The efficacy and sustainability of the CIMBAA transgenic Cry1B/Cry1C Bt cabbage and cauliflower plants for control of lepidopteran pests

Kaliaperumal, R.
THE ENERGY AND RESOURCES INSTITUTE, HABITAT CENTER, LODHI ROAD, NEW DELHI, INDIA
ramesh.kaliaperumal@bayer.com

Russell, D.A.
NATURAL RESOURCES INSTITUTE, UNIVERSITY OF GREENWICH, CHATHAM MARITIME, KENT ME44TB, UK
derek.russell@unimelb.edu.au

Gujar, G.T.
ENTOMOLOGY DIVISION, INDIAN AGRICULTURAL RESEARCH INSTITUTE, PUSA CAMPUS, NEW DELHI, INDIA
gtgujar@yahoo.com

Behere, G.
CESAR, GENETICS DEPT, UNIVERISTY OF MELBOURNE, 3010, AUSTRALIA
ganeshbehere@gmail.com

Dutt, S., Krishna, G.K., Mordhorst, A.
NUNHEMS INDIA PRIVATE LIMITED, BILASPUR PATAUDI ROAD, GURGAON, HARYANA
andreas.mordhorst@bayer.com; sunil.dutt@bayer.com

Grzywacz, D.
NATURAL RESOURCES INSTITUTE, UNIVERSITY OF GREENWICH, CHATHAM MARITIME, KENT ME44TB, UK
d.grzywacz@gre.ac.uk

ABSTRACT
In 2003 the Collaboration on Insect Management for Brassicas in Asia and Africa (CIMBAA) public/private partnership selected the Cry1B/Cry1C Bt protein combination as having the potential to provide effective and sustainable control of diamondback moth (DBM), *Plutella xylostella*. Following transformations and extensive plant selection, insect efficacy trials were undertaken in 2008 to 2010 in north India (Murthal near New Delhi) and south India (near Bengaluru) in large scale screen-house experiments using artificial infestations on the best performing (Elite Event) plant lines and on hybrids produced from them. Plant damage was scored on a scale of 0 (no visible damage) to 4 (plant effectively destroyed). For DBM, cabbage cluster caterpillar (*Crocidolomia binotalis*), cabbage webworm (*Hellula undalis*) and semi-looper (*Trichoplusia ni*) there was zero insect survival and a zero damage score on the Elite Event lines and on their hybrids, while control plants had 50 to 100% insect survival (depending on species, life stage and trials) and damage scores of 3.3 to 4. Cabbage white (*Pieris brassicae*) and cotton leaf worm (*Spodoptera litura*) showed some larval survival and damage scores up to 1.4 (especially in early trials) but no survival to pupation. Screening of DBM populations worldwide (inc. 18 populations for Cry1B and 13 for Cry1C from India) showed mean LC_{50}s close to that of international susceptible strains. To date F2 screening has not identified the presence of resistance genes in DBM in the field. Cry1B resistance was slowly developed artificially in the laboratory but 1C resistance and resistance to the Cry1B/1C combination was harder to develop and had higher fitness costs. The 'resistant' lines showed some extended survival of stunted DBM larvae on dual gene Bt plants but no survival to pupation. There was no cross-resistance between Cry1B and Cry1C. Resistance to both genes was autosomal and recessive. Beneficial insects were demonstrated to have the potential to provide additional mortality on rare surviving insects in Bt fields. Aphids were well controlled for the first 40 days post-transplanting using imidacloprid pelleted onto seed and, if necessary, by 1-2 *Verticillium lecanii* sprays thereafter. Surviving *S. litura* and *Helicoverpa armigera* in Bt sprayed fields were well controlled by one or two applications of their species-specific nucleopolyhedroviruses

Keywords
Cry1B/Cry1C, brassicas, diamondback moth, CIMBAA

INTRODUCTION
Grzywacz *et al*. (2010) summarize the current pest management practices for brassica production in Asia and Africa and review the rationale for the deployment of Bt cabbage and cauliflower. Studies emerging from the Shelton laboratory at Cornell University have shown excellent control of *Plutella xylostella* by *Brassica oleracea* plants carrying a synthetic or modified *Bt* gene (see references in Shelton *et al*. (2008). Transgenic collards with *cry1Ac* or *cry1C* genes showed complete control of susceptible *P. xylostella* larvae (Cao et al. 2005). Additional studies with a *cry1C* gene expressed in broccoli, demonstrated control of Cry1Ac-resistant *P. xylostella* (Cao *et a*l. 1999) and studies with pyramided *cry1Ac* and *cry1C* broccoli plants demonstrated excellent control of both Cry1C-resistant and Cry1Ac-resistant *P. xylostella* (Cao et al. 2002, Zhao *et al*. 2003). However, *P. xylostella* has shown its ability to develop resistance to sprayed Bts in the field (Mau and Gusukuma-Minuto 2004). In developing a Bt-based resistance to *P. xylostella*, it was therefore essential to ensure the sustainability as well as the immediate efficacy of the particular gene combination chosen. Russell *et al.* (2008 and this volume) provide an overview of the Collaboration on Insect Management for Brassicas in Asia and Africa (CIMBAA), the rationale for the choice of the Bt proteins Cry1B and Cry1C and the progress in other areas of the collaboration. Shelton *et al*. (2009) summarize the efficacy of the individual Cry1B and Cry1C purified proteins against the major caterpillar pests of brassicas in Asia, Africa and elsewhere. The

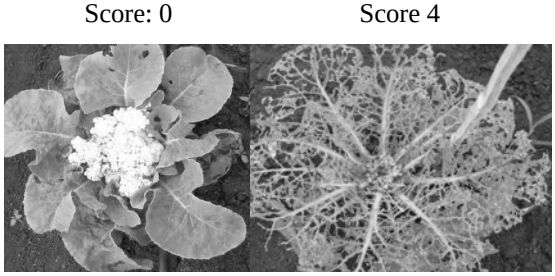
current paper provides detail on the efficacy of CIMBAA transgenic cabbage and cauliflower containing this Cry1B+Cry1C gene combination against these pests and explores its likely sustainability in the face of evolved resistance, with preliminary information on advances in the development of an appropriate IPM context for the control of pest insects not susceptible to these Bts.

EFFICACY OF Bt PLANTS

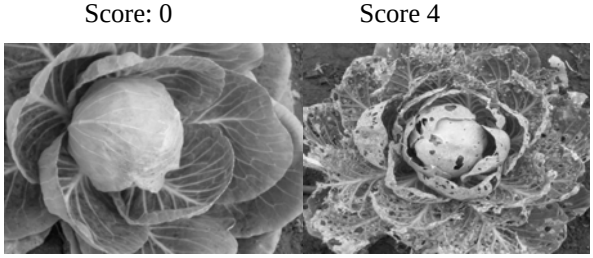
Methods

The various cabbage and cauliflower lines derived from different transformation events were selected for their suitability for commercialization (Elite Events) in large-scale screenhouse trials from 2008-2010 on the CIMBAA private partner (Nunhems India Pvt Ltd) sites at Murthal near New Delhi in the Rabi (Spring) season and near Bengaluru in the Kharif (Autumn-winter) season.

Colonies of the key pest species (P. *xylostella, Crodidolomia binotalis, Hellula undalis, Pieris brassicae* and *Trichoplusia ni*) were maintained at Nunhems facilities or species such as S*podoptera litura and Helicoverpa armigera* were obtained from the laboratory colonies of Pest Control India Ltd. The screen-houses were divided in half, each for cabbage and cauliflower and each half into plots for each pest species with three or more replicated sub plots of 15 plants per species tested in each trial for each line under selection.



a) Cauliflower



b) Cabbage

Figure 1. Damage scoring system from 0 (no damage) to 4 (totally useless for any commercial purpose) as measured 10 days after release of insects (in this case 40 neonates of *S. litura* per plant). a) cauliflower b) cabbage

Insects were released onto each plant using eggs on sheets (*P. xylostella* 50/plant; *H. undalis 15* or 20 per plant; *P. brassicae* 60/plant) or neonate larvae (*C. binotalis* 10 or 15/plant; *T. ni* 10 or 15/plant; *S. litura* 40/plant). Counts were made on surviving larvae on the 5th and 10th days following release (7th and 12th day for *P. xylostella*). Plant damage scores were taken at the second observation, on a scale of 0 (no feeding damage seen) to 4 (plant effectively destroyed for any commercial purpose (Figure 1).

Results

The results for the finally selected Elite Event and the hybrids produced using that line as one parent are presented in Table 1.

In summary, all non-Bt control lines were very heavily damaged by all species. There was no larval survival or damage at all on Bt cabbage or Bt cauliflower or their hybrids for *P. xylostella, C. binotalis* and *H. undalis,* the three most widespread and important caterpillar pests of brassicas, nor for *T. ni* which is a more sporadic problem. There was some survival of *P. brassicae* larvae in 2008 on Bt cabbage but none in 2010 and none in either year on the Bt cauliflower. None of the surviving larvae on Bt cabbage in 2008 survived to pupation. (Note: the 2008 cabbage may still have been segregating for Bt in these early trials). There was 3% survival of *S. litura* larvae on Bt cabbage and its hybrids in 2009 in the N. India trials in 2009 but damage scores were less than one, with even lower survival and damage on cauliflower. Again there was no survival through pupation on either Bt cabbage or Bt cauliflower. Larvae of any species which did survive on Bt plants were small and stunted.

These results suggest that the Cry1B/Cry1C combination is capable of providing excellent control of the major caterpillar pests of cabbage and cauliflower, particularly given that in the artificial screen-house situation there was very strongly reduced natural mortality from parasitoids or predators. (See below for details on IPM strategies for other pests).

SUSTAINABILITY OF Bt PLANTS

Risk of resistance development in diamondback moth

P. xylostella has a long history of development of resistance to insecticides including Bts (Mau and Gusukuma-Minuto 2004) and it has proved possible to select very high levels of resistance to Cry1C in the laboratory (Zhao et al. 2001). For us to have confidence that the Cry1B/Cry1C combination would stand up against the risk of resistance development in a field situation, the following assumptions required to be met (essentially those of the 'high dose-refugia strategy' as used in other commercialized GM crops).

- *Expression of insecticidal protein in all attacked parts of the plant is high relative to the susceptibility of the pest*

- *Genes for resistance to either protein are rare in the field pest population*
- *The stacked proteins are not cross-resisted*
- *Proteins make an equal contribution to mortality, and are approximately equally susceptible to resistance development*
- *Proteins do not act antagonistically*
- *Resistance is functionally recessive*
- *Beneficial insects will contribute to mortality of insects resistant to the Bt proteins*
- *Plant host diversity within the agro-ecosystems will make planted refugia unnecessary*

Expression of insecticidal protein in all attacked parts of the plant is high relative to the susceptibility of the pest

Susceptibility of the major caterpillar pests, including *P. xylostella* to Cry1B and Cry1C protein was reported in Shelton *et al*. (2009). The Energy and Resources Institute, New Delhi (Kaushik *et al*. In Prep) confirmed that the Bt proteins were expressed in all plant tissues (including curds in cauliflowers) and uniform as well as at high enough levels to ensure *P. xylostella* mortality in all tissues and at all plant stages. The screen-house trials described above showed these plants to be effective in caterpillar control, with concerns only for *S. litura* and particularly for *H. armigera*.

Genes for resistance to either protein are rare in the field pest population

Shelton *et al*. (2009) showed that despite the c.100 fold variation in LC_{50}s across *P. xylostella* field populations in USA, China, Australia, Indonesia, Taiwan and India, all LC_{50}s were < 1.0 ppm for Cry1B and <1.2ppm for Cry1C and so no more than seven times the LC_{50} of international standard susceptible strains (0.43 ppm for Cry1B and 0.18 ppm for Cry1C). Screening at the Indian Agricultural Research Institute using the F2 method of Andow and Alstad (1998) of Indian populations from the mountainous north (Katrain, Himachel Pradesh) and the plains (Hosur – Tappi-Manali) was not able to identify resistance genes, although this work is still on-going.

The stacked proteins are not cross-resisted

Using the *P. xylostella* strains selected in the laboratory which were moderately resistant to Cry1B and Cry1C, experiments in Australia and India have showed no cross resistance between Cry1B and Cry1C in either direction (Behere and Gujar in prep.).

Proteins make an equal contribution to mortality and are approximately equally susceptible to resistance development

The range of susceptibilities of different *P. xylostella* populations to the two Cry proteins individually is given in Shelton *et al*. (2008). The mean and range of LC_{50}s for Indian populations is given in Table 2.

Table 2. LC50s of Indian populations of *P. xylostella* and an international susceptible standard (Geneva) strain (data ex Shelton *et al*. 2008)

		Mean LC50 ug/ml pure Cry protein		
	No. Indian populations	'Geneva' strain	Mean of Indian populations	Range of means of Indian populations
Cry1B	18	0.43	0.09	0.01-0.46
Cry1C	13	0.18	0.18	0.01-0.61

For Indian strains, on an average, Cry1B showed 1.6 times the efficacy of Cry1C against *P. xylostella*. However, in the CIMBAA plant lines examined in detail for Bt expression, the proportion of the two proteins produced by the plant averages c. 0.66 Cry1B : 1.0 Cry1C in cabbage and 0.25 Cry1B : 1.0 Cry1C in cauliflower (Kaushik *et al*. in prep). As a happy consequence, their killing efficacy (the product of the expression x the LC_{50}) is therefore in the ratio of Cry1B: Cry1C 1.0:1.0 in cabbage and 0.4:1.0 in cauliflower – probably sufficiently close to contributing equal mortality for the purposes of risk of resistance selection.

Laboratory selections with *P. xylostella* using pure Cry1B and Cry1C proteins separately and together were undertaken at Melbourne University and in the Indian Agricultural Research Institute. Selection was continued though 21 generations for Cry1C and Cry1C+B and for 25 generations for Cry 1B. For Cry1C and Cry1B+C resistance was so hard to select that levels peaked at 8 fold resistance in the 10th generation of selection and declined rapidly thereafter as selection had to be relaxed to allow sufficient survival for breeding. Insects selected with Cry1B protein showed 54-fold resistance over the unselected Australian (Waite) strain of the same genetic background which 304 fold resistance with respect to a field-derived (Queensland) strain and 1,290 fold resistance in comparison with the most susceptible (Hosur) Indian strain.

Resistant insects selected in the laboratory in both Australia and India were very delicate and did not breed well. As soon as selection was relaxed, the resistance levels declined sharply (*i.e*., there was a strong fitness cost of resistance). The fitness cost with Cry1B and Cry1C resistance was in the form of extended larval growth, abnormal development of pupae and adults, and less fecundity in females. For Cry1C and Cry1B+C resistance was very hard to select *i.e*., fitness costs were close to 100%. Cry1B resistance fitness cost was around 60% in the laboratory but would most likely be much more in the field.

Proteins do not act antagonistically

Experiments in Australia and India with Cry1B and Cry1C pure protein used together in ratios around that expressed in the plants (Cry1B:Cry1C, 1:1, 1:2 and 2:1) showed only additive effects on *P. xylostella* mortality.

Resistance is functionally recessive

At the University of Melbourne the Cry1B resistant strain was crossed with the susceptible Australian (Waite and Queensland) strains and with the Indian (Hosur) susceptible strain and the offspring back-crossed to the Cry1B resistant strain. Inheritance of resistance proved to be recessive with dominance co-efficient (h) values of around 0.24-0.29 when tested in different genetic backgrounds. For the much less resistant Cry1C strain, work at IARI suggested an h value of 0.4 (incompletely recessive) and reciprocal crossing of moths resistant to either Cry1B or Cry1C showed resistances to both to be autosomal and functionally recessive. We can conclude that to be resistant to the Bt plants (even to the modest level selected) an insect would require to be homozygous for the resistance gene. This had already been established for Cry1C resistance in experiments in USA.

Survival of 'resistant' diamondback moth on Bt plants

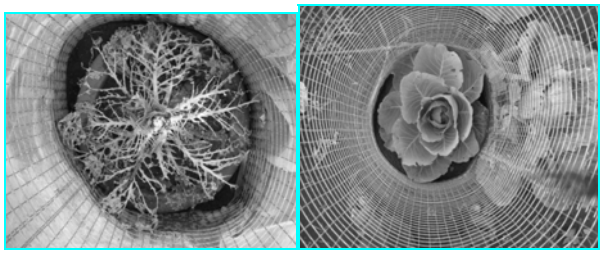
Challenging the three selected 'resistant' strains of *P. xylostella* with transgenic Bt plants in Australia and India produced no significant increased survival of the 'resistant' larvae of the Cry1B strain (>400 fold less susceptible than the Indian field strains) and the Cry1C strain (>20 fold less susceptible than Indian field strains), and only a lengthening of larval life by a few days in a few individuals of the Cry1B+Cry1C selected strain. No insects survived on the Bt plants through pupation to adulthood.

Beneficial insects will contribute to mortality of insects resistant to the Bt proteins

The potential role of natural enemies in removing initial, rare, surviving caterpillars in a transgenic cabbage or cauliflower field was unknown. Would beneficial insects be sufficiently common in Bt brassica fields to play a significant role? This was tested using the methods of Furlong *et al.* (2004 a&b).

The Energy and Resources Institute undertook trials in north (near Delhi) and south (near Bangalore) India, using simulated Bt cabbage and cauliflower fields (spraying Bt on non-transgenic crops) and examining the mortality of artificially placed 'rare' surviving *P. xylostella* in those fields. The presence of beneficial insects was measured in pitfall traps and by examining whole plants taken to the laboratory. Their impact on *P. xylostella* mortality was measured by the use of caged plants which were either partially open to access to predators and parasitoids or fully closed (Figure 2) and which had small numbers of laboratory reared *P. xylostella* released into them as eggs. Predators were collected and tested to ascertain whether they had eaten *P. xylostella* using a Polymerase Chain Reaction (PCR) diagnostic test provided by Dr Furlong from University of Queensland and validated at the University of Melbourne.

a)



b) **c)**

Figure 2. Parasitoid and predator exclusion cages infested with *S. litura* eggs (a) in place in a Bt sprayed field (b) a closed cage showing *S. litura* damage (c) a partially open cage with a lightly damaged plant due to the access to parasitoids and predators.

Major predators were spiders and ants. In the north India, predators were more numerous in simulated Bt fields than in the conventionally sprayed equivalents. Differences were smaller in South India. Mortality in open cages was 12% versus only 1% in the closed cages in the north India, but 39% in open cages in the south India versus 21% in the closed cages. In the north India, parasitoid induced mortality (especially by *Cotesia* sp.) was visible in the simulated Bt fields but not in the conventionally sprayed fields. In the south India, *Oomyzus* sp. was also important and again there was much more activity in the simulated Bt field than in the conventionally sprayed control. It is clear that these parasitoids and predators are having a considerable impact. However, unfortunately there was no background wild population of *P. xylostella* in the control fields in those seasons making interpretation of the results difficult and the trial is being repeated near Delhi in August - September 2011.

Plant host diversity within the agro-ecosystems will make planted refugia unnecessary

Most cabbage and cauliflower fields in the developing world are small, with farmers frequently planting a diversity of crops simultaneously and with cruciferous weeds present on verges, waste areas and sometimes within the crop. Mustard and radish are both *P. xylostella* hosts and are widely grown. Planted areas for each crop are available from the Indian Government Agricultural Statistics and 'ground truthing' searches for weed and crop alternate hosts were made by The Energy and Resources Institute in representative brassica production districts of North India (Sonipat district of Murthal in Haryana and at Hapur in the Ghaziabad district of Uttar Pradesh) and South India (Tehsil Malur in Kolar district of Karnataka). Planting times are naturally variable and mustard planting areas were difficult to ascertain but from radish plantings alone, when compared with the cabbage plus cauliflower areas, it would seem that a 'refuge' area of 45% would be available in Sonipat, 7% in Ghaziabad and 30% in Kolar. Mustard areas are expected to be comparable. Surprisingly, cruciferous weed hosts were not found to be significant in any area examined and no *P. xylostella* were found in systematic sweep netting off the crops.

The distance likely to be moved before mating by a rare, resistant *P. xylostella* individual emerging in a Bt cabbage field is unknown and these issues need further study, but unless market penetration of Bt cabbage and cauliflower in a particular district was very high indeed, it would seem that alternate crop hosts should have a valuable role to play in delaying resistance development.

Conclusion on Bt resistance risks for P. xylostella

Taken together these results suggest that a) development of simultaneous resistance to Cry1B and Cry1C in the field would be very difficult and b) resistant insects would be very 'unfit' in a natural environment c) both parents of a resistant insect would have to pass on a resistance gene, making the substantial non-Bt crop refugia important as a source of insects not carrying the resistance genes. These would be likely to mate with the rare resistant insects emerging from the Bt field and their offspring would be susceptible to the Bt plants, removing the resistance gene from the population gene pool. A version of visual basic resistance risk model (Kranthi and Kranthi 2004) *DBM-Bt-Adapt* has been produced for *P. xylostella* in India and resistance risk management scenarios are under virtual test now.

IPM strategies for Bt crops

Aphids are widely recognized as the second most important group of cabbage and cauliflower pests in the developing world (Grzywacz *et al.* 2010) including India (Badnes-Perez et al 2006) and they are not, of course, susceptible to Bt proteins. The key species for India are *Myzus persicae, Brevicoryne brassicae* and *Lipaphis erysimi.* Amongst the Lepidoptera, *S. litura* is not totally controlled before significant feeding has occurred on the Cry1B/Cry1C plants and *H. armigera,* which appears to be becoming more important as a pest of brassicas, is not at all well controlled. Some alternate strategies are required for these species if the secondary benefits of Bt brassicas in removing insecticidal chemistries which adversely affect beneficial organisms is to be retained. Preliminary studies were undertaken in 2009 and 2010 at the same sites and dates as the caterpillar control trials using Bt (Xentari®) sprays which contain Cry1C as a major component, to mimic transgenic Bt plants in the open field.

Aphids were well controlled for at least the first 45 days post transplanting by using imidacloprid (0.1%) to drench the soil in seedling trays prior to transplanting. However, this has operator health implications and, in any event, many farmers transplant bare rooted, which reduces the efficacy of the treatment. Imidocloprid (as Confidor® 600 FS) experimentally pelleted onto the seed, resolved both those issues resulting in a mean of only 3.7 aphids/plant at 40 days post transplanting as opposed to 90 aphids per plant in the control plots. In the second half of the season, where necessary, one or two sprays of *Verticillium lecanii* (at 45 and 55 days post transplanting) gave satisfactory control until harvest.

S. litura and *H. armigera* were artificially infested onto the sprayed Bt plots. Larvae surviving the Bt sprays were reduced by >90% due to applications of their species-specific NPVs at $1.04x10^9$ PIB. These NPVs are readily commercially available in India. It is likely that only spot spraying of hotspots of these caterpillars in Bt fields would be required, as caterpillars of both species are generally killed or severely stunted when feeding on Bt transgenic plants.

Using open and closed cages with artificial infestations of the pests, of the same type as used for *P. xylostella* (Figure 2) it was possible to separate the impacts of natural enemies, the Bt sprays and the additional mortality due to NPV. For *S. litura*,on both cabbage and cauliflower, mortality was around 30% at 10 days after infestation with neonates in the absence of any of the three mortality factors. Parasitoids and predators raised that mortality to around 70%. Bt sprays alone produced around 55% mortality and with predators and parasitoids this rose to c.90%. Bt + *S. litura* NPV in the absence of natural enemies produced 100% mortality. The addition of natural enemies could not of course increase this, but it did result in higher mortality in the days leading up to the 10 day post-treatment sample date.

Identical experiments with *H. armigera* produced similar results with c. 22% mortality in the absence of natural enemies, Bt or NPV rising to c80% with Bt + natural enemies and >97% mortality with all three factors operating. By 15 days post the start of the experiment this rose to 100% mortality on both cabbage and cauliflower.

The timing of attacks of these secondary pests varies with the geographic locations and timing of the crop and further work on locally adapted IPM packages will be required.

CONCLUSION

The transformation events producing the Cry1B/Cry1C cabbage and cauliflower lines eventually selected as suitable for use in generating commercial hybrids resulted in complete control (100% mortality) of early larval instar diamondback moth (*P. xylostella*), cabbage cluster caterpillar (C. *binotalis*), cabbage webworm (*H. undalis*) and cabbage looper (*T. ni*) caterpillars. Control of cabbage white butterfly (*P. brassicae*) and cabbage leafworm (*S. litura*) was good, with no survival through pupation in either species but resulted in some larval feeding damage.

Under laboratory conditions, resistance to Cry1C and to the Cry1B+Cry1C combination in *P. xylostella* was very difficult to select and unstable in the absence of selection and the resistant insects were difficult to maintain. Cry1B resistance was slightly easier to select but also unstable. There was no cross-resistance between the two proteins, which operated additively. Indian field populations of *P. xylostella* are highly susceptible to both Bt proteins and the F2 screening undertaken to date has not revealed the presence of resistance alleles. Laboratory selected *P. xylostella* several hundred fold more resistant to Cry1B, >20 times more resistant to Cry1C and several time more resistant to the Cry1B+Cry1C combination than Indian field strains, were unable to survive to pupation on the Bt plants. Alternate crop brassicas provide a very useful prospective 'refugia' .

Experimental seed coatings with imidacloprid offer good early season protection against aphids and *V. lecanii* applications are effective thereafter. The available NPVs of *S. litura* and *H. armigera,* in combination with Bt, provides excellent control.

In all, the Cry1B+Cry1C combination offers an excellent new tool in cabbage and cauliflower IPM.

Acknowledgements

The authors would like to thank the dedicated field team at Nunhems stations in India for their painstaking work in insect testing and selection. Dr Gangadhar of Pest Control India Pvt. was most generous with advice and insect material. Dr M. Furlong of University of Queensland provided training and support in the predator/parasite field cage work. Dr V.Khalia and Ms R. Nair of IARI's Entomology Division undertook the Indian resistance assays. The work was partially supported by the Australia-India Strategic Research Fund grant BF020053 and by the UK Department for International Development.

References

Andow DA , Alstad DN 1998 F2 screen for rare resistance alleles. *Journal of Economic Entomology* 91(3): 572-578.

Cao, J., J. D. Tang, N. Strizhov, A. M. Shelton, and E. D. Earle. 1999. Transgenic broccoli with high levels of *Bacillus thuringiensis* Cry1C protein control diamondback moth larvae resistant to Cry1A or Cry1C. *Mol. Breed.* 5:131-141.

Cao J, J-Z Zhao, T.D. Tang, A. M. Shelton and E.D. Earle. 2002. Broccoli plants with pyramided cry 1Ac and cry1C Bt genes control diamondback moths resistant to Cry1A and Cry1C proteins. *Theor. Appl. Genet.* 105: 258-264

Cao J., A.M. Shelton and E.D. Earle. 2005. Development of transgenic collards (*Brassica oleracea* L., var. *acephala*) expressing a cry1Ac or cry1C Bt gene for control of the diamondback moth. *Crop Prot.* 24: 804-813

Furlong, MJ, Shi ZH, Liu SS, Zalucki MP 2004a. Evaluation of the impact of natural enemies on *Plutella xylostella* L. (Lepidoptera: Yponmeutidae) populations on commercial Brassica farms. *Agricultural and Forest Entomology* 6: 311-322.

Furlong, MJ, Shi ZH, Liu YQ, Guo SJ, Lu YB, Liu SS and Zalucki MP 2004b. Experimental analysis of the influence of pest management practice on the efficacy of an endemic arthropod natural enemy complex of the diamondback moth. *Journal of Economic Entomology* 97: 1814-1827

Grzywacz D, Rossbach A, Rauf A, Russell DA, Srinivasan R, Shelton AM 2010 Current control methods for diamondback moth and other brassica insect pests and the prospects for improved management with lepidopteran-resistant Bt vegetable brassicas in Asia and Africa. *Crop Protection* 29: 68-79.

Kranthi KR and Kranthi NR 2004: Modelling adaptability of cotton bollworm *Helicoverpa armigera* (Hubner) to Bt-cotton in India. *Current Science* 87(8): 1096-1107

Mau RFL, Gusukuma-Minuto L 2004. Diamondback moth (Plutella xylostella (L.) resistance management in Hawaii. In Endersby N.M., Ridland P.M. (eds) The Management of Diamondback moth and Other Crucifer Pests, Proceedings of the 4th International Workshop on the management of Diamondback Moth and Other Crucifer Pests. Melbourne University, 26-29 Nov 2001.

Russell DA, Uijtewaal, B, Dhawan V, Grzywacz,D and Kaliaperumal, R. (this volume) Progress and challenges in the Bt brassica CIMBAA public/private partnership.

Russell DA, Uijtewaal B, Shelton AM, Mao C, Srinivasan, R, Gujar GT, Rauf A, Grzywacz D, Gregory P 2008. Bt brassicas for DBM control: The CIMBAA public/private partnership. pp272-289 In Shelton et al (Eds) Proceedings of the 5th International Workshop on the Management of Diamondback moth and other crucifer pests. China Agricultural science and Technology Press.

Shelton AM, Fuchs M, Shotkoski FA 2008 Transgenic vegetables and fruits for control of insects and insect-vectored pathogens. pp249-272 In: Romeis J et al. (eds) Integration of insect-resistant genetically modified crops within IPM programmes. Springer Science.

Shelton AM, Gujar GT, Chen M, Rauf A, Srinivasan R., Kalia V. Mittal A., Kumari A, Kaliaperumal, R, Borkakatti, R, Zhao JZ, Endersby N, Russell D, Wu YD, Uijtewaal B 2009 Assessing the susceptibility of cruciferous Lepidoptera to Cry1Ba2 and Cry1Ca4 for future transgeneic cruciferous vegetables. *Journal of Economic Entomology* 102(6): 2217-2223

Zhao, J. Z., Y. X. Li, H. L. Collins, J. Cao, E. D. Earle, and A. M. Shelton. 2001. Different cross-resistance patterns in the diamondback moth (Lepidoptera: Plutellidae) resistant to *Bacillus thuringiensis* toxin Cry1C. *J. Econ. Entomol.* 94: 1547-1552..

Zhao, J. Z., J. Cao, Y. X. Li, H. L. Collins, R. T. Roush, E. D. Earl, and A. M. Shelton. 2003. Plants expressing two *Bacillus thuringiensis* toxins delay insect resistance compared to single toxins used sequentially or in a mosaic. *Nature Biotech.* 21: 1493-1497.

Table 1. Mean percentage larval survival (MLS) and plant damage score (DS) (*scale 0-4*) of pest species on Elite Event a) cabbage and b) cauliflower and their hybrids in trials near New Delhi (Kharif-Spring) and Bengaluru (Rabi- autumn) 2008-10 *(see text for details.)*

	CABBAGE							
	North India				South India			
	2008		2009		2009		2010	
	MSL	DS	MSL	DS	MSL	DS	MSL	DS
Plutella xylostella **Control**	76.8	3.6	72.8	4.0				
Elite Event	0.0	0.0	0.0	0.0				
Hybrid	-	-	0.0	0.0				
Crocidolomia binotalis **Control**			68.7	4.0	76.0	4.0		
Elite Event			0.0	0.0	0.0	0.0		
Hybrid			0.0	0.0	0.0	0.0		
Hellula undalis **Control**			4.0	4.0	4.0	4.0		
Elite Event			0.0	0.0	0.0	0.0		
Hybrid			0.0	0.0	0.0	0.0		
Trichoplusia ni **Control**			61.5	4.0	70.0	4.0		
Elite Event			0.0	0.0	0.0	0.0		
Hybrid			0.0	0.0	0.0	0.0		
Spodoptera litura **Control**	58.3	3.4	78.0	3.8	57.4	3.6		
Elite Event	1.4	0.2	3.2	0.6	8.6	0.5		
Hybrid	-	-	3.2	0.6				
Pieris brassicae **Control**	83.3	3.8					47.5	4.0
Elite Event	12.3	0.8					0.0	0.2
Hybrid	-	-					1.7	0.7

	CAULIFLOWER							
	North India				South India			
	2008		2009		2009		2010	
	MSL	DS	MSL	DS	MSL	DS	MSL	DS
Plutella xylostella **Control**	76.8	3.3	70.5	4.0				
Elite Event	0.0	0.0	0.0	0.0				
Hybrid	0.0	0.0	0.0	0.0				
Crocidolomia binotalis **Control**			67.0	4.0	84.0	4.0		
Elite Event			0.0	0.0	0.0	0.0		
Hybrid			0.0	0.0	0.0	0.0		
Hellula undalis **Control**			-	4.0	-	4.0		
Elite Event			-	0.0	-	0.0		
Hybrid			-	0.0	-	0.0		
Trichoplusia ni **Control**			83.5	4.0	68.0	4.0		
Elite Event			0.0	0.0	0.0	0.0		
Hybrid			0.0	0.0	0.0	0.0		
Spodoptera litura **Control**	89.6	3.64	71.9	4.0	67.5	4.0		
Elite Event	2.4	1.3	1.3	0.3	6.3	1.3		
Hybrid			1.4	0.4	18.5	2.1		
Pieris brassicae **Control**							51.3	4.0
Elite Event							-	-
Hybrid							1.3	0.1