THE IMPORTANCE OF pH IN FOOD SELECTION BY THE TOBACCO WHITEFLY, *BEMISIA TABACI*

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The tobacco whitefly, *Bemisia tabaci* Gennadius, was found to differentiate between pH values at the level of 0.25. It had a clear preference for media with pH values of 6.0 to 7.25 offered *in vitro*, in both choice and no-choice situations, when "resting whiteflies" or survival was measured. The whiteflies showed a clear preference for a sucrose concentration of 15%. The addition of 10% sucrose to buffers at various pH values did not change the pattern of their pH preference. In *in vivo* experiments whiteflies preferred old cotton leaves (120 days) to younger leaves (60 days). The pH of old leaves was 6.8 while that of young leaves was 5.9. These results may explain the fact that whiteflies attacked cotton plants in commercial fields only late in the season, when the pH values of the cotton leaves exceeded pH 6.
KEY WORDS: pH, *Bemisia tabaci;* artificial feeding; cotton, host selection.

INTRODUCTION

The tobacco whitefly (*Bemisia tabaci* Gennadius) is a polyphagous insect, common to warm climates all over the world (11). It attacks many crops belonging to various botanical families (2, 11). Since 1976 it has become the most serious pest of cotton in Israel (7). The sticky honeydew it excretes causes clumping of cotton fibers and turns them black due to the sooty mold which grows on the honeydew. These two phenomena reduce the quality of the fibers and make them unmarketable (6, 9, 10).

During the first 3 months of cotton growth (April-June) only few whiteflies are found in the cotton fields, although fairly big populations occur at that time on other plants, such as sunflowers, potatoes, cucurbits, ornamentals and weeds. It seems that cotton plants younger than 3 months are not attacked by adult white-

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flies. Some of the possible reasons may be endogenous factors which change with plant age, such as sugar content or acidity (pH values) of the leaves.

Hussein *et al.* (9, 10) claimed that there is a correlation between the attack of whiteflies and the pH of the cotton leaves. They based their hypothesis on the results of multiannual observations in which various cultivars, seasons, and localities (types of soils) were compared. The importance of pH in the diet of insects was demonstrated by Fife and Frampton (5) for a leafhopper and by Auclair (1) for an aphid.

The pH of plant cell extracts may differ from crop to crop: Caldwell (3) found pH values between 5.0 and 6.5 in different plants, and that the pH of an individual crop may change with its age.

In the present study it was intended to confirm that the pH of the leaf cell extract really changes with the age of the cotton plants and to determine the response of whitefly adults to differing pH values. The preference of the whiteflies for plants or leaves of different ages, and thus different pH values, was examined in the laboratory.

MATERIALS AND METHODS

Whiteflies *(Bemisia tabaci)* were collected from cotton plants in a commercial field and reared in cages on potted cotton plants in a glasshouse at 20-33°C, 70-80% RH, and long-day (LD 14:10) conditions. All experiments were carried out with one-day-old females, collected from an emergence-cage into which leaves, infested by "pupae," had been placed a day before.

Leaves of 20 marked cotton plants of cv. 'Acala SJ 2' were sampled at five localities every 10 days for 3 months, from June 7 – when the plants were 60 days old, until September 5 – when they were 150 days old. Five samples of 5-10 young leaves (the first fully expanded leaf) and five samples of 5-10 mature leaves (10-13th nodes from the top of the plant) were collected. The leaves picked in the field were placed in an ice-cooled insulated container, brought to the laboratory within an hour, homogenized in a blender, and the pH of the crude extract was determined by means of a pH Meter (PHM 28 Radiometer, Copenhagen). The pH of each leaf extract sample was determined five times and averaged. Total soluble solids (TSS) was determined by means of a portable Carl Zeiss refractometer. At the same time, the whitefly infestation rate in the field was evaluated visually, as low (I), moderate (II) or heavy (III).

In vitro experiments

All experiments were performed in a "cage" made of a 10-cm-diam glass funnel. A parafilm membrane was stretched four times its original length and width, and fixed over the wide mouth of the funnel. Phosphate, acetic acid, and citric acid buffers, each of varying pH values, were prepared at 0.1 M according to Hale (8) and used as test diets in the pH-preference tests. Droplets of the test diets were put on the membrane and spread out in a thin layer over the surface by covering them with a second piece of parafilm. The membrane did not leak, as ^{14}C glucose added to the droplet could not be detected on the lower surface. The whiteflies, collected by an aspirator into a glass tube that was attached to the stem of the funnel, entered the funnel. They sucked the medium through the membrane (4), as was shown by adding a dye to the medium and observing its presence in the whiteflies' bodies. Twenty whiteflies were used for each of four to six replications. The number of "resting whiteflies," which was used as the criterion of acceptance or preference (13), was counted every 30 min during 6-8 hours. Only whiteflies which were resting motionless on the diet, for 2 min or more, were counted as resting whiteflies. In some experiments the rate of survival of the whiteflies was evaluated after 24, 48 and 72 h. In choice experiments two different media were offered on two halves of the membrane.

In vivo experiments

Cotton plants (cv. 'Acala SJ 2'), 60 or 120 days old, were used. They were grown in the glasshouse in 10-liter plastic buckets. Two leaves, one from each of two plants, were attached side by side, without covering each other, to the upper side of the funnel without detaching them from the plants. A glass plate with a sponge rim was put on top of the leaves to prevent the escape of the whiteflies. The whiteflies thus could choose between the two leaves. Resting whiteflies were recorded every half hour during 6 hours.

RESULTS

The changes in pH values of leaf extracts were measured at five locations during June-August, the most critical months for whitefly attack. In all five fields the pH values increased significantly with the age of the plants, from pH 5.6 to 6.8 (Fig. 1).

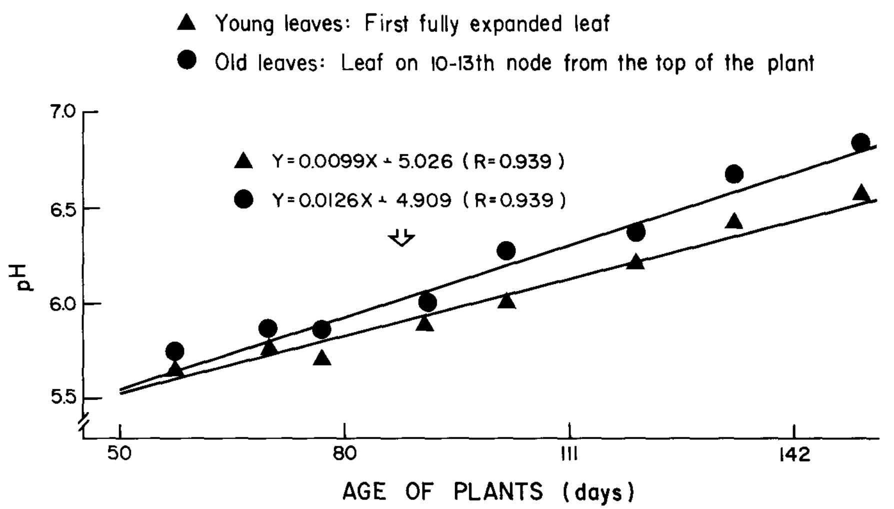

Fig. 1. The changes in pH of cotton leaf extract during the growth season. Each point is the mean of five determinations at each of five locations; 5-10 leaves were used for each determination. Sowing date: April 11, 1979.

The pH changed also within the same plant, with the age of the leaves. The pH of the older leaves was always higher, by 0.1-0.3 units, than that of the younger leaves of the same plant.

A moderate infestation by whiteflies (level II), indicated by an arrow in Fig. 1, was observed when the plants were 90 days old and the pH of the younger leaves exceeded 5.9 and that of the older leaves exceeded 6.0.

The preference of whiteflies for pH 6 and 7 in a paired choice situation is demonstrated in Fig. 2. This held true for a phosphate buffer, as well as for an acetic acid buffer.

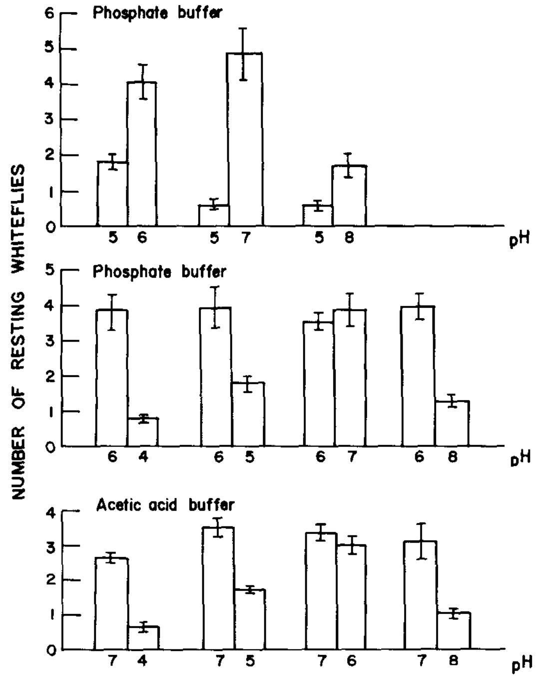

Fig. 2. pH preference of *Bemisia tabaci* adults in a paired choice situation. Twenty whiteflies were used for each of four replications. Resting whiteflies were counted every 30 min during 6 h. Bars indicate S.E.

The effect of pH under a no-choice situation on whitefly preference is shown in Table 1. A large increase in the number of resting whiteflies was observed in all three buffer systems when the pH increased from 5 to 6 or 7; a decrease was observed when the pH changed from 7 to 8. The number of resting whiteflies on each of the three buffers differed and was highest for the phosphate buffer.

A more detailed study of pH preference, with narrower pH intervals, is presented in Fig. 3. The number of resting whiteflies was the highest in the pH range between 6.0 and 7.25. Any pH lower than 5.75 or higher than 7.5 caused a significant reduction in the number of resting whiteflies.

TABLE 1

THE NUMBER OF RESTING *BEMISIA TABACI* ADULTS ON VARIOUS DIETS WITH THREE DIFFERENT BUFFERS (ALL AT 0.1 M), IN A NO-CHOICE SITUATION

pH	*Average number of resting whiteflies*		
	Phosphate buffer	Acetic acid buffer	Citric acid buffer
4	1.9b	1.5d	0.5d
5	2.9b	2.3c	2.8b
6	8.2a	5.7b	4.5a
7	8.4a	6.9a	4.0a
8	2.5b	2.2c	1.0c

The statistical analysis was done for each buffer separately. Figures followed by different letters differ significantly at $P = 0.001$, according to the Newman-Keuls multiple range test.
Twenty whiteflies were used; resting whiteflies were counted every 30 min during 6 h. Four replications were performed.

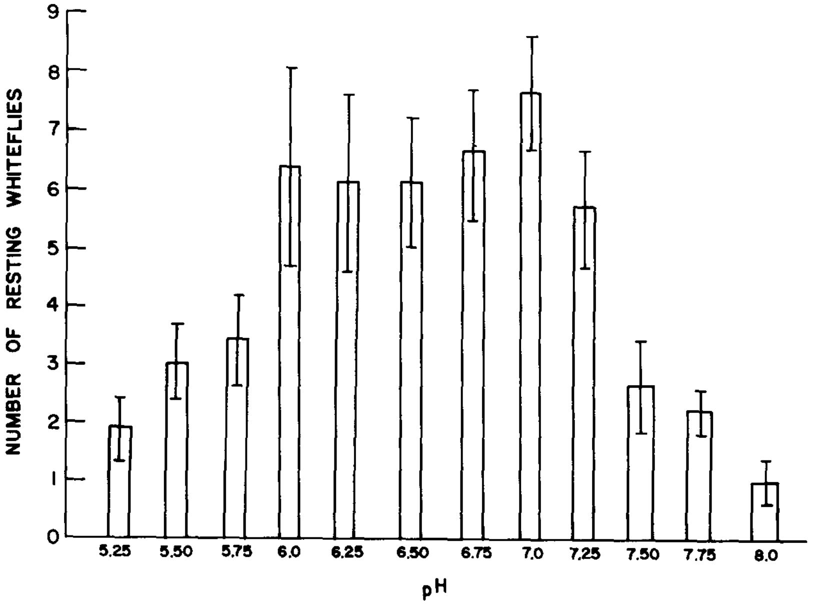

Fig. 3. pH acceptance by *Bemisia tabaci* adults in a no-choice situation. Twenty whiteflies were used for each of four replications. Resting whiteflies were counted every 30 min during 6 h. Bars indicate S.E.

TABLE 2

THE EFFECT OF SUCROSE CONCENTRATION ON THE NUMBER OF RESTING ADULTS OF *BEMISIA TABACI* IN A NO-CHOICE SITUATION

*Sucrose (%)**	*Average number of resting whiteflies*
0	2.5d
5	6.0b
10	6.2b
15	8.2a
20	4.4c
25	3.2d

* In 0.1 M phosphate buffer, pH 6.

Figures followed by different letters differ significantly at $P = 0.001$, according to the Newman-Keuls multiple range test.

Twenty whiteflies were used; resting whiteflies were counted every 30 min during 6 h. Four replications were performed.

TABLE 3

THE INFLUENCE OF 10% SUCROSE, ADDED TO 0.1 M PHOSPHATE BUFFER AT VARIOUS pH VALUES, ON THE NUMBER OF RESTING *BEMISIA TABACI* ADULTS IN A NO-CHOICE SITUATION

pH	*Average number of resting whiteflies*	
	without sucrose	with 10% sucrose
4	1.9b	3.0d
5	2.9b	4.2c
6	8.2a	7.8b
7	9.4a	9.9a
8	2.6b	4.8c

The statistical analysis was done for each column separately. Figures followed by different letters differ significantly at $P = 0.01$, according to the Newman-Keuls multiple range test.

Twenty adult whiteflies were used; resting whiteflies were counted every 30 min during 6 h. Four replications were performed.

The effect of the sugar concentration in the medium, on the preference of the whitefly adults, is shown in Tables 2 and 3. The most attractive sucrose concentration was 15%, but the 5% and 10% solutions were still significantly more attractive than water alone or 20% or 25% sucrose (Table 2). The effect on whitefly adults of adding 10% sucrose to the phosphate buffer at various pH values (4-8) was tested under a no-choice situation (Table 3). It was found that the overall pattern of the whitefly reaction to pH did not change when 10% sucrose was added to the solution, although the attractiveness of the unpreferred pH 4, 5 and 8 media increased somewhat.

The effect of pH in the diet on the survival of the whitefly adults is described in Fig. 4. Without food and water, the whiteflies died within 24 h. The survival of the whiteflies feeding on 10% sucrose was highest at pH 7. On pH 6 the survival was reduced but was still significantly higher than that on pH 4, 5 and 8. After 72 h some whiteflies survived only on media of pH 6 and 7.

In vivo test

The effect of plant age on the numbers of resting whiteflies in a no-choice situation is detailed in Table 4. With increasing age of the plants (60-140 days), pH values increased from 5.9 to 7.0. The number of resting whiteflies increased with plant age from 2.9 to 5.6. TSS fluctuated between 8.6 and 10.2%. In each of the two choice experiments (A and B, Table 5) the number of resting whiteflies (7.2 and 7.1, respectively) was significantly higher on the older leaves of the 120-day-old plants than on the young leaves

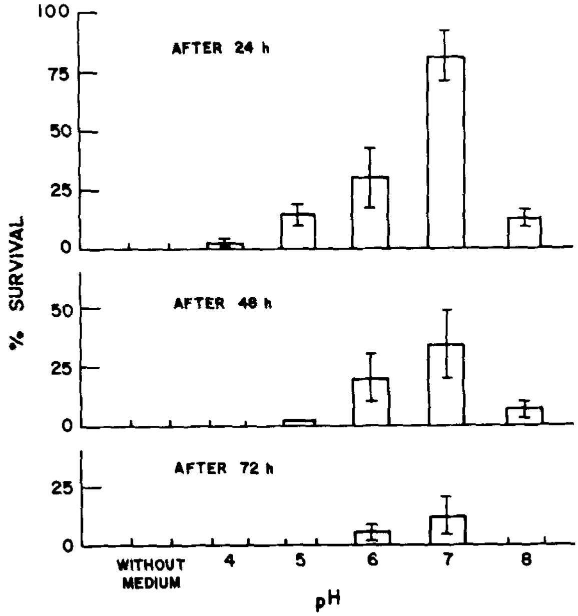

Fig. 4. Percent survival of *Bemisia tabaci* adults as a function of pH values of phosphate buffer with the addition of 10% sucrose. Twenty whiteflies were used for each of four replications. Bars indicate S.E.

of old plants (4.1); the lowest number (2.9) was found on the young leaves of 60-day-old plants. Again, the whitefly preference was in good accord with the pH values of the examined leaves.

TABLE 4

CHANGES WITH PLANT AGE IN TOTAL SOLUBLE SOLIDS (TSS) AND pH OF LEAF EXTRACTS AND IN THE NUMBER OF RESTING WHITEFLIES ON COTTON LEAVES IN A NO-CHOICE EXPERIMENT

Plant age (days)	*TSS (%)*	*pH of leaf extract*	*Average number of resting whiteflies*
60	–	5.9	2.9
95	10.2	6.4	3.6
110	8.9	6.6	3.8
125	8.6	6.7	5.1
140	9.7	7.0	5.6

The plants were sown on different dates and the experiment was done simultaneously. Twenty adult whiteflies were used for each of five replications. Resting whiteflies were counted every 30 min during 8 h.

DISCUSSION AND CONCLUSIONS

In the cases tested so far, allelochemics or plant structure was found to be the most important governing factor for host selection by insects (12). It goes without saying that other factors may affect the attractiveness of the host plants as well. Hussein *et al.* (9) suggested that the attack of cotton by *B. tabaci* is governed mainly by cell sap acidity. Our results confirm the fact that the pH of leaf extract changes with the age of the plant. Plants in these experiments were attacked when they reached the age of 80-90 days from sowing, when the pH of their leaf extracts was close to 6.0. The first question to answer was, obviously, if the whitefly adult detects differences in pH values in its diet.

In vitro experiments using a diet, *via* a membrane, enabled us to separate the effect of pH from the effects of the whole complex of the developing leaf. It was clear that the whiteflies detected differences in pH values and reacted to them. Preliminary experiments using a dye and a radioactive tracer showed that the medium does not leak through the membrane and that whiteflies really sucked the medium through the membrane, as had been shown by Cohen (4), which means that they detected the pH differences while sucking the medium. All three parameters used in this work (testing in choice and no-choice experiments, and survival) indicated that the whiteflies preferred a rather narrow spectrum of pH values (6.0-7.25), regardless of the buffers used. The whiteflies even detected pH differences as small as 0.25 unit. The importance of the pH of the diet was shown also by Auclair (1) for an aphid *(in vitro)* and by Fife and Frampton (5) for a leaf hopper *(in vivo)*. More resting whiteflies were found on phosphate buffer, fewer on acetic acid buffer and least on citric acid buffer; thus, the composition of the buffer had some effect on its attraction. Nevertheless, the overall pattern of pH preference remained the same in all three buffers.

The addition of sucrose affected the attractiveness of the diets but did not change the pH preferred (1). Although 15% sucrose in the medium was preferred by whiteflies, only 10% was used in the experiments, since the soluble sugar contents of the cotton leaves did not exceed 10% and even declined with plant age, from 8 to 4% (A. Marani and U. Mor, personal communication). It seems that the effect of pH, in whitefly preference, was more important than the sugar contents of the diets. This assumption is supported by the results of the laboratory *in vivo* experiment, which showed that the number of resting whiteflies was correlated with the pH of the leaf extracts and leaf age but not with the TSS, *i.e.*, mainly sucrose contents.

The above mentioned results show that when pH is the only varying parameter, the whiteflies will choose the medium *(in vitro)* or leaf *(in vivo)* according to its pH. This also supports the hypothesis of Hussein *et al.* (8, 9) that the whitefly's attack on cotton is correlated with the plant's pH values. These findings may also explain the fact that young cotton plants, less than 90 days old and with a pH below 5.9-6.0, were not heavily attacked in the field although whitefly populations were already present in the neighborhood. The above results also point to the possibility that

TABLE 5

THE PREFERENCE OF *BEMISIA TABACI* ADULTS FOR COTTON LEAVES OF VARIOUS AGES, IN A CHOICE SITUATION

Experiment	*Plant age (days)*	*Leaf age**	*pH of leaf extract*	*Number of resting whiteflies*
A	60	old	5.9a	2.9a
	120	old	6.8b	7.2b
B	120	young	6.5a	4.1a
	120	old	6.8b	7.1b

The statistical analysis was done for each experiment separately. Twenty whiteflies were used for each of 12 replications; resting whiteflies were counted every 30 min during 6 h. Figures followed by different letters differ significantly at $P = 0.01$, according to the Newman-Keuls multiple range test.

*Young leaf = the first fully expanded leaf; old leaf = on 10-13th node from the top of the plant.

selection of cotton plants for low pH values may render them less susceptible to the whitefly's attack, a speculation to be studied further.

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REFERENCES

1. Auclair, J.L. (1967) Effects of pH and sucrose on rearing the cotton aphid, *Aphis gossypii*, on a germ-free and holidic diet. *J. Insect Physiol.* **13:** 431-446.
2. Avidov, Z. (1956) Bionomics of the tobacco whitefly (*Bemisia tabaci* Gennad.) in Israel. *Ktavim* **7:** 25-41.
3. Caldwell, P.C. (1956) Intercellular pH. *Int. Rev. Cytol.* **4:** 229-277.
4. Cohen, S. (1967) The occurrence in the body of *Bemisia tabaci* of a factor apparently related to the phenomenon of "periodic acquisition" of tomato yellow leaf curl virus. *Virology* **31:** 180-183.
5. Fife, J.M. and Frampton, V.L. (1936) The pH gradient extending from the phloem into the parenchyma of the sugar beet and its relation to the feeding behavior of *Eutettix tenellus*. *J. agric. Res.* **53:** 581-593.

6. Fowler, H.D. (1956) Some physiological effects of attack by whitefly *(Bemisia gossypiperda)* and of spraying parathion on cotton in the Sudan Gezira. *Emp. Cott. Grow. Rev.* **33:** 288-299.
7. Gerling, D., Motro, U. and Horowitz, R. (1980) Dynamics of *Bemisia tabaci* (Gennad.) attacking cotton in the coastal plain of Israel. *Bull. ent. Res.* **70:** 213-219.
8. Hale, L.J. (1965) Biological Laboratory Data. Science Paperbacks and Methuen & Co. Ltd., London.
9. Hussein, M.A., Puri, A.N. and Trehan, K.N. (1936) Cell sap acidity and the incidence of whitefly *Bemisia gossypiperda* on cotton. *Curr. Sci.* **4:** 486-487.
10. Hussein, M.A. and Trehan, K.N. (1933) Observations on the life history, bionomics and control of the whitefly of cotton. *Indian J. agric. Sci.* **3:** 701-753.
11. Mound, L.A. and Halsey, S.H. (1978) Whitefly of the World. A Systematic Catalogue of the Aleyrodidae (Homoptera), with Host Plant and Natural Enemy Data. British Museum (Natural History) and John Wiley and Sons, Chichester.
12. Southwood, T.R.E. (1973) The insect/plant relationship – an evolutionary perspective. *in:* Van Emden, H.F. [Ed.] Insect/Plant Relationships. Symp. Royal Entomol. Soc. London No. 6, pp. 3-30. Blackwell Scientific Publications, Oxford.
13. Verschoor van der Poel, P.J.G. and van Lenteren, J.C. (1978) Host plant selection by the greenhouse whitefly *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). *Meded. Fac. Landbouww. Rijksuniv. Gent.* **43:** 387-396.