FINAL PROGRESS REPORT

PROJECT NO.: ARS

TITLE: Sublethal effects of *Bt* and propensity for resistance development in apple leafrollers

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REPORTING PERIOD: 1998-99

ACCOMPLISHMENTS:

1. Refined laboratory protocols for studying prolonged effects on survivors of sublethal exposure to *Bt* δ-endotoxins.

2. Determined lethal concentrations of selected *Bt* preparations containing the *cry* 1ac and *cry* 2a crystal toxins. Primary powders from Ecogen and commercial formulations from Abbott and Ecogen were bioassayed against OBLR and *Pandemis* neonates.

3. Determined sublethal effects of *cry* 1ac and *cry* 2a *Bt* crystal toxins on OBLR and *Pandemis* larvae and pupae, when larvae were first exposed to an LC_{60} for 7 days and then fed on fresh media until death or pupation.

4. Studies were conducted on the propensity for resistance development were performed with OBLR.

Background: The most widely used of all microbial control agents is *Bacillus thuringiensis* (*Bt*). It has been an ideal component in integrated pest management strategies due to its selectivity for and efficacy against targeted organisms including lepidopteran pests of orchards (Lacey and Knight 1998). However, resistance to *Bt* toxins has been reported for several other insects. The most notable example of resistance under field conditions is that of diamond back moth (DBM), *Plutella xylostella*. Widespread resistance to *Bt* in the DBM has changed the perception of crucifer growers on the long term reliability of this bacterium as a microbial control agent; its use now includes strategies for management of resistance. One of the strategies is to use toxins with different modes of action. Most of the commercial formulations used in apple orchards are based on *Bacillus thuringiensis* var. *kurstaki* predominantly containing the *cry* 1ac toxin. Alternative *Bt* preparations contain the *cry* 2a toxin.

Results of earlier bioassay of several *cry* 1 toxins of *Bacillus thuringiensis* against four tree fruit leafroller species revealed good larvicidal activity of most of the toxins and some sublethal effects on development time and pupal weights (Knight et al. 1998). Most of the toxins tested were expressed in *Pseudomonas fluorescens* using the Mycogen CellCap© encapsulation system. Larvae were also continually exposed to toxin.

Toxins in the same general grouping tend to have similar modes of action. In addition to the *cry* 1 toxins, it would also be beneficial to ascertain the effects of the *cry* 2 toxins against leafrollers.

Current studies: A modified bioassay method was used in our studies to limit the amount of toxin consumption to only 7 days. Primary powders of *cry* 1ac and *cry* 2a toxins which were produced in sporulating *B. thuringiensis* were compared for larvicidal activity and sublethal effects against OBLR and *Pandemis* leafroller. After the exposure period, mortality was determined in treated and control larvae and they were removed to fresh media and observed daily until pupation. Pupae were sexed and weighed and individually placed in shell vials until emergence. In addition to daily mortality, development time from neonates to pupae, pupal weight and sex, and duration of the pupal stage were monitored. The efficacy of 4 commercial preparations containing either *cry* 1a or *cry* 2a toxins was determined in laboratory bioassays using a 7 day exposure. All assays were run at 25°C and 16 hours daylight and 8 hours of dark. Bioassays were also conducted with an Ecogen liquid preparation of cry 2 toxin under the same conditions described above for the primary powders, except that a 10 exposure period was used.

Based on mortality assessed after the 7 day exposure to primary powders containing *cry* 1ac and *cry* 2a toxins, OBLR was the more sensitive of the two species. It was also slightly more sensitive to the *cry* 2a toxin than to the *cry* 1ac toxin (Table 1). The susceptibility of *Pandemis* to both toxins was similar.

Cumulative mortality in *Pandemis* and OBLR after exposure to primary powder preparations containing *cry* 1ac or *cry* 2a toxins is presented in Figures 1-5. Significant and in some cases substantial additional mortality was observed between that which was assessed after 7 of exposure and over the next 3 weeks when the larvae were placed on fresh media. Additional mortality in OBLR and *Pandemis* was also observed following the 10 exposure period to the liquid preparation of *cry* 2a toxin (Figures 6 and 7).

In those treated larvae that survived until pupation there was a significant delay in pupation (Tables 2-7), but pupal weights were not significantly different from that of the controls. Likewise the duration of the pupal period was not affected in survivors of sublethal concentrations of *Bt* toxin.

What this could mean in terms of sublethal effects in the orchard is that surviving *Bt*-treated larvae are exposed to natural enemies and other mortality factors for a longer period of time than control larvae. Unfortunately, they may also consume more host plant. Apparently most of the treated larvae are able to repair damage to midgut epithelium. If they survive to the pupal stage they amass the same amount of nutritive reserves, hence the same pupal weight and pupal duration as controls.

Results of bioassays of commercial formulations are reported in Tables 8 and 9. Unlike the assays of primary powders, *Pandemis* larvae were slightly more sensitive than OBLR

to the Lepinox formulation. Similar susceptibilities were observed for OBLR and *Pandemis* to the CryMax formulation (Table 8). Both formulations are based on *Bacillus thuringiensis* var. *kurstaki*.

Similar to results for the Ecogen formulations, *Pandemis* larvae were considerably more sensitive to the Abbott formulations than were OBLR larvae. Its sensitivity to the two formulations was similar. In contrast to assays with primary powders, OBLR was more sensitive to the Dipel formulation than to the Xantari formulation. The Dipel formulation is based on the *kurstaki* variety of *B. thuringiensis* which contains the *cry* 1ac toxin, whereas Xen Tari is based on the *aizawa* variety of *B. thuringiensis* which contains the *cry* 2a toxin.

Attempts to generate resistance in OBLR were made by rearing survivors of an initial LC_{60}, and interbreeding them, exposing their offspring and interbreeding them. In all cases limited survival did not permit tests beyond the F_3 generation. Based on these findings and the low number of generations per annum, it is unlikely that resistance to commercial formulations of *Bt* will be a threat in the foreseeable future. If, however, transgenic plants are utilized that are encoded for single toxins of *Bt*, the threat of resistance development will be sufficiently high to take precautions to avoid it (provision of refugia, high dose expression, alternation of toxins, etc.).

REFERENCES:

Knight, A. L., L. A. Lacey, B. Stockoff and R. Warner. 1998. Activity of *Cry* 1 endotoxins of *Bacillus thuringiensis* for four tree fruit leafroller pest species (Lepidoptera: Tortricidae). J. Agric. Entomol. 15: 93-103.

Lacey, L. A. and A. L. Knight. 1998. Use of entomopathogens for the microbial control of lepidopterous pests of pear and apple orchards. Proc. VII International Colloquium on Invertebrate Pathology and Microbial Control. 23-28 August, 1998. Sapporo, Japan. pp. 194-198.

Table 1. Bioassay of *Bacillus thuringiensis* primary powders containing *cry* 1ac toxin or *cry* 2a toxin against neonate larvae of *Choristoneura rosaceana* and *Pandemis pyrusana*.

	Treatment[1]	Dosage (ng/cm^2)	Mean % Mortality +/- S.E.
OBLR	*cry* 1ac	130	96.7 ± 1.3
		52	91.7 ± 3.2
		26	78.3 ± 6.4
		5.2	36.7 ± 14.8
		0.52	1.7 ± 1.3
	cry 2a	26	98.3 ± 1.3
		5.2	60.0 ± 5.8
		2.6	43.3 ± 2.6
		1.2	26.7 ± 1.3
		0.52	15.0 ± 3.8
Pandemis	*cry* 1ac	260	98.3 ± 1.3
		52	83.3 ± 6.4
		26	83.3 ± 6.4
		13	53.3 ± 1.3
		2.6	23.3 ± 8.3
	cry 2a	260	96.7 ± 1.3
		130	93.3 ± 1.3
		52	86.7 ± 1.3
		26	73.3 ± 1.3
		0.52	6.8 ± 2.6

[1] Control mortality for both OBLR and Pandemis was 1.7 ± 1.3 %.

[2] Means are generated from assays run on three separate dates.

Table 2. Sublethal effects of an LC_{60} of *Bacillus thuringiensis* cry 1ac toxin (primary powder) on *Choristoneura rosaceana* treated as neonate larvae. Exposure to surface treated medium was made at 25°C for 7 days. Survivors were placed on fresh nontreated medium after 7 day exposure until pupation.

treatment	n	mean development time (days from hatch to pupation duration)	mean pupal weight (g)	mean pupal duration (days)
control males	70	21.3429	0.0717	6.7353
control females	66	22.0303	0.1268	7.4531
treated male	11	31.9091	0.0725	6.8889
treated females	15	30.4000	0.1249	7.1818

Table 3. Sublethal effects of an LC_{60} of *Bacillus thuringiensis* cry 2a toxin (primary powder) on *Choristoneura rosaceana* treated as neonate larvae. Exposure to surface treated medium was made at 25°C for 7 days. Survivors were placed on fresh nontreated medium after 7 day exposure until pupation.

treatment	n	mean development time (days from hatch to pupation duration)	mean pupal weight (g)	mean pupal duration (days)
control males	59	22.6102	0.0740	6.2885
control females	55	23.9815	0.1261	5.8800
treated male	16	30.4375	0.0698	7.7143
treated females	17	31.0000	0.1368	6.0000

Table 4. Sublethal effects of an LC_{60} of *Bacillus thuringiensis* cry 1ac toxin (primary powder) on *Pandemis pyrusana* treated as neonate larvae. Exposure to surface treated medium was made at 25˚C for 7 days. Survivors were placed on fresh nontreated medium after 7 day exposure until pupation.

treatment	n	mean development time (days from hatch to pupation duration)	mean pupal weight (g)	mean pupal duration (days)
control males	79	27.9221	0.0598	9.6438
control females	63	26.8254	0.0924	9.7049
treated male	20	31.8000	0.0595	9.1250
treated females	22	33.4545	0.0944	8.4211

Table 5. Sublethal effects of an LC_{60} of *Bacillus thuringiensis* cry 2a toxin (primary powder) on *Pandemis pyrusana* treated as neonate larvae. Exposure to surface treated medium was made at 25˚C for 7 days. Survivors were placed on fresh nontreated medium after 7 day exposure until pupation.

treatment	n	mean development time (days from hatch to pupation duration)	mean pupal weight (g)	mean pupal duration (days)
control males	26	33.9231	0.0529	9.3333
control females	6	33.3333	0.0704	8.6667
treated male	7	30.8571	0.0570	8.0000
treated females	5	27.4000	0.1033	8.4000

Table 6. Sublethal effects of an LC_{60} of *Bacillus thuringiensis* cry 2a toxin (Ecogen liquid) on *Choristoneura rosaceana* treated as neonate larvae. Exposure to surface treated medium was made at 25˚C for 10 days. Survivors were placed on fresh nontreated medium after 10 day exposure until pupation.

treatment	n	mean development time (days from hatch to pupation duration)	mean pupal weight (g)	mean pupal duration (days)
control males	72	20.2917	0.0770	8.2388
control females	80	21.9494	0.1312	7.7917
treated male	21	25.6667	0.0740	7.7500
treated females	28	27.0714	0.1418	7.2692

Table 7. Sublethal effects of an LC_{60} of *Bacillus thuringiensis* cry 2a toxin (Ecogen liquid) on *Pandemis pyrusana* treated as neonate larvae. Exposure to surface treated medium was made at 25˚C for 10 days. Survivors were placed on fresh nontreated medium after 10 day exposure until pupation.

treatment	n	mean development time (days from hatch to pupation duration)	mean pupal weight (g)	mean pupal duration (days)
control males	28	27.2143	0.0535	9.9167
control females	30	27.5667	0.0842	9.3704
treated male	6	38.0000	0.0510	9.3333
treated females	4	32.7500	0.0782	9.5000

Table 8. Bioassay of the Ecogen formulations, Lepinox wdg and CryMax, of *Bacillus thuringiensis* against neonate larvae of *Choristoneura rosaceana* and *Pandemis pyrusana*.

	Treatment[1]	Dosage (ng/cm^2)	Mean % Mortality[2] +/- S.E.
OBLR	Lepinox[3]	260	100.0 ± 0.0
		52	61.7 ± 3.2
		26	40.0 ± 5.8
		13	20.0 ± 1.9
		5.2	13.3 ± 5.1
	CryMax[4]	130	86.7 ± 5.1
		52	68.3 ± 10.9
		26	50.0 ± 1.9
		5.2	15.0 ± 1.8
		2.6	3.3 ± 1.3
Pandemis	Lepinox	130	95.0 ± 1.9
		26	70.0 ± 5.8
		13	16.0 ± 3.2
		5.2	21.7 ± 5.1
		2.6	3.3 ± 2.6
	CryMax	130	100.0 ± 0.0
		39	75.0 ± 1.9
		26	73.3 ± 4.5
		5.2	28.3 ± 2.6
		2.6	20.0 ± 3.8

[1] Control mortality for OBLR was 6.7 ± 3.2 % and pandemis was 3.3 ± 2.6 %.

[2] Means are generated from assays run on three separate dates.

[3] Lepinox wdg is a water dispersible granule containing 15% toxin from *Bacillus thuringiensis* var *kurstaki* strain EG7826.

[4] CryMax is a water dispersible granule containing 15% toxin from *Bacillus thuringiensis* var *kurstaki* strain EG7841.

Table 9. Bioassay of the Abbott Laboratories formulations, Xen Tari and DiPel, of *Bacillus thuringiensis* against neonate larvae of *Choristoneura rosaceana* and *Pandemis pyrusana*.

	Treatment	Dosage (ng/cm^2)	Mean % Mortality +/- S.E.
OBLR	Xen Tari[1]	0	0.8 ± 0.8
		520	91.3 ± 1.3
		260	86.1 ± 3.2
		130	68.3 ± 4.4
		52	28.8 ± 5.2
		5.2	22.7 ± 4.7
	DiPel[2]	0	1.7 ± 1.1
		130	92.5 ± 2.5
		52	69.2 ± 8.7
		26	55.8 ± 10.2
		13	27.5 ± 3.2
		5.2	11.7 ± 3.6
Pandemis	Xen Tari[3]	0	2.9 ± 1.0
		52	75.0 ± 7.2
		26	54.9 ± 10.4
		13	47.5 ± 7.2
		5.2	19.3 ± 4.1
		2.6	5.7 ± 4.1
	DiPel[4]	0	3.8 ± 1.2
		39	92.0 ± 3.0
		26	78.0 ± 3.4
		13	64.0 ± 4.6
		5.2	25.0 ± 4.2
		2.6	15.2 ± 5.2

[1] Means are generated from assays run on three to six separate dates.
[2] Means are generated from assays run on four to six separate dates.
[3] Means are generated from assays run on six to seven separate dates.
[4] Means are generated from assays run on five separate dates.

DiPel is 10.3% active ingredient - *cry*1ac.
Xen Tari is 6.4% active ingredient - *cry*2a.

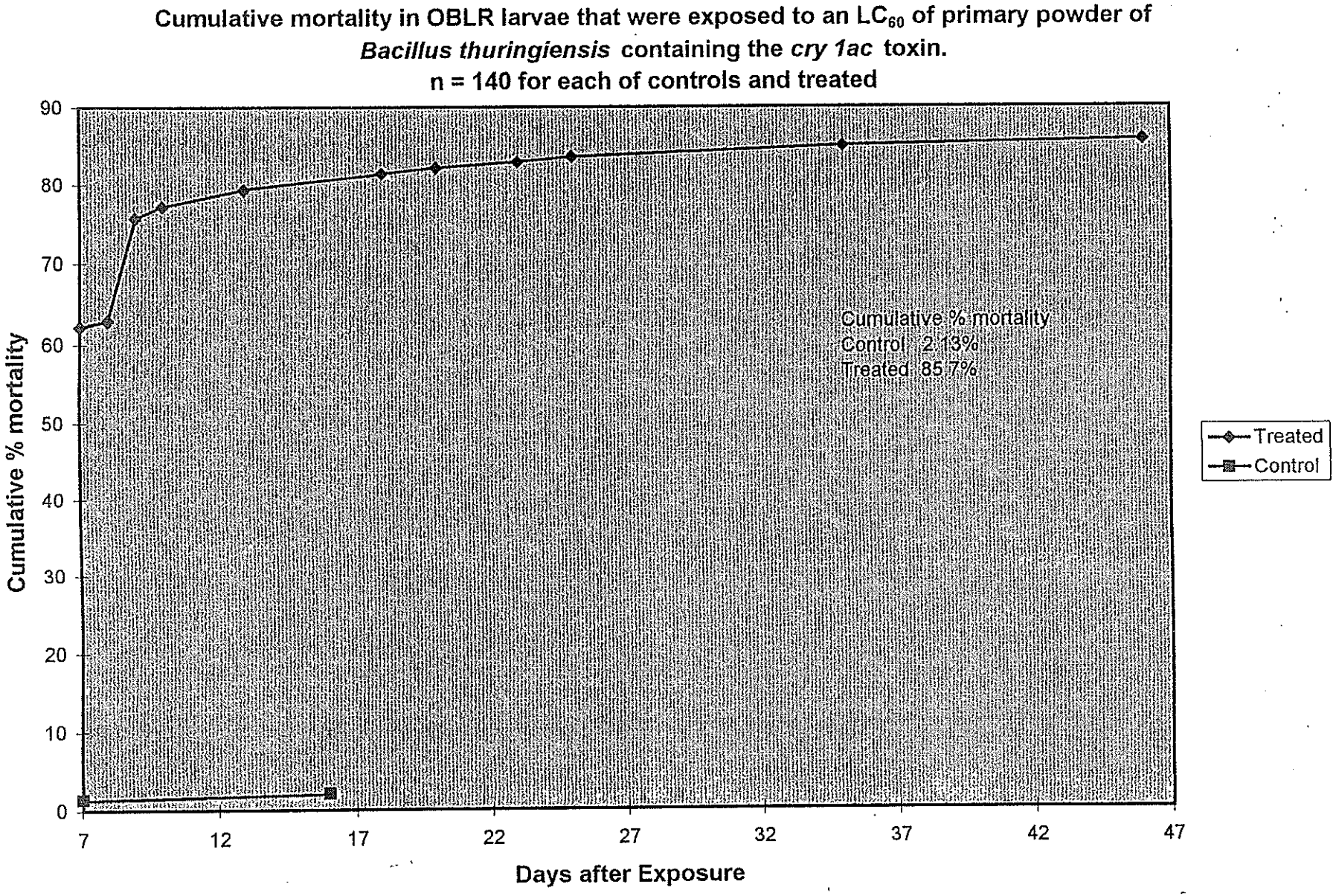
Cumulative mortality in OBLR larvae that were exposed to an LC_{60} of primary powder of *Bacillus thuringiensis* containing the *cry 1ac* toxin.
n = 140 for each of controls and treated
Cumulative % mortality
90
80
70
60
50
40
30
20
10
0
7
12
17
22
27
32
37
42
47
Days after Exposure
Cumulative % mortality
Control 2.13%
Treated 85.7%
Treated
Control

Figure 1

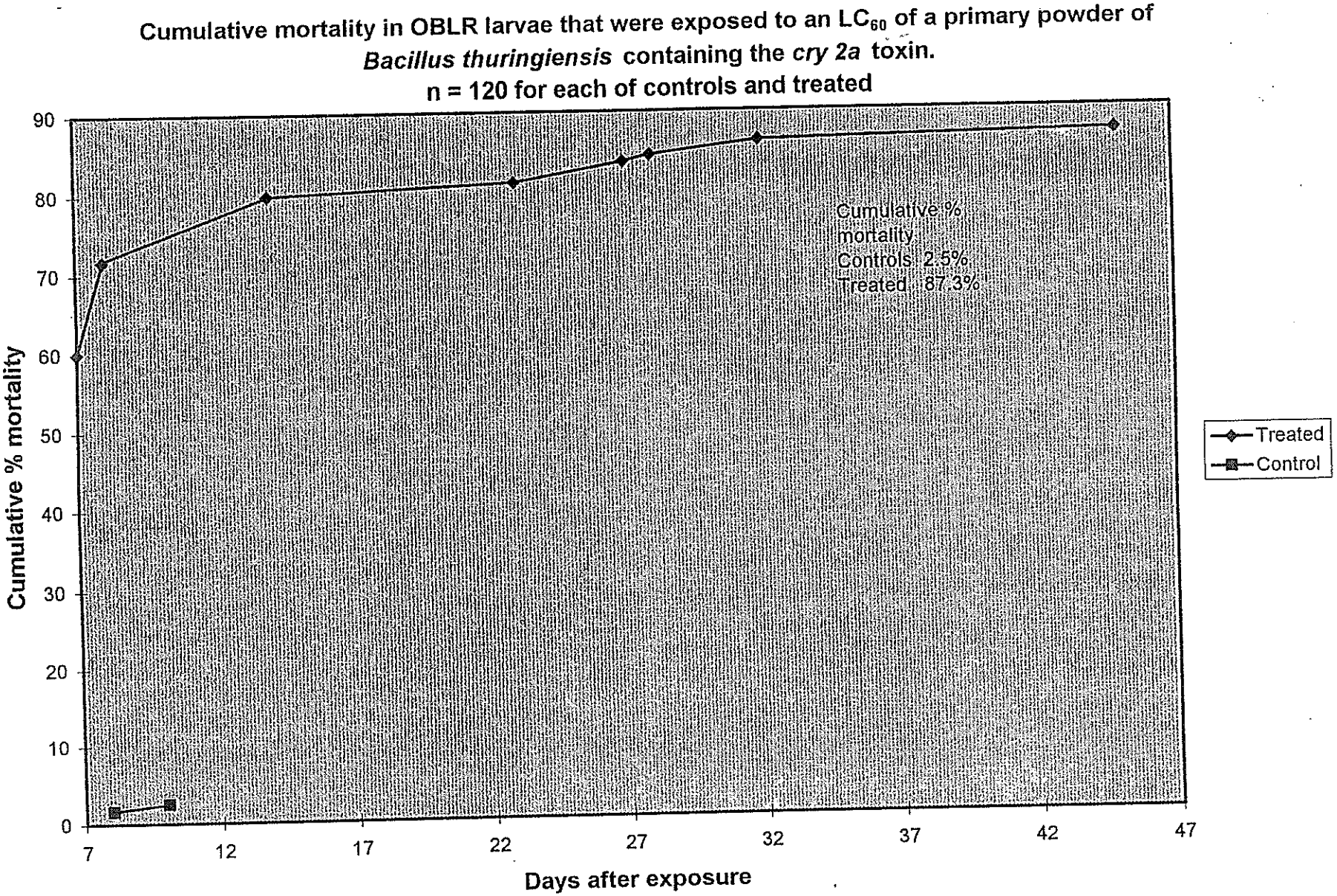
Cumulative mortality in OBLR larvae that were exposed to an LC_{60} of a primary powder of *Bacillus thuringiensis* containing the *cry 2a* toxin.
n = 120 for each of controls and treated
Cumulative % mortality
Controls 2.5%
Treated 87.3%
Cumulative % mortality
Days after exposure
Treated
Control
0
10
20
30
40
50
60
70
80
90
7
12
17
22
27
32
37
42
47

Figure 2

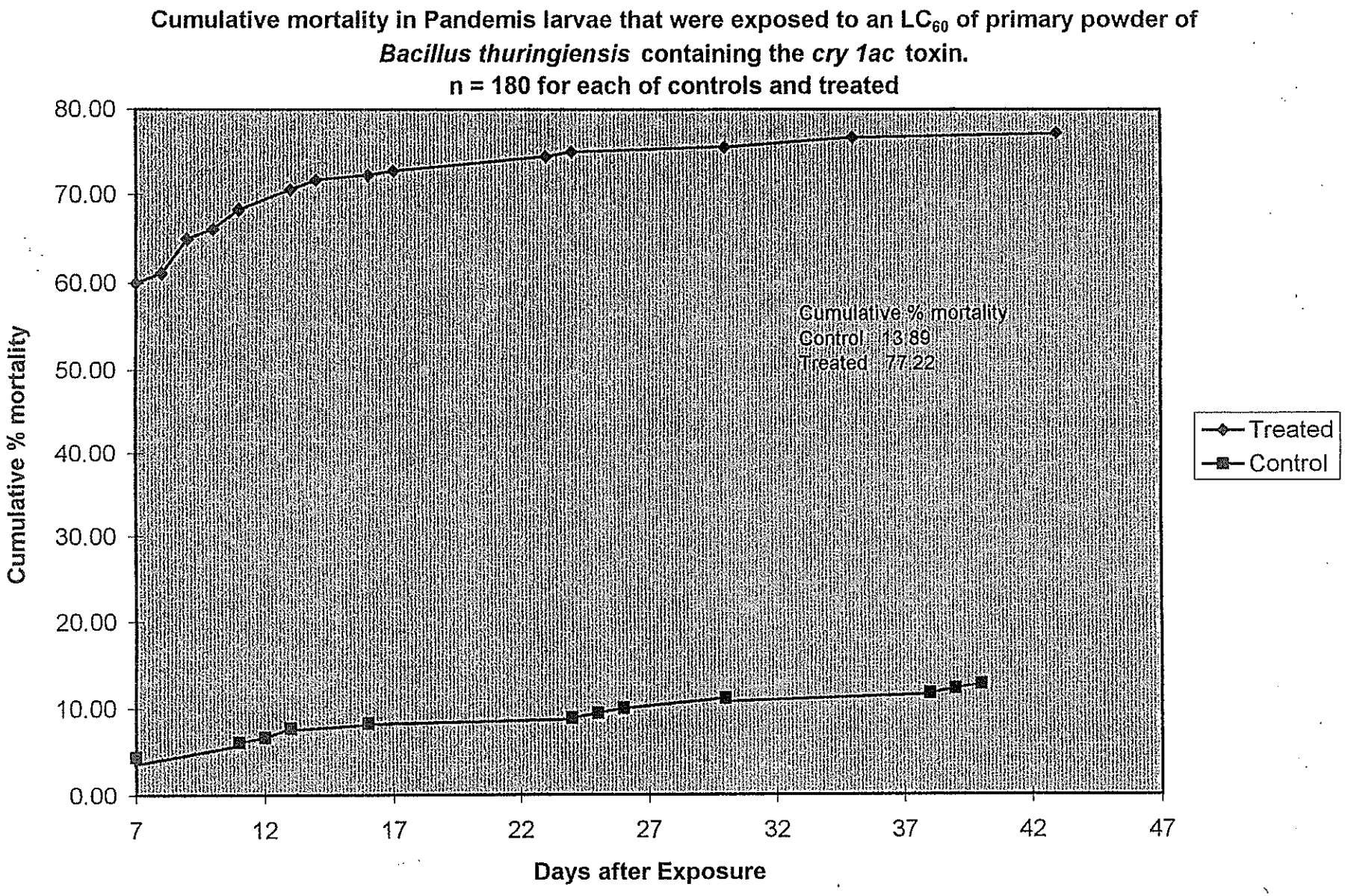
Cumulative mortality in Pandemis larvae that were exposed to an LC_{60} of primary powder of *Bacillus thuringiensis* containing the *cry 1ac* toxin.
n = 180 for each of controls and treated
Cumulative % mortality
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
7
12
17
22
27
32
37
42
47
Days after Exposure
Cumulative % mortality
Control 13.89
Treated 77.22
Treated
Control

Figure 3

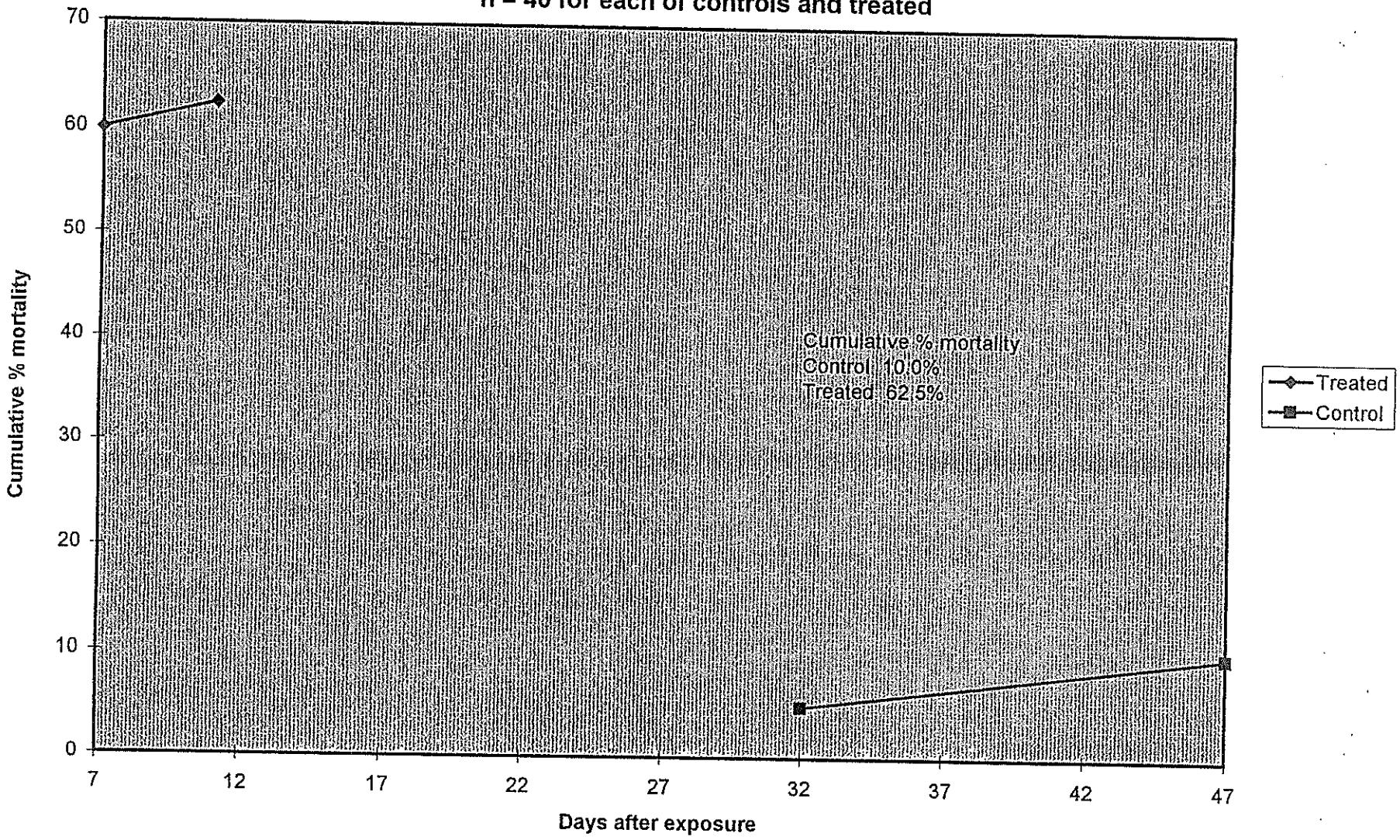
Cumulative mortality in Pandemis larvae that were exposed to an LC_{60} of a primary powder of *Bacillus thuringiensis* containing the *cry 2a* toxin.
n = 40 for each of controls and treated
Cumulative % mortality
70
60
50
40
30
20
10
0
7
12
17
22
27
32
37
42
47
Days after exposure
Cumulative % mortality
Control 10.0%
Treated 62.5%
Treated
Control

Figure 4

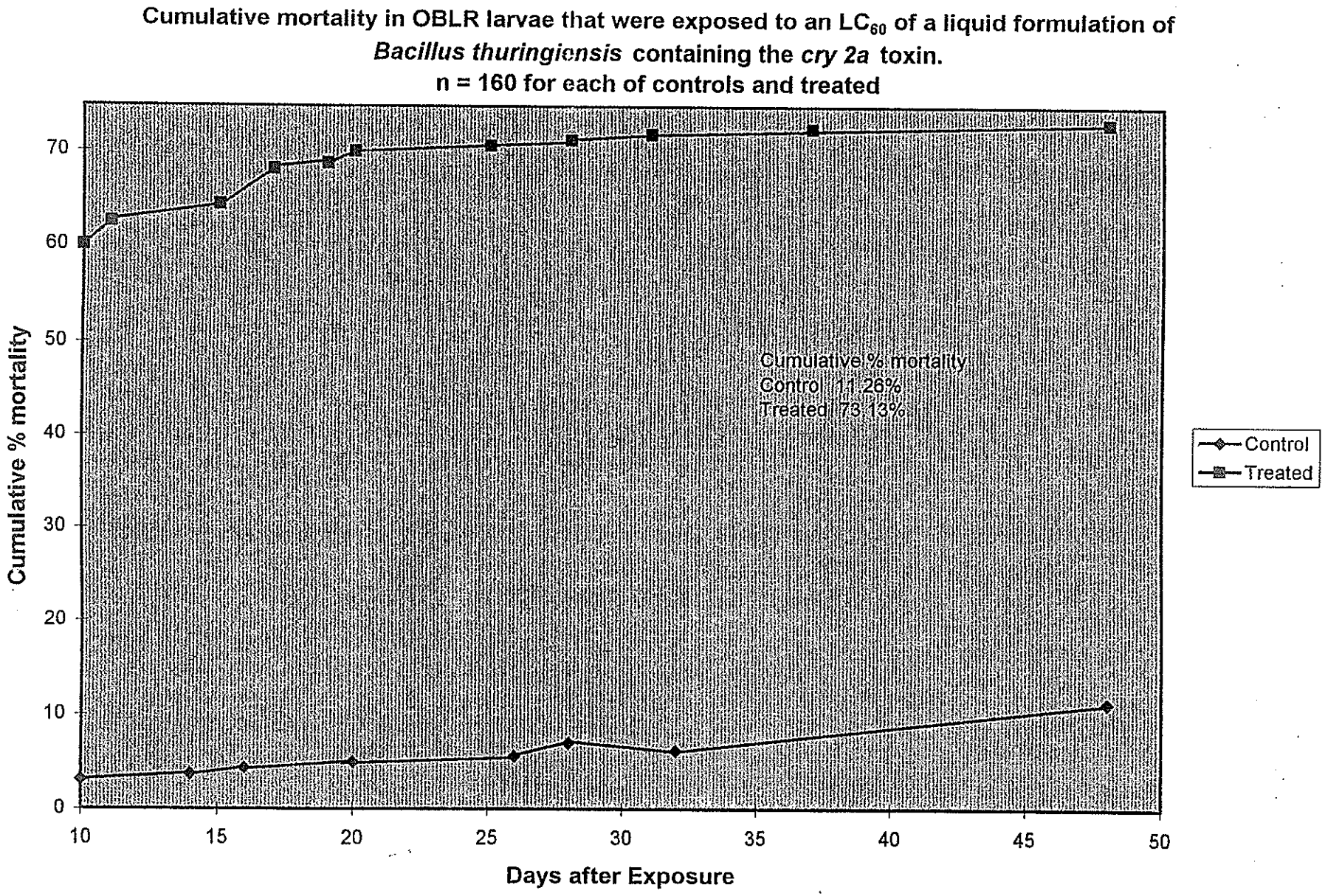
Cumulative mortality in OBLR larvae that were exposed to an LC_{60} of a liquid formulation of *Bacillus thuringiensis* containing the *cry 2a* toxin.
n = 160 for each of controls and treated
Cumulative % mortality
Days after Exposure
Cumulative % mortality
Control 11.26%
Treated 73.13%
Control
Treated
0
10
20
30
40
50
60
70
10
15
20
25
30
35
40
45
50

Figure 5

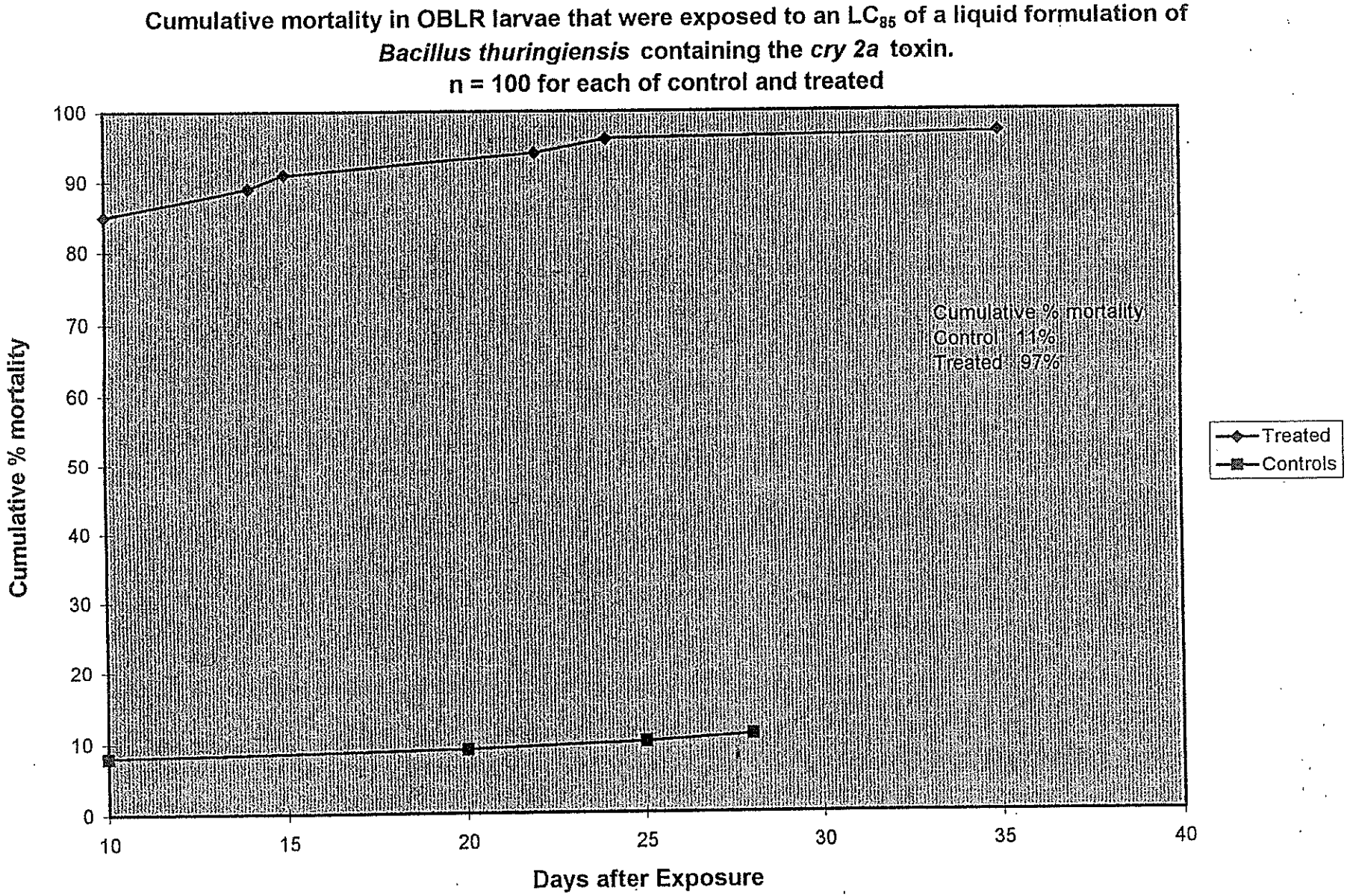
Cumulative mortality in OBLR larvae that were exposed to an LC_{85} of a liquid formulation of *Bacillus thuringiensis* containing the *cry 2a* toxin.
n = 100 for each of control and treated
Cumulative % mortality
100
90
80
70
60
50
40
30
20
10
0
10
15
20
25
30
35
40
Days after Exposure
Cumulative % mortality
Control 11%
Treated 97%
Treated
Controls

Figure 6

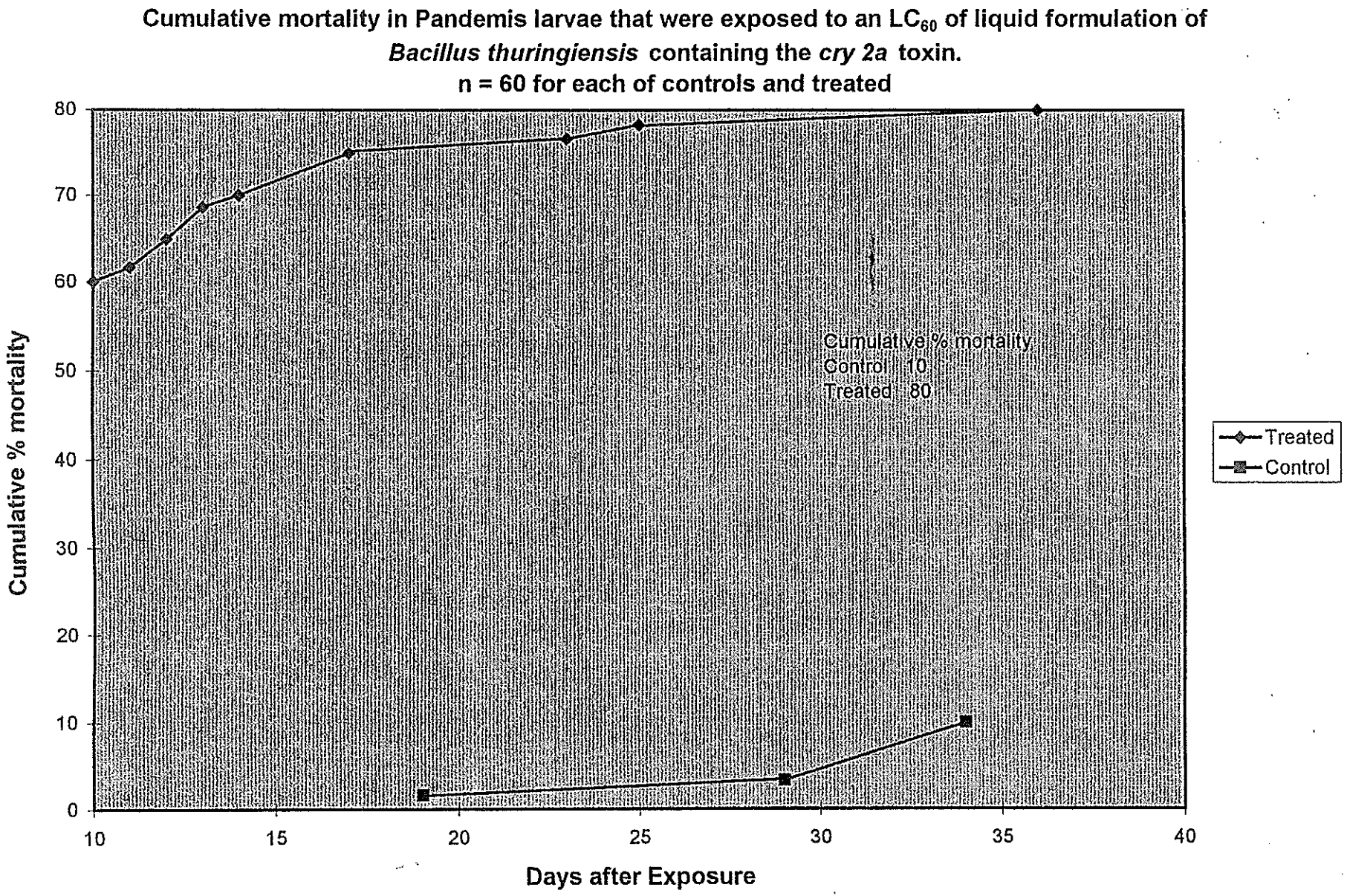
Cumulative mortality in Pandemis larvae that were exposed to an LC_{60} of liquid formulation of *Bacillus thuringiensis* containing the *cry 2a* toxin.
n = 60 for each of controls and treated
Cumulative % mortality
80
70
60
50
40
30
20
10
0
10
15
20
25
30
35
40
Days after Exposure
Cumulative % mortality
Control 10
Treated 80
Treated
Control

Figure 7