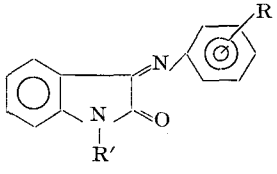
Cysts from a pure line culture of *Schizopyrenus russelli* were used in this work. The amoebae were grown on non-nutrient agar (2.5%; w/v), 0.5% (w/v) NaCl; pH 6.8–7), plates supplied with a 3-day-old culture of *Escherichia coli*, grown on nutrient agar slopes, as food. 3- to 7-day-old cysts were harvested and viable cysts, free from living or dead bacteria, were obtained[6].

A concentration of 2 mg/ml of the test compounds (in 1% agar suspension) was employed for excystment experiments[7]. 50 to 75 cysts were placed as a hanging drop suspension in a cavity slide (25 ± 1 °C), housed in a moist chamber. The percentage excystment was calculated from the count of amoebae and the unexcysted cysts. A cyst was considered excysted only when an amoeba escaped from it and was found moving in the surrounding medium.

For cysticidal activity[7], the test compound was freed from the cysts, after initial treatment, by repeated centrifugation. The cysts were then treated with an excystment agent (*E. coli* extract), after getting rid of trophozoites in cases where excystment had taken place by treating the sample for 2 h with 2% HCl, and the number of cysts excysted were counted. The cysts that did not excyst were presumed to be dead. The number of cysts taken in each experiment varied from 200 to 350. The results are described in the Table.



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Acute toxicity. The acute toxicity tests were done by feeding orally the test compounds (900 mg/kg) to albino rats weighing 20–25 g of either sex and fed on a diet of milk and bread). No mortality was recorded during 7 days of observation.

Summary. A series of isatin-3-anils (with or without a N-piperidino/morpholinomethyl substituent) have been screened for their cysticidal activity against *Schizopyrenus russelli*. Their ability to cause excystment has also been studied.

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Action of *Bacillus thuringiensis* Preparation Against Larch Bud Moth, *Zeiraphera diniana* (Gn.), Enhanced by β-Exotoxin and DDT[1]

The larch bud moth *Zeiraphera diniana* (Guénée), a monovoltine tortricide moth of the subfamily Olethreutinae, is a primary pest of larch in subalpine forests of Austria, France, Italy, and Switzerland[2]. In larch forests, situated at 1400 to 2100 m above sea level, the density of the insect fluctuates in cycles, with gradations of 8–10 years duration[3,4]. Tree defoliation in the phase of culmination impairs the touristic attraction of the forests attacked, reduces wood production considerably[5], and leads to noticeable tree mortality when defoliation takes place in 2 successive years, as for instance in 1973/74. Research is therefore being conducted at our Department to find suitable means to control of this pest.

Large scale applications of insecticides in the culmination phase of a gradation showed that a single application of DDT could reduce larval populations by 97% and thus prevent defoliation for the whole gradation cycle, whereas a single treatment with the organophosphate phosphamidone, causing 93% mortality, could prevent defoliation only in the year of application but not in the succeeding year[6]. The latter treatment caused heavy mortality of birds in the forests treated[7], and since meanwhile DDT has been banned, chemical control offers no solution of the problem of *Z. diniana*.

More than 10 years ago, the author tried selective microbial control of *Z. diniana* by means of biopreparations of *Bacillus thuringiensis* Berliner[8]. Unpublished experiments in 1963 on 3 and 4 ha plots, treated by helicopter[9] at the rate of 40 l per ha, resulted in population reduction of 60–75%. These values, after correction for natural mortality, corresponded to 41% induced mortality on plots treated with Thuricide 90T (7.5 l/ha) or Bactospeine (3.3 kg/ha), and 64% mortality on plots treated with Biospor Hoechst (3.3 kg/ha). Repetition of the treatment with Biospor on the same plot with a dose of 4 kg/ha in 1964 resulted in a 95% reduction of the larval population, corresponding to 90% induced larval mortality. Unfortunately, the producer of Biospor stopped the production of this most promizing preparation in 1965.

Large scale applications of Bactospeine against *Z. diniana* in France have been reported by GRISON et al.[10,11]

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Table I. Results of treatments of 12 plots with per ha 7.5 l of Thuricide 90T (treatment D) and combinations with the β-exotoxin containing supernatant of cultures of *Bacillus thuringiensis*, serotype H1 (E) or DDT (F)

Treatment	Thuricide 90T	β-exotoxin	DDT	Reduction of population (%)	Induced mortality[a] (%)
A	–	–	–	53.2	–
B	–	+	–	58.9	12.1
C	–	–	+	80.1	57.4[a]
D	+	–	–	78.1	53.2[a]
E	+	+	–	91.7	82.3[b]
F	+	–	+	92.1	83.0[b]

Controls: A, untreated; B, β-exotoxin alone; and C, DDT alone. Each plot = 2 ha; 2 plots per treatment. [a]Corrected for natural mortality. Values followed by same letter are not statistically different at 5% level.

and AUER[12]. Population reductions of respectively 60 and 80% were found after treatment of larval populations in the gradation minimum and in the culmination phase.

All experiments reported so far indicate that a single application of *B. thuringiensis*, producing not more than 80% larval mortality in the culmination phase of a gradation, cannot fully prevent visible damage in the year of treatment and can only prevent defoliation in the following year, if the typical 'defense reaction' of the larch trees[13] has been induced by the pest insects in the absence of general damage. Thus more powerful preparations of *B. thuringiensis* would be needed for a good control of *Z. diniana*.

The experiments reported below show a way to enhance the action of commercial preparations of *B. thuringiensis* by the addition of the heatstable β-exotoxin (ET) that is released into the culture medium by some serotypes of the *Bacillus*[14]. The experiments, which showed clear synergistic action of the ET, have been conducted 10 years ago. They are reported because (to the knowledge of the author) no similar experiments have been conducted in Western countries, and because it is hoped that the publication of the results may stimulate more research in this direction. For comparison, the results of experiments with a low dose of DDT and its combination with *B. thuringiensis* are added, since synergism of such combinations has been reported by several authors[15].

Table II. Densities of larval populations on 12 plots before (census No. 1) and after (census No. 2) treatments A–F (see Table I)

Treatment	Plot No.	Census No. 1	Census No. 2	Differences[a]	
				Density	%
A	4	1440	645	795	55.2
	7	1900	917	983	51.7
	Ø	1670	781	889	53.2
B	3	985	394	591	60.0[a]
	11	1522	636	886	58.2[a]
	Ø	1253	515	738	58.9
C	6	1501	285	1216	81.0[b]
	10	1100	233	867	78.8[b]
	Ø	1300	259	1041	80.1
D	2	1130	228	902	79.8[c]
	12	1658	382	1276	77.0[c]
	Ø	1394	305	1089	78.1
E	1	1587	154	1433	90.3
	9	1159	74	1085	93.6
	Ø	1373	114	1259	91.7
F	5	1570	138	1432	91.2[d]
	8	1416	98	1318	93.1[d]
	Ø	1493	118	1375	92.1

Densities are given as mean numbers of larvae per 10 kg of branches (5 trees/plot; 2 kg/tree). [a]Comparison of plots with same treatment: figures followed by same letter are not statistically different at 5% level.

The preparation of *B. thuringiensis* used was Thuricide 90T, a liquid formulation of Stauffer Chemical Company, New York, which, besides the spore/endotoxin complex of *B. thuringiensis*, contained also some ET. Thuricide was applied at the dose of 7.5 l/ha. For additional doses of ET the Péchiney-Progil Company of France furnished a 25 times concentrated supernatant of centrifuged cultures of *B. thuringiensis*, serotype H 1. It was applied at the dosage of 10 l/ha, corresponding to 250 l of supernatant per ha. A 25% emulsion of DDT was applied at the dosage of 0.5 l/ha, i.e. 125 g DDT/ha.

The densities of the larval populations of *Z. diniana* were estimated from samples of branches taken from 60 trees (5 trees/plot) 1 day before treatment (1st census) and 3 weeks after treatment (2nd census). Each census based on 2 samples per tree, 1 kg of branches cut in the upper half and 1 kg in the lower half of the crown[9]. The larvae were counted in the laboratory. At the time of treatment, most larvae were in the 3rd or 4th instar.

Since it was important that the 2nd census was made before the fully grown larvae left the trees for pupation, insects descending or falling from the trees were caught on glue tables placed below some of the trees sampled (1 table per plot). The tables were controlled every 2nd day. The number of bud moth larvae as well as their developmental stages were computed. Rough estimates of the number of other insects caught were noted.

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The initial population density on different trees varied between 70 and 283 larvae per kg of branches, the mean values of the plots between 98 and 190 larvae per kg (Table II). In spite of these large variations, the relative population reductions on plots treated the same way were so similar (Table II) that the results of the replicates could be computed (values of Table I).

The results show that without treatment the populations were reduced by about 50% within 3 weeks (natural mortality). In order to estimate the values of the mortalities induced by the different treatments, the experimental values had to be corrected by applying Abbott's formula. The corrected results in the last column of Table I show that, at the concentrations used, both Thuricide 90T alone and DDT alone induced an additional larval mortality of about 55%, whereas the supernatant (ET) alone had almost no toxic effect. However, in combination with Thuricide, both DDT and ET caused more than 80% mortality. The result of the combination with DDT corresponds to independent action of the chemical insecticide and the biopreparation. This is not true for the combination of Thuricide with ET, where independent action would account for 59% mortality only. Therefore supplemental synergism[15] has to be postulated for this combination.

The controls of the glue tables revealed no differences between the control plots and the plots treated with ET alone. Large numbers of larvae of *Z. diniana*, and relative large numbers of other insect species, descended or fell only from the trees in plots treated with DDT.

Biopreparations of *B. thuringiensis* are used against many lepidopterous insect pests. However, even against susceptible species, as in the case of *Z. diniana*, their use may be limited since sometimes control is not fully satisfactory. Our experiments indicate that this handicap might be overcome by the addition of ET.

Except for one Russian preparation, all commercial preparations of *B. thuringiensis* produced at present are devoid of ET which, in conformation with the rules of biological control, is not wanted because of lack of specificity and its toxicity for vertebrates[16]. The producers therefore either use strains of the serotype H3 which does not synthetize ET or, if strains of the serotype H1 are used, the spore/crystal complex is separated from the culture medium which contains the ET and which is discarded. According to present knowledge, biopreparations based on the spore/crystal complex only are practically devoid of substances which are poisonous for organisms other than larvae of Lepidoptera.

The production of highly specific biopreparations is certainly commendable. However, because of their limited usefulness, the production of more potent preparations containing relatively high amounts of ET should also be considered and studied carefully. Although ET has an oral toxicity for mice similar or slightly higher than DDT[17], the amount of ET needed to produce the same combination effect against *Z. diniana* is at least 10 times less than that of DDT (assuming 50 mg of ET per liter of supernatant[16]). Low doses of ET are probably harmless for insects and other organisms which are not susceptible for the spore/endotoxin complex of *B. thuringiensis*, as suggested by the results of the glue table controls and by the finding that repeated injections of sublethal doses of ET in mice did not lead to toxic effects, i.e. to accumulation[18]. We may therefore expect that the specificity of bacterial preparations is not unduely reduced by the addition of β-exotoxin, whereas the effect of the endotoxin in susceptible species might be considerably enhanced. This has recently been confirmed by ALYOSHINA, though no further proofs have been offered[19].

Summary. Addition of either DDT or the supernatant of a centrifuged liquid culture of *Bacillus thuringiensis*, serotype H1, containing β-exotoxin, enhanced the action of the bacterial preparation Thuricide 90T against larvae of the larch pest *Zeiraphera diniana*, increasing mortality from 53% to more than 80%. Since DDT alone produced 57% mortality, its combined action corresponds to independent action. The preparation of β-exotoxin, on the other hand, had only little effect alone but synergized the action of the bacterial preparation considerably.

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Development of the Electric Discharge in Mormyrid and Gymnotid Fish (*Marcusenius sp.* and *Eigenmannia virescens*)

The recent identification of the environmental factors leading to gonad growth in several species of the two major groups of weakly electric fish (the Mormyriformes of Africa, and the Gymnotoidei of South America) and the repeated successful reproduction of two of these species in captivity[1], has enabled us to carry out a longitudinal study of the development of the electric organ, electroreceptors[2] and the discharge itself. In this paper we present the first results of a detailed study of the ontogeny of electric discharge in the gymnotid *Eigenmannia virescens* and the mormyrid *Marcusenius sp.*

Method. Electrical recording was carried out in a specially constructed glass cell of low capacity (0.90 ml), filled with water of constant conductivity (650 μmho. cm^{-1}) and maintained at constant temperature.

Results. Eigenmannia. The first discharges (Figure 1) were detected on Day 8 and were of very low amplitude (20–30 μV in all specimens) rizing to about 150 μV within 20 min (Figure 2a). The discharge was discontinuous at first and occurred in short bursts which became longer and longer until the fish was continuously discharging after 12 min. Discharge frequency was very low on Day 1

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