REPORT

New knowledge on insect-resistance management for transgenic Bt corn

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ABSTRACT

The technology of transgenic crops involving plants containing insecticidal proteins derived from *Bacillus thuringiensis* (Bt) has to be protected against the intrinsic risk of insect-resistance through pest management strategies, the socalled ´high dose/refuge' strategy the most frequently implemented. It comprises cultivating Bt-transgenic plants which effectively control the pest with a nearby cultivar of non-transgenic plants, known as 'refuge'. This strategy assumes insect oviposition as an event of random distribution between both plant cultivars. In this work, oviposition and the survival of *Spodoptera frugiperda* were examined in Bt-corn and its conventional refuge cultivars in an experimental area for six cultivation seasons. The impact of this strategy on the emergence of insect-resistance was analyzed once applied the simulation models starting from the obtained data. This was the first report of a higher oviposition of *S. frugiperda* in all the conditions tested for Bt-corn over that of the refuge cultivar, when the damage caused by the pest in the refuge was statistically higher. Simulation models showed that this insect behavior accelerates its resistance against the Bt toxin it was exposed to. New key information is provided to optimize the management of insect resistance in Bt transgenic crops, further suggesting new interventions to control the population density of pest in the field. This research granted the 2015 Award of the Cuban National Academy of Sciences.

Keywords: Bacillus thuringiensis, GM crops, high dose/refuge strategy, oviposition behavior, population dynamics, resistance management

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RESUMEN

Nuevos conocimientos para el manejo de la insecto-resistencia en el maíz-Bt. Con el propósito de salvaguardar la tecnología de los cultivos trangénicos con proteínas insecticidas derivadas de *Bacillus thuringiensis* (Bt) frente al riesgo intrínseco de la insecto-resistencia, se emplean diferentes estrategias de manejo, entre ellas la de 'alta dosis/refugio' como la más frecuente. Esta comprende el cultivo de plantas con toxinas Bt que ejercen un control eficaz de la plaga, en combinación con un área sembrada con plantas no transgénicas a Bt en su proximidad (refugio). Esta estrategia asume que la ovoposición ocurre al azar, a través de las plantaciones de Bt y refugio. En este trabajo, se examinó la ovoposición y la supervivencia de *Spodoptera frugiperda*, en plantaciones de maíz-Bt y su refugio en un área experimental durante seis temporadas del cultivo. Tras la aplicación de modelos de simulación a los datos obtenidos, se analizó el impacto de esta estrategia sobre la evolución de la insecto-resistencia. Se reporta por primera vez que la ovoposición por planta de *S. frugiperda* fue superior en todas las condiciones evaluadas en el maíz-Bt, en comparación con el cultivo del refugio, cuando el daño producido por la plaga en el refugio fue significativamente superior. Los modelos de simulación mostraron que este comportamiento del insecto acelera su resistencia a la toxina Bt a la cual se expuso. Se aportan nuevos elementos para optimizar el manejo de la insecto-resistencia en los cultivos-Bt y se sugieren intervenciones dirigidas a controlar la densidad poblacional de la plaga en el campo. Esta investigación mereció el Premio de la Academia de Ciencias de Cuba en el 2015.

Palabras clave: Bacillus thuringiensis, cultivos transgénicos, estrategia altas dosis/refugio, dinámica poblacional, manejo de resistencia

Introduction

Transgenic cultures have become a technological revolution which efficiently increases production yields in agriculture. Aside polemics concerning genetically modified organisms (GMOs), the transgenic crop technology promises unquestionable benefits for developing countries. Currently, up to 181 million hectares of transgenic crops worldwide are cultured [1], with transgenic plants bearing insecticidal toxins of *B. thuringiensis* among the most remarkable examples. Known as Bt crops, they have fostered plague control to the next level, with a more effective pest management strategy, lower use of chemical pesticides and increased productions. Such crops include cotton and corn production as the most economically relevant ones, with the specific transgenic events protecting against Lepidoptera and Coleoptera species.

Nevertheless, pest resistance is the most feared limitation of the extensive use of Bt-based strategies. Resistance is commonly conferred by a specific set of genes providing recessive resistance to high levels of

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Bt [2]. This poses the need for management strategies to prevent and to eradicate the risks for pesticide resistance in the field. One of such strategies comprises the use of the so-called 'high dose/refuge' approach, providing a higher number of adult insects regarded as 'pest' which become susceptible to the pesticide once going out of their conventional cultivar. Those insects then mate with any homocygotic resistant moth which emerged in the Bt-transgenic plants cultivar, leading to an heterocygotic progeny susceptible to the high doses of the Bt toxin [3, 4].

One of the first documented cases of insect-resistance in Bt crops was that of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Bt-Cry1F corn, carrying the TC1507 event, in Puerto Rico [5]. In general, there are two main causes for the emergence of insect resistance: the non-strict adherence to planting the refuge [6, 7], and that Cry1Fa toxin expression in those transgenic plants is either low or variable but insufficient to kill the resistant heterocygotic insects [5, 6, 8].

It was generally assumed that adult pest oviposition occurred randomly for persistence of the refuge. But studies led by the groups of Hellmich, Kjaer and Lei [9-11] established that adult procreating females did not discriminate between transgenic and isogenic line cultures for oviposition. However, these studies only focused on a small number of eggs found in undamaged plants, ignoring any further damage that larva could produce while feeding. Such effect is significant since volatile vegetal compounds released during larval feeding can further dissuade the moth from oviposition in the very same plant, probably reducing the intraspecific competition or its detection by natural enemies which could use the volatile vegetal compounds as stimuli for feeding [12, 13].

Therefore, this work was aimed to test the hypothesis that *S. frugiperda* could change its oviposition preference in response to foliar damage in conventional corn crops as compared to transgenic Bt corn. The open field studies were performed during six climatic dry and wet periods in Cuba, very similar to that occurring in Puerto Rico, where *S. frugiperda* resistance to Bt-Cry1Fa was first reported [5].

Results

The preference for ovoposition of *S. frugiperda* in Bt corn correlates with the level of damage in the refuge

Field data were obtained from 2009 to 2012 during the dry and wet climate periods, in an experimental parcel at the Center for Genetic Engineering and Biotechnology (CIGB; Havana, Cuba). The damage to the crop caused by the fall armyworm was classified according to a four-level scale. The results of the experimental observations revealed that 20 to 25 days after planting, the damage caused by *S. frugiperda* in the transgenic variety FR-Bt1 was almost inexistent, as opposed to that found in the FR-28 non-resistant variety. In fact, the damage found in this conventional variety was high and statistically significant as compared to the transgenic variety ($p < 0.05$; non-parametric Kruskal-Wallis test).

The counts of eggs/plant done in both cultivars during the six evaluated periods evidenced a higher number of ovipositions in the Bt transgenic cultivar. It was predicted before conducting the study that the random oviposition would account for up to 309 in the refuge, but just 145 were found (47 %). When evaluating the abundance of *S. frugiperda* and the damage in the refuge, it was found that the preference for the Bt resistant corn cultivar markedly increases while increasing the damage in the refuge cultivar [14].

The preferential oviposition of *S. frugiperda* in Bt corn plays a significant role in the evolution of insect resistance

Foliar damage by *S. frugiperda* was found as limiting for the oviposition of adult female insects in the non-transgenic, conventional corn cultivar FR-28, leading to a preference for the transgenic cultivar. This ultimately favored the emergence of insect resistance to Bt. This study was partially based on previous unpublished observations under field conditions. The larval density in the refuge was found as determinant for the tendency in oviposition in this experimental model. Difference pest-management regimes were considered, modeling three scenarios: i) the application of one pesticide in the refuge cultivar, at a threshold of 0.25 ovipositions/plant; ii) a natural predation model exclusively in the refuge; and iii) a natural predation model in the refuge cultivar in both, the Bt corn and the conventional corn cultivars.

The frequency of appearance of resistance alleles in the three scenarios tested, corresponding to the random oviposition with recessive resistance, showed that the increase in 10 to 30 % in the refuge area could effectively control insect resistance in the transgenic crop. A less effective but maybe still successful control of the pest could be achieved by applying a pesticide in smaller refuges. Another opposite picture was obtained when the insect resistance alleles were partially dominant, with no possible control to be implemented under the parameters established in the three models tested [14].

The curves representing the behavior of oviposition, when recessive resistance is present and foliar damage is limiting, showed that the emergence of resistance accelerates [14]. The only scenario in which the emergence of insect resistance is still possible to be controlled is that simultaneously managing the density of the pest in the entire cultivar area (both the Bt cultivar and the refuge cultivar simultaneously) with natural predators and increasing the refuge area in 30 % of its estimated size.

Discussion

It was demonstrated the preference of *S. frugiperda* for oviposition in Bt-transgenic plants over those of the refuge non-transgenic cultivar when this last is highly affected [14]. This behavior also has implications for the adequate crop management, to avoid the emergence of insect resistance. Our results also coincide with previous ones but obtained when using conventional cultivars only. For instance, 1979, Sparks demonstrated that, in this species, oviposition changes in response to larval density [15]. Moreover, Signoretti *et al*. [16], in 2012, proved that adult female insects preferred the volatiles compounds of undamaged corn plants.

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Remarkably, we controlled any possible effect of attraction caused by the genetic background of the cultivars used, by using as refuge cultivar the conventional variety FR-28, parental for the transgenic FR-Bt1 variety, with an overall 96.75 % of homology between them. The behavior described for adult females to avoid plants damaged by individuals of the same species for oviposition could be regarded as an adaptive behavior, seeking for protecting the progeny from intra-specific competence [17, 18].

Our findings stress the strategic relevance of adequate crop management procedures to minimize the risks for favoring insect resistance when the refuge cultivar is severely damaged. One possible strategy comprises planting decoy refuges for insect ovoposition, releasing the pressure over the transgenic cultivar. Additionally, the integral pest management strategies also recommend the use of pheromones, natural hosts, among others.

Such strategies are necessary since Bt transgenic crops are not immune to the evolution of the insect-resistance. Hence, minimizing the risk for insect attack further expands its use, quite relevant for the intensive cultivation of economically-relevant Bt-transgenic crops. Moreover, the efficacy of the refuge cultivar could be susceptible for management improvements even for plants systems including one or more Bt toxins as self-insecticides [19-21].

At the same time, the function of the refuge cultivar could be reduced due to a marked preference of the pest for oviposition in healthy, undamaged plants. This is more significant in the presence of multivoltine species or those reproducing themselves under conditions favoring the simultaneous coexistence of different insect generations, further leading to a faster or even irreversible deprivation of the refuge cultivar.

Hence, a more integrated and strong crop management strategy is required, by combining different mechanisms for pest control in an ecosystem-friendly manner, to extend the useful lifetime of Bt technology.

Relevance of the study

It was demonstrated for the very first time that the preference of *S. frugiperda* for oviposition in Bt plants over the conventional susceptible crops is significantly higher when the damage increases in the refuge cultivar, positively correlating with it. This behavior poses relevant implications for the evolution of insect resistance involving a cross-talk between both the transgenic and non-transgenic cultivars. This increased preference for affecting the transgenic cultivar has to be taken into consideration to control the population density of the pest while trying to control the emergence of insect-resistance in Bt plants. This also points towards the relevance of implementing adequate management procedures for Bt-transgenic corn cultivars, which could exert a better control over the pest population density and extend the halftime of this Bt transgenic technology.

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