KEYWORDS: genetically modified organism, Bacillus thuringiensis, ecological impact

The Yin and Yang of Killer Corn

Hardy Hall
Botany, University of British Columbia
Submitted January 2003

Introduction

Bt corn, a genetically modified food (GMO), has been both the poster-child and thorn in the side of the plant biotechnology industry from the late nineties to present. While plants possess and barrage of secondary metabolites to deter insects (Pare and Tumlinson 1999), these natural plant strategies have proven inadequate in fighting invading crop pests, even under the influence of the strong artificial selection pressures of modern breeding programs. Biotechnology, however, has radically transformed crop development, providing immediate solutions to age-old agricultural problems. Bt corn is generated by the simple insertion of an insecticidal protein from a bacterial plant pathogen directly into a corn race. Despite this minor genetic alteration, Bt corn has been mired by complications.

With all its inherent risks, the development and implementation of Bt corn may be an immeasurable social and ecological liability. Current debate in North America has centered on the health and ecological risks associated with the extensive use of Bt corn, while a ban exists on all transgenic crop cultivation in Europe pending a definitive safety assessment of Bt corn. Biotechnology advocates assert that unreasonable fears have arisen from the public's understanding of the motives and methods of the genetic engineer. Opponents of Bt corn use have raised many valid concerns stemming from a lack of evidence supporting Bt corn safety.

Towards a better understanding of the unique risks posed by transgenic Bt corn crops, this review provides a history and habit of the European corn borer, the natural and agricultural role of *Bacillus thuringiensis* as a pathogen and "insecticide", and the biotechnological methods used to harness Bt's insecticidal properties through transgenic means. I present a summary of the major human health and environmental issues and of the current (polarized and partisan) debate around these potential risks.

History and Habit of the European Corn Borer

The European corn borer moth (*Ostrinia nubilalis*) lives most of its life as a caterpillar, burrowing through corn (*Zea mays*) stems. It is thought to have first arrived in Boston, Ma. in the early 1900s with a shipment of corn brooms (Miles *et al*. 2002). Since then, it has spread to the vast majority of North American corn producing regions (De Maagd, 2001), causing significant damage to crop quality and production. Removal of post-harvest corn stubble has been the most popular means of controlling the corn borer populations (Loake 2001), although this method often fails to prevent major outbreaks and localized crop loss. The search for quicker and more cost-effective means of controlling ECB has led researchers to investigate natural enemies of the ECB.

History and Habit of *Bacillus thuringiensis*

Bacillus thuringiensis (Bt) has long been recognized as an insect pathogen, targeting flies (Order Diptera), beetles (Order Coleoptera), and moths/butterflies (Order Lepidoptera). Dormant Bt spores reside on plant matter and are ingested along with plant matter by various insects where they germinate and proliferate. Bt rapidly reproduces and then sporulates just prior to the final instar of the insect larva (Aronson, 2002). During sporulation, the formation of a protective coat for dormancy, results in spores appearing crystal-like during this late stage. In the process, the larva is killed and plant matter is infected for subsequent ingestion

within a two-year maximum dormancy period.

The coevolution of Bt bacterial strains with insect families has led to a wide diversity of unique host-pathogen relationships (De Maagd *et al*. 2001). There are thirty-four known subspecies of Bt that collectively express more than 100 known proteinaceous insecticidal proteins which crystallize in intracellular inclusion bodies (Crickmore *et al*. 1998). Production of these endotoxins correlates closely with the Bt sporulation phase. They have been grouped into two classes; cytolysins (Cyt) and crystal delta-endotoxins (Cry). While Cyt proteins are largely effective towards the insect orders Coleoptera and Diptera, Cry proteins target Lepidopterans (Aronson 2002).

In the 1980's, genetic and molecular discoveries have provided both an evolutionary model for host-specificity and a proposed mechanism of action for Cry and Cyt proteins. In the case of Cry proteins [1], a fatal interaction between endotoxins and their insect victims occurs in the mid-gut of maturing Lepidopteran larvae. There, digestion of a portion of the Bt population results in the rupture of Bt inclusion bodies followed by cleavage of Cry proteins by insect proteases to produce three protein domains which perform various functions resulting in the lysis of the epithelial cell (Hofte and Whiteley 1989). The N-terminal domain is thought to be involved in pore formation, promoting lysis, whereas the C-terminal domain and interior domain are thought to bind to the BTR1 receptor in the epithelial cell membrane, instigating a signal transduction matrix within the target cell resulting in upregulation of other lytic mechanisms (Dorsch *et al*. 2002).

Each Bt strain produces several Cry proteins, forming an arsenal of endotoxins that together define host specificity. Genetic analysis of Bt strains causing insect mortality has shown that toxicity depends on a number of Cry gene loci (Rahardja and Whalon 1995). Insect epithelial cell receptors have co-evolved to elude this 'lock-and-key' toxicity. Thus, specificity is also determined by the BT-R1 variation within and between insect species.

Bt as Biological Control Agent

With the growing knowledge of the molecular basis of Bt pathogenicity, artificial selection techniques have produced Bt strains that target a number of specific pests, such as ECB on the basis of Cry gene arrays. Bt has been recognized for its potential as a biological control agent (BCA) since its first use as a foliar insecticide[2] in France in 1938 (Van Frankenhuyzen 1993). Confidence in Bt strain host specificity has prompted the organic food industry to adopt it as a de sirable chemical insecticide substitute for control of many crop pests [3]. Yet, over-reliance on Bt as the principle insecticide may lead to the development of insect resistance to Cry proteins, and Bt in general (Bourguet *et al*. 2000;Rahardja and Whalon 1995).

While Bt has become a key component in the integrated pest management[4] of many Lepidopterons (like ECB), the commercial application of foliar Bt on corn crops has been limited compared to its theoretical potential as a target-specific and safe insecticide against ECB. Unnecessary crop losses often result from undetected infestations poorly-timed applications of Bt, and undesirable climatic conditions during treatment periods. Bt Cry proteins are sensitive to degradation (light, heat, and desiccation) and don't pe netrate into the affected tissues, thereby limiting their effectiveness at controlling the ECB. Given the poor efficacy of Bt against ECB, largescale spraying of corn is very cost prohibitive.

The Search for a Cry Protein Delivery System

A means penetrating tissue with Bt is required to offer long-term, preventative measures against tunneling insects. It is not feasible to integrate dormant pathogenic bacteria into living, growing plant tissue and the growth, maintenance and proper application of such strains can be viewed as unnecessary inconveniences. Furthermore, Bt becomes toxic only after damage the larvae have developed at the expense of the crop. A significant reduction in the growth of the larvae could be achieved by the direct application of Cry proteins. The cloning and characterization of several crystal-delta endotoxins has created some interesting possibilities. Synthetic insecticides that mimic Cry proteins offer a means to circumvent the limitations of conventional Bt sprays (Berenbaum 1995). However, these synthetic compounds are susceptible to rapid degradation on exposed surfaces, favoring an endogenous expression system in corn.

The lateral transfer of genes from one organism to another by recombinant DNA technologies allows a quick means of adding entirely new traits to crops. Ectopically expressed Cry proteins in corn, offer a cost-effective and practical means of limiting crop loss to ECB. There are several competing recombinant technologies for transforming monocotyledonous plant tissues, cells, or protoplasts[5]. DNA bombardment, a direct method involving a "gene gun" has not been widely applied since multiple copy insertions lead to end-product complications and equipment is costly. Furthermore, DNA bombardment is effective only on cell cultures *in vitro*, not *in planta* due to cellulose and lignin in cell walls which impede glass microtubes. Also, vacuoles are huge and can get ruptured, releasing hydrolases. Silicon carbide- mediated transformation is a "quick and dirty" method whereby cell cultures are vortexed[6] with DNA. Unfortunately, a high degree

of cell damage and low transformation efficiency have prevented the application of this technique on a commercial scale. Electroporation is a common bacterial transformation tool, and can work with some success on protoplasts, yet it has remained a specialized research tool due to poor plant regeneration and low expression levels resulting from deleterious effects of the electric field and cell wall degradation enzymes.

Agrobacterium tumefaciens t-DNA insertion (indirect method) remains the tool of choice for plant biotechnologists. Unlike other transformation methods, this system has not been adapted from animal transformation systems. *A. tumefaciens* is the pathogenic agent in crown gall disease. The cancerous tumours that define this disease arise from a unique bacterial transfor mation mechanism involving the Ti-plasmid[7] which coordinates the random insertion of T-DNA into the chromosome of woody (dicotyledenous) plant cells. T-DNA is known to contain genes which encode and up-regulate two key plant hormones controlling cellular division; auxin and cytokinin. Additionally, TDNA encodes opine synthases for producing the *A.tumefaciens* food supply. T-DNA has been modified such that these pathogenic genes are replaced with genes of interest, while the transformation genes are preserved.

T-DNA is known to stably integrate single copies per cell, making this a very useful transformation tool. *A.tumefaciens* is a dicot-specific pathogen, so transformation efficiency in monocots (grasses, including corn) has been a major technical roadblock. Recently, modifications to the recombinant-specific regions of the Ti-plasmid have enabled the stable integration of Cry genes in corn with an acceptable transformation efficiency (Huckelhoven *et al*. 2000). Cry gene expression was initially low, until researchers recognized that deletions in the C-terminal regions enhanced expression of Cry genes in transgenic Bt corn (Ishida 1996). What started as a theoretical possibility has turned into a major source of revenue for its developers (seed sales) and farmers (insecticide savings and increased productivity). Additionally, the reduction in the wounding rate by herbivory (from ECB) has the added benefit of limiting subsequent infections from opportunistic plant pathogens (Munkvold *et al*. 1997;Rao *et al*. 2000).

The Drawbacks of Bt Corn

The promise of this technology has been largely overshadowed by concerns about the unintended effects of Bt corn to human health and environment. Cry protein toxicity, allergenicity, and lateral transfer of antibiotic resistant marker genes to the microflora of our digestive system threaten to compromise human health. The environment is potentially vulnerable to the toxic effects of Bt corn on non-target organisms, transgenic gene escape to related corn species, and the development of resistance in ECB and other pests. These concerns, however, have not been evident in the use of the *Bacillus thuringiensis* as a foliar spray and may be unique to Bt corn, arising from the complex effects of a constitutive presence of Cry proteins in all tissues or the effects of such expression in context of the plant genome, proteome, and metabolome.

Purified Cry proteins have been shown in many cases[8] to have no apparent toxicity to mammals at levels in great excess of endogenous Cry proteins in Bt corn (Turlings *et al*. 2000). In fact, levels of Cry proteins in excess of 10,000 times endogenous Bt corn levels are not persistent in simulated human gastrointestinal environments. Yet these data are not considered admissible in the safety assessment of Bt corn (Frey *et al*. 2000). Research that affirms the harmful effects of transgenically expressed endotoxin has been criticized for lacking appropriate controls, while studies to the contrary also display a common lack of harmonized design (Frey *et al.* 2000). Cry proteins' acute and chronic toxicity must be measured in the context of Bt corn food or silage[9] products, yet clinical approaches to the assessment of Cry protein toxicity are currently debated.

Allergenicity has been a major health concern accompanying GMOs in general, recently coming to bear on Bt corn. A Cry protein, Cry9c, is banned for human consumption yet was recently discovered in Taco Bell ® taco shells by Greenpeace® and reported in CNN®(Bennett 1995). Following this report, a flurry of 28 claimants reported severe allergic reactions after eating at Taco Bell®. While these claims may have been legitimate instances of allergic reactions, diets are complex and causation is very hard to prove in uncontrolled settings. Subsequent testing of their blood serum with a Cry9c antigen revealed no Cry9c antibodies. Further investigation of experimental procedure revealed that the Cry9c antibodies were derived from *E.coli*, and not from Bt corn, undermining the serum testing process (Frey *et al.* 2000).

Antibiotic resistance marker genes remain as an ecological liability long after they have served their purpose in the development of transgenic corn lines[10]. These markers may move by lateral gene transfer into numerous, and poorly understood microbes that reside in animal or human gastrointestinal tracts. This threatens to compromise clinical and veterinary use of those antibiotics[11]. Although gene transfer is complex and the event is unlikely, there is little *in vivo* data to suggest otherwise. The gastrointestinal environment is very complex and poorly understood as an ecosystem. In general, the risks to human health appear small, based upon what is known about the bacterial endotoxin, its

specificity, and confidence in the processes of plant transformation and screening [12].

As we tinker with the code of life to improve crop production, farmer safety, and cost effectiveness, some argue that we are playing Russian roulette with the environment. While the agricultural industry clashes with the natural environment on many fronts, transgenic crop technology has been tagged as the modern "Pandora's box", with the introduction of foreign genes into crops marking the beginning of a new era of instant evolution. With this abrupt change in crop phenotype, comes abrupt changes in the way that crop interacts with the natural environment. Many claim that Bt corn has already had a measurable and detrimental effect on the ecosystem.

The alleged affects of Bt corn pollen on Monarch butterfly larvae has rocketed to the front pages of ma jor newspapers around the world . Researchers revealed in a laboratory experiment that Monarch butterfly larvae which feed on milkweed leaves dusted with Bt corn pollen suffer a significant decline in fitness (Losey 1999). Subsequent inquiry into the Losey study revealed significant problems in their methodology (Leistner 1993) while the numerous field studies that followed suit showed no adverse effects on Monarch butterflies or the black swallowtail[13] (Shen *et al*. 2000). Longer term field studies under variable conditions are needed to better address this question.

Most recently, the threat of Cry gene introgression into wild populations has been realized by the discovery that 35S promoter (p-35S)[14] sequences from Cry gene fusion insertions have been detected in traditional maize landraces in remote areas of Mexico (Baulcombe 1999). These landraces are an 8000 year MesoAmericn legacy (Cummings 2002), representing both contributors to the corn gene pool and cultural treasure. This startling discovery makes the theoretical risks of transgenic introgression more realistic. Yet this study reveals that there is possibly a high level of gene flow between transgenic crops and wild populations[15]. Hybrids between traditional crops (or wild plants) and modern crops are thought to be less fit than either progenitor and unable to persist, preventing "escaped genes" from fixing in wild populations. Yet, Nature's way is to make the unlikely an actuality, regardless of our risk evaluations.

The development of insect resistance to corn has been a persistent issue in the public forum on trans genic crops. Although resistance to specific Cry proteins can develop in ECB[16], resistance can also develop in crops sprayed with foliar Bt. Furthermore, the use of Bt corn must be viewed as one approach in the larger integrated pest management program. For this reason, this issue has been omitted from discussion.

All environmental issues are diverse, yet similarly unapproachable. Research has largely focused on laboratory experiments in the past. Field experiments reveal more about the true nature of relationships and provide a better assessment of risk, yet are logistically more difficult to conduct.

Transgene effects on Corn plants

The transition from Bt **plants** to Bt **crops** has not been smooth. Much has been debated, and little has been resolved in the realms of human health and the environment. At the heart of this debate has been the biotechnologies themselves. Some molecular issues that create uncertainties on the safety of GMOs including Bt corn are 1) the pleiotropic (indirect) effects on the regulation/expression of the transgene on other genes in the host genome, 2) potentially novel roles of ectop ically expressed proteins, and 3) the creation of entirely new pathways by the addition of one or more metabolic genes (Schubert 2002). There is no way to predict how a plant is affected by the ectopic expression of a transgene, nor can we predict the biological effects of such changes.

Several other confounding factors increase the challenge of predicting ectopic expression effects. Genetic engineers utilize a large number of Cry genes with expression patterns that vary according to the location of the insert or the promoter chosen. Furthermore, there is a broad source of Bt strains to derive Cry genes from, and many varieties of Corn to transform. The case-specific nature of Bt corn studies have impeded integration of knowledge and prevented broad conclusions on the safety of Bt corn. These uncertainties fuel notions of "frankenfoods" or a new spin on "agroterrorism"[17]. While biotechnology advocates discount these fears, science has offered little evidence to discern fact and fiction.

Should we accept Bt corn?

Bt corn has obvious benefits for agricultural production, increasing profit margins through more efficient and consistent corn production and improving the working environment for farmers. In a surplus market, these benefits may be passed on to the consumer as a grocery bill reduction. On a global scale, decreased losses due to herbivory may translate to improved world food supply. Ecosystems are not likely to benefit from Bt corn use since this technology replaces a largely mechanical (nonchemical) control for ECB[18]. The perception of "benefit" weighs heavily on perspective, or bias. These benefits have been used as leverage in the argument to accept a certain level of risk to human health and environment. Thus, acceptable risk is proportional to the perceived benefit.

Lured by potential profit, humanity has a recent history of applying new technologies, such as

insecticides like DDT, before they have been properly tested. While GM foods may be a low risk, they are now subject to heightened public scrutiny in the wake of these mistakes. In response to this, industry and government have agreed that some GM foods, including select Bt corn cultivars are "substantially equivalent" to the accepted, non GM foods. That is, no detectable differences exist between the GM and the non GM foods. Public demand for better support of GM safety (Schenkelaars 2002) has given rise to a new concept of "substantiated equivalence", whereby human health and environmental risks are more clearly defined in long-term clinical trials and field studies[19].

From our hunter-gatherer origins, we have learned through "trial-and-error" what helps us and what harms us. Yet, as our technology acquisition accelerates, complex situations arise faster than can be interpreted. Bt corn is brilliantly simple in design yet enigmatic. The great potential of this transgenic line can not be banned or postponed, yet its extensive use may be jeopardizing both human and environment.

Footnotes

1. These will be ignored for this discussion on Lepidopteran-specific endotoxins
2. sprayed on leaves for ingestion by insects feeding on a plants external surfaces.
3. Bacillus Thuringiensis (Bt) is a biological control which is considered "non-synthetic". As it is non-synthetic, it is allowed under the NOP per NOP Standard 205.206. As the NOP is necessary for any products sold, labeled, or advertised as organic in the United States, OCIA must allow the use of this product for anybody who applies for organic certification (personal correspondence; Brian Kozisek, OCIA, 2002/12/11.)
4. IPM = integrated pest management: a system and philosophy of multiple, coordinated approaches to monitoring and control of target pests with the long-term goal of management, not eradication.
5. Undifferentiated plant cell culture that has been subjected to cell wall degradation enzymes
6. Agitation of suspensions within test tubes using a vibrating mechanism
7. "Ti" = tumour inducing
8. Except those Egyptians, el fayed?
9. Silage = feed for livestock
10. used in transgenic constructs to confirm insertion of the target gene into the corn genome. Transformed corn cells grow on antibiotic -infused growth medium
11. Antibiotic resistance can transfer to animal pathogens via one or several intermediate vectors
12. "screeing" is the process of selection of desirable plants from an array of transformants with variation in trait depending on location and number of t-DNA insertions.
13. a very close relative of the ECB
14. p-35S promoter from Cauliflower mosaic virus (CaMV) is popular for its consistent and constitutive promotion of target gene expression in many plant species
15. In April of 2002, Nature retracted support for this publication on the basis of insufficient evidence for some aspects of the study. The Mexican government has conducted a study that supports their findings, yet this issue is largely unresolved.
16. Populations that feed exclusively on Bt corn crops are likely to be more prone to this.
17. Agroterrorism has traditionally referred to the means to destroy crops, yet now there exists the possibility of crops being utilized to destroy people or the environment.
18. Removal of post-harvest corn stubble doesn't involve pesticide use at all, only fossil-fuel consumption and soil replenishment with fertilizers and organic matter substitutes.
19. Personal correspondence, Brian Ellis

References

1.Agrawal, A.A., Laforsch, C. & Tollrian R. (1999) *Transgenerational induction of defences in animals and plants*. *Nature*, **401**, 60-63.

2.Aronson, A. (2002) *Sporulation and Delta-Endotoxin Synthesis by Bacillus Thuringiensis*. *Cellular and Molecular Life Sciences*, **59**, 417-425.

3.Baulcombe, D.C. (1999) *Fast Forward Genetics Based on Virus-Induced Gene Silencing*. *Current Opinion in Plant Biology*, **2**, 109-113.

4.Bennett, J.W. (1995) *From Molecular-Genetics and Secondary Metabolism to Molecular Metabolites and Secondary Genetics*. *Canadian Journal of Botany- Revue Canadienne De Botanique*, **73**, S917-S924.

5.Berenbaum, M.R. (1995) *The Chemistry of Defense - Theory and Practice*. *Proceedings of the National Academy of Sciences of the United States of America*, **92**, 2-8.

6.Bourguet, D., Genissel, A. & Raymond, M. (2000) *Insecticide Resistance and Dominance Levels*. *Journal of Economic Entomology*, **93**, 1588-1595.

7.Crickmore, N., Zeigler, D.R., Feitelson, J., Schnepf, E., Van Rie, J., Lereclus, D., Baum, J. & Dean, D.H. (1998) *Revision of the nomenclature for*

the Bacillus thuringiensis pesticidal crystal proteins. *Microbiol Mol Biol Rev*, **62**, 807-13.

8. Cummings, C.H. (2002) *Risking corn, risking culture*. *World Watch*, **15**, 8-19.

9. De Maagd, R.A., Bravo, A. & Crickmore, N. (2001) *How Bacillus Thuringiensis Has Evolved Specific Toxins to Colonize the Insect World*. *Trends in Genetics*, **17**, 193-199.

10. Denholm, I., Devine, G.J. & Williamson, M.S. (2002) *Evolutionary genetics. Insecticide resistance on the move*. *Science*, **297**, 2222-3.

11. Dorsch, J.A., Candas, M., Griko, N.B., Maaty, W.S.A., Midboe, E.G., Vadlamudi, R.K. & Bulla, L.A. (2002) *Cry1a Toxins of Bacillus Thuringiensis Bind Specifically to a Region Adjacent to the Membrane-Proximal Extracellular Domain of Bt -R-1 in Manduca Sexta: Involvement of a Cadherin in the Entomopathogenicity of Bacillus Thuringiensis*. *Insect Biochemistry and Molecular Biology*, **32**, 1025-1036.

12. Frey, M., Stettner, C., Pare, P.W., Schmelz, E.A., Tumlinson, J.H. & Gierl, A. (2000) *An Herbivore Elicitor Activates the Gene for Indole Emission in Maize*. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 14801-14806.

13. Hofte, H. & Whiteley, H.R. (1989) *Insecticidal crystal proteins of Bacillus thuringiensis* . *Microbiol Rev*, **53**, 242-55.

14. Huckelhoven, R., Fodor, J., Trujillo, M. & Kogel, K.H. (2000) *Barley Mla and Rar Mutants Compromised in the Hypersensitive Cell Death Response Against Blumeria Graminis F.sp Hordei Are Modified in Their Ability to Accumulate Reactive Oxygen Intermediates at Sites of Fungal Invasion*. *Planta*, **212**, 16-24.

15. Ishida, Y., Saito, H., Ohta, S., Hiei, Y., Komari, T. & Kumashiro, T. (1996) *High efficiency transformation of maize (Zea mays L.) mediated by Agrobacterium tumefaciens*. *Nat Biotechnol*, **14**, 745-50.

15. Leistner, E. (1993) *Development of Secondary Metabolism - Principles of Chemical Evolution According to Aromatization Mechanisms*. *Archiv Der Pharmazie*, **326**, 853-856.

16. Loake, G. (2001) *Plant Cell Death: Unmasking the Gatekeepers*. *Current Biology*, **11**, R1028-R1031.

17. Losey, J.E., Rayor, L.S. & Carter, M.E. (1999) *Transgenic pollen harms monarch larvae*. *Nature*, **399**, 214.

18. Miles, G.P., Samuel, M.A. & Ellis, B.E. (2002) *Suramin Inhibits Oxidant Signalling in Tobacco Suspension- Cultured Cells*. *Plant Cell and Environment*, **25**, 521- 527.

19. Munkvold, G.P., Hellmich, R.L. & Showers, W.B. (1997) *Reduced Fusarium Ear Rot and Symptomless Infection in Kernels of Maize Genetically Engineered for European Corn Borer Resistance*. *Phytopathology*, **87**, 1071-1077.

20. Pare, P.W. & Tumlinson, J.H. (1999) *Plant Volatiles as a Defense Against Insect Herbivores*. *Plant Physiology*, **121**, 325-331.

21. Rahardja, U. & Whalon, M.E. (1995) *Inheritance of resistance to Bacillus thuringiensis subsp. tenebrionis CryIIIA delta-endotoxin in Colorado potato beetle (Coleoptera: Chrysomelidae)*. *J Econ Entomol*, **88**, 21-6.

22. Rao, Mulpuri V., Lee, Hyung- il, Creelman, Robert A., Mullet, John E., and Davis, Keith R. Jasmonic Acid Signaling Modulates Ozone-Induced Hypersensitive Cell Death. Plant Cell 12[9], 1633-1646. 2000/9/1.

23. Schubert, D. (2002) *A different perspective on GM food*. *Nat Biotechnol*, **20**, 969.

24. Schenk, P.M., Kazan, K., Wilson, I., Anderson, J.P., Richmond, T., Somerville, S.C. & Manners, J.M. (2000) *Coordinated Plant Defense Responses in Arabidopsis Revealed by Microarray Analysis*. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 11655-11660.

25. Shen, B.Z., Zheng, Z.W. & Dooner, H.K. (2000) *A Maize Sesquiterpene Cyclase Gene Induced by Insect Herbivory and Volicitin: Characterization of Wild-Type and Mutant Alleles*. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 14807-14812.

26. Turlings, T.C.J., Alborn, H.T., Loughrin, J.H. & Tumlinson, J.H. (2000) *Volicitin, an Elicitor of Maize Volatiles in Oral Secretion of Spodoptera*

Exigua: Isolation and Bioactivity. *Journal of Chemical Ecology* , **26**, 189-202.

27. Van Frankenhuyzen, K. (1993) The challenge of Bacillus thuringiensis. *Bacillus thuringiensis, An environmental biopesticide: Theory and practice* John Wiley & Sons.