



## Research article

# Persistence of seeds, seedlings and plants, performance of transgenic wheat in weed communities in the field and effects on fallow weed diversity



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## ABSTRACT

Introduction of transgenic crops to agriculture has raised concerns about their effects on agro-ecosystems. We compared nine conventional lines of spring wheat with six genetically modified (GM) lines that contained transgenes of resistance against powdery mildew (*Pm3b* gene) or against fungi in general (*Chi* and *Glu* genes). We assessed the persistence and performance of these lines without competition and in experimental weed communities in the field, their seed germination in the laboratory, their survival in fallow plots and effects on post-harvest vegetation in the field.

In competition with weeds, the GM lines showed reduced seed number, plant height and biomass allocation to seeds than their corresponding non-GM lines. No such performance differences were observed without competition. The seedlings of GM lines did not persist longer than those of the corresponding non-GM lines in fallow plots. In the field, GM and non-GM wheat lines had similar performance and persistence and both were able to reproduce in dense weed communities and to survive during winter on fallow plots. Stored in soil in the laboratory, the seeds of GM and non-GM lines either germinated quickly or lost their viability after 3 months. GM and non-GM lines had no differential effects on the structure and diversity of fallow plant communities within the 6-month period of monitoring. Poor seed longevity yet successful plant persistence in weed communities or on fallow plots indicate that not removing a population of growing plants presents a greater risk than allowing the build-up of a soil seed bank regarding the potential escape of transgenic wheat to the environment. Strong varietal differences in persistence point out the importance of case-by-case assessment of new GM varieties and indicate that transgenic traits should preferably be introduced into varieties with low persistence.

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## 1. Introduction

Introduction of transgenic crops to agriculture has raised concerns about their effects on agro- and natural ecosystems. New introduced traits, in particular those conferring resistance to pathogens or abiotic stresses, have a potential to increase weediness or invasiveness of the genetically modified (GM) plants or their offspring leading to the persistence and spread of transgenes within agricultural, uncultivated, or natural areas (Andow and Zwahlen, 2006; Gilbert, 2013; Hails, 2000; Linder and Schmitt, 1994; Purrington and Bergelson, 1995; Quist and Chapela, 2001; Schmitt and Linder, 1994; Snow, 2002). Multiple studies reported

unintended contamination of non-GM seed lots due to seed persistence in soil and GM plant volunteering in subsequent conventional crops or along the transport routes (Andersen et al., 2010; Demeke et al., 2006; Friesen et al., 2003; Hecht et al., 2014; Knispel and McLachlan, 2010; Mallory-Smith and Zapiola, 2008; Schafer et al., 2011). Volunteering and outcrossing of transgenic plants with traditional landraces or wild relatives pose a serious agricultural and environmental problem (Joergensen et al., 2009; Holman et al., 2011; Mercer and Wainwright, 2008; Mortensen et al., 2012; Piñeyro-Nelson et al., 2009). In particular, persistence and spread of the transgenes in agroecosystems and changes in weed management practices due to the introduction of herbicide-resistant crops are known to have direct and indirect effects on biodiversity in fallow fields, often leading to the shifts in the species composition of the weed communities, with resistant genotypes dominating (Culpepper, 2006; Green and Owen, 2011; Harker et al., 2005b; Vencill et al., 2012; Warwick et al., 2009).

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A thorough environmental risk assessment must therefore precede any release of the new transgenic plants to the environment. Such an assessment has to be done on a case-by-case basis and should include evaluation of the direct and indirect risks to the environment which the deliberate release of a new genetically modified organism may pose (EC, 2001; EFSA, 2011). For transgenic crops, their potential to persist and spread in agricultural and wild habitats through gene flow, persistence in the soil seed bank or as volunteers in subsequent conventional crops and outside agricultural fields, as well as the effects on the non-target organisms are among the major ecological risks to be addressed.

Wheat is the most important food crop in temperate climate with 723 million tons world production forecast for 2015 (FAO, 2015). To date, a range of traits has been introduced into wheat plants by the means of biotechnology, including tolerance to abiotic stresses (Bahieldin et al., 2005; Sivamani et al., 2000a; Xue et al., 2004), insect (Altpeter et al., 1999; Stoger et al., 1999) and disease resistance (Altpeter et al., 1999; Bieri et al., 2003; Brunner et al., 2011; Sivamani et al., 2000b; Wang et al., 2014b) and grain quality traits (Vasil et al., 2001). Most of the introduced traits can potentially provide fitness advantages to transgenic plants over their conventional relatives, e.g., through increased plant growth and vigor, fecundity or improved survival under abiotic or biotic stresses (Sivamani et al., 2000a; Snow et al., 2003; Wang et al., 2014a; Xue et al., 2004).

We used nine non-GM and six GM lines or varieties (later simply called lines) of spring wheat (*Triticum aestivum* L.) to study the persistence of plants of these lines in the environment at different stages of their life cycle. The GM lines were genetically modified to be resistant against powdery mildew (*Blumeria graminis* f.sp. *tritici* (DC.) Speer) or had a general quantitative resistance against fungi. A series of experiments was carried out to assess the performance of the non-GM and GM lines without competition and in two different experimental weed communities in the field, their seed longevity in the soil under controlled conditions in the laboratory, seedling volunteering, persistence and over-winter survival in fallow plots in the field and the potential effects of these plants on post-harvest weed communities.

We asked the following questions: (1) how do GM plants perform (in terms of their yield, fitness-related traits and mildew infection) compared to their non-GM counterparts without competition and in the experimental weed communities common for wheat fields and wheat fallows? (2) Can the seeds of wheat persist in soil throughout winter and is seed longevity different between GM and conventional lines? (3) Do GM seedlings appear more often and do they persist longer than those of conventional wheat on post-harvest fallow plots? (4) Are there any post-harvest effects of wheat plants on fallow weed communities?

## 2. Materials and methods

### 2.1. Plant material

We used six transgenic lines derived from two maternal varieties of spring wheat, the Mexican variety Bobwhite and the old Swiss variety Frisal. These two varieties were chosen because they show high transformation efficiency and are susceptible to powdery mildew pathogen (Bieri et al., 2003; Pellegrineschi et al., 2002). Furthermore, they are both susceptible to powdery mildew, yet to different degrees (Bobwhite > Frisal).

Four transgenic lines (*Pm3b*#1–4) were produced by biolistic transformation of Bobwhite in different transformation events. The *Pm3b* gene was cloned from hexaploid wheat (Yahiaoui et al., 2004) under the control of the *Zea mays* L. ubiquitin promoter (Christensen and Quail, 1996) and confers race-specific resistance to powdery mildew. Transformants were selected on mannose-containing media using the phosphomannose isomerase (PMI)-coding gene as selectable marker (Reed et al., 2001). After regeneration of T0 transformants, four independent T1 families were selected. From each T1 family, an offspring pair was further propagated consisting of a homozygous transgenic plant (GM lines *Pm3b*#1–4) and a null-segregant, i.e. a plant that did neither inherit the *Pm3b* transgene nor the selectable marker (control lines Sb#1–4). The ubiquitin promoter from maize ensured high constitutive transgene expression. The seeds used in this study were obtained from homozygous GM and control lines that had passed through five generations of sexual reproduction by self-pollination.

The performance in monoculture under natural powdery mildew infection pressure in the field and the transgene expression of the lines *Pm3b*#1–4 have been described by two companion studies (Brunner et al., 2011; Zeller et al., 2010). *Pm3b*#1–3 lines carried a single copy of the transgene *Pm3b*, and *Pm3b*#4 line carried one full-length and one inactive truncated copy. The transgene expression levels were similar among *Pm3b*#1, *Pm3b*#3 and *Pm3b*#4, while *Pm3b*#2 line showed five times higher levels of expression. Partial gene silencing and consequent segregation in resistance were observed in the *Pm3b*#3 line, where some plants showed high resistance and others were susceptible to mildew (Brunner et al., 2011).

The respective non-transgenic sister lines Sb#1–4 were used as a control to ensure that any somaclonal variations acquired during tissue culturing were shared between transgenic and control lines. In the present study, we also used conventional wheat variety Bobwhite as an additional non-transgenic control (comparator) for *Pm3b* GM lines.

The GM lines derived from the variety Frisal expressed either a barley seed *chitinase* gene (line A9 *Chi*) or both a *chitinase* and a  $\beta$ -1,3-*glucanase* gene (Bieri et al., 2003; Leah et al., 1991). Quantitative

**Table 1**  
Transgenic and control wheat lines used in the experiments.

Line name	Variety (genetic base)	GM/non-GM (control)	Transgene/s conferring resistance
<i>Pm3b</i> #1–4	Bobwhite	GM	<i>Pm3b</i>
Sb#1–4	Bobwhite	Non-GM, null-segregants, control	–
Bobwhite	Bobwhite	sister lines for <i>Pm3b</i> lines	–
		Non-GM, Mexican wheat variety	–
		Bobwhite, mother variety for <i>Pm3b</i> and	
		Sb lines	
A13 <i>Chi/Glu</i>	Frisal	GM	<i>Chitinase</i> and
A9 <i>Chi</i>	Frisal	GM	$\beta$ -1,3- <i>glucanase</i> ( <i>Chi</i> , <i>Glu</i> )
Frisal	Frisal	Non-GM, mother variety for A9 <i>Chi</i> and	<i>Chitinase</i> ( <i>Chi</i> )
		A13 <i>Chi/Glu</i> lines	–
Casana, Fiorina, and Toronit	Casana, Fiorina, and Toronit	Non-GM commercialized Swiss wheat	–
		varieties	

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