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Short communication

Persistence of an oilseed rape transgene in the environment

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ABSTRACT

Transgenic bromoxynil (BX)-resistant spring oilseed rape (*Brassica napus* L.) was grown commercially in Canada only for two crop seasons – 2000 and 2001. We investigated the persistence of suspected BX-resistant oilseed rape volunteers in a 64-ha wheat field in Saskatchewan, Canada in 2007, 7 years after the BX-resistant cultivar BX Armor was grown. A small oilseed rape volunteer population, estimated at less than 100 plants, was observed in three main areas or patches in the field. These patches were located in low-lying areas that were too wet to plant or spray with herbicides in 2007. Viable seed was collected from 35 mature volunteers and F_1 progeny screened with BX at 280 g ai/ha in the greenhouse. Progeny of all of the volunteers were visually rated as BX-resistant; the presence of the *Oxy* 235 transgene in leaf tissue of progeny of all volunteers was confirmed by PCR analysis. This study has documented the longest persistence of oilseed rape volunteers in Canada. Volunteers were not observed in 2008 or 2009, because of drought conditions in spring of both years. Results support the findings from previous studies that persistence of volunteer oilseed rape populations in western Canada is generally ephemeral or transitory in the absence of seed bank immigration. However, this study shows that oilseed rape transgenes can persist in the environment for a number of years even after all cultivars with the conferred trait are removed from the market.

1. Introduction

Most spring oilseed rape (*Brassica napus* L.) in Canada is grown in the semi-arid to sub-humid western prairies. Transgenic herbicide-resistant (HR) oilseed rape comprised 86% of the crop grown on 6.3 million ha in Canada in 2008: 45% glyphosate-resistant and 41% glufosinate-resistant; non-transgenic, imidazolinone-resistant cultivars occupied 13% of the crop area and non-HR cultivars only 1% (Canola Council of Canada, personal communication; Statistics Canada, 2008). Bromoxynil (BX)-resistant oilseed rape (Navigator® system) was grown commercially only in 2000 and 2001. These cultivars contained the *Oxy* transgene conferring BX resistance. The bacterial gene expressed an enzyme that enabled the plant to metabolize BX and ioxynil to non-phytotoxic compounds (CFIA, 1998).

The first cultivar registered in the BX-resistant oilseed rape system was Armor BX in 2000 (McVetty et al., 2001). The cultivar was 2.5% higher yielding than the mean of three checks, Cyclone, AC Excel, and Legend, and was moderately resistant to blackleg disease caused by the fungus *Leptosphaeria maculans* (Desm.) Ces. and de Not. It was adapted to the long- and mid-season oilseed rape-growing areas of western Canada. Four other BX-resistant oilseed rape cultivars were registered after Armor BX: 295BX, Cartier BX, Zodiac BX, and Renegade BX (Canola Council of Canada, 2009).

The BX-resistant cultivars occupied less than 1% of the oilseed rape-planted area in 2000 and 2001 (Beckie et al., 2006). Poor adoption of this HR oilseed rape system was attributed to the narrow spectrum of broadleaf weeds controlled. Bromoxynil needed to be tank-mixed with an acetyl-CoA carboxylase (ACCase) inhibitor for grass weed control; by 2000, there was widespread ACCase inhibitor resistance in wild oat (*Avena fatua* L.) and green foxtail [*Setaria viridis* (L.) Beauv.], the two most abundant grass weeds in the Canadian prairies. BX-resistant cultivars were de-registered by 2004 (CFIA, 2009; Canola Council of Canada, 2009). Because commercial seed is no longer sold, BX-resistant cultivars are not approved and any seed is considered adventitious presence if detected in oilseed rape seedlots.

The abundance of oilseed rape volunteers in cultivated fields has been regularly monitored over the past 20 years. Volunteer oilseed rape, mainly HR, was ranked 12th in relative abundance among residual (post-management) weed species when averaged across Canadian prairie field surveys (mainly cereals) conducted from 2001 to 2003; volunteer density averaged 4.5 plants/m^2 in fields

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where they occurred (Leeson et al., 2005). However, mean relative abundance ranking had declined from 10th position as determined from surveys conducted in the mid-1990s when oilseed rape was mainly non-HR. In contrast, volunteer non-HR spring wheat (*Triticum aestivum* L.) increased in rank from 18th to 8th place from the 1990s to 2000s, suggesting that the HR trait is not a major factor influencing volunteer oilseed rape abundance. Based on estimated crop yield loss, the economic impact of volunteer wheat and oilseed rape is ranked third and eighth, respectively, among weedy species (O'Donovan et al., 2005).

Oilseed rape can produce large volunteer populations because of the large amount of seed lost before and at harvest. In a study in the province of Saskatchewan, Canada in 1999 and 2000, average oilseed rape seed loss during harvest operations was 5.9% of crop yield (3000 viable seeds/m^2) as determined from measurements in 35 growers' fields (Gulden et al., 2003a); yield loss among growers ranged from 3.3 to 9.9% or 9 to 56 times the recommended seeding rate of oilseed rape.

Oilseed rape can persist in the seed bank because of the potential for induction of secondary dormancy (Gulden et al., 2003b, 2004). Volunteers can occur 4–5 years after production (Légère et al., 2001; Simard et al., 2002), although most occur in the first year after oilseed rape (Harker et al., 2006) particularly when there is no seed bank replenishment. The latter study also determined that preventing seed production of oilseed rape volunteer plants in the year following oilseed rape reduced volunteer densities in subsequent years to levels that would not require herbicidal intervention in those crops.

The small area where BX-resistant oilseed rape was grown previously and short time span of cultivation – 2000–2001 – provided a unique opportunity to examine the persistence of the *Oxy* transgene in the oilseed rape seed bank. In 2007, we located two commercial fields in Saskatchewan where BX-resistant oilseed rape had been grown in 2000, with no oilseed rape grown since then. Cultivar Cartier BX was grown in a field in northern Saskatchewan, and cultivar Armor BX in a field in central Saskatchewan. Oilseed rape volunteers sampled in the northern Saskatchewan field were determined not to be BX-resistant (data not shown). However, volunteers in the central Saskatchewan field were confirmed as BX-resistant, as detailed in this report.

2. Materials and methods

The field in central Saskatchewan where cultivar Armor BX was grown in 2000 was located at latitude 51.9° N, longitude 108.1° W. The soil is an Elstow loam (Typic Boroll) with 4.5% organic matter content and pH 6.0, developed on glacio-lacustrine parental material. The topography is moderately sloping (6–9%). The field was managed under a minimum-tillage regime for over 10 years (i.e., one pre-seeding high-disturbance tillage operation using a field cultivator with mounted tine harrows). In 2000 or 2001, no BX-resistant cultivars were grown within at least 10 km of the field based upon grower interviews. Since 2000, oilseed rape was not planted in the field. Barley (*Hordeum vulgare* L.), spring wheat, or lentil (*Lens culinaris* L.) were grown from 2001 to 2006, except in 2002 when the field was fallowed. In 2007, spring wheat was grown. Broadleaf and grass weeds were controlled by postemergence herbicides applied using a tractor-drawn ground sprayer.

The 64-ha field (800 × 800 m) was surveyed in early July, 2007, when volunteer oilseed rape is typically flowering, by walking north–south (N–S) transects spaced 50 m apart. Oilseed rape volunteers were found thinly dispersed (<1 plant/m^2) in three main areas or patches (Figs. 1 and 2). Volunteers in patches BX1 and BX2 were located along the periphery of sloughs (transient small-water bodies); volunteers in patch BX3 were located in a depressional

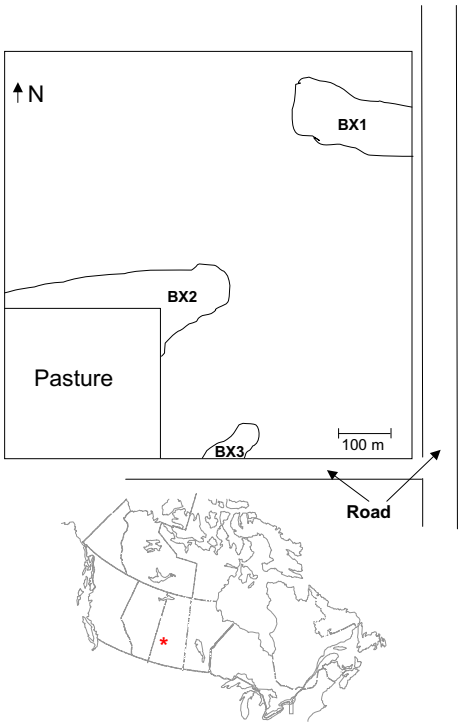

Fig. 1. Location of three putative bromoxynil-resistant oilseed rape patches in a 64-ha wheat field in the province of Saskatchewan, Canada in 2007.

(low-lying) area. Because of excess moisture, these areas had not been sprayed with herbicides during the 2007 growing season.

The total population of oilseed rape volunteers was estimated in early July at 20, 44, and 30 plants in patches BX1, BX2, and BX3, respectively, by counting flowering plants observed from N–S transects spaced 2 m apart (Table 1). Each plant was flagged. In late August immediately prior to wheat harvest, all seed pods were harvested from 50% of plants comprising the population in each patch. A total of 47 randomly selected volunteers were sampled. Pods from each plant were placed in a paper bag and dried for 2 weeks in the laboratory at ambient room temperature. Each plant sample was manually threshed and seeds were stored at ambient

Fig. 2. Putative bromoxynil-resistant oilseed rape volunteers at flowering in a wheat crop in Saskatchewan, Canada in July, 2007 (redroot pigweed, *Amaranthus retroflexus* L., also present).