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Susceptibility, mechanisms of response and resistance to *Bacillus thuringiensis* toxins in *Spodoptera* spp.

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Bioinsecticides based on *Bacillus thuringiensis* have long been used as an alternative to synthetic insecticides to control insect pests. In this review, we focus on insects of the genus *Spodoptera*, including relevant polyphagous species that are primary and secondary pests of many crops, and how *B. thuringiensis* toxins can be used for *Spodoptera* spp. pest management. We summarize the main findings related to susceptibility, midgut binding specificity, mechanisms of response and resistance of this insect genus to *B. thuringiensis* toxins.

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Introduction

The genus *Spodoptera* includes some important polyphagous insect species that are primary and secondary pests of many crops such as asparagus, cabbage, pepper, tomato, lettuce, celery, strawberry, eggplant, sugar beet, alfalfa, cotton, corn and tobacco. From an agronomic point of view, the most important species of this genus are *Spodoptera exigua* (Hübner) (worldwide distributed), *Spodoptera frugiperda* (JE Smith) (native to the tropical regions of the western hemisphere from the USA to Argentina), *Spodoptera littoralis* (Boisduval) (Europe and Africa) and *Spodoptera litura* (Fabricius) (Indo-Australian tropics).

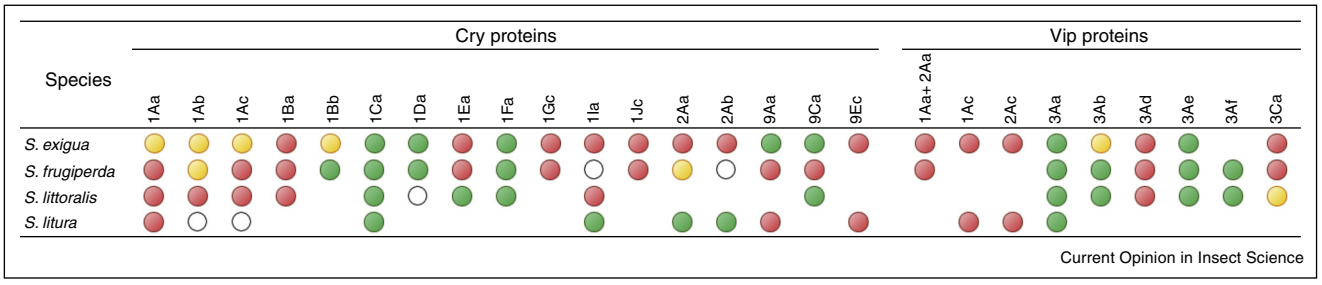
The bacterium *Bacillus thuringiensis* (Bt) produces a wide range of toxins which are active against a number of pest species in the orders Lepidoptera (including *Spodoptera* spp.), Coleoptera, Diptera, Hymenoptera, Hemiptera and Blattaria [1,2]. Bioinsecticides based on this bacterium have been used for years as an alternative to chemical insecticides to control insect pests. One of the most interesting features of *B. thuringiensis* bioinsecticides is their specificity: they are completely safe to vertebrates and do not harm beneficial insects. This specificity is due to the mode of action of their toxins. After ingestion, these toxins are activated by the action of digestive enzymes, bind to specific receptors in the epithelial cells of the midgut and induce pores in the membrane which eventually lead to septicemia and insect death [3]. Because of the large number of publications dealing with the insecticidal spectrum of *B. thuringiensis* toxins, in 1998 a toxin specificity database was created to pull together most information available on this subject (http://www.glfc.cfs.nrcan.gc.ca/bacillus).

In the present paper, we review the current knowledge on the specificity of *B. thuringiensis* toxins against *Spodoptera* spp., their binding to host receptor molecules, as well as the means by which insects from this genus can overcome their toxic effects.

Susceptibility of *Spodoptera* spp. to *B. thuringiensis* toxins

Several papers have determined the specificity of individual *B. thuringiensis* toxins against *Spodoptera* spp. to identify those toxins with the highest potential to be used as bioinsecticides and for the development of transgenic crops for the control of *Spodoptera* spp. Most studies on the genus *Spodoptera* have been carried out with *S. exigua* and *S. frugiperda*, followed by *S. littoralis* and *S. litura*. Bioassays have been carried out with a number of *B. thuringiensis* Cry proteins belonging to the Cry1, Cry2, Cry3, Cry7, Cry8, Cry9, Cry15, Cry22, Cry48/49, and Cry54 families and also with the secretable Vip1, Vip2, and Vip3 proteins [4–6,7•]. To date, a limited number of *B. thuringiensis* proteins have been found active against *Spodoptera* spp., although in some cases, the results of bioassays are contradictory. In Figure 1 we have summarized the activity of individual *B. thuringiensis* toxins which have been tested in at least two *Spodoptera* spp. In general, Cy1Ca, Cry1Fa, Vip3Aa, Vip3Ab, and Vip3Ae are considered to be active against *Spodoptera* spp. (at least to three *Spodoptera* species) [1,5,7•,8]. Other proteins, such as Cry1Bb, Cry1Da, and Vip3Af, have also been reported to be active against at least two *Spodoptera* spp. [1,5,7•,9]. Unfortunately, Cry1A proteins, the most common in commercial *B. thuringiensis* products and in Bt-crops (insect-resistant transgenic crops expressing one

Figure 1



Toxicity of some *B. thuringiensis* toxins against *Spodoptera* spp. The figure includes only those *B. thuringiensis* toxins tested to at least two different insect species. Data from this figure were extracted from the *B. thuringiensis* specificity database (http://www.glfc.cfs.nrcan.gc.ca/bacillus) and later reports [5,6,7•,8,16,26,56]. The activity of the toxins is indicated as strong (green), moderate (yellow), and non-active (red). Cases for which contradictory results were reported are indicated by empty circles.

or more insecticidal proteins from *B. thuringiensis*), have low or no activity against most *Spodoptera* spp. [1,8,10].

Binding of *B. thuringiensis* toxins to the *Spodoptera* midgut

Binding of *B. thuringiensis* toxins to insect midgut receptors is a necessary step for toxicity [9,11,12]. Indeed, toxin binding alteration has been associated with high levels of resistance in insect populations [13]. In this context, if different *B. thuringiensis* toxins share the same binding site, an alteration of this site could confer cross-resistance to all toxins that share the same site. Therefore, the study of the molecules involved in the binding of *B. thuringiensis* toxins is crucial for the correct design of pest control management strategies.

Midgut binding sites

To date, studies performed with brush-border membrane vesicles (BBMV) prepared from midgut tissues and *B. thuringiensis* toxins labeled with either iodine-125 or biotin, have shown that *Spodoptera* spp. have midgut binding molecules that specifically bind Cry1C, Cry1D, Cry1F, Cry1B, Cry1E, Cry1Aa, Cry1Ab, and Cry1Ac [8,9,14–22], Cry9Ca [17], and Vip3A proteins [20,23•]. Despite Cry1A proteins not being very active against *Spodoptera* spp., they also bind with high affinity to midgut binding sites.

Heterologous competition studies have helped determine whether different *B. thuringiensis* toxins share binding sites in the *Spodoptera* midgut. If shared binding is indicated, it may involve all or just some of the relevant sites. Partial competition is an indication of more than one binding site. Studies performed with BBMV from *S. frugiperda* and *S. exigua* have shown that Cry1Ca (one of the most toxic proteins to *Spodoptera* spp.) binds to sites partially shared by Cry1Ba, Cry1Bb, and Cry9Ca, but not by Cry1A proteins or Cry1Ea [9,17,19,21,24]. Cry1Fa was found to compete with labeled Cry1Ca in *S. exigua* and *S. frugiperda* [9]. However, in a more recent work with *S. frugiperda*, these two toxins did not compete to each other in reciprocal heterologous competition experiments [25••]. In *S. littoralis*, Cry1C and Cry1E compete for a common site in addition to their independent binding sites [22].

Cry1Fa does not share binding sites with Cry2Ab and Cry2Ae proteins, but it does with Cry1Ab, Cry1Ac and Cry1A.105 (a chimeric protein with domains I and II of Cry1Ac and domain III almost identical to cry1Fa) in *S. frugiperda* [9,16,20] and with Cry1Ja in *S. exigua* [18]. Cry1Ab and Cry1Ac proteins share two binding sites in *S. exigua*, one of them also shared with Cry1Aa [19]. Vip3 proteins (Vip3Aa and Vip3Af) do not share binding sites with Cry proteins (Cry1Ab, Cry1Ac, Cry1Fa, and Cry1Ia) in *S. frugiperda* [20,23•,26]. The information derived from the heterologous competition binding experiments has been recently revised by Jakka *et al.* [27]. A schematic summary of the binding sites for Cry and Vip proteins in *Spodoptera* spp. is shown in Figure 2.

Molecules identified as functional receptors for *B. thuringiensis* toxins

The identity of some receptor molecules involved in the *B. thuringiensis* toxin binding in *Spodoptera* spp. is known, though much less work has been done compared to other insect species. This section reviews those studies that have demonstrated a functional role of the putative receptor molecules and a schematic summary is given in Figure 2. RNA interference (RNAi) technology has been applied to *B. thuringiensis* toxin receptor studies. Administration of dsRNA to *S. litura* larvae identified an aminopeptidase N (APN) protein as a receptor for Cry1C [28]. Using heterologous protein expression in cultured insect cells and subsequent toxin exposure, it was shown that *S. litura* APN was a receptor for Cry1C but not for Cry1Ac [29]. The role of APN1 in Cry1Ca toxicity was reinforced after the study of a Cry1Ca resistant *S. exigua* strain revealed that resistant insects lacked the expression