RESULTS

4 RESULTS

4.1 Bioassay

4.1.1 Adult leaf-dip bioassay, organophosphates

Early season collections of Egyptian whitefly (strains EGYPT-1a, EGYPT-2 and EGYPT-3) displayed little or no resistance to the organophosphates (profenofos and pirimiphos methyl). In comparison, Israeli strains exhibited 18.3 to 38.9-fold resistance factors to profenofos and ca. 7.6 to 7.7-fold resistance to pirimiphos methyl. The Egyptian whitefly strain collected in December 2001 (EGYPT-1b) was 17.4-fold resistant to profenofos (Table, 2).

4.1.2 Adult leaf-dip bioassay, pyrethroids

Early season collections of Egyptian whitefly (strains EGYPT-1a, EGYPT-2 and EGYPT-3) displayed moderate resistance to both cypermethrin and lambda cyhalothrin (9.7 to 27.4-fold). In comparison, Israeli strains exhibited similar resistance to cypermethrin (23.6 to 33.4-fold) but greater resistance to lambda cyhalothrin (49.9-52-fold). The Egyptian whitefly strain collected in December 2001 (EGYPT-1b) was more resistant to cypermethrin (46.4-fold) than Egyptian strains collected in the early season (Table, 3).

Table (2) Comparative responses of Egyptian and Israeli strains of *Bemisia tabaci* tested against organophosphates.

	Profenofos					Pirimiphos methyl				
Strains	**N.**	**LC_{50}[1]**	**95% CLs**	**Slope**	**RF**	**N.**	**LC_{50}[1]**	**95% CLs**	**Slope**	**RF**
SUDS	622	4.19 a	3.55-4.75	3.5	-	525	19.2 a	16.3- 21.6	6.5	-
ISR-AV	600	164 c	101-236	2.2	38.9	582	146 b	121-173	3.7	7.6
ISR-HC	776	77.1 c	66.3-86.9	3.6	18.3	671	148 b	129-169	2.8	7.7
EGYPT-1a	940	7.48 b	5.60-9.41	2.3	1.8	1229	22.4 a	17.5-28.6	2.9	1.2
EGYPT-1b	479	73.2 c	54.4-118	1.4	17.4	-	-	-	-	-
EGYPT-2	534	5.53 a	3.24-7.21	2.6	1.3	584	25.2 a	18.5-34.4	3.1	1.3
EGYPT-3	912	8.12 b	6.53-9.82	2.3	1.9	880	25.9 a	21.3-31.7	2.8	1.3

[1]All LC_{50}s in ppm. Different letters denote non-overlap of confidence limits ($p < 0.05$).

Table (3) Comparative responses of Egyptian and Israeli strains of *Bemisia tabaci* tested against pyrethroids.

	Cypermethrin					Lambda cyhalothrin				
Strains	**N.**	**LC_{50}[1]**	**95% CLs**	**Slope**	**RF**	**N.**	**LC_{50}**	**95% CLs**	**Slope**	**RF**
SUDS	619	5.23 a	2.50-6.87	1.9	-	877	1.19 a	1.03-1.34	2.4	-
ISR-AV	502	167 cd	107-220	1.5	33.4	597	59.9 d	42.1-75.4	2	49.9
ISR-HC	537	118 c	83.4-149	1.5	23.6	783	62.5 d	51.5-72.5	2.4	52
EGYPT-1a	539	54.5 b	42.3-66.4	2.3	10.9	988	12.1 b	8.15-15.9	1.3	10.2
EGYPT-1b	534	232 d	197-279	1.6	46.4	-	-	-	-	-
EGYPT-2	556	48.5 b	21.6-71.3	1.8	9.7	485	17.6 bc	5.73-28.2	1	14.7
EGYPT-3	763	137 c	108-187	1.5	27.4	686	29.5 c	24.2-34.9	1.5	24.6

[1]All LC_{50}s in ppm. Different letters denote non-overlap of confidence limits ($p < 0.05$).

4.1.3 Systemic bioassay, carbamates

All Egyptian strains were resistant to carbamates, regardless of time of collection. Resistance factors varied from 24.9 to 48.7-fold for carbosulfan and 38.3-83 fold for aldicarb. Israeli strains were only very slightly tolerant (resistance factors of ca. 4) (Table, 4).

4.1.4 Systemic bioassay, imidacloprid

Assays at which the end point of the assay was 48 hrs, gave better discrimination between the strains than those scored at 72 hrs. At 48 hrs, slight 6 to 8-fold resistance was found to imidacloprid in the Israeli strains. Early season collections from Egypt were susceptible. The late season collection (EGYPT-1b) showed the same slight resistance as the Israeli strains (Table, 5).

4.1.5 Egg-dip bioassay, pyriproxyfen

Pyriproxyfen proved very effective against the eggs of all the Egyptian strains, whilst the Israeli strains displayed strong resistance (67.6 to 123.6-fold) (Table, 6).

Table (4) Comparative responses of Egyptian and Israeli strains of *Bemisia tabaci* tested against carbamates.

	Carbosulfan					Aldicarb				
Strains	**N.**	**LC_{50}[1]**	**95% CLs**	**Slope**	**RF**	**N.**	**LC_{50}**	**95% CLs**	**Slope**	**RF**
SUDS	499	4.22 a	3.12-5.44	1.3	-	596	2.17 a	0.75-3.89	1.2	-
ISR-AV	562	12.8 b	9.10-17.6	0.8	3	608	8.26 b	5.64-11.4	0.9	3.8
ISR-HC	515	19.2 b	13.1-28.8	0.7	4.6	539	9.59 b	5.77-14.8	0.6	4.4
EGYPT-1a	641	205 c	102-666	0.5	48.7	812	183 c	87.4-722	0.7	83
EGYPT-1b	719	182 c	95.3-528	0.5	43.3	-	-	-	-	-
EGYPT-2	656	105 c	66.2-200	0.9	24.9	610	84.3 c	47-213	0.6	38.3
EGYPT-3	561	90.4 c	51.0-225	0.5	21.5	733	96.4 c	66.9-156	0.8	43.8

[1]All LC_{50}s in ppm. Different letters denote non-overlap of confidence limits ($p < 0.05$).

Table (5) Comparative responses of Egyptian and Israeli strains of *Bemisia tabaci* tested against imidacloprid (with bioassay end-points at 48 and 72 h).

	Imidacloprid (48hrs)					Imidacloprid (72 hrs)				
Strains	**N.**	**LC_{50}[1]**	**95% CLs**	**Slope**	**RF**	**N.**	**LC_{50}**	**95% CLs**	**Slope**	**RF**
SUDS	717	0.76 a	0.50-1.05	1.4	-	887	0.400	0.233-0.598	1.3	-
ISR-AV	658	4.23 c	2.99-5.53	1.3	5.6	564	1.15	0.397-2.02	1.4	2.9
ISR-HC	782	5.73 c	4.39-7.14	1.3	7.6	595	1.77	0.729-3.03	1.03	4.4
EGYPT-1a	653	0.49 a	0.22-0.81	1.1	0.65	808	0.134	0.039-0.280	0.9	0.36
EGYPT-1b	615	4.64 cb	3.12-6.73	0.6	6	746	0.100	0.032-0.334	1.3	0.25
EGYPT-2	572	0.23 a	0.04-0.63	0.54	0.3	757	0.609	0.370-0.871	1.2	1.52
EGYPT-3	906	2.29 ab	1.69-2.96	0.79	3	-	-	-	-	-

[1]All LC_{50}s in ppm. Different letters denote non-overlap of confidence limits ($p < 0.05$)

Table (6) Effect of pyriproxyfen on zero-to one-day old eggs of *Bemisia tabaci*.

Strain	N.	LD_{50}[1]	95% CLs	Slope	RF
SUD-S	2385	0.005 a	0.004-0.007	1.5	-
ISR-HC	1535	0.622 b	0.362-1.39	0.8	123.6
Ayalon V.	1649	0.344 b	0.210-0.821	0.94	67.6
EGYPT-1a	1738	0.008 a	0.005-0.011	1.9	1.6
EGYPT-2	1242	0.003 a	0.002-0.005	1.4	0.6
EGYPT-3	2351	0.005 a	0.004-0.006	1.5	1
EGYPT-1b	647	0.005 a	0.002-0.009	0.55	1

[1]All LC_{50}s in ppm. Different letters denote non-overlap of confidence limits ($p < 0.05$).

4.1.6 Larval-dip bioassays, organophosphates and pyrethroids

Larval dip bioassays against SUDS illustrated that profenofos and cypermethrin were as active against whitefly nymphs as they were against adults; LC_{50}s of profenofos and cypermethrin were 4.06 & 5.87 ppm in nymphs, (Table, 7) and 4.19 & 5.23 ppm in adults (Tables, 2 and 3 respectively). The nymphs reflected the resistance factors seen in adult bioassays. ISR-AV nymphs were 37.7-fold resistant to profenofos and 15.2-

fold resistant to cypermethrin. Adults of the same strain were 39 and 33-fold resistant to these two compounds, respectively. Neither adults nor nymphs of the strain EGYPT-1a resisted to profenofos, and they exhibited similar resistance factors to cypermethrin (11 and 8-fold, respectively).

4.1.7 Adult leaf-dip bioassay, pyriproxyfen

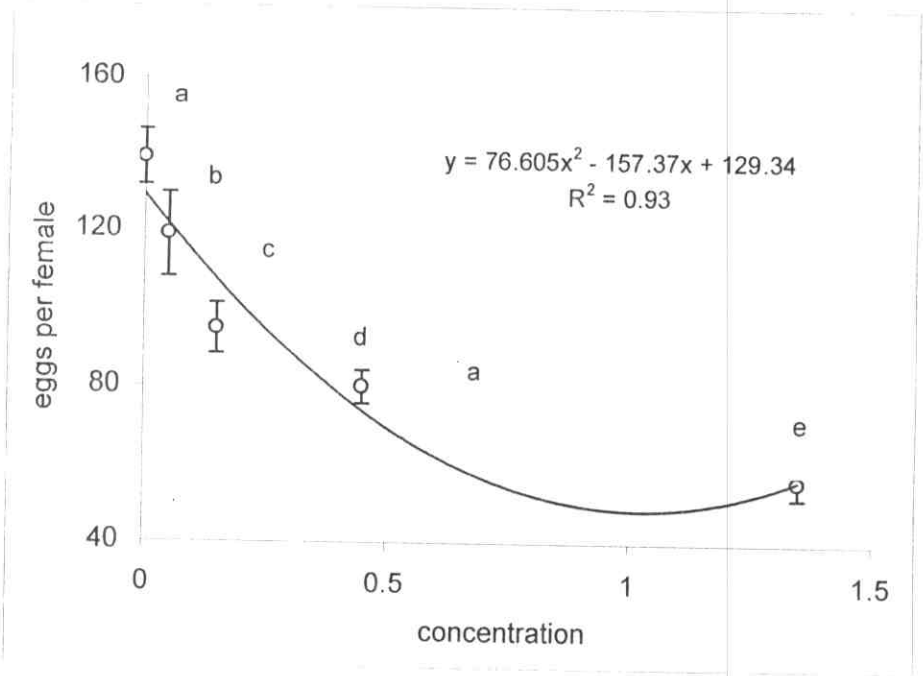
Pyriproxyfen also affected adult fecundity (fig., 11). The mean number of eggs produced by females in the control was 159. The mean number of eggs produced by females exposed to pyriproxyfen declined with increasing concentration. At 0.05 ppm, the mean number of eggs was 118, at 1.35 ppm that number declined to 51 (represented 32% of the control figure).

Table (7) Response of *Bemisia tabaci* nymphs against profenofos and cypermethrin.

	Profenofos					Cypermethrin				
Strains	**N**	**LC_{50}[1]**	**95% CLs**	**Slope**	**RF**	**N**	**LC_{50}**	**95% CLs**	**Slope**	**RF**
SUDS	1407	4.06 a	3.56-4.52	2.9	-	1275	5.87 a	3.85-7.63	2.02	-
ISR-AV	835	151 b	90.4-199	1.6	37.7	940	90.9 b	72.3-108	2.08	15.4
EGYPT-1a	1267	5.54 a	4.46-6.57	2.4	1.4	1164	44.9 c	32.6-55.9	1.95	7.6

[1]All LC_{50}s in ppm. Different letters denote non-overlap of confidence limits ($p < 0.05$).

Fig. (11) The effect of pyriproxyfen on adult fecundity (mean and 95% confidence limits)



Points annotated with a different letter are significantly different (non-overlapping confidence limits, $p < 0.05$) Fitted line is a polynomial in order to allow the data for dose 0 to be fitted (otherwise a linear regression on a log dose scale would be sufficient.

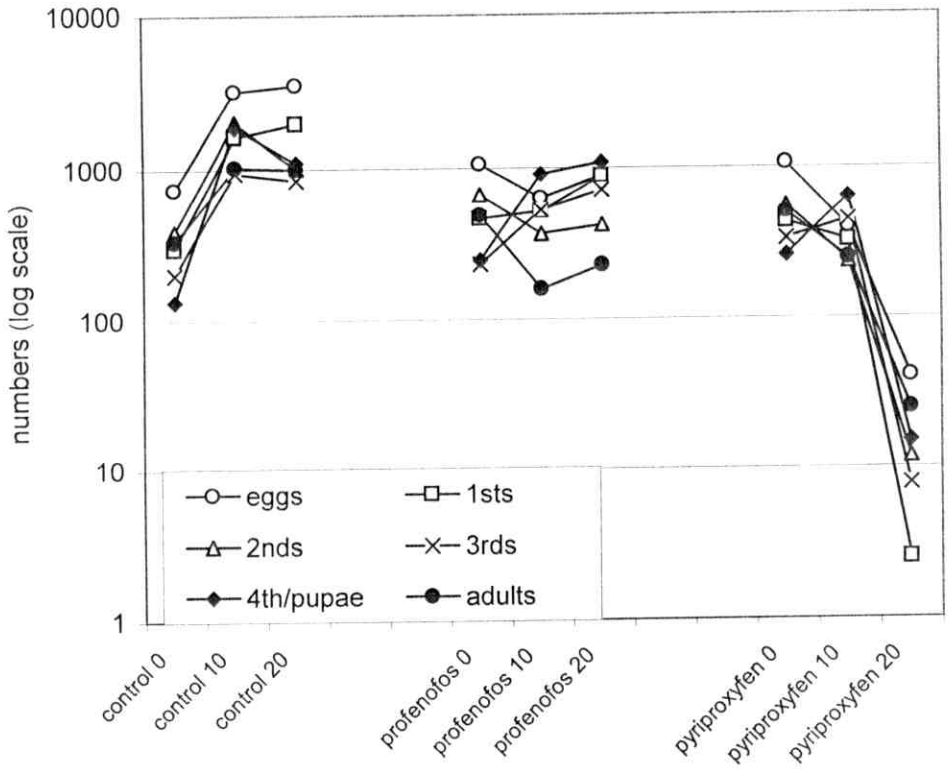
4.2 Field simulator experiments

4.2.1 Effect on adult populations

As expected, the Egyptian strain was completely almost completely controlled by a single application of pyriproxyfen at the recommended field rate (750 ml / 75 litres per ha.) (Fig., 12). After 10 days, a slight decrease in all numbers was noted whilst numbers in the

control simulators has increased considerably. By 20 days, however, the pyriproxyfen treated population had continued to decrease dramatically. Profenofos was initially also effective in causing a decline in numbers, but after 20 days had recovered to pre-treatment levels.

Fig. (12) Effect of pyriproxyfen and profenofos on total whitefly numbers (means and 95% confidence limits)



4.2.2 Effect on age structure of populations

Prior to treatment, the population structure in all the simulators was very similar (Fig., 13a). With more than 30% of the population present as eggs. Ten days after treatment, the population structures of the treated populations had begun to diverge from that of the control (Fig., 13b). In comparison with the control, both treated populations showed a significant decrease in the proportion of eggs and second instars, and an increase in the proportion of late instar / pupae.

After 20 days (Fig., 13c), all treatments had begun to diverge significantly from each other. The age structure of the control population was still similar to that which had been displayed both pre-treatment and 10 days after treatment. In comparison, the profenofos treated population was made up mostly of eggs (40%) and adults (20%). The pyriproxyfen treated population consisted of a relatively small proportion of eggs (20%) and a very small proportion of first instars and adults (4% and 5%, respectively).

Fig. (13 a) Age structure of untreated populations.

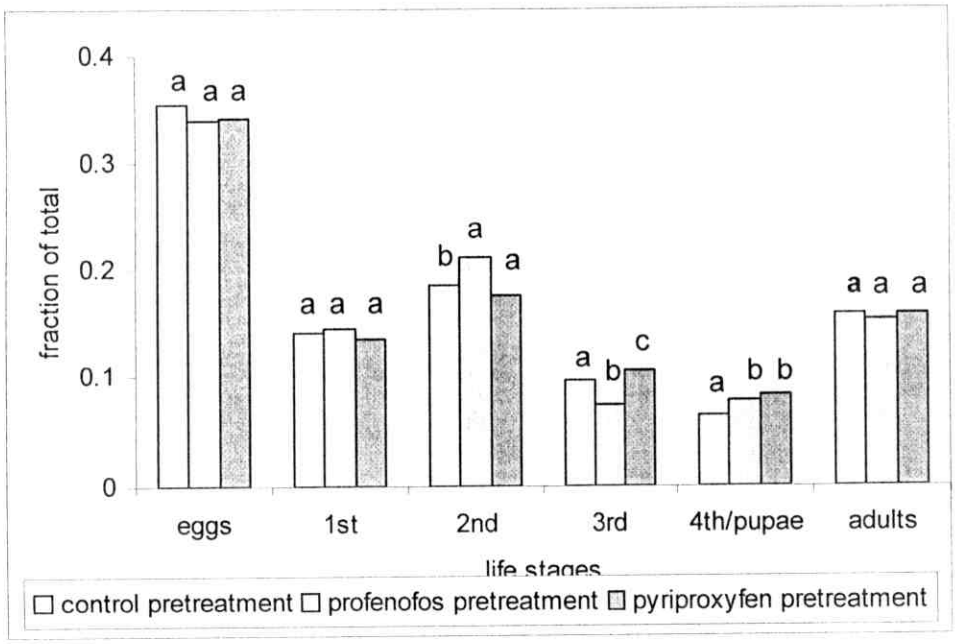


Fig. (13 b) Age structure of treated populations, 10 days after treatment.

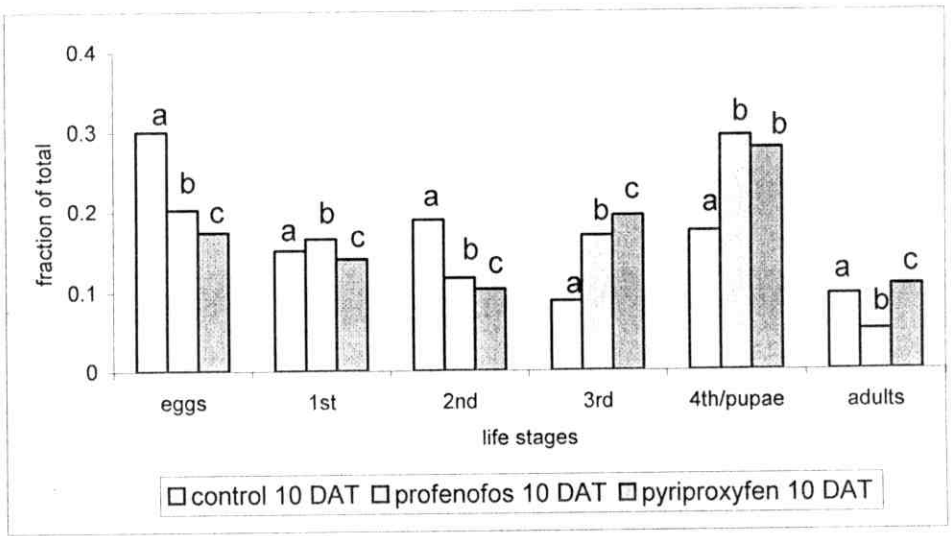
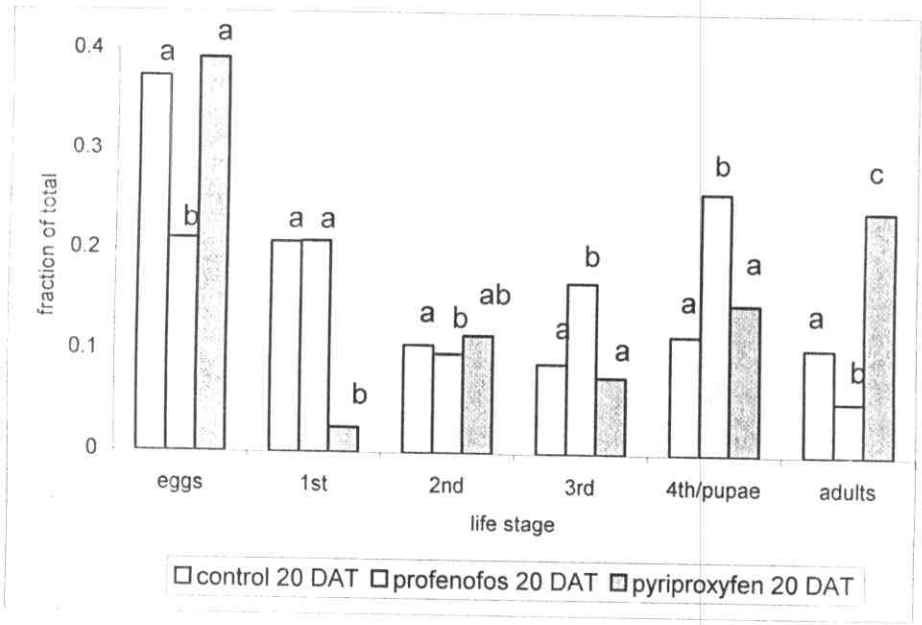


Fig. (13 c) Age structure of treated populations, 20 days after treatment



For any group of bars, annotation with different letters designates significant differences in proportion of that life stage between treatments ($F > 3.84$, $p < 0.05$).

4.3 The effect of pesticides on *B. tabaci* parasitoids

4.3.1 Whitefly population in the absence of insecticide and parasitoids

With no insecticide application and in the absence of parasitoids, *B. tabaci* numbers showed a characteristic pattern whereby after 21 days, the offspring of the initial number began to emerge as adults resulting in a sharp increase in population size (Fig., 14a). By day 51, after the F2 generation has begun to emerge, the population was so large (>20,000 individuals) that the cotton plants began to deteriorate, leading to a decline in population from day 54 onwards.

4.3.2 Whitefly population in the absence of insecticide but with parasitoids

With the addition of 80 female parasitoids, it was clear that the *B. tabaci* population was constrained (Fig., 14d). Parasitoid numbers began to increase from day 15 onwards, and limited the peak adult *Bemisia tabaci* population to ca 2000 individuals (ca. $1/10^{th}$ of the unchecked control population). By the end of the experiment (day 60) the ratio of adult parasitoids to adult whitefly was 1:4 and it is likely that the whitefly population was in decline.

Fig. (14 a) Whiteflies only, no treatment

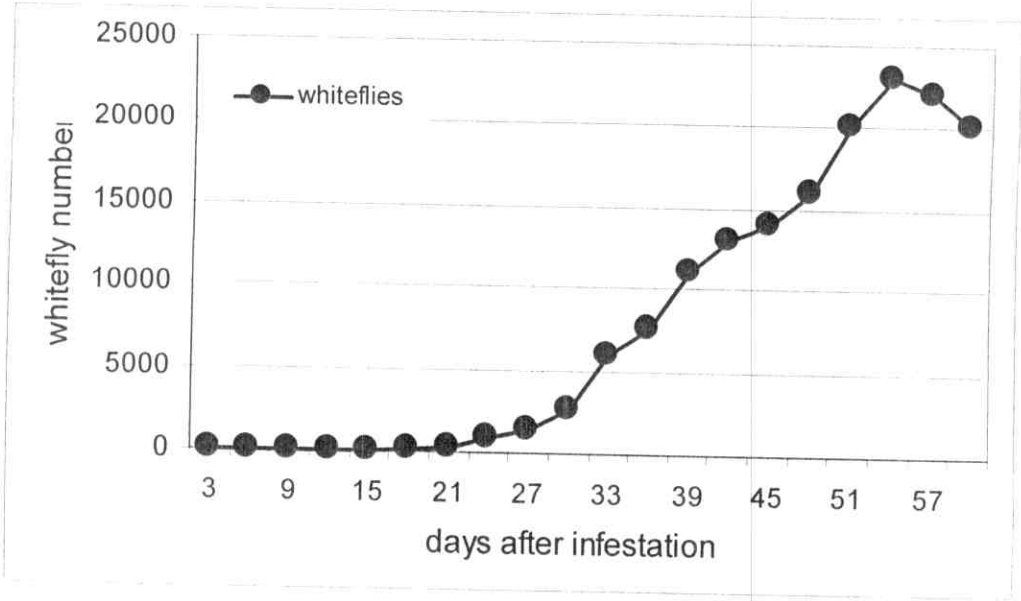
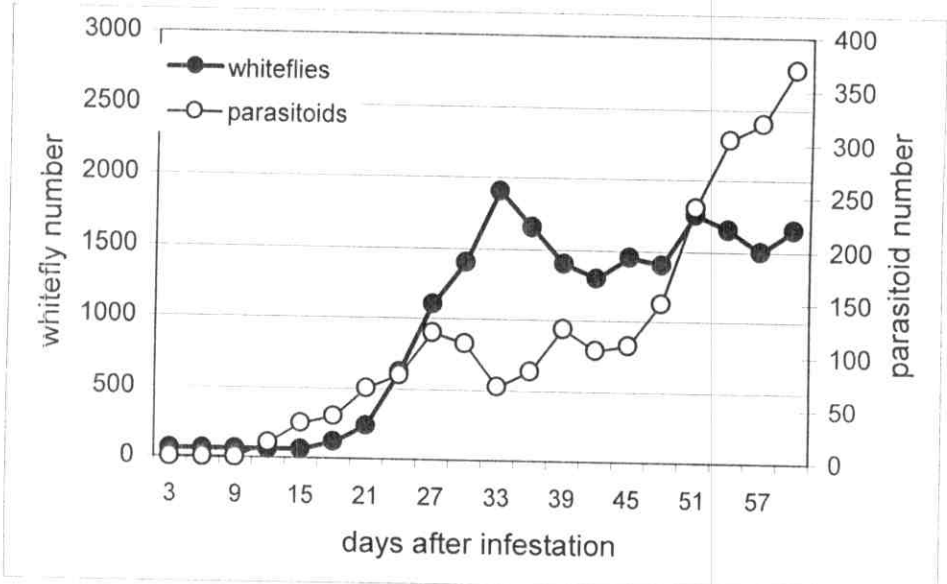


Fig. (14 d) Whiteflies and parasitoids, no treatment



4.3.3 Incorporation of insecticides in the absence of parasitoids

4.3.3.1 Profenofos:

A single application of profenofos on day 28 (Fig., 14b) had a dramatic effect on the population of *B. tabaci.* The population declined to a low, at 45 days, of ca 250 adults. After that time, it began to build again, tripling in size (750 adults) by day 60.

4.3.3.2 Pyriproxyfen:

A single application of pyriproxyfen on day 28 (Figure 14c) had an even more dramatic effect upon the population of *B. tabaci.* The population declined slowly and continuously to a point, at 60 days, when only ca. 30 adults remained.

Fig. (14 b) Whiteflies only, profenofos treated

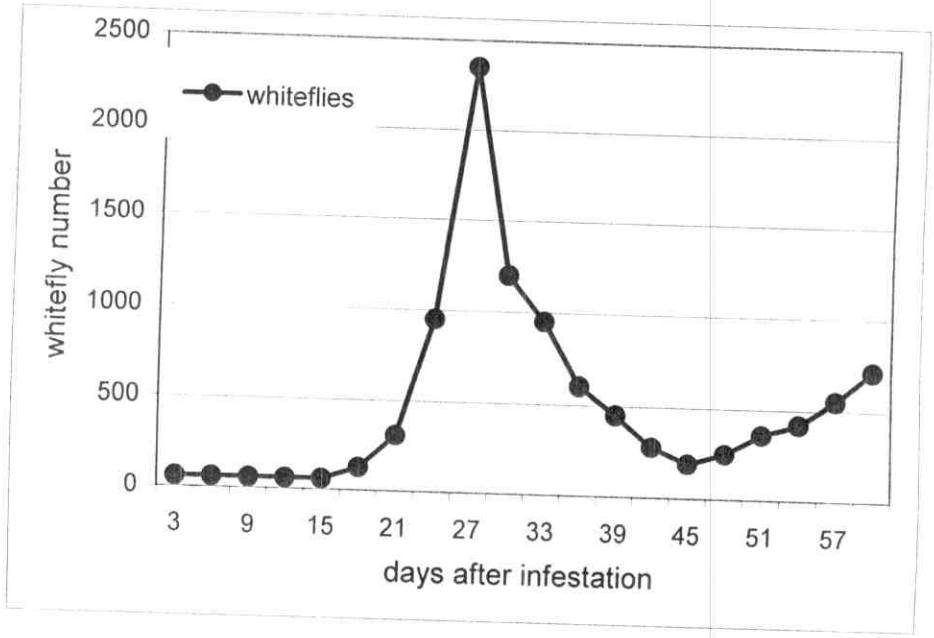
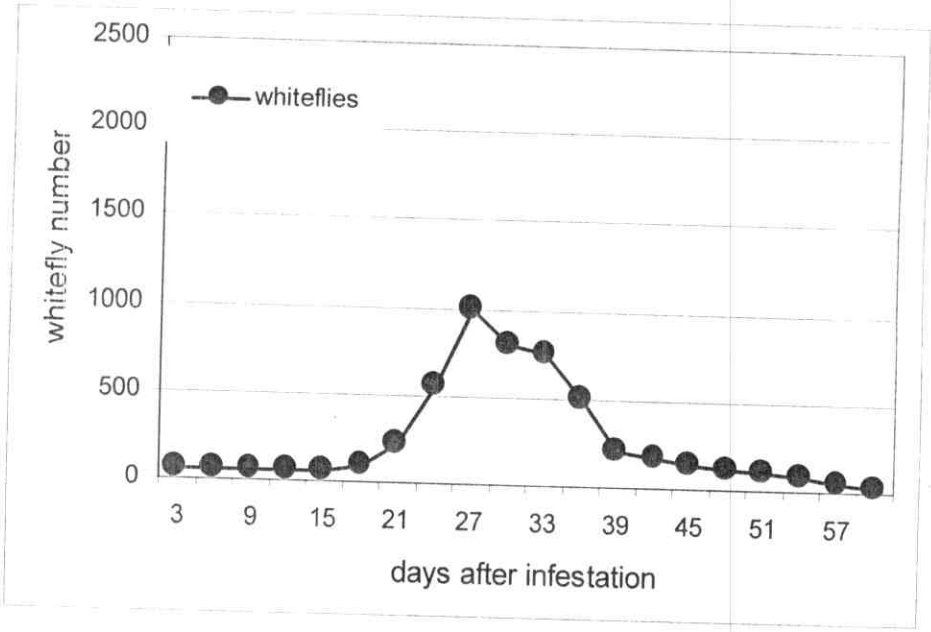


Fig. (14 c) Whiteflies only, pyriproxyfen treated



4.3.4 Incorporation of insecticides in the presence of parasitoids

4.3.4.1 Profenofos:

A single application of profenofos on day 28 (Fig., 14e) had a dramatic effect upon both *B. tabaci* and *E. formosa* populations. From a pre-treatment high of ca 140 parasitoid adults, the parasitoid population dropped to just a few individuals. However, the *B. tabaci* population also dropped dramatically. By day 48 however, both the whitefly and parasitoid populations, from that point onwards, it was clear that even the recovering parasitoid population could not control the whitefly population and, by the end of the experiment (day 60), the population comprised ca 2500 whitefly adults and ca. 250 parasitoid adults (a parasitoid: whitefly ratio of 1:10).

4.3.4.2 Pyriproxyfen:

A single application of pyriproxyfen on day 28 (Fig., 14f) had a far more dramatic effect upon *B. tabaci* than it did upon *E. formosa*. From a pre-treatment high of ca 2000 whitefly adults, the population went into a slow and terminal decline. The parasitoid population however, despite dipping to ca. 70 adults shortly after spraying recovered such that by the end of the experiment ca 200 parasitoid adults remained and just 65 whitefly adults. This is a parasitoid: whitefly ratio of ca 3:1).

Fig. (14 e) Whiteflies and parasitoids, profenofos treated

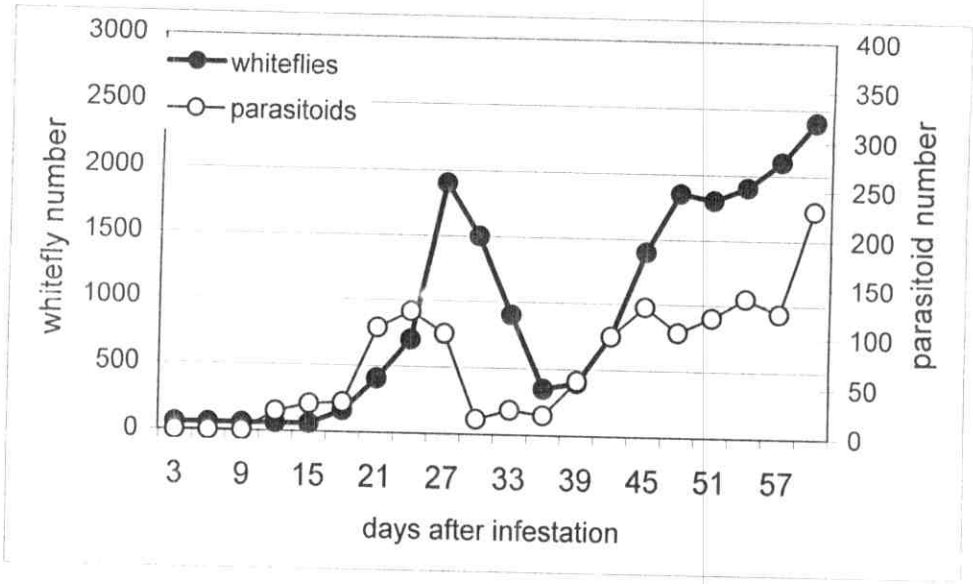
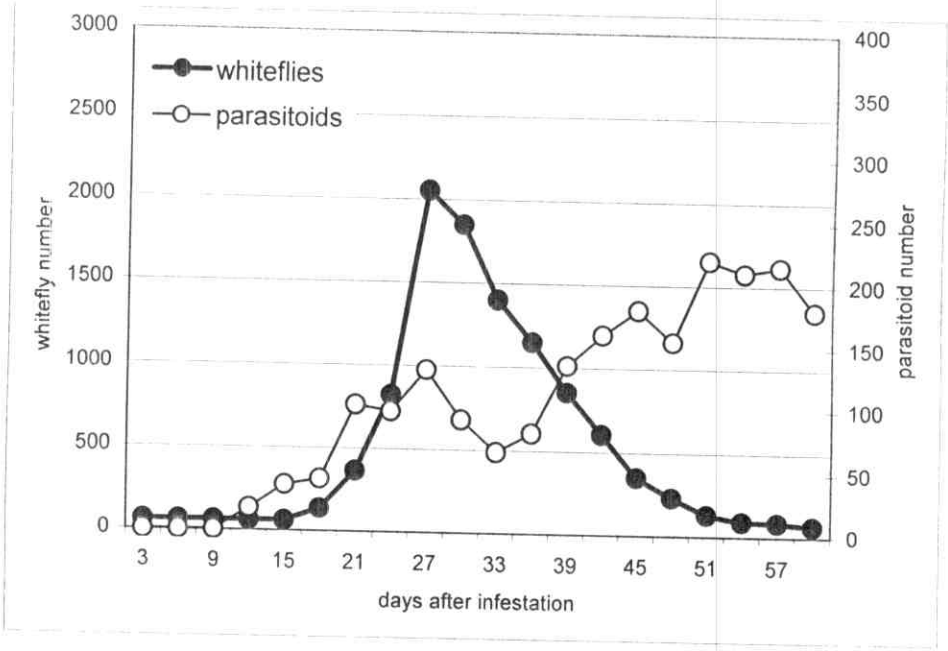


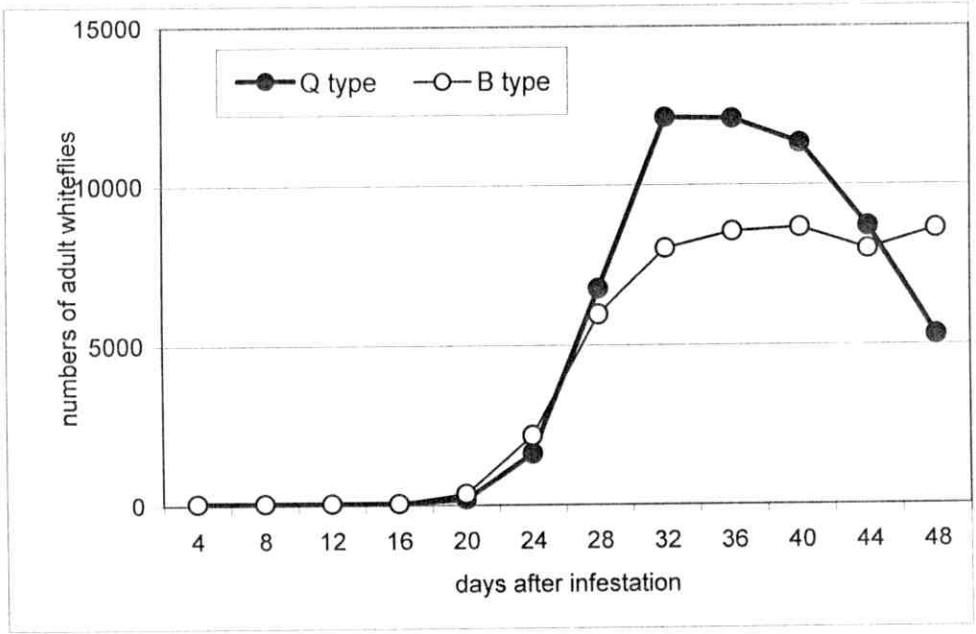
Fig. (14 f) Whiteflies and parasitoids, pyriproxyfen treated



4.3.5 Dispersal of 'B' and 'Q' biotypes under simulator conditions

Prior to connecting the simulators with the perspex tunnels, 20 days after infestation, the two populations showed similar population sizes (Fig 15). The Egypt 1a population comprised ca 360 adults whilst the ISR-HC population was ca 200 adults. Peak population sizes (of the now mixed populations) were reached at day 32 (ca 12000 adults) for the simulator population that initially held the ISR-HC population and day 40 (ca 9000 adults) for the simulator that initially held the Egypt-1a population.

Fig. (15) Relative size of populations in linked simulators.



Bioassay tests with carbosulfan and pyriproxyfen were carried out with both strains (B & Q) after 20, 40 and 60 days. The bioassay results for both simulator populations showed that the 'B' and 'Q' types were certainly mixing between simulators (Table, 8). The LC_{50} values for the Egypt 1a 'B' type showed a high level of susceptibility to pyriproxyfen (0.003 ppm; Table 8) before dispersal by the ISR-HC 'Q' type. Conversely, and as expected, the LC_{50} values for a subset of the ISR-HC population showed a high level of resistance to pyriproxyfen (1.75 ppm; Table 8) before dispersal by the Egypt 1a population. Notably, only the ISR-HC population seemed to be affected by the connection of the simulators. Resistance to pyriproxyfen in this population decreased to 100-fold and finally to just 6-fold. The Egypt 1a population however, remained consistent in its susceptibility, throughout the course of the experiment.

The LC_{50} values for the Egypt 1a 'B' type showed moderate resistance to carbosulfan, as expected, prior to connection of the simulators (177 ppm; Table, 9) and prior to dispersal by the ISR-HC 'Q' type. Conversely, and as expected, the LC_{50} values for a subset of the ISR-HC population showed no resistance to carbosulfan (0.5 ppm; Table, 9). In this instance, after connecting the simulators, resistance to carbosulfan only changed in the Egypt 1a population, decreasing from 42-fold prior to connection, to just 3-fold at the end of the experiment. The response to carbosulfan in the ISR-HC strain remained unchanged.

Table 8. The effect of pyriproxyfen on 'B' and 'Q' biotypes before and after connection of simulators.

	'B' type					'Q' type				
	N.	**LC_{50}[1]**	**95% CLs**	**Slope**	**RF^2**	**N.**	**LC_{50}[1]**	**95% CLs**	**Slope**	**RF^2**
Before connection	1128	0.003 a	0.001-0.006	1.6	0.6	749	1.75 a	0.3-12.3	0.66	350
F1 after connection	555	0.003 a	0.001-0.005	1	0.6	1353	0.5 a	0.2-1.5	0.5	100
F2 after connection	803	0.006 a	0.004-0.008	1	1.2	876	0.03 b	0.02-0.04	0.97	6

[1]All LC_{50}s in ppm. Different letters denote non-overlap of confidence limits ($p < 0.05$).

[2]RFs in comparison to SUDS data (see Table 6)

Table 9. The effect of carbosulfan on 'B' and 'Q' biotypes before and after connection of simulators.

	'B' type					'Q' type				
	N	**LC_{50}[1]**	**95% CLs**	**Slope**	**RF[2]**	**N**	**LC_{50}[1]**	**95% CLs**	**Slope**	**RF[2]**
Before connection	448	176.9 a	57-7455	0.3	42	495	0.5 a	0.007-1.8	0.6	0.12
F1 after connection	248	77.8 ab	32-583	0.44	18.5	497	3.3 a	1.6-5.2	0.8	0.8
F2 after connection	335	13.2 b	4-34	0.36	3	340	0.4 a	0.003-1.8	0.4	0.1

[1]All LC_{50}s in ppm. Different letters denote non-overlap of confidence limits ($p < 0.05$).

[2]RFs in comparison to SUDS data (see Table 4).

4.4 Viability of crossing 'B' and 'Q' type

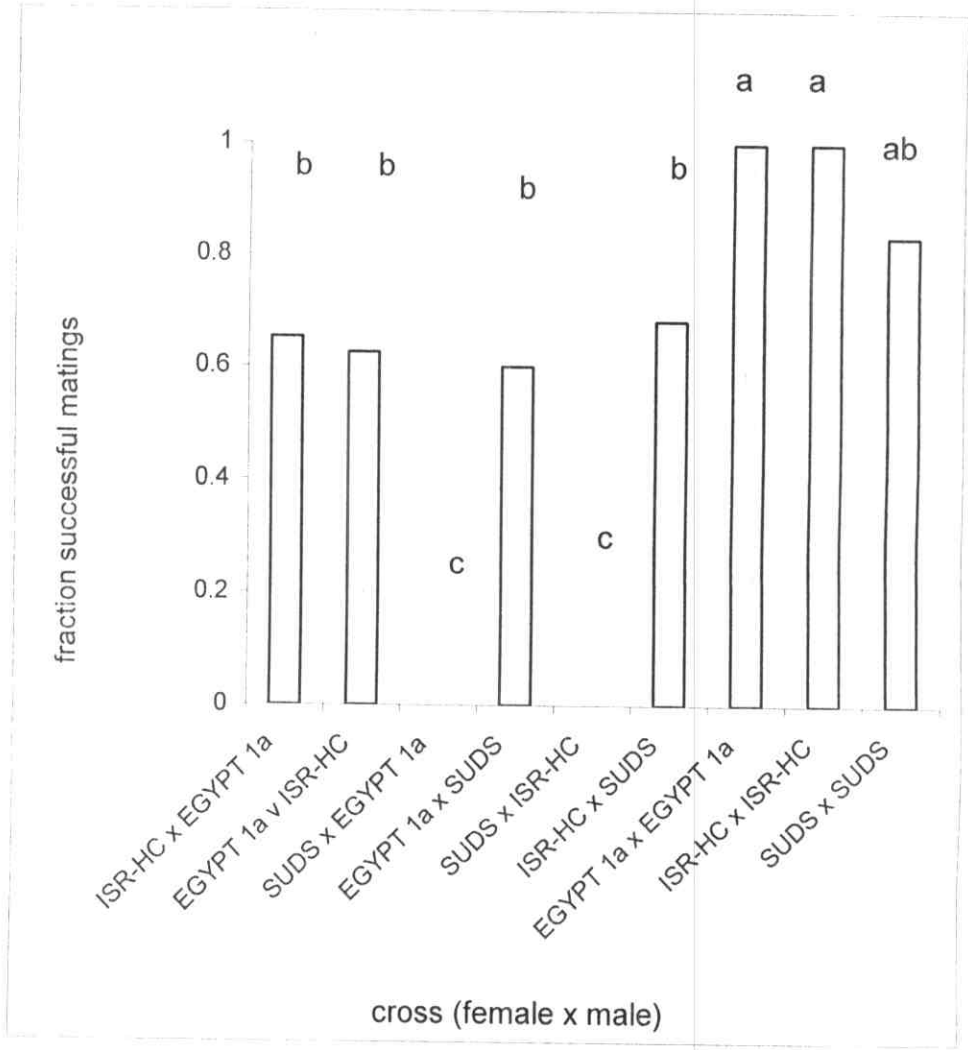
4.4.1 Single pair mating success

It was clear that some mating were successful (i.e. resulted in at least some female progeny) whilst others were not (Figure 16). Crosses involving female SUDS whitefly and male 'B' or 'Q' biotypes were always unsuccessful, although the reciprocal of this (male SUDS whitefly and 'B' or 'Q' females) did result in female progeny. The most successful crosses (100%) success involved intra-biotype crosses between B or Q biotypes. Intra-biotype crosses between SUDS males and females were also highly successful. All other crosses involving inter-biotype matches were also successful (60 – 65%), but less so than intra-biotype crosses between 'B' or 'Q' biotypes.

4.4.2 Female bias in progeny of crosses

Clearly, crosses involving female SUDS whitefly and male 'B' or 'Q' biotypes had a female bias of 0 as these were always unsuccessful. Inter biotype crosses between SUD-S, B or Q whitefly resulted in the highest female bias (0.65, 0.58 and 0.60 respectively). Of the inter-biotype crosses, only that between a 'Q' female and a SUDS male resulted a similarly high female bias (0.55; fig., 17). With the other inter-biotype crosses, all exhibited significantly lower female bias (0.40 to 0.18).

Fig. (16) Fraction of single pair mating that was successful (resulted in female offspring).

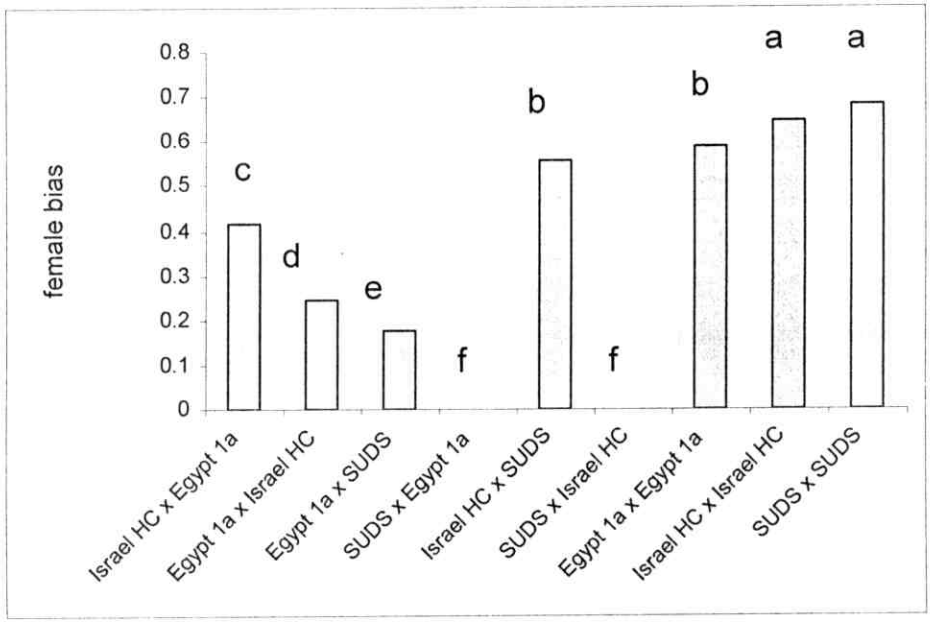


bars annotated with different letter have mating success rates significantly different to each other ($F > 3.84$, $p < 0.05$)

4.4.3 Fecundity of females in single pair mating

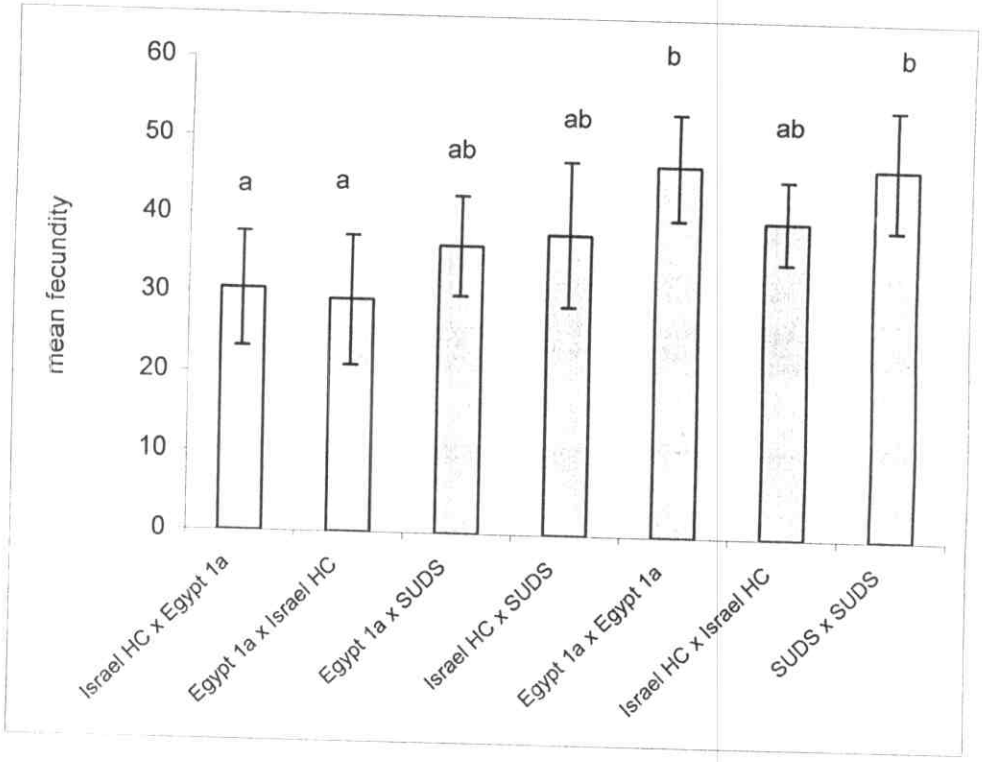
Intra-biotype mating resulted in the highest number of progeny (Fig., 18). 'B', 'Q' and SUDS mating resulted in a mean fecundity of 45, 38 and 44 eggs per female, respectively. The fecundity of intra-biotype crosses between SUDS males and B or Q females was also successful (35 and 37 eggs per female, respectively). The inter-biotype crosses between 'B' and 'Q' biotypes were least successful of all (28 eggs for the B female x Q male cross and 30 eggs for the reciprocal).

Fig. (17) Female bias in offspring of crosses between B and Q biotypes



bars annotated by different letters denote significant differences in X2 comparisons ($F > 3.84$, $p < 0.05$)

Fig. (18) Mean fecundity (and 95% confidence limits) of successfully mated females



Bars annotated with a different letter are significantly different (non-overlapping confidence limits, $p < 0.05$)

4.4.4 Inheritance of resistance

As expected ISR-HC parents were significantly less affected by profenofos than Egypt 1a or SUDS parents (Fig., 19 a and b). Their offspring however, exhibited intermediate responses. Thus resistance to profenofos is clearly a heritable trait, can be transferred to the hybrid offspring of different biotypes and is partially dominant.

As expected ISR-HC parents were significantly less affected by cypermethrin than SUDS parents (Fig., 19 c). Their offspring however, seemed to exhibit an intermediate response, although this was not significantly different to that of the SUDS parents. Thus resistance to cypermethrin is probably a heritable trait, can probably be transferred to the hybrid offspring of B and Q parents and is partially dominant.

Fig. (19 a) Response of offspring of B x Q cross (Israel HC x Egypt 1a) to profenofos

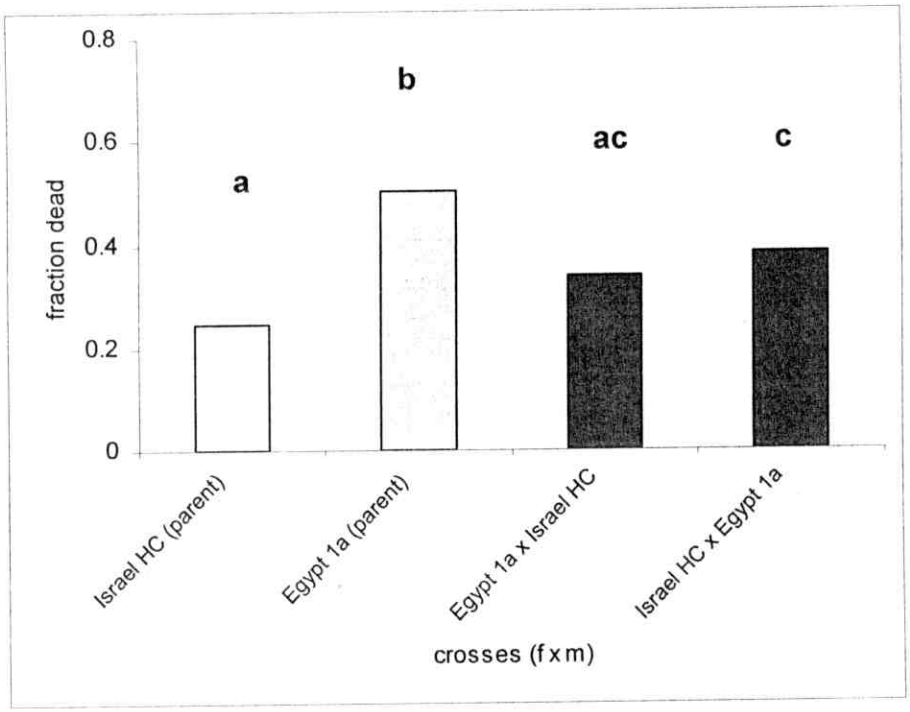


Fig. (19 b) Response of offspring of SUDS x Q cross (SUDS x Israel HC) to profenofos

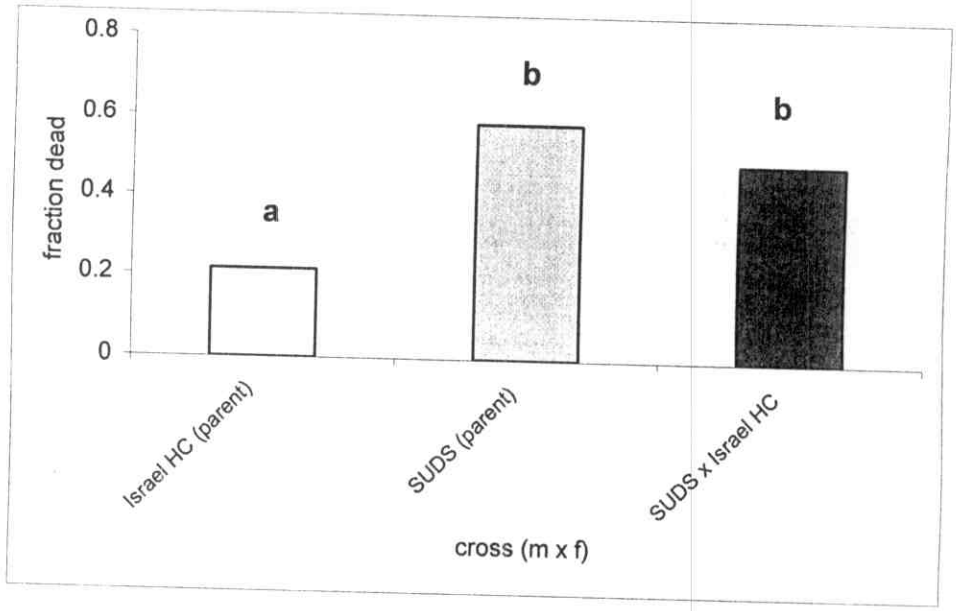
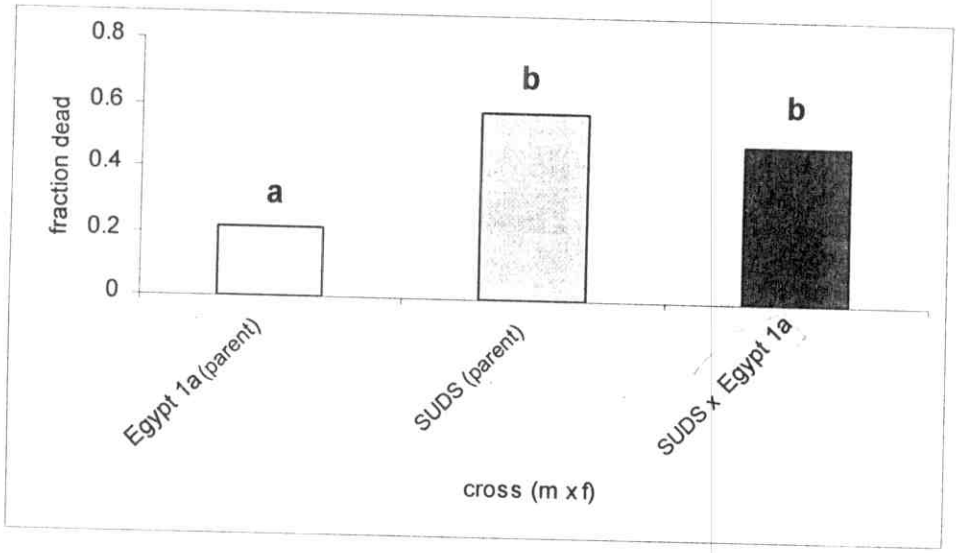


Fig. (19 c) Response of offspring of SUDS x B cross (SUDS x Egypt 1a) to cypermethrin



4.4.5 Identification of hybrids using PAGE techniques

A comparison of the banding patterns between 'B' and 'Q' and SUDS parents and their progeny (Fig., 20) shows that the offspring of single pair mating often show banding patterns different to that of their parents (often with elements of both biotypes exhibited). It is also clear however, that it would be extremely difficult to use this methodology to distinguish hybrids either in the field or in simulator experiments.

In Figure 20a, showing the results of crossing Egypt 1a males ('B') and ISR-HC females ('Q'), 'Q' biotype parents clearly have only one band in common (Q1). 'B' biotype parents clearly exhibit three (B1, B2 and B3). The offspring however, can either look very like the B parent (e.g. on channel 11 of the gel), share attributes of both B and Q parents (i.e channel 7) and exhibit a new banding pattern (i.e. channels 3, 8 and 9). The diagnostic bands for the B and Q biotypes are, in any case, so similar in mobility (also see Fig., 4) that the major bands (Q1 and B1) in these gels seem to combine in hybrids to give one band of intermediate mobility that is indistinguishable from either parental band.

In Fig 20b, showing the results of crossing a SUD-S males and an Egypt 1a females ('B'), SUD-S biotype parents clearly have two bands in common (S1 and S2). 'B' biotype parents again clearly exhibit three bands in common (B1, B2 and B3). The offspring of this cross all looked extremely similar to the 'B'

parent, in that they all appeared to have B1 and B2 bands, but differed in that they were lacking the B3 band.

In no case, could any of those offspring, even alongside the banding patterns that have become accepted as being 'B', 'Q' or SUDS standards, be identified as hybrids. Had they been part of a sample of unknown origin, they would almost certainly have been identified as B or Q types.

Fig. 20a Banding patterns of offspring of Egypt-1a female x SUDS male

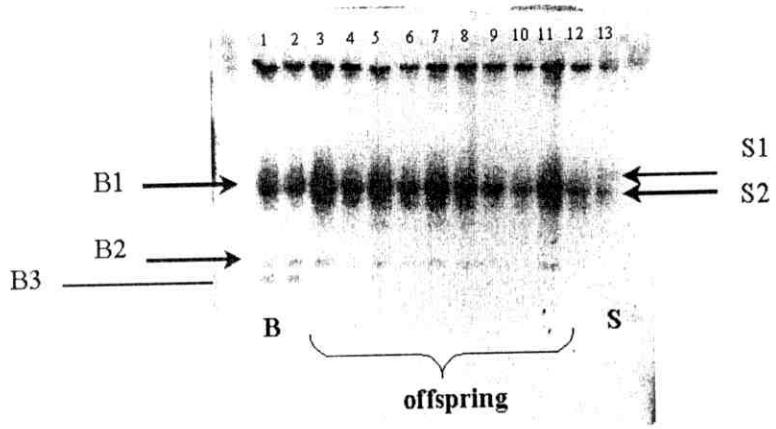


Fig. 20b Banding patterns of offspring of Israel-HC female x Egypt-1a male