8 Mechanism of Cry1Ac Resistance in Cabbage Loopers – A Resistance Mechanism Selected in Insect Populations in an Agricultural Environment

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Summary

The development of resistance to *Bacillus thuringiensis* (Bt) in insect populations in agriculture not only depends on the level of resistance conferred by a selected resistance mechanism, but also on the fitness cost associated with the resistance mechanism under specific ecological and environmental conditions. Bt resistance in the cabbage looper (*Trichoplusia ni*), which was identified by Janmaat and Myers (2003), is a case of Bt resistance evolved in an agricultural system, and is used in this chapter to review and discuss the mechanism of Cry1Ac resistance that is selected in an agricultural environment.

8.1 Introduction

Resistance of insects to pesticide sprays in agriculture has been observed for a century (Melander, 1914). Under selection pressure by pesticide applications, thousands of cases of pesticide resistance in hundreds of arthropod species have been recorded (Mota-Sánchez *et al*., 2008). Since the first report of insect resistance to *Bacillus thuringiensis* (Bt) in 1985 (McGaughey, 1985), the potential for the development of insect resistance to Bt has been well demonstrated by the laboratory selection of various insects with resistance to Bt toxins (Tabashnik, 1994; Ferré and Van Rie, 2002; Bravo and Soberón, 2008). Insect resistance to Bt toxins from both Bt sprays and transgenic Bt crops has now been reported in field populations of a number of species (Tabashnik *et al*., 1990, 2009; Shelton *et al*., 1993; Janmaat and Myers, 2003; van Rensburg, 2007; Downes *et al*., 2010; Storer *et al*., 2010; Zhang *et al*., 2011; Wan *et al*., 2012; Gassmann *et al*., 2014). The occurrence of increasing numbers of cases of field-evolved resistance confirms the potential for the development of insect resistance to Bt toxins in the field and indicates the rising risk of its occurrence with the increasing application of Bt toxins for insect control, if adequate resistance management programmes are not in place.

Laboratory selections of Bt-resistant insect populations have greatly facilitated the study of Bt resistance in insects and enabled the building of the main body of the current understanding of the various mechanisms of Bt resistance (Oppert *et al*., 1997; Gahan *et al*., 2001; Griffitts and Aroian, 2005; Pardo-López *et al*., 2013). Bt-resistant lepidopteran strains established

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by laboratory selections showed resistance to different Bt toxins at different levels, and exhibited various cross-resistance patterns (Ferré and Van Rie, 2002; Tabashnik *et al*., 2003). Biochemical and molecular studies have indicated that resistance to Bt in insects is complex and that the mechanisms of Bt resistance in different insects and strains can be diverse (Griffitts and Aroian, 2005; Heckel *et al*., 2007; Pardo-López *et al*., 2013). For resistance management in agriculture, it is important to understand the resistance mechanisms that may be selected in agricultural systems as a means of conferring resistance in the field. Current understanding of insect resistance to Bt toxins has indicated that laboratory-selected Bt resistance does not always confer resistance to Bt transgenic plants, and that the Bt resistance developed in field insect populations may involve a mechanism different from those found in laboratory-selected resistance (Tabashnik *et al*., 2003; Baxter *et al*., 2005; Zhang *et al*., 2012b).

In the field, cases of insect resistance or increased frequency of resistant alleles to either Bt formulations or Bt crops have been reported in a number of lepidopteran pests, including *Plutella xylostella*, *Trichoplusia ni*, *Busseola fusca*, *Spodoptera frugiperda*, *Helicoverpa zea*, *H. armigera*, *H. punctigera* and *Pectinophora gossypiella* (Tabashnik *et al*., 1990, 2009; Janmaat and Myers, 2003; van Rensburg, 2007; Matten *et al*., 2008; Dhurua and Gujar, 2011; Zhang *et al*., 2011, 2012a; Wan *et al*., 2012). Field-evolved and laboratory-selected resistant insects may exhibit similar resistance characteristics. For example, high-level resistance to the Bt toxins Cry1Ab or Cry1Ac conferred by reduced toxin binding to the host midgut receptors has been found to be the major type of resistance in both laboratory-selected and field-selected resistant insect populations. However, the underlying molecular basis conferring the resistance can be distinctively different between the laboratory-selected and field-selected resistant insect populations (Morin *et al*., 2003; Baxter *et al*., 2005, 2011; Xu *et al*., 2005; Yang *et al*., 2006; Tiewsiri and Wang, 2011; Zhang *et al*., 2012b). Hence, it is crucially important to understand the molecular genetic basis of Bt resistance in insect populations evolved in agricultural situations in order to provide fundamental knowledge for insect resistance management in agriculture.

8.2 Resistance of the Cabbage Looper to the Bt toxin Cry1Ac

The cabbage looper (*T. ni*) is an important agricultural pest that is widely distributed in temperate regions in Africa, Asia, Europe and the Americas. Although *T. ni* is a major pest of cruciferous crops, its hosts include over 160 plants in 36 families, many of which are important crops (Lingren and Green, 1984). *T. ni* is considered to be a secondary pest on cotton in the USA but, if uncontrolled, it could cause severe yield loss as much as 92% (Schwartz, 1983). Bt resistance in *T. ni* populations has been found in commercial greenhouses in British Columbia, Canada, that exhibited various levels of resistance to a sprayable formulation of *B.t. kurstaki* (Btk), DiPel®, of up to 160-fold (Janmaat and Myers, 2003). *T. ni* is one of only two species that have evolved resistance to Bt under selective pressure from Bt sprays in agricultural practice (Tabashnik *et al*., 1990; Shelton *et al*., 1993; Janmaat and Myers, 2003). Thus, Bt-resistant populations of *T. ni* are a unique biological system for studying the mechanisms of field-evolved Bt resistance. The characterization of a Bt-resistant greenhouse population of *T. ni*, GLEN-DiPel, determined that the DiPel-resistance trait was polygenic and incompletely recessive (Janmaat *et al*., 2004). The incompletely recessive inheritance of DiPel resistance in *T. ni* is similar to most cases of insect resistance to Bt. The polygenic inheritance so demonstrated is indicative of multiple resistance mechanisms to the multiple toxins in Bt sprays.

The DiPel-resistant *T. ni* populations were highly resistant to the toxin Cry1Ac, a major Cry toxin in *Btk* (Kain *et al*., 2004). Resistance to Cry1Ac in *T. ni* exhibits typical 'Mode 1' type resistance (Kain *et al*., 2004), i.e. a high level of resistance to one or more Cry1A

toxins, recessive inheritance, reduced binding of one or more Cry1A toxins to the midgut brush border membranes and little or no cross-resistance to Cry1C toxin (Tabashnik *et al*., 1998). Mode 1-type resistance is the most common type of Bt resistance and has been identified in both laboratory-selected and field-evolved resistant strains from numerous insect species (Tabashnik *et al*., 1994, 1998; González-Cabrera *et al*., 2003; Wang *et al*., 2007). Nevertheless, the underlying genetic mechanisms conferring Mode 1-type resistance selected under different situations, e.g. field versus laboratory, can be different (Baxter *et al*., 2005, 2011; Tiewsiri and Wang, 2011). Therefore, studying the mechanism of Bt resistance in *T. ni* will shed light on understanding the development of Bt resistance in field insect populations.

Cry1Ac resistance in *T. ni* is an autosomal monogenic trait (Kain *et al*., 2004; Wang *et al*., 2007). A backcross strain of *T. ni*, GLEN-Cry1Ac-BCS, generated by introgression of the Cry1Ac resistance trait into a susceptible inbred laboratory strain showed a high level of Cry1Ac resistance, similar to that of the original DiPel-resistant GLEN population, and could survive on transgenic Cry1Ac broccoli and Cry1Ac cotton plants. For analysis of the resistance mechanism using comparative biochemical and molecular approaches, it is desirable to have a resistant backcross strain near isogenic to a susceptible strain to facilitate identification of resistance-associated biochemical and molecular alterations. Introgression of the Cry1Ac resistance trait into a highly inbred susceptible laboratory strain has been proven effective in minimizing non-resistance-associated variations and thereby allowing comparative biochemical analysis to identify biochemical and molecular changes that are associated with Bt resistance in *T. ni* (Wang *et al*., 2007; Tiewsiri and Wang, 2011).

8.3 Mechanism of Cry1Ac Resistance in the Cabbage Looper

The intoxication pathways of Bt toxins in insects involve a complex cascade of toxin-midgut protein interactions (Bravo *et al*., 2004; Heckel, 2012; Pardo-López *et al*., 2013). Alteration of any step in the pathway can potentially lead to Bt resistance. It has been reported that the toxicity of Bt toxins in the insect midgut can be affected by reduced solubilization of the Cry protein crystals (Schnepf *et al*., 1998), insufficient proteolytic activation or excessive degradation of Bt toxins by midgut proteinases (Oppert *et al*., 1997; Shao *et al*., 1998; Li *et al*., 2004; Karumbaiah *et al*., 2007), reduced permeability of the midgut peritrophic membrane to the toxin (Hayakawa *et al*., 2004), elevated immune response (Rahman *et al*., 2004) and increased sequestering of toxin in the midgut (Gunning *et al*., 2005). Nevertheless, numerous studies on Bt resistance have indicated that reduced binding of toxins to the midgut brush border membranes is a primary mechanism for high level Bt resistance (Heckel *et al*., 2007; Pardo-López *et al*., 2013). Currently identified midgut proteins that may serve as receptors for Cry toxins include the midgut cadherin, aminopeptidase Ns (APNs), the membrane-bound alkaline phosphatase (mALP), an ABC (ATP Binding Cassette) transporter and several other midgut proteins and glycolipids (Pigott and Ellar, 2007; Pardo-López *et al*., 2013). The identification of Bt resistance in *T. ni* in commercial greenhouses provided an opportunity to investigate Bt resistance mechanisms that may be selected in an agricultural environment.

8.3.1 Midgut proteinases

Midgut proteases in lepidopteran larvae are primarily serine proteinases and the alteration of midgut proteinases could contribute to Bt resistance in insects (Oppert *et al*., 1997; Li *et al*., 2004). In the *T. ni* larval midgut, serine proteinases are highly active at an alkaline pH (pH 10) (Li *et al*., 2009). By SDS-PAGE based proteinase zymographic analysis, midgut proteinase variations could be detected within the original Cry1Ac-resistant greenhouse *T. ni* strain, GLEN-Cry1Ac, and between the resistant and the

susceptible strains; however, the observed variations of the midgut proteinase activity profiles were confirmed not to be associated with Bt resistance (Wang *et al*., 2007). In addition, when an examination was made of both the activation Cry1Ac protoxin and the degradation of activated Cry1Ac by larval midgut fluid from susceptible and resistant strains of *T. ni*, there was no significant difference between the resistant and susceptible strains in either toxin activation and degradation in the midgut (Wang *et al*., 2007). Therefore, alteration of proteinase activities is not the mechanism of Cry1Ac resistance selected in *T. ni* populations in greenhouses.

8.3.2 Midgut esterases

Upregulated production of midgut esterases to bind and sequester Cry1Ac toxin has been reported to be a mechanism of resistance to Cry1Ac in *H. armigera* (Gunning *et al*., 2005). This midgut esterase-mediated resistance mechanism has not been observed in *T. ni*. In the Cry1Ac-resistant *T. ni* strain, the larval midgut esterase activity and esterase isoenzyme composition do not differ from those in its near-isogenic susceptible strain (Wang *et al*., 2007).

8.3.3 Haemolymph melanization activity

Heightened immune response, as determined by *in vitro* haemolymph melanization activity and visualization of melanization in the midgut and the midgut peritrophic membrane, has been proposed to be a mechanism by which Bt resistance is conferred (Rahman *et al*., 2004; Ma *et al*., 2005). In *T. ni*, the *in vitro* melanization activity of haemolymph plasma from both the susceptible and the resistant *T. ni* larvae was determined to be low, and no activity difference was observed between the two strains (Wang *et al*., 2007). Melanization or darkening of the midgut or the peritrophic membrane does not occur in Cry1Ac-resistant *T. ni* larvae.

8.3.4 Binding of Cry1Ac to midgut brush border membranes

Binding of a Cry toxin to the midgut brush border membrane is a key process in the intoxication pathway of Cry toxins. The association of reduced binding of a Cry toxin to the insect midgut brush border membrane with resistance was first observed in a Bt-resistant strain of *Plodia interpunctella* (Van Rie *et al*., 1990). It has become well known that reduced binding of toxins to the midgut brush border membranes is a primary mechanism for high-level Bt resistance (Heckel *et al*., 2007; Pardo-López *et al*., 2013). In *T. ni* larvae, there are specific binding sites in the midgut brush border membranes for Cry1Ac and Cry1Ab (Estada and Ferré, 1994; Iracheta *et al*., 2000; Wang *et al*., 2007). A binding analysis of Cry1Ac and Cry1Ab toxins to the midgut brush border membrane vesicles (BBMVs) confirmed that the toxins bound to these specific binding sites in the BBMVs from the susceptible larvae, but neither Cry1Ab and Cry1Ac bound to the BBMVs from the larvae of the Cry1Ac-resistant strain GLEN-Cry1Ac-BCS (Wang *et al*., 2007). The GLEN-Cry1Ac-BCS larvae were highly resistant to Cry1Ac, but showed no significant cross-resistance to Cry1C (Wang *et al*., 2007). So the resistance to Cry1Ac in *T. ni* is a case of Mode 1-type Bt resistance.

Mode 1-type resistance is conferred by the alteration of the midgut binding sites, or receptors, for Cry1Ac. The midgut cadherin, APNs, mALP and an ABC transporter are the primary midgut proteins that have been proposed to serve as the receptors to interact with Cry toxins in the cascade of the intoxication pathways (Griffitts and Aroian, 2005; Heckel *et al*., 2007; Pardo-López *et al*., 2013). These putative receptor proteins play different physiological functions in the midgut, so alterations to them may result in different types or different levels of negative fitness consequences. Therefore, alterations of the different receptors may differentially respond to selections for Bt resistance in different situations. The Cry1Ac resistance evolved in *T. ni* represents a case of resistance-conferring alteration of midgut

binding sites for Cry1Ac selected in an agricultural environment.

8.3.5 Midgut cadherin

The midgut cadherin is a known Bt toxin-binding protein with high-binding affinity for Cry toxins in the monomeric form (Gómez *et al*., 2003) and serves as an important receptor for Cry toxins (Francis and Bulla, 1997; Nagamatsu *et al*., 1999; Bravo *et al*., 2004). Mutations of the cadherin gene have been identified as linked with resistance to Cry1Ab or Cry1Ac. In a laboratory-selected Bt-resistant *Heliothis virescens* strain, the resistance was found to be associated with disruption of the cadherin gene by insertion of a retrotransposon (Gahan *et al*., 2001). Similar cadherin mutations have also been identified in Cry1Ac-resistant *P. gossypiella* and *H. armigera* (Morin *et al*., 2003; Xu *et al*., 2005). The *T. ni* midgut cadherin, a 194.7 kDa protein with 1733 amino acid residues, shares the same sequence characteristics as other known lepidopteran midgut cadherins, containing 11 cadherin repeats followed by a membrane-proximal domain in the extracellular region, a transmembrane region and a cytoplasmic tail at the C-terminus (Zhang *et al*., 2012b). Sequence motifs identified as Cry toxin-binding regions from other lepidopterans are also present in the *T. ni* cadherin (Zhang *et al*., 2013). The *T. ni* cadherin gene is highly polymorphic. Single nucleotide polymorphisms (SNPs), insertion mutations and deletion mutations have all been identified in the *T. ni* cadherin gene (Zhang *et al*., 2013). In addition to gene sequence polymorphisms, differential splicing of the cadherin transcript also occurs in the expression of the cadherin gene in *T. ni* (Zhang *et al*., 2013).

The high variability of the cadherin in *T. ni* could potentially be the genetic basis for the selection of cadherin-mediated Bt resistance (Zhang *et al*., 2013). However, the Cry1Ac resistance developed in *T. ni* greenhouse populations has been identified as independent of the alteration of the midgut cadherin (Zhang *et al*., 2012b). Genetic linkage analysis of the cadherin alleles with Cry1Ac resistance in *T. ni* determined that the cadherin gene was not genetically associated with greenhouse-selected Cry1Ac resistance in *T. ni*. Analyses of cadherin expression in the *T. ni* midgut at both the mRNA and protein levels further confirmed that there is no quantitative difference of the cadherin between susceptible and Cry1Ac-resistant *T. ni* larvae. Moreover, Cry1Ac binds similarly to the cadherin from the Cry1Ac-susceptible and Cry1Ac-resistant *T. ni* larvae (Zhang *et al*., 2012b). In addition, genetic mapping using amplified fragment length polymorphism (AFLP) markers confirmed that the gene controlling Cry1Ac resistance and the cadherin gene reside on two different chromosomes in *T. ni* (Baxter *et al*., 2011). Thus, the resistance to Cry1Ac evolved in greenhouse populations of *T. ni* is not conferred by cadherin alteration.

It is noteworthy that among the cadherin alleles identified in *T. ni*, some are predicted to lack the membrane domain to localize in the midgut brush border membranes and so lose any function as a receptor for Cry toxins (Zhang *et al*., 2013). Such alleles would be expected to confer cadherin-mediated resistance, but were found to be low in abundance and were not selected for Cry1Ac resistance in *T. ni*. Why these loss-of-function mutations were not selected for resistance to Cry1Ac has yet to be understood, but it is possible that they may be associated with a very strong fitness cost.

8.3.6 Alkaline phosphatase

The midgut mALP from *H. virescens* has been identified as a potential receptor for the Bt toxin Cry1Ac (Jurat-Fuentes and Adang, 2004). This mALP is a glycoprotein glycosylphosphatidylinositol (GPI)-anchored to the midgut brush border membranes, and the terminal GalNAc on mALP serves as the binding site for the toxin. It has been shown that a decreased level of mALP in the midgut directly correlated with resistance to the Bt toxin in *H. virescens*. Additionally, reduced mALP activity has also been found

in Cry-resistant *H. armigera* and *S. frugiperda* (Jurat-Fuentes *et al*., 2011). The mALP in *T. ni* has a predicted molecular weight 61.4 kDa with 564 amino acid residues (Baxter *et al*., 2011). Analysis of mALP activity in the midgut BBMVs from Cry1Ac-susceptible and Cry1Ac-resistant *T. ni* larvae determined that there was no mALP activity change in Cry1Ac-resistant *T. ni* (Wang *et al*., 2007). Similarly, a quantitative comparative proteomic analysis of the midgut BBMV proteins from Cry1Ac-susceptible and Cry1Ac-resistant *T. ni* larvae showed that there was no significant difference in mALP quantity between the two strains (Tiewsiri and Wang, 2011). Genetic mapping of the Cry1Ac resistance has also determined that the mALP gene is not on the same chromosome as the Cry1Ac resistance gene in *T. ni* (Baxter *et al*., 2011). Thus, greenhouse-evolved Cry1Ac resistance in *T. ni* is not associated with the mALP.

8.3.7 Aminopeptidase N

Insect APNs are a multi-gene family of GPI-anchored membrane proteins (Adang, 2013). Midgut APNs are the first identified midgut receptors for Cry toxins (Knight et al., 1994; Sangadala *et al*., 1994; Gill *et al*., 1995). The role of an APN as a receptor for Cry1Ac has been shown by the transformation of *Drosophila*, which was not susceptible to Cry1Ac, with an APN gene from *Manduca sexta*. The resulting transgenic *Drosophila* with the *M. sexta* APN transgene became susceptible to Cry1Ac, indicating the functional role of the APN in Bt toxicity (Gill and Ellar, 2002). In addition, Cry1Ac-induced pore formation in the midgut brush border membranes from *T. ni* larvae was found to depend on the APN activity on the brush border membranes (Lorence *et al*., 1997). In a Cry1C-resistant *Spodoptera exigua* strain, it was found that the expression of one APN was completely lacking (Herrero *et al*., 2005). Therefore, the alteration of APNs could potentially be a mechanism for Bt resistance in insects.

In *T. ni*, six APNs have been identified in the larval midgut by the cloning of complementary DNA (cDNA) and proteomic analysis (Wang *et al*., 2005; Tiewsiri and Wang, 2011). A comparative analysis of proteins in the midgut BBMV proteins from the susceptible and the near-isogenic Cry1Ac-resistant larvae identified that the Cry1Ac-resistant *T. ni* strain lacked a 110 kDa protein from the BBMV proteins (Tiewsiri and Wang, 2011). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) quantitative proteomic analysis and Western blot analysis with APN1-specific antibodies determined that the missing protein was the intact 110 kDa APN1 that Cry1Ac could bind to (Tiewsiri and Wang, 2011). Further LC-MS/MS analysis of midgut BBMV protein bands ranging from 33 to 250 kDa resolved by SDS-PAGE, identified another differentially expressed BBMV protein, APN6, in resistant *T. ni* larvae; APN6 was rare in BBMV proteins from the susceptible strain, but was detected in multiple protein bands with a relatively higher abundance in BBMV proteins from the resistant strain (Tiewsiri and Wang, 2011).

The midgut BBMV proteins from *T. ni* larvae have been globally analysed to identify proteins that are differentially present between Cry1Ac-susceptible and Cry1Ac-resistant *T. ni* larvae; the analysis used the non-gel-based quantitative proteomic technique, iTRAQ (isobaric tags for relative and absolute quantitation)-based 2D-LC-MS/MS analysis (Tiewsiri and Wang, 2011). Over 1400 proteins could be identified from the midgut BBMVs of *T. ni* larvae and their relative abundances were determined. Quantitative analysis of the BBMV proteins from *T. ni* larvae identified two proteins that were significantly different in quantity between Cry1Ac-susceptible and Cry1Ac-resistant *T. ni* – the amounts of APN1 and APN6 in the resistant strain were 0.11 times and 6.0 times, respectively, of those found in the susceptible strain (Tiewsiri and Wang, 2011).

The significant decrease in APN1 and increase in APN6 in the midgut of resistant *T. ni* larvae have been confirmed to be regulated at transcription level. The expression of APN1 and APN6 genes in the

midgut of resistant larvae was downregulated to 2.6% and upregulated to 3900%, respectively, at mRNA level. The other four APNs, APN2–APN5, were found to be unchanged in the resistant *T. ni* larvae at both protein and mRNA levels (Tiewsiri and Wang, 2011). Importantly, Cry1Ac resistance in *T. ni* was determined to be associated with the differential expression of APN1 and APN6 by a linkage analysis (Tiewsiri and Wang, 2011). So the Mode 1-type resistance selected in greenhouse populations of *T. ni* by Bt sprays is associated with differential alteration of APN1 and APN6 in the midgut, which is distinctly different from the cadherin gene mutation-based mechanism previously identified in three laboratory-selected insects (Gahan *et al*., 2001; Morin *et al*., 2003; Xu *et al*., 2005).

Although the greenhouse-selected Cry1Ac resistance in *T. ni* is associated with downregulation of APN1 and upregulation of APN6, genetic linkage analysis of the APN genes with resistance determined that all six APN genes were clustered in one linkage group and had no genetic linkage with resistance (Tiewsiri and Wang, 2011). An additional genetic mapping study of Cry1Ac resistance in *T. ni* further confirmed that the APN genes and the resistance gene are localized on different chromosomes (Baxter *et al*., 2011). Therefore, resistance to Cry1Ac in *T. ni* is controlled by a *trans*-regulatory mechanism, leading to the absence of the full size (110 kDa) toxin-binding APN1 in the midgut brush border membranes and, as a result, the loss of binding sites for the toxin.

8.3.8 ABC transporter

ABC transporters are a large superfamily of transmembrane proteins. A mutation in an ABC transporter gene, *ABCC2*, has been identified to be genetically associated with Cry1Ac resistance in *H. virescens* (Gahan *et al*., 2010). ABC transporter proteins have not been identified as Cry toxin-binding proteins by the biochemical analysis of midgut proteins from any insects, but their functional role as a Cry toxin receptor has been proposed and is supported by experimental data from the functional expression of the *Bombyx mori ABCC2* gene in cell culture and the introduction of a susceptible allele of this gene into a resistant strain of *B. mori* to rescue its susceptibility to Cry1Ab (Atsumi *et al*., 2012; Heckel, 2012; Tanaka *et al*., 2013). The ABCC2 protein from *T. ni*, which is orthologous to the *H. virescens* ABCC2, is a protein of 150 kDa with similar domain architecture and sequence characteristics to the ABCC2 from other lepidopterans (Gahan *et al*., 2010; Baxter *et al*., 2011; Atsumi *et al*., 2012). By genetic mapping, Cry1Ac resistance in *T. ni* was mapped to the *ABCC2* gene locus in a linkage group homologous to *B. mori* chromosome 15 (Baxter *et al*., 2011). Notably, Cry1Ac resistance in *P. xylostella* selected by Bt sprays in open fields has also been mapped to the *ABCC2* locus, but is independent of the cadherin gene (Baxter *et al*., 2005, 2011).

Although Cry1Ac resistance in *T. ni* has been mapped to the *ABCC2* locus region in *T. ni*, whether mutations in *ABCC2* or in another gene in the same region control the resistance and whether or how the altered expression of APN1 and APN6 is conferred by the mutation in an ABC transporter have yet to be understood.

8.4 Conclusion

The intoxication pathways of Bt toxins in insects are complex and the mechanisms of Bt resistance can be diverse. For the sustained application of Bt for insect pest control, it is important to understand the resistance mechanisms that have evolved in insect populations in agricultural situations to provide fundamental knowledge for the management of insect resistance in agriculture. Bt resistance in *T. ni* was selected in an agricultural situation and the resistant *T. ni* could not only survive Bt sprays on vegetable crops, but also on Bt broccoli and Bt cotton plants. Consequently, Bt resistance in *T. ni* is conferred by a mechanism that threatens the continuing success of Bt technology in agriculture.

Cry1Ac resistance in *T. ni* is a typical example of Mode 1-type Bt resistance.

However, the loss of midgut binding sites for Cry1Ac in *T. ni* is associated with downregulation of APN1 and upregulation of APN6, which is different from the cadherin mutation-associated Mode 1-type resistance identified in *H. virescens*, *H. armigera* and *P. gossypiella* (Gahan *et al*., 2001; Morin *et al*., 2003; Xu *et al*., 2005). The midgut cadherin gene in *T. ni* populations is highly polymorphic and differential slicing of its transcripts also occurs. Even so, cadherin-mediated resistance was not selected for Cry1Ac resistance in greenhouse *T. ni* populations. The alteration of APN expression in Cry1Ac-resistant *T. ni* is regulated by a *trans*-regulatory mechanism yet to be known, and the resistance is localized to an ABC transporter gene locus region. Bt resistance in *T. ni* is a unique case for studying the molecular mechanism of Bt resistance that has evolved in agricultural systems. Cases of field-evolved Bt resistance have been increasingly reported, but the detailed molecular mechanisms of such resistance remain to be understood.

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