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Bt-maize effects on biological parameters of the non-target aphid *Sitobion avenae* (Homoptera: Aphididae) and Cry1Ab toxin detection

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Abstract

Bt-maize crop is increasingly used worldwide and the study of ecological side effects is a major subject in this domain. Under laboratory conditions, we determined Bt-maize effects on the non-target aphid *Sitobion avenae* (Fabricius) (Homoptera: Aphididae). We found no significant differences between *S. avenae* on MON810 and the near-isogenic line when alate offspring production, apterous survivorship, longevity, intrinsic rates of natural increase (r_m), finite rates of increase and doubling times were compared. No significant differences were found between treatments for apterous pre-reproductive and reproductive periods. Additionally, we used immunological tests (ELISA) to detect Cry1Ab protein in maize leaves and *S. avenae* nymphs. Results showed that Bt-maize leaves expressed 0.203 (±0.05) μg Cry1Ab/g leaf tissue (Mean ± SEM). No Cry1Ab protein was present in *S. avenae* nymphs developing on Bt or conventional maize. We conclude that Bt-maize does not affect the development of the non-target aphid *S. avenae* and that Cry1Ab toxin quantities in these aphids are nil, suggesting an inconsequential risk for natural enemies of this aphid species.

Keywords: *Sitobion avenae*; ELISA; Bt-maize; Cry1Ab; Non-target insect; Risk assessment

1. Introduction

Genetically modified (GM) crops are becoming an increasingly important feature of agricultural landscapes. A total of 102 million ha of GM crops were planted worldwide in 2006 with GM-maize being one of the most widely grown GM crops [1]. Among GM-maize varieties, Bt-maize MON810, Bt 11 and event 176 have been genetically modified to express the Cry1Ab toxin [2] which is naturally produced by the bacterium *Bacillus thuringiensis* during its sporulation phase [3]. The Cry1Ab toxin presents insecticidal action against the European Corn Borer (ECB) *Ostrinia nubilalis* (H.) (Lepidoptera: Pyralidae) [4]. In susceptible insects, Cry1Ab crystal proteins produce lesions in the midgut epithelium [5] inducing septicemia caused by enteric bacteria of the exposed insects [6].

Although Bt-toxins are generally considered as highly specific, biological modifications on non-target insects have been reported as a result of exposure to Bt-cultivars [7,8]. However, few studies have been developed to assess risk for non-target phytophagous insects. To date, the non-target phytophagous species most thoroughly studied is the monarch butterfly *Danaus plexippus* L. (Lepidoptera: Danaidae) [9–15]. Aphids are one of the most common phytophagous insects found on maize worldwide [16]. Moreover, they are important prey for many natural enemies, which can be negatively affected when feeding on

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toxic-contaminated prey [16] or prey products [17]. For these reasons, aphids are good tools for studying the effects of Bt crops on non-target phytophagous insects.

One way to estimate the potential risk of Bt crops on non-target phytophagous insects is by estimating the level of potential exposure to Bt-toxin in crops. Immunological analyses can be performed to assess the presence of Bt-toxins on exposed insects and plant products. For Bt-maize, quantification of the Cry1Ab toxin in two non-target aphid species, *Rhopalosiphum maidis* (Fitch) and *R. padi* (L.), has shown that no toxin or only traces of the toxin can be found in Bt-maize-exposed aphids [18–20]. The principal reason for this is the absence of Cry1Ab protein translocation into the phloem of Bt-maize [19]; although traces of Cry1Ab protein on aphids have been explained as a result of intracellular plant sap ingestion during puncture probing [19].

On the other hand, effects on aphids can be related with plant modifications other than the Cry1Ab protein expression, such as pleiotropic effects. It is known, for example, that the content of lignine can be higher in some tissues of Bt-maize [21,22] and this could affect attractiveness to aphids because lignine influences water permeability and the strength of particular cell walls [23]. In this way, the impact may be negative (direct action of the toxin), positive (reduction of competition with other insects), or both (changes in nutritional or physiological quality due to pleiotropic effects of transgene expression). For this reason a complete risk assessment of Bt-crops for non-target aphids also requires the measurements of effects on biological parameters which could be affected by modifications of plant characteristics. Effects of Bt-maize on biological parameters have only been assessed on the aphid *R. padi* [20,24,25]. For this species no effects were found in the first approaches [20,24], although some effects were reported in a posterior study according to the alate or apterous condition of the aphids [25].

Additional to *R. padi*, other aphid species can colonize maize such as *Sitobion avenae* (Fabricius) and *Metopolophium dirhodum* (Walker) [26,27]. *Sitobion avenae* is a major pest of cereals on Europe [28] displaying all life cycle forms known for aphids [29,30]. Generally, it remains on the same host [31,32] and its dispersal induction depends on crowding and food quality [33].

In this context, studies considering these species are warranted because they will contribute to the knowledge of Bt-maize effects on aphid community. For the aphid *R. padi* available information is not consistent about effects of Bt-maize on biological parameters [20,24,25] which indicate that these effects cannot be completely excluded. Studying the effects on more than one aphid species is important in terms of population dynamics, because modifications on biological traits of some species, and thus, on interspecific relationships could result in population dynamic variations [34,35], which justify the study of additional relevant species in the system.

In the present study, we aimed to assess the effects of Bt-maize plants regarding survivorship, demographic parameters and developmental duration periods on the non-target aphid *S. avenae* (F.) (Homoptera: Aphididae). This species can be present in maize crops and cause direct damage and transmit viruses (mainly maize dwarf mosaic virus, MDMV) [26]. We also quantified levels of Cry1Ab toxin in aphids using immunological tests (ELISA) to estimate direct exposure to Cy1Ab protein.

2. Materials and methods

2.1. Biological materials

Standard maize seedlings (AW956 Dekalb Monsanto) were used to rear the host aphid colony of *S. avenae*. For the Bt-maize treatment, the variety 'Novelis', event MON810 (expressing Cry1Ab toxin) was used. For the non-Bt-maize treatment the variety 'Nobilis', a conventional cultivar (Isogenic variety of event MON810), was used. Experiments were carried out when seedlings had 5–6 leaves. Maize was grown in a climatized room at $T = 23 \pm 2$ °C, RH $= 40 \pm 10\%$ with a 16:8 LD photoperiod. Seeds were placed two by two in individual pots ($10 \times 9 \times 8.5$ cm) containing potting mix and watered twice a week with a fertilizer solution.

The *S. avenae* colony was reared using maize seedlings (5–6 leaves) in a room under controlled conditions $T = 23 \pm 2$ °C, RH $= 40 \pm 10\%$ and 16:8 LD. The original strain (c85) was provided by the "Biologie des Organismes et des Populations Appliquée à la Protection des Plantes" (INRA, Rennes, France).

2.2. Effects of Bt-maize on Sitobion avenae development

Alate aphids from the mass reared colony were placed in groups of three in a circular arena (height 0.8 cm; diameter 1 cm) clipped on one maize leaf. Clip cages were randomly assigned to different maize plants and leaves, with a total of 40 clip cages per treatment (i.e. Bt and conventional maize). After 48 h alate aphids were removed and newly laid nymphs were left for 6 days allowing them to establish themselves on plants. After this period of time, each apterous aphid was individually isolated in a clip cage and observed every 2 days. Individuals which became alate were not considered for observation (4.46% and 8.08% of the total offspring for Non-Bt and Bt treatments, respectively).

Apterous aphids were checked to determine survival, pre-reproductive period (i.e. the period of time from birth until the beginning of their reproduction period) and longevity. For each treatment, the offspring were counted and removed on each observation day. Recorded values were divided by the number of living adults to estimate daily fecundity. For each treatment, the intrinsic rate of natural increase r_m was calculated according to the Lotka equation [36] $\Sigma e^{-r_m \chi} l_\chi m_\chi = 1$, where χ is the age, l_χ the age-specific survival and m_χ the age-specific fecundity.