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COMPARATIVE PATHOGENICITY OF VARIOUS
STRAINS OF BACILLUS THURINGIENSIS (B.T.)
FOR THE SPRUCE BUDWORM - PROGRESS REPORT

by

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SUMMARY

This report outlines the work completed to date on a screening programme designed to determine the relative potencies of various B.t. strains for the spruce budworm. The bioassay technique follows closely that of Dulmage et al. (1). After several initial test series, the following configuration was adopted:

- 5 - 3-fold dilutions per sample
- 30 - 3rd or 4th instar larvae per dilution
- 5 larvae per cup
- 30 + larvae on diet w/o B.t. as a check for natural mortality

None of the 32 sample isolates tested to date appear to be significantly more potent than HD-1-S-1971 for the spruce budworm. Fifteen of the samples tested are significantly less potent than the reference strain.

There is evidence that the potency of some strains has decreased over time.

ANALYSIS OF THE DATA

Data was analysed using a single and parallel line probit analysis computer programme supplied by the Applied Statistics and Scientific Computing Branch, D.O.E. This programme is very thorough and rejects any bioassays which do not conform to certain statistical assumptions.

Potency ratios (relative potencies) are calculated using HD-1-S-1971 as a reference strain in a parallel line analysis. Potency ratio estimates from replicates are combined by calculating the weighted means of the log potency ratios, using the reciprocals of their variances as weights as suggested by Finney (2).

EVIDENCE OF DECLINE IN POTENCY OVER TIME

Although the sample isolates are in a powder form and are stored in the dark at about 2°C, there is some evidence of a decline in potency over time. There is no indication of a change in the potency of the reference strain, HDL-S-1971. The result of this decline in the potency of the samples is an increase in IC50's and a decrease in potency ratios over time (See table 2).

TEST SERIES #	3	4	5	6	7	8	9	12	13	14
IC50 OF REFERENCE (PPM)	15	3	20	8	10	14	9	6	12	8

REFERENCES

- 1) Dulmage, H.T., Martinez, A.J. and Pena, T., 1976.
Bioassay of *Bacillus thuringiensis* (Perliner) Delta-Endotoxin
Using the Tobacco Budworm; USDA Technical Bulletin No. 1528
- 2) Finney, D.J., 1952 - A Statistical Treatment of the Sigmoid
Response Curve; Cambridge University Press.

Table 1
Results to date

REPORT
OF PROGRESS IN B.T. SCREENING PROGRAMME TO MAR 30, 1977

HD NO.	SAMPLE NO.	MEAN LC50	POTENCY RATIO	95 PER CENT C.L. FOR P.R.	NO. OF BIOASSAYS
269	R749A	1(1)	1.755	(0.86 - 3.58)	1
124	R760B	6(2)	1.641	(0.79 - 3.43)	2
72	R636B	9(2)	1.393	(0.78 - 2.49)	1
301	R756B	10(1)	1.133	(0.59 - 2.16)	1
283	R750B	22(2)	1.130	(0.84 - 1.52)	2
1	R763A	(0)		(-)	0
73	R636C	59(2)		(-)	0
136	R762B	103(1)		(-)	0
175	R754C	10(2)	0.917	(0.56 - 1.50)	3
232	R639C	3(1)	0.910	(0.47 - 1.74)	1
147	R754A	12(1)	0.705	(0.14 - 3.52)	1
211	R690A	11(1)	0.696	(0.18 - 2.75)	1
241	R663A	4(1)	0.640	(0.34 - 1.21)	1
270	R749B	18(1)	0.600	(0.21 - 1.69)	1
52	R584B	12(1)	0.598	(0.23 - 1.57)	1
317	R757A	(0)	0.595	(0.39 - 0.90)	2
7	R763B	16(1)	0.579	(0.26 - 1.28)	1
137	R761A	17(1)	0.500	(0.22 - 1.14)	1
198	R763C	22(1)	0.389	(0.17 - 0.88)	1
15	R622B	24(2)	0.343	(0.13 - 0.91)	1
59	R645A	10(1)	0.257	(0.12 - 0.53)	1
260	R714B	13(1)	0.197	(0.09 - 0.42)	1
203	R755C	3(2)	0.178	(0.04 - 0.84)	1
6	R655A	(0)	0.165	(0.05 - 0.57)	1
184	R755A	(0)	0.162	(0.02 - 1.37)	1
250	R758A	96(1)	0.122	(0.07 - 0.21)	1
109	R649B	118(2)	0.093	(0.05 - 0.17)	1
123	R762A	108(1)	0.049	(0.01 - 0.16)	1
135	R760C	(0)	0.039	(0.01 - 0.19)	1
107	R648B	(0)	0.037	(0.01 - 0.24)	1
110	R759A	(0)	0.034	(0.01 - 0.19)	1
83	R658A	(0)	0.023	(- 0.20)	1
224	R756A	(0)	0.012	(- 1.65)	1
99	R646A	54(1)	0.009	(- 0.07)	2
149	R754B	(0)	0.005	(-39.18)	1

MEAN LC50 = THE AVERAGE LC50 IN PPM (MICROGRAMS PER GRAM OF DIET) ESTIMATED BY SINGLE LINE ANALYSIS. FIGURE IN BRACKETS IS THE NO. OF BIOASSAYS USED IN DETERMINING THIS AVERAGE.

POTENCY RATIO = WEIGHTED MEAN POTENCY OF THE SAMPLE RELATIVE TO THE HD-1-S-1971 STANDARD. ESTIMATED BY PARALLEL LINE ANALYSIS.

NO. OF BIOASSAYS = THE NUMBER OF BIOASSAYS USED TO DETERMINE THE POTENCY RATIO.

Table 2
Changes in Potency Between Replicates

	<u>LC50's (in ppm)</u>		<u>DIRECTION OF CHANGE</u>	
	<u>REP. 1</u>	<u>REP. 2</u>		
HD-124	6.8	5.2	-	
HD-72	0.6	16.8	+	
HD-283	20.0	24.9	+	The probability of
HD-73	17.4	101.4	+	getting 5 '+' s and 1 '--'
HD-175	2.5	18.2	+	by chance alone is 0.035
HD-15	22.8	24.7	+	
HD-109	106.4	129.2	+	

	<u>Potency Ratios</u>		<u>DIRECTION OF CHANGE</u>	
	<u>REP. 1</u>	<u>REP. 2</u>		
HD-124	2.2	1.4	-	
HD-283	1.2	0.44	-	
HD-175	4.6	0.14	-	The probability of
	0.14	0.65	+	getting 5 '--' s and 1 '+'
HD-317	0.96	0.28	-	by chance alone is 0.109
HD-99	0.063	0.0015	-	

Table 3
Diet Used for Bioassays

SPRUCE BUDWORM DIET INGREDIENTS

WATER	1000 ml
CASEIN	140 g
KCH, 4M	20 ml
ALPHACEL	20 g
SALT MIXTURE W	40 g
SUCROSE	140 g
WHEAT EMBRYO	120 g
ASCORBIC ACID	16 g
VITAMIN DIET FORTIFICATION MIXTURE	40 g in 100 ml of water

ANTIMICROBIALS

BENOMYL (100% A.I.)	1 g
METHYL PARABEN	6 g
* FORMALIN (37%)	2 ml
* AUREOMYCIN	1.25 g

AGAR

GRANULATED AGAR	50 g
¹ WATER	2200 ml

* EXCLUDED FROM DIET USED FOR BIOASSAYS

¹ Agar plus 1100 ml of water is autoclaved at 15 pounds per square inch for 15 min. The remaining 1100 ml of water is mixed with melted agar before addition of agar to other ingredients.

Reference: Crisdale, D. 1973 - 'Large Volume Preparation and Processing of a Synthetic Diet for Insect Rearing'; Can. Ent. 105.