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INTERACTION OF BACILLUS THURINGIENSIS VAR. KURSTAKI WITH SELECTED EMULSIFIERS, SOLVENTS AND AN ORGANOPHOSPHATE INSECTICIDE

by

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ABSTRACT

The compatibility of *Bacillus thuringiensis* var. *kurstaki (B.t.)* with 12 emulsifiers, 2 solvents and a commercial chemical insecticide were studied by way of their effects on spore germination, vegetative cell replication and physiological reactions.

Five additives, viz. EMCOL AL6940 and N-500B TOXIMUL - MP8, SPONTO-541 and ATLOX 3409 F, at 1 ppm concentration significantly inhibited spore germination. All additives tested at this concentration inhibited vegetative cell replication. Mixtures of these 5 additives with *B.t.* should be avoided in practice.

The data indicate that 10,000 ppm of acephate has no apparent effect, on several key physiological reactions of *B. thuringiensis kurstaki*.

RÉSUMÉ

La compatibilité de *Bacillus thuringiensis* var. *kurstaki (B.t.)* avec 12 émulsifiants, 2 solvants et un insecticide chimique commercial a été étudiée au moyen de l'effet de ces composés sur la germination des spores, la division cellulaire par voie végétative et les réactions physiologiques.

A la concentration de 10^{-6}, 5 additifs (EMCOL AL6940 et N-500B, TOXIMUL - MP8, SPONTO-541 et ATLOX 3409 F) ont notablement réduit la germination des spores. A cette concentration, tous les additifs ont nui à la subdivision cellulaire végétative. Dans la pratique, on devrait donc éviter de mélanger ces 5 additifs avec *B.t.*

D'après les données, 10 000 x 10^{-6} d'acéphate n'ont aucun effet significatif sur plusieurs réactions physiologiques vitales de *B. thuringiensis kurstaki*.

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INTRODUCTION

The strategy of applying mixtures of insect pathogens and low concentrations of chemical pesticides in pest insect control is a logical one in cases where the pathogens alone do not give acceptable crop protection. Such a strategy has been reported to enhance the effectiveness of *Bacillus thuringiensis* and nuclear polyhedrosis viruses under both laboratory and field conditions (Benz 1971; Chen *et al.* 1974; Morris 1975a, Morris and Armstrong 1975; Morris 1977a, b; Vail *et al.* 1980 and Jaques and Morris (in press).

Prior to the application of mixtures of insect pathogens and chemical pesticides, however, the compatability of the two agents should be ascertained. *B. thuringiensis* is known to be compatible with some insecticides in terms of their effect on spore germination, vegetative cell replication and parasporal crystal (Sutter *et al.* 1971; Altathawy and Abaless 1972; Morris 1972; Chen *et al.* 1974; Morris 1975b; Roa and Rana 1977 and Morris 1977c). Some chemical pesticides were reported to be stimulatory to *B. thuringiensis* growth (Chen *et al.* 1974; Morris 1975b and Roa and Rana 1977) due presumably to the metabolism of the insecticide by the bacteria in culture.

Morris (1977c) found that: 1. Carbamates as a group were less toxic to *B. thuringiensis* than were organophosphates, pyrethrins, chlordimeform and urea derivatives and 2. Technical formulations were less harmful to the bacteria than were wettable powders (WPs) and emulsifiable concentrates (ECs) implying that emulsifiers and other surfactants normally added to wettable powders and emulsifiable concentrates of chemical insecticides may be bacteriocidal or bacteriostatic. Earlier reports (Morris 1969, 1975b) indicated that the additives Lovo 192, Atlox and Triton X-100 affected the rate of *B.t.* sporulation and crystal synthesis and inhibited the bacterial metabolism of hexokinase and invertase.

Since *B.t.* is applied over forests as water suspensions, the addition of WPs and ECs of chemical pesticides to tank mixes is simpler than adding technical concentrates since the latter are usually not water-soluble. The objective of the present study was to determine which emulsifiers, solvents and surfactant commonly used in the formulation of WPs and ECs of chemical pesticides presently used in forests are the least inhibitory to *B.t.* germination and growth and thus more compatible with the pathogen. The effect of 10,000 ppm technical acephate® on key physiological reactions of *B.t.* was also investigated. This insecticide was previously reported to be compatible with *B.t.* in terms of spore germination and vegetative cell replication (Morris, 1975b; Morris 1977c).

MATERIALS AND METHODS

Emulsifiers

The emulsifiers selected for testing (Table 1) were components of emulsifiable concentrates of the insecticides Pyrenone spray, Sumithion, Pyrocide, SBP 1382, PH-60-40 (difluorobenzuron) and Dylox and represented a wide variety of chemical compounds. A single lot of Thuricide 16B (*B. thuringiensis* var. *kurstaki*, Sandoz, Inc., Homestead, Florida) was used in all tests.

In a series of preliminary tests, all adjuvants were added in concentrations of 0 (control), 10, 100, 1000, 10,000 and 100,000 ppm to a 1/100 dilution of Thuricide in trypticase soy broth. One hundred ml of the broth cultures were placed in 250 ml flasks and incubated in a water bath shaker for 24 hr at 29°C at which time wet mounts were examined with phase contrast optics for the presence of vegetative cells. If there were no cells, a calibrated loopful (0.01 ml) was spread evenly on trypticase soy agar surface to determine if the additives had killed the spores or had simply delayed their germination. The concentrations of adjuvants selected for the main tests were 1.0 ppm and those which showed only slight growth in the broth, or if no growth in broth, some growth on solid agar when transferred from broth. Concentrations below those selected were completely inhibitory.

In the main tests, the bacteria were cultured as above in concentrations of the adjuvants listed in Table 2. The pH of the cultures were recorded at 2 and 24 hr incubation time. Low pHs of some bacterial cultures are known to affect physiological integrity of bacterial spores (Rode and Foster 1960).

At 24 hr of incubation, four smears from each culture were prepared on clean slides and stained (Smirnoff 1962). The numbers of spores, crystals and vegetative cells were counted under oil immersion in 10 fields per slide and the spore/crystal and cell/crystal ratios calculated. Experiments were replicated four times.

The hypothesis that the ratios of spores to crystals and cells to crystals within a culture containing adjuvant was similar to the corresponding control without adjuvant was tested by the non-parametric Mann-Whitney test.

Acephate

Pure cultures of *B.t.k.* were isolated from an HD-1-S-1971 standard obtained from H. Dulmage, U.S.D.A. and grown on trypticase soy agar. These cultures were used to inoculate trypticase soy broth with and without 10,000 ppm concentrations of technical acephate (94% active ingredient). This concentration of acephate approximates that used in aerial application of *B. thuringiensis*-acephate tank mixes against the spruce budworm, *Choristoneura fumiferana*. The cultures were incubated in a water bath shaker for 6 and 24 hrs at 30°C and used to perform the physiological reaction tests listed following standard procedures.

RESULTS AND DISCUSSION

Emulsifiers and solvents

Vegetative cells were not present in smears of the commercial product before incubation in culture media and the ratio of spore/crystal was 1.0 based on counts of 160 microscopic field on 16 smears stained by the Smirnoff (1962) method. A similar ratio has been reported for commercial Dipel (Morris 1977d). The ratio of vegetative cells/crystals in 24 hr cultured untreated Thuricide was 4.61 indicating a high level of germination and growth. The spores counted in these tests were all originally introduced ones since sporulation of *B. thuringiensis* vegetative cells normally takes 3-4 days of incubation in liquid culture compared with 24 hr incubations in the present tests.

The highest concentrations of additive used in the main tests (Table 2) were those which nearly completely inhibited germination and replication as indicated in the preliminary microscopic observations. For example EMCOL-AL3940 at 1000 ppm completely inhibited bacterial growth in liquid culture with no growth when transferred to solid media, so 100 ppm was chosen for the main test. The highest concentration tested, however, was 100,000 ppm which was considered far beyond the concentrations which would be used in practical application.

All the additives tested significantly inhibited bacterial replication at 1 ppm concentration but only EMCOL-AL6940 and N-500B, TOXIMUL MP8, SPONTO 541 and ATLOX 3490F produced significant inhibition to bacterial spore germination at the same concentration. SORPOL 3081 and SPONTO 1362 inhibited germination at 10 ppm but not at 1 ppm. This suggests a growth stimulation phenomenon referred to above. With the exception of these two compounds, there was a general dose-response trend within treatment groups for spore/crystal and crystal/cell ratios. There was no dose-response in terms of spore, crystal and cell counts, however, since the actual amounts of cultures used to make the smears were different.

Although the additives at the highest concentration tested were generally inhibitory, it is apparent that some were relatively more toxic than others. Based on the concentrations producing complete inhibition, the most bacteriocidal compounds tested were PENICK EM-2-7810RS, SORPOL-3081, and SPONTO-1362 which were completely inhibitory at 100 ppm. The next most toxic group included EMCOL AL3940, EMCOL-N500B, PENICK-EM-1-7809 RS, SORPOL-SM 100S, SORPOL-3082, TOXIMUL-MP8, SPONTO 541 and ATLOX-3409F. Lowest toxicities were recorded for EMCOL-H44C and SORPOL-2495G.

The inhibitory effects noted were not attributable to pH of the culture media. It is known that pHs of 1-5 of bacterial cultures containing anionic or cationic surfactants can cause a release of dipicolinic acid from spores thereby affecting their germination (Rode and Foster 1960). In the present tests, however, all pHs were in the 5.5-7.1 range which closely parallels the normal pH fluctuations (4.8-neutrality) observed during the exponential growth of *B.t.* in simple semi-defined media (Rogoff and Yousten 1969). Nor would the numbers of parasporal crystal be affected since they are only soluble under highly alkaline conditions (Cooksey 1971).

Damaging effects of other surfactants on bacterial spores have been noted previously. Rode and Foster (1960) reported that cationic and anionic surfactants markedly potentiated thermal killing of *B. megatherium* and *B. subtilis* spores. Tween 80, a non-toxic surfactant, at 0.05% appeared to increase the permeability of microbial vegetative cell wall (Reese and Maguire 1969) but had no apparent deleterious effect on the germination of *B. thuringiensis* var. *galleriae* spores or parasporal crystal (Morris 1975). Lovo 192 (amine strearate), Igepal (nonionic nonyl phenoxypolyoxyethelene) and Triton 1956B (alkyd resin) were reported to affect the rate of sporulation and parasporal crystal production of *B. thuringiensis* var. *galleriae* depending on the composition of the growth media and to produce fundamental changes in the metabolism of the vegetative cells (Morris 1969).

The differential toxicities within related groups of emulsifiers (e.g., EMCOL and SORPOL) are probably attributable to the chemical composition of the compounds. SORPOL 3082, for example contains xylene which is known to be bacteriostatic. Xylene is lacking in SORPOL-2495G which is considerably less bacteriostatic. The solvent, ATLOX 3409F (anionic-nonionic blend of dodecylbenzene sulfonate polyoxyethylene ethers) at 100 ppm in the present tests affected spore/crystal/cell ratios only moderately compared with the corresponding control (11/41/48 vs 4/21/76). ATLOX-8916 (polyoxyethylene solution esters of mixed fatty and resin acids), however, at the same concentration and culture pH had a drastic effect on the ratios (2/98/9 vs 14/29/57 (Morris 1975)).

It is generally accepted that some insect pest species are more susceptible to the spore-cyrstal complex of *B.t.* than to either component alone. In cases where the spores play an important role, it is incumbent on the user of commercial *B.t.* products to avoid the addition of agents to the tank mixes which could be harmful to the bacteria thereby reducing the effectiveness of the product. The emulsifiers and solvents reported here are examples of additives which should be avoided. The present results support Morris' (1975b, 1977c) previous conclusion that in choosing a chemical pesticide for combining with *B.t.* in control operations, those chemicals containing bacteriostatic or bacteriocidal surfactants should be avoided in favour of technical formulations which do not contain such additives.

Acephate

Of the 30 tests conducted at 6 hr and 24 hr each (Table 3) only the 24 hr Voges-Proskauer reaction showed a physiological change in the presence of acephate, suggesting that the bacteria had lost its ability to produce acetyl-methyl carbinol. This fermentation reaction, however, is variable in many species and is not considered to be as significant a biochemical tests as are salicin and sucrose fermentations (Norris and Burges, 1965; Heimpel, 1967). Of some significance was the fact that var. *kurstaki* used in the present tests did not produce acid reactions in galactose and mannose but did in salicin. The opposite reactions occurred for *B. thuringinesis* var. *galleriae* as earlier reported (Morris, 1969, 1972).

Based on the tabulated results, it is concluded that acephate at 10,000 ppm will have no significant effect on the key physiological reactions of *B. thuringiensis*. This supports previously published data on compatibility of the two agents in terms of bacterial spore germination and replication.

REFERENCES

ALTAHTAWY, M.M. and I.M. ABALESS. 1972. Compatibility of the bio-insecticide Thuricide 90TS Flowable with insecticides used in the chemical control of *Spodoptera littoralis* (Boisd.). Bull. Entomol. Soc. Egypt, Econ. Ser. VI:239-245.

BENZ, G. 1971. Synergism of microorganisms and chemical insecticides. *In* H.D. Burges and N.W. Hussey (Eds.), Microbial control of insects and mites, pp. 327-355. Academic Press, N.Y.

CHEN, K., B.R. FUNKE, J.T. SCHULZ, R.B. CARLSON and F.I. PROSHOLD. 1974. Effects of certain organophosphate and carbamate insecticides on *Bacillus thuringiensis*. J. Econ. Entomol. 67:471-473.

COOKSEY, K.E. 1971. The protein crystal toxin of *Bacillus thuringiensis*: Biochemistry and mode of action. *In* H.D. Burges and N.W. Hussey (Eds.), Microbial control of insects and mites, pp. 247-274. Academic Press, N.Y.

HEIMPEL, A.M. 1967. A taxonomic key proposed for the species of "crystalliferous bacteria". J. Invertebr. Pathol. 9:364-375.

JACQUES, R.P. and O.N. MORRIS. (in press). Integration: Compatibility of pathogens with other methods of pest control and with different crops. *In* H.D. Burges (Ed.), Microbial control of insects, mites and plant diseases: Vol. 2. Academic Press, N.Y. 36 ms. pp.

MORRIS, O.N. 1969. Susceptibility of several forest insects of British Columbia to commercially produced *Bacillus thuringiensis*. 1. Studies on the physiological properties of some commercial products. J. Invertebr. Pathol. 13:134-146.

MORRIS, O.N. 1972. Susceptibility of some forest insects to mixtures of commercial *Bacillus thuringiensis* and chemical insecticides, and sensitivity of the pathogen to the insecticides. Can. Ent. 104:1419-1425.

MORRIS, O.N. 1975a. Susceptibility of the spruce budworm, *Choristoneura fumiferana*, and the white-marked tussock moth, *Orgyia leucostigmata*, to *Bacillus thuringiensis*--chemical insecticide combinations J. Invertebr. Pathol. 26:193-198.

MORRIS, O.N. 1975b. Effect of some chemical insecticides on the germination and replication of commercial *Bacillus thuringiensis*. J. Invertebr. Pathol. 26:199-204.

MORRIS, O.N. and J.A. ARMSTRONG. 1975. Preliminary field trials with *Bacillus thuringiensis*--chemical insecticide combinations on the integrated control of the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). Can. Ent. 107:1281-1288.

MORRIS, O.N. 1977a. Long-term study of the effectiveness of aerial application of *Bacillus thuringiensis* acephate combinations against the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). Can. Ent. 109:1239-1248.

MORRIS, O.N. 1977b. Long-term effects of aerial applications of virus-fenitrothion combinations against the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). Can. Ent. 109:9-14.

MORRIS, O.N. 1977c. Compatibility of 27 chemical insecticides with *Bacillus thuringiensis* var. *kurstaki*. Can. Ent. 109:855-864.

MORRIS, O.N. 1977d. Relationship between microbial numbers and droplet size in aerial spray applications. Can. Ent. 109:1319-1323.

NORRIS, J.R. and H.D. Burges. 1965. The identification of *Bacillus thuringiensis*. Entomophaga 10:41-47.

RAO, M.V.R. and R.S. RANA. 1977. Interaction of *Bacillus thuringiensis* var. *thuringiensis* with commonly used chemical insecticides. Indian J. Microbial. 17:9-12.

REESE, E.T. and A. MAGUIRE. 1969. Surfactants as stimulants of enzyme production by microorganisms. Appl. Microbiol. 17:242-245.

RODE, L.J. and J.W. FOSTER. 1960. The action of surfactants on bacterial spores. Arch. Microbiol. 36:67-94.

ROGOFF, M.J. and A.A. Yousten. 1969. *Bacillus thuringiensis*: Microbial considerations. Ann. Rev. Microbiol. 23:357-386.

VAIL, P.V., R.E. SEAY and J. DEBOLT. 1980. Microbial and chemical control of the cabbage looper on fall lettuce. J. Econ. Entomol. 73:72-75.

SMIRNOFF, W.A. 1962. A staining technique for differentiating spores, crystals and cells of *Bacillus thuringiensis*. J. Insect Pathol. 4:384-386.

SUTLER, G.R., A.D. ABRAHAMSON, E.W. HAMILTON and I.D. BICK. 1971. Compatibility of *Bacillus thuringiensis* var. *thuringiensis* and chemical insecticides. 1. Effect of insecticides on bacterial replication rate. J. Econ. Entomol. 64:1348-1350.

TABLE 1

List of emulsifiers and solvents tested for their compatibility with *Bacillus thuringiensis* var. *kurstaki*

Compound	Chemical nature	Source
EMCOL-AL 3940	Ethylene glycol fatty acid ester	McLaughlin Gormley King Co. U.S.A.
EMCOL-H 44C	Blend of polyethelene glycol esters of tall oil fatty acids and modified monoglyceride	MGK Co. U.S.A.
EMCOL-N 500B	Blend of oil soluble sulfonates with poly-ethylene ethers	MGK Co. U.S.A.
PENICK EM-1-7809RS	Triton X-180, 80% and Triton X-190, 20%	S.B. Penick Co. U.S.A.
PENICK EM-2-7810RS	Toximul D, 80% and Toximul H, 20%	S.B. Penick Co. U.S.A.
SORPOL SM-100S	Blend of polyoxyethylene styryl phenyl ether, polyoxyethylene styryl phenyl ether polymer, polyoxyethylene fatty acid derivative and alkylaryl sulfonate	Sumitomo Chem. Co. Ltd. Japan
SORPOL 3081	Blend of polyoxyethylene polyoxypropylene block polymer, polyoxyethylene alkyl aryl ether and alkyl aryl sulfonate	Sumitomo Chem. Co. Ltd. Japan
SORPOL 3082	Blend of polyoxyethylene polyoxyprophylene block polymer, sorbitan alkylate, alkyl aryl sulfonate and xylene	Sumitomo Chem. Co. Japan

(continued)

TABLE 1 (concluded)

List of emulsifiers and solvents tested for their compatibility with *Bacillus thuringiensis* var. *kurstaki*

Compound	Chemical nature	Source
SORPOL 2495G	Blend of polyoxyethylene alkyl aryl ether sulfate and alkyl aryl sulfonate	Sumitoma Chem. Co. Japan
TOXIMUL MP8	Blend of aniogenic (sulfonates) and non-iogenic emulsifiers	Stephan Chem. Co. U.S.A.
SPONTO 541	NA	FMC Corp. U.S.A.
SPONTO 1362	NA	FMC Corp. U.S.A.
THFA (75-464) Solvent	Tetrahydroflur-furyl alcohol	Chemagro Corp. U.S.A.
ATLOX 3409 F Solvent	Anionic-nonionic blend of dodecyl-benzene sulfonate/ polyoxyethylene ethers	Atlas Chem. Industries U.S.A.

NA = Not available

Table 2

Effect of emulsifiers and solvents on the germination and growth of *Bacillus thuringiensis* var. kurstaki[1]

Adjuvants	Conc. (ppm)	Culture pHs at 0 and 24 hr incubation	Mean counts/slide[2] ± SE			Median Spore/crystal ratio	Median Cell/crystal ratio
			Spores	Crystals	Cells		
SORPOL-3081	1	5.8, 7.0	74 ± 22	204 ± 36	336 ± 31	0.22*	1.88*
	10	5.7, 7.1	39 ± 6	176 ± 16	270 ± 25	0.17*	1.56*
SORPOL-3082	1	5.9, 7.0	45 ± 10	196 ± 22	343 ± 40	0.10*	1.96*
	100	5.9, 6.9	46 ± 7	188 ± 22	112 ± 24	0.17*	0.43*
SORPOL-2495G	1	5.8, 6.9	31 ± 10	144 ± 21	197 ± 14	0.09*	1.53*
	100,000	5.9, 6.9	242 ± 35	374 ± 60	124 ± 25	0.63*	0.25*
TOXIMUL-MP8	1	6.1, 6.9	85 ± 15	289 ± 59	544 ± 51	0.29*	2.29*
	100	6.0, 6.9	127 ± 16	282 ± 21	206 ± 18	0.42*	0.71*
SPONTO-541	1	5.7, 7.1	56 ± 10	167 ± 35	227 ± 31	0.33*	2.22*
	100	6.3, 6.9	50 ± 10	205 ± 26	209 ± 62	0.17*	0.56*
SPONTO-1362	1	5.7, 7.0	86 ± 14	169 ± 19	213 ± 27	0.38*	1.33*
	10	5.7, 7.0	46 ± 13	131 ± 17	190 ± 28	0.13*	1.85*

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Table 2

Effect of emulsifiers and solvents on the germination and growth of *Bacillus thuringiensis* var. kurstaki[1]

Adjuvants	Conc. (ppm)	Culture pHs at 0 and 24 hr incubation	Mean counts/slide[2] ± SE			Median Spore/crystal ratio	Midean Cell/crystal ratio
			Spores	Crystals	Cells		
EMCOL-AL6940	1	5.9, 7.1	49 ± 9	205 ± 25	181 ± 23	0.21*	0.89*
	100	5.8, 7.1	72 ± 12	213 ± 27	222 ± 28	0.29*	0.95*
EMCOL-H44C	1	6.0, 7.0	42 ± 15	167 ± 29	310 ± 27	0.09*	2.13*
	100,000	ND	43 ± 7	187 ± 28	312 ± 34	0.19*	1.22*
EMCOL-N500B	1	5.7, 7.1	68 ± 17	234 ± 22	345 ± 43	0.16*	1.36*
	100	6.3, 6.9	53 ± 5	270 ± 29	91 ± 28	0.17*	0.08*
PENICK-EM1-7809RS	1	5.8, 7.0	36 ± 11	175 ± 30	215 ± 32	0.07*	1.11*
	100	5.8, 7.0	57 ± 12	273 ± 41	69 ± 24	0.15*	0.06*
PENICK-EM2 7810RS	1	5.6, 6.9	39 ± 9	228 ± 33	190 ± 25	0.09*	0.94*
	10	5.5, 7.0	45 ± 9	178 ± 27	229 ± 23	0.20*	1.27*
SORPOL-SM100S	1	5.8, 6.9	65 ± 20	192 ± 26	233 ± 24	0.14*	1.25*
	100	5.6, 7.0	28 ± 4	272 ± 20	184 ± 37	0.08*	0.70*

.....continued

Table 2

Effect of emulsifiers and solvents on the germination and growth of *Bacillus thuringiensis* var. kurstaki[1]

Adjuvants	Conc. (ppm)	Culture pHs at 0 and 24 hr incubation	Mean counts/slide[2] ± SE			Median Spore/crystal ratio	Median Cell/crystal ratio
			Spores	Crystals	Cells		
THFA (75-464)	1	6.0, 6.9	28 ± 7	220 ± 40	272 ± 47	0.08*	1.80*
	10,000	5.7, 7.0	49 ± 9	197 ± 27	206 ± 38	0.21*	1.15*
ATLOX 3409F	1	6.1, 6.9	106 ± 26	344 ± 32	620 ± 36	0.15*	1.87*
	100	ND, 6.9	147 ± 23	519 ± 30	625 ± 48	0.19*	1.13*
Pooled controls	-	6.1, 6.9	18 ± 4	98 ± 11	358 ± 21	0.11	4.61

[1] Cultured in trypticase soy broth. Four replicates

[2] Ten microscopic fields per slide

ND = No data taken by error

* Ratios followed by astericks are significantly different from corresponding ratios in control cultures (Mann-Whitney test)

.....Concluded

Table 3

Changes in vegetative cell metabolism of *Bacillus thuringiensis* var. *kurstaki* cultured with 10,000 ppm of the organophosphate insecticide, acephate, for 24 hr.[1]

Tests	Control untreated bacteria	Treated bacteria
Arabinose	0	0
Cellobiose	A	A[3]
Dulcitol	0	0
Fructose	A	A
Galactose	0	0
Glucose	A	A
Inositol	0	0
Inulin	0	0
Lactose	0	0
Maltose	A	A
Mannose	0	0
Melibiose	0	0
Raffinose	0	0
Rhamnose	0	0
Ribose	A[2]	A[2]
Salicin	A	A
Sorbitol	0	0
Sorbose	0	0
Sucrose	A[5]	A[5]
Trehalose	A	A
Xylose	0	0
Aesculin	A	A
Indole	+	+
Gelatin liquefaction	-	-

Litmus milk	Peptonization with alkalkne surface	Peptonization with alkaline surface
Methyl Red	+	-
Voges-Proskauer	+	+
Nitrate reduction (dextrose)	+	+
Starch hydrolysis	+	+

[1]No difference between control and treated at 6 hr.

0 = no change (alkaline); A^3 = acid production in 3 days;

+ = positive reaction; = negative reaction.