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RESEARCH ARTICLE

Seed bank persistence of genetically modified canola in California

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Abstract
Introduction Canola, which is genetically modified (GM) for tolerance to glyphosate, has the potential to become established as a new glyphosate resistant weed, thus reducing the effectiveness of glyphosate.
Methods Volunteer from dormant canola seeds produced thousands of plants per hectare in the fourth year (2011) following a 2007 crop harvest. This occurred with no additional canola seed production since the 2007 harvest.
Results Volunteer plants following harvests of annual crops are typically only a problem for the first year after harvest. In California, glyphosate is the core herbicide on over a million hectares of high value row, tree, and vine crops and new glyphosate resistant weeds reduce the effectiveness of glyphosate.
Conclusions The combination of dormant seed and herbicide resistance makes GM glyphosate-resistant canola a new and difficult California weed which was first observed in the winter of 2009.

Keywords Genetically modified · Canola · Glyphosate · *Brassica napus* · Resistance · Seed dormancy · Volunteer

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1 Introduction

Canola is known for shattering large amounts of seed before and during harvest (Mallory-Smith and Zapiola 2008). When shattered canola seed is buried, seed can enter a "secondary dormancy" (Lutman et al. 2003; Gruber et al. 2004). Even if all volunteer canola is controlled before it produces seed in the first year following canola, seedlings will continue to emerge for many years from dormant seed (Squire et al. 2011; Knispel and McLachlan 2010; Pessel et al. 2001; Begg et al. 2008). Most of this dormant seed emerges in the first 4 years (Crawley and Brown 2004; Lutman et al. 2003), but in Sweden some canola emerged 10 years after burial in the soil following harvest (D'Hertefeldt et al. 2008). In the UK, the seedbank of GM tolerant canola was studied for 4 years, and models predicted 95% seed loss after approximately 9 years (Lutman et al. 2005). Within an oilseed rape field, seed rain was found to deposit several thousand seed per square meter, but after just 3 years, average seed density had declined to about 200 seeds m^{-2} (Begg et al. 2008). Most annual crops only produce volunteer crop plants during the year following production.

Vehicle movement has been implicated as a main source of canola seed transport and the infestation of new sites along roadways. In Manitoba, Canada, 93–100% of the escaped canola along roadsides and field edges was observed to be herbicide-tolerant (Knispel and McLachlan 2010), similar to the proportion of herbicide tolerant canola produced in the region. They observed these populations were relatively distinct, indicating very little propagule exchange among populations. Canola has limited natural seed dispersal, and thus seed transport has been observed to be the main mechanism for dispersal along roadsides (Knispel and McLachlan 2010; Crawley and Brown 2004; von der Lippe and Kowarik 2007). Paved road surfaces and areas close to grain elevators were more likely to contain populations of herbicide tolerant canola, than were dirt roads or areas further from grain elevators (Knispel and McLachlan 2010). In Germany, feral canola seed was collected between June and October, with no seed found the remainder of the year (von der Lippe and Kowarik 2007). Since seed production from canola plants occurred much later than June in that area, they theorized that spillage during seed transport was the mechanism of seed deposition.

Glyphosate-resistant canola is a weed because of its ability to produce a significant percentage of secondary dormant seed when seed is buried after harvest (Lutman and Lopez-Granados 1998). There are no studies of glyphosate resistant dormant seed in California with its Mediterranean climate.

Canola is used to produce high-quality oil. Canola varieties are selections from several mustard species, but most varieties produced in the United States originated from *Brassica napus*, commonly called rapeseed mustard. Rapeseed mustard and other mustard species, to which canola is related, are present in California as wild weeds (Hickman 1993).

Canola is the most important oilseed crop in Canada (Harker et al. 2000), planted on millions of hectares of farmland and more recently has become an important crop in the northern United States. In these areas, canola is commonly grown in rotation with wheat where several phenoxy herbicides can be widely used to control volunteer canola. In California, with the presence of cotton, grapes, and other phenoxy sensitive crops, the use of phenoxy herbicides is restricted. This limits the available herbicides for controlling glyphosate resistant canola. California's diverse agriculture and restrictions on phenoxy herbicide use makes "weedy" glyphosate-resistant canola control much more difficult than in Canada and the northern cereal growing areas of the United States.

2 Materials and methods

In 2007, a variety trial was done on a Butte County farm (−121°49′E, 39°41′ N) with four GM canola varieties. This was the second year canola, or any other oil seed rape crop, had been planted on this farm. The first oil seed rape planting was on 6 ha in the 1980's. Ten years of detailed GPS weed mapping through 2010 found no oil seed rape from the 1980's or any wild *B. napus* or *B. rapa* on the 325-ha farm, and thus canola volunteers in the 0.4-ha area were assumed to be from the 2007 variety trial.

Three of the varieties in the 2007 variety trial were tolerant to glyphosate and one to glufosinate. Each variety was planted in 2.4×10.7 m plots with four replications. The total planted area was 0.04 ha.

The crop history of the area from 2006 to 2011 is shown in Table 1. The area was left fallow from June 2007 through October 2008 to allow for the destruction of volunteer canola seedlings before any seed was produced. Canola germinated with fall and winter rainfall and was destroyed several times each year with shallow (10–15 cm) tillage.

Table 1 Crop history of 0.04 ha 2007 GM canola trial area and the surrounding 0.4 ha

Date	Crop
December 2006–June 2007	Canola (surrounding 0.4 ha fallow)
July 2007–November 2008	Fallow
December 2008–March 2009	Wheat
April 2009–May 2011	Fallow

Assuming all volunteer canola was germinated and destroyed, wheat was planted in November 2008. Following planting, hundreds of canola plants germinated in the wheat. To prevent canola seed production, the wheat and canola were destroyed before the canola produced seed.

Since late winter 2009, the area has been kept fallow and weeds controlled with a combination of tillage and herbicides, mostly glyphosate. The clean fallow 0.4-ha area made counting canola volunteers very simple and effective. Because of the control of all volunteer canola before any seed production, all canola volunteers are from the seed produced in the spring of 2007.

Each year since canola harvest, volunteer canola seedlings have been visually estimated or counted several times and then destroyed in a 0.4-ha area including and surrounding the initial trial area. Canola volunteers were counted and destroyed as seedlings. The counted canola plants were pulled and counted in groups of ten and then the number of groups of ten counted at the end, plus any remaining less than ten.

In addition to the plot area, GM glyphosate-resistant canola populations along county roads leading to the farm were identified by applications of glyphosate or by Enviro-Logic test strips for the presence of the CP4 EPSPS protein.

3 Results

The numbers of canola seedlings from the initial 0.04-ha planting were initially visually estimated as shown in Table 2

Table 2 GM canola volunteer counts in the 0.04 ha 2007 trial planting and in the surrounding 0.4 ha area

Date	Canola (no. estimated or counted)	Canola (no. per ha)
July 2007–June 2008	Thousands	Ten thousands
July 2008–June 2009	Hundreds	Thousands
July 2009–June 2010	Hundreds	Thousands
July 2010–June 2011	372	9,193

and later were counted as the numbers decreased to lower numbers. The fallow area surrounding the original 0.04 ha planted area is ten times larger allowing for good estimations of the scattered emerging canola seedlings. The number of canola per hectare in Table 2 is calculated based on the original 0.04-ha planted area since this was the source of all germinating seed. During the fourth year of volunteer emergence, a total of 372 seedlings emerged, which was 9,193 per hectare.

Only a few GM canola plants were found on this farm outside of the 0.4-ha plot area. These were found in the combine loading area and along roadsides where the combine was hauled. These plants were not included in the counts in Table 2.

4 Discussion

Weedy glyphosate-resistant canola is an unintentional consequence of producing a glyphosate-tolerant crop, not glyphosate resistance occurring from repeated use of glyphosate for weed control in the field.

In addition to the plot area, the transportation of farm equipment to and from this 2007 trial scattered some GM canola along county roads and state highways. This scattering of seed along the roadside is typical in field crop production. However, unexpected reproducing roadside populations of canola were found during the winter of 2009 as shown in Figs. 1 and 2.

Mowing and herbicide application have both been observed to increase herbicide-tolerant canola frequency (Knispel and McLachlan 2010). The timing of these operations could influence feral canola. Mowing during flowering would be expected to reduce seed set and future populations. Likewise, treating with an effective herbicide would also reduce feral canola, but mowing or herbicide treatments performed too early in the season may remove competing vegetation. Soil disturbance resulted in a reduction in the feral canola population in the sampling year (Knispel and McLachlan 2010). However, Crawley and Brown (2004) observed an increased frequency in feral canola the year following soil disturbance. They hypothesized that feral canola benefited by the removal of competing vegetation, which might also explain the observation by Knispel and McLachlan (2010) that canola increased following mowing or herbicide treatment, which would also reduce competition.

Roadside GM canola is established in Japan along roads leading from 13 harbors to inland canola processing plants (Kawata et al. 2009). Other glyphosate resistant crops, corn and cotton, have been widely planted over the past 10 years in California, but have not become established along roadsides as reproducing weeds.

Aggressive control with effective herbicides and hand pulling of escapees along some of the county roads has resulted in control, if not eradication. Some state highways with more limited control efforts have canola populations along their roadsides. Disturbance of roadside soil may promote secondary dormant seed through shallow burial.

Wild types of rapeseed mustard are not common weeds in California's agricultural fields or on roadsides. Currently these wild types are well controlled by glyphosate.

Fig. 1 Roadside glyphosate resistant canola growing with competition from other weeds removed by roadside glyphosate applications

Fig. 2 Glyphosate resistant canola surviving a recently applied roadside glyphosate application

The seed dormancy of canola makes it a difficult weed to control, persisting in a field where it was once planted for years. Shattered rape seeds have almost no dormancy, but when buried, particularly under dry conditions, rape seeds can enter a secondary dormancy which can persist for many years (Lutman et al. 2003; Gruber et al. 2004). Even if all volunteer canola is controlled before it produces seed in the first year following canola, seedlings will continue to emerge for many years from dormant seed (Squire et al. 2011; Knispel and McLachlan 2010; Pessel et al. 2001; Begg et al. 2008). Canola's glyphosate resistance in combination with canola's seed dormancy makes it a challenging weed for roadsides, orchards, vineyards, fallow fields, and glyphosate resistant crop fields, or anywhere where glyphosate is an important herbicide.

Glyphosate is the most common (California Department of Pesticide Regulation 2009) and valuable herbicide in California agriculture. Stephen Powles of the University of Western Australia has described glyphosate as "a once-in-a-century herbicide" (Powles and Preston 2006). Glyphosate is effective on many broadleaf and grassy weeds, both annual and perennial, with extensively proven animal and environmental safety. If glyphosate is a "once in a century herbicide," a replacement herbicide for glyphosate is likely decades into the future. Each time another weed, for example, ryegrass (Powles and Preston 2006), develops resistance to glyphosate, it makes weed control more complicated, more expensive, and decreases the value of glyphosate. If glyphosate-resistant canola spreads along roadsides and into orchards and fields, it will make glyphosate less valuable in those places. It will also result in the use of additional herbicides, adding both economic and environmental costs.

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