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Coexistence in the Case of a Perennial Species Complex: The Potential Challenges of Coexistence between GM and Non-GM *Prunus* Species

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The characteristics of *Prunus* species highlight the complexity of maintaining coexistence between genetically modified (GM) and non-GM *Prunus* in the case of commercial production of GM *Prunus* species and have implications for proposed low-level presence (LLP) policies. These characteristics include genetic diversity, genetic bridging capacity, inter- and intra-specific genetic compatibility, self sterility (in most species), high frequency of open pollination, insect-assisted pollination, perennial nature, tendency to escape from cultivation, and the existence of ornamental and roadside *Prunus* species. GM plum is the first *Prunus* species to be deregulated, yet the current plum production system is not designed to prevent gene flow at the level required to prevent LLP of given GM traits especially when it does not take into account the implication of the opportunity for trait movement across the *Prunus* species complex. The commercialization of one GM *Prunus* species may create coexistence and LLP issues for the commercial production of many non-GM *Prunus* species.

Key words: *Prunus*, coexistence, low-level presence (LLP), ferality, GM plum, biosafety, metapopulation.

Introduction

Although genetic modification (GM) is compelling because of the opportunity to facilitate the inclusion of unique traits, there are concerns regarding the safety of GM or the safety of GM-facilitated traits, which has lead to asynchronous deregulation globally and trade issues related to the adventitious presence (AP) of GM crops (Tranberg, 2013). Plant-to-plant gene movement is facilitated by wind, pollinators, animals, volunteer and feral populations, human transport of seeds, and human error in seed handling (Van Acker, 2012). Once transgenes escape into the environment they are difficult, if not impossible, to retract. This can be seen in cases of invasive plants or the asymmetric gene flow that can lead to genetic assimilation and consequently increase the risk of wild taxa extinction (Marvier & Van Acker, 2005). In recent years there also has been an increasing number of reports on the potential risks of GM plants to the environment, often facilitated by intra- and inter-specific transgene movement (Van Acker, 2012).

The first GM plum variety (European plum, *Prunus domestica* L.) resistant to *plum pox virus* (PPV, a potyvirus that affects all stone fruit species and causes a disease called Sharka) called “C5 plum or Honey Sweet” (Scorza et al., 1994), was deregulated in the United States (Scorza et al., 2007). With the first GM *Prunus* deregulated there is a need to consider the possibility of transgene movement among *Prunus* species (Table 1; see also Cici & Van Acker, 2010).

Prunus species have been grown throughout the world for centuries. This genus contains ornamental plants, fully fertile hybrids (sour cherry, *P. cerasus* L.), invasive plants (black cherry; *P. serotina* Ehrh., in Europe), and stone fruits (plum [*P. domestica* L., *P. insititia* L., and *P. cerastifera* Ehrh.]; apricot [*P. armenica* L.]; peach [*P. persica*, L. Batsch.]; cherry [*P. avium* L. and *P. cerasus* L.] and almond [*P. communis* Archang]). The world-wide harvested area of *Prunus* fruit trees in 2012 was approximately 6.8 million hectares. Among commercial *Prunus* species, plums are the most commonly cultivated, with a global total cultivated area of near 2.7 million hectares (FAOSTAT, 2012).

Coexistence in the Context of a Species Complex

A number of *Prunus* species are the result of natural hybridization and polyploidy (see examples in Knight, 1969). In some *Prunus* species, hybridization has caused the production of progeny which are more tolerant to certain ecological conditions, as is the case for the plum × apricot crosses for example. Many ornamental cherries with showy flowers and sour cherry are also the result of hybridization (Kuitert, 1999). Some *Prunus* species in the subgenera Lithocerasus (Microcerasus) have the ability to hybridize with species in the Pruno-

Table 1. Center of origin, scale of commercial production, and recognized cross compatibility of common global commercial *Prunus* species.

Common commercial *Prunus* crops	Center of origin	Global commercial scale	Examples of cross compatibility [a]
Plum	Caspian and Black Seas and North America	Cultivated area[b]=2.5 M ha; Global production value $14B (US); Global trade value $1B (US)	Cherry plum × Japanese plum; European plum and Japanese plum; Japanese plum and American plum; plum × apricot; plum × cherry; plum × peach and nectarine
Apricot	Western Asia and China	Cultivated area=0.5 M ha; Global production value $3.4B (US); Global trade value $0.54 (US)	sand cherry × apricot; plum × apricot; almond × apricot; apricot × peach
Peach	China	Cultivated area=1.3 M ha; Global production value $11B (US); Global trade value $1.7B (US)	peach × almond; peach × nectarine; peach × plum; peach × apricot; peach × cherry
Almond	Central Asia to Mediterranean	Cultivated area=1.8 M ha; Global production value $8.4B (US); Global trade value $2.6B (US)	almond × peach
Cherry	Central Asia	Cultivated area=0.3M ha; Global production value $4.7B (US); Global trade value $0.8B (US)	sweet × sour cherry; cherry × plum; cherry × apricot, cherry × peach

[a] *adapted from Cici and Van Acker (2010)*
[b] *2009 estimates from FAO*

phora (plums and apricot) and Amygdalus (peach and almond) subgenera to produce fertile progeny (Table 1; Rehder, 1947).

Among *Prunus* species, plums have the most genetic diversity and they may act as a genetic hub in the *Prunus* complex (Watkins, 1976). Most plums are self-incompatible or partially self-compatible. The anthers of plum flowers do not extrude but remain within closed flower buds, and pollination will not happen before flowers open (Szabo, 2003). Interspecific fertilization is common in plums (Cici & Van Acker, 2010). Cultivated plums can readily hybridize with wild plum species (Watkins, 1976). They also hybridize with apricot (Knight, 1969), cherry (Knight, 1969), and peach or nectarine (Ramming, 2004). For example, Joseph Rullo (US Patent No. US PP19,842 P3, 2009) found that using only one limb of a Japanese plum tree, hand pollination using sweet cherry pollen produced 200 whole seeds, five of which produced completely viable seedlings. Crosses between cherry plum (from the Euprunus section of the Subgenera Prunophora) and *P. pumila* L. (from Subgenera Lithocerasus) have been shown to result in a fertile hybrid called *P.*X *cistena* N. E. Hansen. (Knight, 1969).

According to published reports on the deregulated GM plum, the GM virus-resistance trait is easily transferred to progeny via pollen, and hybrids produced from GM plum parents have been shown to be effectively PPV-resistant (Scorza et al., 2007). Successful transgene flow from GM to non-GM plum trees has been recorded at a distance of 520m at a rate of 2 out of 2,950 seeds (APHIS Petition 04-264-01p). Although this gene-flow level is low (0.067% or ≈ 6 in 10,000), it would be sufficient to cause market issues in jurisdictions where given events have not yet been deregulated (Lamb & Booker, 2011). Recently, Scorza et al. (2013) completed more than a decade-long field-based study examining the potential for pollen-mediated gene flow (PMGF) from GM plum to non-GM plum. Their results suggest that although PMGF is possible, the extent is low. They found successful PMGF from GM to non-GM plum in only 4 of 11 years, and in the years when there was measurable PMGF it was at low levels (from 0.215% to 0.017% at distances from 134m to more than 300m, respectively) on the basis of individual embryos tested. This type of research is essential for informing practical low-level presence (LLP) and coexistence policy creation; in the case of *Prunus*, it should be carried out for PMGF within the species complex, not just plum to plum. There are no published reports or studies on the potential for pollen-mediated transgene flow from GM plum to other compatible *Prunus* species, but the intercompatibility of European plum and other *Prunus* species has been relatively well documented (Cici & Van Acker, 2010; Knight, 1969; Szabo, 2003; Table 1).

Implications in LLP and Coexistence Policy Development

LLP is defined as the unintended presence at low levels of a GM crop that has been approved in accordance with the Codex Plant Guidelines for food in at least one country but not in the country of import (Tranberg, 2013). Understanding the importance of effectively managing the international trade of GM plum (or other *Prunus* species) and minimizing the trade impacts caused by LLP is an important issue that should be addressed in policy. Given the length of experience in cultivating GM crops worldwide, it is now commonly known that crop-to-crop transgene movement via pollen and/or seed occurs within a complex of sub-populations that exist across agricultural and semi-natural landscapes. These sub-populations include crops, volunteers, and feral (or escaped) stands (Bagavathiannan & Van Acker, 2008). Without very strict containment practices, transgenes can move among these sub-populations, which taken together comprise a metapopulation with respect to a given transgene. As in other commercial crops where GM has been introduced—including canola (*Brassica napus* L.), wheat (*Triticum aestivum* L.), and alfalfa (*Medicago sativa* L.; Van Acker, 2012)—for plum there is also a potentially active metapopulation with respect to GM traits. One key difference between plum and these other crops is the extension of the metapopulation beyond the single crop species. The characteristics of *Prunus* species—including genetic diversity, genetic bridging capacity, inter- and intra-compatibility, self sterility (in most species), high frequency of open pollination, insect-assisted pollination, perennial nature, complex phenotypic architecture (canopy height, heterogeneous crown, number of flowers produced in an individual plant), tendency to escape from cultivation, and the existence of ornamental and roadside species within the genera—suggest that there is a tremendous and especially complicated ability for pollen-mediated gene movement among *Prunus* species (Cici & Van Acker, 2010). Risk assessments and consideration of practical LLP policy related to the release of a GM *Prunus* species such as plum, for example, should not only consider the role of feral, ornamental, and wild species populations in transgene movement but also other commercially produced *Prunus* species within the region, and their respective metapopulation constituents including wild types and also compatible ornamental relatives.

Less than half a century ago, some *Prunus* tree species were still classified as weeds in the United States (Holm, Pancho, Herberger, & Plucknett, 1979). These were mostly plum and cherry species. Today, one of these weeds (black cherry; *P. serotina* Ehrh., 4x) is also considered an invasive weed in Europe. Black cherry is a human-facilitated invasive *Prunus* and—in light of the existence of an extensive genetically compatible *Prunus* species complex—it is an example that is relevant in terms of both biosafety and coexistence considerations for GM *Prunus* species. The *Prunus* species listed as possible weeds by Holm et al. (1979) is not complete given that it is only US based and we know that the center of origin for most *Prunus* species is Asia minor (around the Caspian and Black seas) and China.

The discovery of GM flax in shipments of flax from Canada to Europe in 2009 highlighted the challenges that asynchronous approvals of GM crops around the world pose in a world that is reliant on global trade in agricultural commodities and food (Lamb & Booker, 2011). The GM flax case is especially interesting because GM flax was never commercially produced in Canada, yet it appeared in a shipment of commercially produced flax (Lamb & Booker, 2011). The lessons from this case are important and should draw attention to the vulnerability of international agriculture and food trade to very low AP of GM material. Internationally, AP is recognized as the impetus for a need to develop LLP policy, and it is commonly recognized that AP includes events that may have been deregulated in one jurisdiction but not in another, so is directly relevant to LLP (this is in contrast to the unusual definition of AP proposed by Tranberg [2013]). The example of AP in flax is very relevant to the consideration of GM *Prunus*, where there may be an even greater risk of AP in commercial crops given the existence of a sexually compatible complex of commercial species.

For LLP considerations in plum and more broadly in *Prunus*, there will be unique practical considerations. A special challenge for any segregation scheme is participation by relevant actors. In the case of coexistence and LLP for GM traits, this is problematic when the traits are essentially invisible and when the key control points for limiting trait movement are at the receiver end, the receiver is typically not expecting the AP, the receiver is not testing or monitoring for AP, and the receiver is not part of any sort of scheme or contract (Van Acker, McLean, & Martin, 2007). If governments are working with sector, producer, or industry organizations to develop awareness and monitoring approaches (e.g., Tranberg, 2013), in the case of *Prunus* they will need to broaden their efforts to include all commercial *Prunus* species, even in the case of only one *Prunus* species GM event being commercialized in any jurisdiction that is part of a global trade chain. They may also need to include surveys and monitoring of roadside wild-type species, and there may be a special issue with gene escape into perennial wild types (e.g., black cherry) growing in unmanaged areas (Cici & Van Acker, 2010).

Beyond LLP, the case of the *Prunus* species complex is concerning for biosafety considerations, especially those related to protecting the integrity of genetic resources in centers of origin (Table 1). This may be mitigated to some extent, however, given that the *Prunus* species are perennials and outcrossing does not transform the parent plant (see below). On the positive side for *Prunus* species generally, the commercial product is relatively large, is often shipped whole, and is often consumed whole. This can facilitate retraction of the product more easily than may be the case for common grain and oilseed crops, especially in comparison to small seeded crops that can form persistent seedbanks, such as canola (Van Acker, 2012). In addition, the persistence of any AP in commercial *Prunus* production may be low because even if individual flowers are pollinated with GM pollen and the resulting fruits are hybrids containing the GM trait, the trees remain true breeding and non-GM (Scorza et al., 2013). For the subsequent year, therefore, farmers should be able to address and mitigate the situation if they are able to identify the source of the AP.

Global food trade is constantly expanding and so too is the deregulation and commercialization of GM crops, including tree fruits. LLP is intended to recognize and serve this situation. Therefore, information to aid the development and efficacy of LLP policy and schemes is valuable. Current plum production systems world-wide are not designed to prevent gene flow and likely are not ready to prevent gene flow at the level required to prevent LLP of given GM traits. Given the species complex associated with plums, many other commercial *Prunus* species may be at risk of market harm due to AP of GM traits if there is broad production of GM plums. To maintain GM-free *Prunus* species, facilitate the coexistence of GM and non-GM commercial *Prunus* production, and make effective LLP policy, policy makers need to make conscientious efforts to understand and respect the mechanisms of GM trait movement in this species complex and perhaps use it as an example to guide further coexistence and LLP considerations.

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