BIOLOGICAL CONTROL METHODS AND BREEDING FOR RESISTANCE AGAINST THE SUNFLOWER MOTH *(HOMOEOSOMA NEBULELLUM HB.)*

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The sunflower moth (*Homoeosoma nebulellum Hb., Lepidoptera Phycytidae)* is an important pest in Europe that feeds on the seeds (achenes) and causes severe losses. The knowledge of its biology, ethology and natural enemies is of great importance not only for Europe as the information may be useful against the related Homoeosoma electellum Hulst. that causes similar problems in the USA. Against both species the ectoparasitic braconid *Habrobracon hebetor Say.* (Syn.: *H. vernalis Szépligeti.)* (Hymenoptera: Braconidae) may be of importance whereas for the breeding for resistance the phytomelanin (carbon) layer in the husk of achenes may be important. This secondary metabolite constitutes a disorganised layer of varying thickness between the hypoderm and lamellar layers during ripening. This layer hinders the chewing of Homoeosoma larvae and prevents their entering into the achenes.

According to prolonged domestic researches the different genotypes of common sunflower hybrids were classified into 5 different sensitivity groups by the location and form of phytomelanin layer related to the resistance against the sunflower moth *Homoeosoma nebulellum HB:*

1. The phytomelanin forms a more or less compact layer above the sclerenchyma and can be found as greater and smaller spots or stripes in the upper cells of the epicarpium and hypoderm.
2. The phytomelanin forms „multi layers" in the lower one (two) cell layer of hypoderm and the upper cells of sclerenchyma which becomes thinner above the ray parenchyma cells.
3. The phytomelanin forms a single layer above the sclerenchyma, and is missing above the ray parenchyma cells.
4. The phytomelanin can be found in a single layer at the border of sclerenchyma and it can be observed as closely packed small spots in the cross-section pictures.
5. The phytomelanin forms a single layer which can be observed as big spots but is missing at several places not only above the ray parenchyma cells.

Introduction

The sunflower has hundreds of different pests. However significant agricultural damage is caused by only a few species (Schulz 1978.). Moreover insects which propagate diseases may also have a great importance (Zimmer and Hoes, 1978.). Amongst other insects the *Homoeosoma* genus (Lepidoptera, Phycitidae) causes the most difficulties on the four sunflower growing continents. The Homoeosoma nebulellum HB. damages in Europe and Asia, the *Homoeosoma heinricni Pastr.* in South America; and the *Homoeosoma electellum Hulst.* in Mexico, USA and Canada.

The resistance of European sunflowers against *Homoeosoma nebulellum HB* was worked out 40-45 years ago in the Soviet Union by the help of inter-species hybridisation (sunflower cultivar x *Helianthus tuberosus subsp. purpurellus* Cockerell, Verbalov-Skoric 1988). The resistance is due to the carbon compound rich phytomelanin (=carbon) layer. The black colour of achenes is in positive correlation with the phytomelanin content. The intactness of achenes of "shelled" (high phytomelanin content) sunflower cultivars against the damage of sunflower moth demonstratively shows the resistance of plants against pests (according to Scsegolev 1951.) He calls those sunflower cultivars "shelled" in the achenes of which a peculiar black coloured, mostly carbon containing (phytomelanin) layer is formed between the sclerenchyma and phellem. The phytomelanin layer being formed in the fruit wall of achenes protect the seeds from damage caused by insects. According to Saharov (1935) the 3rd-instar larvae feeds on the pollen, inner parts of flower and the corolla for 1-2 days (the 1st-instar and 2nd-instar larvae solely) then after 23 days gnawing trough the fruit wall of achenes they strive inside the seeds. According to Scsegolev (1951) the phytomelanin layer begins to develop 3-4 days after flowering. The flowering of sunflower plants lasts generally for 8 days. So in these sunflowers after 11-12 days a fully developed phytomelanin layer constitutes. So by the time the caterpillars begin to feed on the achenes, a well developed phytomelanin layer blocks their way. Caterpillars eat only the epidermis and the suberin layer but cannot penetrate the phytomelanin layer thereby they can't damage the seed either.
Later, on the shelled sunflower species (hybrids) caterpillars feed on the receptaculum (discus) and on the involucral bracts (squama) which has less influence on seed-crop.

The North-American Homoeosoma electellum is much more virulent than its European cognate species the *Homoeosoma nebulellum HB.* thus the efforts in developing such resistance has great importance (Horváth 1993.)

According to Rogers (1980) for resistance-improving the following species can be used as possible resistance sources: ***Helianthus arizonensis, H. ciliaris, H. pumilu·, H. resinosus, H. rigidus x H. laetiforus, H. silphioides and H. smithii.*** According to Horváth (1991) and Horváth & Bujáki (1992a, 1992b) ***Homoeosoma nebulellum HB*** is the only pest against this kind of resistance-improving (phytomelanin carbon layer in the achenes) can be used successfully. Its an interesting circumstance that in those species and hybrids (striped for human or bird food) or in their restorer lines in which it wasn't successful to present resistance genes, the ***Orobanche cumana Wallr.*** sensitivity appears as a "related feature" (Horváth 1989, 1991, 1993).

Materials and methods

For plough-land experiments we used 13 sunflower hybrids in four repeated experiments, on 35 square meter pro experiments (beside the code numbers we report the moth sensitivity numbers of 1992 plough-land experiments in parallel, from 1 to 5 in increasing sensitivity order)

Serial number (code)	name of sunflower	plough-land moth sensitivity number*	classification of phytomelanin layer**	Orobanche sensitivity number***
1	IH-NK-81	5	3	5
2	IH-NK-173	3	4	3
3	NS-H-45 RM	2	2	2
4	NS-H-26 RM	2	4	2
5	Topflor	4	3	4
6	OD-121	1	1	1
7	OD-122	1	1	1
8	OD-106	1	1	1
9	Bonosus	5	5	5
10	S-277	4	5	4
11	Viki	2	2	2
12	Blumix	3	2	3
13	U-55 E	3	2	3

* moth sensitivity number
** classification by the form and location of phytomelanin layer
*** Orobanche sensitivity number

number	meaning
1	resistance
2-3	tolerance
4-5	sensitivity

moth sensitivity number	moths on one sunflower head	Orobanche sensitivity number	parasites on one sunflower plant
1	none	1	none
2	1 - 2	2	1 - 2
3	2 - 3	3	5 - 6
4	3 - 5	4	6 - 10
5	6 or more	5	11 or more

The plough-land experiments were carried out by "time shifted" sowing, June (10/06.). In this case (according to Horváth and Bujáki, 1992) the 3ard-instar larvae damage seeds in an extremely high degree. Thus the differences between the different hybrids and candidate hybrids are well discernible (Horváth and Bujáki 1992.).
Parallel with plough-land experiments each hybrids were subject to laboratory phytomelanin layer analysis. We identified the variation of the form and location of phytomelanin layer in the different hybrids with the moth sensitivity number as we report it in chapter "Results". By this comparison the positive correlation between moth sensitivity number (1-5) and the type of form and location of phytomelanin layer (1-5 group) is well discernible, and results can be directly used in practice.

Results

The phytomelanin layer is a poliacetilene complex (Szabó 1989.). This seconder metabolism forms a disorganised black or dark brown coloured layer of different thickness and strength at the border of hypoderm and sclerenchyma during ripening. We presume that there are big differences between the species and hybrids in the ability of seconder metabolism. That's why not the accurate quantification of phytomelanin but rather its location, developing type, appearance in time and frequency inside the fruit wall that reflect best of all the genetically determined ability of seconder metabolism of the species (hybrids). The frequency of phytomelanin in the fruit wall can be evaluated and examined horizontally and vertically. Our present experiment gives a vertical view of the location and developing type of phytomelanin layer by the samples from fruit wall of ripe sunflower achenes (Pataky 1992). On basis of 13 samples the achenes were classified into 5 groups (according to location and appearance of phytomelanin layer). This classification gives a close positive correlation with the ploughland moth sensitivity numbers (numbers in brackets) by the following:

1. The most compact above all is formed in the 6th, 7th and 8th (moth sensitivity number 1) samples. In the 6th sample an upper and a lower layer, having developed at the border of sclerenchyma can be observed. The epidermis (exocarpium) is pigmented. In the 7th sample there is a layer at the border of sclerenchyma which can be found also above the ray parenchyma and in the radial and tangential cell walls too. The epidermis is pigmented (fig 1.) In the 8th sample the layer is continuous above the sclerenchyma but not continuous below the epidermis. Both epidermis and the hypodermis are pigmented.

2. In the 11th (moth sens. no. 2) 12th (moth sens. no. 3) 13 th (moth sens. no. 3) and 3rd (moth sens. num. 2) samples the phytomelanin layer forms a single layer which is located above the sclerenchyma and is relatively compact. In the 3rd sample the phytomelanin layer is double but becomes thinner above the wide ray parenchyma. The 12th and 13th samples contain more or less phytomelanin above the narrow ray parenchyma fields too. The epidermis and hypodermis are pigmented. In the 13th sample the phytomelanin layer forms a continuous thin layer, the sclerenchyma is dissected by several narrow ray parenchyma fields and is very thin.

3. In the 5th (moth sens. no. 4) and lst (moth sens. no. 5) samples the phytomelanin layer is single and becomes thinner above the ray parenchyma cells. The 5th sample has a more slack structure than sample lst has, the lumen of sclerenchymatic cells are bigger but their cell walls are thicker.

4. In the 2nd (moth sens. no. 3) and 4th (moth sens. no. 2) samples the phytomelanin forms a single layer at the border of sclerenchyma and is constituted by small areas. In the 2nd sample the layer is double in some places but grows thinner above the ray parenchyma cells.

5. In the 10th (moth sens. no. 4) and 9th (moth sens. no. 5) samples the phytomelanin layer can be found only above the sclerenchyma and forms a thin layer. It appears in big spots in the cross-section pictures but is missing in several places not only above the ray parenchyma. The epidermis is pigmented in both sample and the hypoderm is pigmented in the 9th sample.

Except for the first group (in which the phytomelanin layer is the most compact and the moth resistance is the most distinct) the single groups cannot be sharply distinguished by the location and development of phytomelanin layer but they tally well with the empiric observations of plough-land moth sensitivity.

Discussion

According to the 1991 and 1992 plough-land experiments it is proved that, sunflower moth (*Homoeosoma nebulellum HB.)* generation which was called partial 3rd-instar generation in the literatures previously extremely increases at the time shifted (the first 10 days of June) sowing (absence of parasites!) and is active till the end of October (Horváth and Bujáki 1992). Because amongst the 3 generation this is the "most stronger" one (sometimes on one sunflower head 20-30 caterpillars damages the achenes). This circumstance queries the present behaviour of moth resistant sunflower cultivars. We have to draw attention again to the optimal sowing time of sunflower hybrids (mid-April - beginning of May), which avoids lst and 2nd moth generation and practically keep away from the 3rd generation (at the worst, in case of ramifying local cultivars and restorer lines of long growing time). The phytomelanin layer analysis gives reliable information for modernising the resistance-improving and for the selection of lines with advantageous features.

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