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Understanding Agricultural Species Metapopulation Biology and Ecology and the Implications for Coexistence in Low Level of Presence Scenarios

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The intraspecific movement of novel traits in agriculture involves a metapopulation that includes cropped, volunteer and feral subpopulations as well as a latent population comprised of any viable seed anywhere within the agro-ecosystem and agricultural supply chain. An assessment of novel trait movement risk or containment requirements relies on a good understanding of volunteer and feral populations as well as a deep understanding of supply chain operations, processes, protocols and equipment. For coexistence scenarios where threshold levels are low, an assessment of the potential success of trait containment will be overestimated if there is a poor understanding of the species metapopulation. The biology and ecology of cropped species varies with respect to the potential for intraspecific trait movement and some species, such as alfalfa, may pose a much greater challenge in terms of trait containment than spring wheat, for example, although agronomic practices do mitigate these differences and one should not assume that species nature alone will determine trait containment. We show examples of this for canola, spring wheat and alfalfa and explain the implications for coexistence in low level of presence scenarios.

Key words: coexistence, genetic engineering, GE, low-level presence, metapopulation.

Introduction

The advent of genetic engineering has heightened awareness of the challenges and potential risks that can come with the development of GE traits in plants. Many risks associated with the release of GE crops are related to trait movement, and this is especially true for the movement of traits within and among farming systems and agricultural supply chains (Marvier & Van Acker, 2005). The inability to contain GE traits may jeopardize export markets, thus creating an unfavorable economic climate and uncertainties among exporters (Bagavathiannan, Spok, & Van Acker, 2011; Van Acker & Bagavathiannan, 2011). In cases where traits cannot be readily contained, technology developers and regulators need to be very cautious about which traits are allowed, not only for widespread commercial release but also for cultivation in small, contained plots. Cooperative initiatives among international regulatory agencies could help establish protocols for effective containment of traits that warrant zero tolerance within specific production and supply chains (Bagavathiannan & Van Acker, 2009b).

In North America, we have more than a decade of experience with commercial production of GE crops. This experience has provided two key lessons (Marvier & Van Acker, 2005): (1) when GE crops are grown outside on a commercial scale, the movement of GE traits beyond their intended destinations is certain and the risk of escape increases with the scale of production; (2) full retraction of escaped GE traits is unlikely.

These points support the need for caution and serious consideration where there is a hope or expectation of coexistence and commercial segregation, especially for situations where a GE trait is regulated. Trait movement is especially complex within large agricultural supply chains that involve many actors and living elements across an active landscape (Van Acker, McLean, & Martin, 2007). Traits may persist and move among living populations of plants—including feral and volunteer plants—and among latent populations in seed that may exist in a myriad of places within the production and supply chain. In any case, the role of volunteer and feral populations and latent seed populations in trait persistence and movement can be substantive. As such, this needs to be well recognized and understood for trait risk-assessment purposes and for the consideration of commercial coexistence or segregation schemes.

Crop species range in terms of their biology and ecology in relation to intraspecific trait movement and the challenges of trait containment. Wheat (*Triticum* *aestivum* L.) may represent the least challenging scenario, whereas species such as alfalfa (*Medicago sativa* L.) and canola (*Brassica napus* L.) may be the most challenging. Placement along this continuum depends very much on the biology and ecology of these species in the context of agronomy and farming practices and the nature of the supply chains they move through. In this article we will explore the nature of these crops in relation to trait containment and coexistence.

Canola

GE canola has been grown in Western Canada since 1995. Currently, well over 90% of the canola grown in Western Canada is GE. By the year 1998 (4 years after the start of cultivation) GE traits were stacking within volunteer canola plants (Hall, Topinka, Huffman, Davis, & Good, 2000), and by 2007 the stacking of GE traits in escaped (and possibly feral) roadside populations of canola had also been documented (Knispel, McLachlan, Van Acker, & Friesen, 2008). This was very strong empirical evidence of the effectiveness of the complete dynamic of the GE traits within the canola metapopulation and through the agricultural supply chain. Recently there was evidence of GE canola having moved through broad areas within the United States (Schafer et al., 2011), primarily along the Canada-US border and along grain transportation routes. In addition, GE canola has been found commonly in shipping ports in both exporting and receiving countries (Yoshimura, Beckie, & Matsuo, 2006).

The movement of GE traits within the canola metapopulation is a function of its biology and ecology (as well as the way in which canola is farmed), the farming systems it is part of, and also the way in which canola is handled in the supply chain—including the production of seed. There has been so much intraspecific GE trait movement in canola in Western Canada that farmers in this region have come to expect the appearance of unintended traits in their canola (Friesen, Nelson, & Van Acker, 2003). Many elements come together to allow for extensive movement of GE traits in canola.

- GE herbicide-tolerant canola was granted unconfined release when it was deregulated in Canada.
    - This meant that there were no requirements for containing the GE traits or GE canola (Canadian Food Inspection Agency, 1995).
- A large number of acres of GE canola have been grown in fields across Western Canada.
- There is a relatively high frequency of canola in crop rotations in Western Canada (Thomas, Leeson, & Van Acker, 1999).
- There are large volunteer canola populations in fields in Western Canada (Leeson, Thomas, & Hall, 2002a; Leeson, Thomas, Andrews, Brown, & Van Acker, 2002b).
    - Volunteer canola commonly survives to flowering at significant occurrence densities in a significant proportion of fields in Western Canada (Harker et al., 2006; Leeson et al., 2002a, 2002b).
    - Volunteer canola can persist until, emerge in, and flower in subsequent canola crops (Leeson et al., 2002a, 2002b; Simard & Legere, 2003).
- Plant to plant outcrossing rates in canola are relatively high (Cuthbert & McVetty, 2001).
- The current canola pedigreed seed production system was not designed to prevent gene flow at the level required to prevent low-level presence of given GE traits (Friesen et al., 2003).

Wheat

Estimates of outcrossing rates in wheat are dependent on synchrony of flowering between pollen donors (males) and pollen receptors (females), the presence of receptive females, and the availability of single dominant nuclear marker genes to facilitate detection of outcrossing (Willenborg & Van Acker, 2008). With the proposed introduction of GE wheat in North America, there was considerable interest in the outcrossing capability in wheat, and Waines and Hegde (2003) noted that there was enough evidence to show that it regularly occurred and varied depending on the situation including the variety. As in canola, there is an active metapopulation with respect to traits in wheat growing areas. There exist very similar conditions to canola in Western Canada that could facilitate extensive GE trait movement in wheat. These conditions include

- a large number of acres of wheat grown in all agricultural regions of Western Canada (up to 10 million ha annually);

- the relatively high frequency of wheat in crop rotations in Western Canada, higher than for canola (Thomas et al., 1999);
- the high population levels of volunteer wheat in average fields in Western Canada (Leeson et al., 2002a, 2002b);
- volunteer wheat commonly survives to flowering at significant occurrence densities in a significant proportion of fields in Western Canada (Harker et al., 2005; Leeson et al., 2002a, 2002b);
- empirical evidence shows that wheat is as persistent as canola both in terms of quantity (density) and frequency (% of fields), and it can persist to a measurable level for up to five years (Beckie, Hall, & Warwick, 2001; Harker et al., 2005);
- volunteer wheat can persist until, emerge in, and flower in subsequent wheat crops (Beckie et al., 2001; Harker et al., 2005);
- outcrossing in wheat is possible from plant to plant within a commercial crop (Matus-Cadiz, Hucl & Dupuis, 2007; Waines & Hegde, 2003); and
- the current wheat pedigreed seed production system was not designed to prevent gene flow at levels required to prevent GE trait movement (Gaines, Preston, Byrne, Henry, & Westra, 2007).

There is empirical evidence of the presence of specific traits in certified seed of wheat. Gaines et al. (2007) showed that the non-GE trait conferring tolerance to imidazolinone herbicides in wheat ("IMI"-tolerance trait) has been found in certified conventional wheat seed lots in the United States at levels of up to 11% only 2 to 3 years after commercial release. This adventitious presence (AP) occurred despite the fact that certified wheat seed is grown and handled under strict segregation regimens.

Alfalfa

Alfalfa is the most important forage species in North America, and GE alfalfa has been deregulated in both the United States and Canada. As an outcrossing perennial, the ecology and biology of alfalfa is unique among deregulated GE crops. The potential for intraspecific GE trait movement in alfalfa has been studied and considered (Van Deynze et al., 2008), but there has been limited study of the nature of roadside populations and the role they can play in GE trait movement.

The life-history characteristics of alfalfa suggest that it is a candidate species for high gene flow and ferality potential (Bagavathiannan & Van Acker, 2008). Alfalfa cultivars are typically selected for persistence under grass mixtures, providing traits that favor persistence in roadsides—including the ability to fix nitrogen, presence of deep tap roots, drought and cold tolerance, perenniality, high genetic diversity, and fast regrowth potential (Bagavathiannan & Van Acker, 2009a). Alfalfa populations are commonly observed in roadsides and unmanaged habitats, particularly in alfalfa growing regions (Bagavathiannan, Gulden, & Van Acker, 2011b; Fitzpatrick, Reisen, & McCaslin, 2003; Kendrick, Pester, Horak, Rogan, & Nickson, 2005; Prosperi, Jenczewski, Angevain, & Ronfort, 2006). In studies in Manitoba, roadside alfalfa populations were found not to be genetically distinct from typical commercial alfalfa cultivars (Bagavathiannan, Julier, Barre, Gulden, & Van Acker, 2010), indicating that these were typical escapes from cultivation. Such escape could happen during farming activities (i.e., planting, harvesting, transport operations, etc.) or through intentional planting in roadsides, which is not uncommon in North America.

The demography of roadside alfalfa suggests that it is capable of establishing self-perpetuating populations in roadside habitats, with key facilitating elements being persistent seedbanks, successful seedling recruitment, and adult reproductive success (Bagavathiannan, Gulden, Begg, & Van Acker, 2010). Alfalfa grows well despite limited nutrient levels in roadside habitats (Drenovsky, Martin, Falasco, & James, 2008). Mowing affects the reproductive success of roadside alfalfa, but roadsides are typically not completely mowed (Bagavathiannan, 2009). Roadside alfalfa populations are very similar in growth performance to cultivated alfalfa, except for fecundity where cultivated alfalfa can produce more than three times as much seed. Nevertheless, the levels of seed production are likely sufficient to perpetuate these populations (Bagavathiannan, Gulden, et al., 2010). In Western Canada, average hard seed content for cultivated alfalfa seed ranged from 14 to 37% (Fairey & Lefkovitch, 1991). Alfalfa seed had a good ability to overwinter in roadside conditions (overwintering mortality ranged from only 14 to 24%; Bagavathiannan, Gulden, et al., 2010), but there can be high levels of winter mortality for seedlings (> 80% after 2 years; Bagavathiannan, Gulden, et al., 2010) especially for seedlings emerging near mother plants, as would be expected (Jennings & Nelson, 1991; Rumbaugh, 1982). Alfalfa recruits very well in a typical grass sward, and—although herbicide (2,4-D) applications can effectively control all emerged alfalfa plants—some dispersed seeds remain as a dormant seedbank, allowing recruitment in subsequent years (Bagavathiannan, Gulden, & Van Acker, 2011a).

There is evidence that roadside alfalfa populations experience selection pressure for adaptive traits, including winter survivability, rhizome production, and prostrate growth habit—traits that favor persistence in unmanaged habitats (Bagavathiannan, Julier, et al., 2010). Matrix modeling (Bagavathiannan, Begg, Gulden, & Van Acker, In press) suggests that typical roadside feral alfalfa populations will persist, and the likelihood for extinction is minimal, especially under current roadside management regimes. Long-term persistence of alfalfa populations in pastures and rangelands has been reported by several authors (Kilcher & Heinrichs, 1965; Pearse, 1965; Rumbaugh & Pedersen, 1979). Seed immigration generally increases equilibrium densities, but it is not an absolute requirement for sustaining populations. In a timely-mowing scenario, the survival of the population was dependent on seed immigration, but only if local seed production was completely prevented.

There is an abundance of evidence that alfalfa can readily establish and persist in roadside habitats without managed cultivation and can in this manner act as a reservoir for GE traits. There remains little information on the seedbank dynamics of roadside alfalfa populations, and estimates of persistence are not robust. Alfalfa seed dispersal is poorly understood, yet its nature has substantive implications for population establishment, growth, and sustenance. Studies on the nature of feral alfalfa remain rare. In addition, the likelihood of persistence, spread, and invasion of feral populations can be influenced by introduced traits (Claessen, Gilligan, Lutman, & Van den Bosch, 2005a; Claessen, Gilligan, & Van den Bosch, 2005b), and this has not been well studied in alfalfa or in other GE crops.

Managing Metapopulations for Trait Containment

Considerations of intraspecific trait movement for GE traits in crops is very different from considerations of trait movement in natural or even semi-natural scenarios because the traits can reside and move in supply chains managed by people. Human error can cause escape and persistence and it is unpredictable. In the United States, there have been a number of documented cases of trait escape involving human error, including the ‘Starlink’ case (Marvier & Van Acker, 2005). Three years after this discovery and after the execution of a massive recall effort, traces of the Starlink protein could still be found within both food and feed handling streams in the United States (US Department of Agriculture [USDA], 2003). The Starlink case showed that full retraction of traits (and their products) from complex and massive commercial food and feed systems is difficult—and perhaps impossible. Another example of human-mediated trait escape is the Prodigene case—also in the United States—where corn genetically modified to produce a vaccine that prevents diarrhea in pigs was discovered in a commercial grain elevator in Iowa (Gillis, 2002). Upon investigation, the USDA found that the company who owned this GE corn (Prodigene) had failed to comply with US federal regulations requiring that the company destroy volunteer GE corn growing in subsequent crops. This error required that 13,600 t of contaminated soybean be destroyed to prevent further contamination of food or feed supply chains. This case demonstrated insufficient trait containment oversight on the part of the company (Prodigene). Between 2000 and 2008, the USDA documented six cases of regulated novel traits escaping beyond their intended containment spaces within the United States (US Government Accountability Office [USGAO], 2009). The mechanism for escape ranged from cross pollination and commingling of seed, to cross pollination and uncontrolled volunteers, to misidentified seed. In the three remaining cases, the mechanism of escape has not been determined according to the USDA. In Canada, there also have been cases of human error leading to trait escape, including the inadvertent release of the GT200 event of Roundup Ready® canola (resistance to glyphosate herbicide; Demeke, Perry, & Scowcroft, 2006). The company’s response to the mistake was swift and effective, but the case demonstrated the possibility for these types of mistakes to occur, and they highlight the challenges of trait containment and the diligence that must be employed in order to effectively contain traits.

A key element in supply chain management of trait containment is keeping track of seed. Viable seed can be considered a latent population within an active metapopulation (Van Acker & Bagavathiannan, 2011), and any cache of seed is a seedbank potentially moving into and out of grain and seed streams. Managing seed and grain handling systems for segregation requires dedication of processes and equipment and strict adherence to protocol, and maintaining purity standards below 1% is often prohibitive in terms of cost (Van Acker et al., 2007). Current isolation distance requirement for certified seed production in Canada may or may not be sufficient for trait containment programs (Canadian Seed Growers' Association [CSGA], 2003). These isolation distances are designed to achieve variety purity (within limits) but not necessarily genetic purity (or the prevention of GE trait entry). As such, and given the evidence of long-distance pollen-mediated gene flow (PMGF) in many crops including alfalfa and canola (Fitzpatrick et al., 2003; Rieger, Lamond, Preston, Powles, & Roush, 2002; St. Amand, Skenner, & Peaden, 2000), isolation distances may need to be revisited. However, the appropriate isolation distance may be dictated by the nature of the GE trait and the level of AP allowed.

For alfalfa, hay fields need to be managed properly and cut regularly before flowering. In a survey in Southern Manitoba, we found flowering plants in many hay fields, with flowering synchrony occurring between feral populations and hay fields in 1/3 of cases (Bagavathiannan, 2009).

Producers who wish to maintain crops as GE-free will need to make conscientious efforts to do so and need to better understand the routes and mechanisms of GE trait movement (Van Acker et al., 2007). The identification and management of feral population will be required, particularly in scenarios where AP thresholds are very low.

Some GE traits may facilitate the persistence of feral populations, including traits favoring adaptation including drought and salt tolerance and pest and disease resistance. In addition, traits that confer herbicide resistance may be a concern if the associated herbicide is broad spectrum (e.g., glyphosate) and used to control weeds along roadways, right-of-ways, and volunteer GE plants in subsequent crops.

Conclusions

Stewardship and coexistence programs for GE crops need to consider the entire metapopulation in GE trait confinement and in coexistence plans and protocols. The degree to which feral populations need to be managed and other stewardship practices should be implemented will depend on the nature of risk posed by the GE trait in question and the resultant allowable AP threshold level in GE-sensitive production systems. Total confinement (and achieving zero-tolerance) of GE traits under normal commercial field conditions is likely not practical, and alfalfa (for example) is not a good candidate crop for traits that require absolute containment. For traits that may not warrant total containment, establishing acceptable and practically attainable threshold levels is essential for successful coexistence of GE and GE-sensitive crop production systems in agricultural landscapes. Further, enactment and enforcement of appropriate regulations is vital.

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