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Short communication. Mechanisms in competition, under laboratory conditions, between Spanish biotypes B and Q of *Bemisia tabaci* (Gennadius)

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Abstract

Laboratory studies were carried out on aspects affecting competition between biotypes B and Q of whitefly [*Bemisia tabaci* (Gennadius)]: mating behaviour, egg viability and pyriproxyfen resistance. Pairs of biotype B females and males spent more time as courting couples than pairs with Q males, regardless of female biotype. Mixed pairs of biotype Q females and biotype B males spent more time as courting couples than biotype Q pairs. Egg viability from the different crosses and from single females was similar. Pyriproxyfen application to a mixture of whiteflies with a biotype ratio B/Q of one gave a reduction in the ratio because of higher resistance of the Q biotype to the insecticide. The data agrees with the observed displacement of biotype Q by biotype B whiteflies under laboratory conditions, and *vice versa* in the field.

Additional key words: courtship behaviour, egg viability, insecticide resistance, whiteflies.

Resumen

Nota corta. Mecanismos en la competencia, en condiciones de laboratorio, de los biotipos españoles B y Q de *Bemisia tabaci* (Gennadius)

Se han estudiado en laboratorio aspectos relacionados con la competencia entre biotipos B y Q de *Bemisia tabaci* (Gennadius): comportamiento de cortejo, viabilidad de huevos y resistencia a pyriproxyfen. Se observó un cortejo más prolongado en parejas formadas por machos y hembras de biotipo B que en parejas con macho Q independientemente del biotipo de la hembra, así como entre hembras Q y machos B que en parejas de hembras y machos Q. La viabilidad de los huevos procedentes de los distintos cruzamientos y de hembras solas fue semejante. La aplicación de pyriproxyfen a una mezcla de moscas blancas con una proporción de biotipos B/Q igual a uno produjo un descenso de esta proporción debido a la mayor resistencia del biotipo Q al insecticida. Estos datos concuerdan con el desplazamiento del biotipo Q por el B en condiciones de laboratorio, y viceversa en el campo.

Palabras clave adicionales: comportamiento de cortejo, moscas blancas, resistencia a insecticidas, viabilidad de huevos.

In recent years the distribution of *Bemisia tabaci* biotype Q has increased worldwide (Brown *et al.*, 2005; Khasdan *et al.*, 2005). In Spain a decrease in biotype B paralleled an increase of biotype Q *B. tabaci* (Simón *et al.*, 1999). However, in the laboratory biotype B displaced biotype Q whiteflies and insecticide resistance has been suggested as the reason for the field biotype ratio (Pascual and Callejas, 2004). One of the parameters studied was the sex ratio (females/males) of the F_1 populations from single and mixed biotype cultures. The data suggested that reproductive interference (Reitz and Trumble, 2002) may have contributed to competitive displacement because the sex ratio of the F_1 individuals of biotype Q was higher in single than in mixed cultures with biotype B. In this work courtship behaviour of single and mixed biotype whitefly pairs was observed to determine if reproductive interference actually occurred. The possible contribution of egg viability to the displacement observed, under laboratory conditions, was also studied. The effect of pyriproxyfen on the biotype ratio of mixed cultures of whiteflies as well as pyriproxyfen resistance in both biotypes was studied to confirm that resistance

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of biotype Q whiteflies can explain its dominance in the field.

B. tabaci whiteflies, biotypes B and Q, were reared on tomato (*Lycopersicon esculentum* Mill.) plants cv. Marmande in a growth chamber at 25 ± 2ºC, 60 ± 10% rh with 16L:8D. The same tomato cultivar and incubation conditions were used in all experiments. Whitefly colonies were analysed monthly by RAPD-PCR to check their biotype (see Pascual and Callejas, 2004, for details).

Single and mixed biotype whitefly pairs and single females of each biotype were established in cages each containing one cut tomato leafleat. There were 10 replicates for each cross or single female. Whitefly couples were observed, on average 7 times per day until the female in the couple died. Courtship behaviour was determined by parallel orientation of males and females and the courtship ratio was calculated by dividing the number of times the whiteflies were observed courting by the total number of observations. Whiteflies were transferred to cages containing fresh leaflets twice a week. Leaflets separated from whiteflies were maintained in the growth chamber for 14 d. Eggs and nymphs were counted and the percentage egg viability was calculated.

Mixed biotype cultures were established on tomato seedlings which had been sprayed to run-off with pyriproxyfen at 0.0, 0.05, 0.2 and 1 mg active ingredient (AI) L^{-1}. The biotype ratio (number of B biotype whiteflies/number of Q biotype whiteflies) was one. A total of 24 whiteflies (50% females) were established per plant, with four replicates per insecticide dose. After F_1 whiteflies emerged, B and Q biotypes were determined by RAPD-PCR.

Analysis of variance was determined out on courtship ratio and percentage egg viability. Courtship ratio data were transformed by sqrt(x + 0.05), and the percentage egg viability by arcsin(x/100) prior to analysis of variance. Means were separated by a HSD Tukey test at a 95% confidence level. A t-test was also carried out to compare percentage egg viability from B and Q females. Contingency table analysis was used to compare biotype ratios of F_1 whiteflies from cultures treated with different doses of pyriproxyfen. Analyses used the STATGRAPHICS Plus package (Statistical Graphics Corp., 1997).

For the insecticide resistance test, tomato seedlings with one-day-old eggs were immersed in pyriproxyfen solution at 0.0, 0.001, 0.002, 0.005, 0.01 and 0.02 mg AI L^{-1}. There were 5 replicates per insecticide dose and biotype. After 14 d incubation the number of eggs and nymphs in 50 individuals was determined. Baseline susceptibility was established for each biotype by probit analysis from POLO-PC (LeOra, 1987).

There were significant differences in courtship ratio, depending on the cross type ($F = 7.95$, $p = 0.0001$) (Table 1). In crosses between females and males of the B biotype the courtship ratio was significantly higher than that in crosses between females and males of biotype Q. Differences in courtship time do not necessarily mean differences in copulation. Perring *et al.* (1994) documented courtship behaviour between silverleaf whitefly and sweetpotato whitefly without copulation occuring. However, Pascual and Callejas (2004) observed that the sex ratio (females:males) of B biotype progeny was higher than in the Q biotype progeny. Knowing that *B. tabaci* always exhibits haplo-diploidy, i.e., males (haploid) are produced parthenogenetically from unfertilized eggs and females (diploid) are produced from fertilized eggs (Byrne and Bellows, 1991), the data indicates that in this work a higher courtship time was probably related to higher copulation and egg fertilization. Other authors have compared the courtship time in biotype B whiteflies with other biotypes of *B. tabaci*. Perring *et al*. (1994) found that biotype A males and females spend less than 33% of the time in precopulatory courtship behaviour than biotype B males and females. This is similar to these results: the courtship ratio was more than 3 times higher in biotype B than in biotype Q. However, in Australian mating studies of *B. tabaci*, the proportion of time spent courting was the same in pairs of the exotic B biotype as in pairs of indigenous eastern (EAN) populations (De Barro and Hart, 2000). The proportion of time spent courting in both cases (B × B and EAN × EAN pairs) was similar to values obtained here for biotype B.

The courtship ratio was significantly higher in B × B couples than in B × Q crosses. In the presence of B and

Table 1. Courtship ratio in crosses between biotypes B and Q of *Bemisia tabaci* (n = 10)

Type of cross (female × male)	Courtship ratio (mean ± SE)
B × B	0.33 ± 0.05a
Q × Q	0.09 ± 0.02c
B × Q	0.14 ± 0.03bc
Q × B	0.22 ± 0.04ab

Data followed by the same letter are not significantly different. Turkey's HSD test.

Q males together it is possible that Q males block courtship and mating between B biotype females and males. However, a previous study observed that the sex ratio of B biotype whiteflies was the same in both single and mixed biotype cultures (Pascual and Callejas, 2004). This means that the courtship ratio between B biotype females and Q biotype males is not only lower than between B biotype females and males, but it does not affect normal copulation of B biotype whiteflies. On the other hand, the courtship ratio was significantly higher in Q × B crosses than in Q × Q couples. Thus, in the presence of B biotype males courtship and mating behaviour between Q biotype couples would probably be affected, and this would produce the significant reduction in the sex ratio of Q biotype progeny previously observed in mixed cultures of Q and B biotype whiteflies (Pascual and Callejas, 2004). Overall these and previous results support the view that reproductive interference is a competition mechanism playing a role in the competitive displacement of biotype Q by biotype B under laboratory conditions. Perring *et al.* (1994) also reported that B biotype males of *B. tabaci*, by the nature of their biotype having longer courtships prior to copulation, effectively block courtship and mating of *B. tabaci* (biotype A) females by *B. tabaci* males. This leads to the displacement of biotype A by biotype B whiteflies. In *B. tabaci* from Australia, De Barro and Hart (2000) studied mating interactions between different biotypes. They found that mixed biotype pairs spent more time courting than single biotype pairs. Females and males of different biotypes were unable to recognize the difference and entered into courtship. As a result the mixed pair could have reduced ability to complete courtship with successful copulation and so continue to devote time to courtship. However, the present results for Spanish whiteflies indicate that biotype B females recognized the difference, i.e., the courtship ratio was higher in B × B couples than in B × Q couples. However, the Q biotype females could not, i.e. the courtship ratio was lower in Q × Q than in Q × B couples.

Egg viability from females alone or females crossed with males of the same or a different biotype did not differ (Table 2). However, values were always higher for the B biotype than for the Q biotype females. A t-test showed that the mean percentage egg viability of B biotype females (89.68 ± 3.20) was higher than for Q biotype females (71.66 ± 7.64) ($p = 0.02$).

Pascual and Callejas (2004) showed that mortality of Q biotype immature instars was significantly higher than that of B biotype instars. The present work shows that death of eggs contributes to higher immature mortality on tomato plants cv. Marmande. The values are in the range of those reported by Tsai and Wang (1996) on tomato for *B. argentifolii* (B biotype *B. tabaci*).

Table 2. Percentage egg viability from crosses between biotypes B and Q of *Bemisia tabaci* and females alone

Type of cross (female × male)	Percentage egg viability (mean ± SE)
B × B	92.05 ± 1.86
Q × Q	72.12 ± 8.38
B × Q	92.05 ± 2.23
Q × B	80.26 ± 13.21
B females alone	84.94 ± 9.51
Q females alone	58.17 ± 19.21

Data are mean of five replicates.

Application of pyriproxyfen to mixed biotype whitefly cultures affected the biotype ratio (B/Q) of F_1 individuals. The values were 38/2, 34/0, 19/1 and 3/6 for 0.0, 0.05, 0.2 and 1 mg AI L^{-1}, respectively. Contingency table analysis of the biotype ratio of F_1 individuals showed that treatment with pyriproxyfen at 1 mg AI L^{-1} significantly decreased the biotype ratio ($\chi^2 = 42.18$, $df = 3$, $p < 0.00001$). The biotype ratio of F_1 individuals did not differ between control plants and those sprayed with insecticide at 0.05 and 0.2 mg AI L^{-1} ($\chi^2 = 1.76$, $df = 2$, $p = 0.42$).

The decrease in the biotype ratio when pyriproxyfen was applied at 1 mg AI L^{-1} must be a consequence of a difference in the baseline susceptibility of both biotypes. Table 3 shows the results of probit analysis. Mortality data were best described by separate, parallel lines (same slope), for biotypes B and Q, which indicates a significant difference in the susceptibility of both biotypes, with biotype B being the more susceptible. This criterion was considered prevalent compared to a lack of significant difference in lethal concentrations (the 95% CL overlap). It has been shown that the diffe-

Table 3. Susceptibility of *Bemisia tabaci* eggs, biotypes B and Q, to pyriproxyfen

Biotype	n	Slope ± SE	LC_{50}[1] (95% CL)	LC_{90}[1] (95% CL)	Relative potency (95% CL)
B	1,474	1.82 ± 0.08	0.001 (0.001-0.002)	0.007 (0.004-0.014)	1.00
Q	2,439	1.82 ± 0.10	0.002 (0.002-0.003)	0.012 (0.009-0.017)	1.73 (1.09-2.77)

[1] Dose in mg AI L^{-1}. [2] CL: confidence limit.

rence in lethal doses is not always an useful criterion (Robertson *et al*., 1995). Both LC_{50} values are similar but are slightly lower than that of Devine *et al*. (1999) for a susceptible *B. tabaci* strain.

The populations studied here have been maintained, without insecticide pressure, since they were established in the laboratory in 1994. Thus, these values indicate a small, but higher, inherent resistance of the Q biotype of *B. tabaci* compared to the B biotype. The importance of insecticide resistance in the balance between biotypes was shown in studies with field populations of *B. tabaci* by Nauen *et al.* (2002), Rauch and Nauen (2003) and Horowitz *et al.* (2005). Although the difference in inherent sensitivity between both biotypes was small, biotype Q also probably has a higher ability to develop resistance in the field because of its higher intrapopulational genetic diversity (Moya *et al*., 2001).

This work confirms that the biological potential of the B biotype of *B. tabaci* is higher than that of Q biotype under laboratory conditions. The decrease in the B biotype, in the field, is explained by modification of the competition conditions caused by the use of insecticides. This favours biotype Q because of its higher resistance.

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