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Transgenic glyphosate-resistant canola (*Brassica napus*) can persist outside agricultural fields in Australia

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ABSTRACT

In the last two decades the cultivation of transgenic crops has steadily increased worldwide. In Western Australia transgenic glyphosate-resistant canola (GR) has been cultivated since 2009. This study was conducted to examine the potential for transgene persistence outside agricultural fields after commercialization of GR crops. Propagule pressure, population fluctuations and reproductive output of GR canola plants have been assessed in semi-natural (roadside) and natural environments over consecutive years. The estimation of demographic parameters (plant survival and fecundity) suggest that GR canola has low likelihood to become invasive, as plants are subjected to biological and abiotic stressors likely limiting the fitness. This was particularly evident in a natural environment in which a propagule of 300 GR canola plants accidentally introduced by a wind storm could persist for three years before extinction. Thus, in natural areas GR canola populations did not show a positive population turnover and declined overtime. Conversely, on roadsides the significant correlation ($r = 0.975$) between mean plant fecundity (seed rain) and the soil seedbank density in the following year suggests that local recruitment contributed to canola persistence for at least three years. As, no individual GR plants were found with stacked genes for multiple herbicide resistance we suggest that GR volunteer canola plants can be controlled by simple mixture of herbicide modes of action different to glyphosate although an integrated management including mechanical control operations would be the optimal strategy.

1. Introduction

The cultivation of transgenic glyphosate herbicide-resistant (GR) crops has been the most rapidly adopted crop technology in modern agriculture. Introduced in 1996 in USA, GR crops have generated benefits to farmers (e.g. simplified and effective weed control, profit, etc.) (Duke, 2015). At present, transgenic crops are grown on an area in excess of 180 million ha (James, 2014) comprising 80% of GR crops as the dominant trait (Green, 2014).

GR canola (*Brassica napus* L.) is widely planted in Canada (Beckie et al., 2006) and more recently and to a lesser extent in Australia. All varieties of cultivated canola retain vestiges of wild traits such as a degree of seed shattering and secondary seed dormancy which allowed plants to disperse and seed to persist within the soil seedbank prior to domestication (Hall et al., 2005). Thus, it is important to understand how transgenic traits can affect plant fitness, whether they can increase crop plant weediness and what are the mechanisms allowing movement of transgenic traits into nearby natural ecosystems (reviewed by Chèvre et al., 1997; Chapman and Burke, 2006; Warwick et al., 2009).

In Europe studies showed little seed movement from canola crops to adjacent fields, but roadside canola populations were evident from repeated spillages from trucks transporting seed to delivery points (Crawley and Brown, 1995; Devos et al., 2009). Similarly, in Japan and Canada spillages of imported herbicide-resistant canola varieties have occurred along roadsides connecting harbors to oil factories (Yoshimura et al., 2006; Kawata et al., 2009). Other studies in the U.S. and Europe confirm that transgenic herbicide-resistant canola can establish on roadsides (Schafer et al., 2011; Munier et al., 2012) and persist for several years (Pessel et al., 2001).

In agricultural fields, volunteer canola plants can infest subsequent crops and the problem is exacerbated if volunteer plants are able to stack traits for resistance to multiple herbicides through sequential crossing (Hall et al., 2000). Secondary dormancy contributes to the persistence of *B. napus* seeds in the soil seed-bank (Gruber et al., 2004) with an estimated persistence of seeds in the soil seed-bank up to four years or longer depending on the level of disturbance (Lutman et al., 2003). Australian studies confirmed a more rapid decline of the seed-bank in a minimum

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versus no-till system and indicated a complete decline of the soil seedbank over 3.5 years with no germination recorded in either tillage system (Baker and Preston, 2008). The risk of gene escape from cropped fields through intra-specific crop-to-crop cross-pollination of canola between fields can occur at considerable distance at low frequencies (Rieger et al., 2002). In Australia the risk of inter-generic crop-to-weed gene flow with major cruciferous weeds such as *Raphanus raphanistrum* L. is also very unlikely (reviewed by Rieger et al., 1999, 2001). Canola is a poor competitor and therefore its presence or persistence on roadsides is often related to recurrent seed spillages (Yoshimura et al., 2006). However, where glyphosate is applied to roadsides for weed control, GR canola could survive and persist on roadsides or corridors between farms and grain delivery sites (Knispel and McLachlan, 2010). In Western Australia (WA) spillages of GR canola have been reported (McCauley et al., 2012; D. Bowran, unpublished data), but the persistence of GR canola outside agricultural fields in disturbed areas such as roadsides or natural environments has not been investigated (i.e. GR segregation analysis and progeny tests).

This study took advantage that in 2009 for the very first time GR transgenic canola was commercially grown in WA crop fields. From 2009 we monitored over four consecutive years a site near Quairading, WA, where transgenic GR canola was first grown on a 50 ha field in 2009. In 2012 we also surveyed roadsides near one grain delivery site for transgenic GR canola to assess the ability of transgenic canola plants to persist on WA roadsides. Here we report the potential for populations of GR canola to persist in bushland or road margins in the south-western Australian environment.

2. Materials and methods

2.1. Roadside survey of volunteer canola plants

In October 2012 a canola population that had established from truck-vectored spillages on a 3500 m roadside (RS) transect was surveyed near a grain storage point that accepts deliveries of GR (CBH, Perth Metro grain center, −31.9746, 115.9849, Western Australia). Along 38 roadside sub-transects (approximately 92 m in length starting exactly from the facility's main gate), flowering volunteer canola plants were counted on road margins and/or in the 6 m-wide unpaved median strip. The number of fertile canola siliquae produced by each individual was counted for subsequent estimation of seed production. GPS coordinates were taken at the beginning of each sub-transect. The total plant number for each sub-transect was divided by the corresponding area surveyed in order to calculate plant density (plants m^{-2}). The total number of plants found along a 3500 m transect, divided into 500 m distances (0–500 m, 501–1000 m, 1001–1500 m, 1501–2000 m, 2001–2500 m, 2501–3000 m, 3001–3500 m) is presented. Twenty seeds per siliquae was the calculated mean number of several fertile siliquae ($n > 50$) inspected during the surveys. We emphasized that the majority of surveyed plants were observed growing in isolation or at much lower mean densities (<1 plant m^{-2}) than in agricultural field conditions in southern Australia (50–100 plants m^{-2}). Thus, canola volunteer plants were not substantially affected by intra-specific competition and we assumed the seed number produced by each siliquae on mother plants contained 20 viable seeds (Buzza, 1979).

2.2. Survey of volunteer canola plants in natural areas

A 50 ha GR canola (variety: Hyola 502 RoundUp Ready) crop at Wamenusking, Quairading, WA (−32.0106120; 117.4024570) was windrowed prior to harvest in October 2009. A wind storm the next day moved GR canola plants into immediately adjacent areas of remnant natural bushland (two areas of natural land NL1 and NL2, each approx. 2 ha). Subsequent careful inspections of the two areas shortly after the wind storm revealed several siliquae had been already detached from mother canola plants and had dispersed seeds scattered on the ground as the plants moved into these bushland areas. Native vegetation mainly consisted of *Eucalyptus* spp. woodland as described by Keighery et al. (2001). Since 2010, in spring when canola plants are flowering (August–September), we have carefully monitored and counted any canola individuals present in these two bushland areas to assess the persistence of transgenic canola plants in remnant bushland. At natural land site NL1 an estimated 300 transgenic GR canola plants were wind-transported in late 2009 approximately 50–100 m from the closest field edge. Some seeds released from those canola plants resulted in established plants that in 2010 produced seed although in 2010 the growing season April–October rainfall (160 mm) was significantly lower than average (330 mm). Plants were counted and geo-referenced and leaf material collected from plants to test for the presence of the *CP4-EPSPS* transgene. Again in 2011 there were canola plants which established, flowered and produced seed in the bushland site. Plants were counted and geo-referenced and their morphological traits were measured (height, number of branches, number of fertile siliquae) to estimate plant fecundity as seed production. The survey of canola plants continued in the spring of 2012 and 2013, a total of four consecutive years after the unique movement of the 2009 wind-dispersed GR canola plants into the bushland site from the nearby windrowed GR canola. At the other natural land paddock NL2 site no canola plants were ever evident between 2010 and 2014. No canola was grown in the fields nearby NL1 and NL2 in the following years.

2.3. Glyphosate assays to confirm presence of GR roadside canola

Approximately 220 individual seed samples (one individual plant per sample) were randomly collected from canola individuals growing along the 3500 m roadside transect surveyed, labeled with a unique number, and kept in separate paper bags. The seeds of each sample were subsequently scattered in flat trays (one 20×30 cm tray per sample) containing standard potting mix (50% river sand, 25% peat, 25% pine bark), watered as required (>80% field capacity) and fertilized weekly with KNO_3 (50 mg kg^{-1} potting mix). Emerging seedlings (two-leaf stage) were treated with a lethal dose of glyphosate (600 g glyphosate ha^{-1}). The treatment was applied as RoundUp PowerMax® (Nufarm, Melbourne, VIC, Australia) (540 g a.e. L^{-1}) with a cabinet track sprayer mounted with twin flat-fan nozzles and delivering a water volume of 120 L ha^{-1} per pass at a pressure of 200 kPa. Surviving canola plants were counted 28 days after spraying and a second glyphosate treatment, identical to the first, was then applied (plants were at the 6-leaf stage, prior to bud formation). A final count of survivors was conducted 28 days after the second glyphosate treatment. A commonly grown non-transgenic, commercial canola variety (TT Thunder) was also sprayed with glyphosate as a susceptible control. In addition, a transgenic GR canola variety (RR Hyola) was included as a positive GR control.

As triazine-resistant and imidazolinone-resistant canola varieties are grown in WA, sub-samples of plants surviving both glyphosate treatments, now at the rosette stage, were evaluated for resistance to either of two different herbicide modes of action applied at the recommended rates: atrazine (a photosystem II inhibitor) at 2000 g ha^{-1} and imazamox + imazapyr (acetolactate synthase inhibitors) at 17 + 8 g ha^{-1}. A total of 823 and 698 plants surviving glyphosate were treated with atrazine or imazamox + imazapyr, respectively.