EFFICACY TESTS OF *Bacillus thuringiensis* FOR STRATEGIC BIO-CONTROL OF *Metisa plana*

by: RAMLAH ALI A S, MAHADI N M and BASRI M W

JUNE 2001

113

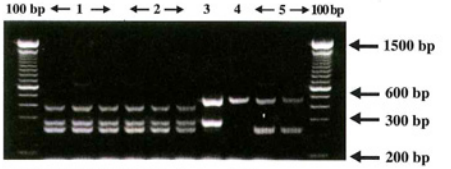
MPOB TT No. 98

MPOB INFORMATION SERIES (formerly known as PORIM Information Series) ISSN 1511-7871

Amongst the oil palm insect pests, bunch moth, nettle caterpillars and bagworm (*Mahasena corbetti*) are easily controlled with *Bacillus thuringiensis* (Mohd Basri *et al.*, 1994). Bio-control of *M.plana* using *B. thuringiensis* requires essentially the following preliminary laboratory tests:

ANALYSIS OF *cry* GENES IN *B. thuringiensis (Bt)*

Determination of *cry* genes of *Bt* using specific primers is essential for detecting its toxin combination. Each *Bt* isolate produces several mixtures of crystalliferous toxins during sporulation (Zelany *et al.*, 1995: Vasquez *et al.*, 1995). These crystals vary in shape from typically bipyramidal, spherical, rhomboid, rectangular to irregular (Zelany *et al.*, 1995).

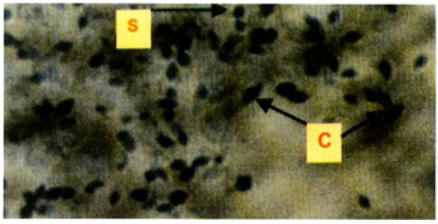


PCR Screening of Toxin Genes of Bt

Combination of *cry* genes for different *Bt* (1, 2, 3, 4 and 5). The sizes for the cry genes are *cry 1Aa*, 724 bp; *cry 1Ab*, 238 bp; *cry 1Ac*, 487 bp; *cry1B*, 830 bp; *cry1C*, 288 bp; *cry 1E*, 883 bp; *cry 1F* , 368 bp and *cry2A/2B*, 1070 bp.

These crystals called the Cry proteins are classified Cry 1 to Cry 4, according to their specific toxicity towards insect orders and DNA homology (Hofte and Whiteley, 1989). The gene that codes for the Cry protein is named *cry* gene (Kalman *et al.*, 1993). Determination of *cry* genes in *Bt* is a rapid evaluation of insecticidal activity (Mahadi *et al.*, 1998). Knowledge on the *cry* gene content can be exploited to overcome *Bt* resistant problem.

PRODUCTIVITY OF TOXINS

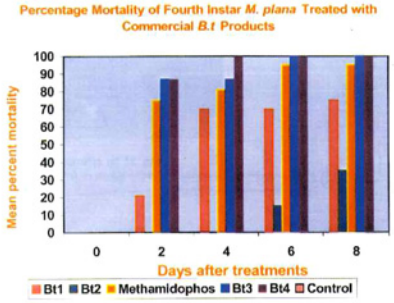


Efficacious Bt *produces plenty of crystal toxin from its* cry *genes: crystals (c) and spores (s).*

The *cry* genes must be well expressed to form crystal toxins sufficient for 'quick knock-off effect' of *M. plana*.

MORTALITY TEST

Comparative mortality showing well formulated *Bt* 3 and 4 are comparable to methamidophos and far superior than other *Bt* products. Detailed bioassay of *Bt* with probit analysis will give the exact dose required for effective bio-control of *M. plana*.



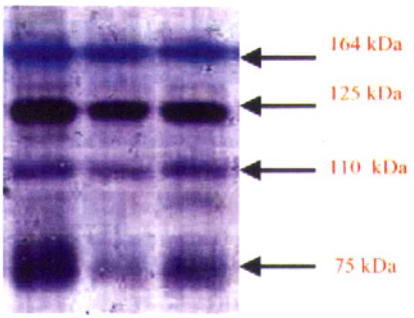
ISSN 1511-7871

9 771511 787001

Malaysian Palm Oil Board, Ministry of Primary Industries, Malaysia
P. O. Box 10620, 50720 Kuala Lumpur, Malaysia. Tel: 03-89259155, 89259775, Homepage: http://mpob.gov.my Telefax: 03-89259446

TEST ON THE PRESENCE OF RECEPTOR FOR BINDING WITH CRY PROTEINS

The efficacy of *Bt* requires receptors called the brush border membrane vesicle (bbmv) in *M. plana* for binding with Cry proteins.



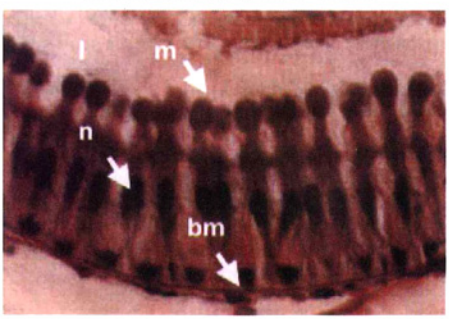
In vitro *binding of* cry *protein with bbmv of* M. plana.

Susceptibility to *Bt toxin* is directly proportional to (Ramlah Ali, 2000):

- No. of bbmv proteins;
- Amount of bbmv; and
- Binding irreversibility.

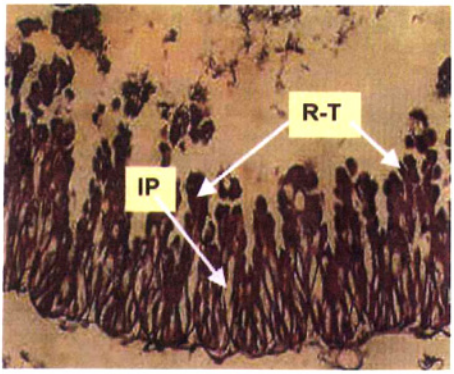
Changes and reduction in receptors (bbmv subsequently, lead to reduced binding of toxin and insect resistance (Ballester *et al.*, 1999).

TEST ON *in situ* EFFECT OF *Bt*



Normal mid-gut epthelia of M. plana: *lumen (l), microvilli (m), nucleus (n) and basement membrane (bm).*

Normal mid-gut epithelia of *M. plana* on basement membrane with microvilli on the luminal side.



Binding of toxin with bbmv M. plana *and destruction of mid-gut lining. Bbmv bound with toxin forming receptor-toxin complex (R-T), ion-pores (IP).*

Binding of Cry protein with bbmv of *M. plana* forming dark receptor-toxin (R-T) complex (Ramlah Ali, 2000).

In vivo insertion of toxin into membrance of *M. plana* forms ion-pore which is indicated as *in vitro* irreversible binding (Ramlah Ali, 2000).

The pore leads to leakage of electrolytes, osmotic imbalance and cell lyses (Ramlah Ali, 2000; Schnepf *et al.*, 1998).

ALL THE ABOVE TESTS ARE AVAILABLE IN THE FORM OF SERVICES FOR CHEMICAL/PESTICIDE COMPANIES AT A NEGOTIABLE COST OF RM 40 000 INCLUSIVE OF CONSULTANCY.

REFERENCES

BALLESTER, V; GRANERO, F; TABASHNIK, B E; MALVAR, T and FERRE, J (1999). Integrative model for binding of *Bacillus thuringiensis* toxins in susceptible and resistant larvae of the diamondback moth (*Plutella xylostella*). *Appl. Environ. Microbiol., 65(4)*: 1413-1419.

HOFTE, H and WHITELEY, H R (1989). Insecticidal proteins of *Bacillus thuringiensis. Microbiological Reviews, 53*: 242-255.

KALMAN, S; KIEHNE, K L; LIBS, J L and YAMAMOTO, T (1993). Cloning of a novel cryIC-type gene from a strain of *Bacillus thuringiensis* subsp. *galleriae. Appl. Environ. Microbiol., 59 (4)*: 1131-1137.

MAHADI, N M; HASTOWOS, S and DEAN, D H (1998). Application of multiplex PCR for rapid determination of cryI genes profiles of new *Bacillus thuringiensis isolates. J. Microbiol. & Biotech., 8(5)*: 1-6.

MOHD BASRI,W; SITI RAMLAH, A A and NORMAN, K (1994). Status report on the use of *Bacillus thuringiensis* in the control of some of oil palm pests. *Elaeis, 6(2)*: 82-101.

RAMLAH ALI, A S (2000). Mechanism of action of *Bacillus thuringiensis* d-endotoxins: studies on binding of d-endotoxins to brush border membrane vesicle of *Metisa plana* (Wlk). Ph. D thesis. Universiti Kebangsaan Malaysia.

SCHNEPF, H E; CRICKMORE, N; van RIE, J; LERECLUS, D; BAUM, J; FEITELSON, J; ZEIGLER, D R and DEAN, D H (1998). *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol. & Mol. Biol. Rev., 62(3)*: 775- 806.

VASQUEZ, M; PARRA, C; HUBERT, E; ESPINOZA, P; THEODULOZM, C and BASSO, L M (1995). Specificity and insecticidal activity of Chilean strains of *Bacillus thuringiensis. J. Invertbr. Pathol., 66*: 143-148.

ZELAZNY, B; STEPHAN, D and HAMACHER, J (1995) . Irregular crystal formation in some isolates of *Bacillus thuringiensis. J. Invertebr. Pathol., 63*: 229-234.

For more information kindly contact:

Director-General
MPOB
P.O. Box 10620
50720 Kuala Lumpur, Malaysia.
Tel: 03-89259155, 89259775,
Homepage: http://mpob.gov.my
Telefax: 03-89259446