CRDC 177c.
K-2001.

2001-11-21

ACRI
Myal Vale
Narrabri
NSW2390

RE:Report on trip to the UK attending the Resistance 2001 Conference.

To: Mr Ralph Schulze
Director
CRDC
Narrabri
NSW2390

Dear Ralph,

Firstly, I wish to express my gratitude to CRDC for funding my trip to attend the conference at the Institute for Arid Crop Research (IACR), Rodhamsted, UK.

The conference was very beneficial to me, especially enabling me to interact with the scientists around the world who work on various disciplines pertaining to resistance detection and managements .

Please find attached the research paper that I presented at the conference .

Yours sincerely ,

Dr Ho T. Dang

Resistance to *Bacillus thuringiensis* delta-endotoxin CryIAc in Australian *Helicoverpa armigera.* (Lepidoptera: Noctuidae)

Ho T Dang[1] and Robin Gunning[2]

Introduction:

H. armigera and *H. punctigera* are important pests of cotton. in Australia. Transgenic cotton (containing Cry1Ac) has been commercially grown in Australia for 5 years. The susceptibility of both species to *Bacillus thuringiensis* (Bt) toxins in Australia field populations has been monitored since 1993.

This paper reports results of Bt resistance monitoring for the 2000/2001 cotton season as compared with results from previous seasons. Our studies indicate the development of resistance in *H. armigera* and possible esterase mediated mechanism of resistance in *H.armigera* .

Methods:

1. Bioassays:

Helicoverpa eggs were collected throughout the cotton season from cotton and other summer crops in cotton growing regions in Australia. Eggs were then transferred onto an artificial diet, hatched and maintained to early third instar larvae. Larvae were then transferred onto a "testing diet" into which a discriminating dose of a Bt product had been incorporated (Fig 1). Dipel®, Xentari® and MVP® were used for screening (Table 1). Larval mortality was assessed after 7 and 10 days of exposure to the testing diet, respectively. The discriminating dose for each product was the LC99 for each species. Large sample sizes were monitored. (In the 2000/2001 cotton season total numbers tested larvae was 20,413 and 6,837 for *H. armigera* and *H. punctigera*, respectively).

H.armigera surviving the discriminating doses of MVP (3 ul/ml diet) were retained and bred to form resistant strains (Silver F1 and Silver F2). Single–pair mating was carried out from the F1 population of Silver strain. Neonates produced by the single–pair mating F2 isolines were tested for survivorship on young leaves of transgenic cotton plants. Mortality was assessed at five days after introduction.

A Cry1Ac susceptible, laboratory strain (KO) was obtained from Dr Ray Ackhurst (CSIRO Entomology). Silver F2, KO plus a field strain from Emerald, Queensland, were bioassayed with MVP and full dose/mortality curves obtained. Data were analysed by probit analysis (Gillespie,1995:probit 5 for window P38).

2. Whole plant bioassays:

Larvae from resistant, Silver (F4) and KO strains were placed on caged plants under greenhouse conditions. Two neonates were introduced into each caged plant at 50 days after seeding (squaring stage). Larval and pupal survivorship monitored and pupal weight recorded.

3.Bioassay of field populations:

In collaboration with Monsanto Australia, larval mortality was assessed using F1 progeny of field collected, over wintering pupal populations from different cotton

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growing regions in Australia (collected in August, 2001). Diet containing MVP (3ul/ml) was used for testing and mortality was assessed at after 10 days .

4. Resistance Mechanism Studies:

Susceptible and Silver F2 *H.armigera* homogenates were incubated with concentrations of purified Cry1Ac. Total esterase activity was detected using 1-naphthyl acetate as a substrate. Incubates were also run on polyacrylamide gels and stained for esterase activity.

Results:

Susceptibility of field populations of Australian *H. armigera* to Dipel® and Xentari® has not changed .There was, however, a significant shift in susceptibility of *H. armigera* to MVP® (containing CryIAc toxin) in 2000-2001 season (Table 2, Figures 2 and 3).

Compared to the laboratory susceptible *H. armigera*, the Silver selected F1 and F2 strains were 118 and 187 fold less susceptible to MVP, respectively. Field strains showed smaller resistance factors (Table 3). Laboratory selection for two generations at the discriminating dose of MVP (3ul/ml diet) resulted in significant increase in resistance to Cry1Ac, from 77 fold (Emerald F4) to 187 fold (SF2).

Mortality of neonates from SF2 isolines tested on young leaves of transgenic cotton plants is shown in figure 6. Among the fourteen isolines, five had 0% mortality, seven had mortality ranging from 2.5 to 7.9%, one had 20% mortality and one had 80% mortality. The F2 population resulting from mass mating, the field strain and susceptible strain had 2.5, 20 and 81.9 percent mortality, respectively. The results indicate that the resistance may be partial dominant. However, only two generations selected at the discriminating dose of MVP (3 ul/ml diet) and populations are still highly heterozygous. Thus the results obtained at this stage should be interpreted with caution with respect to the genetics of resistance.

Whole plant bioassay data (Table4) show the survival of Silver strain F4 on transgenic and conventional cotton plants, with 10.75% of neonates surviving through to pupation on Ingard plants . Susceptible larvae (KO strain) did not survive beyond the first week after introduction onto Ingard plants.

Table 5 shows discriminating dose diet MVP bioassay data from recently collected field populations. The results indicate the varying survival of different populations according to selection pressure. Average survival of six field populations was about 9.2%, these figures are higher than the average survival observed in the 2000–01 cotton season (7.1%).

Polyacrylamide gels (Figure 4) show that larvae from resistant strain (Silver F1) had increased esterase activity compared with the susceptible strain (KO). There were also considerable differences in esterase banding patterns between the susceptible and resistant strains. Our data (Figures 4 and 5), show that Cry1Ac inhibited esterase activity in the resistant strain, whereas there was no detectable binding of Cry1Ac to esterase in the susceptible strain. Esterases are adhesive molecules and have already been shown to sequester insecticides in *H. armigera.* It is likely that the reduced susceptibility to Cry1Ac is at least in part, due to esterase sequestration of Cry1Ac in *H. armigera.*

Conclusion :

The change in susceptibility of Australian *H. armigera* to Cry1Ac is great concern to the Australian cotton industry that depends on the use of transgenic cotton as a fundamental component for IPM .Currently,145.000 ha or 30 % of the Australian cotton acreage is planted with transgenic cotton. Our studies on the inheritance and mechanisms of the reduced susceptibility in *H. armigera* to Cry1Ac are continuing.

Figure 1: Testing with a discriminating dose of Dipel®, Xentari® or MVP®

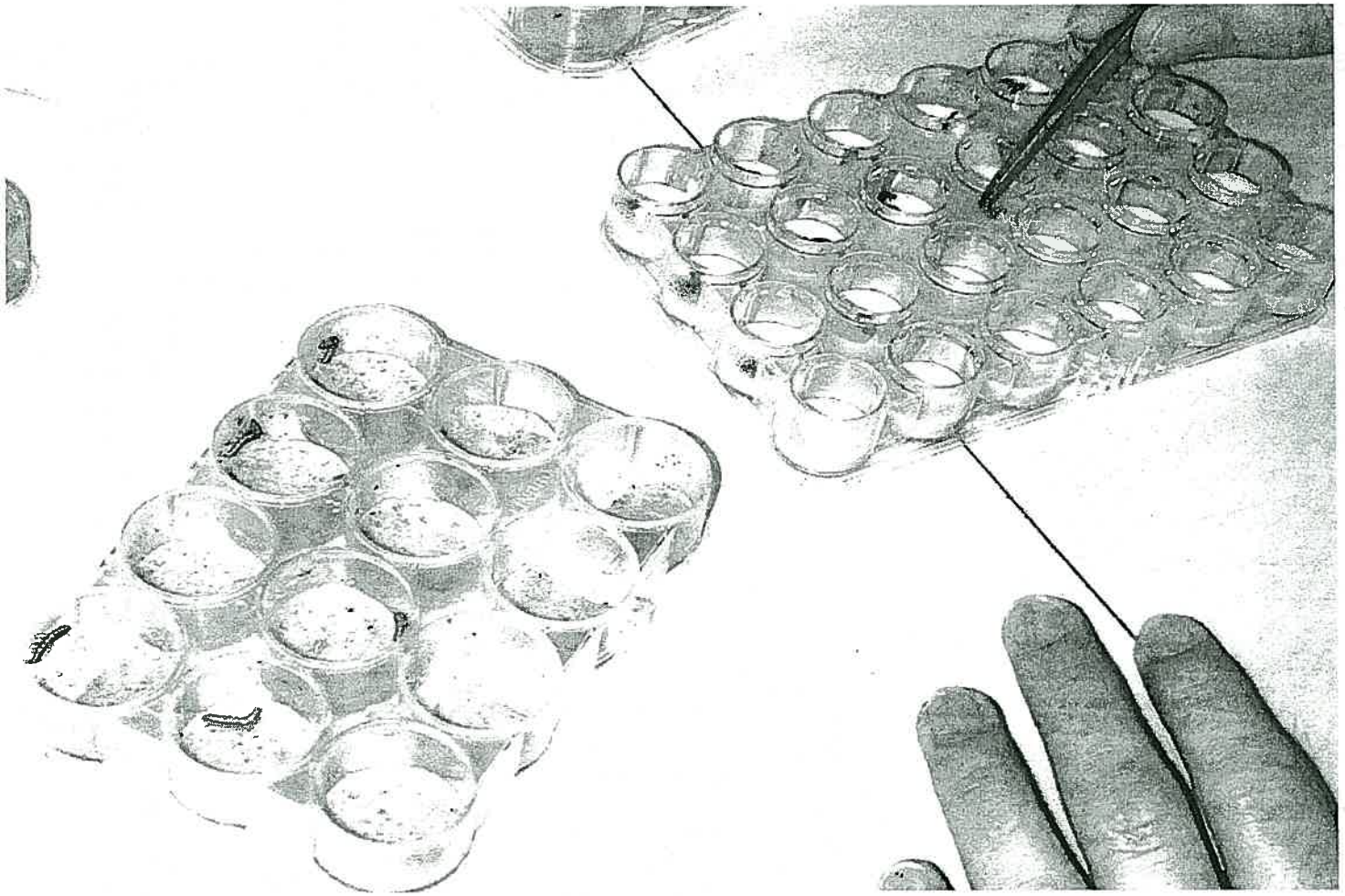

Table 1: Products tested against *H.armigera* and *H.punctigera* in the Bt Resistance Monitoring Survey(DD=Discriminating Dose)

Dipel® (DD=2mg/ml)
Cry 1Aa, Cry 1Ab, Cry 1Ac, Cry2A, Cry2B, spore

Xentari® (DD=2mg/ml)
Cry 1Aa, Cry 1Ab, Cry1C, Cry1D, Cry 2B, spore

MVP/MVP2® (DD=3ul/ml)
Cry 1Ac encapsulated in dead *seudomonas flourescens* cells

Table 2: Summary of resistance monitoring survey 1996 - 2001

Bt Product	Year/1	*H. armigera*		*H. punctigera*	
		% Survival	Number Tested	% Survival	Number Tested
Dipel®	1996/97	0.3	6149	0.5	1788
	1997/98	0.7	7580	1.3	1699
	1998/99	0.6	9974	1.4	974
	1999/00	0.7	14295	0.2	1496
	2000/01	1.0	5143	0.5	1393
Xentari®	1996/97	0.4	4980	0.5	1155
	1997/98	0.2	3130	0.4	974
	2000/01	0.8	3698	0.6	1059
MVP®	1997/98*	0	2575	0	1217
	1999/00**	2.6	11275	2.3	1884
	2000/01**	7.1	11572	3.7	4385

* Data based on screening with neonates

** Data based on screening with 3rd instar larvae

1/Results of 96-99 survey from Dr N.W.Forester .

Figure 2: Percent survival of *H. armigera* (MVP - 3ul/ml)

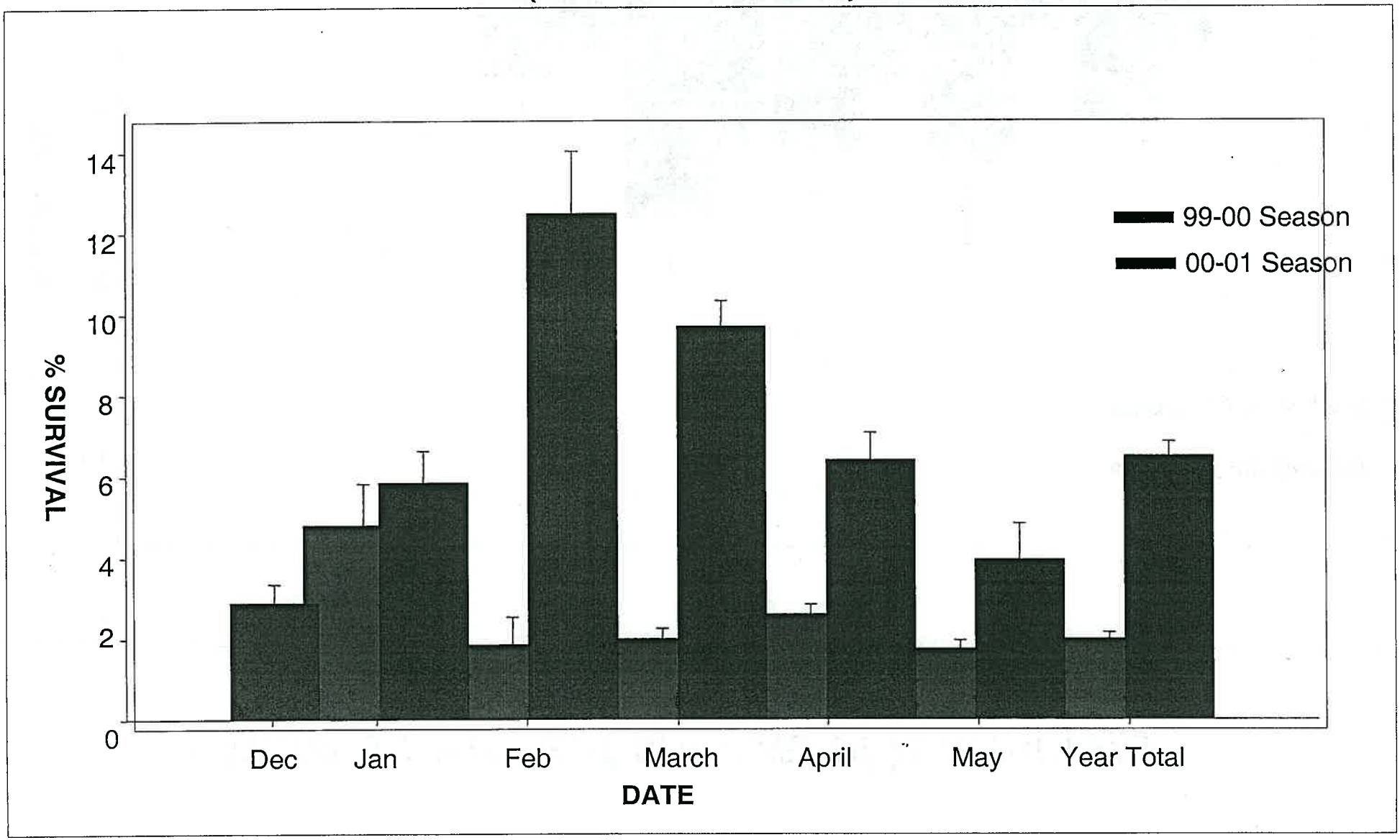

Figure 3 : Percent survival of H.armigera *(Dipel -2mg/ml)*

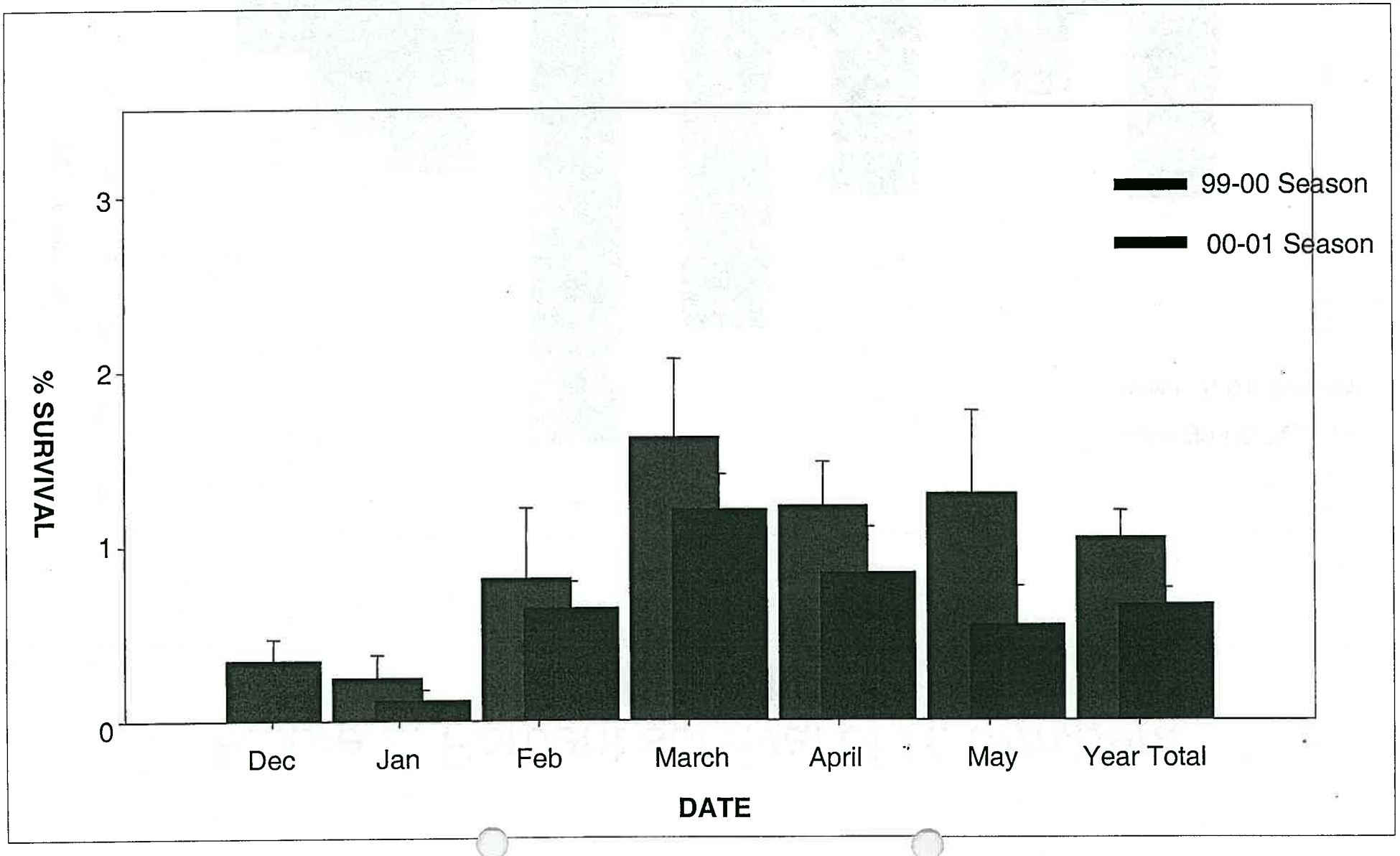

Table 3: LC99, LC50 and Resistance Factor (RF) of selected and field strains of *H. armigera* tested against MVP*

Strain	Selection	LC99	LC50	RF**
1. Silver F1	+	201	3.099	118 fold
2. Silver F2	+	321	4.033	187
3. Emerald F3 (Field)	-	48	0.665	28
4. Emerald F4	-	132	1.303	77
5. Ko (Susceptible)	-	1.71	0.084	1

* Slopes of probit line for strain 1 to 5 are 1.06, 1.02, 1.04, 0.96 and 1.48, respectively

** Resistance Factor based on LC99

Figure 4: Polyacrylamide gels showing effects of Cry1Ac on esterase activity in Cry1Ac - susceptible and Cry1Ac- resistant Australian *H. armigera*

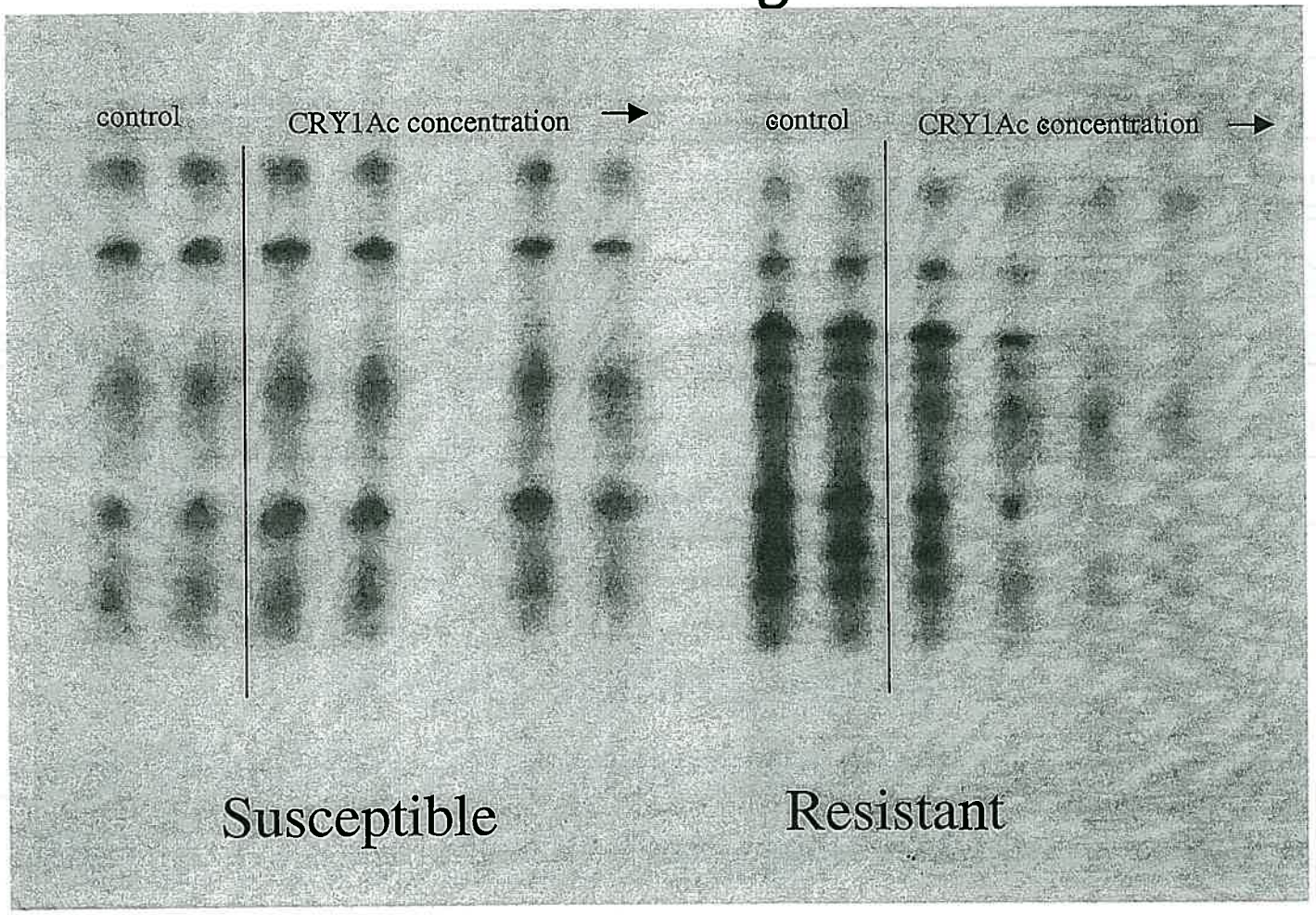

Figure 5: Inhibitory effects of Cry1Ac on total esterase activity in Cry1Ac -susceptible and Cry1Ac-resistant Australian *H. armigera*

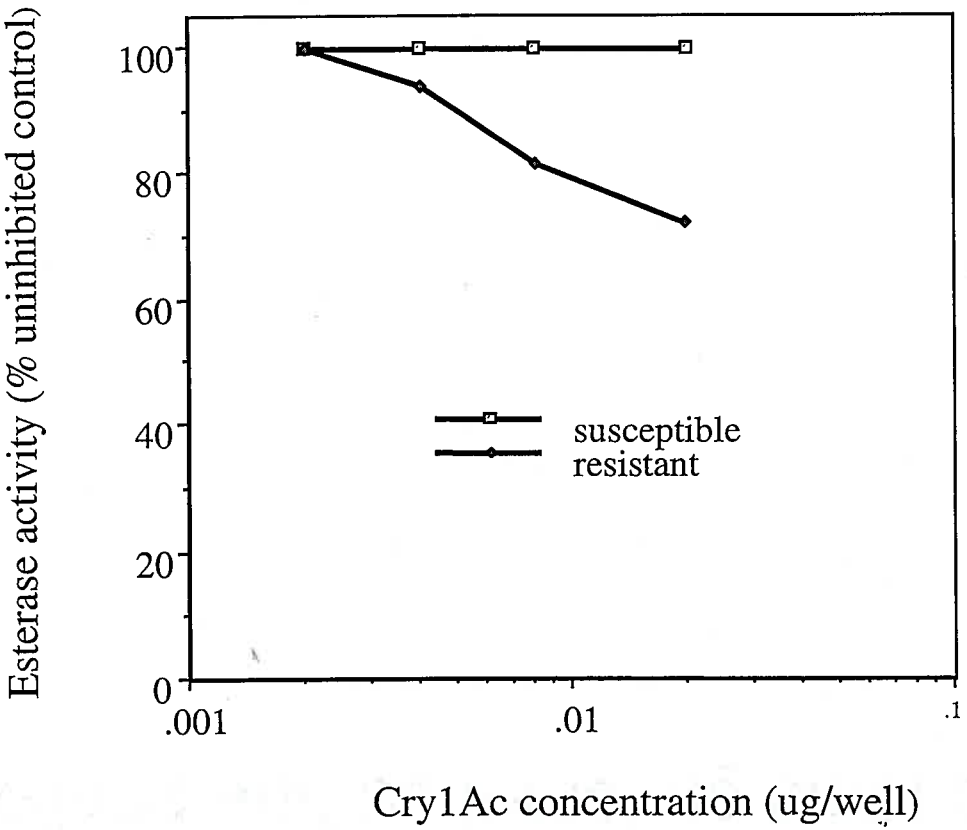

Figure 6: Results of leaf bioassay of the F2 single pair mating isolines and bulk-mating strains of *H. armigera*

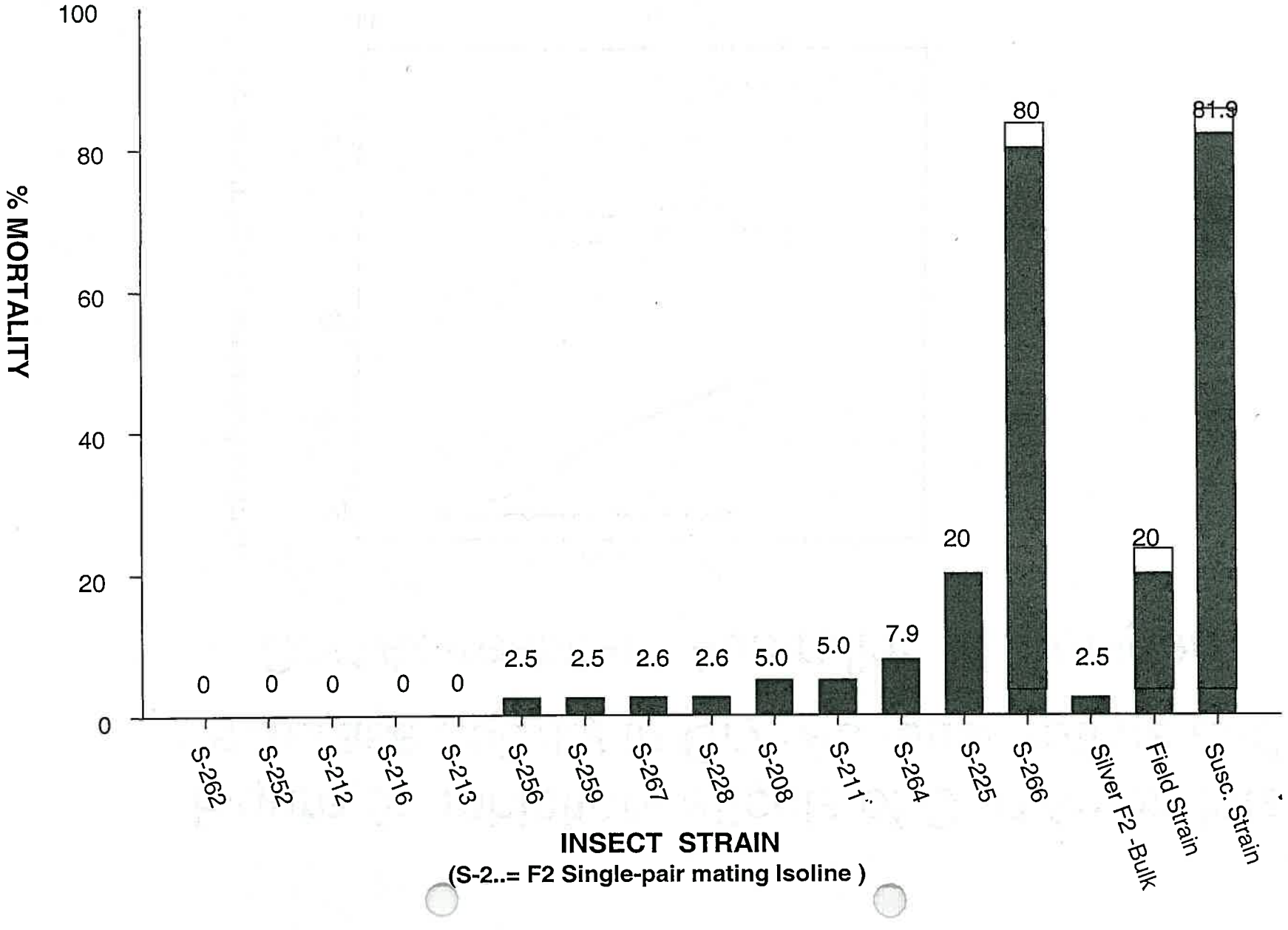

Table 4: Percentage survivorship of *H. armigera* on greenhouse plants[1]

Insect	Plant Type[2]	No. of Plants	Larval Survival		Pupal Survival	Pupal Weight (mg)
			7 DAI[3]	14 DAI		
Silver F4	Ingard	200	25.50	13.25	10.75	325.1
Silver F4	Convention	40	92.0	80.0	55.0	327.1
KO	Ingard	40	1.25	0	0	-
KO	Convention	40	82.0	78.0	57.0	304.5

1. Two first instar larvae were introduced to each caged plant at 50 days old.
2. Ingard: Sioct 289i, Convention: Sicot 189

3.DAI=Day after introduction .

Table 5: Percent survivorship of *H. armigera* field and laboratory strains tested in September 2001[1]

Insect Colony[2]	No. Tested	% Survival
Thebo	308	9.09
Kununurra	162	8.64
Agriland	256	16.8
Warren	330	4.55
Boggabri	42	4.76
Dalby	481	11.23
SF4–Lab strain	96	43.7
KO–Lab strain	72	0

1. Third instar larval tested with diet containing MVP (3 ul/ml diet), mortality assessed at 10 days after introduction.
2. Field strains are F1 progeny of pupae collected from cotton fields in August, 2001. Laboratory strain: SF4 (Silver F4, resistant strain) and KO (susceptible strain).