WHY IS *BACILLUS THURINGIENSIS* SUBSP. *ISRAELENSIS* (*BTI*) A RELEVANT MICROBIAL MOSQUITO CONTROL AGENT IN YUGOSLAVIA? *(Abstract only)*

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The major part of the Panonian Plain is rich agricultural soil which has been well known for its high wheat, maize, sugar beet and vegatable production. These crops are regularly treated with large quantities of fertilizer and a variety of pesticides. The only virgin, non-contaminated sites are rural fields, gravitating towards the rivers and sites regularly flooded by two major rivers: the Danube and the Tisa river. This inundation area, which is a favorable mosquito breeding site for the most common and abundant species, *Aedes vexans,* has been the subject of mosquito control in Yugoslavia for more than 20 years. Ecological demands and concern for the environment have been truly acknowledged and require a more friendly, environmently sustainable approach than conventional chemical larvicides and adulticides. The entomofauna of the rural region has been irreversibly suppressed with malathion, deltamethrin or lambdacyhalothrin adulticides, whereas most of the aquatic organisms were affected by temephos treatments. Our studies showed that in many mosquito habitats the most susceptible immature stages of water insects (Ephemeroptera, Odonatoptora, Heteroptera, Coleoptera, and Diptera: Chironomidae, as well as Collembola: *Podura*) tolerated different formulations of *Bti* treatments. Concomitantly, the applied *Bti* rates achieved very high efficacy in controlling larval stages of *Aedes, Anopheles* and *Culex* species. However, environmental factors influence the effectiveness of *Bti* to some extent. According to our results low water temperature (5°C) yielded up to 10-fold higher LC_{50} and LC_{90} values compared with those at high temperatures (25°C). Decrease in *Bti* efficacy was related to increase in larval density and sunlight intensity, as a linear function.

SUSCEPTIBILITY OF NATURAL POPULATIONS OF MALARIA MOSQUITOES TO THE TOXINS OF *BACILLUS THURINGIENSIS* SUBSP. *ISRAELENSIS* (*BTI*) *(Abstract only)*

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Studies on the susceptibility of larval populations of the *Anopheles maculipennis* complex to *Bti* toxins were carried out in different regions of Russia. It has been shown that susceptibility of *An. messeae* Fall. to toxins is related to the form of arm 2R of chromosome 2. Individuals with genotypes $2R_{11}$ appeared to be most susceptible and those with genotypes $2R_{00}$–$3L_{00}$ — least susceptible. $2R_1$ and $3L_1$ inversions seem to reduce protective mechanisms inactivating microbial toxins in mosquitoes. Larvae of *An. messeae* with genotypes $2R_{11}$–$3L_{01(11)}$ demonstrated the highest rate of filter-feeding. However, larvae of the sibling species *An. beklemishevi* Steg. et Kab. with a high rate of filtration were less susceptible to the toxins. Thus, the relation between the rate of filter-feeding and the susceptibility to *Bti* toxins in malaria mosquitoes appeared to be an intraspecific characteristic. The different influence of *Bti* treatments upon a number of the comb teeth on stigmal plate of surviving larvae and its fluctuating asymmetry has been noted. In *An. messeae*, the females with predominance on the right side were less susceptible than the ones with predominance on the left side. The reaction of *An. beklemishevi* larvae differed significantly from that of

An. messeae. Under experimental conditions females of *An. messeae* and males of *An. beklemishevi* appeared to be less susceptible to *Bti* than males of *An. messeae* and females of *An. beklemishevi.* At high experimental density both sexes of *An. beklemishevi* and males of *An. messeae* with sex chromosome XL_1 demonstrated lower susceptibility to *Bti* toxins. The susceptibility to *Bti* was 2–4 times higher in larval populations from the western part of the country than that in those from the eastern part.

MOSQUITO POPULATIONS IN THE GAZA REGION AND THEIR CONTROL BY *BACILLUS THURINGIENSIS* SUBSP. *ISRAELENSIS (By title only)*

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SENSIBILITY OF MEADOW MOTH (*PYRAUSTA STRITICALIS*) LARVAE TO *BACILLUS THURINGIENSIS* SUBSP. *KURSTAKI* MORPHOVARS *(Abstract only)*

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Bacillus thuringiensis subsp. *kurstaki* (*Btk*) strains of only K-1 crystovar are the base of the well-known bioinsecticides such as Dipel and Lepidocide. However, Jaquet et al. (1987) and MacIntosh et al. (1990) showed up to 15-fold differences in the insecticidal activities between strains of *Btk* K-73 and K-1 crystovars against a range of insects such as: *Heliothis virescens, H. zea, Mamestra brassicae, Ostrinia nubilalis* and *Agrotis ipsilon.* We studied meadow moth (*Pyrausta striticalis*) larval sensibility to *Btk* K-1 and K-73 crystovars. In our previous investigation we found simple biochemical criteria for distinguishing between K-1 and K-73 crystovars. The *Btk* strains were divided into six morphovars (R, X, M, M, r, m) differing in their ability to synthesize enzymes, hemolysins and bacteriocine-like substances. The model strains had been grown on the nutrient agar and on the liquid medium until spores and crystals were completely formed. Purified suspensions of spores and crystals as well as culture liquid (CL) were fed to the second stage meadow moth caterpillar. The meadow moth rearing had been maintained in the laboratory for 58 successive generations. The bioassays demonstrated that purified spores and crystals of all morphovars differed a little in the insecticidal activity. At the same time their CLs increased the toxicity according to morphovars. The activity of the CL of m morphovar of K-73 crystovar was three and a half times less than that of r morphovar strains of K-73 crystovar. The insecticidal activity of the CL of X morphovar strains of K-1 crystovar was two and a half times higher than that of r morphovar strains and four times less than that of R morphovar strains of K-1 crystosubsp. Thus, the by-metabolites as the additional factors of virulence appeared to be able to increase the sensibility of meadow moth larvae to *Btk.*

ISOLATION AND CHARACTERIZATION OF STRAINS

IDENTIFICATION, CHARACTERIZATION AND NUCLEOTIDE SEQUENCING OF NOVEL *CRY*-TYPE GENES FROM *BACILLUS THURINGIENSIS* STRAINS *(Abstract only)*

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Two pairs of universal oligonucleotide primers were designed to probe the most conserved regions of all known *cryI*-type gene sequences, so that the amplified PCR fragments of the DNA template from *Bacillus thuringiensis* strains may contain all possible *cryI*-type gene sequences. The restriction fragment length polymorphism (RFLP) patterns of the PCR-amplified fragments revealed that 14 distinct *cry*-type genes have been identified from 20 *B. thuringiensis* strains. Interestingly, five *cry*-type genes and seven *cry*-type genes have been detected from *B. thuringiensis* subsp. *morrisoni* HD-12 and *B. thuringiensis* subsp. *wuhanensis*, respectively. Among them, at least four *cry*-type genes are novel. Some novel *cry*-type genes were cloned. The restriction maps and partial nucleotide sequences of those clones further confirmed that those *cry*-type genes are really novel. The nucleotide sequence of one of them from *B. thuringiensis* subsp. *wuhanensis* has been completed. By use of the Pileup program in the Genetics Computer Group Sequence Analysis Software Package to analyze the position of this *cry* gene in the evolutionary dendrogram of *cry* genes, we found that the *cry* gene had only 43 to 70% homology with all known *cry* gene sequences, and this novel *cry*-type gene may be positioned between *cryIF* and *cryIB* genes. Characterization of this novel *cry*-type in *Escherichia coli* and in *B. thuringiensis* strains is being carried out.

STRAIN DIVERSITY OF *BACILLUS THURINGIENSIS* IN THAILAND *(Abstract only)*

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A collection of 299 *Bacillus thuringiensis* (*Bt*) isolates was obtained from soil samples of rice-growing areas, rice stemborer, rice dust and rice bran from various rice mills in Thailand. According to the classification by serological methods, these isolates were classified into 14 subspecies. Among those, 13 subspecies were previously reported as follows: *kurstaki (H3abc), alesIAi (H3ac), kenyae (H4ac), galleriae (H5ab), canadensis (H5ac), entomocidus (H6ab), aizawai (H7), tolworthi (H9), kumamotoensis (Hl8), tochigiensis (Hl9), neoleonensis (H24), mexicanensis (H27),* and *leesis (H33)*, and the additional one proved to be a new serovar, *chanpaisis (H46).* Moreover, some of them were classified as non-serotypable strains which will be further analyzed and additional new strains may be obtained from them.

The most encountered subspecies isolated, in ranking order, are *kurstaki, kenyae* and *galleriae* with 109, 54 and 44 isolates, respectively. The regions where *Bt* is mostly found are northern and central cultivated lands of high humidity and fertility. *Bt* can be isolated from soil samples, rice bran and stemborers. However, in the southern and eastern regions with salty water, *Bt* is scarcely found. Most isolates can be obtained from rice bran only.

It therefore can be seen that *Bt* strains are diverse throughout the country, especially in the highly fertile lands with reduced insecticide application such as fertile forests, National Forest Parks, etc. A large number and diversity of *Bt* was then expected to be found in these localities.

GENOME TYPING OF *BACILLUS THURINGIENSIS* SEROVARS AND STRAINS
(Abstract only)

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Twenty-five *Bacillus thuringiensis* (*Bt*) standard serovars, 12 special strains and 19 commercial strains compared with 2 *B. cereus* (*Bc*) and 2 *B. sphaericus* (*Bs*) strains were analyzed by AP-PCR (arbitrarily primed-PCR), a technology using total DNAs as templates and two arbitrary primers, respectively. Genome typing was made on the basis of the electrophoretic fingerprints of AP-PCR products. It shows common characteristics and polymorphisms among different serovars, biovars and even different strains belonging to the same serotype. Most different serovars produce distinct banding patterns except that serovars *dakota, kumamotoensis* and *colmeri* and serovars *kurstaki* and *galleriae* produce relatively similar patterns, respectively. Biovars belonging to the same serotype such as biovars *dendrolimus* and *sotto* are distinguished from each other. Moreover, some special strains can still be differentiated as well. For example, strain 7501 (H4a4c), which produces irregular sphere crystals and has low toxicity to *Heliothis armigera,* differs from other Btken-Ag strains with high toxicity. Interestingly, a nonflagellated *Bt* strain 140 generates fingerprints similar to those of serovar *kurstaki* and *galleriae*. Since AP-PCR fingerprints are related to the diversity of *Bt* in H antigens, biochemical properties, crystal shapes and insecticidal activities, we have concluded that AP-PCR is a simple, rapid, reliable and direct genetically based diagnostic method for the differentiation and identification of *Bt* strains. In addition, we also found that the fingerprints of 2 *Bc* and 2 *Bs* are drastically different from those of *Bt* strains.

THE NEW APPROACH TO *BACILLUS THURINGIENSIS* STRAIN SELECTION
(Abstract only)

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The reseachers dealing with microorganisms encounter many difficulties owing to inconstancy of the properties of these organisms. Inevitable strain subculturing leads to microbial population heterogeneity. Especially the problems of scale-up of aerobic fermentations need attention. Little is known, however, about the interactions between microorganisms and their environment. *Bacillus thuringiensis* (*Bt*) is a pathogen of a large range of insects, including lepidopterans, dipterans and coleopterans. For many years, we have dealt with the problem of *Bt* taxonomy and selection. First of all we are studying newly isolated *Bt* strains, old stock strains, and as many of their variants as possible; then we select correlating properties that are common to all these strains, for the purpose of delineating the subspecies which these strains represent. A convenient method to plate *Bt* strains on empirically selected nutrient media was developed and used to differentiate widely by appearence a few morphovars within the subspecies *galleriae, kurstaki* and *israelensis.* Morphovars differed by proteolytic, phospholipase (A or C) and hemolytic activities, pathogenicity and specificity. We demonstrated cross-antagonism between different morphovars of the same subspecies. The spore-crystal mixtures of different morphovar strains were studied by means of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). The results show that SDS-PAGE can be a valuable tool in the detection of protein profiles of morphovars. Further work is required to elucidate the parameters which mediate specificity.

GENE EXPRESSION

ASPECTS OF PROTOXIN GENE REGULATION *(Abstract only)*

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Most *Bacillus thuringiensis* (*Bt*) subspecies which produce Cry1 protoxins deposit different amounts of several prototoxins in a single inclusion. Each protoxin has its own specificity profile and there may be synergistic interactions contributing to the overall toxicity profile. At the cellular level, protoxin gene composition can change due to the instability of certain protoxin encoding plasmids as well as transfer of such plasmids by cell mating. The *cryI* genes are transcribed primarily during sporulation and each contains dual overlapping promoters utilizing forms of RNA polymerase containing specific mother cell sigma factors. This ensures a constant rate of protoxin synthesis throughout much of sporulation. Differential control depends upon unique upstream regions and the binding of a novel transcription factor, the E2 subunit of pyruvate dehydrogenase. This protein binds with different affinities to specific sites in these upstream sequences and appears to have been recruited to signal post exponential metabolism. Mutations in the binding sites which altered the affinity for E2 also affected expression of a *lacZ* fusion but only when cells had been grown on poor carbon sources (such as TCA cycle intermediates). These elaborate control systems are apparently required both for inclusion assembly and for modulating protoxin composition in response to the environment.

THE EFFECTS OF *CRYIVB* TERMINATOR FRAGMENTS ON EXPRESSION OF THE CHLORAMPHENICOL ACETYLTRANSFERASE GENE IN *BACILLUS THURINGIENSIS (Abstract only)*

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The *cryIVB* gene of *B. thuringiensis* subsp. *israelensis* encodes its 130 kDa δ-endotoxin. The terminator region of this gene has been purposed to enhance toxin production by RNA stabilization. To study this proposal, the *Cla*I-*Bam*HI fragment harbouring the terminator region of the gene was cloned into *Escherichia coli* cloning vector pGEM7-Zf(+). This recombinant plasmid was designated pGBT8 and was used to construct three additional plasmids. The first (pGBT8.1) contained 221 bp of the distal portion of the *cryIVB* gene plus its terminator. The second (pGBT8.2) contained the complete 127 bp *cryIVB* terminator. The third (pGBT8.3) contained 86 bp of the *cryIVB* gene and only half of the *cryIVB* terminator. These three fragments were further subcloned into vector pTFM6 at a position 5′ to the terminator for its chloramphenicol acetyltransferase gene (*cat-86*). Thus three double terminator constructs were obtained and designated pTT1, pTT2, and pTT3, respectively. In order to study the effects of the single *cryIVB* terminator, a derivative of pTT2 was constructed where the normal *cat-86* terminator was deleted. This was designated pTT2A. The presence of cloned terminator regions in the newly constructed plasmids was confirmed by restriction patterns and Southern blot hybridization. All the recombinant plasmids were first transformed into *Bacillus subtilis* MIII3 and subsequently into *Bacillus thuringiensis* c4Q272 using electroporation. Strains of *Bacillus* were determined by measuring the specific activity of chloramphenicol acetyltransferase (CAT) at various growth phases. Results showed that the *cryIVB* terminator had a stimulatory effect on *cat-86* activity in *B. thuringiensis* subsp. *israelensis* c4Q272.

CLONING AND EXPRESSION OF *BACILLUS THURINGIENSIS CRYIIA* GENE IN *ANABEANA (Abstract only)*

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The mosquito specific *cryIIA* gene was cut out from pGEM1 with *Bam*HI and ligated into the *Bam*HI site of an *Escherichia coli*-cyanobacterium shuttle plasmid pRL25C. The recombinant plasmid was transformed into *E. coli* HB101 which bears plasmid pRL528, a helper plasmid carrying mob and genes for *Eco*47II and *Ava*I methylase. Then three parents: *E. coli* HB101 bearing the recombinant plasmid and helper plasmid pRL528; *E. coli* HB101 bearing RP4; and *Anabeana* PCC 7120, a filamentous cyanobacterium which can fix nitrogen and can be fed by mosquito larvae in water, were put together to do triparent matings. Both Southern blot and Western blot demonstrated that the recombinant plasmid existed and expressed in the positive conjugant of *Anabeana* PCC 7120. But the expression of the *cryIIA* gene with its original promoter in transgenic *Anabeana* PCC 7120 was too low to kill mosquito larvae. The research probed the way to construct mosquito-killing and nitrogen-fixing bifunctional *Anabeana* with the crystal protein genes of *Bacillus thuringiensis* subsp. *israelensis* and lay the foundation for further work. This research was supported by a grant of UNDP/World Bank/WHO–Special Program for Research and Training in Tropical Diseases.

RESTRICTION MAP OF THE 125 KB PLASMID OF *BACILLUS THURINGIENSIS* SUBSP. *ISRAELENSIS* CARRYING THE MOSQUITO LARVICIDAL GENES *(Abstract only)*

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Mosquitoes and black flies are vectors of many human infectious diseases. One of the best biocontrol agents against their larvae is the bacterium *Bacillus thuringiensis* subsp. *israelensis* [serovar H14 (*Bti*)]. Its mosquito larvicidal activity is included in five polypeptides of a parasporal crystalline body (δ-endotoxin), CryIV, A-D, and CytA (134, 128, 78, 72 and 27 kDa in size), encoded by the respective genes which are highly expressed during sporulation. These, and all the other genetic elements responsible for toxicity, are located on one of the largest (125 kb) plasmids of *Bti*.

The large plasmid containing all δ-endotoxin genes was isolated from *Bti*, restricted by *Bam*HI, *Eco*RI, *Hin*dIII, *Kpn*I, *Pst*I, *Sac*I and *Sal*I, and cloned as appropriate libraries in *Escherichia coli*. The libraries were screened for inserts containing recognition sites for *Bam*HI, *Sac*I and *Sal*I. Each was labeled with ^{32}P and hybridized to Southern blots of gels with fragments generated by cleaving the plasmid by several restriction endonucleases, to align at least two fragments of the relevant enzymes. All nine *Bam*HI fragments and all eight *Sac*I fragments were mapped in two overlapping linkage groups (with total sizes of about 76 and 56 kb, respectively). The homology observed between some fragments is apparently a consequence of the presence of transposons and repeated insertion sequences. Four δ-endotoxin genes (*cryIV, B-D* and *cytA*) and two for regulatory polypeptides (of 19 and 20 kDa) were localized on a 21 kb stretch of the plasmid; without *cytA*, they are placed on a single *Bam*HI fragment. This convergence enables sub-cloning of δ-endotoxin genes (excluding *cryIVA*, localized on the other linkage group) as an intact natural fragment.

MOLECULAR BIOLOGY AND GENETIC ENGINEERING

MECHANISM OF MEMBRANE PERMEATION BY CYT (*BTI*) AND CRY δ-ENDOTOXINS *(Abstract only)*

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The insecticidal δ-endotoxins are produced by *Bacillus thuringiensis* (*Bt*) bacteria during sporulation. Two major families of δ-endotoxins have been identified, namely, the Cry and the Cyt (*Bti*) toxins. Both families are assumed to act *via* the formation of transmembrane pores in the midguts of the susceptible insects. However, the Cry toxins are hypothesized to exert their toxic activity *via* a receptor mediated process, while the activity of the Cyt toxins is assumed to result from direct lipid-protein interactions. The X-ray structure of toxins from both families have been resolved recently by Ellar and coworkers and the structures support their hypothesized mechanism of action. We are using various spectroscopic methods combined with a synthetic peptide approach to investigate the mode of action of the pore forming domains of both toxins. Peptides corresponding to structural elements of the toxins have been synthesized, site-specifically labeled with various fluorescent probes, and structurally and functionally characterized. Our results give direct experimental evidence for oligomeric pore formation mechanisms for both toxins and suggest structural models for the membrane insertion and assembly of several monomers to form the active pore.

PHENOTYPE OF *BACILLUS SPHAERICUS* CONTAINING *BTI* TOXIN GENES *(Abstract only)*

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A 6 kb *Msc*I fragment of the 72 mDa plasmid of *Bti* was cloned into plasmid pTV51Ts at a site immediately following the promotorless β-galactosidase gene and inserted into the chromosome of *B. sphaericus* 2362. Several transformed clones expressed both β-galactosidase and *Bti* toxin genes, but many more expressed the toxin genes alone, apparently utilizing internal *Bti* promoters. Many clones showed impaired growth, sporulation, and germination, together with altered spore morphology. The most active clones were 8–12 times more toxic to *Aedes aegypti* larvae than the wild type *B. sphaericus*.

MEMBRANE ORGANIZATION OF THE HELICES FROM THE PORE FORMING DOMAIN OF δ-ENDOTOXIN *(Abstract only)*

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To gain insight into the toxic mechanism and the structural features of the pores formed by *B. thuringiensis* δ-endotoxin, the membrane interaction and the orientation within membranes of its seven helices were studied. Peptides with sequences corresponding to the seven helices of the toxin were synthesized and characterized. Six of the helices, namely helices a2–a7, were found to bind phospholipid vesicles, suggesting that they may have a structural role in the pores formed by the toxin. To study the structure of the helices and their orientation relative to the normal of phospholipid membranes, ATR-FTIR spectroscopy was used. The dichroic ratio of the amide I bands of the peptides reconstituted to oriented phospholipid membranes, indicated different orientations for the various peptides. While some of the peptides (a4, a5) were preferentially oriented in a transmembrane orientation, the rest of the helices were oriented randomly or nearly parallel to the surface of the lipid membranes. Taken together, our results are consistent with an "umbrella" model for the structure of the pores formed by the toxin. The model involves the insertion of some segments as an helical hairpin, where the others are open on the membrane surface like the ribs of an umbrella.

THE 19 KDA PROTEIN OF *BACILLUS THURINGIENSIS* SUBSP. *ISRAELENSIS* DID NOT PROTECT *ESCHERICHIA COLI* CELLS FROM THE LETHAL EFFECT OF CYTA *(Abstract only)*

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The bacterium *Bacillus thuringiensis* subsp. *israelensis* produces large amounts of δ-endotoxin that is lethal to mosquito and black fly larvae, vectors of many human infectious diseases. Larvicidity is included in polypeptides of a parasporal crystalline body (δ-endotoxin) produced during sporulation, which is composed of five polypeptides, CryIV, A-D, and CytA (134, 128, 78, 72 and 27 kDa in size), encoded by the respective genes. In addition to its mosquitocidal activity, CytA is also hemolytic and cytotoxic. The crystals formed in various *B. thuringiensis* species are protease-resistant. Crystallization of CryIIA (71 kDa), CryIVD (72 kDa) and CytA (27 kDa) require accessory proteins, in contrast to other Cry proteins such as the 130 kDa CryI, that spontaneously form biologically active inclusions. The genes *cryIIA* and *cryIVD* are organized each in an operon with genes not involved in toxicity (*orf2* and *p20*, respectively). Both Orf2 and P20 may act as chaperones to initiate, facilitate, or stabilize crystal formation. The protein P20 stabilizes CytA in *Escherichia coli;* they form a complex together, which seems to act as a scaffold facilitating assembly of CytA into the protease-resistant oligomers. Expression of cloned *cytA* in the absence of P20 is lethal to *E. coli* cells. Survival in the presence of P20 indicates that the lethal action of CytA is abolished by facilitated crystallization. It has been proposed recently that P19, encoded by a new gene (*p19*), mapped upstream of *cryIVD,* is also involved in the crystallization process of CytA. This hypothesis predicts that P19 will also protect *E. coli* cells from the lethal action of CytA. In this study, *p20* and *p19* were isolated separately and cloned in pairwise combinations with *cytA* by inducible expression vectors into *E. coli.* While P20 protected the host cells from the lethal action of CytA, P19 failed to do so. This result rules out the hypothesis that P19 is involved in the crystallization process of CytA.

ENGINEERING OF *SPODOPTERA*-RESISTANT PLANTS USING *CRYIC (Abstract only)*

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A synergistic anti-*Spodoptera* effect was obtained by co-applying *Escherichia coli* producing CryIC (756aa) and ChiAII (endochitinase) to young larvae of *Spodoptera littoralis.* The latter is encoded by *Serratia marcescens chiAII* and capable of perforating the chitinic peritrophic membrane lining the lumen of the insect midgut and increasing toxicity.

The 3′-truncated *cryIC* and *chiAII* were cloned in plant expression cassettes in *E. coli-Agrobacterium*-plant shuttle vectors and introduced into alfalfa, tobacco, and potato. Transgenic plants were developed carrying *chiAII* and *pat* [conferring resistance to the non-selective herbicide "Basta" (glufosinate)] linked to the mannopine synthase dual promoter and *cryIC* driven by the CaMV 35S promoter and four tandemly arranged 35S enhancers. Transgenic alfalfa possessing *chiAII* and *cryIC* transgenes were found to be more resistant to young larvae of *S. littoralis* than plants carrying the *cryIC* gene alone. However, the level of *cryIC* transcripts in these relatively resistant plants was below the detection level of Northern blot analysis.

To improve *cryIC* expression in plant cells, a plant-like *cryIC* was synthesized by a novel method developed in the course of this study. This synthetic *cryIC* was hardly expressed in *E. coli.* Transgenic alfalfa and tobacco plants carrying this synthetic gene were resistant to all instar larvae of *S. littoralis* and *S. exigua* and expressed a high level of CryIC (0.1–0.2%). All the transgenic plants were also resistant to the herbicide "Basta" and therefore can be directly and easily integrated into agronomic cultivars by routine breeding programs.

AUTHOR INDEX

NOTES FOR AUTHORS

Israel Journal of Entomology, a peer-reviewed journal, publishes original contributions in all areas of entomology. Authors are entirely responsible for statements, whether of fact or opinion.

MANUSCRIPTS

Manuscripts, in standard English only, are considered on the understanding that their contents would not be published elsewhere. If a preliminary announcement relating to the contents of the paper has already been published, this must be stated.

Papers should be concisely written. Consulting the latest issue of the Journal and the "Style Manual for Biological Journals" is highly recommended. Manuscripts should be submitted in triplicate, typed double spaced on one side of a page only, leaving 3-cm margins on both sides, together with a 3.5" or 5.25" IBM-compatible diskette. The name and version of the wordprocessor used should be indicated. For programs other than WordPerfect, Wordstar, MS Word or Einstein, an ASCII version of the file should be included. The title of the paper should be informative, but preferably not exceed twenty words. An abstract provided at the beginning of the paper will indicate the main aspects of the subject, to be followed by 5–7 key words. Words which are to be italicized in print, such as scientific names, should be italicized in the manuscript or underlined with a single solid line. No more than three categories of subheadings are allowed. Footnotes to text should be kept to a minimum.

SPELLING

Spelling and terminology should be consistent throughout. Scientific names, on first mention, should be followed by the name of the first describer, written in full. When referring to paired organs in morphological descriptions, the singular form should be used. Locality records should preferably be followed by coordinates. Names of localities in Israel will be given as they are transliterated in the "Israel Touring Map" (1:250,000) and "List of Settlements," published by the Survey of Israel, Ministry of Labour. Regions in Israel and nearby areas should follow the "Fauna Palaestina" map (as in Theodor, O. 1975. Fauna Palaestina, Insecta I: Diptera Pupipara. The Israel Academy of Sciences and Humanities, Jerusalem).

REFERENCES

1. In the text, reference to the literature should conform to the "name-and-date" system, e.g., Williams (1929); (Bodenheimer, 1938); Jones and Smith (1950). Unpublished references are to be cited as author followed by either (personal communication), (unpublished) or (in press). Only the latter category will appear in the list of references, together with the title of the periodical to which the paper was submitted for publication.

2. When reference is made to taxonomic descriptions, or to quoted passages, the relevant page number(s) should follow the year, e.g., Brown (1939:25).

3. Where three or more authors are concerned, reference is made only to the first, followed by "et al." and the year, e.g., Thomson et al. (1945).

4. The list of references will be given at the end of the article, according to the following examples, *with the titles of all periodicals unabbreviated and italicized.*

Bergman, E.D. 1976. The future of insecticides — a problem of human environment. *Israel Journal of Entomology* 11:5–14.

Taylor, L.R. and Palmer, J.M.P. 1970. Aerial sampling. In: Aphid Technology. Edit. H.F. van Emden. Academic Press, London. pp. 125–138.

TABLES

Tables should be kept to a minimum, typed on separate sheets, and their approximate position should be indicated in the manuscript. The same data should not be given both in tables and graphs.

ILLUSTRATIONS

Only high-quality photographs and drawings, mounted for reproduction, will be accepted. Originals should be sent only after acceptance of the manuscript for publication. Each figure and photograph should be identified on the back, in pencil, with the author's name and figure number. A typed list of captions is required. Photographs should be submitted on glossy paper, not smaller than 6 × 9 cm. Drawings should be prepared so as to allow a maximum of 30–50% reduction.

TAXONOMY

1. Comprehensive treatments of taxa (genera, families, etc.) will receive higher priority over partial treatments. Partial lists of species or faunistic lists, not accompanied by proper keys or references to such keys, will receive lower priority. Keys should be dichotomic, with two alternatives for each character, and preferably illustrated.

2. Authors must comply with the requirements of the International Code of Zoological Nomenclature and with the published Opinions of the International Commission.

3. The following abbreviations should be adopted: ***n. gen.*** – new genus; ***n. sp.*** – new species; ***n. comb.*** – new combination of names; ***n. syn.*** – denotes synonymy established for the first time; ***n. stat.*** – will be used to indicate a new change in rank of a name; ***nomen nudum, nomen dubium, nomen novum*** are not abbreviated.

4. In treating the taxonomy of a described taxon, the following form is essential for the beginning of a chapter.

Filippia oleae (Costa, 1832)
(Fig. 1)

Coccus oleae Costa, 1832:21; Green, 1868:42 (biology).
Filippia oleae Fernald, 1903:13 (catalog); Hall, 1943:50 (hosts list).

The full references to the above citations should be given in the REFERENCES section.

5. New taxa must be distinguished from related taxa.

6. In describing new species, the complete data of the type-series, together with the collection(s) in which it is deposited, will be recorded verbatim as follows:

MATERIAL EXAMINED. Holotype ♀, ISRAEL: Jerusalem, 14.v.1956, on *Ficus carica*, G. Levi (BMNH). Paratypes, 20♀, same data as holotype (USNM); Tel Aviv, 3.v.1962, on *Acacia* sp., G. Brown (1♂, 8♀; TAU).

Records of specimens other than type series will be listed at the end of each relevant chapter in a similar manner, but see "SPELLING" above.

7. Authors are required to deposit all type-material in nationally or internationally recognized institutions (not private collections).

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