hybrids. Both interpretations are probably equally valid, although, there are no known comparative studies of sunflower hybrids to show that there are major differences in the chemical composition of hybrid sunflower florets or other parts.

Development time increased significantly relative to the rearing diet (F=57.7; df=7,16; P=0.001) when 100 g of acetone-extracted or unextracted florets were added to the diet. In this respect, there was an indication of resistance to the banded sunflower moth, but the effect was not related to an acetone-soluble factor from florets because the results were the same with intact or acetone extracted florets.

Ray flower diet. The addition of 100 g of acetone extracted or unextracted ray flowers to the diet did not significantly affect mortality of banded sunflower moth larvae compared with larvae fed the rearing diet (F=0.5; df=7,16; P=0.5, Table 1). Mortality in this case was not related to acetone-extractable components in ray flowers. The development time of larvae reared on diet prepared with ray flowers was significantly increased (F=41.0; df=7,16; P= 0 .001) by nearly 6 days over larvae fed the rearing diet (Table 1). The increase in development time was not related to acetone-extractable material from ray flowers because the effect was seen with both intact and extracted of ray flowers. The increase in development time caused by the introduction of ray flowers (or florets) into the diet may have resulted from the activity of sunflower components that are soluble in other solvents or because of some subtle change that substitution of these sunflower parts might have in the nutritional value or palatability of the diet that slowed feeding or assimilation of nutrients.

Table 1: Effect of acetone extracted and unextracted sunflower seed, florets, ray flowers, and leaf material on mortality and development time of banded sunflower moth larvae (mean (SEM)

Diet treatment	Total development time (days)	% mortality	n*
100 g unextr. seed (rearing diet)	24.9 ± 0.4a	44.1 ± 4.0a	3
100 g acetone extr. seed	21.7 ± 0.1a	19.2 ± 3.1b	3
100 g unextr. florets	33.1 ± 0.2c	15.0 ± 3.1b	3
100 g extr. florets	30.8 ± 0.3c	11.7 ± 4.3b	3
100 g unextr. ray flowers	29.6 ± 0.3c	48.0 ± 5.6a	3
100 g extr. ray flowers	29.9 ± 0.2c	36.0 ± 4.9a	3
100 g unextr. leaf	23.7 ± 0.3a	33.0 ± 3.4a	3
100 g extr. leaf	23.1 ± 0.1a	35.0 ± 4.1a	3

*n = replicates of 100 insects

Means within columns with the same letter are not significantly different (P = 0.05; SNK mean separation)

Leaf diet. Mortality (F=0.8 ; df=7,16; P=0.4) and development time (F=2.1; df=7,16; P=0.1) of larvae fed test diets that contained 100 g of unextracted or acetone-extracted leaf material in place of sunflower seed were not significantly different from the rearing diet (Table 1). Mortality on the leaf diet or the rearing diet was in the 35 to 45% range, respectively. The basis for the mortality of larvae fed these diets must have been different from the seed diet because acetone extraction of leaves had no significant effect on mortality, while acetone extraction of seed did have a significant effect.

CONCLUSIONS

In summary, the significant findings of this study were 1) seeds appeared to have an acetone extractable factor with larvicidal effects, 2) florets were not larvicidal, but the development time on diet with florets was significantly increased compared with the rearing diet, 3) the development time on diet with ray flowers significantly increased, 4) mortality on the leaf diet was as high as mortality on the rearing diet possibly indicating resistance in sunflower leaf, and 5) the effects of ray flowers or leaves on mortality and development, like that of florets, seemed to be based on different factors than for seed because the results were not significantly changed when either intact or acetone extracted ray flowers or leaf material were added to the diet. Possible explanations for the different responses of *C. hospes* and *H. electellum* are that banded sunflower moth larvae have adapted to different sunflower components more readily than *H. electellum* and were resistant to kauranoid components or sunflower hybrids differ in the distribution of these compounds. However, there are no published reports to support differences in the distribution of kauranoid compounds or other chemical compounds in sunflower hybrids. The presence of acetone soluble components in seed that increased mortality or of components in florets or ray flowers that increased development time have potential in the development of control strategies for this pest insect.

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RESISTENCIA A LA POLILLA DE BANDAS (*Lepidoptera: cochylidae*) DE COMPONENTES EN LA SEMILLA DE GIRASOL FALSOS PETALOS Y HOJAS

RESUMEN

Las flores de híbridos de girasol se informó quie contienen dos compuestos kauronoides solubles en acetone que confieren resistencia, en la pomada efecto larvacidad y contra la polilla de girasol *Homeosoma ellectellum* (Hulst). El objetivo de este estudio fué determinar si la semilla, falsos petalos o flores de un híbrido de girasol, contienen kuranoides u otros compuestos que confieran resistencia a la larva de la polilla de bandas de girasol *Cochylis hospes* Walsingham. La mortalidad y tiempo de desarrollo de la larva criada con dietas preparadas con semilla sin extraer (dieta de crianza) flores o material de hojas fueron comparadas con la mortalidad y desarrollo de larvas alimentadas con dietas complementarias que contenian semilla, flores, o falsos pétalos o material de hojas extraidos con acetona como ingrediente testigo. Descubrimientos importantes en este estudio fueron los siguientes: 1) La mortalidad larval decreció significativamente cuando las larvas fueron alimentadas con dieta preparada con semilla extraida con acetona en comparación con dietas con semilla no extraida. 2) La sustitución de las flores en la dieta no fué larvicida, aunque la sustitución de flores en la dieta resultó en un incremento significativo del tiempo de desarrollo de larvas. 3) El tiempo de desarrollo con la dieta con falsos pétalos se incrementó significativamente. 4) La mortalidad sobre la dieta con hojas que tan alta como con la dieta de crianza y 5) Los efectos de flores, falsos pétalos u hojas sobre la mortalidad o tiempo de desarrollo de *C. hospes* no estuvieron relacionados con componentes solubles de acetona de girasol porque los resultados en cada caso fueron similares cuando tanto flores, falsos pétalos o material de hojas, extraidos o intactos fueron sustituidos en la dieta. La diferencia en respuesta obtenida aquí por *C. hospes* a las obtenidas por la polilla de girasol *H. ellectellum* pueden reflejar diferencias de especies en adaptación a la planta huesped, o pueden haber diferencias significativas en la composición química de diferentes híbridos de girasol. La selección de girasol para componentes que confieren resistencia en la forma de incremento de mortalidad y tiempo de desarrollo de *C. hospes* podría ser de utilidad para un control mayor de esta plaga.

RÉSISTANCE DE LA GRAINE, DES FLEURONS, DES FLEURS LIGULÉES ET DES FEUILLES DE TOURNESOL À LA MOUCHE RAYÉE DU TOURNESOL *(Lepidoptera cochylidae)*

RÉSUMÉ

On a montré que les fleurons d'hybrides de tournesol contiennent deux composés "kauranoides" solubles dans l'acétone qui confèrent, sous forme d'effets larvicides, la résistance à la mouche du tournesol *Homeosoma electellum* (Hulst). L'objet de cette étude est de déterminer si la graine, les fleurons, les fleurs ligulées ou les feuilles d'un hybride de tournesol contiennent des composés "kauranoides" solubles dans l'acétone ou d'autres composés qui confèrent la résistance aux larves de la mouche rayée du tournesol *Cochylis hospes* Walsingham. La mortalité et le temps de développement des larves élevées sur des rations préparées à partir de graines (alimentation de base), de fleurons, de fleurs ligulées, ou de feuilles non extraites a été comparée à la mortalité et au développement de larves nourries avec un régime complémentaire à base de graines, de fleurons de fleurs ligulées ou de matériel foliaire extraits par l'acétone. Les résultats marquants de cette étude sont les suivants: 1) la mortalité larvaire diminue significativement lorsque les larves sont nourries à base d'un régime préparé à partir de graines extraites par l'acétone comparativement à un régime à base de graines non extraites, 2) la substitution des fleurons dans la ration n'a pas d'effet larvicide, bien que celle ci conduise à un accroissement significatif du temps de croissance larvaire, 3) le temps de développement sur le régime à base de fleurs ligulées est significativement augmenté, 4) la mortalité dans le régime à base de feuilles est aussi élevée que dans le régime de base (sans extraction) et 5) les effets des fleurons, des fleurs ligulées ou des feuilles sur la mortalité ou la durée de développement de C. *hospes* ne sont pas liés à la présence des composés solubles dans l'acétone chez le tournesol, car les résultats restent identiques lorsqu'il y a substitution de fleurons, de fleurs ligulées ou de feuilles extraites par l'acétone, par le même matériel intact.

Les différences de réponse trouvées ici pour C. *hospes* et celles rapportées pour la mouche du tournesol *H. electellum* refléteraient des différences, entre espèces, d'adaptation à la plante hôte ou bien résulteraient de différences significatives dans la composition chimique des différents hybrides de tournesol. La sélection du tournesol pour des constituants qui confèrent la résistance sous forme d'une augmentation de mortalité et de la durée de développement de C. *hospes* pourrait être utile pour un meilleur contrôle de ce ravageur.