Journal of Asia-Pacific Entomology 18 (2015) 657–668

Contents lists available at ScienceDirect

Journal of Asia-Pacific Entomology

journal homepage: www.elsevier.com/locate/jape

Original Article

Effects of transgenic cabbage expressing Cry1Ac1 protein on target pests and the non-target arthropod community under field conditions

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ARTICLE INFO

Article history:
Received 7 February 2015
Revised 15 July 2015
Accepted 28 July 2015
Available online 5 August 2015

Keywords:
Cabbage
Non-target organism
Pieris rapae
Plutella xylostella
Transgenic crop

ABSTRACT

Under field conditions, we investigated how transgenic *Bt* cabbage expressing the insecticidal Cry1Ac1 protein affects two target Lepidoptera species—*Plutella xylostella* (Plutellidae) and *Pieris rapae* (Pieridae)—as well as the structure of the local non-target arthropod community. When exposed to *Bt* cabbage Line C30, both species were significantly less abundant than when in the presence of the non-transgenic control. Transgenic line C24 had no apparent influence on those target populations. Multivariate analyses (PerMANOVA and NMDS) showed that composition of the non-target community was affected by sampling date but not by cabbage genotype. These results suggest that transgenic cabbage expressing Cry1Ac1 protein can be effective in controlling *P. xylostella* and *P. rapae* in the field and that its cultivation does not adversely affect non-target arthropods.

Introduction

Since 1996, transgenic crops expressing insecticidal protein originating from *Bacillus thuringiensis* (*Bt*) have been commercially available for agricultural pest management (James, 2013). Farmers have adopted the use of these insect-resistant *Bt* crops because this practice reduces the need for chemical pesticides while also increasing crop quality and yields (Betz et al., 2000; Clark et al., 2005). However, concerns remain about the potential adverse effects of such crops on non-target arthropods. For example, *Bt* maize pollen and detritus have had detrimental impacts on the survival and growth of the non-target lepidopteran *Danaus plexippus* (Hansen Jesse and Obrycki, 2000; Mattila et al., 2005) and the non-target leaf-shredding trichopteran *Lepidostoma liba* (Chambers et al., 2010).

Cabbage (*Brassica oleracea* L. var. *capitata*) is an economically important crop widely attacked by many insect pests. In particular, leaf feeding by the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae), and the small white, *Pieris rapae* (Lepidoptera: Pieridae), can greatly reduce productivity and quality (Chen et al., 2008a). Although farmers usually apply conventional insecticides to control those pests in cabbage fields, their use can account for 25–30% of the total production cost (Dadang and Djoko, 2009).

Several *Brassica* crops, including cabbage (Metz et al., 1995; Yi et al., 2013), oilseed rape (*B. napus*; Stewart et al., 1996), broccoli (*B. oleracea* var. *italica*; Zhao et al., 2000; Cao et al., 2002), and collard (*B. oleracea* var. *acephala*; Cao et al., 2005), have been transformed to express *Bt* proteins for controlling lepidopteran pests. Ramachandran et al. (1998) showed that transgenic canola expressing Cry1Ac protein effectively controlled its target pest *P. xylostella* in the field. The impacts of transgenic *Brassica* crops on non-target organisms such as herbivores (Howald et al., 2002), predators (Tian et al., 2013), and parasitoids (Schuler et al., 2004; Chen et al., 2008b) have also been studied in the laboratory. However, relatively few studies have examined target and non-target species at the population or community level under field conditions (Lang and Otto, 2010).

In the present study, we asked two questions: (1) How effective is *Bt* cabbage in controlling *P. xylostella* and *P. rapae* in the field? (2) Does *Bt* cabbage expressing Cry1Ac1 protein influence non-target arthropods? To answer these, we compared the abundance of both pests and the community structures of non-target arthropods in the presence of transgenic *Bt* and non-transgenic cabbage.

Materials and methods

Plant materials and field experiments

Seedlings of transgenic and non-transgenic cabbage lines were provided by the Biotechnology Institute of Nongwoo Bio Company Ltd.,

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http://dx.doi.org/10.1016/j.aspen.2015.07.015
1226-8615/

Korea. Two transgenic lines of *Bt* cabbage (C24 and C30), derived from inbred, non-transgenic Line AD126, carry *cry1Ac1*gene (GeneBank accession no. AY126450, Park et al., 2003) under the control of the cauliflower mosaic virus *35S* promoter and the *nos* terminator selection. The transgenic lines also contain the neomycin phosphotransferase II gene (*nptII*) for kanamycin (Harn et al., 2011).

All studies from 2011 to 2013 were conducted in the same experimental field at the Korea Research Institute of Bioscience and Biotechnology (KRIBB), Cheongwon-gun, Chungcheongbuk-do, Korea (36°43′N, 127°26′E; elevation, 35 m). The first trials ran from May to July of 2011. Cabbage is usually ready for transplanting when seedlings have five or six true leaves (Andaloro et al., 1983). Here, 7-week-old plants were placed in the field on 27 May 2011and mature cabbages were harvested on 15 July 2011. The three genotypes (C24, C30, and the AD126 control) were arranged in a 3 × 3 Latin square design (3 replicated plots per cabbage line). Each 6 × 6 m plot had six planting rows mulched with black plastic film to control weeds. In each row, 12 seedlings were planted 50 cm apart (72 plants per plot). Each plot was separated by 1 m. Conventional insecticides and fungicides were not sprayed during the study period. For the second trial, 7-week-old seedlings were transplanted on 7 September 2012. Harvesting occurred on 9 November 2012. The same experimental design and practices were followed in both years.

Because plants from Line C24 did not reduce the number of target pests as effectively as those from C30 during either 2011 or 2012, we focused only on the impact of Line C30 for two additional studies conducted in 2013. The first involved 7-week-old seedlings from Lines C30 and AD126 transplanted on 25 April. Mature cabbages were harvested on 2 July 2013. For this, a randomized block design with five replicate plots was adopted. Plot size, number of rows, number of plants per plot, and distance between plots were the same as in 2011 and 2012. In the final trial, seedlings were transplanted on 30 September 2013, followed by harvesting on 22 November. The experimental design was the same as in Spring 2013. Again, conventional insecticides and fungicides were not sprayed during the study periods.

Monitoring of arthropod community

We measured the population densities of our two target pest species as well as non-target arthropods on or near plants from the three lines. For direct counts, three plants were chosen within the center four rows in each replicated plot during Spring 2011, Autumn 2012, and Spring and Autumn 2013. Their aboveground parts were examined weekly, with tallies of insects recorded six times per trial between 3 June and 8 July in 2011, 27 September and 2 November in 2012, 23 May and 28 June in 2013, and 11 October and 15 November in 2013. The counted insects were not removed from the plants.

Arthropod samples were collected from two yellow sticky traps (250 mm × 150 mm) positioned 30 cm above the second and fourth rows of each plot. Traps were replaced weekly from 12 October to 2 November 2012, 23 May to 28 June 2013, and 11 October to 15 November 2013. They were stored in a freezer (−20 °C) until all sampled arthropods were identified.

Statistical analysis

Multiple observations per plot were averaged and the plot mean was used in statistical analyses. Repeated measures ANOVAs (RMANOVAs) were employed to test for significant differences in the abundance of the target species between *Bt* and non-*Bt* control plants. Normality

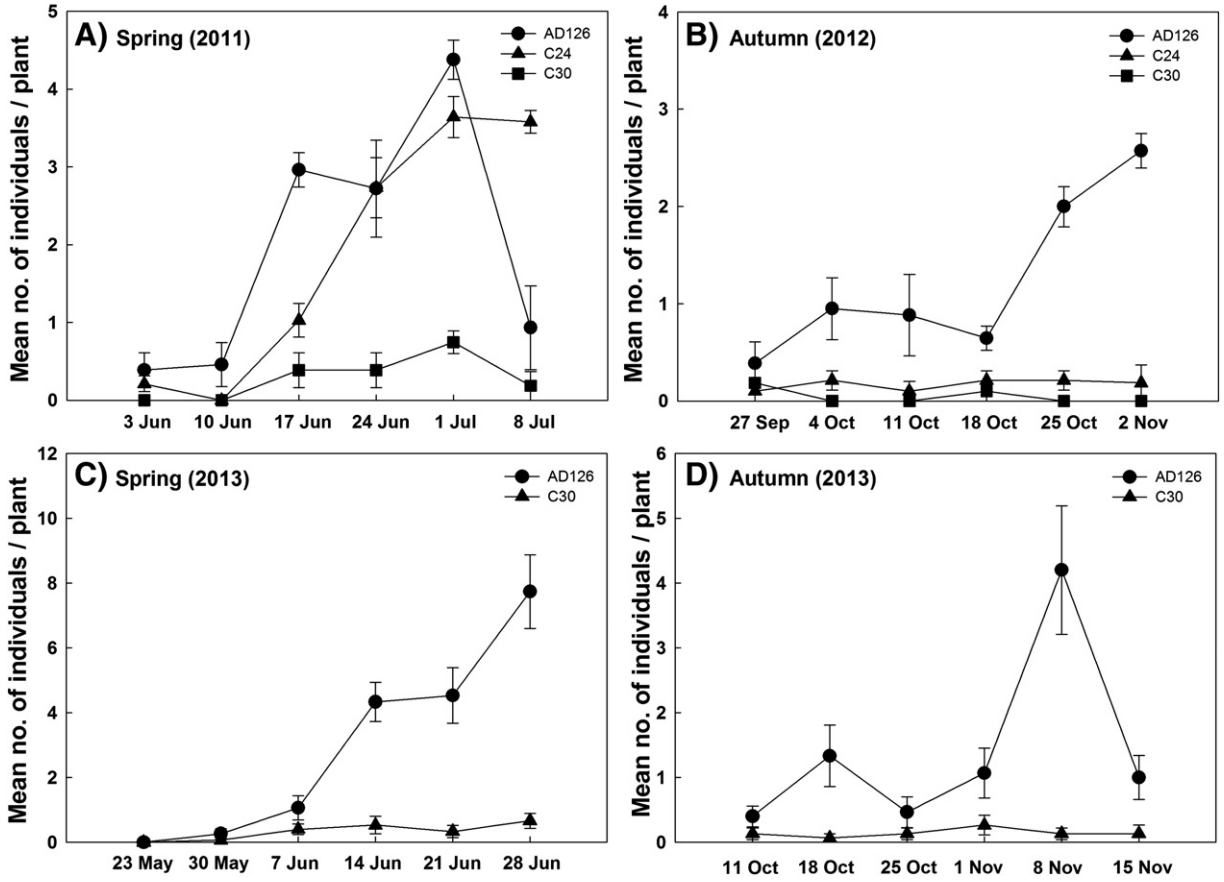

Fig. 1. Abundance (mean number of individuals per plant) of *Plutella xylostella* in cabbage field assessed by visual counts in (A) Spring 2011, (B) Autumn 2012, (C) Spring 2013, and (D) Autumn 2013. Bars represent one standard error of mean.