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Research article

Relationships between selectivity of *Aphis gossypii* Glover and *Aphis craccivora* Koch to sucrose and maltose and their resistance to pirimicarb

M. M. A. Rizk, Y. A. Darwish*, S. A. Eraky, Amal H. A. Atta

Plant Protection Department, Faculty of Agriculture, Assiut University, 71526 Assiut, Egypt

Abstract

Pirimicarb LC_{50} base line was determined by the dipping technique in successive concentrations of the trade form of pirimicarb in water. The LC_{25} was used for the selection every month through a year. In case of 10 sucrose solutions versus water, 8 counts indicated that aphids did not prefer the sucrose solution. After 6 hours the cotton aphid could not discriminate between the sugar solution and water. The discrimination between sugars was enhanced by adding 10% maltose. It was observed that the dark forms of the cotton aphid, *A. gossypii* and light forms of the cowpea aphid, *A. craccivora* attracted to the higher sucrose solution than the other forms. The cotton aphid, *A. gossypii* has a light color as the basic color, transforming under reversible condition to dark green, whereas, the basic color of cowpea aphid, *A. craccivora* usually black in color, transforming under stress to light brownish. This reversed evidence explained the differences in response towards sugar of the two aphid species.It may be concluded that the dark morphs of *A. gossypii*, and light morphs of *A. craccivora* which are considered pirimicarb resistant preferred high sugar levels. In other meaning, the present study proved that dark morphs of the cotton aphid and light forms of the cowpea aphid were resistant to pirimicarb and this resistance was positively correlated with the levels of sugars in the plants. Therefore, plant breeders are requested to pay efforts in screening process of resistant plants to produce plants with low sugar contents to increase the effectiveness of pirimicarb against both species of aphids.

Key words: Cotton aphid, Cowpea aphid, Sugars, Color forms, Choice-chamber, Insecticide resistance

* **Corresponding author:** yousefdarwish11@yahoo.com

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Introduction

Problems associated with aphid in Egyptian cotton include yield reductions due to the large early season infestations, effects of honeydew on fiber quality at late season infestations and transmitting of plant virus diseases to cotton and other economic plants. Resistance to insecticides was found in at least 20 species of aphids including *A. gossypii* (Georghiou 1981). Resistance of *A. gossypii* to carbamates was first reported by Kung et al. (1964) who attributed this phenomenon to some morphological and physiological characteristics. The cotton aphid can produce a small, yellow morph varies from its normal, larger, multi-colored morph (Paddock 1919). Kerns and Gaylor (1992) reported that sub-lethal doses of insecticides affected color morph frequency of cotton aphid with certain insecticides causing an increase in dark morphs. In general, the light morphs of the cotton aphid act as a festival form occurs under unfavorable conditions, such as high temperatures or an unfavorable host plant. The cotton aphids are often able to reproduce more rapidly when treated with insecticides applied against other pests. With the extensive use of pirimicarb for aphid's control, the reproductive capacity increased up to 30% as compared with that of the check (Rongai et al. 1998). The dark morphs of the cotton aphid are able to increase their reproductive capabilities (Kring 1959). The sudden shift from the green morph to the red one is common as reported in insecticide resistance data, (Masukwedza et al. 2013). The cotton aphid also displays another type of polymorphism that is important in the life cycle. El-Ghareeb and Nasser (1993), studied the insecticidal susceptibility and esterase activity of three color forms of *Hyalopterus pruni* Geoffrey (Homoptera: Aphididae) and they recorded susceptibility of the three color forms against pirimicarb insecticide and found that the red color was the least susceptible aphid and the pale green aphid was the most susceptible one while the dark green aphid was moderately susceptible. The dietary requirements of different aphid species were extensively studied by several investigators (Auclair (1966) and (1967), Mittler (1967) and (El-Sayed et al. 1977). Varying levels of sucrose and maltose in the aphid's diet influence population growth. Auclair (1966) and (1967) reported 20 grams sucrose and 10 grams maltose as an ideal daily diet. He concluded that any long term variation from this diet results in a shortening of the aphids life span. Leser et al. (1992) and Leser (1994) attempted to compile the results of these tests and others into a comprehensive management guide for producers.

The present study is an attempt to find out an answer to an important question: are the dark morphs of the cotton aphids (insecticide resistant) prefer certain sugar/s differed from the light morphs? The answer of this question is discussed in the present investigation.

Materials and methods

Maintenance of aphids: The stock populations of the cotton aphid, *A. gossypii* and the cowpea aphid, *A. craccivora* were collected from cucurbitaceous and legume plants grown in the farm of the Faculty of Agriculture, Assiut University, Egypt, brought into the laboratory at the Plant Protection Department.

Insecticide selection: Pirimicarb LC_{50} base line was determined by the dipping technique in successive concentrations of the trade form of pirimicarb dissolved in water. The LC_{25} was used for the selection every month through a year. The insecticide selection was stopped wherever the aphid population seemed to be in stress. The light and dark forms of each aphid species were separated and used as a base line of Pirimicarb resistance. These procedures resulted in four groups of aphids, light and dark forms selected by Pirimicarb Plates (1, 2).

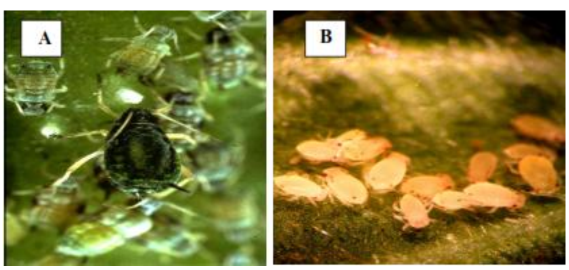

Plate 1: The cotton aphid, *Aphis gossypii* Glover: dark morph (A) and light morph (B).

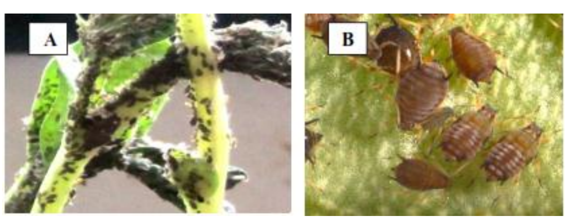

Plate 2: The cowpea aphid, *Aphis craccivora* (Koch): dark morph (A) and light morph (B).

Preparation of the feeding cages and nutrient solutions: The used containers, cages and choice chambers were thoroughly washed, soaked in 10 % aqueous formalin for 2-3 hours, washed aging, dried, then frequently subjected to ultra-violet radiation for 2 hours before reuse. Avoiding the breaking down of the complex diets, Zeis filters were used for sterilization. Sterilized containers, large one (100 cc) for the long run and small one (1.8 cc) (carpule vials, Plate 2) for daily use. Complex diets were stored in deep freeze at -20ºC.

Bayer Carpule^R syringe (Plate 3) was used to transfer the diet from the small/stock carpule vial (which is special for the syringe) to the feeding choice chamber cage without opening this vial. With the help of these syringes, the diets could be used and re-stored without contaminations since they have not been exposed to air. The present procedure was modified after El-Sayed et al. (1977).

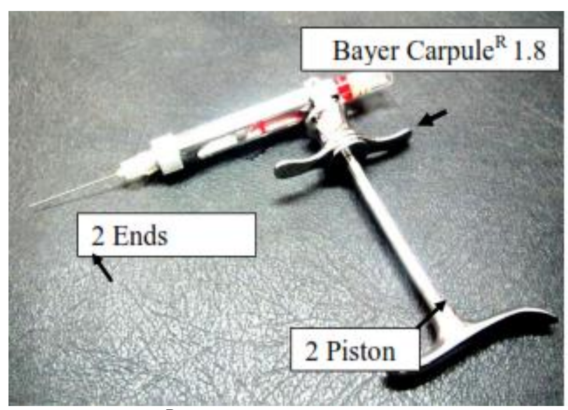

Plate 3: Carpule^R syringe used to transfer the diet solution from the small/stock carpule vial to the feeding choice chamber.

Discrimination between two different solutions: In these experiments, aphids of the light and dark forms of 2-3 days old nymphs were allowed to discriminate between the two different feeding fluids in a choice chamber (plate 4). Sucrose solutions of 10, 15, 20, 25, 30, 35 and 40% and concentrations of 15, 20, 25, 30 % sucrose plus 10% maltose were prepared in water versus water and preserved for use when needed.

In all experiments, the number of aphids under each liquid was recorded. The counts were made after ½, 1, 2, 3, 4, 5, 6, 12, and 24 hours. Percentage of aphids settled under each fluid versus water was calculated. Also, the ratio between aphid settled under certain fluids and that settled under water in each unit was also calculated. χ^2 was used to test the hypothesis assumption that aphids were equally distributed under the two liquids in the choice chamber. The experimental units were kept under room conditions with yellow artificial light.

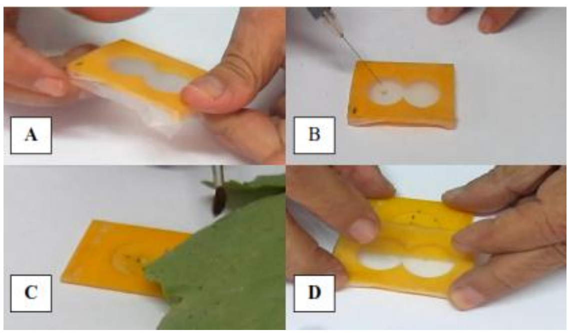

Plate 4: Preparation of choice chamber; stretching a Para-film membrane (A), deposing liquids by mean of Carpule^{R} syringe (B), transferring individuals into the bottom part of choice chamber (C) and adjust the two parts of choice chamber (D).

Results

The cotton Aphids

Light form: Table (1) showed the results of the discrimination between different solutions of sucrose versus water in choice chambers. In case of 10 sucrose solutions versus water, 8 counts indicated that aphids did not prefer the sucrose solution, while in case of 15-30% sucrose solution 58-67% of the aphids settled under the sucrose solutions. After 6 hours aphids did not discriminate between the sugar solutions and water. The average count after 24 hours indicates that the light colored form was not able to discriminate between any sucrose solution and water, except at 20% sucrose concentration where 58.0% of aphids were settled.

Table (2) indicated that 20 % sucrose plus 10% maltose being an ideal combination under which 78% of aphids were settled. While 30% sucrose solution alone insignificantly attracted 54.75% (Table 1), meanwhile, 30% sucrose plus 10% maltose significantly attracted 58% of light aphids (Tale 2). It was found that light colored aphids could not discriminate between water and 35% sucrose solution with or without maltose.

Dark form: Table (3) showed that 58.75% and 60.38 % of the dark colored morphs of the cotton aphid were settled under 25 and 30% sucrose solutions. The statistical analysis proved that the dark morph was not able to discriminate between 10 – 20 % sucrose solutions versus water. The same results were observed in case of sucrose solutions with more than 30%. Table (4) showed that the sucrose concentrations of 20, 25, and 30% plus 10% maltose versus water enhanced attractions of dark morphs. The average count for more than 30% plus 10 % maltose solution showed insignificant discrimination by dark colored form.

Table 1: Percentages of 2-3 days old light form nymphs of the cotton aphid, *A. gossypii* settled under different concentrations of sucrose solutions in choice-chambers versus water at successive observation times.

Sucrose (%)	Access time (hours) ½	1	2	3	4	6	12	24	Average
10	43	44	46	46	49	50	50	42	46.25
15	44	46	45	54	55	(59)	52	45	50
20	52	56	(58)	(62)	(64)	(64)	55	53	(58.00)
25	48	52	55	(62)	(65)	(67)	50	50	56.13
30	57	55	48	52	(64)	(67)	50	45	54.75
35	40	42	44	48	52	54	40	35	44.38
40	38	39	42	43	40	42	40	30	39.25

Percentages in brackets denote that the χ^2 value calculated for a pair of values (% under sucrose & % under water) exceeds the tabulated value of 3.84 (significant).

Table 2: Percentages of 2-3 days old light form nymphs of the cotton, aphid *A. gossypii* settled under different concentrations of sucrose solutions plus 10% maltose in choice-chambers versus water at successive observation times.

Sucrose % + 10 % Maltose	Access time (hours) ½	1	2	3	4	6	12	24	Average
15	53	(60)	(60)	(62)	(66)	(67)	(68)	(62)	(62.25)
20	56	(63)	(68)	(70)	(77)	(78)	(80)	(70)	(78)
30	55	(58)	(58)	(58)	(60)	(62)	57	55	(58)
35	44	50	48	50	52	53	52	43	49.00

Percentages in brackets denote that the χ^2 value calculated for a pair of values (% under sucrose & % under water) exceeds the tabulated value of 3.84 (significant).

Table 3: Percentages of 2-3 days old dark form nymphs of the cotton aphid, A. gossypii settled under different concentrations of sucrose solutions in choice-chambers versus water at successive observation times.

Sucrose (%)	Access time (hours) ½	1	2	3	4	6	12	24	Average
10	42	43	45	46	54	50	50	42	46.5
15	43	44	50	54	55	56	52	45	49.88
20	50	56	(58)	(62)	(62)	(62)	53	52	57.13
25	50	(61)	(60)	(64)	(67)	(68)	50	50	(58.75)
30	55	(62)	(64)	(65)	(70)	(72)	50	45	(60.38)
35	40	(60)	(64)	(65)	52	54	40	35	51.25
40	38	42	47	50	40	42	40	30	41.13

Percentages in brackets denote that the χ^2 value calculated for a pair of values (% under sucrose & % under water) exceeds the tabulated value of 3.84 (significant).

Table 4: Percentages of 2-3 days old dark form nymphs of the cotton aphid, *A. gossypii* settled under different concentrations of sucrose solutions plus 10% maltose in choice-chambers versus water at successive observation times.

Sucrose %+ 10% maltose	Access time (hours) ½	1	2	3	4	6	12	24	Average
20	54	58	(62)	(63)	(62)	(64)	66	52	(60.125)
25	56	(62)	(65)	(66)	67	(68)	(68)	50	(62.75)
30	57	(65)	(68)	(68)	(75)	(80)	(77)	(58)	(68.5)
35	50	55	(64)	(66)	52	54	50	40	53.875

Percentages in bracket denote that the χ^2 value calculated for a pair of values (% under sugar & % under water) exceeds the tabulated value of 3.84 (significant).

The cowpea aphids

Black form: The results of the black form of cowpea aphid showed the same trend in the light form of the cotton aphid. Table (5) showed the results of the discrimination between different solutions of sucrose versus water in choice chambers. From this table it is shown that 10% sucrose solutions versus water, in all successive times (8 counts) indicated that aphids did not prefer the sucrose solution. After 6 hours, aphids were not able to discriminate between the sugar solution and water. The average count after 24 hours indicates that the black colored form was not able to differentiate between any of sucrose solutions and water, except 20 % sucrose solution was able to attract 58.6% of aphids. This result seemed to be statistically significant. Table (6) showed that the discrimination between sucrose solutions were enhanced by adding 10% maltose solution. The average count indicated that 15, 20, and 30% sucrose plus 10% maltose solutions significantly attracted 58.25, 71/00 and 58.75 % of the light colored form, respectively. It was found that 20 % sucrose and 10% maltose solutions being an ideal combination under which 71% of aphids were settled, whereas 20% sucrose solution alone attracted 58.63 % of light colored aphids (Table 5). More than 30 % of sucrose plus 10 % of maltose solutions insignificantly attracted 53.36 % of light colored form (Table 6).

Light form (brownish): Table (7) showed that 71.75% of light morph cowpea aphids preferred 30% sucrose solution, whereas they could not distinguish between sucrose in low concentration (10%) and high concentration (40%) versus water. When maltose was added to sucrose versus water, it synergized the selectivity of sugar solution versus water. Table (8) indicated that the most favorable solution is consisted of 20% sucrose and 10% maltose.

Discussion

The cotton aphid, *A. gossypii* has a light color as a basic color, transforming under reversible conditions to dark green, whereas, the basic color of the cowpea aphid, *A. craccivora* usually black in color, transforming under stress to light brownish. The present results explain the differences in response towards sugar of tested aphid species. It may be concluded that the dark morphs of *A. gossypii*, and light morphs (brownish) of *A. craccivora* which considered pirimicarb resistant preferred high sugar levels. The present study showed that both aphid species were able to discriminate between the pair of testing solutions after a very short period (30 minutes), indicating that *A. gossypii* and *A. craccivora* don't have a threshold of response as suggested by Mittler (1967) for *M. persicae*, whose response to a sucrose solution containing 6 amino acids mixture was strongly affected by period of access to a pure sucrose solution before the tests. Our results also showed that the discrimination between sugar solution versus water was enhanced by adding 10% maltose solution. The behavior of the dark colored form of the cotton aphid and light form of the cowpea aphid towards the combination of sucrose solutions plus 10% maltose was enhanced.

Table 5: Percentages of 2-3 days old black form nymphs of the cowpea aphid *A. craccivora* settled under different concentrations of sucrose solutions in choice-chambers versus water at successive observation times.

Sucrose (%)	Access time (hours) ½	1	2	3	4	6	12	24	Average
10	46	48	48	49	49	50	50	42	47.75
15	48	48	48	54	55	(60)	52	46	51.38
20	56	(58)	(58)	(64)	(66)	(64)	52	51	(58.63)
25	48	52	56	(63)	(65)	(68)	44	46	55.25
30	54	54	50	52	(64)	(68)	50	44	54.50
35	40	42	48	48	52	54	40	36	45.00
40	36	38	42	44	40	40	40	32	39.00

Percentages in brackets denote that the χ² value calculated for a pair of values (% under sucrose & % under water) exceeds the tabulated value of 3.84 (significant).

Table 6: Percentages of 2-3 days old dark form nymphs of the cowpea aphid *A. craccivora* settled under different concentrations of sucrose+10% maltose solutions in choice-chambers versus water at successive observation times.

Sucrose % + 10 % Maltose	Access time (hours) ½	1	2	3	4	6	12	24	Average
15	46	(58)	(64)	(58)	(68)	(64)	(60)	48	(58.25)
20	(58)	(63)	(68)	(72)	(77)	(78)	(82)	(70)	(71.00)
30	(59)	(58)	(58)	(58)	(60)	(62)	(60)	55	(58.75)
35	(62)	50	48	50	(58)	(60)	(58)	43	53.63

Percentages in brackets denote that the χ² value calculated for a pair of values (% under sugar & % under water) exceeds the tabulated value of 3.84 (significant).

Table 7: Percentages of 2-3 days old light form nymphs of the cowpea aphid, *A. craccivora* settled under different concentrations of sucrose solutions in choice-chambers versus water at successive observation times.

Sucrose (%)	Access time (hours) ½	1	2	3	4	6	12	24	Average
10	46	47	57	(58)	(62)	(64)	(60)	(59)	56.88
15	52	55	(59)	(59)	(65)	(67)	57	56	(58.25)
20	50	(75)	(75)	(80)	(72)	(60)	(65)	(62)	(67.38)
25	50	50	(70)	(75)	(70)	(80)	(75)	(70)	(68.75)
30	(62)	(63)	(72)	(73)	(76)	(77)	(76)	(75)	(71.75)
35	(58)	(62)	(65)	(60)	(70)	(75)	(76)	(65)	(66.38)
40	(41)	44	48	55	(58)	(60)	(58)	55	52.50

Percentages in brackets denotes that the χ² value calculated for a pair of values (% under sucrose & % under water) exceeds the tabulated value of 3.84 (significant).

Table 8: Percentages of 2-3 days old dark form nymphs of the cowpea aphid, *A. craccivora* settled under different concentrations of sucrose+10% maltose solutions in choice-chambers versus water at successive observation times.

Sucrose % + 10 % Maltose	Access time (hours) ½	1	2	3	4	6	12	24	Average
15	50	50	(63)	(61)	(64)	(67)	(65)	(62)	(60.25)
20	(64)	(66)	(74)	(74)	(78)	(80)	(78)	(78)	(74.00)
25	(62)	(63)	(72)	(73)	(76)	(77)	(76	(75)	(71.75)
30	(58)	(59)	(71)	(70)	(72)	(74)	(72	(70)	(68.25)

Percentages in brackets denotes that the χ² value calculated for a pair of values (% under sugar & % under water) exceeds the tabulated value of 3.84 (significant).

In a study of Blayne Reed (2000), the results of leaf sugar analysis from the cotton genotypes in the screening test showed major genotype differences in amounts of sucrose and maltose existed in the leaves. He reported that the results of leaf sugar analysis from the cotton genotype, *Gossypium arboretum* showed much higher levels of sucrose and maltose. Visual observations of the aphids surviving on *G. arboretum* confirms that they were all of the light colored morphs or intermediate colored morphs, but never were dark colored moths. In addition, these aphids appeared smaller and less healthy than the light colored morphs on other cotton genotypes. The author suggested that the lack of dark aphid morphs on *G. arboretum* might be due presence of a mild form of antibiosis that did not cause immediate death of the insects. This evidence proves that the sucrose and maltose contents of cotton leaves play an important role in plant resistance to cotton aphids. Slosser et al. (2004) observed negative linear relationship between change in aphid numbers and sugar ratio; population growth was limited by high levels of glucose and fructose in cotton leaves. They indicated also that the percentage of dark-colored aphids was negatively correlated with temperature and day length and positively correlated with leaf moisture and nitrogen and the sucrose/ glucose ratio. These findings in addition to those of Blayne Reed (2000) are in close agreement with the results of the present investigation. Therefore, plant breeders must take these evidences into consideration attempting to transfer the genes responsible for the low levels of sucrose and maltose from resistant cotton genotypes to the cotton varieties with high yield.

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