Annotated Bibliography on Environmental and Ecological Impacts from Transgenic Plants III: Insect Resistance

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Bourguet, D., J. Chaufaux, et al. (2003). "Frequency of alleles conferring resistance to Bt maize in French and US corn belt populations of the European corn borer, Ostrinia nubilalis." Theoretical and Applied Genetics **106**(7): 1225-1233.

> Farmers, industry, governments and environmental groups agree that it would be useful to manage transgenic crops producing insecticidal proteins to delay the evolution of resistance in target pests. The main strategy proposed for delaying resistance to Bacillus thuringiensis (Bt) toxins in transgenic crops is the high-dose/refuge strategy. This strategy is based on the unverified assumption that resistance alleles are initially rare (<10(-3)). We used an F-2 screen on >1,200 isofemale lines of Ostrinia nubilalis Hifter (Lepidoptera: Crambidae) collected in France and the US corn belt during 1999-2001. In none of the isofemale lines did we detect alleles conferring resistance to Bt maize producing the Cry1Ab toxin. A Bayesian analysis of the data indicates that the frequency of resistance alleles in France was <9.20 x 10(-4) with 95% probability, and a detection probability of >80%. In the northern US corn belt, the frequency of resistance to Bt maize was <4.23 x 10(-4) with 95% probability, and a detection probability of >90%. Only 95 lines have been screened from the southern US corn belt, so these data are still inconclusive. These results suggest that resistance is probably rare enough in France and the northern US corn belt for the high-dose plus refuge strategy to delay resistance to Bt maize.

Carriere, Y., C. Ellers-Kirk, et al. (2003). "Long-term regional suppression of pink bollworm by Bacillus thuringiensis cotton." Proceedings of the National Academy of Sciences of the United States of America **100**(4): 1519-1523.

> Despite the potentially profound impact of genetically modified crops on agriculture and the environment, we know little about their long-term effects. Transgenic crops that produce toxins from Bacillus thuringiensis (Bt) to control insects are grown widely, but rapid evolution of resistance by pests could nullify their benefits. Here, we present theoretical analyses showing that long-term suppression of pest populations is governed by interactions among reproductive rate, dispersal propensity, and regional abundance of a Bt crop. Supporting this theory, a 10-year study in 15 regions across Arizona shows that Bt cotton suppressed a major pest, pink bollworm (Pectinophora gossypiella), independent of demographic effects of weather and variation among regions. Pink bollworm population density declined only in regions where Bt cotton was abundant. Such long-term suppression has not been observed with insecticide sprays, showing that transgenic crops open new avenues for pest control. The debate about putative benefits of Bt crops has focused primarily on short-term decreases in insecticide use. The present findings suggest that long-term regional pest suppression after deployment of Bt crops may also contribute to reducing the need for insecticide sprays.

Coombs, J. J., D. S. Douches, et al. (2003). "Field evaluation of natural, engineered, and combined resistance mechanisms in potato for control of colorado potato beetle." Journal of the American Society for Horticultural Science **128**(2): 219-224.

> The colorado potato beetle, Leptinotarsa decemlineata Say (Coleoptera: Chrysomelidae), is the leading insect pest of potato (Solanum tuberosum L.) in northern latitudes. Host plant resistance has the potential use in an integrated pest management program for control of colorado potato beetle. During the 1998 and 1999 seasons, field studies were conducted to compare natural (leptine glycoalkaloids and glandular trichomes), engineered (Bt-cry3A and Bt-cry5 transgenic potato lines), and combined (Bt-cry5+glandular trichomes) plant resistance mechanisms of potato for control of colorado potato beetle. Nine different potato clones representing five different host plant resistance mechanisms were evaluated under natural colorado potato beetle infestation at the Montcalm Research Farm in Entrican, Michigan. The Bt-cry3A transgenic lines, the high leptine line (USDA8380-1), and the high foliar glycoalkaloid line (ND5873-15) were most effective for controlling defoliation by colorado potato beetle adults and larvae. The Bt-cry5 line (SPc5-G2) was not as effective as the Bt-cry3A transgenic lines ('Russet Burbank Newleaf,' RBN15, and YGa3.1). The glandular trichome (NYL235-4) and Bt-cry5+glandular trichome lines proved to be ineffective. Significant rank correlations for the potato lines between the two years were observed for egg masses, second and third instar, and fourth instar seasonal cumulative mean number of individuals per plant, and defoliation. Egg mass and first instar seasonal cumulative mean number of individuals per plant were not strong indicators of host plant resistance in contrast to second and third instars or adults. Based on these results, the Bt-cry3A transgenic lines, the high leptine line, and the high total glycoalkaloid line are effective host plant resistance mechanisms for control of colorado potato beetle.

Davidson, M. M. and A. J. Conner (2003). "Solanum weeds as hosts for Phthorimaea operculella: implications for resistance management of genetically modified potatoes (Solanum tuberosum)." New Zealand Journal of Crop and Horticultural Science **31**(2): 91-97.

> Foliage from Solanum species found within or near potato fields in New Zealand was compared to potato (Solanum tuberosum) foliage for its ability to support growth of larvae of Phthorimaea operculella (potato tuber moth, PTM). Solanum species included S. laciniatum (poroporo), S. physalifolium (hairy nightshade), S. chenopodioides (velvety nightshade), S. nigrum (black nightshade), and S. dulcamara (bittersweet). In detached leaf bioassays larval growth was greater on velvety nightshade and poroporo than on foliage from the potato cultivar, 'Iwa', in the first year, but only larval growth on poroporo foliage was greater in the second year ($P < 0.05$). Larval growth on bittersweet foliage was similar to larvae on 'Iwa' foliage. No larvae survived on black nightshade beyond 5 days in both

years. From a field survey, no PTM larval mines were found on any foliage of hairy, velvety, or black nightshade plants in or within 100 in of a field trial of transgenic PTM-resistant potato plants from a survey undertaken in the third summer. Over the entire survey, an average of 0.18 mines/plant were recorded on poroporo plants, all of which were <200 mm(2), compared to an average of 3.8 mines/non-transgenic potato plants (volunteer potato plants >100 m from the field trial and non-transgenic potato plants of the cultivars 'Iwa', 'Red Rascal', and breeding line 2390, used as experimental controls within the field trial). This study demonstrated that, under no-choice conditions, PTM larvae could grow on alternative Solanum species. However, PTM exhibited a clear preference for oviposition and development on potato plants in the field.

Genissel, A., S. Augustin, et al. (2003). "Initial frequency of alleles conferring resistance to Bacillus thuringiensis poplar in a field population of Chrysomela tremulae." Proceedings of the Royal Society of London Series B-Biological Sciences **270**(1517): 791-797.

Globally, the estimated total area planted with transgenic plants producing Bacillus thuringiensis (Bt) toxins was 12 million hectares in 2001. The risk of target pests becoming resistant to these toxins has led to the implementation of resistance-management strategies. The efficiency and sustainability of these strategies, including the high-dose plus refuge strategy currently recommended for North American maize, depend on the initial frequency of resistance alleles. In this study, we estimated the initial frequencies of alleles conferring resistance to transgenic Bt poplars producing Cry3A in a natural population of the poplar pest Chrysomela tremulae (Coleoptera: Chrysomelidae). We used the F-2 screen method developed for detecting resistance alleles in natural pest populations. At least three parents of the 270 lines tested were heterozygous for a major Bt resistance allele. We estimated mean resistance-allele frequency for the period 19992001 at 0.0037 (95% confidence interval = 0.00045-0.0080) with a detection probability of 90%. These results demonstrate that (i) the F-2 screen method can be used to detect major alleles conferring resistance to Bt-producing plants in insects and (ii) the initial frequency of alleles conferring resistance to Bt toxin can be close to the highest theoretical values that are expected prior to the use of Bt plants if considering fitness costs and typical mutation rates.

Genissel, A., J. C. Leple, et al. (2003). "High tolerance against Chrysomela tremulae of transgenic poplar plants expressing a synthetic cry3Aa gene from Bacillus thuringiensis ssp tenebrionis." Molecular Breeding **11**(2): 103-110.

Hybrid poplars (Populus tremula x Populus tremuloides) have been genetically engineered via Agrobacterium tumefaciens, to express a synthetic cry3Aa gene derived from the native Bacillus thuringiensis subsp. tenebrionis cry3Aa gene. The presence and the expression of the transgene have been verified in four transgenic poplar lines, using

Southern, northern and western analyses. The transgenic poplar's toxicity towards the phytophagous beetle Chrysomela tremulae (Coleoptera, Chrysomelidae) has been assessed on six month-old greenhouse-grown selected plants in laboratory conditions. Laboratory experiments consisted of feeding tests of fresh detached leaves on C. tremulae at all developmental stages. Our results indicate that the transgenic poplar leaves, expressing a Cry3Aa protein amount in a range of 0.05-0.0025% of total soluble protein, were definitely deleterious for C. tremulae, regardless of the developmental stage.

Gore, J., B. R. Leonard, et al. (2003). "Influence of agronomic hosts on the susceptibility of Helicoverpa zea (Boddie) (Lepidoptera : Noctuidae) to genetically engineered and non-engineered cottons." Environmental Entomology **32**(1): 103-110.

Field and laboratory studies evaluated the influence of selected crop hosts on Helicoverpa zea (Boddie) population dynamics in relation to genetically engineered Bt (Bollgard) and non-Bt cottons. Host specific H. zea colonies were initiated with a colony originally collected from sweet corn. The colony was allowed to complete one generation on meridic diet then split into cohorts and allowed to complete one generation on field corn, grain sorghum, soybean, cotton, or meridic diet in individual 29.5 ml plastic cups. During the first part of the study, larval developmental times, pupal weights, and survival were measured. H. zea survival was higher on meridic diet and grain sorghum than on soybean and cotton. Development of H. zea larvae was faster on field corn than all other larval diets. Also, H. zea required a longer period of time to complete development on cotton than on the other hosts. Pupal weights were higher on meridic diet than the plant hosts. Pupal weights of H. zea that completed larval stadia on cotton were lower than on the other larval diets. Neonates (F-1) from each of the host specific colonies (200 per colony) were exposed to Bt and non-Bt cottons. Mortality of second generation H. zea on non-Bt and Bt cottons was measured at 96 h. H. Zea larvae from the cotton colony had higher mortality on non-Bt cotton than the other host specific colonies except the grain sorghum colony. On Bt cotton, larvae from the corn colony had a higher level of mortality than larvae from the soybean and grain sorghum colonies. These data provide valuable information for evaluating the contribution of cultivated hosts as additional, alternative refugia in Bt cotton resistance management plans.

Greenplate, J. T., J. W. Mullins, et al. (2003). "Partial characterization of cotton plants expressing two toxin proteins from Bacillus thuringiensis: relative toxin contribution, toxin interaction, and resistance management." Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie **127**(6): 340-347.

Laboratory studies were performed to characterize the lepidopteran toxicity of cotton plants expressing two different toxin proteins from Bacillus thuringiensis (Bt), in order to assess insect resistance management implications of a commercial, two-toxin transgenic cotton. An independent and additive interactive effect of two Bt delta-endotoxins expressed by the transgenic cotton variety 15985 was demonstrated by examining the responses of Heliothis virescens (F.), Helicoverpa zea (Boddie), and Spodoptera frugiperda (J.E. Smith) larvae to field- or greenhouse-grown tissue from genetic near-isolines, which expressed Cry1A only, Cry2Ab only, or both toxins. In all cases, the Cry2Ab component was the larger contributor to total toxicity in the two-toxin isoline. Toxin-specific, quantitative enzyme-linked immunosorbent assay (ELISA) tests confirmed that the levels of each toxin in tissues of the two-toxin isoline were not statistically different ($P > 0.05$) from the levels found in the corresponding tissues of the respective single-toxin isoline. Resistance management considerations were discussed. Considering the additive interaction of toxins, a relatively simple insect resistance-monitoring procedure was proposed for the monitoring of commercial cotton varieties expressing both toxins.

Hibbard, B. E., D. P. Duran, et al. (2003). "Post-establishment movement of western corn rootworm larvae (Coleoptera : Chrysomelidae) in Central Missouri corn." Journal of Economic Entomology **96**(3): 599-608.

If registered, transgenic corn, Zea mays L., with corn rootworm resistance will offer a viable alternative to insecticides for managing Diabrotica spp. corn rootworms. Resistance management to maintain susceptibility is in the interest of growers, the Environmental Protection Agency, and industry, but little is known about many aspects of corn rootworm biology required for an effective resistance management program. The extent of larval movement by the western corn rootworm, Diabrotica virgifera virgifera LeConte, that occurs from plant-to-plant or row-to-row after initial establishment was evaluated in 1998 and 1999 in a Central Missouri cornfield. Post-establishment movement by western corn rootworm larvae was clearly documented in two of four treatment combinations in 1999 where larvae moved up to three plants down the row and across a 0.46-m row. Larvae did not significantly cross a 0.91-m row after initial host establishment in 1998 or 1999, whether or not the soil had been compacted by a tractor and planter. In the current experiment, western corn rootworm, larvae moved from highly damaged, infested plants to nearby plants with little to no previous root damage. Our data do not provide significant insight into how larvae might disperse after initial establishment when all plants in an area are heavily damaged or when only moderate damage occurs on an infested plant. A similar situation might also occur if a seed mixture of transgenic and isoline plants were used and if transgenic plants with rootworm resistance are not repellent to corn rootworm larvae.

Horner, T. A. and G. P. Dively (2003). "Effect of MON810 Bt field corn on Helicoverpa zea (Lepidoptera : Noctuidae) cannibalism and its implications to resistance development." Journal of Economic Entomology **96**(3): 931-934.

Pairs of Helicoverpa zea (Boddie) larvae reared on diet-incorporated MON810 transgenic leaf tissue of field corn (Zea mays L.) were observed in the laboratory to characterize effects of sublethal levels of Bacillus thuringiensis variety kurstaki (Bt) Cry1Ab endotoxins on cannibalistic behavior and mortality. Feeding on sublethal levels of Bt corn reduced the frequency of cannibalistic behaviors exhibited by H. zea when uneven instars were paired together. Exposure to the Bt endotoxin had no significant effect on when cannibalistic mortality occurred or the level of mortality as a result of cannibalism. Assuming that H. zea larvae reared on nonBt corn tissue behaved in a similar way that resistant larvae would if feeding on Bt tissue, sublethal effects of Cry1Ab intoxication may reduce the chances of successful cannibalism by susceptible larvae and thus play a disproportionate role in the survival of multiple ear infestations. Furthermore, cannibalistic encounters could result in partially resistant larvae feeding on nontoxic food, thus temporarily providing an escape from exposure to the Bt endotoxin. These behavior alterations could increase the selective differential between susceptible individuals and those carrying resistance genes.

Horner, T. A., G. P. Dively, et al. (2003). "Effects of MON810 Bt field corn on adult emergence of Helicoverpa zea (Lepidoptera : Noctuidae)." Journal of Economic Entomology **96**(3): 925-930.

A 3-yr study (1996-1998) was conducted to evaluate the effects of MON810 Bt corn on Helicoverpa zea (Boddie) emergence and to determine whether delayed larval development as a result of Bt intoxication results in higher levels of diapause induction and pupal mortality. In the 1997 study, there was no difference in prepupal mortality between corn types, although significantly more prepupae from Bt plots than from non-Bt plots died in emergence buckets before constructing pupal chambers in 1998. In all years, significantly fewer moths emerged from prepupae collected from Bt plots, suggesting that effects of the expressed Cry1Ab extended to the prepupal and pupal stages. Late plantings of corn showed the greatest reductions in moth emergence from Bt corn because environmental conditions were more conducive to trigger diapause at the time H. zea was developing in these plantings. This was supported by a significantly greater proportion of diapausing pupae remaining in the ground in the late plantings of both Bt and non-Bt corn. For April and early May plantings, larval feeding on Bt corn delayed the time to pupation, although there was no significant difference in moth emergence between corn types for those larvae that successfully pupated. Although Bt expression had less impact on the proportion of moths emerging, the actual number of moths emerging from Bt corn was significantly reduced because fewer larvae reached pupation. Delays in adult emergence, along with significant reductions in adult emergence from MON810 Bt corn, should reduce the rates of colonization in soybean and other late host crops but may also result in asynchrony of mating between individuals emerging from Bt and non-Bt corn. This, in turn, may contribute to the evolution of resistance to Bt corn.

Martel, C., A. Rejasse, et al. (2003). "Host-plant-associated genetic differentiation in Northern French populations of the European corn borer." Heredity **90**(2): 141-149.

The phytophagous insects that damage crops are often polyphagous, feeding on several types of crop and on weeds. The refuges constituted by noncrop host plants may be useful in managing the evolution in pest species of resistance to the Bacillus thuringiensis toxins produced by transgenic crops. However, the benefits of these refuges may be limited because host-plant diversity may drive genetic divergence and possibly even host-plant-mediated sympatric speciation. The European corn borer, Ostrinia nubilatis Hubner (Lepidoptera: Crambidae), is the main pest of maize in Europe and North America, where it was introduced early in the 20th century. It has a wide host range but feeds principally on mugwort (Artemisia vulgaris L.) and maize (Zea mays L.). O. nubilalis is found on mugwort only in the northern part of France, whereas it is found on maize throughout France. The extent of genetic variation at allozyme markers was investigated in populations collected from the two host plants over the entire geographical distribution of the European corn borer on mugwort in France. Allelic differentiation between pairs of populations and hierarchical analyses of pools of samples from each host plant indicate that the group of populations feeding on maize differed from the group of populations feeding on mugwort. Our results suggest (1) host-plant-related divergent selection at the genomic region surrounding the Mpi locus and (2) limited gene flow between the populations feeding on mugwort and those infesting maize fields. These data indicate that adults emerging from mugwort would not be useful for managing the evolution of resistance to the B. thuringiensis toxins in European corn borer populations.

Morin, S., R. W. Biggs, et al. (2003). "Three cadherin alleles associated with resistance to Bacillus thuringiensis in pink bollworm." Proceedings of the National Academy of Sciences of the United States of America **100**(9): 5004-5009.

Evolution of resistance by pests is the main threat to long-term insect control by transgenic crops that produce Bacillus thuringiensis (Bt) toxins. Because inheritance of resistance to the Bt toxins in transgenic crops is typically recessive, DNA-based screening for resistance alleles in heterozygotes is potentially much more efficient than detection of resistant homozygotes with bioassays. Such screening, however, requires knowledge of the resistance alleles in field populations of pests that are associated with survival on Bt crops. Here we report that field populations of pink bollworm (Pectinophora gossypiella), a major cotton pest, harbored three mutant alleles of a cadherin-encoding gene linked with resistance to Bt toxin Cry1Ac and survival on transgenic Bt cotton. Each of the three resistance alleles has a deletion expected to eliminate at least eight amino acids upstream of the putative toxin-binding region of the cadherin protein. Larvae with two resistance alleles in-any combination were resistant, whereas those with one or none were susceptible to Cry1Ac. Together with previous evidence, the results reported here identify the cadherin genie as a leading target for DNA-based screening of resistance to Bt crops in lepidopteran pests.

Storer, N. P., S. L. Peck, et al. (2003). "Spatial processes in the evolution of resistance in Helicoverpa zea (Lepidoptera : Noctuidae) to Bt transgenic corn and cotton in a mixed agroecosystem: a biology-rich stochastic simulation model." Journal of Economic Entomology **96**(1): 156-172.

> A simulation model is developed to examine the role of spatial processes in the evolution of resistance in Helcoverpa zea populations to Bt corn and Bt cotton. The model is developed from the stochastic spatially explicit Heliothis virescens model described by Peck et al. (1999), to accommodate a spatial mix of two host crops (corn and cotton), and to reflect the agronomic practices, as well as the spatial and temporal population dynamics of H.; ea, in eastern North Carolina. The model suggests that selection for resistance is more intense in Bt cotton fields than in Bt corn fields. It further suggests that local gene frequencies are highly dependent on local deployment levels of Bt crops despite the high mobility of the adult insects. Region-wide average gene frequencies depend on the region-wide level of Bt deployment, so incomplete technology adoption slows the rate of resistance evolution. However, on a local scale, H. zea populations in clusters of fields in which Bt use is high undergo far more rapid evolution than populations in neighboring clusters of fields in which Bt use is low. The model suggests that farm-level refuge requirements are important for managing tile risk of resistance. The model can be used as an aid in designing plans for monitoring for resistance by suggesting the appropriate distribution of monitoring locations, which should focus on areas of highest Bt crop deployment. The findings need to be placed in the context of the input parameters. many of which are uncertain or highly variable in nature, and therefore, a thorough sensitivity analysis is warranted.

Storer, N. P., S. L. Peck, et al. (2003). "Sensitivity analysis of a spatially-explicit stochastic simulation model of the evolution of resistance in Helicoverpa zea (Lepidoptera : Noctuidae) to Bt transgenic corn and cotton." Journal of Economic Entomology **96**(1): 173-187.

> The sensitivities of a model simulating the evolution of resistance in Helicoverpa zea to Bt toxins in transgenic crops were investigated by examining effects of each of the model parameters on the frequency of resistance alleles after 8 yr. The functional dominance of resistance alleles and the initial frequency of those alleles had a major impact on resistance evolution. The Survival of susceptible insects on the transgenic crops and

the population dynamics of the insect, driven by winter survival and reproductive rates, were also important. In addition, agricultural practices including the proportion of the acreage planted to corn, and the larval threshold for spraying cotton fields affected the R-allele frequency. Many of these important parameters are inherently variable or cannot be measured with accuracy, so model output cannot be interpreted as being a forecast. However, this analysis is useful in focusing empirical research on those aspects of the insects' life system that have the largest effects on resistance development, and indicates ways in which to improve products and agricultural practices to increase the expected time to resistance. The model can thus be used as a scientific basis for devising a robust resistance management strategy for Bt crops.

Vacher, C., D. Bourguet, et al. (2003). "Modelling the spatial configuration of refuges for a sustainable control of pests: a case study of Bt cotton." Journal of Evolutionary Biology **16**(3): 378-387.

> The 'high-dose-refuge' (HDR) strategy is widely recommended by the biotechnology industry and regulatory authorities to delay pest adaptation to transgenic crops that produce Bacillus thuringiensis (Bt) toxins. This involves cultivating nontoxic plants (refuges) in close proximity to crops producing a high dose of Bt toxin. The principal cost associated with this strategy is due to yield losses suffered by farmers growing unprotected, refuge plants. Using a population genetic model of selection in a spatially heterogeneous environment, we show the existence of an optimal spatial configuration of refuges that could prevent the evolution of resistance whilst reducing the use of costly refuges. In particular, the sustainable control of pests is achievable with the use of more aggregated distributions of nontransgenic plants and transgenic plants producing lower doses of toxin. The HDR strategy is thus suboptimal within the context of sustainable agricultural development.

Zhang, N. Y., S. Linscombe, et al. (2003). "Out-crossing frequency and genetic analysis of hybrids between transgenic glufosinate herbicide-resistant rice and the weed, red rice." Euphytica **130**(1): 35-45.

> The potential of transferring herbicide resistance from transgenic rice (Oryza sativa L.) varieties to sexually compatible weeds is of paramount importance for development of effective weed control strategies. The objective of this research was to determine the genetic control and frequency of natural outcrossing between a transgenic, glufosinate-resistant rice line and a Louisiana biotype of red rice (Oryza sativa L.). Molecular and phenotypic data showed that outcrossing in field plots between a non-transgenic purple marker line and red rice did occur within one field season, but at a low rate of <1%. Similarly, molecular and phenotypic data demonstrated that glufosinate resistance was transferred from the transgenic line to the red rice biotype in the field within one year at a low frequency of 0.3%. Compared to parental lines, the transgenic-red

rice hybrids were extremely late, tall, and never set seed during the normal field season. Genetic analyses in all F-2 populations showed glufosinate resistance behaved in a Mendelian fashion as a single, dominant gene. Presence of the bar gene for glufosinate resistance did not increase fitness or seed fecundity in hybrids or subsequent progeny. The genetic analyses and outcrossing results from this study suggest that an effective management program can be developed to prolong the usefulness of transgenic, glufosinate herbicide technology.

Cao, J., J. Z. Zhao, et al. (2002). "Broccoli plants with pyramided cry1Ac and cry1C Bt genes control diamondback moths resistant to Cry1A and Cry1C proteins." Theoretical and Applied Genetics **105**(2-3): 258-264.

> This study was undertaken to determine the effects of pyramiding two Bacillus thuringiensis (Bt) genes in the same plant on the production of Bt proteins and the control of diamondback moths (DBM, Plutella xylostella) resistant to one or the other protein. Broccoli lines carrying both cry1Ac and cry1C Bt genes were produced by sexual crosses of cry1Ac- and cty1C-transgenic plants. Plants containing both genes were selected by tests for resistance to kanamycin and hygromycin, and confirmed by PCR analysis for the Bt genes. Both cry1Ac and cry1C mRNAs were detected in the hybrid lines, and Cry1Ac and Cry1C proteins were stably produced at levels comparable to the parental plants. Plants producing both Cry1Ac and Cry1C, proteins caused rapid and complete mortality of DBM larvae resistant to Cry1A or Cry1C, and suffered little or no leaf damage. These plants, in combination with the resistant DBM populations available, will allow greenhouse or field studies of resistance management strategies involving gene pyramiding.

Douches, D. S., W. Li, et al. (2002). "Development of Bt-cry5 insect-resistant potato lines 'Spunta-G2' and 'Spunta-G3'." Hortscience **37**(7): 1103-1107.

> The potato tuber moth (Phthorimaea operculella Zeller) is the primary insect pest of cultivated potato (Solanum tuberosum L) in tropical and subtropical regions, causing both foliar and tuber damage. In contrast, the Colorado potato beetle (Leptinotarsa decemlineata Say) is the most important insect pest in the northern potato production latitudes. The codon-modified Bacillus thuringiensis Bt-cry5 gene (revised nomenclature cry1Ial), specifically toxic to Lepidoptera and Coleoptera, was transformed into cultivar Spunta using an Agrobacterium vector to provide resistance to both potato tuber moth and Colorado potato beetle. The Bt-cry5 gene was placed downstream from the constitutive CaMV35S promoter. Two transgenic 'Spunta' clones, G2 and G3, produced high levels of mortality in first instars of potato tuber moth in detached-leaf bioassays (80% to 83% mortality), laboratory tuber tests (100% mortality), and field trials in Egypt (99% to 100% undamaged tubers). Reduced feeding by Colorado potato beetle first instars was also observed in detached-leaf bioassays (80% to 90% reduction). Field trials in the United States demonstrated that

the horticultural performance of the two transgenic lines was comparable to 'Spunta'. These Bt-cry5 transgenic potato plants with high potato tuber moth resistance have value in integrated pest management programs.

Dres, M. and J. Mallet (2002). "Host races in plant-feeding insects and their importance in sympatric speciation." Philosophical Transactions of the Royal Society of London Series B-Biological Sciences **357**(1420): 471-492.

The existence of a continuous array of sympatric biotypes-from polymorphisms, through ecological or host races with increasing reproductive isolation, to good species-can provide strong evidence for a continuous route to sympatric speciation via natural selection. Host races in plant-feeding insects, in particular, have often been used as evidence for the probability of sympatric speciation. Here, we provide verifiable criteria to distinguish host races from other biotypes: in brief, host races are genetically differentiated, sympatric populations of parasites that use different hosts and between which there is appreciable gene flow. We recognize host races as kinds of species that regularly exchange genes with other species at a rate of more than ca. 1% per generation, rather than as fundamentally distinct taxa. Host races provide a convenient, although admittedly somewhat arbitrary intermediate stage along the speciation continuum. They are a heuristic device to aid in evaluating the probability of speciation by natural selection, particularly in sympatry. Speciation is thereby envisaged as having two phases: (i) the evolution of host races from within polymorphic, panmictic populations; and (ii) further reduction of gene flow between host races until the diverging populations can become generally accepted as species. We apply this criterion to 21 putative host race systems. Of these, only three are unambiguously classified as host races, but a further eight are strong candidates that merely lack accurate information on rates of hybridization or gene flow. Thus, over one-half of the cases that we review are probably or certainly host races, under our definition. Our review of the data favours the idea of sympatric speciation via host shift for three major reasons: (i) the evolution of assortative mating as a pleiotropic by- product of adaptation to a new host seems likely, even in cases where mating occurs away from the host; (ii) stable genetic differences in half of the cases attest to the power of natural selection to maintain multilocus polymorphisms with substantial linkage disequilibrium, in spite of probable gene flow; and (iii) this linkage disequilibrium should permit additional host adaptation, leading to further reproductive isolation via pleiotropy, and also provides conditions suitable for adaptive evolution of mate choice (reinforcement) to cause still further reductions in gene flow. Current data are too sparse to rule out a cryptic discontinuity in the apparently stable sympatric route from host-associated polymorphism to host-associated species, but such a hiatus seems unlikely on present evidence. Finally, we discuss applications of an understanding of host races in conservation and in managing adaptation by pests to control strategies, including those involving biological control or transgenic parasite-resistant plants.

Ferre, J. and J. Van Rie (2002). "Biochemistry and genetics of insect resistance to Bacillus thuringiensis." Annual Review of Entomology **47**: 501-533.

Bacillus thuringiensis (Bt) is a valuable source of insecticidal proteins for use in conventional sprayable formulations and in transgenic crops, and it is the most promising alternative to synthetic insecticides. However, evolution of resistance in insect populations is a serious threat to this technology. So far, only one insect species has evolved significant levels of resistance in the field, but laboratory selection experiments, have shown the high potential of other species to evolve resistance against Bt. We. have reviewed the current knowledge on the biochemical mechanisms and genetics of resistance to Bt products and insecticidal crystal proteins. The understanding of the biochemical and genetic basis of resistance to Bt can help design appropriate management tactics to delay or reduce the evolution of resistance in insect. Populations.

Gould, F., N. Blair, et al. (2002). "Bacillus thuringiensis-toxin resistance management: Stable isotope assessment of alternate host use by Helicoverpa zea." Proceedings of the National Academy of Sciences of the United States of America **99**(26): 16581-16586.

Data have been lacking on the proportion of Helicovera zea larvae that develop on noncotton host plants that can serve as a refuge from selection pressure for adaptation to transgenic cotton varieties that produce a toxin from the bacterium Bacillus thuringiensis. We found that individual H. zea moths that develop as larvae on cotton and other plants with C-3 physiology have a different ratio of C-13 to C-12 than moths that develop on plants with C-4 physiology, such as corn. We used this finding in determining the minimum percentage of moths that developed on noncotton hosts in two cotton-growing areas. Our results indicate that local corn can serve as a refuge for H. zea in midsummer. Our results contrast dramatically with the prevailing hypothesis that the large majority of late-season moths are produced from larvae feeding on cotton, soybean, and other C-3 plants. Typically, <50% of moths captured in August through October have isotope ratios indicative of larval feeding on C-3 plants. In one October sample, 100% of the moths originated from C-4 hosts even though C-4 crops were harvested at least 1 mo earlier, and no common wild C-4 hosts were available. These findings support other research indicating that many late-season H. zea moths captured in Louisiana and Texas are migrants whose larvae developed on corn in more northern locations. Our isotope data on moths collected in Texas early in the season indicate that the majority of overwintering H. zea do not originate from cotton- feeding larvae and may be migrants from Mexico. Non-Bt corn in Mexico and the U.S. corn belt appears to serve as an important refuge for H. zea.

Guse, C. A., D. W. Onstad, et al. (2002). "Modeling the development of resistance by stalk-boring Lepidoptera (Crambidae) in areas with irrigated transgenic corn." Environmental Entomology **31**(4): 676-685.

> The population dynamics and population genetics of two bivoltine species of corn borers are modeled in a hypothetical region of irrigated transgenic and nontransgenic corn. European corn borer, Ostrinia nubilalis (Hubner), adults were assumed to disperse throughout the landscape for both mating and oviposition. Southwestern corn borer, Diatraca grandiosella Dyar, adults were assumed to have very localized dispersal behaviors. Resistance developed quickly in both species when the allele for resistance to the transgenic toxin was dominant. When the allele for resistance was not dominant and few or none of the heterozygous larvae survived the toxin, the behaviors of adult insects determined the speed of resistance development. With block refuges of 10-40% the European corn borer developed resistance within 15-38 yr, but the southwestern corn borer never developed resistance within the 100 yr simulated. A row- strip refuge configuration (lid not change the time for resistance to develop in the European corn borer; however, row- strip refuges cannot be recommended for the southwestern corn borer. Uncertainty about adult behaviors in irrigated corn led us to examine the potential impact of behavior on resistance development, Adult behaviors influenced resistance development more than refuge size. For instance, if the first flight of moths exhibit random mating and uniform oviposition throughout the landscape and the second flight exhibits localized mating and oviposition, resistance developed at least five times faster in the southwestern corn borer population and three times slower in the European corn borer population compared with our standard assumptions. We discuss the implications of adult behavior, refuge configuration, refuge placement within the landscape and year-to-year relocation on resistance management plans.

Hillier, J. G. and A. N. E. Birch (2002). "A bi-trophic mathematical model for pest adaptation to a resistant crop." Journal of Theoretical Biology **215**(3): 305-319.

> A version of the Lotka-Volterra predator-prey model with logistic crop growth is modified to explore the rate of adaptation of a herbivore to a pest-resistant crop. This provides a phenotypic model for the evolution of resistance in a population comprising three different pest types each defined by differing parameter values for respiration rate and crop palatability. Expressions estimating the rates of increase of the fitter pest types are obtained as a function of the food qualities, and respiration and mortality rates. Potential strategies for delaying the rate of adaptation with regard to the expressions derived above, via the use of pest-susceptible refugia and natural enemies, are discussed. Although the model is formulated as one in which a single gene is the factor conferring resistance it can be interpreted and used independently of this. (C) 2002 Elsevier Science Limited. All rights reserved.

Huang, F. N., L. L. Buschman, et al. (2002). "Survival of Kansas Dipel-resistant European corn borer (Lepidoptera : Crambidae) on Bt and Non-Bt corn hybrids." Journal of Economic Entomology **95**(3): 614-621.

> The Kansas Dipel-resistant and susceptible European corn borer, Ostrinia nubilalis(Hubner), were evaluated in the greenhouse on different Bt transgenic events expressed in corn hybrids. There were important differences in the resistance offered by the different Bt event corn hybrids. Hybrid comparison tests indicate that these Dipel-resistant first-instar European corn borer were not able to survive to adulthood on whorl-stage MON810, Bt11, or 176 Bt event corn plants. Third instars did not survive to adulthood on whorl-stage MON810 or Bt11. event corn plants but a small number of fifth instars were found on whorl-stage DBT418 plants infested with Dipel-resistant larvae. First and third instars of these Dipel-resistant European corn borers caused more leaf-feeding damage and more tunneling on whorl-stage Bt-corn plants than did the Dipel-susceptible European corn borers. However, in the single Bt corn hybrid test, there was no survival of the Dipel-resistant European corn borers on DK580BtX or MAX454 Bt plants 35 to 42 d after they had been infested with first instars. These results demonstrate that the current Kansas selection of Dipel- resistant European corn borer strain cannot establish reproducing populations in the tested Bt corn lines and hybrids.

Hurley, T. M., S. Secchi, et al. (2002). "Managing the risk of European corn borer resistance to Bt corn." Environmental & Resource Economics **22**(4): 537-558.

> New pesticidal crops are taking advantage of advances in genetic engineering. For example, corn has been engineered to express Bt proteins that are toxic to the European corn borer. These crops are effective pest management tools for United States growers. However, there is concern that pests will develop resistance to these crops resulting in the increased use of more hazardous pesticides. The purpose of this paper is to develop a stochastic dynamic bioeconomic simulation model to help guide regulatory policy designed to mitigate the threat of resistance to new pesticidal crops. The model is used to evaluate the insect resistance management guidelines mandated by the United States Environmental Protection Agency for the use of Bt corn in the Midwestern United States.

Ives, A. R. and D. A. Andow (2002). "Evolution of resistance to Bt crops: directional selection in structured environments." Ecology Letters **5**(6): 792-801.

> Widespread use of transgenic crops that express an insecticidal endotoxin from Bacillus thuringiensis increases the risk of evolution of resistance by the European corn borer and other insect pests. To delay resistance evolution, the high-dose refuge strategy is being implemented for Bt maize and Bt cotton. We develop a general modelling framework to understand the invasion and spread of alleles conferring resistance. We show that at least three processes are involved in explaining the effectiveness of the

high-dose refuge strategy: the intensity of selection, assortative (non-random) mating due to spatial subdivision, and variation in male mating success also due to spatial subdivision. Understanding these processes leads to a greater range of possible resistance management tactics. For example, efforts to encourage adults to leave their natal fields may have the unwanted effect of speeding rather than slowing resistance evolution. Furthermore, when Bt maize causes high mortality to susceptible target pests, spraying insecticides in refuges to reduce pest populations may not greatly disrupt resistance management.

Khanna, H. K. and S. K. Raina (2002). "Elite indica transgenic rice plants expressing modified Cry1Ac endotoxin of Bacillus thuringiensis show enhanced resistance to yellow stem borer (Scirpophaga incertulas)." Transgenic Research **11**(4): 411-423.

Bt-transgenics of elite indica rice breeding lines (IR-64, Pusa Basmati-1 and Karnal Local) were generated through biolistic of Agrobacterium-mediated approaches. A synthetic cry1Ac gene, codon optimised for rice and driven by the maize ubiquitin-1 promoter, was used. Over 200 putative transformants of IR-64 and Pusa Basmati-1 and 26 of the Karnal Local were regenerated following use of the hpt (hygromycin phosphotransferase) selection system. Initial transformation frequency was in the range of 1 to 2% for particle bombardment while it was comparatively higher (similar to 9%) for Agrobacterium. An improved selection procedure, involving longer selection on the antibiotic-supplemented medium, enhanced the frequency of Bt- transformants and reduced the number of escapes. Molecular evaluation revealed multiple transgene insertions in transformants, whether generated through biolistic or Agrobacterium. In the latter case, it was also observed that all genes on the T-DNA do not necessarily get transferred as an intact insert. Selected Bt-lines of IR-64 and Pusa Basmati-1, having Bt-titers of 0.1% (of total soluble protein) and above were evaluated for resistance against manual infestation of freshly hatched neonate larvae of yellow stem borers collected from a hot spot stem borer infested area in northern India. Several Bt-lines were identified showing 100% mortality of larvae, within 4-days of infestation, in cut-stem as well as vegetative stage whole plant assays. However, there was an occasional white head even among such plants when assayed at the reproductive stage. Results are discussed in the light of resistance management strategies for deployment of Bt-rice.

Laxminarayan, R. and R. D. Simpson (2002). "Refuge strategies for managing pest resistance in transgenic agriculture." Environmental & Resource Economics **22**(4): 521-536.

When the application of pesticides places selective evolutionary pressure on pest populations it can be useful to plant refuge areas: crop areas intended to encourage the breeding of pests susceptible to the pesticide. Renewed interest in refuge areas has arisen with recent advances in biotechnology and genetically modified (GM) crops. This paper uses a simple model of evolution of pest population and pest resistance to characterize the socially optimal refuge strategy to manage pest resistance. We show both that the establishment of refuge areas might best be delayed until resistance becomes an important concern, and that the use of refuge areas in the long-run use will not be optimal if the fitness cost of resistance does not exceed the discount rate.

Liu, Y. B., B. E. Tabashnik, et al. (2002). "Oviposition on and mining in bolls of Bt and non-Bt cotton by resistant and susceptible pink bollworm (Lepidoptera : gelechiidae)." Journal of Economic Entomology **95**(1): 143-148.

Transgenic cotton that produces insecticidal crystal protein Cry1Ac of Bacillus thuringiensis (Bt) has been effective in controlling pink bollworm, Pectinophora gossypiella (Saunders). We compared responses to bolls of Bt cotton and non-Bt cotton by adult females and neonates from susceptible and Cry1Ac- resistant strains of pink bollworm. In choice tests on caged cotton plants in the greenhouse, neither susceptible nor resistant females laid fewer eggs on Bt cotton bolls than on non-Bt cotton bolls, indicating that the Bt toxin did not deter oviposition. Multiple regression revealed that the number of eggs laid per boll was negatively associated with boll age and positively associated with boll diameter. Females also laid more eggs per boll on plants with more bolls. The distribution of eggs among bolls of Bt cotton and non-Bt cotton was clumped, indicating that boll quality rather than avoidance of previously laid eggs was a primary factor in oviposition preference. Parallel to the results from oviposition experiments, in laboratory no-choice tests with 10 neonates per boll, the number of entrance holes per boll did not differ between Bt cotton and non-Bt cotton for susceptible and resistant neonates. Also, like females, neonates preferred younger bolls and larger bolls. Thus, acceptance of bolls by females for oviposition and by neonates for mining was affected by boll age and diameter, but not by Bt toxin in bolls. The lack of discrimination between Bt and non-Bt cotton bolls by pink bollworm from susceptible and resistant strains indicates that oviposition and mining initiation are independent of susceptibility to Cry1Ac.

Livingston, M. J., G. A. Carlson, et al. (2002). "Use of mathematical models to estimate characteristics of pyrethroid resistance in tobacco budworm and bollworm (Lepidoptera : Noctuidae) field populations." Journal of Economic Entomology **95**(5): 1008-1017.

Genetic models have been used to examine the evolution of insecticide resistance in pest species subject to data and assumptions regarding genetic, biological, and operational parameters. We used time-series data on pyrethroid tolerance and simple genetic models to estimate underlying genetic and biological parameters associated with resistance evolution in tobacco budworm, Heliothis virescens (F.), and bollworm, Helicoverpa zea

> (Boddie), Louisiana field populations. Assuming pyrethroid resistance is conferred by one gene at one locus in both species, inheritance of pyrethroid resistance was partially dominant in the tobacco budworm and partially recessive in the bollworm. Relative fitness estimates indicated that fitness costs associated with resistance selected against resistance alleles in the absence of selection pressure in the tobacco budworm, but not in the bollworm. In addition, relative fitness estimates obtained using the indirect method outlined in this study were similar in magnitude to estimates obtained using traditional direct approaches.

Losey, J. E., M. E. Carter, et al. (2002). "The effect of stem diameter on European corn borer behavior and survival: potential consequences for IRM in Bt-corn." Entomologia Experimentalis Et Applicata **105**(2): 89-96.

> The ability of non-crop plants to support complete development of insect pests is an important factor for determining the impact of those plants on resistance management programs for transgenic crops. We assessed the effect of one physical factor, plant stem diameter, on the ability of plants to support full development of the European corn borer (ECB), Ostrinia nubilalis Hubner (Lepidoptera: Crambidae), the target pest of transgenic Bt-corn. In the field, European corn borer larvae were significantly more likely to tunnel and survive in plants with larger stem diameters. Larvae were 40 x more likely to survive on corn, the largest plant tested, compared to many of the smaller plants. In the laboratory, larvae were more likely to survive in and less likely to abandon the largest diet-filled artificial stems that varied only in stem diameter. In conditions simulating those that an ECB larvae would encounter upon abandoning a host, larvae survived up to three weeks and were able to locate corn as a new host with a significantly higher frequency than would be expected if they were foraging randomly. These results indicate that the probability of ECB larval survival to maturity on a plant other than corn is relatively low and thus these smaller stemmed non-corn plants may not make a substantial contribution to the pool of susceptible adults. Conversely, since more mature larvae are not as susceptible as neonates, any larvae that partially develop on non-corn plants and subsequently colonize Bt-corn may not be exposed to a lethal dose of the toxin. Since some proportion of the individuals that survive could be partially resistant heterozygotes the presence of non-corn host plants could facilitate the development of resistant ECB populations.

Onstad, D. W., C. A. Guse, et al. (2002). "Modeling the development of resistance by stalk-boring lepidopteran insects (Crambidae) in areas with transgenic corn and frequent insecticide use." Journal of Economic Entomology **95**(5): 1033-1043.

> We simulated the population dynamics and population genetics of two bivoltine species of corn borers, the European corn borer, Ostrinia nubilalis (Hubner), and the southwestern corn borer, Diatraea grandiosella

Dyar, in a hypothetical region of irrigated transgenic and nontransgenic corn where insecticide was applied only to the nontransgenic refuge crop. Over the 100-yr time horizon, resistance developed quickly in both species and to both transgenic corn and the insecticide when the allele for resistance to the respective toxin was dominant. When the allele for transgenic resistance was not dominant and the refuge location was constant over the time horizon, spraying the refuge to control southwestern corn borer had no effect on how quickly resistance to the transgenic corn developed. In contrast, the European corn borer developed resistance to transgenic corn much sooner when the refuge was sprayed once per year, and the time to 3% resistance allele frequency decreased as efficacy of the insecticide increased. Only when the refuge was treated less than once every 5 yr (10 generations) did the frequency of application decline enough to permit resistance management for the European corn borer to approximate the effectiveness of an unsprayed refuge. A consistently sprayed refuge <40% of the corn acreage was an inadequate resistance management strategy for the European corn borer even when a low efficacy insecticide (70% mortality) was used. When assumptions about European corn borer adult behavior were changed and the adults behaved similarly to adult southwestern corn borer, the development of resistance to the transgenic crop was slowed significantly.

Shea, K., H. P. Possingham, et al. (2002). "Active adaptive management in insect pest and weed control: Intervention with a plan for learning." Ecological Applications **12**(3): 927-936.

A major problem in insect pest and weed management is uncertainty. Managers are faced with three main types of uncertainty: uncertainty about biological and environmental processes, and observational uncertainty. Active adaptive management (AAM) is management with a deliberate plan for learning about the managed system, so that management can be improved in the face of uncertainty. We discuss the potential benefits of applying AAM to insect pest and weed control with reference to a number of examples. We first address the possible uses for AAM in biological control, with particular reference to agent selection and release. We also propose applying AAM methods to resistance management and to spatial strategies for pest control. We conclude with an overview of AAM, a discussion of some of the potential limitations to its use in pest management, and the possibilities for increased implementation of AAM in the future.

Wu, K. M., Y. Y. Guo, et al. (2002). "Evaluation of the natural refuge function for Helicoverpa armigera (Lepidoptera : Noctuidae) within Bacillus thuringiensis transgenic cotton growing areas in north China." Journal of Economic Entomology **95**(4): 832-837.

The density of Helicoverpa armigera (Hubner) populations on Bacillus thuringiensis Berliner (Bt) transgenic cotton, corn. peanut. and soybean: differences in its development on Bt cotton and common (nontransgenic) cotton and the potential for mating among populations from Bt cotton fields and other crop fields were investigated in the suburbs of Xinxiang City (Henan Province) and Langfang City (Hebei Province) in the southern and northern parts of north China, respectively, Although development of H. armigera on Bt cotton was much slower than on common cotton. there was a still high probability of mating between populations from Bt cotton and other sources due to the scattered emergence pattern of H. armigera adults, and overlap of the second and third generations. In a cotton and corn growing region. early and late planted Corn provided Suitable refugia for the third and fourth generations of H armigera, but not for the second generation. In a cotton and soybean/peanut mix system, noncotton crops provided a natural refugia from the second- to fourth-generation H, armigera, but function of the refuge would closely depend on the proportion of Bt cotton. Consequently. it may be necessary to compensate the original mixed cropping patterns in different areas for delaying resistance development of H. armigera to Bt cotton.

Wu, K. M., Y. Y. Guo, et al. (2002). "Resistance monitoring of Helicoverpa armigera (Lepidoptera : Noctuidae) to Bacillus thuringiensis insecticidal protein in China." Journal of Economic Entomology **95**(4): 826-831.

Sensitivities of Helicoverpa armigera (Hubner) field populations to Bacillus thuringiensis (Bt) insecticidal protein Cry1Ac were monitored during 1998-2000 in China. A total of 41 strains was sampled, and most of them were collected from Bt cotton planting regions. The range of IC50 values (concentration producing 50% inhibition of larval development to third instar) among different populations in 1998, 1999, and 2000 were 0.020-0.105 mug/ml. 0.016-0.099 mug/ml, and 0.016- 0.080 mug/ml, respectively. Diagnostic concentration studies (IC99) showed that the percentage of individuals reaching third instar ranged from 0 to 4.35% with only eight of the 41 tested populations showing values above 0%. Also interesting was a trend over successive years in which fewer populations contained individuals that survived the diagnostic concentration by reaching third instar. Considering these data, it was determined that the field populations sampled during the 3-yr study were susceptible to Cry1Ac protein and that movement toward resistance among H. armigera populations was not apparent.

Zhao, J. Z., Y. X. Li, et al. (2002). "Examination of the F-2 screen for rare resistance alleles to Bacillus thuringiensis toxins in the diamondback moth (Lepidoptera : Plutellidae)." Journal of Economic Entomology **95**(1): 14-21.

A synthetic laboratory population of the diamondback moth, Plutella xylostella (L.), was used to test the F-2 screen developed for detecting the frequency of rare resistance alleles to Cry1Ac and Cry1C toxins of Bacillus thuringiensis (Bt). Of the 120 single-pair matings set up, 106 produced enough F-2 families for screening of Cry1Ac or Cry1C resistance alleles using both transgenic broccoli and an artificial diet overlay assay with a diagnostic dose. When using Bt broccoli plants as the F-2 screen method, only one F-2 family was detected for Cry1Ac resistance and no family was detected for Cry1C resistance. Six families were detected for either Cry1Ac or Cry1C resistance using the diet assay. The survivors in the diagnostic diet assay were crossed with the resistant individuals to confirm their resistance genotypes. Four F-2 families were confirmed to contain one copy of an allele resistant to Cry1Ac in the original single-pairs and four other F-2 families contained an allele resistant to Cry1C. Our results suggest that using transgenic plants expressing a high level of a Bt toxin in an F-2 screen may underestimate the frequency of resistance alleles with high false negatives, or fail to detect true resistance alleles. The diagnostic diet assay was a better F-2 screen method to detect alleles, especially for the Cry1Ac resistance with monogenic inheritance in the diamondback moth. The estimated probabilities of false positives and false negatives were 33 and 1%, respectively, for detecting Cry1Ac resistance at the allele frequency of 0.012 using the diagnostic diet assay. Careful validation of the screening method for each insect-crop system is necessary before the F-2 screen can be used to detect rare Bt resistance alleles in field populations.

Bohorova, N., R. Frutos, et al. (2001). "Novel synthetic Bacillus thuringiensis cry1B gene and the cry1B-cry1Ab translational fusion confer resistance to southwestern corn borer, sugarcane borer and fall armyworm in transgenic tropical maize." Theoretical and Applied Genetics **103**(6-7): 817-826.

In order to develop a resistance management strategy to control tropical pests based on the co-expression of different toxins, a fully modified Bacillus thuringiensis cr1B gene and the translational fusion cry1B-cry1Ab gene have been developed. Both constructs were cloned under the control of a maize ubiquitin-1 or a rice actin-1 promoter and linked to the bar gene driven by the CaMV 35S promoter. Immature embryos from the tropical lines CML72, CML216, and their hybrids, were used as the target for transformation by microprojectile bombardment. Twenty five percent of the transformed maize plants with cry1B expressed a protein that is active against southwestern corn borer and sugarcane borer. Ten percent of the transgenic maize expressed single fusion proteins from the translational fusion gene cry1B-1Ab and showed resistance to these two pests as well as to the fall armyworm. Transgenic maize plants that carried the cry1B gene in T1 to T3 progenies transmitted trangenes with expected Mendelian segregation and conferred resistance to the two target insects. Molecular analyses confirmed the cn, genes integration. the copy number, the size of protein(s) expressed in maize plants, the transmission, and the inheritance of the introduced cry gene. These new transgenic products will provide another recourse for reducing the build-up of resistance in pest populations

Cao, J., A. M. Shelton, et al. (2001). "Gene expression and insect resistance in transgenic broccoli containing a Bacillus thuringiensis cry1Ab gene with the chemically inducible PR-1a promoter." Molecular Breeding **8**(3): 207-216.

> We produced 49 broccoli plants (Brassica oleracea L. ssp. italica) containing a Bacillus thuringiensis cry1Ab gene under control of the chemically inducible PR-1a promoter from tobacco. Most of them showed substantial or complete control of neonate diamondback moth larvae, regardless of whether the transgene was induced or not. Ten plants were selected for detailed study via northern and western analysis and insect bioassays. They expressed the cry1Ab gene and gave complete insect control when treated with the chemical inducers INA (2,6-dichloroiso-nicotinic acid) or BTH (1,2,3- benzothiadiazole-7-carbothioic acid S-methyl ester); however, leaves treated with water alone were also partially or completely protected from insect damage. Transgenic progeny plants showed greater inducibility than primary transformants at the molecular level. Two progeny lines produced cry1Ab mRNA and Cry1Ab protein and gave insect control only after induction, both when detached leaves and intact plants were tested. The relevance of these results to resistance management strategies is discussed.

Caprio, M. A. (2001). "Source-sink dynamics between transgenic and non-transgenic habitats and their role in the evolution of resistance." Journal of Economic Entomology **94**(3): 698-705.

> The interaction of population dynamics and movement among two habitat types (toxic transgenic fields and nontoxic refuge fields) on the evolution of insecticide resistance was examined in two different simulation models. The two models were developed to test the hypothesis that increasing habitat grain from fine-grained to coarse-grained, and the resultant increase in nonrandom mating, would increase the rate of local adaptation, here the evolution of resistance. The first model, a complex, stochastic spatially explicit model, altered. habitat grain by varying adult dispersal rates between habitat patches. In contrast to the expectation that increasing patch isolation and increasing the coarseness of the habitats would increase the rate of resistance evolution, intermediate levels of dispersal actually delayed resistance by as much as fivefold over the range of dispersal levels observed. Source-sink dynamics related to ovipositional patterns and the related population dynamics appear to explain the results. A simple deterministic model was developed to abstract out the separate impacts of mating and ovipositional behaviors. This model showed qualitatively the same results, although under similar assumptions it predicted much longer delays in resistance evolution. In this model, nonrandom mating alone always increased the rate at which insects adapted to transgenic crops, but nonrandom mating in combination with nonrandom oviposition could significantly delay resistance evolution. Differences between the two models may be due to

the population regulation incorporated in the spatially explicit model. The models clearly suggest that resistance management programs using untreated refuges should not over-emphasize random mating at the cost of making the habitat too fine-grained.

Carriere, K., T. J. Dennehy, et al. (2001). "Large-scale management of insect resistance to transgenic cotton in Arizona: Can transgenic insecticidal crops be sustained?" Journal of Economic Entomology **94**(2): 315-325.

A major challenge for agriculture is management of insect resistance to toxins from Bacillus thuringiensis (Bt) produced by transgenic crops. Here we describe how a large-scale program is being developed in Arizona for management of resistance to Bt cotton in the pink bollworm, Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechiidae), and other insect pests of cotton. Financial support from growers makes this program possible. Collaboration between the Arizona Cotton Research and Protection Council, the University of Arizona, and government agencies has led to development of resistance management guidelines, a remedial action plan, and tools for monitoring compliance with the proposed guidelines. Direct participation in development of resistance management policies is a strong incentive for growers to invest in resistance management research. However, more research, regularly updated regulations, and increased collaboration between stakeholders are urgently needed to maintain efficacy of Bt toxins in transgenic crops.

Carriere, Y., C. Ellers-Kirk, et al. (2001). "Fitness costs and maternal effects associated with resistance to transgenic cotton in the pink bollworm (Lepidoptera : Gelechiidae)." Journal of Economic Entomology **94**(6): 1571-1576.

Transgenic cotton producing a Bacillus thuringiensis (Bt) toxin is widely used for controlling the pink bollworm. Peretinophora gossypiella (Saunders). We compared performance of pink bollworm strains resistant to Bt cotton with performance of their susceptible counterparts on non-Bt cotton. We found fitness costs that reduced survival on non-Bt cotton by all average of 51.5% in two resistant strains relative to the susceptible strains. The survival cost was recessive in one set of crosses between a resistant strain and the susceptible strain from which it was derived. However, crosses involving an unrelated resistant Laid susceptible strain indicated that the survival cost could be dominant. Development time oat non-BI cotton did not differ between the two related resistant and susceptible strains, A slight recessive cost affecting development time was suggested by comparison of the unrelated resistant and susceptible strains. Maternal effects transmitted by parents that had eaten Bt-treated artificial diet as larvae had negative effects on embryogenesis, adult fertility, nr both, and reduced the ability of neonates to enter cotton bolls. These results provide further evidence that fitness costs associated with the evolution of resistance to BL cotton are substantial in the pink bollworm.

Carriere, Y., C. Ellers-Kirk, et al. (2001). "Overwintering cost associated with resistance to transgenic cotton in the pink bollworm (Lepidoptera : Gelechiidae)." Journal of Economic Entomology **94**(4): 935-941.

> Fitness costs associated with resistance to transgenic crops producing toxins from Bacillus thuringiensis (Bt) may have important effects on the evolution of resistance. We investigated overwintering costs in pink bollworm, Pectinophora gosypiella (Saunders), strains with different degrees of resistance to Bt cotton. Frequency of resistant individuals in a strain was not associated with induction of diapause or emergence from diapause in early winter. Emergence from diapause in the spring was 71% lower in three highly resistant strains than in two heterogeneous strains from which the resistant strains were derived. This underestimates the overwintering cost because the frequency of the resistance allele was relatively high in the heterogeneous strains. Emergence in the spring in hybrid progeny from crosses between the resistant and heterogeneous strains was greater than in resistant strains but did not differ from susceptible strains, showing that the overwintering cost was recessive to some extent.

Carriere, Y., C. Ellers-Kirk, et al. (2001). "Predicting spring moth emergence in the pink bollworm (Lepidoptera : Gelechiidae): Implications for managing resistance to transgenic cotton." Journal of Economic Entomology **94**(5): 1012-1021.

> Cultural control methods have been central in the southwestern United States for reducing pink bollworm, Pectinophora gossypiella (Saunders), damage to cotton. Nevertheless, it is not clear at present how such methods could be integrated within the novel pest management framework allowed by introduction of cotton producing a toxin from Bacillus thuringiensis (Bt) for pink bollworm control. Using, statewide pheromone trapping and climatic data in conjunction with deterministic simulation models, we investigated whether manipulation of cotton planting date and use of other cultural control methods could represent valuable tactics for control of the pink bollworm in Arizona. Accumulation of heat units from one January accurately predicted the rate of pink bollworm emergence from diapause in 15 cotton-producing regions. Significant variation in rate of emergence from diapause was present among regions, with earlier emergence at higher altitudes. Most adults emerge from diapause too early to reproduce successfully on cotton, a phenomenon known as suicidal emergence. A method for prediction of the fraction of suicidal emergence resulting from adoption of a given cotton planting date is presented. Results from simulation models suggest that manipulation of planting date and implementation of other control cultural methods, reduce the rate of application of insecticides and delay the evolution of resistance to Bt cotton in the pink bollworm.

Carriere, Y. and B. E. Tabashnik (2001). "Reversing insect adaptation to transgenic insecticidal plants." Proceedings of the Royal Society of London Series B-Biological Sciences **268**(1475): 1475-1480.

> The refuge-high-dose strategy for delaying insect adaptation to transgenic plants produces non-transgenic plants that enable survival of susceptible individuals. Previous theoretical work has suggested three requirements for success of the refuge- high-dose strategy: a low initial frequency of the resistance allele, extensive mating between resistant and susceptible adults and recessive inheritance of resistance. In order to understand an observed decrease in resistance frequency and improve the potential for managing resistance better, we used analytical and simulation models for exploring the conditions that prevent or reverse the evolution of resistance, even when resistance is not rare initially Assuming random mating and recessive or nearly recessive inheritance of resistance, the factors favouring reversal of resistance are non-recessive costs of resistance, low initial resistance allele frequency, large refuges, incomplete resistance and density-independent population growth in refuges.

Chaufaux, J., M. Seguin, et al. (2001). "Chronic exposure of the European corn borer (Lepidoptera : Crambidae) to CrylAb Bacillus thuringiensis toxin." Journal of Economic Entomology **94**(6): 1564-1570.

> Transgenic corn expressing the insecticidal toxin from Bacillus thuringiensis Berliner is gaining support as an effective control technology for use against lepidopteran pests, particularly European corn borer, Ostrinia nubilalis Hubner (Lepidoptera: Crambidae). However, there is concern that widespread adoption of transgenic plants will rapidly lead to B. thuringiensis toxin resistance, Thus, long-term selection of O. nubilalis populations with the Cry1Ab B. thuringiensis toxin has been undertaken in several laboratories in the United States and in Europe. We present results from two independent selection experiments performed in laboratories at the University of Nebraska and at the Institut National de In Recherche Agronomique in France. Although the protocols and methods used by the two laboratories were different, the results were comparable. The highest level of resistance occurred at generation 7 (14-fold), generation 9 (13-fold), and generation 9 (32-fold) for three different strains. For each strain, the level of resistance fluctuated from generation to generation, although there were consistently significant decreases in toxin susceptibility across generations for all selected strains. These results suggest that low levels of resistance are common among widely distributed O. nubilalis populations.

Gahan, L. J., F. Gould, et al. (2001). "Identification of a gene associated with bit resistance in Heliothis virescens." Science **293**(5531): 857-860.

> Transgenic crops producing insecticidal toxins from Bacillus thuringiensis (Bt) are widely used for pest control. Bt- resistant insect strains have been

studied, but the molecular basis of resistance has remained elusive. Here, we show that disruption of a cadherin-superfamily gene by retrotransposon- mediated insertion was linked to high levels of resistance to the Et toxin Cry1Ac in the cotton pest Heliothis virescens. Monitoring the early phases of Bt resistance evolution in the field has been viewed as crucial but extremely difficult, especially when resistance is recessive. Our findings enable efficient DNA-based screening for resistant heterozygotes by directly detecting the recessive allele.

Gonzalez-Cabrera, J., S. Herrero, et al. (2001). "High genetic variability for resistance to Bacillus thuringiensis toxins in a single population of diamondback moth." Applied and Environmental Microbiology **67**(11): 5043-5048.

The long-term benefit of insecticidal products based on Cry toxins, either in sprays or as transgenic crops, is threatened by the development of resistance by target pests. The models used to predict evolution of resistance to Cry toxins most often are monogenic models in which two alleles are used. Moreover, the high-dose/refuge strategy recommended for implementation with transgenic crops relies on the assumption that the resistance allele is recessive. Using selection experiments, we demonstrated the occurrence in a laboratory colony of diamondback moth of two different genes (either allelic or nonallelic) that confer resistance to Cry1Ab. At the concentration tested, resistance was dominant in one selection line and partially recessive in the other. Resistant insects from the two selection lines also differed in their cross- resistance patterns. The diamondback moth colony was derived from a field population from the Philippines, which originally showed a different resistance phenotype. This is the first time that an insect population has been directly shown to carry more than one gene conferring resistance to the same Cry toxin.

Hunt, T. E., L. G. Higley, et al. (2001). "Dispersal of adult European corn borer (Lepidoptera : Crambidae) within and proximal to irrigated and non-irrigated corn." Journal of Economic Entomology **94**(6): 1369-1377.

The European corn borer, Ostrinia nubilalis (Hubner), causes economic damage to corn, Zea mays L., throughout the Corn Belt. Because this insect has become the primary target of Bacillus thuringiensis Berliner (Bt) transgenic corn, current efforts addressing the management of O. nubilalis resistance to Bt corn require information on adult European corn borer dispersal and factors affecting its dispersal, In 1998 we conducted mark-release-recapture, release-recapture, and caged-mating studies to directly measure and compare local dispersal patterns of O. nubilalis adults within and proximal to irrigated and non- irrigated cornfields. Releases of marked adults were made corresponding to the first and second flight of O. nubilalis in eastern Nebraska. Adult dispersal was significantly different between irrigated and non-irrigated cornfields. Released adults tended to remain in and near irrigated cornfields, but dispersed out of and away from non-irrigated cornfields. When released at the edge of the cornfield, neither male nor unmated female O. nubilalis displayed an initial tendency to move out of irrigated corn and into the mixed smooth bromegrass (Bromus inermis Leyss) and broadleaf-weed field edge. Mating efficiency in a late-season cornfield was not significantly different than in dense foxtail (Setaria spp.). Generally, we found that adult O. nubilalis dispersal may vary depending on variables such as action-site availability and agronomic practices and their interaction with O. nubilalis life history.

Liu, Y. B., B. E. Tabashnik, et al. (2001). "Effects of Bt cotton and Cry1Ac toxin on survival and development of pink bollworm (Lepidoptera : Gelechiidae)." <u>Journal of Economic Entomology</u> **94**(5): 1237-1242.

> We evaluated the effects of Bacillus thuringiensis (Bt) toxin Cry1Ac on survival and development of a susceptible strain and laboratory-selected resistant strains of pink bollworm, Pectinaphora gossypiella (Saunders). For susceptible and resistant strains tested on artificial diet, increases in Cry1Ac concentration reduced developmental rate and pupal weight. In greenhouse tests, survival of resistant larvae on transgenic cotton that produces Cry1Ac (Bt cotton) was 46% relative to their survival on non-Bt cotton. In contrast, Bt cotton killed all susceptible larvae tested. F, hybrid progeny of resistant and susceptible adults did not survive on Bt cotton, which indicates recessive inheritance of resistance. Compared with resistant or susceptible larvae reared on non-Bt cotton, resistant larvae reared on Bt cotton had lower survival and slower development, and achieved lower pupal weight and fecundity. Recessive resistance to Bt cotton is consistent with one of the basic assumptions of the refuge strategy for delaying resistance to Bt cotton. Whereas slower development of resistant insects on Bt cotton could increase the probability of mating between resistant adults and accelerate resistance, negative effects of Bt cotton on the survival and development of resistant larvae could delay evolution of resistance.

Liu, Y. B., B. E. Tabashnik, et al. (2001). "Genetics of pink bollworm resistance to Bacillus thuringiensis toxin Cry1Ac." <u>Journal of Economic Entomology</u> **94**(1): 248-252.

> Laboratory selection increased resistance of pink bollworm (Pectinophora gossypiella) to the Bacillus thuringiensis toxin Cry1Ac. Three selections with Cry1Ac in artificial diet increased resistance from a low level to >100-fold relative to a susceptible strain. We used artificial diet bioassays to test F-1 hybrid progeny from reciprocal crosses between resistant and susceptible strains. The similarity between F-1 progeny from the two reciprocal crosses indicates autosomal inheritance of resistance. The dominance of resistance to Cry1Ac depended on the concentration. Resistance was codominant at a low concentration of Cry1Ac, partially recessive at an intermediate concentration, and completely recessive at a high concentration. Comparison of the artificial diet results with previously reported results from greenhouse bioassays shows that the high

concentration of Cry1Ac in bolls of transgenic cotton is essential for achieving functionally recessive inheritance of resistance.

Morse, R. J., T. Yamamoto, et al. (2001). "Structure of Cry2Aa suggests an unexpected receptor binding epitope." Structure **9**(5): 409-417.

Background: Genetically modified (GM) crops that express insecticidal protein toxins are an integral part of modern agriculture. Proteins produced by Bacillus thuringiensis (Bt) during sporulation mediate the pathogenicity of Bt toward a spectrum of insect larvae whose breadth depends upon the Bt strain. These transmembrane channel-forming toxins are stored in Bt as crystalline inclusions called Cry proteins. These proteins are the active agents used in the majority of biorational pesticides and insect-resistant transgenic crops. Though Bt toxins are promising as a crop protection alternative and are ecologically friendlier than synthetic organic pesticides, resistance to Bt toxins by insects is recognized as a potential limitation to their application. Results: We have determined the 2.2 Angstrom crystal structure of the Cry2Aa protoxin by multiple isomorphous replacement. This is the first crystal structure of a Cry toxin specific to Diptera (mosquitoes and flies) and the first structure of a Cry toxin with high activity against larvae from two insect orders, Lepidoptera (moths and butterflies) and Diptera. Cry2Aa also provides the first structure of the proregion of a Cry toxin that is cleaved to generate the membrane-active toxin in the larval gut. Conclusions: The crystal structure of Cry2Aa reported here, together with chimeric-scanning and domain-swapping mutagenesis, defines the putative receptor binding epitope on the toxin and so may allow for alteration of specificity to combat resistance or to minimize collateral effects on nontarget species. The putative receptor binding epitope of Cry2Aa identified in this study differs from that inferred from previous structural studies of other Cry toxins.

Nault, B. A. (2001). "Survival and fecundity of Bt-susceptible Colorado potato beetle adults after consumption of transgenic potato containing Bacillus thuringiensis subsp tenebrionis Cry3A toxin." Entomologia Experimentalis Et Applicata **101**(3): 265-272.

Survival and fecundity of Colorado potato beetle adults, Leptinotarsa decemlineata (Say), that had or had not fed previously on non-transgenic potato before exposure to transgenic potato containing the Bacillus thuringiensis subsp. tenebrionis Cry3A toxin (Bt) was investigated. In the laboratory, < 5% of first-generation adults survived after two weeks when restricted to Bt foliage since eclosion, but over 85% of adults that had fed initially on non-Bt potato survived exposure to Bt potato for two weeks. In field experiments, less than 0.5% of adults that were exclusively provided Bt potato plants survived overwinter, whereas 44% to 57% survived overwinter when fed non-Bt potato plants for two weeks before being provided Bt potato as a final pre-overwintering host. Survival through the winter increased as the duration of initial feeding on non-Bt potato increased and was similar for beetles provided either tubers or Bt potato plants as a final pre-overwintering host. Only overwintered beetles that fed initially on non-Bt potato before encountering either tubers or Bt potato as a final pre-overwintering host laid eggs the following spring. Survival and reproduction of potato beetle adults after colonizing Bt potato fields should not be adversely affected as long as they have had sufficient time to feed initially on non-Bt potato. Implications for how potato production practices in the Mid-Atlantic US may affect the utility of general resistance management plans for Bt potato are discussed.

Onstad, D. W., C. A. Guse, et al. (2001). "Modeling the dynamics of adaptation to transgenic corn by western corn rootworm (Coleoptera : Chrysomelidae)." Journal of Economic Entomology **94**(2): 529-540.

A simulation model of the population dynamics and genetics of the western corn rootworm, Diabrotica virgifera virgifera LeConte, was created for a landscape of corn, soybean, and other crops. Although, the model was created to study a 2-locus problem for beetles having genes for resistance to both crop rotation and transgenic cool, during this first phase of the project, the model was simulated to evaluate only resistance management plans for transgenic corn. Allele expression in the rootworm and toxin dose in the corn plant were the two most important factors affecting resistance development. A dominant resistance allele allowed quick evolution of resistance to transgenic corn, whereas a recessive allele delayed resistance >99 yr. With high dosages of toxin and additive expression, the time required to reach 3% resistance allele frequency ranged from 13 to >99 yr. With additive expression, lon er dosages permitted tire resistant allele frequency to reach 3% in 2-9 yr with refuges occupying 5-30% of the land. The results were sensitive to delays in emergence by susceptible adults and configuration of the refuge (row strips versus blocks).

Rausher, M. D. (2001). "Co-evolution and plant resistance to natural enemies." Nature **411**(6839): 857-864.

Co-evolution between plants and their natural enemies is generally believed to have generated much of the Earth's biological diversity. A process analogous to co-evolution occurs in agricultural systems, in which natural enemies adapt to crop resistance introduced by breeding or genetic engineering. Because of this similarity, the investigation of resistance mechanisms in crops is helping to elucidate the workings of co-evolution in nature, while evolutionary principles, including those derived from investigation of co- evolution in nature, are being applied in the management of resistance in genetically engineered crops.

Reed, G. L., A. S. Jensen, et al. (2001). "Transgenic Bt potato and conventional insecticides for Colorado potato beetle management: comparative efficacy and non-target impacts." Entomologia Experimentalis Et Applicata **100**(1): 89-100.

Field studies were conducted in 1992 and 1993 in Hermiston, Oregon, to evaluate the efficacy of transgenic Bt potato (Newleaf(R), which expresses the insecticidal protein Cry3Aa) and conventional insecticide spray programs against the important potato pest, Leptinotarsa decemlineata (Say), Colorado potato beetle (CPB), and their relative impact on non-target arthropods in potato ecosystems. Results from the two years of field trials demonstrated that Newleaf potato plants were highly effective in suppressing populations of CPB, and provided better CPB control than weekly sprays of a microbial Bt-based formulation containing Cry3Aa, bi-weekly applications of permethrin, or early- and mid-season applications of systemic insecticides (phorate and disulfoton). When compared with conventional potato plants not treated with any insecticides, the effective control of CPB by Newleaf potato plants or weekly sprays of a Bt-based formulation did not significantly impact the abundance of beneficial predators or secondary potato pests. In contrast to Newleaf potato plants or microbial Bt formulations, however, bi-weekly applications of permethrin significantly reduced the abundance of several major generalist predators such as spiders (Araneae), big-eyed bugs (Geocorus sp.), damsel bugs (Nabid sp.), and minute pirate bugs (Orius sp.), and resulted in significant increases in the abundance of green peach aphid (GPA), Myzus persicae (Sulzer) - vector of viral diseases, on the treated potato plots. While systemic insecticides appeared to have reduced the abundance of some plant sap-feeding insects such as GPA, lygus bugs, and leafhoppers, early and mid-season applications of these insecticides had no significant impact on populations of the major beneficial predators. Thus, transgenic Bt potato, Bt- based microbial formulations and systemic insecticides appeared to be compatible with the development of integrated pest management (IPM) against other potato pests such as GPA because these CPB control measures have little impact on major natural enemies. In contrast, the broad-spectrum pyrethroid insecticide (permethrin) is less compatible with IPM programs against GPA and the potato leafroll viral disease.

Sequeira, R. V. and C. L. Playford (2001). "Abundance of Helicoverpa (Lepidoptera : Noctuidae) pupae under cotton and other crops in central Queensland: Implications for resistance management." Australian Journal of Entomology **40**: 264-269.

Planting of refuge crops and post-harvest cultivation of soil in winter are key elements of resistance management strategies (RMS) for Bt-transgenic (Ingard(lozenge)) and conventional cottons in southern Queensland and New South Wales. As part of a larger project to examine the feasibility of growing Ingard(lozenge) in central Queensland (CQ), field assessments were conducted during the 1996-97 growing season to examine the adequacy of the southern Bt-cotton RMS under local environmental conditions. The suitability of cotton and a number of other field crops as refuges for Bt-cotton was assessed in terms or their relative

Helicoverpa pupal productivity. The practicality and potential effectiveness of post-harvest cultivation under CQ conditions were also assessed. Field assessments show that pigeon pea has the greatest potential as a refuge for Bt-cotton. Unsprayed cotton, sorghum and maize also produced substantially high pupal densities and hence are suitable refuge options, but they will require larger areas to be planted relative to pigeon pea. Post-harvest cultivation in cotton fields is largely ineffective for resistance management under CQ conditions. A Bt-cotton RMS for CQ is proposed. The CQ strategy includes refuge crop options contained in the southern strategy and the use of late season trap-crops of pigeon pea as an alternative to post-harvest cultivation.

Storer, N. P., J. W. Van Duyn, et al. (2001). "Life history traits of Helicoverpa zea (Lepidoptera : Noctuidae) on non-Bt and Bt transgenic corn hybrids in Eastern North Carolina." Journal of Economic Entomology **94**(5): 1268-1279.

Transgenic varieties of field corn that express the Cry1Ab B. thuringiensis (Bt) toxin in ear tissue present the potential of reducing ear feeding by the com earworm, Helicoverpa zea (Lepidoptera: Noctuidae), and for reducing the size of populations of the insect infesting other host crops. Life, history parameters of H. zea feeding on ears of conventional and Bt field corn varieties were measured in field plots in eastern North Carolina in 1997 and 1998. Transformation events investigated were Mon-810 and Bt-11. Bt corn was found to cause a steady mortality of larvae during development, but permitted approximate to 15-40% survival to the prepupal stage compared with non-Bt corn. Mortality of prepupae and pupae from Bt corn was also higher than from non-Bt com, reducing overall adult production by 65-95% The larvae that did survive grew more slowly on Bt than on non-Bt corn, and produced pupae that weighed 33% less. Pupation and adult eclosion were delayed by 6-10 d by feeding on Bt com ears. Com varieties, expressing Bt in ear tissue have the potential to reduce H. zea ear feeding by up to 80%, and the potential to reduce populations emerging from ear-stage corn fields to infest cotton, soybean and other crops by around 75%. To have a measurable effect on area-wide populations, Bt corn varieties would need to be planted in large proportions of corn fields, Extensive planting of varieties such as those tested here, having only moderate effects on H. zea, would raise concerns about rapid evolution of resistance.

Tan, S. J., X. F. Chen, et al. (2001). "Can other host species of cotton bollworm be non-Bt refuges to prolong the effectiveness of Bt-cotton?" Chinese Science Bulletin **46**(21): 1804-1808.

The potential ecological risks of Bacillus thurigiensis (Bt) insecticides and Bt-crops have caused increasing concern since their commercial release in the field, among which pests' resistance to Bt-crops is the major ecological risk. Refuge tactic, which can produce sensitive populations, has proved to be a key and sound resistance management strategy in

USA and Australia; however, no tactics have been performed in China where Bt-cotton is mostly planted with other host crops of cotton bollworm. Genetic variation and gene flow among different host populations of the cotton bollworm Helicoverpa armigera were analyzed using PCR fingerprinting method. The results show that maize and castor-oil plant, as well as cotton can take effect as refuges to prevent resistance of cotton bollworm to Bt-cotton, while peanut and sesame are not as suitable for planting with Bt-cotton as refuges in the field as low gene flow was detected among populations on peanut, sesame and Bt cotton.

Tang, J. D., H. L. Collins, et al. (2001). "Greenhouse tests on resistance management of Bt transgenic plants using refuge strategies." Journal of Economic Entomology **94**(1): 240-247.

Experimental evaluation of the effectiveness of resistance management tactics is vital to help provide guidelines for the deployment of transgenic insecticidal crops. Transgenic broccoli expressing a Cry1Ac gene of Bacillus thuringiensis (Bt) and the diamondback moth, Plutella xylostella (L.), were used in greenhouse tests to evaluate the influence of size and placement of nontransgenic refuge plants on changes in resistance allele frequency and pest population growth. In the first test with an initial Cry1Ac-resistance (R) allele frequency of 0.007, P. xylostella were introduced into cages with the following treatments: 0, 3.3, 10, 20, and 100% refuge plants. Results after four generations showed that resistance could be delayed by increasing the proportion of refuge plants in the cage. Population growth was also influenced by refuge size with the highest populations occurring in treatments that had either no refuge plants or all refuge plants. In the second tests, we evaluated the effect of refuge placement by comparing 20% separate and 20% mixed refuges. P. xylostella with an initial frequency of resistant alleles at 0.0125 were introduced into cages and allowed to cycle; later generations were evaluated for resistance and population growth. Separating the refuge had a pronounced effect on delaying resistance and slowing establishment of resistant larvae on Bt plants. Combining information from both trials, we found a strong negative correlation between the number of larvae on Bt plants and the mortality of the population in leaf dip bioassays. Results from larval movement studies showed that separate refuges delayed resistance better than mixed refuges because they conserved relatively more susceptible alleles than R alleles and did not increase the effective dominance of resistance.

Zhao, J. Z., Y. X. Li, et al. (2001). "Different cross-resistance patterns in the diamondback moth (Lepidoptera : Plutellidae) resistant to Bacillus thuringiensis toxin CryIC." Journal of Economic Entomology **94**(6): 1547-1552.

Two strains of the diamondback moth, Plutella xylostella (L.), were selected using Cry1Cprotoxin and transgenic broccoli plants expressing a Cry1C toxin of Bacillus thuringiensis (Bt). Both strains were resistant to

Cry1C but had different cross- resistance patterns. We used 12 Bt protoxins for cross- resistance tests, including Cry1Aa, Cry1Ab, Cry1Ac, Cry1Bb, Cry1C, Cry1D, Cry1E, Cry1F, Cry1J, Cry2Ab, Cry9Aa, and Cry9C. Compared with the unselected sister strain (BCS), the resistance ratio (RR) of one strain (BCS-Cry1C-1) to the Cry1C protoxin was 1,090-fold with high level of cross-resistance to Cry1Aa, Cry1Ab, Cry1Ac, Cry1F, and Cry1J (RR > 390-fold). The cross-resistance to Cry1A, Cry1F, and Cry1J in this strain was probably related to the Cry1A resistance gene (s) that came from the initial field population and was caused by intensive sprayings of Bt products containing Cry1A protoxins. The neonates of this strain can survive on transgenic broccoli plants expressing either Cry1Ac or Cry1C toxins. The other strain (BCS-Cry1C-2) was highly resistant to Cry1C but not cross-resistant to other Bt protoxins. The neonates of this strain can survive on transgenic broccoli expressing Cry1C toxin but not Cry1Ac toxin, The gene(s) conferring resistance to Cry1C segregates independently from Cry1Ac resistance in these strains. The toxicity of Cry1E and Cry2Ab protoxins was low to all of the three strains. The overall progress of all work has resulted in a unique model system to test the stacked genes strategy for resistance management of Bt transgenic crops.

Acciarri, N., G. Vitelli, et al. (2000). "Transgenic resistance to the Colorado potato beetle in Bt- expressing eggplant fields." Hortscience **35**(4): 722-725.

Colorado potato beetle (CPB; Leptinotarsa decemlineata-Say) is a serious pest because it has developed resistance against insecticides. Three transgenic eggplant (Solanum melongena L,) lines bearing a mutagenized Bacillus thuringiensis; Berl, gene coding for the Cry3B toxin, and the nontransformed control DR2- line were tested in field trials to assess their insect resistance. The transgenic lines 3-2, 6-1, and 9-8 were tested at two different locations in a randomized complete-block design. Samples were taken biweekly to assess the level of CPB and the presence of other insects. At harvest, total yield and fruit number per plot were recorded. Two transgenic lines showed high levels of resistance at both locations, as measured by CPB abundance and yield, Fruit production was almost twice as great in the highly resistant lines (3-2 and 9-8) as in the nontransformed control. The 6-1 transgenic line showed an intermediate Level of resistance: it was similar to the control under heavy CPB pressure and was comparable to the other transgenic lines under milder infestations. Analysis by double antibody sandwich-enzyme linked immunosorbent assay (DAS- ELISA), performed on different tissues, revealed a fewer amount of Cry3B protein in the 6-1 transgenic line than in lines 3-2 and 9-8, No detrimental effects on nontarget arthropods (including the chrysomelid Altica) were evident. Field observations confirmed that Bt may be able to control CPB infestation in eggplant, representing a potential effective and environmentally safe means of pest control.

Alinia, F., B. Ghareyazie, et al. (2000). "Effect of plant age, larval age, and fertilizer treatment on resistance of a cry1Ab-transformed aromatic rice to lepidopterous stem borers and foliage feeders." Journal of Economic Entomology **93**(2): 484-493.

> The resistance of vegetative, booting, and flowering stage plants of a variety of an aromatic rice, Oryza sativa L., transformed with a Bacillus thuringiensis Berliner cry1Ab gene under control of the maize phosphoenolpyruvate carboxylase (PEPC) promoter was evaluated against four lepidopterous lice pests-the stem borers Chilo suppressalis (Walker) (Lepidoptera: Crambidae) and Scirpophaga incertulas (Walker) (Lepidoptera: Pyralidae), and the foliage feeders Cnaphalocrocis medinalis Guenee (Lepidoptera: Pyralidae) and Naranga aenescens Moore (Lepidoptera: Noctuidae). Plants of the cry1Ab-transformed line (no. 827) were more resistant to young, larvae of S. incertulas. C. suppressalis, and C. medinalis than control plants at the vegetative stage but not at the flowering stage. Survival of 10-d-old stem borer larvae did not differ on cry1Ab plants and control plants at either the vegetative or flowering stage, but the development of 10-d-old C. suppressalis larvae was retarded on the vegetative stage cry1Ab plants. Immunological analysis also showed an apparent decline in Cry1Ab titer in leaf blades and leaf sheaths at the reproductive stage. In experiments comparing three fertilizer treatments (NPK, PK, and none), there was a significant interaction between fertilizer treatment and variety on larval survival only in whole-plant assays at booting stage with C. suppressalis. On cry1Ab plants, larval survival did not differ significantly among the three fertilizer levels, whereas on control plants survival was highest with the NPK treatment. cry1Ab plants tested at the sixth and seventh generations after transformation were more resistant than control plants to N. aenescens and C, suppressalis, respectively, suggesting that gene silencing will not occur in line 827. The results of the experiments are discussed in terms of resistance management for B. thuringiensis toxins in rice.

Andow, D. A., D. M. Olson, et al. (2000). "Frequency of resistance to Bacillus thuringiensis toxin Cry1Ab in an Iowa population of European corn borer (Lepidoptera : Crambidae)." Journal of Economic Entomology **93**(1): 26-30.

> The refuge plus high-dose strategy for resistance management assumes that the frequency of resistance alleles is low. We used an F-2 screen to estimate the frequency of resistance to transgenic corn that produces Bacillus thuringiensis Berliner Cry1Ab toxin (Bt corn) in an Iowa population of European corn borer, Ostrinia nubilalis (Hubner). We also proposed a modification to the statistical analysis of the F-2 screen that extends its application for nonuniform prior distributions and for repeated sampling of a single population. Based on a sample of 188 isofemale lines derived from females caught at light traps during the 2nd flight of 1997, we show with 95% confidence that the frequency of resistance to Bt corn was <3.9 x 10(-3) in this Iowa population. These results provide weak evidence that

the refuge plus high-dose strategy may be effective for managing resistance in O. nubilalis to Bt corn. Partial resistance to Cry1Ab toxin was found commonly. The 95% CI for the frequency of partial resistance were [8.2 x 10(-4), 9.4 x 10(-3)] for the Iowa population. Variable costs of the method were $14.90 per isofemale line. which was a reduction of 25% compared with our initial estimate.

Bourguet, D., M. T. Bethenod, et al. (2000). "Gene flow in the European corn borer Ostrinia nubilalis: implications for the sustainability of transgenic insecticidal maize." Proceedings of the Royal Society of London Series B-Biological Sciences **267**(1439): 117-122.

Strategies proposed for delaying resistance to Bacillus thuringiensis toxins expressed by transgenic maize require intense gene flow between individuals that grew on transgenic and on normal (referred to as refuges) plants. To investigate gene flow in the European corn borer, Ostrinia nubilalis (Hubner), the genetic variability at 29 sampled sites from France was studied by comparing allozyme frequencies at six polymorphic loci. Almost no deviations from Hardy-Weinberg expectations occurred, and a high stability of allelic distribution was found among samples collected in the same site over two or three different generations, indicating a high stability of the genetic structure over time. The overall genetic differentiation was low at the region and whole country level, suggesting a high and homogeneous gene flow. These results are discussed in relation to the sustainability of transgenic insecticidal maize.

Bourguet, D., M. T. Bethenod, et al. (2000). "Host-plant diversity of the European corn borer Ostrinia nubilalis: what value for sustainable transgenic insecticidal Bt maize?" Proceedings of the Royal Society of London Series B-Biological Sciences **267**(1449): 1177-1184.

The strategies proposed for delaying the development of resistance to the Bacillus thuringiensis toxins produced by transgenic maize require high levels of gene flow between individuals feeding on transgenic and refuge plants. The European corn borer Ostrinia nubilalis (Hubner) may be found on several host plants, which may act as natural refuges. The genetic variability of samples collected on sagebrush (Artemisia sp.), hop (Humulus lupulus L.) and maize (Zea mays L.) was studied by comparing the allozyme frequencies for six polymorphic loci. We found a high level of gene flow within and between samples collected on the same host plant. The level of gene flow between the sagebrush and hop insect samples appeared to be sufficiently high for these populations to be considered a single genetic panmictic unit. Conversely the samples collected on maize were genetically different from those collected on sagebrush and hop. Three of the six loci considered displayed greater between-host-plant than within- host-plant differentiation in comparisons of the group of samples collected on sagebrush or hop with the group of samples collected on maize. This indicates that either there is genetic isolation of the insects feeding on maize or that there is host-plant divergent selection at these three loci or at linked loci. These results have important implications for the potential sustainability of transgenic insecticidal maize.

Bourguet, D., A. Genissel, et al. (2000). "Insecticide resistance and dominance levels." Journal of Economic Entomology **93**(6): 1588-1595.

Dominance has been assessed in different ways in insecticide resistance studies, based on three phenotypic traits: the insecticide concentration required to give a particular mortality (D-LC) mortality at a particular insecticide dose (D- ML), and fitness in treated areas (D-WT). We propose a general formula for estimating dominance on a scale of 0 to 1 (0 = complete recessivity and 1 = complete dominance). D-LC, D-ML, and D-WT are not directly related and their values depend on genetic background and environmental conditions. We also show that pest management strategies can have die consequence to increase D-WT via the selection of dominance modifiers. Studies on resistance to Bacillus thuringiensis toxins provide the ultimate example of the complexity of the definition of the concept of dominance. Almost all studies have focused on calculation of D-LC, which provides little information about the efficiency of pest management programs. For instance, one assumption of the high dose/refuge strategy is that Bacillus thuringiensis resistance must be effectively recessive (i.e., D-ML must be close to zero). However, D-WT, rather than D-ML, is relevant to the resistance management strategy. Therefore, we strongly suggest that the time has come to focus on fitness dominance levels in the presence and absence of insecticide.

Caprio, M. A. and D. M. Suckling (2000). "Simulating the impact of cross resistance between Bt toxins in transformed clover and apples in New Zealand." Journal of Economic Entomology **93**(2): 173-179.

Simulations were conducted to guide development of resistance management strategies aimed at prolonging the usable life of B. thuringiensis (Bt) endotoxins in multiple cropping situations, where different crops expressing Bt endotoxins are host plants for a common pest. We used the New Zealand apple and clover model ecosystem to explore the relative impact on the rate of resistance development of varying levels of cross-resistance between different toxins expressed in these 2 potentially Bt- transformed crops. These 2 crops are hosts for a complex of leafrollers in New Zealand, including the lightbrown apple moth, used here as the model pest. Cross-resistance was varied between 0.0 and 0.5 (zero to partial cross-resistance) to allow for the case in which selection by one plant has a potential effect on resistance to the toxin in another plant. The largest factor affecting the evolution of resistance was the total habitat area occupied by transgenic orchards. The proportion of the clover habitat that was transformed was also an important. factor, even in the absence of cross-resistance. The effect of increasing the proportion of the second transformed crop (clover) acted on resistance

evolution mainly by reducing the external refuge of susceptibility for the transgenic orchards. Hence, the ecological implications of reducing the available source of susceptible insects from clover, which can help to dow resistance development in the orchard ecosystem, had a more significant impact than the presence of cross-resistance. Partial cross-resistance between different toxins in the separate crops was overall of relatively minor importance. These simulations have implications for deployment decisions for individual transformed crops in multiple cropping systems, where there is the potential for the crops to serve as refuges for each other. These decisions may need to focus less on cross-resistance between toxins, than on economic trade-offs between the relative roles of individual crops as refugia maintaining susceptibility in the system as a whole.

Davis, P. M. and D. W. Onstad (2000). "Seed mixtures as a resistance management strategy for European corn borers (Lepidoptera : Crambidae) infesting transgenic corn expressing Cry1Ab protein." Journal of Economic Entomology **93**(3): 937-948.

Dispersal of neonate European corn borers, Ostrinia nubilalis (Hubner), in seed mixtures of transgenic corn expressing Cry1Ab protein (Bt+) and nontransgenic corn (Bt-) was evaluated in a 2-yr field study. The main objective was to determine if larval dispersal limits the effectiveness of seed mixtures as a resistance management strategy. Mixtures evaluated included (1) all Bt+ plants, (2) every fifth plant Bt- with remaining plants Bt+, (3) every fifth plant Bt+ with remaining plants Bt-, and (4) all Bt- plants. The transformation events MON 802 (B73 BC1F2 X Mo17) and MON 810 (B73 BC1F1 X Mo17), which express the Cry1Ab endotoxin isolated from Bacillus thuringiensis subsp. kurstaki, were used as the sources of Bt+ seed in 1994 and 1995 respectively (YieldGard, Monsanto, St. Louis, MO). At corn growth stage V6-V8, subplots within each mixture (15-20 plants each) were infested so that every fifth plant in mixtures 1 and 4, every Bt- plant in mixture 2, and every Bt+ plant in mixture 3 received two egg masses. Larval sampling over a 21-d period indicated increased neonate dispersal off of Bt+ plants, reduced survival of larvae that dispersed from Bt+ plants to Bt- plants, and a low incidence of late-instar movement from Bt- plants to Bt+ plants. Computer simulations based on mortality and dispersal estimates from this study indicate that seed mixtures will delay the evolution of resistant European corn borer populations compared with uniform planting of transgenic corn. However, resistant European corn borer populations likely will develop faster in seed mixes compared with separate plantings of Bt and non-Bt corn.

Dirie, A. M., M. B. Cohen, et al. (2000). "Larval dispersal and survival of Scirpophaga incertulas (Lepidoptera : Pyralidae) and Chilo suppressalis (Lepidoptera : Crambidae) on cry1Ab-transformed and non-transgenic rice." Environmental Entomology **29**(5): 972-978.

Sowing seed mixtures of transgenic and nontransgenic plants is one approach to establishing refuges for resistance management of crops transformed with Bacillus thuringiensis (Bt) toxins. We studied larval dispersal and survival of two rice stern borers, Scirpophaga incertulas (Walker) and Chilo suppressalis (Walker), to evaluate the potential effectiveness of seed mixtures for resistance management of Bt rice, Oryza sativa L. Experiments were conducted with two cry1Ab-transformed rice varieties, 'IR58' and 'Tarom Molaii', and corresponding nontransgenic controls. During 0-24 and 24-48 h after egg hatch, the proportion of C. suppressalis larvae dispersing from the natal plant did not differ between transgenic and control plants for either rice variety Dispersal by S. incertulas did not differ between transgenic and control plants of IR58 during either 0-24 or 24-48 h, but a greater proportion of S. incertulas larvae dispersed from transgenic Tarom Molaii than from control plants 24-48 h after eclosion. Larvae that dispersed 0-24 h after eclosion on IR58 and 0-24 and 24-48 h after eclosion on Tarom Molaii were collected and transferred to stern pieces of control plants. No differences in survival were detected between larvae that dispersed from transgenic or control plants 24 (IR58) or 72 h (Tarom Molaii) after dispersal. Because both stem borer species move among plants during larval development, seed mixtures may not be the most effective approach to maintaining refuges for resistance management. However, additional experiments to compare the dispersal and fitness of Bt-resistant and -susceptible stem borer larvae are needed.

Hommel, B. and B. Pallutt (2000). "Evaluation of herbicide resistance from a point of view of integrated plant protection within a system of a 4-field crop rotation including glufosinate-resistant rape and maize." Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz-Journal of Plant Diseases and Protection: 411-420.

The present study evaluates, from a point of view of integrated plant protection, short-term and longterm crop protection effects found in a long-term trial with transgenic herbicide- resistant winter rape and maize. The trial started in 1996 and includes a 4-field crop rotation. Main points of consideration are the use of injury and control thresholds, control of volunteer rape, and an ecological evaluation of established and new herbicide strategies. Outcrossing of herbicide resistance is part of accompanying agroecological research. Use of LIBERTY (Glufosiant-ammonium) in rape and maize led to similar results as standard herbicides. There were no significant differences in yields. Systems with herbicide-resistant rape and maize have a smaller biological risk potential than conventional systems. Outcrossing rates in rape fields were clearly below 1%. Higher rates were observed only in directly neighbouring fields.

Jin, R. G., Y. B. Liu, et al. (2000). "Development of transgenic cabbage (Brassica oleracea var. Capitata) for insect resistance by Agrobacterium tumefaciens-mediated transformation." In Vitro Cellular & Developmental Biology-Plant **36**(4): 231-237.

> Transgenic head cabbage (Brassica oleracea var. capitata), resistant to diamondback moth (Plutella xylostella) larvae, was developed through Agrobacterium tumefaciens-mediated transformation with Bacillus thuringiensis (Bt) cry genes using a modified procedure. Factors important for transformation included cabbage cultivar; preculture: and coculture of explants on a callus initiation medium; use of appropriate amount; and delay in initial application of selective agents. A total of 15 independent transformed lines with over 100 plants were obtained from several transformation experiments, representing an overall transformation efficiency of similar to 1%. Cabbage plants transformed with a synthetic Bt gene, cry1Ab3, were all resistant to larvae of the diamondback moth, whereas all plants transgenic for cry11a3, a wild-type Bt gene, were susceptible. As a first step towards testing the hypothesis that reduced exposure of Bt to target insects would delay the evolution of insect resistance to Bt, cry1Ab3 expression was put under the transcriptional control of the soybean wound-inducible vspB promoter and transgenic cabbage was obtained. Insect bioassay showed that such plants were all resistant to diamondback moth even without induction for the expression of Bt.

Layton, M. B. (2000). "Biology and damage of the tarnished plant bug, Lygus lineolaris, in cotton." Southwestern Entomologist: 7-20.

> Tarnished plant bug (TPB), Lygus lineolaris (Palisot de Beauvois), is a key pest of cotton in many states. Although this pest can damage cotton throughout most of the growing season, economic damage is most likely to occur during the period from first square through early bloom due to feeding on small squares and subsequent abscission of these squares. During this period, excessive damage by high TPB populations may result in reduced yields or delayed maturity. However, current research suggests that cotton can tolerate low levels of TPB damage without sustaining yield loss. Most states recommend monitoring both numbers of insects and percent square retention in order to obtain information on which to base TPB management decisions. The relative importance of TPB as a key pest of cotton is increasing due to a number of changes in cotton insect management systems. These include: development of insecticide resistance in TPB, boil weevil eradication, transgenic Bt cotton, and the development and availability of more target-specific foliar-applied insecticides.

Milks, M. L. and D. A. Theilmann (2000). "Serial selection for resistance to a wild-type and to a genetically modified nucleopolyhedrovirus in Trichoplusia ni." Biological Control **19**(3): 283-289.

> In previous work, cabbage loopers (Trichoplusia ni) evolved 22- fold resistance to the single nucleocapsid nucleopolyhedrovirus of T. ni

(TnSNPV) after 26 generations of selection with the virus. The goal of the present study was to determine if T. ni could evolve resistance to the recombinant Autographa californica multiple nucleocapsid nucleopolyhedrovirus (AcMNPV- AaIT) that; expresses an insect specific neurotoxin and to determine if it was influenced by prior development of resistance to TnSNPV. To answer these questions, the T. ni line that had been exposed to TnSNPV was divided into two sublines at generation 27. One of them was serially selected for resistance to AcRMNPV-AaIT (subline TnSNPV/AcMNPV-AaIT), while the other one was mock infected with distilled water (subline TnSNPV/H2O). The same was done with the line that was used as a control from generations 1 to 26 (subline H2O/AcMNPV-AaIT and subline H2O/H2O). After 17 generations of selection with AcMNPV-AaIT, T. ni that had not been previously exposed to TnSNPV evolved only twofold resistance to AcMNPV-AaIT, However, those that had been selected with TnSNPV evolved fourfold resistance to AcMNPV-AaIT. Exposure to AcMNPV-AaIT conferred cross-resistance to TnSNPV in only one subline, subline H2O/AcMNPV-AaIT. Resistance to AcMNPV-AaIT did not affect the developmental time, pupal weight, egg production, or percentage of egg hatch of T. ni. (C) 2000 Academic Press.

Shelton, A. M., J. D. Tang, et al. (2000). "Field tests on managing resistance to Bt-engineered plants." Nature Biotechnology **18**(3): 339-342.

Several important crops have been engineered to express toxins of Bacillus thuringiensis (Bt) for insect control. In 1999, US farmers planted nearly 8 million hectares (nearly 20 million acres) of transgenic St crops approved by the EPA. Bf- transgenic plants can greatly reduce the use of broader spectrum insecticides, but insect resistance may hinder this technology. Present resistance management strategies rely on a "refuge" composed of non-Bt plants to conserve susceptible alleles. We have used Bf-transgenic broccoli plants and the diamondback moth as a model system to examine resistance management strategies. The higher number of larvae on refuge plants in our field tests indicate that a "separate refuge" will be more effective at conserving susceptible larvae than a "mixed refuge" and would thereby reduce the number of homozygous resistant (RR) offspring. Our field tests also examined the strategy of spraying the refuge to prevent economic loss to the crop while maintaining susceptible alleles in the population. Results indicate that great care must be taken to ensure that refuges, particularly those sprayed with efficacious insecticides, produce adequate numbers of susceptible alleles. Each insect/Bt crop system may have unique management requirements because of the biology of the insect, but our studies validate the need for a refuge. As we learn more about how to refine our present resistance management strategies, it is important to also develop the next generation of technology and implementation strategies.

Stewart, S. D. and M. B. Layton (2000). "Cultural controls for the management of

Lygus populations in cotton." Southwestern Entomologist: 83-95.

> We have attempted to review the potential and limitations of cultural approaches for control of Lygus populations in cotton, including management of alternate hosts and use of host plant resistance. Other than insecticides, cultural approaches seem the most likely of the currently available insect-management tactics to help in the management of Lygus populations. The use of transgenic or nectariless cotton cultivars that are resistant to Lygus could be easily implemented if they were available. Other kinds of cultural controls, such as management of alternate hosts, may not be adopted on a large scale unless they are part of an area-wide management program or until additional evidence of cost efficiency is demonstrated Adoption of cultural approaches will be largely influenced by the availability and efficacy of alternative management practices, including insecticides, and other socioeconomic factors. Cultural approaches are best suited in "soft" systems, with low use of disruptive insecticides, where natural enemies are relied upon most heavily to maintain cotton pests below economically damaging levels.

Tabashnik, B. E., A. L. Patin, et al. (2000). "Frequency of resistance to Bacillus thuringiensis in field populations of pink bollworm." Proceedings of the National Academy of Sciences of the United States of America **97**(24): 12980-12984.

> Strategies for delaying pest resistance to genetically modified crops that produce Bacillus thuringiensis (Bt) toxins are based primarily on theoretical models. One key assumption of such models is that genes conferring resistance are rare. Previous estimates for lepidopteran pests targeted by Pt crops seem to meet this assumption. We report here that the estimated frequency of a recessive allele conferring resistance to Pt toxin Cry1Ac was 0.16 (95% confidence interval = 0.05-0.26) in strains of pink bollworm (Pectinophora gossypiella) derived from 10 Arizona cotton fields during 1997. Unexpectedly, the estimated resistance allele frequency did not increase from 1997 to 1999 and Pt cotton remained extremely effective against pink bollworm. These results demonstrate that the assumptions and predictions of resistance management models must be reexamined.

Venette, R. C., W. D. Hutchison, et al. (2000). "An in-field screen for early detection and monitoring of insect resistance to Bacillus thuringiensis in transgenic crops." Journal of Economic Entomology **93**(4): 1055-1064.

> We present a field-based approach to detect and monitor insects with resistance to insecticidal toxins produced ty transgenic plants. Out objective is to estimate the phenotypic frequency of resistance in a population by relating the densities of insects on genetically transformed plants to densities on nontransformed plants. We focus on European corn borer, Ostrinia nubilalis (Hubner), in sweet corn, Zea mays L., expressing Cry1Ab from Bacillus thuringiensis subsp. kurstaki Berliner to illustrate principles underlying the method. Thr probability of detecting one or more

rare, resistant larvae depends on sample size, the density of larvae on nontransformed plants. and an assumed frequency of resistant phenotypes in a given population. Probability of detection increases with increases in sample size, background density, or the frequency of resistant individuals. Following binomial probability theory, if a frequency of 10(-4) is expected. 10(3)-10(4) samples must Le collected from a B. thuringiensis (Bt) crop to have at least a 95% probability of locating one or more resistant larvae. In-field screens using transgenic crops have several advantages over traditional laboratory-based methods, including exposure to a large number of feral insects, discrimination of resistant individuals based on Bt dosages expressed in the field, incorporation of natural and Bt-induced mortality factors, simultaneous monitoring for more than one insect species, and ease of use. The approach is amenable to field survey crews working in research, extension, and within the seed corn industry. Estimates of the phenotypic frequency of resistance front the in-field screen can Le useful for estimating Initial frequency of resistant alleles. Bayesian statistical methods are outlined to estimate phenotype frequencies, allele frequencies, and associated confidence intervals from field data. Results of the approach are discussed relative to existing complementary methods currently available for O. nubilalis and corn earworm, Helicoverpa zea (Boddie).

Walker, K. A., R. L. Hellmich, et al. (2000). "Late-instar European corn borer (Lepidoptera : Crambidae) tunneling and survival in transgenic corn hybrids." Journal of Economic Entomology **93**(4): 1276-1285.

Field studies were conducted in 1996 and 1997 to determine injury by and survival of late-instar European corn borer, Ostrinia nubilalis (Hubner), on genetically altered Bacillus thuringiensis Berliner corn, Zea,mays L. Cry1Ab events 176, Bt11, MON810, and MON802; Cry1Ac event DBT418; and Cry9C event CBH351 were evaluated. Plants of each corn hybrid were manually infested with two third-, fourth-, or fifth-instar O. nubilalis. Larvae were held in proximity to the internode of the plant above the ear with a mesh sleeve. Lan ae were put on tie plants during corn developmental stages V8, V16, R1, R3, R4, R5, and R6. This study shows that not all B. thuringiensis hybrids provide the same protection against O. nubilalis injury. Hybrids with B. thuringiensis events Bt11, MON810, MON802, and CHB351 effectively protected the corn against tunneling by late-instar O, nubilalis. Event 176 was effective in controlling late-instar O. nubilalis during V12 and V16 corn developmental stages; however, significant tunneling occurred by fourth instars during R3 and R5. Event DBT418 was not effective in controlling late-instar O. nubilalis during corn vegetative or reproductive stages of development. Whether the B, thuringiensis hybrids satisfied high- and ultrahigh-dose requirements is discussed.

Way, M. J. and H. F. van Emden (2000). "Integrated pest management in practice - pathways towards successful application." Crop Protection **19**(2): 81-103.

Examples from perennial and annual crops in temperate and tropical conditions are used to illustrate the research and development approaches that have contributed to use and integration of host plant resistance and biological, cultural and chemical controls. The evidence shows how successes in IPM have depended upon classical experimental approaches continually responding to changing constraints and to novel discoveries, which are being applied increasingly efficiently and intelligently to farm practice. Future developments are discussed in the context of past experience and new technologies. Recent developments of important new approaches that could help revolutionize management of some pest complexes, in particular genetic engineering, semiochemicals and bioinsecticides, are discussed. Much attention has been devoted to strategic modelling in the IPM context which aims to provide novel insights, but there is little evidence of its value to practical IPM; instead it could be used unwisely to encourage accumulation of unnecessary information. In contrast, tactical models are proving increasingly valuable in forecasting the need for and timing of applied controls. Whilst there have been some outstanding developments in practical application of IPM in many developed countries where the ultimate goal is to decrease over-reliance on conventional insecticides, evidence shows that in many developing countries, where the goal is an ecologically sound mix of non-chemical and chemical methods, there remains a crucial need for much more appropriate research and implementation, especially in small farm conditions. (C) 2000 Elsevier Science Ltd. All rights reserved.

Zhang, T. H. and C. M. Tang (2000). "Commercial production of transgenic Bt insect-resistant cotton varieties and the resistance management for bollworm (Helicoverpa arinigera Hubner)." Chinese Science Bulletin **45**(14): 1249-1257.

There are currently three kinds of transgenic St insect- resistant cotton germplasm lines, Shanxi 94-24, Zhongxin 94 and R19, in China. They showed high resistance to the neonate larvae of bollworm (Helicoverpa armigera). Transgenic St insect-resistant cotton varieties or hybrids have been bred using the three kinds of germplasm lines as parents. Our researches reveal that there exist different expressions in resistant level at different developmental stages in the three categories of germplasm lines. When neonate larvae are fed with leaves of cotton plant at the seeding stage with less than 10 leaves on the main stem, the mortality of the neonate larvae is 100%, but the resistance level will decline at later season. When St gene has been transferred to the cotton genome, it can be steadily transferred to the progeny, the level of resistance to bollworm keeps fundamentally uniform. Such insects as tobacco budworm (Heliothis virencens) in laboratory directive selection are very apt to produce resistance to the Bt insecticidal crystal protein. From the present crop system of cotton region in the Yangtze and Yellow River Valleys, and the expression characteristic of transgenic Bt resistant cotton, we suggest that the resistance to toxin protein in bollworm is not apt to be produced if the transgenic St insect-resistant cotton varieties are released and grown in the regions except in the Xinjiang cotton region. The managing strategies to delay or retard the resistance are discussed.

All, J., H. R. Boerma, et al. (1999). "Interactions in entomology: Utilization and management of new genetic techniques for insect control in southern field crops." Journal of Entomological Science **34**(1): 2-7.

Alyokhin, A. V. and D. N. Ferro (1999). "Modifications in dispersal and oviposition of Bt-resistant and Bt-susceptible Colorado potato beetles as a result of exposure to Bacillus thuringiensis subsp tenebrionis Cry3A toxin." Entomologia Experimentalis Et Applicata **90**(1): 93-101.

Laboratory strains of Colorado potato beetle, Leptinotarsa decemlineata (Say), physiologically resistant and susceptible to Bacillus thuringiensis (Berliner) subsp. tenebrionis Cry3A toxin were reared to adults on caged potato plants. Influence of three different diets (transgenic potatoes, regular potatoes, and regular potatoes followed by the transgenic potatoes) on beetle mortality, fecundity, and flight behavior were: tested under laboratory conditions. A computer-linked flight mill system was used to quantify beetle flight, and dissections were performed to determine the level of flight muscle development. Susceptible beetles continuously fed on transgenic foliage suffered heavy mortality, did not develop flight muscles, and did not produce any eggs. Resistant beetles continuously fed on transgenic foliage were capable of flight and reproduction; however, it took them longer to initiate flight behavior, and their fecundity was lower than fecundity of other treatments. In both strains, detrimental effects became significantly less severe when the beetles were allowed to feed on regular foliage prior to toxin ingestion. In the resistant strain, ingestion of Cry3A toxin significantly increased flight activity, indicating that physiological resistance was probably reinforced by the behavioral escape from toxic environments. No such response was observed for susceptible beetles. When fed on regular foliage, resistant Colorado potato beetles engaged in significantly fewer flights than susceptible beetles. Behavioral differences between resistant and susceptible beetles observed in the present study are likely to affect gene flow between transgenic crops and adjacent refugia, and should be taken in consideration when designing resistance management plans for transgenic potato crops.

Alyokhin, A. V. and D. N. Ferro (1999). "Reproduction and dispersal of summer-generation Colorado potato beetle (Coleoptera : Chrysomelidae)." Environmental Entomology **28**(3): 425-430.

Colorado potato beetle dispersal and reproduction was investigated under field and laboratory conditions. Movement and mating of newly emerged summer-generation Colorado potato beetle adults was monitored in the field using a mark-recapture technique, and beetle mating within experimental plots was recorded. The number of degree-days (DD) required for the beetles to become reproductive was tested in an environmental chamber using 10 degrees C as a developmental threshold. A computer-linked flight mill system was used to quantify. the influence of mating on the flight behavior of male and female beetles. Adult dispersal started within the first 24 h after eclosion from the pupae, but a significant proportion of newly emerged beetles stayed close to the place of their larval development until reaching reproductive maturity. The beetles required at least 34 DD before mating produced viable offspring, and females did not start laying eggs until a minimum of 51 DD after eclosion. Mating had a pronounced effect on beetle night, decreasing flight activity of the females and increasing night activity of the males. Enhancing gene now between beetles surviving on transgenic plants and susceptible beetles in refugia should be advantageous in managing beetle resistance to transgenic plants.

Ballester, V., F. Granero, et al. (1999). "Integrative model for binding of Bacillus thuringiensis toxins in susceptible and resistant larvae of the diamondback moth (Plutella xylostella)." Applied and Environmental Microbiology **65**(4): 1413-1419.

Insecticidal crystal proteins from Bacillus thuringiensis in sprays and transgenic crops are extremely useful for environmentally sound pest management, but their long-term efficacy is threatened by evolution of resistance by target pests. The diamondback moth (Plutella xylostella) is the first insect to evolve resistance to B. thuringiensis in open-field populations. The only known mechanism of resistance to B, thuringiensis in the diamondback moth is reduced binding of toxin to midgut binding sites. In the present work we analyzed competitive binding of B. thuringiensis toxins Cry1Aa, Cry1Ab, Cry1Ac, and Cry1F to brush border membrane vesicles from larval midguts in a susceptible strain and in resistant strains from the Philippines, Hawaii, and Pennsylvania. Based on the results, we propose a model for binding of B. thuringiensis crystal proteins in susceptible larvae with two binding sites for Cry1Aa, one of which is shared with Cry1Ab, Cry1Ac, and Cry1F. Our results show that the common binding site is altered in each of the three resistant strains. In the strain from the Philippines, the alteration reduced binding of Cry1Ab but did not affect binding of the other crystal proteins. In the resistant strains from Hawaii and Pennsylvania, the alteration affected binding of Cry1Aa, Cry1Ab, Cry1Ac, and Cry1F, Previously reported evidence that a single mutation can confer resistance to Cry1Ab, Cry1Ac, and Cry1F corresponds to expectations based on the binding model. However, the following two other observations do not: the mutation in the Philippines strain affected binding of only Cry1Ab, and one mutation was sufficient for resistance to Cry1Aa, The imperfect correspondence between the model and observations suggests that reduced binding is not the only mechanism of resistance in the diamondback moth and that some, but not all, patterns of resistance and cross-resistance can be predicted correctly from the results of competitive binding analyses of susceptible strains.

Bolin, P. C., W. D. Hutchison, et al. (1999). "Long-term selection for resistance to bacillus thuringiensis Cry1Ac endotoxin in a Minnesota population of European corn borer (Lepidoptera : Crambidae)." Journal of Economic Entomology **92**(5): 1021-1030.

> Transgenic corn, expressing the insecticidal delta-endotoxin of Bacillus thuringiensis Berliner, provides high levels of control of some lepidopteran pests, particularly the European corn borer, Ostrinia nubilalis (Hubner). However, resistance to B. thuringiensis has been documented recently in laboratory colonies of agronomically important Lepidoptera, including O. nubilalis. For the past 4 yr, we have selected for Cry1Ac resistance in a population of O. nubilalis from southeastern Minnesota. Increasing resistance to B. thuringiensis was noted after only 8 generations of selection, with a peak at 162-fold resistance, based on comparisons of LC50S to a nonselected parental strain. This resistance was found to decrease at the same rate in the absence of B. thuringiensis selection, with one selected colony becoming nearly as sensitive to the Cry1Ac toxin as the nonselected colony after 9 generations without exposure to B. thuringiensis. The most resistant of the colonies, S-I, was only marginally cross-resistant to Cry1Ab, yet another selected colony, S-IV, did demonstrate a 16-fold cross-resistance, In addition, larvae from the S-IV colony had significantly greater weight gain when feeding on diet incorporated with B. thuringiensis-transgenic corn than did larvae from the nonselected parental colony. These findings emphasize the need for careful deployment of B. thuringiensis corn to preserve this effective pest management technology.

Cao, J., J. D. Tang, et al. (1999). "Transgenic broccoli with high levels of Bacillus thuringiensis Cry1C protein control diamondback moth larvae resistant to Cry1A or Cry1C." Molecular Breeding **5**(2): 131-141.

> A synthetic Bacillus thuringiensis (Bt) cry1C gene was introduced into broccoli (Brassica oleracea ssp. italica) by Agrobacterium-mediated transformation. Twenty-one Cry1C transgenic plants were regenerated from 400 hypocotyl and petiole explants. Variable amounts of stable steady-state cry1C mRNA accumulated in different transgenic plants. Cry1C protein (up to 0.4% of total soluble protein) was produced in correlation with the cry1C mRNA levels. Leaf section and whole- plant bioassays were done using diamondback moth (DBM) larvae from lines susceptible to Bt or resistant to Cry1A or Cry1C proteins (Cry1A(R) or Cry1C(R), respectively). Plants with high levels of Cry1C protein caused rapid and complete mortality of all three types of DBM larvae with no defoliation. Plants with lower levels of Cry1C protein showed an increasing differential between control of susceptible of Cry1A(R) DBM. This study

demonstrated that high production of Cry1C protein can protect transgenic broccoli not only from susceptible or Cry1A(R) DBM larvae but also from DBM selected for moderate levels of resistance of Cry1C. The Cry1C-transgenic broccoli were also resistant to two other lepidopteran pests of crucifers (cabbage looper and imported cabbage worm). These plants will be useful in studies of resistance management strategies involving multiple transgenes.

Frutos, R., C. Rang, et al. (1999). "Managing insect resistance to plants producing Bacillus thuringiensis toxins." <u>Critical Reviews in Biotechnology</u> **19**(3): 227-276.

Insect-resistant transgenic plants have become an important tool for the protection of crops against insect pests. The acreage of insecticidal transgenic plants is expected to increase significantly in the near future. The bacterium Bacillus thuringiensis is currently the source of insecticidal proteins in commercial insect-resistant transgenic plants and will remain the most important source during the next decade. Insect resistance to B. thuringiensis Cry toxins is the main problem. Only one species; the diamondback moth, has evolved a resistance to B. thuringiensis-based formulations under field conditions. However, many other insect species were selected for resistance under laboratory conditions, indicating that there is a potential for evolution of resistance in most major pests. Many studies were conducted to elucidate the mode of action of the Cry toxins, the mechanisms and genetics of resistance, and the various factors influencing its development. This article reviews insect resistance to B. thuringiensis insecticidal proteins and related aspects, including the development of insect-resistant transgenic plants, B. thuringiensis toxins, their mode of action, mechanisms, stability, and genetics of resistance and management strategies for delaying resistance.

Hellmich, R. L., L. S. Higgins, et al. (1999). "Oviposition by European corn borer (Lepidoptera : Crambidae) in response to various transgenic corn events." <u>Journal of Economic Entomology</u> **92**(5): 1014-1020.

Oviposition preference by European corn borers, Ostrinia nubilalis (Hubner), for or against transgenic corn would influence amounts of refuge required for resistance management. The objective of this research was to determine if various Bacillus thuringiensis (Bt) corn transgenic events influence a. nubilalis oviposition. All commercially available events (currently 5) were evaluated, plus I experimental event. Results from 3 independent studies are reported, including 3 field-cage experiments with vegetative corn, 2 field-cage experiments with reproductive corn, and 2 field experiments with natural O. nubilalis on reproductive corn. In each case, Bt corn hybrids are compared with their near isogenic hybrids by counting numbers of egg masses on each plant type. More extensive comparisons were made in 3 of the experiments by determining the number, size, and location of egg masses on the corn hybrids. Moths laid more egg masses on Bt corn than on non-Bt corn in 1 cage experiment. These results, however, were not found in any of the ether experiments. There is evidence that suggests cage effects influence moth oviposition more than Bt protein. Four of the 5 cage experiments and 2 field experiments indicate that the tested Bt events do not influence a. nubilalis oviposition. Larval injury to isogenic corn during the vegetative stage did not influence adult oviposition during the corn reproductive stage when compared with Bt corn and noninjured isogenic corn. Based on these experiments, suggestions are made for future studies that use natural a. nubilalis rather than a. nubilalis in cages.

Hilder, V. A. and D. Boulter (1999). "Genetic engineering of crop plants for insect resistance - a critical review." Crop Protection **18**(3): 177-191.

Genetically engineering inherent crop resistance to insect pests offers the potential of a user-friendly, environment- friendly and consumer-friendly method of crop protection to meet the demands of sustainable agriculture in the 21st century. Work to date has concentrated on the introduction of genes for expression of modified Bacillus thuringiensis (Bt) toxins. Impressive results on the control of Bt-susceptible pests have been obtained in the laboratory and the field, and the first commercial Bt transgenic crops are now in use. A main alternative approach exploits plant-derived insect control genes. Enhanced resistance to a wide spectrum of pests has been demonstrated in laboratory trials of transgenics expressing various protease inhibitors, lectins, etc, and some promising field trials have been carried out, but the scale of effects produced by plant-derived insect control genes has not been deemed convincing enough to lead to serious attempts at commercialization. Both classes of compounds have limitations: there have been serious failures in resistance to targeted pests in Bt cotton; most plant-derived resistance factors produce chronic rather than acute effects; and many serious pests are simply not susceptible to known resistance factors. We have analysed the characteristics which would be desirable in an ideal transgenic technology: these include being environmentally benign, relatively inexpensive to develop, with a potentially wide spectrum of activity (although targetable at pests and not beneficials), generated by a flexible technology that allows any insect site to be targeted and readily adaptable so that alternatives can be produced as required. We are developing such a technology based on the expression of single-chain antibody genes in crop plants which would be compatible with the likely trends in pesticide discovery using biology-driven target-based methods. The importance of a changed, more socially responsible attitude in this sector is emphasised as is the need for much improved presentation of the benefits and need for responsible deployment of genetically engineered crops. (C) 1999 Elsevier Science Ltd. All rights reserved.

Hoy, C. W. (1999). "Colorado potato beetle resistance management strategies for transgenic potatoes." American Journal of Potato Research **76**(4): 215-219.

> NewLeaf potatoes could provide substantial ecological and economic benefits to potato growers. A concern with NewLeaf potatoes, however, is that Colorado potato beetle may develop resistance to the Bt endotoxin because of the intense selection pressure imposed by its constant presence in the transgenic crop. Before these cultivars were released, however, entomologists throughout North America conducted biological research needed for a strategy to prevent or delay resistance in Colorado potato beetle. This paper describes the considerations and research behind the proposed strategy. Because NewLeaf potatoes express a relatively constant and high concentration of Bt endotoxin throughout the foliage of each plant, reducing selection for resistance must come from planting standard potatoes as a refuge for susceptible beetles. Avoiding resistance through random mixtures of NewLeaf and standard potatoes was evaluated in laboratory and field studies. The conclusion from this research was that the seed mixture would not provide an effective refuge. The most effective alternative refuge was judged to be a block of standard potatoes planted in the same field as the NewLeaf potatoes. By allowing susceptible beetles to survive in their fields, potato growers might sustain the benefits of very effective controls.

Huang, F. N., L. L. Buschman, et al. (1999). "Susceptibility of different instars of European corn borer (Lepidoptera : Crambidae) to diet containing Bacillus thuringiensis." Journal of Economic Entomology **92**(3): 547-550.

> The relative susceptibility of different instars of the European corn borer, Ostrinia nubilalis, (Hubner), to a commercial formulation of Bacillus thuringiensis Berliner subsp. kurstaki, Dipel ES, was determined using diet incorporation. European corn borers exposed to Dipel during the 1st instar were much more susceptible than were older larvae. No significant differences in susceptibility were found among 3rd, 4th, and 5th instars at doses of 0.03-0.81 mi of Dipel per kilogram of diet. At high concentrations (2.43 ml/km of diet), 5th instars had significantly lower mortality than did 3rd or with instars. The LC50 and LC90 of the 5th instars were 98.1- and >168.2-fold higher, respectively, than those of Ist instars. These findings should be accounted for during the development of a resistance management strategy for the use of B. thuringiensis toxin as a microbial insecticide and possibly, for Bt-expressing transgenic corn. Differential susceptibility of different instars of European corn borer to B. thuringiensis suggests that the "ultra high-dose" or "high-dose" resistance management strategy should be defined in terms of thr largest and the least susceptible stage, because larvae may have opportunities to grow and develop on non-Bt-corn or alternate hosts before they attack the Bt-corn plants.

James, R. R., B. A. Croft, et al. (1999). "Susceptibility of the cottonwood leaf beetle (Coleoptera : Chrysomelidae) to different strains and transgenic toxins of Bacillus thuringiensis." Environmental Entomology **28**(1): 108-115.

Populus spp. (which include cottonwoods, aspens, and poplars) are important sources of wood, wood filer (pulp), and biofuels throughout the world, and are often intensively managed in short rotation stands. The cottonwood leaf beetle, Chrysomela scripta F., is a major pest of Populus throughout North America. It would be difficult to breed insect resistance into these trees using traditional plant breeding techniques because of their long generation time (4-8 yr); however, insect resistance could be produced through genetic engineering. Toxins from Bacillus thuringiensis (Berliner) genes have negligible nontarget effects and are amenable to genetic engineering. We tested the toxicity of 16 B. thuringiensis preparations to identify genes that produce toxins effective against the cottonwood leaf beetle. B. thuringiensis preparations that contained spores were found to be only moderately more virulent than isolated toxins. Strains that produced Cry3A, Cry3B, and Cry8B caused 97-100% mortality in 1st and 2nd instars, with a mean time to death of 1-5 d. Mature larvae were less susceptible to these toxins than were neonates; however, the toxicity of 1 moderately active strain was not affected Ly larval age. Adults were not as sensitive as larvae to any of these toxins. Cropping with trees genetically engineered to produce B. thuringiensis toxins could lead to the evolution of toxin resistance in cottonwood leaf beetles, We found beetles from 3 different U.S. states varied significantly in their susceptibility to Cry3A. This variation means that regional differences in control levels could occur in the field, and that the potential for evolution of B. thuringiensis-resistance may already exist in some populations of this insect. Therefore, it is very important that before genetically engineered poplars are used extensively, management strategies be developed and implemented to prevent the evolution of resistance to B, thuringiensis in the cottonwood leaf beetle.

Lynch, R. E., B. R. Wiseman, et al. (1999). "Evaluation of transgenic sweet corn hybrids expressing CryIA(b) toxin for resistance to corn earworm and fall armyworm (Lepidoptera : Noctuidae)." Journal of Economic Entomology **92**(1): 246-252.

Many of the lepidopterous insects which attack sweet corn, Zen mays L., are susceptible to insecticidal proteins produced by Bacillus thuringiensis ssp. kurstaki (Berliner) (Btk). Transgenic sweet corn expressing a synthetic cry gene for production of a Btk-insecticidal protein may provide a more environmentally acceptable means of sweet corn production. Eight transgenic sweet corn hybrids containing a synthetic gene for CryIA(b) protein production (BT11 event) were evaluated for resistance to the corn earworm, Helicoverpa tea (Boddie), and fall armyworm, Spodoptera frugiperda (J. E. Smith). Laboratory tests revealed that all Btk sweet corn hybrids were highly resistant to leaf and silk feeding by neonate 3 and 6 d old corn earworm larvae. Ear damage in the field to the Btk sweet corn hybrids caused by corn earworm was negligible. All Btk sweet corn hybrids, except Btk 95-0901, were moderately resistant to leaf and silk feeding by the fall armyworm. Survival and weight gain were reduced when neonates were fed excised whorl leaves of the Btk plants. Weight gain, but not survival, was reduced when 3- and 6-d-old fall armyworm larvae were fed excised whorl leaves of the Btk plants. Btk sweet corn hybrids appear to be ideal candidates for use in integrated pest management (IPM) programs for both the fresh and processing sweet corn markets, and their use should drastically reduce the quantity of insecticides currently used to control these pests in sweet corn. With appropriate cultural practices, it is highly unlikely that Btk sweet corn will contribute to the development of resistance to Btk proteins in these insects because of the high toxicity of the Cry proteins expressed in these sweet corn hybrids and the harvest of sweet corn ears from fields before larvae can complete development.

Lynch, R. E., B. R. Wiseman, et al. (1999). "Management of corn earworm and fall armyworm (Lepidoptera : Noctuidae) injury on a sweet corn hybrid expressing a cryIA (b) gene." <u>Journal of Economic Entomology</u> **92**(5): 1217-1222.

Research was conducted to evaluate transgenic sweet corn containing a modified cryIA(b) gene from Bacillus thuringiensis (Bt) and a minimal number of insecticide applications for management of injury to ears by corn earworm, Helicoverpa tea (Boddie), and fall armyworm, Spodoptera frugiperda (J. E. Smith). GH-0937 (Bt+), Bonus (Bt-), and Silver Queen (Bt-) sweet corn hybrids were planted 15 April, 1 May, 15 May, 1 June, and 15 June 1997 to provide different intensities of insect pressure as populations increased during the season. During ear development, methomyl was applied in 0, 1, 3, or 5 applications beginning when 50% of the plants were silking. GH- 0937 exhibited a high level of resistance to leaf feeding in the whorl stage by fall armyworm and to ear injury by the corn earworm and fall armyworm. With extreme insect pressure on plants for the 15 June planting, as evidenced by the extensive damage to Bonus and Silver Queen in the whorl stage, injury to ears of GH-0937 averaged only 7.9 cm(2) of kernel damage when no insecticide was applied, and was reduced to an average of only 1.7 cm(2) of kernel damage with 5 applications of methomyl. In this same test, Silver Queen and Bonus averaged 323 and 168 cm(2) of kernel damage, respectively, with no insecticide applications, and 172 and 50 cm(2), respectively, with 5 applications of methomyl. Injury to ears on GH-0937 was not eliminated, but was minimal and confined to a few kernels at the ear tip. Most of the observed injury was From feeding by nitidulid beetles, or to an occasional large fall armyworm larva that migrated from a susceptible plant to an untreated ear of GH-0937 where it fed before trying. Bt sweet corn offers an excellent opportunity to develop more environmentally compatible approaches for sweet corn production.

Onstad, D. W. and C. A. Guse (1999). "Economic analysis of transgenic maize and nontransgenic refuges for managing European corn borer (Lepidoptera :

Pyralidae)." Journal of Economic Entomology **92**(6): 1256-1265.

> We simulated the population dynamics and population genetics of Europe;In corn borer, Ostrinia nubilalis (Hubner), and damage to maize in a hypothetical region containing transgenic and nontransgenic maize and no other crops. The model assumes that the same level of refuge for resistance management is used every year over 15-20 yr and that no European corn borers immigrate into the region over the same period. When complete mixing across blocks between generations is assumed, the transgenic block significantly lowers damage to maize in the refuges. For most scenarios without toxin-titer decline during maize senescence, a 20% refuge is a robust, economical choice based on current value. At extremes of initial pest density or crop value (price X expected yield), refuge levels as low as 8% or as high as 26% can be superior. Nontransgenic maize can be planted as strips (at least 6 rows per strip) within a field or as separate but adjacent blocks to be effective at delaying resistance and providing economic returns at a 20% refuge level. With toxin-titer decline during senescence, the model results are sensitive to several biological parameters and assumptions with a 10% refuge level offering a robust, economic choice.

Parker, C. D. and R. G. Luttrell (1999). "Interplant movement of Heliothis virescens (Lepidoptera : Noctuidae) larvae in pure and mixed plantings of cotton with and without expression of the Cry1Ac delta-endotoxin protein of Bacillus thuringiensis Berliner." Journal of Economic Entomology **92**(4): 837-845.

> Laboratory and field studies were conducted during 1993 and 199a to quantify interplant movement of Heliothis virescens (F.) larvae in pure and mixed plantings of cotton, Gossypium hirsutum L., with ('Event 531') and without ('Coker 312') the expression of CrylAc delta-endotoxin protein of Bacillus thuringiensis Berliner. Field studies were conducted with neonate, 4-, and 7-d-old larvae placed on 3-plant experimental units and observed at 24, 48, 72, and 96 h after inoculation of larvae. Combining larval movement across observations of neonates, 4-, and 7-d-old larvae, an estimated 52% of the larvae on pure plantings of Coker 312 had moved at least 1 plant by the cumulative time required to reach the age of 10 d. More larvae placed on Event 531 cotton moved to an adjacent plant (13% of the neonates had moved at least 1 plant within 24 h) than those placed on Coker 312 (0% of the neonates had moved at least 1 plant within 24 h). When larvae were placed on Event 531 plants, an estimated 82% of the larvae had moved to an adjacent plant by cumulative age of 10 d. Collectively, these data indicate that movement of larvae from plant to plant increases with larval age and occurs more rapidly for larvae placed on Event 531 cotton than on Coker 312. Previous studies have suggested that resistance to B. thuringiensis could develop more rapidly in insects exposed to seed mixtures of plants with and without endotoxin if larvae move between plants and if an external refuge exists. These data provide evidence of larval movement between plants in seed mixtures.

Peck, S. L., F. Gould, et al. (1999). "Spread of resistance in spatially extended regions of transgenic cotton: Implications for management of Heliothis virescens (Lepidoptera : Noctuidae)." Journal of Economic Entomology **92**(1): 1-16.

> Through the use of a stochastic, spatially explicit, simulation model, we explored factors that may influence the regional development of resistance in Heliothis virescens (F.) to a Bacillus thuringiensis (Bt) delta-endotoxin in transgenic cotton. The model represents the age structure of adults and larvae, plant to plant movement of larvae within a field, migration of adults among fields, plant type-genotype specific selection, and development time. We find that the spatial scale and the temporal pattern of refuges can have a strong effect on the development of resistance to B. thuringiensis in transgenic cotton. The time to resistance was in general significantly longer in regions where the same fields were used as a refuge year after year, compared with regions where the refuge fields are changed randomly from year to year. Spring movement of emerging adults onto wild hosts delays resistance if the movement is far enough from the field in which pupae overwintered. Increases in the summer migration rate and the distance moved delayed resistance development up to a point at which higher rates do not further delay resistance. Refuges were an effective strategy for delaying resistance. We found that delays in larval development time on Bt cotton may either increase or decrease the rate of resistance development, depending on complex interactions with the length of the growing season. Larval movement between Bt and non-Bt plants was found to increase the rate at which resistance developed but this may be ameliorated with increasing mortality costs associated with larval movement.

Riebe, J. F. (1999). "The development and implementation of strategies to prevent resistance to B.t.-expressing crops: an industry perspective." Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie **21**(2): 101-105.

> Genes for the production of various Bacillus thuringiensis (B.t.) proteins have been incorporated into plants through the techniques of biotechnology. Since 1995, corn, cotton, and potato seed products containing B.t. genes have been introduced to the market. Simultaneous to the development and introduction of B.t. plants, tremendous research efforts were made by industry, academic, and government scientists to devise product-specific insect resistance management (IRM) programs. IRM has become an essential part of the development and regulatory review process for B.t.-expressing plants, as distinct from other conventional chemistries. Despite this unprecedented effort, the long-term success of insect-protected crops or other pest control tools ultimately depends on the transfer of information to, and adoption by, individual growers. The effective implementation of proactive IRM plans will require integration and interaction between regulators, manufacturers of the

technology, seed companies, agri-chemical distributors and dealers, agricultural educators, and end-users of the technology.

Salomon, R. (1999). "The evolutionary advantage of breeding for tolerance over resistance against viral plant disease." Israel Journal of Plant Sciences **47**(3): 135-139.

A tolerant strain of a crop permits the propagation of an entire virus population, thus exerting a much lower selection pressure in favor of a virus mutant overcoming resistance. One may suppose that a tolerant crop may build up a large virus reservoir and therefore increase the chance of emergence of a more virulent type. However, the tolerant crop exerts a much lower selective pressure for a given virus line, and therefore the new virulent type remains a very low proportion of the total virus population. Susceptible crops, into which resistance to a given virus was bred, may be immune to this virus infection, thus preventing the buildup of a large virus reservoir. However, other host crops around it, or wild weeds facilitate propagation of this virus. This peripheral buildup of virus reservoir in the vicinity of the resistant crop creates a strong selection pressure for the propagation of the mutants overcoming resistance. Therefore, the planting of monoculture of a resistant crop accelerates the emergence of virulent virus lines that overcome this resistance. There is no reason to assume that resistance introduced into transgenic plants will be different. In the long run, tolerant crops may have the advantage over resistant crops, preserving for a longer time conditions in which virus infection causes only minimal damage. The production of tolerance to virus infection is an approach not yet favored by breeders and seed producers. However, it may be regarded as a less expensive, additional route to reduce the damage to crops inflicted by viral diseases.

Stein, J. (1999). "Insect resistance management in transgenic maize." Abstracts of Papers of the American Chemical Society **217**: 091-AGRO.

Tabashnik, B. E., A. L. Patin, et al. (1999). "Dispersal of pink bollworm (Lepidoptera : Gelechiidae) males in transgenic cotton that produces a Bacillus thuringiensis toxin." Journal of Economic Entomology **92**(4): 772-780.

To delay evolution of pest resistance to transgenic cotton that produces Bacillus thuringiensis toxin Cry1Ac (Bt cotton), refuges composed of cotton that does not produce Cry1Ac (non-Bt cotton) are mandated by the United States Environmental protection Agency. One critical assumption of this strategy is that susceptible adults emerging from non-Bt cotton refuges mate with resistant adults emerging from Bt cotton, which requires movement of adults between Bt cotton and non-Bt cotton. To better understand movements of pink bollworm, the key lepidopteran pest of cotton in Arizona, we analyzed the distribution of wild mates and dispersal of sterile males during 1997 on a 259-ha block of a commercial farm containing 76 ha of Bt cotton (69%) and 34 ha of non-Bt cotton (31%). The distribution of 28,397 wild males caught in pheromone traps between 22 May and 20 September suggests that many moved at least 400 m from non-Bt cotton to Bt cotton, yet movement was not sufficient to distribute wild males randomly between Bt cotton and non-Bt cotton. The average number of wild males caught per trap in non-Bt cotton was nearly double that for Bt cotton. Of the estimated 176,000 sterile males released on 8 dates from 18 June through 4 July, we captured 4,447 in pheromone traps between 19 June and 24 July, which represents a recovery rate of 2.5%. The results show that 66-94% of captured sterile males dispersed 400 m or less from the release sites. Along with previously published data, the results reported here suggest that, to promote mating between susceptible and resistant adults, refuges for pink bollworm should be close to Bt cotton.

Zhang, Z. L., X. Chen, et al. (1999). "Studies on insect resistance of Bt transplastomic plants and the phenotype of their progenies." Acta Botanica Sinica **41**(9): 947-951.

Insecticidal protein gene CryIA(c) from Bacillus thuringiensis (Bt. toxin gene) was placed under the control of psbA5'- and 3'- regulatory regions of rice(Oryza sativa L.) chloroplast to construct Bt expression cassette, which was ligated with selectable marker aadA cassette and homology regions of tobacco (Nicotiana tabacum L.) chloroplast genome to generate transformation vector pTRS8. Leaves of tobacco plant cv. NC89 were transformed with particle bombardment method, plastid transformants were selected by their resistance to 500 mg/L of spectinomycin. Some transplastomic plants were toxic to the third-instar larvae of Helicoverpa tea, and the growth of the survived insects was remarkably inhibited. Genetic and molecular analyses of T-1 and T-2 progenies of plants with highly efficient insect resistance showed that Bt toxin gene had been inherited in progenies, and spectinomycin resistance was inherited maternally.

Andow, D. A. and D. N. Alstad (1998). "F-2 screen for rare resistance alleles." Journal of Economic Entomology **91**(3): 572-578.

The refuge plus high-dose resistance management strategy for transgenic crops producing Bacillus thuringiensis Berliner crystal protein toxins (Bf crops) assumes that resistance is rare and functionally recessive. We propose an F-2 screening procedure to estimate the frequency of rare resistance alleles in natural insect populations and acquire them for Further testing. The procedure preserves genetic variation in isofemale lines and concentrates the resistance alleles in homozygous genotypes where they can be detected, whether they are recessive or dominant. Bayesian statistics for estimating allele frequency and credibility intervals, and the probability that the screen will not detect a resistance allele are derived. Compared with other methods for detecting and estimating resistance alleles, the F-2 screen will be efficient and accurate, extend the sensitivity of allele-frequency estimation for recessive traits by more than an order of magnitude compared with a discriminating-dose assay, and detect all resistance traits segregating in the initial sample of field-collected insects rather than a subset that responds most rapidly to laboratory selection.

Andow, D. A., D. N. Alstad, et al. (1998). "Using an F-2 screen to search for resistance alleles to Bacillus thuringiensis toxin in European corn borer (Lepidoptera : Crambidae)." Journal of Economic Entomology **91**(3): 579-584.

> We present an application of an F-2 screening method for recovering and estimating the frequencies of rare alleles that confer insect resistance to a transgenic corn variety producing Bacillus thuringiensis Berliner crystal protein toxin (Bt corn). Based on a sample of 91 female Ostrinia nubilalis (Hubner) we show with 95% confidence that the frequency of B. thuringiensis resistance alleles from a wild Minnesota population is <0.013. This is an upper limit to the estimated allele frequency and does not provide clear evidence that 1 of the assumptions of the refuge plus high-dose strategy will or will not be met. With additional sampling, a more precise estimate of resistance allele frequency could be obtained that would clearly support or refute 1 of the assumptions of the refuge plus high-dose strategy. Variable costs of the screening method were $19.70 per female line, but these could be reduced by improved collecting, rearing; and handling methods.

Bolin, P. C., W. D. Hutchison, et al. (1998). "Monitoring for European corn borer (Lepidoptera : Crambidae) resistance to Bacillus thuringiensis: Logistical considerations when sampling larvae." Journal of Agricultural Entomology **15**(3): 231-238.

Brown, T. M., P. K. Bryson, et al. (1998). "Pyrethroid-resistant Helicoverpa zea and transgenic cotton in South Carolina." Crop Protection **17**(5): 441-445.

> Failures to control Helicoverpa tea (Boddie) on cotton in South Carolina were associated with resistance to pyrethroid insecticides. Resistance to cyhalothrin, cypermethrin, and permethrin was observed in a colony collected as larvae from cotton in Estill, SC, in September 1996. Cyhalothrin resistance was expressed as an incompletely dominant trait. In 1997, resistance to cyhalothrin was observed in males captured in pheromone traps in Estill and nearby Ulmer. Resistance was also observed in Cameron and Holly Hill (Santee), SC, 150 km to the north, in adults reared from larvae collected from cotton. In contrast, all moths trapped in the Pee Dee River drainage 300 km to the north were susceptible. Putative mechanisms for resistance as related to improved monitoring of population genetics and implications for control of pests on transgenic insecticidal cotton are discussed. (C) 1998 Elsevier Science Ltd. All rights reserved.

Gould, F. (1998). "Sustainability of transgenic insecticidal cultivars: Integrating pest genetics and ecology." <u>Annual Review of Entomology</u> **43**: 701-726.

> This review examines potential impacts of transgenic cultivars on insect population dynamics and evolution. Experience with classically bred, insecticidal cultivars has demonstrated that a solid understanding of both the target insect's ecology and the cultivar's performance under varied field conditions will be essential for predicting area-wide effects of transgenic cultivars on pest and natural enemy dynamics. This experience has also demonstrated the evolutionary capacity of pests for adaptive response to insecticidal traits in crops. Biochemical and genetic studies of insect adaptation to the Bacillus thuringiensis (Bt) toxins expressed by currently marketed transgenic cultivars indicate a high risk for rapid adaptation if these cultivars are misused. Theoretical and practical issues involved in implementing strategies to delay pest adaptation to insecticidal cultivars are reviewed. Emphasis is placed on examining the "high dose"/refuge strategy that has become the goal of industry and regulatory authorities.

Hellmich, R. L., R. L. Pingel, et al. (1998). "Influencing European corn borer (Lepidoptera : Crambidae) aggregation sites in small grain crops." <u>Environmental Entomology</u> **27**(2): 253-259.

> Reliable methods to attract European corn borer, Ostrinia nubilalis (Hubner), adults to small grain crops could be used to aggregate moths into small well-defined areas for control purposes or could be used in a resistance management program for delaying potential O, nubilalis resistance to transgenic corn. The objective of this research was to determine whether small-grain crops could be managed to influence O. nubilalis aggregation behavior. In farmer-managed oat, Avena sativa (L,.), fields, more O, nubilalis adults were attracted to high- density patches of oat compared with standard patches of oat; no difference was found between patches of high-density oat and brome, Bromus spp. Numbers of O, nubilalis moths found in 6 barley,, Hordeum vulgare (L.), and legume treatments (1995),and 4 oat/legume treatments (1996) were significantly different. The highest number of O.,nubilalis adults were observed in barley planted with alfalfa, Medicago sativa (L.),followed by barley planted with crimson clover, Trifolium incarnatum (L.),barley planted with berseem, Trifolium, alexandrinum (L.) barley planted with black medic, Medicago lupulina (L.), barley alone, and barley planted with Lespedeza, Lespedeza stipulacea (Maximowicz), Double-planted oat attracted the highest number of O. nubilalis adults followed by oat planted with crimson clover, oat planted with alfalfa, and single-planted oat. Each study suggests that there is a positive correlation between moth aggregation and canopy area. Suggestions are made that timing canopy closure of a small-grain crop with peak O. nubilalis night should maximize O, nubilalis aggregation and should thereby increase the efficacy of any control measures.

Jutsum, A. R., S. P. Heaney, et al. (1998). "Pesticide resistance: Assessment of risk and the development and implementation of effective management strategies." Pesticide Science **54**(4): 435-446.

> Insecticides, fungicides and herbicides are critical to successful crop production, but the development of pesticide resistance is a continual threat, especially to many of today's selective toxophores with specific binding sites. In order to manage resistance effectively, an assessment of genetic, ecological and operational risk factors is required, which must then be translated into meaningful local strategies that can be implemented through appropriate labelling of products and education of end users. Assessing resistance risk is a fundamental part of the development process for new molecules and is increasingly becoming a requirement of registration alongside toxicological and environmental risk data. Laboratory studies, including elucidation of target sites and metabolic degradation pathways, mutagenesis, computer models and cross- resistance tests, and field studies, including establishment of baseline sensitivities and evaluation of anti-resistance strategies, all play a part in such assessment. The challenge is then to devise management strategies which are relevant to local practice and actually reduce selection pressure to a point where product life is preserved. A preventative strategy should be in place at time of launch and for most pesticides, regional co-operation between all interested parties, of the kind advocated by the Resistance Action Committees of GCPF (Global Crop Protection Federation), increases the chance of success. Implementation of strategies via a universal product labelling system, already practised in some herbicide markets, is seen as a key way to improve product usage patterns. Monitoring resistance levels in field populations after product launch enables any fine tuning of tactics over time, for example in response to new technologies such as transgenic varieties being introduced. The limited successes in resistance management in Australia, Zimbabwe, Europe and USA are not so easily achieved in small-holder farming in developing countries, as exemplified by continuing problems in parts of India and China. Emphasis must be given to the education of growers and dealers in IRM and IPM, and improved extension services, in order to bring about a more sustainable approach to crop protection. (C) 1998 Society of Chemical Industry.

Lenormand, T. and M. Raymond (1998). "Resistance management: the stable zone strategy." Proceedings of the Royal Society of London Series B-Biological Sciences **265**(1409): 1985-1990.

> The different strategies of insecticide resistance management that have been formulated so far consist of delaying the appearance and spread of resistance genes. In this paper, we propose a strategy that can be used even if resistance genes are already present. This strategy consists of applying insecticides in an area smaller than a certain critical size, so that gene flow from the untreated area, combined with the fitness cost of the

resistance genes, prevents its frequency reaching high equilibrium value. A two-locus model was analysed numerically to determine population densities at equilibrium as a function of selection coefficients (insecticide selection, fitness costs of resistance genes and dominances), gene flow and size of the treated area. This model indicates that there is an optimal size for the treated area where a minimal and stable density reach equilibrium, and where resistance genes cannot invade. This resistance management strategy seems applicable to a large variety of field situations, but eventually it may encounter obstacles due to a modifier which reduces the fitness costs of resistance genes.

Maqbool, S. B., T. Husnain, et al. (1998). "Effective control of yellow stem borer and rice leaf folder in transgenic rice indica varieties Basmati 370 and M 7 using the novel delta-endotoxin cry2A Bacillus thuringiensis gene." Molecular Breeding **4**(6): 501-507.

> Transgenic rice indica varieties Basmati 370 and M 7 expressing the novel cry2A (Bt) insecticidal gene were generated by particle bombardment. Molecular and biochemical analyses in R-0 and R-1 populations confirmed stable integration and expression of this novel Bt transgene. We estimated that the gene product was expressed up to 5% of total leaf protein. Insect feeding bioassays demonstrated that the Cry2A protein was effective against the yellow stem borer and the rice leaf folder, two major rice pests in the Indian Subcontinent. This is the first report of the control of major rice pests using this specific Bt gene. The cry2A gene can now be used in combination with other insecticidal genes for pyramiding resistance against insect pests. This will delay, or perhaps in combination with integrated pest management practices, prevent evolution of insect populations resistant to single insecticidal genes.

Onstad, D. W. and F. Gould (1998). "Do dynamics of crop maturation and herbivorous insect life cycle influence the risk of adaptation to toxins in transgenic host plants?" Environmental Entomology **27**(3): 517-522.

> Because host-plant chemistry is dynamic, chemical defenses are dynamic, and senescence in plants causes many proteins to decompose after flowering, laboratory and field studies on transgenic crops performed over only part of a plant generation or part of a season may not provide sufficient data to evaluate strategies for resistance management. As an example, we focused on the recent introduction of transgenic corn to control European corn borer, Ostrinia nubilalis (Hubner) (Lepidoptera: Pyralidae). We gathered data from literature on the life cycle of European corn borer and con, crop maturation to study the role that phenological relationships may play in resistance evolution. In addition, we simulated hypothetical titer declines resulting in increased survival in European corn borer populations infesting transgenic corn using a model of population dynamics and genetics. The relationship between European corn borer hatching period and corn maturation varies greatly from site to site and

year to year. The peak of the hatching period in the late summer generation of the European corn borer occurs at or after the average midpoint of the dough stage of corn. The last larvae tend to hatch after the dough stage is past and after the midpoint of the dent stage. In simulations where 5% of a region is planted with nontransgenic corn in separate refuge plots and 95% is planted with transgenic corn, complete loss of titer as a result of senescence produces resistance at the 3% resistance-allele level after 5-42 yr, which is less than the 83 yr predicted by the standard model for resistance development when the transgenic corn loses no titer as a result of senescence. We hypothesize that genetically engineered toxins will often decrease in leaf and stem titer as crops reach maturation. The insects feeding and surviving on a crop during its senescence may have important consequences for the population genetics of the breakdown of host plant resistance.

Ramachandran, S., G. D. Buntin, et al. (1998). "Greenhouse and field evaluations of transgenic canola against diamondback moth, Plutella xylostella, and corn earworm, Helicoverpa zea." Entomologia Experimentalis Et Applicata **88**(1): 17-24.

Canola (Brassica napus L.) cultivars Oscar and Westar, engineered with a Bacillus thuringiensis (Bt) cryIA(c) gene, were evaluated for resistance to lepidopterous pests, diamondback moth, Plutella xylostella L. (Plutellidae) and corn earworm, Helicoverpa tea (Boddie) (Noctuidae) in greenhouse and field conditions. In greenhouse preference assays conducted at vegetative and flowering plant stages, transgenic plants recorded very low levels of damage. A 100% diamondback moth mortality and approximate to 90% corn earworm mortality were obtained on transgenic plants in greenhouse antibiosis assays. The surviving corn earworm larvae on transgenic plants had reduced head capsule width and body weight. Mortality of diamondback moth and corn earworm were 100% and approximate to 95%, respectively, at different growth stages (seedling, vegetative, bolting, and flowering) on the transgenic plants in greenhouse tests. In field tests conducted during 1995-1997, plots were artificially infested with neonates of diamondback moth or corn earworm or left for natural infestation. Transgenic plants in all the treatments were highly resistant to diamondback moth and corn earworm larvae and had very low levels of defoliation. Plots infested with diamondback moth larvae had greater damage in both seasons as compared with corn earworm infested plots and plots under natural infestation. After exposure to defoliators, transgenic plants usually had higher final plant stand and produced more pods and seeds than non-transgenic plants. Diamondback moth injury caused the most pronounced difference in plant stand and pod and seed number between transgenic and non-transgenic plants. Our results suggest that transgenic canola could be used for effective management of diamondback moth and corn earworm on canola.

Ramachandran, S., G. D. Buntin, et al. (1998). "Movement and survival of diamondback moth (Lepidoptera : Plutellidae) larvae in mixtures of nontransgenic and transgenic Canola containing a cryIA (c) gene of Bacillus thuringiensis." Environmental Entomology **27**(3): 649-656.

> Knowledge about the movement and survival of diamondback moth, Plutella xylostella (L.) in mixtures of nontransgenic and transgenic canola, Brassica napus (L.), containing a synthetic cr) IA(c) insecticidal gene from the bacterium Bacillus thuringiensis Berliner would have implications in designing resistance management strategies. In diamondback moth larval movement studies neonates moved 72 h after infestation and other instars moved within 24 h. Diamondback moth larval movement and survival were studied in pure stands and mixtures of 80:20, 60:40, 40:60, and 20:80 transgenic and nontransgenic canola plants, respectively, that were arranged in rows within cages in a greenhouse. On the 6th d, no larvae survived in the pure stand of transgenic canola, and few survivors were recorded on transgenic canola plants in mixtures. In mixtures, the percentage of adult emergence decreased as the proportion of transgenic plants increased. All 4 instars were killed when they fed exclusively on transgenic plants. None of the 2nd, 3rd, and 4th instars were able complete their life cycle when they fed on transgenic canola plants for 48 h and were subsequently transferred to nontransgenic plants. The results suggest that in seed mixtures diamondback moth larvae would move from transgenic to nontransgenic plants before acquiring lethal doses of toxin, indicating a higher possibility of resistance development. These results suggest that a seed mixture strategy may not be an effective strategy against diamondback moth to prevent or reduce the rate of resistance development against transgenic canola. However, transgenic and nontransgenic plants could be grown in separate rows with a wider row spacing (strip planting) to minimize the rate of resistance development.

Ramachandran, S., G. D. Buntin, et al. (1998). "Survival, development, and oviposition of resistant diamondback moth (Lepidoptera : Plutellidae) on transgenic canola producing a Bacillus thuringiensis toxin." Journal of Economic Entomology **91**(6): 1239-1244.

> We measured responses of diamondback moth, Plutella xylostella L., to transgenic and nontransgenic canola, Brassica napus L. Transgenic canola expressed a cry1Ac gene of Bacillus thuringiensis Berliner resulting in 238 +/- 29 ng of Cry1Ac protein per milligram of total extractable protein in leaves. We tested 2 Hawaiian strains of diamondback moth: NO-QA was resistant to Cry1Ac and LAB-PS was susceptible. Larval and pupal durations, pupal weights, and adult emergence of the 2 strains were similar on nontransgenic canola, but differed significantly on transgenic canola. Transgenic canola killed all larvae tested from the susceptible strain. In contrast, for the resistant strain, no differences occurred between transgenic and nontransgenic canola in larval survival and head capsule width at day 5, percentage

pupation, pupal weight, percentage adult emergence, and extent of defoliation. For both the susceptible and resistant strains of diamondback moth, no differences were detected between transgenic and nontransgenic canola in feeding initiation or oviposition preference. The lack of discrimination between transgenic and nontransgenic canola by neonates and ovipositing females indicates that host choice behavior is independent from susceptibility to Cry1Ac. Development of resistant diamondback moth on transgenic canola without any adverse effects provides an example of a pest that has completely overcome high levels of a B. thuringiensis toxin expressed by a genetically engineered plant.

Roush, R. T. (1998). "Two-toxin strategies for management of insecticidal transgenic crops: can pyramiding succeed where pesticide mixtures have not?" Philosophical Transactions of the Royal Society of London Series B-Biological Sciences **353**(1376): 1777-1786.

Transgenic insect-resistant crops that express toxins from Bacillus thuringiensis (Bt) offer significant advantages to pest management, but are at risk of losing these advantages to the evolution of resistance in the targeted insect pests. All commercially available cultivars of these crops carry only a single Bt gene, and are particularly at risk where the targeted insect pests are not highly sensitive to the Bt toxin used. Under such circumstances, the most prudent method of avoiding resistance is to ensure that a large proportion of the pest population develops on non-transgenic 'refuge' hosts, generally of the crop itself. This has generated recommendations that 20% or more of the cotton and maize in any given area should be nontransgenic. This may be costly in terms of yields and may encourage further reliance on and resistance to pesticides. The use of two or more toxins in the same variety (pyramiding) can reduce the amount of refuge required to delay resistance for an extended period. Cross-resistance among the toxins appears to have been overestimated as a potential risk to the use of pyramids land pesticide mixtures) because cross-resistance is at least as important when toxicants are used independently. Far more critical is that there should be nearly 100% mortality of susceptible insects on the transgenic crops. The past failures of pesticide mixtures to manage resistance provide important lessons for the most efficacious deployment of multiple toxins in transgenic crops.

Sadras, V. O. (1998). "Herbivory tolerance of cotton expressing insecticidal proteins from Bacillus thuringiensis: responses to damage caused by Helicoverpa spp, and to manual bud removal." Field Crops Research **56**(3): 287-299.

Transgenic cotton varieties expressing Cry IA(c) insecticidal proteins from Bacillus thuringiensis ('Bt cotton') remain vulnerable to non-lepidopteran insects. In addition, they are susceptible to lepidopteran pests when the efficacy of Bt toxins falls because of ontogenetic and/or environmental factors. Hence the importance of knowing to what extent Bt cotton is able to tolerate damage. The degree of tolerance of Bt cotton to actual and simulated insect damage was assessed in three field experiments. Exp. 1 compared the effects of Helicoverpa spp. (Lepidoptera: Noctuidae) on the growth, development and yield of Bt cotton with those of its near isogenic non-Bt counterpart in two genetic backgrounds (Siokra V15, Sicala V2) under two regimes of chemical control of insects (S1: nine insecticide applications during the growing cycle, S2: six insecticide applications). Exp. 2 compared insecticide-protected Bt crops with crops manually damaged to simulate (a) early-season loss of vegetative buds, (b) loss of flowerbuds, and (c) loss of both vegetative and reproductive buds. Also using manual damage, Exp. 3 evaluated the effect of timing of flowerbud loss on the yield and maturity time of insecticide-protected Bt crops. In Exp. 1, well-protected Bt crops (S1) yielded 24% more than their less-protected counterparts (S2). The less protected crops had, however, substantially more immature fruit at the end of the season highlighting a considerable potential for recovery. Poor soil conditions, interacting with season length accounted for the difference between potential and actual compensation in crops that were exposed to almost continuous damage by Helicoverpa spp. Under more favourable growing conditions, maturity was delayed but yield of damaged Bt crops was unaffected by discrete episodes of simulated herbivory (Exps. 2 and 3). The introduction of B. thuringiensis genes into cotton does not seem to have reduced the considerable capacity of the crop to tolerate insect damage, and this attribute should be considered in the development of pest management strategies for Bt crops. (C) 1998 Elsevier Science B.V.

Stapel, J. O., D. J. Waters, et al. (1998). "Development and behavior of Spodoptera exigua (Lepidoptera : Noctuidae) larvae in choice tests with food substrates containing toxins of Bacillus thuringiensis." Biological Control **11**(1): 29-37.

Developmental time and behavior of Spodoptera exigua (Hubner) larvae and their pupal weight were investigated in dual-choice arenas containing Bacillus thuringiensis Berliner (Bt) toxins. In both artificial diet and cotton leaf-choice tests with Bt formulation MVP (containing delta endotoxins var. Kurstaki), the mean proportion of larvae on Bt-free diets was higher than on Bt-containing diets. Artificial diet tests further showed that larvae were more often found on diets where MVP was applied on the diet surface than on diets where the formulation was mixed in the diet. In leaf-choice tests with the Bt transgenic cotton line 'C 1076' and the nontransgenic 'C 312', more larvae were found on nontransgenic cotton leaves, Also, when feeding damage was measured in the leaf experiments, feeding damage was more frequently observed on Bt-free than on Bt-containing leaves in MVP and 'C 1076' choice tests, Leaf- choice tests with the Bt formulations Dipel ES (containing B. thuringiensis var. Kurstaki) and Xentari (containing B. thuringiensis var. Aizawai) and the transgenic Bt line 'C 531' showed patterns of larval behavior and feeding damage different than those obtained in the other leaf tests, The survival rate of larvae on MVP was comparable to the larval survival on control tests containing only Bt-free diets, However, lower pupal weight and longer developmental time were observed. Experiments with neonate S. exigua larvae on MVP-treated cotton plants demonstrated that the number of larvae remaining on the plant was negatively correlated with concentration and exposure time. The consequences and opportunities for behavior adaptation to Bt in pest management are discussed. (C) 1998 Academic Press.

Tabashnik, B. E., Y. B. Liu, et al. (1998). "Insect resistance to Bacillus thuringiensis: uniform or diverse?" Philosophical Transactions of the Royal Society of London Series B-Biological Sciences **353**(1376): 1751-1756.

Resistance to the insecticidal proteins produced by the soil bacterium Bacillus thuringiensis (Bt) has been documented in more than a dozen species of insect. Nearly all of these cases have been produced primarily by selection in the laboratory, but one pest, the diamondback moth (Plutella xylostella), has evolved resistance in open-field populations. Insect resistance to Bt has immediate and widespread significance because of increasing reliance on Bt toxins in genetically engineered crops and conventional sprays. Furthermore, intense interest in Bt provides an opportunity to examine the extent to which evolutionary pathways to resistance vary among and within species of insect. One mode of resistance to Bt is characterized by more than 500-fold resistance to at least one Cry1A toxin, recessive inheritance, little or no cross-resistance to Cry1C, and reduced binding of at least one Cry1A toxin. Analysis of resistance to Bt in the diamondback moth and two other species of moths suggests that although this particular mode of resistance may be the most common, it is not the only means by which insects can attain resistance to Bt.

Verkerk, R. H. J., S. R. Leather, et al. (1998). "The potential for manipulating crop-pest-natural enemy interactions for improved insect pest management." Bulletin of Entomological Research **88**(5): 493-501.

This review identifies key ways in which manipulations of the crop environment based on detailed understanding of tritrophic interactions can contribute to improvements in the control of insect pests. Such approaches are likely to be of particular benefit against those pests, notably certain species of Lepidoptera and aphid, which are difficult to control with insecticides because of insecticide resistance or suppression of natural enemies. Particular attention is given to the compatibility (or otherwise) of partial plant resistance and biological control, citing examples which support contrasting tritrophic theories. Other areas considered and supported with examples include the use or effects of allelochemicals, refugia, intercropping, crop backgrounds, fertilization regimes, parasitoid conditioning (by host plants) and transgenic crops. Examples of manipulations involving use of selective insecticides which show compatibility with biological methods are also included owing to their possible suitability in integrated crop management programmes.

Westedt, A. L., D. S. Douches, et al. (1998). "Evaluation of natural and engineered resistance mechanisms in Solanum tuberosum for resistance to Phthorimaea operculella (Lepidoptera : Gelechiidae)." Journal of Economic Entomology **91**(2): 552-556.

Potato tuber moth, Phthorimaea oprrculella Zeller, is a highly destructive pest of cultivated potato, Solanum tuberosum L., and is responsible for damage to both leaf and tuber tissues. Host plant resistance is a central component to developing ten integrated pest management program to control potato tuber moth. This research tested the efficacy of a codon-modified CryV-Bacillus thuringiensis (CryV-Bt) gene constitutively expressed in potato and the combined effect of CryV-Bt expression with natural host plant resistance mechanisms in potato. 'Lemhi Russet' and 2 lines with host plant resistance mechanisms, USDA8380-1 (leaf leptines) and L235-4 (glandular trichomes), along with the CryV-Bt-transgenic lines of each of these 3 genotypes were examined. Detached leaf bioassays were conducted to examine control of potato tuber moth. Nontransformed Lemhi Russet and L235-4 were susceptible to potato tuber moth, while 54% potato tuber moth mortality was found when first instar larvae fed on USDA8380-1 leaves. High levels of expression occurred in the CryV-Bt transgenic Lines, with up to 96% potato tuber moth mortality. These transgenic lines provide a germplasm base to examine combined insect-resistance mechanisms as a means to achieve durable host plant resistance.

Davis, P. M. and S. B. Coleman (1997). "European corn borer (Lepidoptera: Pyralidae) feeding behavior and survival on transgenic corn containing CryIA(b) protein from Bacillus thuringiensis." Journal of the Kansas Entomological Society **70**(1): 31-38.

The effect of short-term exposure of European corn borer (Ostrinia nubilalis (Hubner)) larvae to transgenic corn expressing cryIA(b) protein from Bacillus thuringiensis subsp. kurstaki (Btk) on leaf consumption and larval survival were compared to negative controls in laboratory bioassays. Neonates and fourth instars were allowed to feed for one of three time periods (1994 bioassays: 1, 4: or 24 hours; 1995 bioassays: 24, 48, or 72 hours) on leaf sections of either cryIA(b)-expressing or control leaf sections before being transferred to artificial diet. Field-grown plants of the transgenic corn lines MON802 and MON810 were used as the source of leaf material in 1994 and 1995, respectively (YieldGard(TM) genes, Monsanto). Leaf source and exposure time significantly affected consumption ratings of neonates with little feeding detected on cryIA(b) leaf material during the first 24 hours during 1994 or during the first 72 hours in 1995. In 1994, cryIA(b) content in leaves did not affect neonate survival at transfer (97%) or after 2 weeks on diet (87%). In contrast, only

35-40% of neonates were alive after 24 and 48 hour feeding periods and no larvae survived 72 hours in 1995. Of the live neonates transferred to diet, 53% and 26% died within 2 weeks for cryIA(b) and control leaves, respectively. For periods of 24 hours or more, at least 90% of the fourth instars initiated feeding, regardless of cryIA(b) content. Cumulative consumption ratings were 46-67% lower for larvae exposed to cryIA(b) expressing leaves; however, survival rates at transfer or after two weeks on diet were not affected by leaf source. Implications for resistance management are discussed.

Georghiou, G. P. and M. C. Wirth (1997). "Influence of exposure to single versus multiple toxins of Bacillus thuringiensis subsp. israelensis on development of resistance in the mosquito Culex quinquefasciatus (Diptera: Culicidae)." Applied and Environmental Microbiology **63**(3): 1095-1101.

The impending widespread use of transgenic crop plants encoding a single insecticidal toxin protein of Bacillus thuringiensis has focused attention on the perceived risk of rapid selection of resistance in target insects. We have used Bacillus thuringiensis subsp. israelensis toxins as a model system and determined the speed and magnitude of evolution of resistance in colonies of the mosquito Culex quinquefasciatus during selection for 28 consecutive generations with single or multiple toxins, The parental strain was synthesized by combining approximately 500 larvae from each of 19 field collections obtained from the states of California, Oregon, Louisiana, and Tennessee. At least 10,000 larvae were selected in each generation of each line at an average mortality level of 84%. The susceptibilities of the parental and selected lines were compared in parallel tests in every third generation by using fresh suspensions of toxin powders. The normal toxin complement of B. thuringiensis subsp, israelensis consists of four toxins, CryIVA, CryIVB, CryIVD, and CytA. Resistance became evident first in the line that was selected with a single toxin (CryIVD), attaining the highest level (resistance ratio [RR], >913 at 95% lethal concentration) by generation F- 28 when the study was completed. Resistance evolved more slowly and to a lower level (RR, >122 by F-25) in the line selected with two toxins (CryIVA+CryIVB) and lower still (RR, 91 by F- 28) in the line selected with three toxins. (CryIVA+CryIVB+CryIVD), Resistance was remarkably low (RR, 3.2) in the line selected with all four toxins, The results reveal the importance of the full complement of toxins found in natural populations of B. thuringiensis subsp. israelensis as an effective approach to resistance management.

Gould, F., A. Anderson, et al. (1997). "Initial frequency of alleles for resistance to Bacillus thuringiensis toxins in field populations of Heliothis virescens." Proceedings of the National Academy of Sciences of the United States of America **94**(8): 3519-3523.

The risk of rapid pest adaptation to an insecticide is highly dependent on the initial frequency of resistance alleles in field populations. Because we have lacked empirical estimates of these frequencies, population-genetic models of resistance evolution have relied on a wide range of theoretical estimates. The recent commercialization of genetically engineered cotton that constitutively produces an insecticidal protein derived from the biocontrol agent, Bacillus thuringiensis (Bt) has raised concern that we lack data needed to quantify the risk of insect pests such as Heliothis virescens rapidly adapting to this ecologically valuable class of toxins. By individually mating over 2,000 male H. virescens moths collected in four states to females of Bt toxin-resistant laboratory strain, and screening F-1 and F-2 offspring for tolerance of the toxic protein, we were able to directly estimate the field frequency of alleles for resistance as 1.5 x 10(-3). This high initial frequency underscores the need for caution in deploying transgenic cotton to control insect pests. Our single-pair mating technique greatly increases the efficiency of detecting recessive resistance alleles. Because alleles that decrease target site sensitivity to Bt toxins and other insecticides are often recessive, this technique could be useful in estimating resistance allele frequencies in other insects exposed to transgenic insecticidal crops or conventional insecticides.

Hardee, D. D. and W. W. Bryan (1997). "Influence of Bacillus thuringiensis-transgenic and nectariless cotton on insect populations with emphasis on the tarnished plant bug (Heteroptera: Miridae)." Journal of Economic Entomology **90**(2): 663-668.

Comparison of Bacillus thuringiensis transgenic and nectariless cottons with non-B. thuringiensis commercial varieties >2-yr showed no significant differences in number of beneficials, bollworm [Helicoverpa zea (Boddie)], and tobacco budworm [Heliothis virescens (F.)] eggs, fruiting sites per plant, and percentage square set. Significantly more tarnished plant bugs, Lygus lineolaris (Palisot de Beauvois), and percentage of crazy cotton were observed in B. thuringiensis 757 (Coker 312 background in 1994) and in Coker 312 variety in 1995 compared with other varieties, suggesting no effect of B. thuringiensis gene insertion in cotton on the number of tarnished plant bugs. Transgenic cotton had significantly fewer bollworms and budworms, cabbage loopers [Trichoplusia ni (Hubner)], and percentage of damaged squares than the other varieties, 75% as many beet armyworms [Spodoptera exigua (Hubner)], and had no effect on fall armyworm [Spodoptera frugiperda (J. E. Smith)]. The transgenic character itself did not cause an increase of any insect population, but consultants and producers are encouraged to monitor other insect pests, especially boil weevil, Anthonomus grandis grandis Boheman, and tarnished plant bug. These pests may cause economic damage when fewer or no sprays are used for bollworm and budworm in cotton. Nectariless MD51 had fewer plant bugs than other varieties both years, but also lower yield. These results suggest transgenic cotton is a useful tool in the management of certain lepidopterous pests.

Huang, F. N., R. A. Higgins, et al. (1997). "Baseline susceptibility and changes in susceptibility to Bacillus thuringiensis subsp. kurstaki under selection pressure in European corn borer (Lepidoptera: Pyralidae)." Journal of Economic Entomology **90**(5): 1137-1143.

> Baseline susceptibility of European corn borer larvae, Ostrinia nubilalis (Hubner), to a commercial formulation of Bacillus thuringiensis Berliner Subsp. kurstaki (Dipel ES), was determined for colonies from 3 different geographic locations. The KS-NE colony, collected near Manhattan in northeast Kansas, was more tolerant to Dipel than those collected from near St. John in south central Kansas (KS-SC colony) and colonies provided by the USDA Corn Insects Laboratory in Ames, IA (Iowa colony). Five colonies of European corn borers were exposed to laboratory selection using a meridic diet incorporating Dipel ES. Decreasing susceptibility of European corn borer to Dipel ES was observed in each colony selected. In the colony collected in south central Kansas (KS-SC), the LC50 and survival of larvae at specific concentrations increased more rapidly than it did in the other colonies. After only 3 selected generations, the LC50 of this colony increased 35.8- fold, and after 7 selected generations, it reached 72.9-fold. The LC(50)s of the 3 Iowa colonies increased 25.0- to 35.2-fold after 9-14 selected generations. The LC(50)s of the colony collected in northeast Kansas increased 16.2-fold after 4 selected generations and it reached 35-fold after 6 selected generations. This indicates that European corn borer populations can respond rapidly to intense selection pressure with a commercial formulation of Bacillus thuringiensis. The LC(90)s of the 5 colonies also increased significantly in a pattern similar to that observed in the LC(50)s, but the increases in LC(90)s were not as large as the increases for the LC(50)s. The bioassay data suggest that a dose of approximate to 2.4 mi Dipel per kilogram of diet could be used as a preliminary discriminating dose. Neonates from field-collected insects could be placed on diets incorporating this concentration of Dipel to monitor for changes and regional differences in susceptibility among field populations of European corn borer. Studies on the genetic basis, the mechanism of action, and the stability over time (in the absence of selection pressure) of this change in susceptibility remain to be carried out.

Jansens, S., A. vanVliet, et al. (1997). "Transgenic corn expressing a Cry9C insecticidal protein from Bacillus thuringiensis protected from European corn borer damage." Crop Science **37**(5): 1616-1624.

> The European corn borer, Ostrinia nubilalis (Hubner), is a devastating insect pest in the corn (Zea mays L.) growing regions of North America and Europe. Field evaluations in the USA and Belgium showed that transgenic corn events expressing Cry9C, an insecticidal crystal protein from Bacillus thuringiensis subsp, tolworthi, very effectively control both generations of the European corn barer. Second to fourth instar larvae fed

with leaf material of event CBH351 expressing the Cry9C protein air died within 4 d. Cry9C events, expressing high levers of the insecticidal protein, showed minimal stalk tunneling after heavy artificial infestations. Event CBH351 tested in plots containing only Cry9C transgenic plants had 0.14- and 0.09-cm tunneling per stalk compared with more than 30- and 23-cm tunneling par stalls Tor the negative controls, inn the Belgium and Iowa field trial, respectively. In plots containing 30% non-transgenic plants the event CBH351, showed only 1.45-cm tunneling per stalk. Leaf, tassel, and pith tissue contained 39.0, 17.4, and 84.8 mu g Cry9C protein mg(-1) soluble protein, respectively, in analyses conducted at harvest of the Belgium trial. The implications of Cry9C use for resistance management strategies sue discussed.

Johnson, M. T., F. Gould, et al. (1997). "Effect of an entomopathogen on adaptation of Heliothis virescens populations to transgenic host plants." Entomologia Experimentalis Et Applicata **83**(2): 121-135.

The role of an entomopathogen in evolution of herbivore adaptation to partially resistant host plants was examined using a tritrophic system in the laboratory. We hypothesized that a pathogen should interact with herbivore behavior to accelerate herbivore adaptation to toxic plants: individuals not adapted to toxin tend to move more on toxic plants, and therefore are more likely to encounter a lethal dose of pathogen, further increasing the probability that they will be eliminated by selection. Heliothis virescens (F.) (Noctuidae) was selected for adaptation to transgenic tobacco containing a sublethal concentration of Bacillus thuringiensis Berliner toxin under two treatment regimes: larvae placed on plants treated with infective conidia of the entomopathogenic fungus, Nomuraea rileyi (Farlow) Samson, and larvae placed on plants without fungus. Selection was initiated with a genetically heterogeneous strain created by crossing two laboratory strains of H. virescens, one not adapted to B. thuringiensis toxin, and one highly adapted (>1000-fold) to toxin. This cross was performed four times to create four independent populations. Selection was initiated with F2 offspring from each cross and continued for 8-10 consecutive generations. Adaptation to toxin within each treatment population was quantified every generation by measuring survival and growth of larvae on artificial diet containing a low concentration of B. thuringiensis toxin. In three of four replicates, H. virescens populations exposed to N. rileyi adapted to B. thuringiensis toxin more quickly than populations not exposed. These results supported our hypothesis that the pathogen should accelerate adaptation to toxic plants. However, this hypothesis was contradicted by the result in one replicate, in which the population not exposed to fungus adapted to toxic plants faster. This opposite result could not be explained, but it suggests that there may be substantial variation in herbivore evolution in tritrophic systems. H. virescens populations selected in the presence of fungus and in the absence of fungus did not differ in feeding or in mortality when placed on leaf disks treated with conidia. Thus, populations exposed to N. rileyi on plants for 7-8 generations displayed neither physiological nor behavioral adaptation to N. rileyi.

Liu, Y. B. and B. E. Tabashnik (1997). "Experimental evidence that refuges delay insect adaptation to Bacillus thuringiensis." Proceedings of the Royal Society of London Series B-Biological Sciences **264**(1381): 605-610.

Theoretical projections suggest that refuges from exposure can delay insect adaptation to environmentally benign insecticides derived from Bacillus thuringiensis, but experimental tests of this approach have been limited. We tested the refuge tactic by selecting two sets of two colonies of diamondback moth (Plutella xylostella) for resistance to B. thuringiensis subsp. aizawai in the laboratory. In each set, one colony was selected with no refuge and the other with a 10% refuge from exposure to B. thuringiensis subsp. aizawai. Bioassays conducted after nine selections were completed show that mortality caused by B. thuringiensis subsp. aizawai was significantly greater in the refuge colonies than in the no-refuge colonies. These results demonstrate that the refuges delayed the evolution of resistance. Relative to a susceptible colony, final resistance ratios were 19 and eight for the two no-refuge colonies compared to six and five for the refuge colonies. The mean realized heritability of resistance to B. thuringiensis subsp. aizawai was 0.046 for colonies without refuges, and -0.002 for colonies with refuges. Selection with B. thuringiensis subsp. aizawai decreased susceptibility to B. thuringiensis toxin Cry1Ab, but not to Cry1C or B, thuringiensis subsp. kurstaki. Although the ultimate test of refuges will occur in the field, the experimental evidence reported here confirms modelling results indicating that refuges can slow the evolution of insect resistance to B. thuringiensis.

Nayak, P., D. Basu, et al. (1997). "Transgenic elite indica rice plants expressing CryIAc delta- endotoxin of Bacillus thuringiensis are resistant against yellow stem borer (Scirpophaga incertulas)." Proceedings of the National Academy of Sciences of the United States of America **94**(6): 2111-2116.

Generation of insect-resistant, transgenic crop plants by expression of the insecticidal crystal protein (ICP) gene of Bacillus thuringiensis (Bt) is a standard crop improvement approach, In such cases, adequate expression of the most appropriate ICP against the target insect pest of the crop species is desirable. It is also considered advantageous to generate Bt-transgenics with multiple toxin systems to control rapid development of pest resistance to the ICP, Larvae of yellow stem borer (YSB), Scirpophaga incertulas, a major lepidopteran insect pest of rice, cause massive losses of rice yield, Studies on insect feeding and on the binding properties of ICP to brush border membrane receptors in the midgut of YSB larvae revealed that cryI4b and cryI4e are two individually suitable candidate genes for developing YSB-resistant rice, Programs were undertaken to develop Bt-transgenic rice with these ICP genes independently in a single cultivar, A cryIAe gene was reconstructed and placed under control of the maize ubiquitin 1 promoter, along with the first intron of the maize ubiquitin 1 gene, and the nos terminator, Tile gene construct was delivered to embryogenic calli of IR64, an elite indica rice cultivar, using the particle bombardment method Six highly expressive independent transgenic ICP lines were identified, Molecular analyses and insect-feeding assays of two such lines revealed that the transferred synthetic cryI4e gene was expressed stably in the T-2 generation of these lines and that the transgenic rice plants were highly toxic to YSB larvae and lessened the damage caused by their feeding.

Perez, C. J., A. M. Shelton, et al. (1997). "Managing diamondback moth (Lepidoptera : Plutellidae) resistance to foliar applications of Bacillus thuringiensis: Testing strategies in field cages." Journal of Economic Entomology **90**(6): 1462-1470.

Three resistance management strategies for field-sprayed commercial formulations of Bacillus thuringiensis Berliner subspecies were tested in field cages during the dry and rainy seasons of 1995 in Honduras. A local field population of Plutella xylostella (L.) with a 21-fold resistance to B. thuringiensis subsp. kurstaki (Javelin), but no resistance to B. thuringiensis subsp. aizawai (Xentari), was selected for 5-6 generations with 16 field applications of a high (1.12 kg/ha) or low (0.3 kg/ha) dose of Javelin, a high or low dose of Javelin in the presence or absence of a refuge (25%), and Xentari (1.12 kg/ha). Resistance to Javelin increased approximate to 1.9-4.4 times, but was significant only with the 1.12 kg/ha rate of Javelin irrespective of the presence or absence of a refuge. Field selection with Javelin at 0.3 kg/ha or Xentari did not cause a significant increase in resistance to B. thuringiensis subsp. kurstaki, nor did P. xylostella selected with Xentari evolve resistance to B. thuringiensis subsp. aizawai. During the same period, the LC50 of Javelin in P. xylostella left unselected did not decrease. Although the rate of resistance increase was lower for lower doses of Javelin, a smaller proportion of marketable cabbage was produced in comparison with higher doses of Javelin or Xentari. Our data suggest that the deliberate inclusion of a refuge may reduce the proportion of marketable produce, any may affect use of this resistance management strategy in both sprayed B. thuringiensis and transgenic crops expressing B. thuringiensis toxins.

Porter, D. R., J. D. Burd, et al. (1997). "Greenbug (Homoptera: Aphididae) biotypes: Selected by resistant cultivars or preadapted opportunists?" Journal of Economic Entomology **90**(5): 1055-1065.

Future advances in wheat, Triticum aestivum L., and sorghum, Sorghum bicolor (L.) Moench, resistance to greenbug, Schizaphis graminum (Rondani), will likely come from introduction of resistance transgenes into high-performance cultivars. First- generation resistance transgenes will be single genes that impart antibiosis traits (similar to Bacillus thuringiensis endotoxins in transgenic corn, Zea mays L.). This approach to pest management is incompatible with interpretations of simulation models that predict that deployment of antibiosis resistance controlled by single genes drives the development of new, virulent pest biotypes. This dichotomy must be addressed if full advantage is to be taken of the new, powerful tools of molecular biology for plant protection against insects. In this study, the specific insect-plant interactions of greenbugs on wheat and sorghum were examined to understand the relationship between the deployment of plant resistance and the development of new greenbug biotypes. From this analysis, there was no relationship between the use of resistant wheat and the development of new greenbug biotypes. For sorghum, with only 3 of the 11 biotypes could there be any correlation between the use of resistant hybrids and the development of new biotypes. Even with these 3 biotypes, no clear cause-and-effect relationship was established. Based on analysis of these specific insect-plant interactions, we propose that future plant resistance efforts focus on the use of the most effective resistance genes, despite past predictions of what effect these genes may have on greenbug population genetics.

Santos, M. O., M. J. Adang, et al. (1997). "Testing transgenes for insect resistance using Arabidopsis." Molecular Breeding **3**(3): 183-194.

One possible strategy to delay the selection of resistant insect populations is the pyramiding of multiple resistance genes into a single cultivar. However, the transformation of most major crops remains prohibitively expensive if a large number of transgene combinations are to be evaluated. Arabidopsis thaliana is a potentially good plant for such preliminary evaluations. We determined that four major agricultural pests, Spodoptera exigua, Helicoverpa tea, Pseudoplusia includens, and Heliothis virescens grew as well when feeding on 'Landsberg Erecta' Arabidopsis as they did on plants of 'Cobb' soybean. Landsberg Erecta was then transformed with either a synthetic Bacillus thuringiensis cryIA(c) gene, or the cowpea trypsin inhibitor gene. Transformed plants were crossed to produce plants transgenic for both genes. Following quantification of transgene expression, the four caterpillar species were allowed to feed on wild-type plants, plants expressing either cryIA(c) or the cowpea trypsin inhibitor gene, or plants expressing both. Both genes reduced growth of the species tested, but cryIA(c) was more effective in controlling caterpillar growth than the cowpea trypsin inhibitor gene. The resistance of plants with both transgenes was lower than that of plants expressing the cryIA(c) gene alone, but higher than that of plants expressing the only the CpTI gene. This could be due to a lower concentration of Cry protein in the hemizygous F-1 plants. Thus, If the cowpea trypsin inhibitor had any potentiation effect on cryIA(c), this effect was less than the cryIA(c) copy number effect. Alternatively, expression of the trypsin inhibitor gene could be antagonistic to the function of the cryIA(c) gene. Either way, these results suggest that the combined use of these two genes may not be effective.

Tabashnik, B. E. (1997). "Seeking the root of insect resistance to transgenic plants." Proceedings of the National Academy of Sciences of the United States of America **94**(8): 3488-3490.

Tabashnik, B. E., Y. B. Liu, et al. (1997). "One gene in diamondback moth confers resistance to four Bacillus thuringiensis toxins." Proceedings of the National Academy of Sciences of the United States of America **94**(5): 1640-1644.

Environmentally benign insecticides derived from the soil bacterium Bacillus thuringiensis (Bt) are the most widely used biopesticides, but their success will be short-lived if pests quickly adapt to them. The risk of evolution of resistance by pests has increased, because transgenic crops producing insecticidal proteins from Bt are being grown commercially, Efforts to delay resistance with two or more Bt toxins assume that independent mutations are required to counter each toxin. Moreover, it generally is assumed that resistance alleles are rare in susceptible populations, We tested these assumptions by conducting single-pair crosses with diamondback moth (Plutella xylostella), the first insect known to have evolved resistance to Bt in open field populations, An autosomal recessive gene conferred extremely high resistance to four Bt toxins (Cry1Aa, Cry1Ab, Cry1Ac, and Cry1F). The finding that 21% of the individuals from a susceptible strain were heterozygous for the multiple-toxin resistance gene implies that the resistance allele frequency was 10 times higher than the most widely cited estimate of the upper limit for the initial frequency of resistance alleles in susceptible populations. These findings suggest that pests may evolve resistance to some groups of toxins much faster than previously expected.

Tabashnik, B. E., Y. B. Liu, et al. (1997). "Global variation in the genetic and biochemical basis of diamondback moth resistance to Bacillus thuringiensis." Proceedings of the National Academy of Sciences of the United States of America **94**(24): 12780-12785.

Insecticidal proteins from the soil bacterium Bacillus thuringiensis (Bt) are becoming a cornerstone of ecologically sound pest management. However, if pests quickly adapt, the benefits of environmentally benign Bt toxins in sprays and genetically engineered crops will be short-lived, The diamondback moth (Plutella xylostella) is the first insect to evolve resistance to Bt in open-field populations, Here we report that populations from Hawaii and Pennsylvania share a genetic focus at which a recessive mutation associated with reduced toxin binding confers extremely high resistance to four Bt toxins, In contrast, resistance in a population from the Philippines shows multilocus control, a narrower spectrum, and for some Bt toxins, inheritance that is not recessive and not associated with reduced binding, The observed variation in the genetic and biochemical basis of resistance to Bt, which is unlike patterns documented for some

> synthetic insecticides, profoundly affects the choice of strategies for combating resistance.

Tang, J. D., S. Gilboa, et al. (1997). "Inheritance, stability, and lack-of-fitness costs of field- selected resistance to Bacillus thuringiensis in diamondback moth (Lepidoptera: Plutellidae) from Florida." Journal of Economic Entomology **90**(3): 732-741.

> A colony of Plutella xylostella. (L.), established from crucifer fields in Florida, was used to investigate resistance to Bacillus thuringiensis Berliner subsp. kurstaki. From an initial level of >1,500-fold resistance fell within 3 generations in the absence of selection to approximate to 300-fold compared with susceptible larvae. Unlike previous cases of resistance to B. thuringiensis P. xylostella, resistance in our Florida colony was stable at approximate to 300-fold without additional selection in the laboratory. High levels of resistance (>1,000-fold) recurred after a single exposure to B. thuringiensis subsp. kurstaki in the 4th generation. High levels of resistance did not recur after a nd selection in the 8th generation. Cage studies and genetic analysis of F-1 larvae and backcross progeny, where the resistant parents were characterized by stable levels of resistance, showed that resistance mas an incompletely recessive, autosomal trait probably controlled by a single allele that did not confer detectable levels of reduced fitness in the absence of exposure to B. thuringiensis. As one of the few studies to demonstrate stable resistance to B. thuringiensis subsp. kurstaki from insects that were collected from the field and not subject to further selection in the laboratory our results clearly emphasize the need to develop specific resistance management strategies for B. thuringiensis before there is widespread evolution of resistance.

Trisyono, A. and M. E. Whalon (1997). "Fitness costs of resistance to Bacillus thuringiensis in Colorado potato beetle (Coleoptera: Chrysomelidae)." Journal of Economic Entomology **90**(2): 267-271.

> We compared fitness components of a Bacillus thuringiensis CryIIIA delta-endotoxin resistant and susceptible strains of Colorado potato beetle, Leptinotarsa decemlineata (Say). The resistant strain had been selected for 35 generations, and the resistance ratio was >700-fold compared with the susceptible strain. The F-36 Of both strains was used in this study. We found that the viability of eggs produced by resistant and susceptible females were high (97.8 +/- 3.7 and 98.3 +/- 2.9%, respectively), but eggs of the resistant strain tended to have longer viability than that of the susceptible strain (5.7 +/- 0.3 and 5.2 +/- 0.2 d, respectively). Slower larval development was associated with resistance to CryIIIA delta-endotoxin in Colorado potato beetle. In addition, resistant females produced 60% fewer eggs than susceptible females. The resistant females also exhibited a shorter oviposition period and fewer eggs per egg mass (16.3 +/- 2.4 versus 27.8 +/- 11.3). These results are discussed together with various resistance management strategies for Colorado potato beetle controlled

by conventional B. thuringiensis sprays and transgenic plants.

Wirth, M. C., G. P. Georghiou, et al. (1997). "CytA enables CryIV endotoxins of Bacillus thuringiensis to overcome high levels of CryIV resistance in the mosquito, Culex quinquefasciatus." Proceedings of the National Academy of Sciences of the United States of America **94**(20): 10536-10540.

Cry proteins produced by Bacillus thuringiensis are selective biodegradable insecticides used increasingly in bacterial insecticides and transgenic plants as alternatives to synthetic chemical insecticides, However, the potential for development of resistance and cross-resistance in target insect populations to Cry proteins used alone or In combination threatens the more widespread use of this novel pest control technology, Here we show that high levels of resistance to CryIV proteins in larvae of the mosquito, Culex quinquefasciatus, can be suppressed or reduced markedly by combining these proteins with sublethal quantities of CytA, a cytolytic endotoxin of B. thuringiensis. Resistance at the LC95 level of 127-fold for a combination of three CryIV toxins (CryIVA, B, and D), resulting from 60 generations of continuous selection, was completely suppressed by combining sporulated powders of CytA in a 1:3 ratio with sporulated powders of a CryIVA, CryIVB, and CryIVD strain. Combining the CytA strain with a CryIVA and CryIVB strain also completely suppressed mosquito resistance of 217-fold to the latter toxins at the LC95 level, whereas combination of CytA with CryIVD reduced resistance in a CryIVD-selected mosquito strain from greater than 1,000-fold to less than 8-fold. The CytA/CryIV model provides a potential molecular genetic strategy for engineering resistance management for Cry proteins directly into bacterial insecticides and transgenic plants.

Hokkanen, H. M. T. and C. H. Wearing (1996). "Assessing the risk of pest resistance evolution to Bacillus thuringiensis engineered into crop plants: A case study of oilseed rape." Field Crops Research **45**(1-3): 171-179.

Existing and novel Bacillus thuringiensis toxin-coding genes may be utilised in transgenic oilseed rape to control many of the important insect pest species on the crop, worldwide. Location-specific ecological crop assessment must be used to evaluate the need and possibilities for managing resistance evolution in the target pests, and to ensure sustainability of the control strategy. In some cases, at least, it appears that durable control could be attained in oilseed rape by an integrated control approach, including the use of transgenic crop and among it, about 10% of non-transformed seed to provide refugia for susceptible insect genotypes. Seed mixtures or strip intercropping would be easy to handle also in practice.

Ives, A. R. (1996). "Evolution of insect resistance to Bacillus thuringiensis-transformed plants." Science **273**(5280): 1412-1413.

Michaud, D., B. NguyenQuoc, et al. (1996). "Response of digestive cysteine proteinases from the Colorado potato beetle (Leptinotarsa decemlineata) and the black vine weevil (Otiorynchus sulcatus) to a recombinant form of human stefin A." Archives of Insect Biochemistry and Physiology **31**(4): 451-464.

> The effects of the cystatins, human stefin A (HSA) and oryzacystatin I (OCI) on digestive cysteine proteinases of the Colorado potato beetle (CPB), Leptinotarsa decemlineata, and the black vine weevil (BVW), Otiorynchus sulcatus, were assessed using complementary inhibition assays, cystatin- affinity chromatography, and recombinant forms of the two inhibitors. For both insects, either HSA and OCI used in excess (10 or 20 mu M) caused partial and stable inhibition of total proteolytic (azocaseinase) activity, but unlike for OCI the HSA-mediated inhibitions were significantly increased when the inhibitor was used in large excess (100 mu M). As demonstrated by complementary inhibition assays, this two-step inhibition of the insect proteases by HSA was due to the differential inactivation of two distinct cysteine proteinase populations in either insect extracts, the rapidly (strongly) inhibited population corresponding to the OCI-sensitive fraction. After removing the cystatin-sensitive proteinases from CPB and BVW midgut extracts using OCI- (or HSA-) affinity chromatography, the effects of the insect "non-target" proteases on the structural integrity of the two cystatins were assessed. While OCI remained essentially stable, HSA was subjected to hydrolysis without the accumulation of detectable stable intermediates, suggesting the presence of multiple exposed cleavage sires sensitive to the action of the insect proteases on this cystatin. This apparent susceptibility of HSA to proteolytic cleavage may partially explain its low efficiency to inactivate insect OCI-insensitive cysteine proteinases when not used in large excess. It could also have major implications when planning the use of cystatin-expressing transgenic plants for the control oi coleopteran pests. (C) 1996 Wiley-Liss, Inc.

Whalon, M. E. and D. L. Norris (1996). "Resistance management for transgenic Bacillus thuringiensis plants." Biotechnology and Development Monitor(29): 8-12.

> Bacillus thuringiensis (Bt) toxin genes have been genetically engineered into dozens of plant species. By expressing a Bt toxin within their tissues, the plants protect themselves from some insect pests without farmers resorting to pesticide sprays. In addition, plant tissues otherwise difficult to reach with conventional pesticides can now be protected. However, to conserve the effectiveness of Bt plants, management measures are essential to control the build-up of resistance by the target insects.

Alstad, D. N. and D. A. Andow (1995). "Managing the Evolution of Insect Resistance to Transgenic Plants." Science **268**(5219): 1894-1896.

> The evolution of resistance in pests such as the European corn borer will imperil transgenic maize varieties that express insecticidal crystal proteins of Bacillus thuringiensis. Patchworks of treated and untreated fields can

delay the evolution of pesticide resistance, but the untreated refuge fields are likely to sustain heavy damage. A strategy that exploits corn borer preferences and movements can eliminate this problem. Computer simulation indicates that this approach can delay the evolution of resistance and reduce insect damage in the untreated fields of a patchwork planting regime.

Bauer, L. S. (1995). "Resistance - a Threat to the Insecticidal Crystal Proteins of Bacillus-Thuringiensis." Florida Entomologist **78**(3): 414-443.

Insecticidal crystal proteins (also known as delta-endotoxins) synthesized by the bacterium Bacillus thuringiensis Berliner (Bt) are the active ingredient of various environmentally friendly insecticides that are 1) highly compatible with natural enemies and other nontarget organisms due to narrow host specificity, 2) harmless to vertebrates, 3) biodegradable in the environment, and 3) highly amenable to genetic engineering. The use of transgenic plants expressing Bt delta- endotoxins has the potential to greatly reduce the environmental and health costs associated with the use of conventional insecticides. The complex mode of action of Bt is the subject of intensive research. When eaten by a susceptible insect delta-endotoxin crystals are solubilized in the midgut; proteases then cleave protoxin molecules into activated toxin which binds to receptors on the midgut brush border membrane. Part of the toxin molecule inserts into the membrane causing the midgut cells to leak, swell, and lyse; death results from bacterial septicemia. Insecticides formulated with Bt account for less than 1% of the total insecticides used each year worldwide because of high cost, narrow host range, and comparatively low efficacy. Environmental contamination, food safety concerns, and pest resistance to conventional insecticides have caused a steady increase in demand for Bt- based insecticides. The recent escalation of commercial interest in Bt has resulted in more persistent and efficacious formulations. For example, improved Bt-based insecticides have allowed management of the diamondback moth, Plutella xylostella (L.). Unfortunately this has resulted in the evolution of resistance to delta-endotoxins in P. xylostella populations worldwide. The recent appearance of Bt resistance in the field, corroborated by the results of laboratory selection experiments, demonstrates genetically-based resistance in several species of Lepidoptera, Diptera, and Coleoptera. The genetic capacity to evolve resistance to these toxins is probably present in all insects, and the heritability, fitness costs, and stability of the resistance trait are documented in several insect populations. In two strains of Bt-resistant lepidopteran species, mechanisms of resistance involve reductions in the binding of toxin to midgut receptors. Research on other resistant strains suggests that other mechanisms are also involved. Unfortunately, the high stability of the resistance trait, as well as broad spectrum cross- resistance to other delta-endotoxins, undermines many potential options for resistance management. Genetically engineered plants, expressing delta-endotoxin continuously and at ultrahigh doses, ensure intense and rapid selection of the target insect population. The efficacy of transgenic plants can be preserved only by developing an integrated pest management program that is designed specifically to reduce selection pressure by minimizing exposure to Bt and increasing other mortality factors, thereby slowing the rate of pest adaptation to Bt.

Gould, F., A. Anderson, et al. (1995). "Selection and Genetic-Analysis of a Heliothis-Virescens (Lepidoptera, Noctuidae) Strain with High-Levels of Resistance to Bacillus-Thuringiensis Toxins." Journal of Economic Entomology **88**(6): 1545-1559.

> Heliothis virescens (F.), tobacco budworms eggs were collected from 3 adjacent counties in North Carolina. A laboratory strain (YDK) was established from these eggs using precautions to avoid loss of genetic diversity. A subset of this laboratory strain (YHDS) was selected on artificial diet containing the Bacillus thuringiensis (Berliner) toxin, CryIA(c). In the first 12 episodes of selection only moderate resistance (approximate to 7- to 8-fold) was found. However, after 19 episodes of selection the strain had developed >500-fold resistance to the CryIA(C) toxin. Further selection lead to higher levels of resistance with the greatest resistance ratio recorded being approximate to 10,000-fold. The YHD2 strain was cross-resistant to CryIA(a), CryIA(b) and CryIF. We also found some resistance to CryIB, CryIC and CryIIA, but the level of resistance to these toxins was more moderate. Reciprocal genetic crosses between the resistant and control strains indicated that resistance to CryIA(c) and to CryIA(b) was partially recessive, but that the low level of resistance to CryIIA was more dominant. Progeny from backcrosses of FI larvae to the resistant parent were placed on artificial diet containing a concentration of CryIA(b) that had previously been found to slow the growth of F-1 larvae. When these larvae were weighed after 10 d, two clearly demarcated size classes were found in approximate to 1:1 ratios as is expected in backcrosses when a single locus (or a set of tightly linked loci) is coding for a major component of a recessive trait. Adults that developed from the larger size class of larvae were mated and their offspring inherited the ability to grow well on CryIA(b). As expected from the single locus model, one quarter of the offspring from matings of the small backcross larvae grew well on CryIA(b). Results of this selection experiment indicate that the initial frequency of this resistance trait could be approximately 10(-3), but field tests will be needed to confirm this rough estimate.

Hoy, C. W. and G. Head (1995). "Correlation between Behavioral and Physiological-Responses to Transgenic Potatoes Containing Bacillus-Thuringiensis Delta- Endotoxin in Leptinotarsa-Decemlineata (Coleoptera, Chrysomelidae)." Journal of Economic Entomology **88**(3): 480-486.

> Proposed resistance management strategies for Colorado potato beetle, Leptinotarsa decemlineata (Say), in transgenic potato crops containing

Bacillus thuringiensis delta-endotoxin include growing a mixture of transgenic and nontransgenic seed. The success of the strategy would be affected by behavioral responses of Colorado potato beetle to tile transgenic foliage in a mixture, and the correlation between behavioral response and physiological resistance. We reared full-sib families of Colorado potato beetle larvae from single mated pairs and measured behavioral response and physiological susceptil,ility; of larvae from each family to transgenic foliage. The behavioral assay consisted of a graded series of endotoxin concentrations, given by potato lines with different endotoxin expression levels. The physiological assay consisted of a single small transgenic leaf disk containing a dose estimated to be approximately the LD(50) for the parent generation. Significant genetic components were observed in the variation in both characters. Heritability estimates were particularly high for the physiological response. The correlation between the two characters was significant. More behaviorally responsive larvae tended to be more physiologically resistant. The implications for the seed mixture strategy are that larvae moving onto transgenic foliage in a mixture are more likely to return to the nontransgenic foliage if they are more resistant; indirect selection for physiological resistance could result. Because indirect selection for physiological resistance is possible for adult beetles as well as larvae, similar studies should be conducted on adults. The assumed benefits of seed mixtures, providing a refuge for susceptible beetles, also should be verified.

Kennedy, G. G. and M. E. Whalon (1995). "Managing Pest Resistance to Bacillus-Thuringiensis Endotoxins - Constraints and Incentives to Implementation." Journal of Economic Entomology **88**(3): 454-460.

Technological advances in combination with societal concerns over the potential for adverse health and environmental effects of chemical pesticides are operating to increase the use of Bacillus thuringiensis endotoxins in crop protection. Accompanying this increased use will be an increase in the intensity of selection for endotoxin resistance in a number of important pest species, To preserve the value of B. thuringiensis endotoxins in crop protection, it will be necessary to implement resistance management measures. This article analyzes the roles of pest control crises, societal values. level of awareness about resistance and resistance management, political action, economic considerations, competing technologies and products, and the difficulty of assessing the success of resistance management as constraints and incentives for the implementation of resistance management by the B. thuringiensis industry and by end users of B. thuringiensis products. Based on this analysis and a consideration of the attributes of new technologies that favor their ready adoption, we concluded that individual farmers have limited incentive to adopt resistance management technologies for B. thuringiensis endotoxins, and that the greatest incentive lies with the B. thuringiensis industry. However. the implementation of a coordinated, industry-wide, B.

thuringiensis resistance management effort is likely to be constrained by competition among segments of the B. thuringiensis industry vested in different technologies (sprays versus transgenic plants) and among producers of B. thuringiensis products using the same technology. It is further concluded that successful implementation of resistance management for B. thuringiensis endotoxins will likely require that the B. thuringiensis industry prepackage resistance management technologies with their products, and that these prepackaged resistance management strategies do not add significantly to the costs or complexity of pest control by the end user.

McBride, K. E., Z. Svab, et al. (1995). "Amplification of a Chimeric Bacillus Gene in Chloroplasts Leads to an Extraordinary Level of an Insecticidal Protein in Tobacco." Bio-Technology **13**(4): 362-365.

Bacillus thuringiensis (Bt) crystal toxins are safe biological insecticides, but have short persistance and are poorly effective against pests that feed inside plant tissues. Production of effective levels of these proteins in plants has required resynthesis of the genes encoding them. We report that amplification of an unmodified crylA(c) coding sequence in chloroplasts up to similar to 10,000 copies per cell resulted in the accumulation of an unprecedented 3-5% of the soluble protein in tobacco leaves as protoxin. The plants were extremely toxic to larvae of Heliothis virescens, Helicoverpa zea, and Spodoptera exigua. Since the plastid transgenes are not transmitted by pollen, this report has implications for containment of Bt genes in crop plants. Furthermore, accummulation of insecticidal protein at a high level will facilitate improvement in the management of Bt resistant insect populations.

Metz, T. D., R. T. Roush, et al. (1995). "Transgenic Broccoli Expressing a Bacillus-Thuringiensis Insecticidal Crystal Protein - Implications for Pest Resistance Management Strategies." Molecular Breeding **1**(4): 309-317.

We used Agrobacterium tumefaciens to transform flowering stalk explants of five genotypes of broccoli with a construct containing the neomycin phosphotransferase gene and a Bacillus thuringiensis (Bt) gene [CryIA(c) type] optimized for plant expression. Overall transformation efficiency was 6.4%; 181 kanamycin-resistant plants were recovered. Of the 162 kanamycin-resistant plants tested, 112 (69%) caused 100% morality of 1st-instar larvae of a Bt-susceptible diamondback moth strain. Southern blots of some resistant transformants confirmed presence of the Bt gene. Selected plants that gave 100% mortality of susceptible larvae allowed survival of a strain of diamondback moth that had evolved resistance to Bt in the field. F-1 hybrids between resistant and susceptible insects did not survive. Analysis of progeny from 26 resistant transgenic lines showed 16 that gave segregation ratios consistent with a single T-DNA integration. Southern analysis was used to verify those plants possessing a single T-DNA integration. Because these transgenic plants kill susceptible larvae and F, larvae, but serve as a suitable host for resistant ones, they provide an excellent model for tests of Bt resistance management strategies.

Nault, B. A., P. A. Follett, et al. (1995). "Assessing Compensation for Insect Damage in Mixed Plantings of Resistant and Susceptible Potatoes." American Potato Journal **72**(3): 157-176.

Plant mixtures have been proposed for pesticidal transgenic potatoes as a means to reduce selection intensity favoring resistant insect genotypes. Colorado potato beetle, Leptinotarsa decemlineata (Say), defoliation was simulated in mixed plantings of susceptible and resistant potato "mimics" to evaluate yield compensation. Various mixtures of susceptible and resistant potato were planted at two densities and two locations in eastern North Carolina. Resistant plants were undamaged throughout the season whereas susceptible plants were completely defoliated by hand either during early or late bloom. The ability of non-defoliated plants to compensate for neighboring defoliated plants was investigated through single- plant and small-plot field experiments for 2 years. Yield compensation for defoliated plants by neighboring non- defoliated plants was not evident in our studies. Yield of two potato plants, positioned on either side of a defoliated plant, was not different from yield of two potato plants positioned on either side of a non-defoliated potato plant. Compensation in mixtures of resistant and susceptible potato was not evident using several non-linear regression analyses. A negative linear relationship existed between yield and an increasing percent of susceptible plants in the mixture for all planting densities, at each location, every year.

Caprio, M. A. (1994). "Bacillus-Thuringiensis Gene Deployment and Resistance Management in Single-Tactic and Multi-Tactic Environments." Biocontrol Science and Technology **4**(4): 487-497.

The effectiveness of two resistance management strategies (RMS) for transgenic crops was examined via simulation in single- tactic (all fields in a population use the same strategy) and multi-tactic (fields either employ transgenic plants or are treated by foliar Bacillus thuringiensis (Bt) applications) environments. The life tables and response to selection used in the simulations resembled those of a simplified, heliothine- like species. In single-tactic environments, the refuge strategy was the most effective approach for delaying the evolution of resistance to toxin genes incorporated into plants. Resistance was delayed most when pure stands of transgenic plants were used with no refugia and the reproductive capacity of the insect was low because the entire population became extinct, though this result may be dependent on the small population size examined (nine fields). With higher levels of reproduction, however this strategy resulted in rapid evolution of resistance. When the insect populations were managed in the refuge fields (but not with the Bt toxin gene), resistance developed more rapidly as migration from the refuge area decreased with the population size. The seed mixture strategy in which 11.1% of the plants in a field were non-transgenic, was intermediate in effectiveness and less affected by changes in assumptions of the net rate of increase of the insect. The unmanaged refuge strategy was also the most effective strategy in the multi-tactic simulations. Assuming minimal costs are associated with implementation of RMS in transgenic fields, cooperation by transgenic producers always increased the durability of the toxin gene. Cooperation with a RMS by foliar Bt producers also increased the durability of the toxin gene, but did not compensate for the reduction in the number of sprays/year. In this system, there did not appear to be justification for compliance by foliar producers in a RMS based solely on reducing the number of treatments/year (other strategies of reducing pesticide use may be less affected). Introduction of transgenic plants into the system increased the durability of the toxin gene if producers rising foliar products did not adopt the RMS, but decreased durability when compared with simulations of foliar producers (without transgenic crops) who had adopted a RMS. A simple, deterministic model was used to estimate the potential effect of inbreeding within subpopulations on resistance development. Variance in gene frequencies due to genetic drift and inbreeding resulted in more rapid selection for recessive alleles than would be predicted from single field models.

Ebora, R. V., M. M. Ebora, et al. (1994). "Transgenic Potato Expressing the Bacillus-Thuringiensis Cryia(C) Gene Effects on the Survival and Food-Consumption of Phthorimea-Operculella (Lepidoptera, Gelechiidae) and Ostrinia-Nubilalis (Lepidoptera, Nochuidae)." <u>Journal of Economic Entomology</u> **87**(4): 1122-1127.

Second generation transgenic potatoes were grown from tubers of transgenic plants expressing the Bacillus thuringiensis CryIA(c) gene. Leaf disks from transgenic and untransformed potato plants were tested against the tubermoth Phthotimea operculella (Zeller), a major pest of potato, and European com borer, Ostrinia nubilalis (Hubner), which can use potato as an alternative host. Ten percentage of mortality of first-instar P. operculella was observed after 48 h of feeding on leaf disks from transgenic plants. Bioassays also showed that second- instar P. operrulella is slightly less capable of surviving on leaf disks from transgenic plants than on untransformed plants after 240 h of feeding. The amount of feeding of P. operculella larvae was also less, as indicated by lighter weight of the dried frass. Bioassay showed that second-instar European com borer is less capable of surviving on leaf disks from transgenic plants than those from untransformed plants. Preference tests showed that leaf disks from transgenic plants were less preferred than those from untransformed plants by third-instar com borer after 24 h of exposure. Prolonged exposure showed that leaf disks from transgenic and untransformed plants were equally preferred by com borer larvae. These transgenic plants can be used together with untransformed plants or transgenic plants (or both) expressing high quantity of B. thuringiensis insecticidal protein to devise insect resistance management strategies.

Forrester, N. W. (1994). "Resistance Management Options for Conventional Bacillus- Thuringiensis and Transgenic Plants in Australian Summer Field Crops." Biocontrol Science and Technology **4**(4): 549-553.

Conventional Bacillus thuringiensis (Bt) is being increasingly used for control of Helicoverpa spp. in Australian cotton. Five years ago, the annual useage of Bt in cotton was less than 1O 000 l. It is now about 200 000 l but still represents only about 0.5 of a spray in cotton which normally receives 6-8 conventional insecticide sprays for Helicoverpa control each season. Bt use in other field crops has been minimal. The present risk for development of resistance to Bt in Helicoverpa spp, is, therefore, low. However any increased use of conventional Bt on cotton or extended registrations to alternative Helicoverpa host crops could increase the risk significantly. Susceptible bioassay baselines and discriminating doses for conventional Bt on both H. armigera and H. punctigera are currently being established. The aim is to assess the natural variability in bioassay response (diet incorporation technique using early third instars) in a number of strains of both species from a number of geographical areas. This will allow monitoring of the impact of increased Bt use and/or the introduction of transgenic cotton on potential resistance problems. The ecology of H. armigera and H. punctigera is discussed in this paper, particularly in relation to the differential resistance risk of the two species where H. punctigera essentially 'manages its own resistance'. This is extended to the development of a possible resistance management strategy for H. armigera on transgenic cotton which will rely on continual dilution of rare resistant mutants by large numbers of unsprayed susceptibles allowed to breed in designated refugia areas. This high-dose/high-immigration resistance management approach will essentially maintain resistant H. armigera individuals as rare, hopefully functionally recessive heterozygotes. Two other factors will be critical for the success of this strategy. (1) strains of conventional Bt with different toxin profiles (e.g. Bt subsp. aizawai) should be used on non-transgenic alternative crop hosts of H. armigera (e.g. sorghum, sunflowers, grain legumes, maize, tomatoes and oilseeds), and (2) development of transgenic alternative crop hosts of H. armigera (especially sorghum and maize) should concentrate on toxins other than those from Bt.

Gould, F. (1994). "Potential and Problems with High-Dose Strategies for Pesticidal Engineered Crops." Biocontrol Science and Technology **4**(4): 451-461.

We lack an empirical basis on which to judge the expected durability of crops that express one or more insecticidal proteins and must therefore rely upon theoretical population genetic models in assessing how best to delay pest adaptation to these toxins. A number of studies using such models indicate that expression of toxins at very high levels could slow pest adaptation to a crawl if the ecology and genetics of the pest and cropping system fit specific assumptions. These assumptions relate to: (1) inheritance of resistance factors; (2) ecological costs of resistance factors; (3) behavioral response of larvae and admits to the toxins; (4) plant-to-plant movement of larvae; (5) adult dispersal and mating behavior; and (6) distribution of host plants that do and do not produce the toxin(s). This paper includes a discussion of whether the biology of insect pests of a number of cropping systems that are targets for toxin-expressing plants fit assumptions that are conducive to slowing pest adaptation. Emphasis is placed upon new data regarding the behavior and movement of larvae and the mating structure of populations. New theoretical work that examines the importance of natural enemy mortality and fitness costs of resistance is presented. This paper also discusses how the complexity of developing resistance management strategies increases when multiple pests and multiple crops are considered. Finally, the problems associated with developing resistance management strategies in poor and developing countries are described.

Heckel, D. G. (1994). "The Complex Genetic-Basis of Resistance to Bacillus-Thuringiensis Toxin in Insects." Biocontrol Science and Technology **4**(4): 405-417.

Recent studies have shown that the genetic basis of resistance to Bacillus thuringiensis (Bt) toxin in insects is far from simple. In this respect, it is similar to resistance to chemical insecticides, which is conferred by multiple physiological mechanisms under independent genetic control. Several lines of evidence for a complex genetic basis for resistance in tobacco budworm Heliothis virescens and diamondback moth Plutella xylostella are reviewed. This suggests that a genetic approach based on linkage mapping, capable of simultaneously analyzing multiple physiological mechanisms, will be very useful in dissecting the complex trait of resistance. In Heliothis, the existence of separate, independently asserting resistance genes has already been confirmed by linkage analysis with marker loci. This approach assists in the measurement of the relative potency of different resistance genes present in the same strain. Additionally, it facilitates comparative studies of the independent acquisition of homologous resistance mechanisms by different species, Perhaps most important from a resistance management standpoint, it directly addresses the question of whether cross-resistance is due to separate loci or the same locus. Differing properties of the diverse physiological mechanisms capable of conferring resistance yield different outcomes when contrasting scenarios of transgene toxin deployment are considered. These genetic issues have important ecological implications because they affect the rate of resistance evolution in the pests, an understanding of which is essential to the development of sensible deployment strategies of transgenic crop plants to maximize their efficacy over the long term.

McGaughey, W. H. (1994). "Implications of Cross-Resistance among Bacillus-Thuringiensis Toxins in Resistance Management." Biocontrol Science and Technology **4**(4): 427-435.

> Insect resistance is now recognized as a serious threat to the long-term usefulness of Bacillus thuringiensis (Bt) toxins in pest management. Because of the great diversity among Bt. toxins found in nature, one of the most tempting resistance management strategies is to use two or more of these toxins in mixtures, rotations or sequences. Cross-resistance among toxins and the ability of insects to develop resistance to multiple toxins will limit the success of this approach. Studies have shown that there are large differences in the cross-resistance spectrum of the insect species that have been selected for resistance using single toxins or simple mixtures. Other studies have demonstrated that some species can readily develop resistance to mixtures of toxins, and that the use of strains of Bt that produce a wide array of toxins can cause broad- spectrum resistance to most Bt toxins. These factors tend to be specific to individual insect species and must be considered when attempting to manage insect resistance using multiple toxin strategies. Polygenic inheritance and the existence of multiple mechanisms of resistance may be involved in broad-spectrum resistance, and may limit the use of multiple toxin strategies for managing resistance.

McGaughey, W. H. (1994). "Problems of Insect Resistance to Bacillus-Thuringiensis." Agriculture Ecosystems & Environment **49**(1): 95-102.

> Insect resistance to Bacillus thuringiensis (Bt) has been a recognised problem for only about 6 years. It now seriously threatens both conventional and gene transfer uses for this environmentally safe biological insecticide. Since 1985, the potential for resistance has been demonstrated in at least five insect species, and high levels of resistance among field populations have been reported in one species. In two moth species, Plodia interpunctella and Plutella xylostella, the potential for resistance is widespread among diverse populations and laboratory studies suggest that it can progress to high levels within only a few generations. The mechanism of resistance in these species involves a change in binding affinity of the insects' midgut membrane that is specific for the particular toxin type used in selecting the resistant population. Recognition of the inevitability of Bt resistance in insects has led to increased research on deployment strategies that might delay or prevent its evolution. Although resistance to Bt toxins expressed in genetically engineered plants has not been reported yet, it is imperative that resistance management tactics be developed before resistance reduces the pest control value of Bt. Currently, the focus of strategies for managing resistance is on techniques that minimise selection pressure, such as providing untreated refuges, and on the use of multiple toxins in various mixture, mosaic, rotational, or sequential patterns. Experimental data are

needed to support the value of these approaches in different pest and cropping systems.

Metz, T. D., R. Dixit, et al. (1994). "Analysis of Bt Insecticidal Crystal Protein Expression in Transgenic Brassica-Oleracea - Applications to Insect Resistance Management." Journal of Cellular Biochemistry: 89-89.

Roush, R. T. (1994). "Managing Pests and Their Resistance to Bacillus-Thuringiensis - Can Transgenic Crops Be Better Than Sprays." Biocontrol Science and Technology **4**(4): 501-516.

Insecticidal toxins from Bacillus thuringiensis (Bt) can now be deployed either in sprays or transgenic plants. Some entomologists and environmentalists have argued that the sprays are preferable to plants because they are less likely to cause resistance. However Bt sprays are not generally competitive with chemical insecticides and seem unlikely to displace them. In contrast, transgenic plants appear to be sufficiently effective to displace chemicals, making such planes attractive from the standpoint of environmental protection. Further, simulation models using data from the diamondback moth and a laboratory experiment using the Indianmeal moth suggest that under at least some circumstances, transgenic plants bearing only one Bt gene may be more effective than sprays for delaying resistance to Bt. Resistance in a laboratory-selected strain of the Colorado potato beetle is especially interesting because a strain that can survive Bt sprays and develop to maturity cannot develop successfully on transgenic plants, not even on some showing very low expression. This suggests that more mechanisms are available for resistance to sprays than to transgenic plants, perhaps making resistance to sprays more 'evolutionarily available'. When the use of two toxin genes is considered, simulation models suggest that transgenic plants may be much move durable than sprays of similar efficacy.

Tabashnik, B. E. (1994). "Evolution of Resistance to Bacillus-Thuringiensis." Annual Review of Entomology **39**: 47-79.

Vandersalm, T., D. Bosch, et al. (1994). "Insect Resistance of Transgenic Plants That Simultaneously Express Modified Bacillus-Thuringiensis Cryia(B) and Cryic Genes - a Resistance Management Strategy." Biocontrol Science and Technology **4**(4): 483-483.

Vandersalm, T., D. Bosch, et al. (1994). "Insect Resistance of Transgenic Plants That Express Modified Bacillus-Thuringiensis Cryia(B) and Cryic Genes - a Resistance Management Strategy." Plant Molecular Biology **26**(1): 51-59.

Tobacco and tomato plants were generated exhibiting insect resistance due to the introduction of modified cryIA(b) and cryIC genes of Bacillus thuringiensis. Limited modifications at selected regions of the coding sequences of both genes are sufficient to obtain resistance against

> Spodoptera exigua, Heliothis virescens and Manduca sexta. The criteria used to modify both genes demonstrate that the removal of sequence motifs potentially resulting in premature polyadenylation and transcript instability causes increased insect resistance. The expression of a cryIC-cryIA(b) fusion resulting in protection against S. exigua, H. virescens and M. sexta demonstrates the potential of expressing translational fusions, not only to broaden the insect resistance of transgenic plants, but also to simultaneously employ different gene classes in resistance management strategies.

Wearing, C. H. and H. M. T. Hokkanen (1994). "Pest Resistance to Bacillus-Thuringiensis - Case-Studies of Ecological Crop Assessment for Bt Gene Incorporation and Strategies of Management." Biocontrol Science and Technology **4**(4): 573-590.

> An ecological assessment is made of the suitability of apples and kiwi fruit in New Zealand for transformation with genes from Bacillus thuringiensis (Bt). The assessment focuses on the risk of pests of these crops developing resistance to the Bt toxins expressed in the plants or in Bt used as a biopesticide. The key target pests which were assessed were tortricids. The most important ecological features of the crops and their pests which influenced the assessment were the host range of the pests, the availability of refugia of susceptible insects on non-transformed host plants within and around the crop, the mobility of the pests enabling the flow of genes for susceptibility into the selected pest populations, the ratio of susceptible to selected pest individuals, and insect behaviour in relation to Bt gene expression in the crop. Strategies are proposed for the management of transformed apple and kiwi fruit to maintain pest susceptibility to Bt, and if these are followed, the crops are considered suitable for transformation. It is essential that these Bt crops are deployed within an integrated pest management programme. The greatest risk of resistance to Bt is associated with polyphagous leaf rollers which also attack white clover, a crop which is a further candidate for transformation with Bt genes. This crop and its pest complex require full assessment of resistance risk before this step is taken.

Wigley, P. J. and C. N. Chilcott (1994). "Present Use of, and Problems with, Bacillus-Thuringiensis in New-Zealand." Agriculture Ecosystems & Environment **49**(1): 45-50.

> The use of Bacillus thuringiensis (Bt) as a foliar spray to Control insect pests has received scant attention from New Zealand growers apart from application to kiwifruit during the period of bee pollination. Major problems underlying this lack of use are the very high standards of quarantine and quality demanded of export produce, the relatively high cost of Bt and its shortcomings in efficacy and persistence. A substantial increase in use appears unlikely unless there is a significant reduction in synthetic pesticide use on export crops. In contrast, rapid progress on research into

plant transformation in New Zealand has led to proposals to insert Bt genes into an increasing range of crops. Although transgenic plants show great potential for pest control, there is concern that continual expression of Bt proteins in plant tissue will increase the intensity of selection for pest resistance. A particular concern is the possibility that Bt-resistant cosmopolitan pests may spread between different transgenic crops. A plan to combat this problem through the integrated use of different Bt genes in different crops is suggested.

Wigley, P. J., C. N. Chilcott, et al. (1994). "Conservation of Bacillus-Thuringiensis Efficacy in New-Zealand through the Planned Deployment of Bt Genes in Transgenic Crops." Biocontrol Science and Technology **4**(4): 527-534.

Although the use of Bacillus thuringiensis (Bt) as a foliar spray is minimal within New Zealand at present, a considerable effort is being made genetically to incorporate Bt delta- endotoxin genes into a wide range of important crops, including pasture species, perennial horticultural crops and annual field crops. For several years it has been clear that the major threat to the sustained efficacy of Bt ill New Zealand is the evolution of insect resistance. Recognition of this threat has prompted consideration of a range of strategies for preventing and/or managing its possible evolution in pests of specific crops. However, such strategies do not address the critical issue of how we might limit the consequences of Bt resistance if it did evolve, particularly the evolution of resistance in polyphagous pests. Highly mobile polyphagous pests, such as leafroller species, might develop resistance to Bt on one transgenic crop and then disperse, nullifying the effectiveness of a wide range of Bt transgenic crops expressing the same or similar Cry proteins. Pests with resistance to CryIA proteins in transgenic plants might also display significant resistance to Bt biopesticides. To prevent such an eventuality in New Zealand we propose a plan for deploying Bt genes among crops according to an assessment of the risk and consequences of Bt resistance evolving in the insect complexes on these crops. The major elements of this plan are: (i) assess the risk of Bt resistant insects evolving and dispersing out of the crop to infest others; (ii) characterize the diversity of Bt protein binding sites in the guts of key polyphagous pests; (iii) use the above information to deploy Bt genes among different transgenic crops in a pattern that minimizes the chance that any future Bt resistant cosmopolitan pests will be broadly cross-resistant to other Bt crops and/or Bt biopesticides.

Whalon, M. E., D. L. Miller, et al. (1993). "Selection of a Colorado Potato Beetle (Coleoptera, Chrysomelidae) Strain Resistant to Bacillus-Thuringiensis." Journal of Economic Entomology **86**(2): 226-233.

For the first time, resistance to the CRYIIIA coleopteran specific delta endotoxin of Bacillus thuringiensis var. san diego or tenebrionis is reported. Colorado potato beetles, Leptinotarsa decemlineata (Say), were

selected in the laboratory after exposure in potato fields in Michigan. After 12 generations of selection, the selected strain was 59 times more resistant than the unselected strain and 24-35 times more resistant than other susceptible or insecticide-resistant strains. We observed no cross-resistance between organophosphate, carbamate, or pyrethroid resistance and B. thuringiensis resistance. No difference in movement induced by B. thuringiensis or initial feeding behavior was observed between selected and unselected strains, although a shift toward petiole feeding behavior was observed in adults (of both strains) that were stressed by B. thuringiensis. The selected strain oviposited at a reduced rate of 0.1 +/- 0.15 (mean +/- SD) egg masses per day) on treated foliage; the unselected strain was oostatic. Based on these results, it is recommended that resistance management strategies be developed and deployed for both conventionally applied B. thuringiensis products and B. thuringiensis transgenic plants.

Gould, F., A. Martinezramirez, et al. (1992). "Broad-Spectrum Resistance to Bacillus-Thuringiensis Toxins in Heliothis-Virescens." <u>Proceedings of the National Academy of Sciences of the United States of America</u> **89**(17): 7986-7990.

Evolution of pest resistance to insecticidal proteins produced by Bacillus thuringiensis (Bt) would decrease our ability to control agricultural pests with genetically engineered crops designed to express genes coding for these proteins. Previous genetic and biochemical analyses of insect strains with resistance to Bt toxins indicate that (i) resistance is restricted to single groups of related Bt toxins, (ii) decreased toxin sensitivity is associated with changes in Bt- toxin binding to sites in brush-border membrane vesicles of the larval midgut, and (iii) resistance is inherited as a partially or fully recessive trait. If these three characteristics were common to all resistant insects, specific crop-variety deployment strategies could significantly diminish problems associated with resistance in field populations of pests. We present data on Bt-toxin resistance in Heliothis virescens, a major agricultural pest targeted for control with Bt-toxin- producing crops. A laboratory strain of H. virescens developed resistance in response to selection with the Bt toxin CryIA(c). In contrast to other cases of Bt-toxin resistance, this H. virescens strain exhibits cross-resistance to Bt toxins that differ significantly in structure and activity. Furthermore, the resistance in this strain is not accompanied by significant changes in toxin binding, and resistance is inherited as an additive trait when larvae are treated with high doses of CryIA(c) toxin. These findings have important implications for Bt-toxin-based pest control.

Mallet, J. and P. Porter (1992). "Preventing Insect Adaptation to Insect-Resistant Crops - Are Seed Mixtures or Refugia the Best Strategy." <u>Proceedings of the Royal Society of London Series B-Biological Sciences</u> **250**(1328): 165-169.

Transgenic crops expressing insecticidal toxins could soon provide safe,

clean and effective means of pest control, but their usefulness will be short-lived if insects adapt to the toxins. Two planting strategies are among those that have been recommended to delay crop failure: susceptible insects could be conserved by planting either 'refugia', i.e. separate fields of toxic and toxin-free crop, or 'seed mixtures' of toxic and toxin-free plants in the same fields. However, we show that if insects can move from plant to plant, seed mixtures may actually hasten insect resistance compared with pure stands of toxic plants. Insect movement causes an increase in effective genetic dominance which can counteract reduced selection due to the mixture. This failure of seed mixtures is likely under just those conditions, low genetic dominance of resistance, which predict a good chance for resistance to the toxin to evolve slowly. Seed mixtures, unlike refugia, are therefore failure prone. This result also suggests potential problems with a third strategy, tissue-specific expression of toxins, which essentially provides a mixture of toxin-free and toxin- containing tissues on the same plant. However, better information and modelling are urgently required to evaluate alternative means of slowing insect adaptation to resistant crop plants. Legislation for toxin-free refugia may provide one of the best available means for conserving insect susceptibility.

Vanrie, J., H. Vanmellaert, et al. (1992). "Mechanism of Insect Resistance to Bacillus-Thuringiensis in Plodia Interpunctella and Plutella-Xylostella." Acs Symposium Series **505**: 191-199.

The mechanism of resistance to Bacillus thuringiensis insecticidal crystal proteins (ICPs) was investigated in a laboratory-selected and a field-selected insect strain. In both cases, insect resistance appeared to be due to changes in ICP receptors, suggesting that alterations in ICP receptors are a general mechanism by which insects can adapt to B. thuringiensis. The absence of cross resistance to ICPs other than those present in the selecting agent and the finding that these ICPs bind to distinct receptors indicate that the use of ICP mixtures or multiple ICP expressing transgenic plants may be a valuable resistance management tactic.

Gould, F. and A. Anderson (1991). "Effects of Bacillus-Thuringiensis and Hd-73 Delta-Endotoxin on Growth, Behavior, and Fitness of Susceptible and Toxin-Adapted Strains of Heliothis-Virescens (Lepidoptera, Noctuidae)." Environmental Entomology **20**(1): 30-38.

A commercial formulation of Bacillus thuringiensis var. kurstaki Berliner (Dipel 2X) and purified HD-73 delta-endotoxin were evaluated for effects on the growth, behavior, and fitness of susceptible and toxin-adapted strains of Heliothis virescens (F.) by incorporation of the materials into artificial diets at varying concentrations. Neonate larvae were placed in experimental arenas where they could choose a diet containing toxin and a control diet, or were placed in arenas where the only diet available contained a toxin. The difference between the strains in growth and survival was much more pronounced when larvae could not choose to feed on the control diet. Both strains avoided moderate and high concentrations of the toxins. At the lowest concentration of HD-73, neither strain avoided the diet that contained the endotoxin. At the two lowest concentrations of Dipel 2X, only the susceptible strain avoided the diet containing Dipel. Data on growth rate, survival, and fecundity of the two strains reared on HD-73 delta-endotoxin were used to set parameters of a single-locus genetic model to predict the rate at which H. virescens populations would adapt to B. thuringiensis var. kurstaki or its endotoxin when exposed to it in choice and no-choice situations. Results from the model generally predict much more rapid adaptation in no-choice situations.