Chapter 9
Transgenic Vegetables and Fruits for Control of Insects and Insect-Vectored Pathogens

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Abstract Fruits and vegetables are major components of a healthy diet, but are subject to severe pest pressure. Approximately 30% of all insecticides applied worldwide are used to control insects affecting vegetables and fruits. Transgenic, or more commonly referred to as genetically modified (GM), vegetables and fruits offer unique opportunities for controlling insects and the pathogens they transmit. Aphid transmitted viruses have been particularly difficult to manage by tactics aimed at reducing aphid populations and in many cases there has not been virus resistant plant germplasm. Farmers in the USA have benefited from having GM virus resistant squash and papaya available to them as tools in their overall IPM programs. In the USA, *Bt* sweet corn has proven effective for control of Lepidoptera and continues to be accepted in the fresh market. However, the best opportunities for GM vegetables and fruits are in developing countries where 83% of the world's population lives, the majority of vegetables and fruits are produced and pest problems are most acute.

9.1 Introduction

Vegetables and fruits are essential for well-balanced diets since they supply many of the essential nutrients not found in many of the staple crops. Additionally, there is compelling evidence that a diet rich in vegetables and fruits can lower the risk of heart disease, strokes and several forms of cancer, as well as improve gastrointestinal health and vision (HSPH, 2007).

Of the total worldwide production of vegetables and fruits in 2004, China (36.6%) and India (9.2%) produce the largest shares, with the USA (5.0%) a distant third (FAOSTAT, 2007). In 2006, world production of vegetables was 903,405,299

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metric tonnes (MT), while production of fruit was 526,496,050 MT. China was the leading producer of vegetables (49.6% of the world's vegetables or > 5-fold the production of the next leading country, India). China was also the world's leading producer of fruits, with 17.7% of the world's production, followed by India with 8.3%. The expansion of vegetable production in China has been particularly significant at almost 6% per year over the last 20 years. Expansion of vegetable production has exceeded 3% per annum in other developing countries in Asia and in developed countries. Worldwide, the area of arable land devoted to vegetables is expanding at 2.8%; higher than fruits (1.75%), oil crops (1.47%), root crops (0.44%) and pulses (0.39%), and at the expense of cereals (−0.45%), and fiber crops (−1.82%).

Many vegetables and fruits are consumed near where they are produced, especially in China and India. Besides local marketing, there is considerable movement of vegetables on the world market, and this includes fresh and processed vegetables. The European Union (EU), followed by North America and Japan, are the world's most important fresh produce import regions. To some extent, countries vary in their standards of acceptable pest management practices and this can affect imports, especially of GM products.

Production of fruits and vegetables is becoming attractive for many poor farmers worldwide because it is profitable. Farmers involved in production of fruits and vegetables usually earn much higher farm incomes compared to cereal producers, with per capita farm income up to 5-fold higher (Lumpkin et al., 2005). Horticultural crops are generally more knowledge and capital intensive than cereal crops and they suffer from many biological stresses including insects, diseases and weeds. Because of their diversity within and between plant families, their pest complexes are far more varied and complex compared to field crops. Considerably fewer resources have been directed at improving their production and pest management options compared to staple crops such as rice, wheat and maize (Lumpkin et al., 2005). Vegetables and fruits are high value commodities with high cosmetic standards, and the main method for control has been the frequent use of pesticides. In the case of insecticides, nearly 30% of the worldwide $8.1 billion annual insecticide market is applied to fruits and vegetables (Krattiger, 1997). Insecticides are regularly applied to control a complex of insects that cause damage by feeding directly on the plant or by transmitting pathogens that harm plants.

Genetic modification of vegetables and fruits for management of insects and insect-transmitted pathogens has provided some successes, and several examples are worth noting besides those covered under potatoes (Grafius and Douches, chapter 7) and maize (Hellmich et al., chapter 5). The literature suggests that more GM vegetables are commercialized (or soon to be so) than GM fruits. This is understandable since vegetables are annuals and require the purchase of new seed, ensuring a continuing market for seeds. Consequently, in this chapter more emphasis will be placed on GM vegetables. What follows is not meant to be a comprehensive review, but an effort to provide insights into some important projects and the issues they represent. Unlike other examples of GM insect-resistant plants discussed in other chapters of this book (i.e. cotton and maize), GM insect-resistant vegetables and fruits have often been

developed through partnerships between the private and public sector. This is likely the result of vegetables and fruits being "minor crops" that are not grown on such extensive areas and generally have limited research and extension resources compared to field crops. Private-public partnerships help leverage resources and create new opportunities for moving much needed technologies forward. In this chapter, several projects on insect-resistant GM plants are described and the ways in which these technologies are being incorporated into IPM programs are discussed.

9.2 Insect Transmitted Virus Protected Vegetables and Fruits

9.2.1 Plant Viruses and Their Vectors

Viruses can substantially reduce production and quality of vegetable and fruit crops and are becoming increasingly problematic worldwide. Many plant viruses are vectored by insects, including aphids, whiteflies, thrips and leafhoppers. Aphid-vectored viruses are particularly problematic because many are transmitted in a non-circulative and non-persistent manner (Zitter et al., 1996; Gonsalves, 1998). This means that a very short time, i.e. a few seconds or minutes, is sufficient for aphids to acquire virus particles when probing on infected plants. A similarly short time period is enough for aphids to release virus particles when probing on healthy plants. The primary injury caused by aphid-vectored viruses arises not from the direct feeding damage of the aphids but from their ability to allow the virus to enter the plant and initiate the disease. Control has focused on using insecticides to control the vectors (aphids, whiteflies, thrips) or tactics such as mulches or barrier crops that lessen the likelihood of the vector landing on the crop (Hooks and Fereres, 2006). Successes with both strategies have been variable.

Host plant resistance should be the foundation of IPM (Kennedy, chapter 1; Naranjo et al., chapter 6). However, virus-resistant germplasm has not been available for many important vegetable and fruit crops. In addition to developing plants that directly resist insect feeding or development, another successful application of agriculture biotechnology is the development of plants that resist insect-transmitted viruses.

Virus resistance can be achieved by applying the concept of pathogen-derived resistance (Sanford and Johnston, 1985). The insertion of a virus gene fragment into a susceptible plant can activate RNA silencing, a potent defense mechanism against viruses (Voinnet, 2005). This mechanism confers virus resistance, providing a high degree of nucleotide sequence homology between the virus-derived transgene and the challenge virus. Resistance can be engineered against multiple viruses if gene fragments from different viruses are fused within a single expression cassette or pyramided within a single T-DNA region of a binary plasmid. The coat protein (CP) is commonly used to engineer virus resistance (Fuchs and Gonsalves, 2007). Described below are two examples of virus-resistant transgenic crop cultivars expressing CP genes.

9.2.2 Papaya

Papaya ringspot virus (PRSV) is a major virus affecting papaya (*Carica papaya* L.) worldwide that causes foliar mosaic, distortion, and plant stunting. PRSV also causes ringspots on fruits and affects fruit yield and quality. No useful resistance is known in the *Caricaceae* family. In Hawaii, PRSV caused production to fall from 58 million pounds in 1992 to 24 million pounds by 1998 (Fuchs and Gonsalves, 2007). Orchard scouting and elimination of PRSV-infected trees is routinely used to limit the spread of the virus, but these approaches are only moderately successful (Gonsalves, 1998). In the early 1980s, a team of scientists characterized PRSV, used rDNA to isolate and clone a gene for the CP of the virus, introduced the gene into plant cells using particle bombardment and created the first transgenic fruit for virus resistance (Fig. 9.1). Genetically modified papaya resistant to PRSV was commercially released in Hawaii in 1998 (Gonsalves, 1998) and has had a tremendous socio-economic impact. The adoption rate of virus-resistant transgenic papaya was rapid and widespread. Transgenic papaya cultivars were planted on more than half of the total papaya production area (480 ha) in 2004 in Hawaii (Fuchs and Gonsalves, 2007; Shankula, 2006). Since the release of PRSV-resistant papaya cultivars, papaya production in Hawaii has reached a level similar to that before PRSV became epidemic in the 1980s (Shankula, 2006).

In addition to Hawaii, China recommended the commercialization of PRSV-resistant transgenic papaya in late 2006 (James, 2006). Efforts are underway to commercialize PRSV-resistant papaya cultivars for the Philippines where the majority of the crop is consumed locally (Hautea et al., 1999; ABSP II, 2007). Through a partnership between Philippine public institutions, Monsanto and the Malaysian Agricultural Research Development Institute, a local Philippine variety was made resistant to PRSV (Hautea et al., 1999; ABSP II, 2007). Contained trials were completed in 2006 and confined trials are underway to assess the safety and efficacy of the new variety. In addition to the Philippines, field tests with PRSV-resistant papaya cultivars have been conducted in Thailand (Gonsalves et al., 2006), Brazil (Souza et al., 2005) and Jamaica (Tennant et al., 2005).

9.2.3 Squash

Yield losses due to viruses in the USA often range from 20% to 80% in summer squash (*Cucurbita pepo* L.) with a reported $2.6 million economic loss in the state of Georgia in 1997 (Gianessi et al., 2002). Three of the most important viruses affecting squash production are *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* (WMV), and *Cucumber mosaic virus* (CMV) (Zitter et al., 1996). No summer squash cultivar with satisfactory resistance to CMV, ZYMV and WMV has yet been developed by conventional breeding (Gaba et al., 2004; Munger, 1993). Control of squash viruses has focused on cultural practices, including delayed transplanting relative to aphid flights, use of reflective film mulch to repel aphids,

Fig. 9.1 Papaya plant on the left was infected by papaya ringspot virus while plant on right was genetically engineered to be resistant to the virus (Photo by J. Ogrodnick)

and application of stylet oil (used to reduce virus transmission) in combination with insecticides to reduce aphid vector populations (Perring et al., 1999). In the state of Georgia, it is estimated that ten applications of stylet oils and insecticides are made routinely to control aphids and, hence, limit virus incidence and transmission (Gianessi et al., 2002).

Squash expressing the CP gene of ZYMV and WMV was exempted from regulation in the US in 1994 and was released thereafter (Tricoli et al., 1995; Acord, 1996). In addition, squash expressing the CP gene of ZYMV, WMV and CMV was deregulated and commercialized in 1995 (Medley, 1994). Subsequently, numerous squash types and cultivars have been developed by crosses and back crosses with the two initially deregulated lines. This material is highly resistant to infection by one, two or three of the target viruses, i.e. CMV, ZYMV and WMV (Arce-Ochoa

et al., 1995; Clough and Hamm, 1995; Fuchs and Gonsalves, 1995; Tricoli et al., 1995; Fuchs et al., 1998; Schultheis and Walters, 1998). The adoption of virus-resistant squash cultivars is steadily increasing in the USA. In 2005, the adoption rate was estimated at 12% (approximately 3,100 ha) across the country with the highest rates in New Jersey (25%), Florida (22%), Georgia (20%), South Carolina (20%) and Tennessee (20%) (Shankula, 2006). Virus-resistant transgenic squash has allowed growers to achieve yields comparable to those obtained in the absence of viruses with a net benefit of $22 million in 2005 (Shankula, 2006).

9.2.4　Other Crops

In China, tomato and pepper resistant to CMV through expression of the viral CP gene have also been released (Shotkoski, personal communication). However, limited information is available on their adoption rate. There is an ongoing project on CMV resistant tomato in Indonesia and the Philippines (ABSP II, 2007). Since research on the development of virus-resistant transgenic plants is making substantial progress, it is anticipated that more crops will be released in the future (Fuchs and Gonsalves, 2007).

9.2.5　Virus-Resistant Plants and IPM

Virus-resistant transgenic squash and papaya are facilitating the implementation of IPM practices because insecticides directed to control arthropod vector populations are reduced or eliminated. This is particularly true in squash for which applications of stylet oil and insecticides are made routinely to control aphids in an effort to limit virus incidence and transmission (Gianessi et al., 2002).

　　Virus-resistant plants can be a major tool in IPM. Virus-resistant transgenic squash limits virus infection rates by restricting challenge viruses, reducing their titers, or inhibiting their replication and/or cell-to-cell or systemic movement. Therefore, lower virus levels reduce the frequency of acquisition by vectors and subsequent transmission within and between fields. Consequently, virus epidemics are substantially limited. Recently, it has been shown that commercial transgenic squash resistant to ZYMV and WMV does not serve as a virus source for secondary (i.e. within field) spread (Klas et al., 2006). Virus-resistant transgenic plants are particularly valuable if no genetic source of resistance has been identified or if host resistance is difficult to transfer into elite cultivars by traditional breeding approaches due to genetic incompatibility or links to undesired traits. In such cases, engineered resistance may be the only viable option to develop virus-resistant cultivars. This is well illustrated by PRSV-resistant papaya. Engineered resistance may also be the only approach to develop cultivars with multiple sources of resistance. This has been the case for squash resistant to CMV, ZYMV and WMV.

In Hawaii, an additional IPM benefit developed because of PRSV-resistant transgenic papaya. Prior to the introduction of PRSV-resistant transgenic papaya, growing papaya was no longer viable despite area-wide efforts to eradicate infected trees in order to limit the propagation of the virus. The cultivation of PRSV-resistant transgenic papaya cultivars dramatically reduced the incidence of PRSV in many areas allowing some growers to return to growing non-GM papaya, which is important for the high value Japanese market in which GM papaya is not allowed. Growers have been able to use the PRSV-resistant transgenic papaya cultivars as a trap crop (Shelton and Badenes-Perez, 2006) by growing it as a border around the non-GM crop and allowing it to cleanse viruliferous aphids of PRSV (Fuchs and Gonsalves, 2007). Thus, the Hawaian papaya industry can now produce and market both transgenic and conventional papaya in the same field, and even organic papaya in adjacent fields if other organic practices are performed. This is a case in which organic agriculture directly benefits from GM crops, which are not allowed as part of the organic production philosophy.

9.3 *Bt* Vegetables and Fruits

Bacillus thuringiensis (*Bt*) genes that encode for Cry proteins that control insect pests have been transferred into a wide variety of crop plants, but only *Bt* maize and cotton are grown commercially on a large scale (James, 2007). However, there are tremendous opportunities to use *Bt* for controlling insect pests in several fruit and vegetable crops. Potatoes have been discussed previously (Grafius and Douches, chapter 7).

9.3.1 *Sweet Corn*

Presently the only *Bt* vegetable crop commercially available in the USA is *Bt* sweet corn. Of the 262,196 ha of sweet corn (fresh and processing) grown in the USA in 2006 (NASS, 2007), it is estimated that <5% is *Bt* sweet corn expressing Cry1Ab endotoxin (Event Bt11). Processors have avoided growing *Bt* sweet corn because of concerns about export markets, so it has been grown only as a fresh market crop. Studies in New York have shown it to be very effective against the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), providing 100% clean ears when no other lepidopteran species were present and >97% when the two noctuids, *Spodoptera frugiperda* (Smith) (fall armyworm), and *Helicoverpa zea* (Boddie) (corn earworm), were also present (Musser and Shelton, 2003). Studies in other states have shown that *Bt* sweet corn provided consistently excellent control of the lepidopteran pest complex (Lynch et al., 1999; Sorenson and Holloway, 1999; Burkness et al., 2001; Hassell and Shepard, 2002; Speese et al., 2005). However, experiences indicate that under very high pressure by *H. zea*, supplemental

sprays of synthetic insecticides often are required and that removal of broad-spectrum sprays targeting Lepidoptera have resulted in damage from other species. Studies conducted by Dively and colleagues (unpublished) under high *H. zea* pressure in Maryland have indicated superior control, compared to Bt11, with sweet corn expressing both Cry1Ab endotoxin (Bt11 event) and the vegetative insecticidal protein VIP3A (MIR 162 event). By using appropriately timed insecticide applications with *Bt* sweet corn varieties, fresh market sweet corn growers in North Carolina have been able to extend their production later into the season when populations of *H. zea* and *S. frugiperda* are generally too high to control satisfactorily with insecticide applications alone (G.G. Kennedy, personal communication). Similar findings have been reported in South Carolina (Hassell and Shepard, 2002). Even when two insecticide sprays are required on Bt11 sweet corn (e.g., for late-season control of *H. zea*), an economic assessment in Virginia found a gain of $1,777/ha for fresh-market sweet corn vs. non-*Bt* sweet corn sprayed up to six times with pyrethroid insecticides (Speese et al., 2005).

Similar to results described by Hellmich et al. (chapter 5) on maize, *Bt* sweet corn has proven to be soft on the major predators of *O. nubilalis*, including the ladybeetles *Coleomegilla maculata* (DeGeer) and *Harmonia axyridis* (Pallas), and the hemipteran *Orius insidiosus* (Say) (Musser and Shelton, 2003; Hoheisel and Fleischer, 2007) and a complex of epigeal coleopterans (Leslie et al., 2007). Overall, *Bt* sweet corn was much better at preserving these predators while controlling *O. nubilalis* than were the commonly used insecticides lambda-cyhalothrin, indoxacarb and spinosad. Results from these studies led to the development of a decision guide for sweet corn growers that uses information on biological control and can advise them on the economic return of using various options, including *Bt* sweet corn (Musser et al., 2006).

While *Bt* sweet corn can replace the traditional method of controlling Lepidoptera with broad-spectrum insecticides, it may also allow secondary pests to arise. In Florida, the corn silk fly, *Euxesta stigmatias* Loew (Diptera: Ulidiidae), has become problematic on *Bt* sweet corn and requires treatment (Nuessly and Hentz, 2004). In some states dusky sap beetles, *Carpophilus lugubris* Murray (Coleoptera: Nitidulidae), normally controlled by foliar insecticides, have become more problematic (Dowd, 2000). In Minnesota and Pennsylvania, corn rootworm beetles or Japanese beetles clipping silks have recently become more prevalent, requiring at least one insecticide application for ear protection (W. Hutchison and S. Fleischer, personal communication). Nevertheless, the use of *Bt* sweet corn has proven to be very effective against the targeted, key pests (Lepidoptera) and plantings of *Bt* sweet corn continue to rise in the USA, with new *Bt* fresh-market hybrids being released each year.

9.3.2 Brassica Vegetables, the Diamondback Moth and Other Lepidoptera

Brassica vegetables include cabbage, cauliflower, broccoli, Brussels sprout (*Brassica oleracea*); turnip, Chinese cabbage, pak choi (*B. rapa*) and mustards (*B. nigra, B. juncea, B. carinata*). In 2005, the area of cabbages harvested worldwide was 3,136,540 ha

with an additional 983,730 ha of cauliflower and broccoli (FAOSTAT, 2007). Of this total, 80% was grown in developing countries. Cabbages and cauliflower are important vegetable cash crops for low-income farmers throughout Asia, Africa, Latin America and the Caribbean. They serve as important staple dietary items and are high in folate, vitamins B and C and other micronutrients (HSPH, 2007).

Lepidopteran larvae are the most problematic insect pests of vegetable brassicas on a worldwide basis. One species in particular, the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is considered the most destructive insect pest and has severely limited brassica production, especially in resource-poor regions (Talekar and Shelton, 1993) (Fig. 9.2). *Plutella xylostella* now occurs wherever brassicas are grown and causes losses to the world economy of about US$1 billion yearly (Talekar and Shelton, 1993). Losses of cabbage and cauliflower due to *P. xylostella* frequently reach 90% without the use of insecticides (CIMBAA, 2007). Even with frequent use of insecticides, substantial losses occur and threaten food security. In tropical areas where pest pressure is high, it is not uncommon to apply insecticides every other day. Such intense use of insecticides poses hazards to farmers, consumers and the environment and has caused populations of this insect to become resistant to most of the major insecticides.

9.3.3   Bt Brassica Vegetables, a Model System

Cry1 *Bt* genes have been introduced into several *Brassica* species, conferring resistance to *P. xylostella* and other Lepidoptera (Earle et al., 2004; Paul et al., 2005). Early work in a collaborative program (Earle and Shelton at Cornell University) on

Fig. 9.2 Cauliflower in India devastated by the Diamondback moth, *Plutella xylostella*, despite nearly 50 sprays of insecticides (Photo courtesy of Nunhems India, Inc.)

Bt brassica vegetables used cytoplasmic male sterile broccoli, *Brassica oleracea* L. subsp. *italica*, transformed using *Agrobacterium tumefaciens* strain LBA4404 containing the binary vector pMON10517-1 (Metz et al., 1995). The latter carried the neomycin phosphotransferase gene and a full-length, synthetic, *B. thuringiensis* Cry1Ac-like gene, derived from HD-73. Progeny were produced by pollinating transformed plants with Green Comet hybrid broccoli and were used in our experiments. In addition to introducing *cry1Ac* into plants, similar methods were employed to produce plants expressing a *cry1C* gene (Cao et al., 1999) and pyramided plants expressing both *cry1A* and *cry1C* genes (Cao et al., 2002). Strains of *P. xylostella* that had developed resistance in the field to Cry1A, Cry1C or both were used with the three types of *Bt* broccoli to conduct the studies highlighted below.

Much of our initial interest in *Bt* brassicas was as a research tool to study Insecticide Resistance Management (IRM) strategies (Metz et al., 1995) because *P. xylostella* is the only insect to have developed resistance to Cry proteins under field conditions (Tabashnik et al., 2003). This resistance evolved from foliar sprays of *Bt* products and not to *Bt*-transgenic brassicas, which are not yet commercially available. However, field collected Cry1-resistant populations of *P. xylostella,* when combined with *Bt* brassicas, resulted in a model system to study IRM strategies for other *Bt* crops such as maize and cotton (Bates et al., 2005b; Ferré et al., chapter 3).

Use of this model system over the last 15 years has led to the following key findings that have implications for commercialized *Bt* crops and those yet to come. Studies have confirmed the importance of refuges (Ferré et al., chapter 3) in maintaining susceptible alleles in the population (Shelton et al., 2000; Tang et al., 2001); demonstrated the superiority of using dual gene (pyramided) *Bt* plants compared to introducing single gene plants in a mosaic or sequential fashion (Zhao et al., 2003); demonstrated the increased speed of resistance evolution to dual gene *Bt* plants if they are grown in association with single *Bt* gene plants that express one of the same proteins (Zhao et al., 2005); demonstrated the potential usefulness of inducible promoters in plants for creating a refuge in time or space (Bates et al., 2005a); and demonstrated the lack of toxicity by a Cry1 toxin to a hymenopteran endoparasitoid (Chen et al., 2008). This last finding supports the idea that previous reports showing harm to parasitoids by Cry1 toxins were likely due to poor host quality (sick or dying insects) rather than toxicity to the parasitoid (Romeis et al., 2006). While these findings from this model insect-*Bt* plant system have been helpful for understanding IRM and biological control in the currently commercialized *Bt* crops (maize and cotton), they have also prepared the way for the introduction of commercialized *Bt* brassica vegetables. In fact, there is far more information available about IRM and non-target effects for *Bt* brassicas prior to their commercialization than was the case for *Bt* maize or *Bt* cotton.

9.3.4   *Commercializing Bt Cauliflower and Cabbage*

It was the ability of the high expressing pyramided *Bt* plants to delay the evolution of *Bt* resistance in *P. xylostella* populations that led to the formation of a

private-public partnership called the Collaboration on Insect Management for Brassicas in Asia and Africa (CIMBAA, 2007) in 2003. This partnership involves Nunhems, a major vegetable breeding company located in The Netherlands, and the following public partners: the Asian Vegetable Research and Development Center in Taiwan (AVRDC), the Centre for Environmental Stress and Adaptation Research at the University of Melbourne in Australia, Cornell University in the USA, and the Natural Resources Institute, University of Greenwich – UK. CIMBAA includes additional international institutions to address specific research aspects of the project. The initial goal of CIMBAA is to make the dual-*Bt* technology available in varieties that are optimally adapted to growing conditions for cabbage and cauliflower in India. Once the developed material meets the regulatory standards for efficacy and environmental and human health, the bioengineered plants will be submitted to the regulatory system of India. Although India is the first country of interest, other countries have also expressed interest in joining CIMBAA. The ownership of the material, the regulatory dossier and any intellectual property rights (IPR) owned or jointly licensed by the collaborating parties will, to the extent possible, be transferred into public hands for dissemination, without technology fees, to brassica breeders in the developing world. These breeders will then be free to grow the material directly or breed the trait into their own varieties for sale and consumption. Nunhems' commercial interest in CIMBAA is to be the first company to produce the high value hybrids while the public side is focused on helping disseminate the technology into areas where it is most needed.

The initial technology discussions focused on which proteins should be expressed in the plants. Because some populations of *P. xylostella* are reported to have already evolved resistance to Cry1A in sections of India (Mittal et al., 2007), other genes were selected. After considerable discussion, it was decided that the CIMBAA plants would use *cry1C* and *cry1B* genes, because they had been shown to be effective against *P. xylostella* and cross-resistance between the two toxins was not detected (Zhao et al., 2001). Additionally, studies had shown that resistance to Cry1C in *P. xylostella* is polygenic (Zhao et al., 2000), making it more difficult for the insect to evolve resistance. Because the material would be transferred to brassica breeders, it was important that the integrity of the material be maintained. Therefore, Nunhems focused on placing the two genes so closely linked on the chromosome that they would not be separated in conventional breeding programs, thus ensuring that lines originating from them would contain the pyramided genes.

As of January, 2008, over 25 field populations of *P. xylostella* have been collected throughout India and several other countries and tested in the laboratory and found to be very susceptible to both proteins (Gujar and Shelton, unpublished). Likewise, breeding efforts by Nunhems have produced cabbage and cauliflower lines that express both proteins at levels sufficient to control not only populations of susceptible *P. xylostella* but also populations of *P. xylostella* that are resistant to Cry1C (Shelton et al., unpublished). No populations of *P. xylostella* have developed resistance to Cry1B so they are not able to be tested. Although *P. xylostella* is the chief target of the CIMBAA plants, other Lepidoptera may also be problematic in

India and other future locations for CIMBAA products, so it is appropriate that they also be tested. Laboratory studies, conducted with the purified proteins, confirmed that the following Lepidoptera are very susceptible to at least one of the proteins: *Pieris rapae* (L.) (Pieridae), *P. brassicae* (L.) (Pieridae), *Crocidolomia binotalis* Zeller (Pyralidae), *Hellula undalis* (Fabricius) (Pyralidae). On the other hand, laboratory studies have shown that two other Lepidoptera species that may feed on cabbage are much less susceptible: *Spodoptera litura* (Fabricius) (Noctuidae) and *Helicoverpa armigera* (Hübner) (Noctuidae). Laboratory and greenhouse studies with CIMBAA breeding lines have shown excellent control of *P. xylostella* and *P. rapae*, and there are ongoing studies with other pest species as well as non-target organisms.

9.3.5   Bt Cauliflower and Cabbage Within IPM

CIMBAA is committed to introducing dual *Bt* gene plants into an overall Integrated Pest Management (IPM) Program. While Lepidoptera, especially *P. xylostella*, are the primary pests, aphids can also be problematic and must be controlled in a fashion that does not compromise the use of the *Bt* plants. Therefore, selective neonicitinoid insecticides, applied as a seed treatment or drench, are being considered as part of the overall strategy for the CIMBAA plants. Use of more broad-spectrum insecticides could potentially disrupt biological control agents such as predators and parasitoids that help control the different pests, as well as affecting other non-target arthropods. Studies on an important predator, *Chrysoperla carnea* (Neuroptera: Chrysopidae), have shown that it is not harmed by either Cry1B or Cry1C when it is fed either protein directly (J. Romeis, unpublished). However, testing the effects of either protein against a hymentopteran parasitoid is more problematic because the parasitoid feeds internally on the host's tissues. Thus, rigorous studies require populations of the host that are resistant to the toxin so host-quality mediated effects can be excluded. Using Cry1C-resistant *P. xylostella*, our studies have shown that its important parasitoid, *Diadegma insulare* (Hymenoptera: Ichneumonidae), is not harmed when it parasitizes *Bt*-fed *P. xylostella* larvae (Chen et al., 2008). This is in stark contrast to parallel studies that demonstrated the parasitoid was harmed by commonly used insecticides.

A major concern about introducing *Bt* brassicas for control of *P. xylostella* is the potential for the evolution of resistance. While models (Roush, 1997a) and greenhouse studies (Zhao et al., 2003) have shown the wisdom of the dual gene approach compared to single *Bt* genes, there should be extra caution about using *Bt* plants for *P. xylostella* management since it has demonstrated its ability to develop resistance to Cry toxins in the field, albeit when applied as foliar sprays. However, an alternative approach to using *Bt* plants would be to spray conventional insecticides, but these have generally failed even after only 3 years of use because of resistance evolution (Bates et al., 2005b). Spraying formulated *Bt* insecticides is another option, but the risk of resistance evolution is greater with *Bt* sprays than with *Bt* plants because sprays create a mosaic of toxin concentrations on plants, which is not the

case with *Bt* plants that more uniformly express a high dose of the Cry toxins. On sprayed plants, the insect population is subjected to a mosaic of doses, many of which are sublethal to heterozygotes in the insect population. It is the survival of the heterozygotes that drives resistance evolution and this can explain why *P. xylostella* resistance to a *Bt* protein developed faster with foliar sprays than when the insects were exposed to high dose *Bt* plants (Roush, 1997b).

   As the CIMBAA plants are being developed, it will be important to verify that they are expressing season-long high doses of both toxins. It will also be important that other strategies be included in the overall management of *P. xylostella*. These strategies include the conservation of natural enemies, crop destruction at the end of the season to reduce population spread, and regular monitoring of susceptibility to the toxins. In many places where CIMBAA products will be grown, it will be difficult to promote the idea of planting refuges of non-*Bt* brassicas, although this is one of the major requirements for IRM in some countries and may account for the present lack of resistance evolution to *Bt* crops (Tabashnik et al., 2003; Ferré et al., chapter 3, but see Matten et al., chapter 2 for a recent case of putative field resistance). While it has been demonstrated that *Bt* plants can, when used over multiple generations, drive down populations of *P. xylostella* (Shelton et al., 2008), other IPM tactics should also be utilized. As an overall strategy, growers should be encouraged to grow non-brassica crops close by that will not serve as hosts for *P. xylostella*. Long-term studies in the broccoli production area of Mexico have shown the value of crop diversity in the landscape for managing *P. xylostella* over the long term (Hoy et al., 2007). *Bt* brassicas will be a tremendous tool for farmers worldwide, but they should be incorporated into a larger IPM program.

9.3.6   Eggplant

Eggplant (*Solanum melongena* L.) is a popular vegetable crop grown in many countries throughout the subtropics and tropics on a total of 1,857,230 ha in 2006 (FAOSTAT, 2007). It is commonly known as *brinjal* in India (510,000 ha) and Bangladesh (64,208 ha) and is the most popular vegetable grown in the Philippines (20,000 ha). The crop is often considered a "poor man's vegetable" and is mainly cultivated on small family farms. It is an important source of nutrition and cash income for many resource-poor farmers. Eggplant is an annual plant attacked by a number of devastating diseases (*Phomopsis* blight, *Verticillium* wilt, and several viruses [Chen et al., 2002]), and insects (including thrips, cotton leafhopper, jassids and aphids); however, the most damaging is the eggplant fruit and shoot borer (FSB), *Leucinodes orbonalis* Guenée (Lepidoptera: Crambidae) (Fig. 9.3). Infestation is caused by adults migrating from neighboring fields, from eggplant seedlings, or from previously grown eggplants in the same planting area. Damage from *L. orbonalis* starts at the nursery stage and continues after crop transplanting until harvest. Losses have been estimated to be 54–70% in India and Bangladesh and up to 50% in the Philippines (ABSP II, 2007).

Fig. 9.3 Eggplant infested by the eggplant fruit and shoot borer, *Leucinodes orbonalis* (Photo by A.M. Shelton)

Recommended insect pest management practices include the prompt manual removal of wilted shoots, trapping male moths using pheromones to prevent mating, ensuring regular crop rotation and using nylon net barriers. These methods, however, are not widely adopted by farmers because of time and resource constraints or lack of awareness (K. Vijayraghavan, personal communication). There are no known eggplant varieties resistant to the borer, so the use of insecticide sprays continues to be the most common control method used by farmers. Fruit and shoot borer are only vulnerable to sprays for a few hours before they bore into the plant. Therefore, farmers spray insecticides as many as 80 times over a 7-month cropping season (AVRDC, 2001). Farmers may even spray every other day, particularly during the fruiting stage (K. Vijayraghavan, personal communication). In Asia, chemical spraying for this insect accounts for 24% of the total cost of production (ABSP II, 2007). Intensive use of insecticides raises serious concerns for environmental and human health. A study conducted in the Jessore District of Bangladesh found that "98% of farmers felt sickness and more than 3% were hospitalized due to various complexities related to pesticide use" (AVRDC, 2003).

9.3.7   Bt Eggplant

Transformation of eggplant with *cry1Ac* was done by the Maharashtra Hybrid Seeds Company Limited (Mahyco) under a collaborative agreement with Monsanto,

and the first *Bt* transgenic eggplant with resistance to *L. orbonalis* (FSB) (FSBR-eggplant) was produced in 2000. The first contained trial for the elite event was undertaken in 2002, and in 2003 a collaboration was initiated with the Cornell University-led Agricultural Biotechnology Support Program II (ABSP II) funded by the United States Agency for International Development (USAID). ABSP II developed a consortium of private and public sector partners to develop FSBR-eggplant for resource-poor farmers. The strategy is that Mahyco will make its profit by selling hybrids while the public sector institutions in India, Bangladesh, the Philippines and other countries will distribute open pollinated lines at a much reduced cost. The potential economic, social and environmental benefits are discussed in detail by Qaim et al. (chapter 12).

9.3.8   *Bt Eggplant Within IPM*

From an IPM standpoint, there are many benefits as well as some concerns about the use of FSBR-eggplant. The first concern is the potential for the insect to develop resistance to FSBR-eggplant (for a detailed discussion on the factors that influence resistance evolution, see Ferré et al., chapter 3). In the case of *L. orbonalis*, an IRM plan was developed and submitted to the Indian Genetic Engineering Approval Committee (GEAC), which is responsible for environmental approval of activities involving transgenic products. The plan consists of a high dose-refuge strategy in which 5% of an area should be planted to non-*Bt* eggplant at the same time as the main planting. The refuge can be treated with another insecticide but not with *Bt*, and the crop in the refuge must not be destroyed at harvest so that susceptible alleles will be maintained in the *L. orbonalis* population. An intended IRM strategy is that seeds of *Bt* and non-*Bt* plants will be distributed together but in different packets, thus facilitating the refuge strategy. Training guides for *Bt* eggplant have been developed and will distributed when the product is commercialized. These training guides emphasize the need for growers to pay attention to any secondary insect pests, such as aphids and leafhoppers, as well as pathogens that will not be controlled by *Bt* eggplant, thus reconfirming the idea that the *Bt* eggplant is a component in the overall IPM program. In the future it may be possible to incorporate other types of insect-resistance genes into *Bt* eggplant. For example, Ribeiro et al. (2006) has described a genetically modified eggplant line expressing oryzacystatin, an inhibitory protein of cystein proteinases, that has a negative impact on population growth and mortality rates of the aphids, *Myzus persicae* (Sulzer) and *Macrosiphum euphorbiae* (Thomas).

In each country, designated agencies have overall responsibility for IRM (in India it is the Department of Biotechnology in collaboration with State Agricultural Universities and other agencies). Plans are being developed on how they will coordinate a monitoring program and who will pay for it. One proposal is that the assays will be performed in the universities, agricultural research institutes and by the company (Mahyco) with each using the same methods. Multi-location field trials

of FSBR-eggplant are being conducted in 2007 and 2008 and commercial release of FSBR-eggplant is anticipated to be in 2009 in India.

9.3.9   Biosafety and Food Safety Issues

As the first transgenic food crop to be commercialized in India, *Bt* eggplant has had to undergo several regulatory tests to ensure food and environmental safety. In the developed world, conducting such tests is expensive and time-consuming. As eggplant is not a major crop in North America or Europe, India's regulatory agencies have insisted on the generation of in-country data. This has included both laboratory and field tests to demonstrate field efficacy, safety to beneficial insects, sheep and humans. For additional details see: http://www.envfor.nic.in/divisions/csurv/geac/macho.htm.

Developing in-country safety data requires appropriate laboratories and skills. India, because of its early work with *Bt* cotton, has been able to apply its own expertise to develop such data. Despite such developments, an NGO in India challenged the commercialization via a Supreme Court case that was later dismissed. Such hurdles can cause major delays and substantially increase the cost for product commercialization.

9.3.10   Warranites, Indemnity and Damages

These may become a major issue in the transfer of any proprietary gene based technologies. In the case of *Bt* eggplant, it was essential to include stewardship warranty and infringement claims. Many public supported programs in India and in other developing countries are unaware of how to structure such agreements, and this may delay the development of much needed technologies. In the *Bt* eggplant project, ABSP II was able to facilitate these agreements so that both parties (the donor of the technology and recipient) understood clearly their roles and responsibilities.

9.4   Challenges and Opportunities for Transgenic Vegetables and Fruits for Control of Insects and Insect-Vectored Pathogens

9.4.1   Plant Development

Vegetables and fruits are considered minor crops and traditionally have had fewer resources channeled to them compared to the staple crops. While it is becoming less expensive to create GM crops for pest management, developing a marketable

product and a regulatory package remains costly. Development and regulatory costs can be more readily recouped if the product is grown on an extensive area, as would be done with staple crops, but which is not generally the case for individual fruit and vegetable crops. For example, the large agriculture biotechnology companies have for the most part abandoned the development of GM vegetable and fruit crops because of the high costs associated with product development and deregulation. For vegetables, there are many varieties of the same crop and the half-life of a particular variety can be quite limited. Introducing a GM trait into a breeding program can be complicated and cost prohibitive, especially in crops where backcrossing is difficult or impossible (e.g. potatoes). In most countries, deregulation of a GM trait is event specific. For many vegetable crops, it is not possible to develop a single GM event that can be converted into many different varieties of a single vegetable species via conventional breeding. For example, *Brassica* contains about 100 species, including rapeseed, cabbage, cauliflower, broccoli, Brussels sprouts, turnip and various mustards. No single parent exists that can be used to backcross the transgene into the many different types of *Brassica* species. Individual events would have to be developed for most of the different crop types and deregulation of more than one event for a single protein is problematic for most business models. For the few transgenic vegetable crops that are being developed, novel or unconventional strategies have been employed to bring the crops to market.

For GM papaya, Gonsalves and his colleagues undertook much of the work without large financial backing from industry. They and Cornell (Gonsalves' institution) were able to develop freedom to operate (FTO) policies with little or no cost because the companies that held patents believed there was little financial incentive for them in papaya. Another approach is for a company to piggy-back its vegetable work with larger scale crops. This is essentially what was done with *Bt* sweet corn and *Bt* maize. A third approach is to develop the private-public partnerships in which the private sector would focus on selling hybrids to higher end producers while the public sector would focus on resource-poor farmers. The roles and the financial responsibilities of each partner need to be clearly defined and the eggplant model serves as a good example of a private-public partnership (for a detailed description, see Medakker and Vijayaraghavan, 2007).

9.4.2 Stewardship and IPM

Production of vegetables and fruits in industrialized and developing countries tends to be on smaller areas and in more diversified holdings than staple crops like rice and maize. Thus, they often operate in more complex agricultural systems in which insects may move from one crop to the next within the same farm. How this will impact the use and effects of GM plants in the agricultural landscape can be complex (Storer et al., chapter 10). If multiple GM insect-resistant plants are grown within the same area and if a polyphagous insect is exposed to the same *Bt* protein expressed in the different species, this will challenge the conventional IRM strategies developed

for cotton and maize. Thoughtful consideration will be needed before choosing which toxins vegetable plants should express, and the selection should be based not only on what will be an effective toxin against the target insect but what toxins are already in use in other crops that may be hosts for the target insect. Additionally, the difficulty of sampling insect populations for resistant alleles will take on a higher level of complexity in a diversified vegetable system. Further consideration should also have to be given to the effects on non-target organisms within diversified GM plantings. In a study conducted in the northeastern USA, Hoheisel and Fleischer (2007) investigated the seasonal dynamics of coccinellids and their food (aphids and pollen) in a farm system containing plantings of *Bt* sweet corn, *Bt* potato and GM insect-resistant squash. Their results indicated that the transgenic vegetable crops provided conservation of cocinellids and resulted in a 25% reduction in insecticides. In a similar study with these same crops, Leslie et al. (2007) compared the soil surface dwelling communities of Coleopetera and Formicidae in the transgenic crops and their isolines and found no differences in species richness and species composition, but did find the transgenic vegetables required fewer insecticide applications. Such results bode well for GM plants within vegetable IPM systems.

In small, diversified vegetable plantings typical of those found throughout developing countries, the challenges for regulatory oversight of GM plants are immense. Farmers will likely save GM seed, move GM seed between locations, and some GM products may move into markets that do not permit these products. These concerns will be lessened if GM plants are consumed locally and in accordance with national biosafety regulatory policies. However, it is likely that violations will occur and this will challenge legal systems.

It is clear that GM vegetables and fruits can offer novel and effective ways of controlling insects and the pathogens they transmit. It is equally clear that such technology must be introduced within the context of IPM. While each vegetable and fruit has its own set of one of more key pests, other pests can also be problematic. Traditional broad-spectrum insecticides often controlled a suite of pest insects. Thus, when *Bt* (or other GM) vegetables and fruits are introduced into production systems, other methods of control will have to be applied or developed for secondary pests. Because the present GM technologies have proven to be less harmful to natural enemies, biological control of secondary pests may be more achievable but other tactics such as the use of selective insecticides (applied either as seed treatments or foliar sprays) may be necessary (Romeis et al., chapter 4).

9.5   Conclusions

GM vegetables and fruits can have a major role in the management of insects and the diseases they transmit. However, to date they have largely played a secondary role compared to the large areas planted to cotton and maize and have generally been under the radar of those opposed to biotechnology. In the USA where labeling of GM products is not required, virus resistant squash and papaya and *Bt* sweet corn

are consumed by the public with little or no thought about the role these crops have played in managing difficult pests. When the markets have allowed the production of GM plants, farmers have readily adopted the technology as part of their pest management practices and this is likely to continue with GM vegetables and fruits.

What will be of great interest and importance for the future of GM vegetables and fruits will be the course set by developing countries. In 2007 about 83% of the world's 6.3 billion population lived in developing countries, and this proportion is expected to increase rapidly in the next several decades. Nearly 46% of the world's vegetables and fruits are grown in China and India, two countries that account for nearly 40% of the world's population and where pest problems are severe. Both countries have readily adopted *Bt* cotton (Naranjo et al., chapter 6) and it is likely that *Bt* rice will be commercialized in China in the near future (Cohen et al., chapter 8). Acceptance of GM crops in these two countries will make it more likely they will adopt GM vegetables and fruits. This in turn will likely hasten their adoption in other parts of the world and allow farmers to use this technology in their overall IPM programs. With the eventual acceptance of GM technology, it is expected that the costs associated with deregulation will become more affordable and that the biotech industry will become more interested in developing GM vegetables and fruits, especially for the developing countries.

References

ABSP II (Agricultural Biotechnology Support Project II), 2007. http://www.absp2.cornell.edu/ (accessed 1 December 2007).

Acord, B.D., 1996. Availability of determination of non-regulated status for a squash line genetically engineered for virus resistance. Federal Register 61: 33484–33485.

Arce-Ochoa, J.P., Dainello, F., Pike, L.M., and Drews, D., 1995. Field performance comparison of two transgenic summer squash hybrids to their parental hybrid lineage. HortScience 30: 492–493.

AVRDC (Asian Vegetable Research and Development Center), 2001. Fact Sheet: Eggplant Fruit and Shoot Borer. www.avrdc.org/LC/eggplant/borer.html (accessed 1 December 2007).

AVRDC (Asian Vegetable Research and Development Center), 2003. Technical Bulletin #29: Socio-economic Parameters of Eggplant Pest Control in Jessore District of Bangladesh. http://www.avrdc.org/pdf/TB29.pdf (accessed 1 December 2007).

Bates, S.L., Cao, J., Zhao, J.-Z., Earle, E.D., Roush, R.T., and Shelton, A.M., 2005a. Evaluation of a chemically inducible promoter for developing a within-plant refuge for resistance management. Journal of Economic Entomology 98: 2188–2194.

Bates, S.L., Zhao, J.-Z., Roush, R.T., and Shelton, A.M., 2005b. Insect resistance management in GM crops: Past, present and future. Nature Biotechnology 23: 57–62.

Burkness, E.C., Hutchison, W.D., Bolin, P.C., Bartels, D.W., Warnock, D.F., and Davis, D.W., 2001. Field efficacy of sweet corn hybrids expressing a *Bacillus thuringiensis* toxin for management of *Ostrinia nubilalis* (Lepidoptera: Crambidae) and *Helicoverpa zea* (Lepidoptera: Noctuidae). Journal of Economic Entomology 94: 197–203.

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

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

Chen, M., Zhao, J.-Z., Colins, H.L., Earle, E.D., Cao, J., and Shelton, A.M., 2008. A critical assessment of the effects of Bt transgenic plants on parasitoids. PLoS ONE 3(5): e2284. doi:10.1371/journal.pone.0002284.

Chen, N.C., Kalb, T., Talekar, N.S., Wang, J.F., and Ma, C.H., 2002. AVRDC Training Guide: Suggested Cultural Practices for Eggplant. http://www.avrdc.org/LC/eggplant/practices.pdf (accessed 1 December 2007).

CIMBAA (Collaboration on Insect Management for Brassicas in Asia and Africa), 2007. http://www.cimbaa.org/ (accessed 1 December 2007).

Clough, G.H., and Hamm, P.B., 1995. Coat protein transgenic resistance to watermelon mosaic and zucchini yellow mosaic virus in squash and cantaloupe. Plant Disease 79: 1107–1109.

Dowd, P.F., 2000. Dusky sap beetles and other kernel damaging insects in Bt and non-Bt sweet corn in Illinois. Journal of Economic Entomology 93: 1714–1720.

Earle, E.D., Cao, J., and Shelton, A.M., 2004. Insect-resistant transgenic Brassicas. In: Biotechnology in Agriculture and Forestry, Vol. 54, E.C. Pua and C.J. Douglas, eds., Springer, Berlin/Heidelberg/New York, pp. 227–252.

FAOSTAT, 2007. http://faostat.fao.org/ (accessed 1 December 2007).

Fuchs, M., and Gonsalves, D., 1995. Resistance of transgenic squash Pavo ZW-20 expressing the coat protein genes of zucchini yellow mosaic virus and watermelon mosaic virus 2 to mixed infections by both potyviruses. BioTechnology 13: 1466–1473.

Fuchs, M., and Gonsalves, D., 2007. Safety of virus-resistant transgenic plants two decades after their introduction: Lessons from realistic field risk assessment studies. Annual Review of Phytopatholology 45: 173–202.

Fuchs, M., Tricoli, D.M., McMaster, J.R., Carney, K.J., Schesser, M., McFerson, J.R., and Gonsalves, D., 1998. Comparative virus resistance and fruit yield of transgenic squash with single and multiple coat protein genes. Plant Disease 82: 1350–1356.

Gaba, V., Zelcer, A., and Gal-On, A., 2004. Cucurbit biotechnology - the importance of virus resistance. In vitro Cellular & Developmental Biology - Plant 40: 346–358.

Gianessi, L.P., Silvers, C.S., Sankula, S., and Carpenter, J.E., 2002. Virus resistant squash. In: Plant Biotechnology: Current and Potential Impact for Improving Pest Management in U.S. Agriculture. An Analysis of 40 Case Studies. National Center for Food and Agricultural Policy, Washington, DC, USA.

Gonsalves, D., 1998. Control of papaya ringspot virus in papaya: A case study. Annual Review of Phytopathology 36: 415–437.

Gonsalves, D., Vegas, A., Prasartsee, V., Drew, R., Suzuki, J., and Tripathi, S., 2006. Developing papaya to control papaya ringspot virus by transgenic resistance, intergeneric hybridization, and tolerance breeding. Plant Breeding Reviews 26: 35–78.

Hassell, R., and Shepard, B.M., 2002. Insect populations on *Bacillus thuringiensis* transgenic sweet corn. Journal of Entomological Science 37: 285–292.

Hautea, R., Chan, Y.K., Attathom, S., and Krattiger, A.F., 1999. The Papaya Biotechnology Network of Southeast Asia: Biosafety Considerations and Papaya Background Information. ISAAA Brief No. 11, International Service for the Acquisition of Agri-Biotech Applications, Ithaca, NY, USA.

Hoheisel, G.A., and Fleischer, S.J., 2007. Coccinellids, aphids, and pollen in diversified vegetable fields with transgenic and isoline cultivars. Journal of Insect Science 7: 64. http://www.insectscience.org/7.61/.

Hooks, C.R., and Fereres, A., 2006. Protecting crops from non-persistently aphid-transmitted viruses- a review on the use of barrier plants as a management tool. Virus Research 120: 1–16.

Hoy, C.W., McCully, J.E., Laborde, J.A., Vargas-López, A., Bujanos-Muñiz, R., and Rangel, E., 2007. The linkage between integrated pest management and agroecosystem management- a case study in the Bajio, Mexico. American Entomologist 53: 174–183.

HSPH (Harvard School of Public Health), 2007. Fruits and Vegetables. www.hsph.harvard.edu/nutritionsource/fruits.html (accessed 1 December 2007).

James, C., 2007. Global Status of Commercialized Biotech/GM Crops: 2007. ISAAA Brief No. 37, International Service for the Acquisition of Agri-Biotech Applications, Ithaca, NY, USA.

Klas, F.E., Fuchs, M., and Gonsalves, D., 2006. Comparative spatial spread overtime of Zucchini yellow mosaic virus (ZYMV) and Watermelon mosaic virus (WMV) in fields of transgenic squash expressing the coat protein genes of ZYMV and WMV, and in fields of non-transgenic squash. Transgenic Research 15: 527–541.

Krattiger, A.F., 1997. Insect Resistance in Crops: A Case Study of *Bacillus thuringiensis* (Bt) and Its Transfer to Developing Countries. ISAAA Brief No. 2, International Service for the Acquisition of Agri-Biotech Applications, Ithaca, NY, USA.

Leslie, T.W., Hoheisel, G.A., Biddinger, D.J., Rohr, J.R., and Fleisher, S.J., 2007. Transgenes sustain epigeal insect biodiversity in diversified vegetable farm systems. Environmental Entomology 36: 234–244.

Lumpkin, T.A., Weinberger, K., and Moore, S., 2005. Increasing income through fruit and vegetable production opportunities and challenges. Draft Note for Consultative Group on International Agriculture Research Science Forum, CGIAR Priorities: Science for the Poor. http://www.globalhort.org/downloads/pdf/forum-agm2005.pdf (accessed 1 December 2007).

Lynch, R., Wiseman, B., Sumner, H., Plaisted, D., and Warnick, D., 1999. Management of corn earworm and fall armyworm (Lepidoptera: Noctuidae) injury on a sweet corn hybrid expressing a cryIA(b) gene. Journal of Economic Entomology 92: 1217–1222.

Medakker, A., and Vijayaraghavan, V., 2007. Successful commercialization of insect-resistant eggplant by a public–private partnership: Reaching and benefiting resource-poor farmers. In: Intellectual Property Management in Health and Agricultural Innovation: A Handbook of Best Practices, A. Krattiger, R.T. Mahoney, L. Nelsen et al., eds., MIHR, Oxford, UK/PIPRA, Davis, CA, USA. www.ipHandbook.org (accessed 1 December 2007).

Medley, T.L., 1994. Availability of determination of non-regulated status for virus resistant squash. Federal Register 59: 64187–64189.

Metz, T.D., Roush, R.T., Tang, J.D., Shelton, A.M., and Earle, E.D., 1995. Transgenic broccoli expressing a *Bacillus thuringiensis* insecticidal crystal protein: Implications for pest resistance management strategies. Molecular Breeding 1: 309–317.

Mittal, A., Kumari, A., Kalia, V., Singh, D., and Gujar, G.T., 2007. Spatial and temporal baseline susceptibility of Diamondback Moth, *Plutella xylostella*, to *Bacillus thuringiensis* spore crystal mixture, purified crystal toxins and mixtures of cry toxins in India. Biopesticides International 3: 58–70.

Munger, H.M., 1993. Breeding for viral resistance in cucurbits. In: Resistance to Viral Diseases of Vegetables: Genetics and Breeding, M.M. Kyle, ed., Timber Press, Portland, OR, USA, pp. 8–43.

Musser, F.R., and Shelton, A.M., 2003. Bt sweet corn and selective insecticides: Their impacts on sweet corn pests and predators. Journal of Economic Entomology 96: 71–80.

Musser, F.R., Nyrop, J.P., and Shelton, A.M., 2006. Integrating biological and chemical controls in decision-making: European corn borer control in sweet corn as an example. Journal of Economic Entomology 99: 1538–1549.

NASS (National Agricultural Statistics Service), 2007. Vegetables: 2006 Annual Summary. http://usda.mannlib.cornell.edu/usda/nass/VegeSumm//2000s/2007/VegeSumm-01-26-2007.pdf (accessed 1 December 2007).

Nuessly, G.S., and Hentz, M.G., 2004. Contact and leaf residue activity of insecticides against the sweet corn pest *Euxesta stigmatias*. Journal of Economic Entomology 97: 496–502.

Paul, A., Sharma, S.R., Sresty, T.V.S., Devi, S., Bala, S., Kumar, P.S., Saradhi, P.P., Frutos, R., and Altosaar, I., 2005. Transgenic cabbage resistant to diamondback moth. Indian Journal of Biotechnology 4: 72–77.

Perring, T.M., Gruenhagen, N.M., and Farrar, C.A., 1999. Management of plant viral diseases through chemical control of insect vectors. Annual Review of Entomology 44: 457–481.

Ribeiro, A.P.O., Pereira, E.J.G., Galvan, T.L., Picano, M.C., Picoli, E.A.T., da Silva, D.J.H., and Fari, M.G., 2006. Effect of eggplant transformed with oryzacystatin gene on *Myzus persicae* and *Macrosiphum euphorbiae*. Journal of Applied Entomology 130: 84–90.

Romeis, J., Meissle, M., and Bigler, F., 2006. Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. Nature Biotechnology 24: 63–71.

Roush, R.T., 1997a. Managing resistance to transgenic crops. In: Advances in Insect Control, N. Carozzi and M. Koziel, eds., Taylor & Francis, London, UK, pp. 271–294.

Roush, R.T., 1997b. Managing risk of resistance in pest to insect-tolerant transgenic crops. In: Commercialization of Transgenic Crops: Risk, Benefit and Trade Consideration. Proceedings of a workshop, Canberra, 11–13 March 1997, G.D. McLean, P.M. Waterhouse, G. Evans and M.J. Gibbs, eds., Cooperative Research Center for Plant Sciences and Bureau of Statistics, Canberra, Australia, pp. 259–271.

Sanford, J.C., and Johnston, S.A., 1985. The concept of parasite-derived resistance - deriving resistance genes from the parasites own genome. Journal of Theoretical Biology 113: 395–405.

Schultheis, J.R., and Walters, S.A., 1998. Yield and virus resistance of summer squash cultivars and breeding lines in North Carolina. HortScience 8: 31–39.

Shankula, S., 2006. Quantification of the Impacts on US Agriculture of Biotechnology-Derived Crops Planted in 2005. http://www.ncfap.org/ (accessed 1 December 2007).

Shelton, A.M., Tang, J.D., Roush, R.T., Metz, T.D., and Earle, E.D., 2000. Field tests on managing resistance to Bt-engineered plants. Nature Biotechnology 18: 339–342.

Shelton, A.M., and Badenes-Perez, F.R., 2006. Concept and applications of trap cropping in pest management. Annual Review of Entomology 51: 285–308.

Shelton, A.M., Hatch, S.L., Zhao, J.-Z., Chen, M., Earle, E.D., and Cao, J., 2008. Suppression of diamondback moth using Bt-transgenic plants as a trap crop. Crop Protection 27: 403–409.

Sorenson, K.A., and Holloway, C.W., 1999. Corn earworm and European corn borer control with Bt treated corn, Brunswick and Sampson Co., NC, 1998. Arthropod Management 24: 429–430.

Souza, M.T., Jr., Nickel, O., and Gonsalves, D., 2005. Development of virus resistant transgenic papayas expressing the coat protein gene from a Brazilian isolate of *Papaya ringspot virus*. Fitopatologia Brasileira 30: 357–365.

Speese III, J., Kuhar, T.P., Bratsch, A.D., Nault, B.A., Barlow, V.M., Cordero, R.J., and Shen, Z., 2005. Efficacy and economics of fresh-market Bt transgenic sweet corn in Virginia. Crop Protection 24: 57–64.

Tabashnik, B.E., Carriere, Y., Dennehy, T.J., Morin, S., Sisterson, M.S., Roush R.T., Shelton, A.M., and Zhao, J.-Z., 2003. Insect resistance to transgenic Bt crops: Lessons from the laboratory and field. Journal of Economic Entomology 96: 1031–1038.

Talekar, N.S., and Shelton, A.M., 1993. Biology, ecology and management of the diamondback moth. Annual Review of Entomology 38: 275–301.

Tang, J.D., Collins, H.L., Metz, T.D., Earle, E.D., Zhao, J.-Z., Roush, R.T., and Shelton, A.M., 2001. Greenhouse tests on resistance management of Bt transgenic plants using refuge strategies. Journal of Economic Entomology 94: 240–247.

Tennant, P., Ahmad, M.H., and Gonsalves, D., 2005. Field resistance of coat protein transgenic papaya to *Papaya ringspot virus* in Jamaica. Plant Disease 89: 841–847.

Tricoli, D.M., Carney, K.J., Russell, P.F., McMaster, J.R., Groff, D.W., Hadden, K.C., Himmel, P.T., Hubbard, J.P., Boeshore, M.L., and Quemada, H.D., 1995. Field evaluation of transgenic squash containing single or multiple virus coat protein gene constructs for resistance to cucumber mosaic virus, watermelon mosaic virus 2, and zucchini yellow mosaic virus. BioTechnology 13: 1458–1465.

Voinnet, O., 2005. Induction and suppression of RNA silencing: Insights from viral infections. Nature Reviews Genetics 6: 206–221.

Zhao, J.-Z., Collins, H.L., Tang, J.D., Cao, J., Earle, E.D., Roush, R.T., Herrero, S., Escriche, B., Ferre, J., and Shelton, A.M., 2000. Development and characterization of diamondback moth resistance to transgenic broccoli expressing high levels of Cry1C. Applied Environmental Microbiology 66: 3784–3789.

Zhao, J.-Z., Li, Y., Collins, H.L., Cao, J., Earle, E.D., and Shelton, A.M., 2001. Different cross-resistance patterns in the diamondback moth resistant to *Bacillus thuringiensis* toxin Cry1C. Journal of Economic Entomology 94: 1547–1552.

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

Zhao, J.-Z., Cao, J., Collins, H.L., Bates, S.L., Roush, R.T., Earle, E.D., and Shelton, A.M., 2005. Concurrent use of transgenic plants expressing a single and two Bt genes speeds insect adaptation to pyramided plants. Proceedings of the National Academy of Sciences of the USA 102: 8426–8430.

Zitter, T.A., Hopkins, D.L., and Thomas, C.E., 1996. Compendium of Cucurbit Diseases. APS Press, St. Paul, MN, USA.