Risk assessment of gene flow from genetically engineered virus resistant cassava to wild relatives in Africa: An expert panel report

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INTRODUCTION  
Cassava (*Manihot esculenta* Crantz) is a major source of food and income in Africa, where its starchy tuberous roots are a staple food, and the foliage is used for human consumption and livestock feed. More than 250 million people in Africa and nearly a billion people globally rely on cassava for food and income (OECD 2009; Gbadegesin et al. 2013). Studies on cassava production and utilization in eastern Uganda and western Kenya found cassava contributed more than any other single crop to household income, with 63% of households selling cassava products to generate income for the family (Fermont et al. 2010). Cassava crop production faces a number of challenges from diseases and pests, including severe losses in yield and quality due to virus infection. Two major viral diseases infect cassava in Africa: Cassava Mosaic Disease (CMD) and Cassava Brown Streak Disease (CBSD) (Legg et al. 2014; OECD 2014). The viruses that cause these diseases are transmitted by whiteflies (*Bemisa tabaci*) and persist through the stem cuttings used to propagate the plants in farmers’ fields. These two virus diseases are currently the most significant threat to cassava production in East and Central Africa (ECA) with massive economic losses and impacts on food security that could spread across the African continent and globally (Thresh 1997; Kanju et al. 2003; 2007; Legg et al. 2014).

CMD has been the main disease constraint for the crop historically. This disease has been triggered by the emergence and spread of at least eight species of geminiviruses (Legg et al. 2014). Symptoms of CMD include severe mosaic leaf chlorosis and deformation of the leaves, with resulting reduction in storage root yields. CBSD was first reported in the 1930s and in the last decade has re-emerged as a major threat to cassava production (Alicai 2007, Legg et al. 2014). CBSD in East Africa is caused by two species of ipomovirus, Cassava Brown Streak Virus (CBSV) and Ugandan Cassava Brown Streak Virus (UCBSV). While symptoms of CBSD are not always detected in the above-ground plants because the disease has limited effects on plant growth and appearance, this disease causes necrotic rot of the storage roots, the primary edible portion of the crop, resulting in partial to complete spoilage, yield loss, and thus loss of food (Hillocks et al. 2001; Legg et al. 2014). Because of the recent re-emergence and severity of CBSD, it is considered one of the seven most dangerous crop diseases impacting food security in the world today (Pennisi 2010).

Conventional and molecular breeding efforts are underway to combat both of these diseases in cassava, including inter-varietal cassava crosses as well as crossing cassava with other *Manihot* species. A limited number of varieties with varying levels of conventionally-bred CMD and CBSD resistance/tolerance have been identified and released. Breeders have been successful in addressing the challenge of CMD, resulting in the release of many high yielding cassava varieties that are resistant or tolerant to the causal geminiviruses (Rabbi et al. 2014; Okogbenin et al. 2012). Three CMD resistance loci have been described, but in all cases the underlying molecular mechanisms remain unknown. CMD1 was introgressed from M. glaziovii and is known to be polygenic (Fregene et al. 2000), while CMD2 is derived from a monogenic locus found in multiple accessions of West African landraces (Akano et al. 2002). CMD3 was recently described in the cultivar TMS97/2205 in which CMD2 is combined with an additional locus on the same chromosome, resulting in very high levels of resistance to CMD (Okogbenin et al. 2012). In all cases however, these improved cultivars are susceptible to CBSD. The best cassava cultivars are, at best, tolerant and not resistant to CBSD under field conditions (Legg et al. 2011). A number of cassava varieties tested showed susceptibility to either UCBSV or CBSV or both (Winter et al. 2010). Recent studies have shown some cassava varieties, for example NASE 3 (Ogwok et al. 2014) and Kaleso (Maruthi et al. 2014), to be resistant to infection by UCBSV (Kabanyolo isolate) but not to CBSV. A few varieties are considered tolerant to CBSD in the field due to reduced symptoms on shoot material and minimal damage within the storage roots. Cultivars in this category include; Kalulu, Kigoma Red, Namikonga, Kiroba, Naliendele and Nanchinyanya derived from breeding programs at Amani in Tanzania (Jennings 2003, Rwegasira and M.E. Re 2012).

Even with conventionally bred resistant/tolerant varieties, adoption of new varieties remains a challenge because farmers have strong preference for certain varieties with familiar taste, quality and yield. Genetic engineering is being pursued as an alternative method to introduce highly effective disease resistance into desirable varieties already known to farmers (Legg et al. 2014). One such project, Virus Resistant Cassava for Africa (VIRCA), is using genetic engineering to introduce sequences derived from the viruses that cause the diseases into cassava to make the plants resistant via RNA interference (RNAi) (Taylor el al. 2012; Odipio et al. 2014). The cassava varieties chosen by VIRCA for engineering are those with which African farmers already have a high level of experience and preference. The current goal of the VIRCA project is to develop cassava varieties that are resistant to CBSD and CMD, using transgenes in the case of CBSD and conventional resistance in the case of CMD. With inherent resistance to CMD present in East African farmer preferred cultivars, the VIRCA project has focused on integrating transgenic RNAi technology for resistance to CBSD into these cultivars in order to provide farmers with planting materials resistant to both of these devastating diseases.Genetically engineered cassava genotypes are currently being screened by the VIRCA project for efficacy of virus resistance and performance in field trials.

Genetically engineered (GE) crops are the subject of environmental risk assessment, as well as food safety assessment, before they are approved for general release in the environment. Environmental risk assessments consider whether the GE crops are as safe as conventionally bred crops, and are conducted case-by-case based on what is known about the biology of the crop, the introduced trait, and the receiving environment (OECD 1993; National Research Council 2002; Craig et al. 2008). One of the key considerations in environmental risk assessment for GE crops involves gene flow from the crop under cultivation to wild relatives that exist in the region and persist outside of agriculture (in this case, also referred to as ‘free-living’ or ‘naturalized’) and whether the persistence of the transgene in populations of the wild relative will result in a more serious adverse effect on the environment than gene flow from the non-GE crop (e.g., Hokanson et al. 2010). Other types of gene flow (i.e., crop-to-crop gene flow or crop-to-weed gene flow) are also the subject of risk assessment (e.g., Légère 2005). The focus of this report is on gene flow from cassava to wild relatives in Africa.

Gene flow occurs naturally between cultivated plant species and compatible wild relatives, and its occurrence is not unusual when these plants grow in proximity to each other (Ellstrand 2003). Considerably less frequently, crop alleles become established in free-living wild or weedy populations (Ellstrand et al. 2013). In about a dozen cases, spontaneous hybridization between traditionally improved crops has led to the evolution of increased weediness (Ellstrand et al. 2010). In fewer still has gene flow from crops increased the extinction risk of rare taxa (Ellstrand 2003). Nonetheless, before the advent of GE crops and their requirements for regulatory approval, there was little interest in the potential environmental risks related to this natural transfer of genes. There are also some concerns about introduction of transgenes via gene flow, particularly into landraces and native species in centers of origin, that have more to do with cultural objections than environmental risks.

The goal of risk assessment is to characterize risk by considering the likelihood and consequence of harmful effects following an activity. According to the most current discussions surrounding internationally accepted approaches to risk assessment of genetically modified plants, the use of proper problem formulation in the initial stages of a risk assessment is important when considering the potential risks of GE crops (Raybould 2006; Hokanson et al. 2010; Wolt et al. 2010; Huesing et al. 2011; Gray 2012; Tepfer et al. 2013; Tepfer et al. 2015). The first part of problem formulation is to determine the protection goals governing the risk assessment, and what effects would be regarded as harmful according to these goals (e.g., Garcia-Alonso and Raybould 2014). When possible harmful effects have been clearly established, the plausible pathways, or scenarios, by which these harms could occur, are considered. A pathway that could lead to harm can be broken down into steps, and the likelihood of each step occurring can be assessed. Existing information, i.e, scientific knowledge, can be used to determine the likelihood of each step in the pathway and to determine when additional empirical information might be useful or necessary (Romeis et al. 2009; Tepfer et al. 2015). If a step in the pathway is likely to occur, it does not necessarily lead to the conclusion that harm is likely, when another step that is a causal link in the pathway is unlikely (Raybould 2010). (See Figure 1.)

This report is the outcome of a VIRCA-sponsored workshop focused specifically on risk assessment of gene flow from transgenic cassava with resistance to Cassava Brown Streak Disease (CBSD) to wild relatives in Africa. To evaluate the situation, experts (the authors) on the subjects of gene flow, risk assessment, and cassava biology and breeding, assembled to consider the existing information to assess the potential for and consequences of gene flow from CBSD-resistant GE cassava to ‘wild relatives’ in Africa. The workshop focused on identifying existing information to assess whether the steps in the pathway to harm are likely or unlikely, refuting or corroborating hypotheses of ‘no harm’ to the environment, for the purposes of risk assessment.

Bringing local experts together with individuals who have specific expertise and experience in risk assessment issues, to follow this problem formulation approach, can be an efficient and effective way to thoroughly consider existing information for the purpose of risk assessment. This has been demonstrated in similar workshops for other crops and traits (Hokanson et al. 2010, Huesing et al. 2011). However, in these cases, these experts are scientists making judgments about scientific questions and whether further data are needed or worthwhile to answer, recognizing as scientists that all hypotheses can always be subjected to extra testing and experimentation. Therefore, the conclusions from all of these workshops reflect a scientific point of view, and are not a judgment of whether the extant data are sufficient, or not, to meet the regulatory requirements of specific countries or of the treaties to which they are signatories.

Here we summarize the risk assessment discussion of the experts under a series of questions that were posed at the workshop. Although the subject of the discussion was CBSD resistant cassava, much of this report is relevant to risk assessment of gene flow for other GE traits in cassava in Africa.

WORKSHOP DISCUSSION SUMMARY

**Question 1: What is the potential for the transgene to escape from virus resistant (VR) cassava and persist in sexually compatible free-living populations in Africa? Are additional studies needed to address this question?**

All *Manihot* species are native to the New World, and in Africa the only free-living populations of a *Manihot* species cross-compatible with cultivated cassava (*Manihot esculenta)* are *M. glaziovii* Muell. Arg., a naturalized species originally introduced into Africa for rubber production (Paterson et al. 1998; Halsey et al. 2008; OECD 2014). The cultivated cassava species itself does not survive well as an escape from cultivation, and it is not found in free-living populations in Africa (Halsey et al. 2008). Information about the frequency or distribution of *M. glaziovii* in Africa is limited.

Natural hybrids between cassava and *M. glaziovii* occur in Africa (Nichols 1947; Jennings 1957; Lefevre 1988; Beeching et al. 1993). They are sometimes what are referred to as ‘tree cassava’ (Wanyera et al 1994). Based mainly on morphological data, it seems that *M. glaziovii* and cassava x *M. glaziovii* hybrids generally occur infrequently as isolated single individuals or less frequently as isolated clusters of a few individuals, and apparent hybrids are more common in West Africa than in East Africa (Wanyera 1993; Wanyera et al. 1994). Based on the ability of breeders to backcross and self from the F1 generation to subsequent generations, post-F1 production should be possible in nature (Andersson and de Vicente 2010; Wanyera et al. 1994; OECD 2014). However, information about naturally occurring post-F1 reproduction is even more limited, and therefore the frequency of gene introgression is unknown. Given the rarity of apparent hybrids, advanced generation hybrids are thought to be rare as well.

The panel agreed that while *M. glaziovii* is not found in large numbers, if it were present in proximity to cassava cultivation, hybridization is likely to occur with CBSD resistant cassava and that the VR gene would be likely to persist in the wild populations (whether the allele is neutral or not, due to recurrent gene flow – see Ellstrand 2003). The panel also agreed that more information about the distribution of *M. glaziovii* in proximity to cassava fields, and about the reproduction by F1s (and, if they naturally occur, any later generation hybrids) in natural populations, would allow a better prediction about the level of persistence. The panel identified two hypotheses for testing, which would support the null hypotheses of ‘no harm’ to the environment (essentially to say ‘gene flow does not occur, therefore there are no consequences’): 1) *M. glaziovii* is not found in proximity to cassava; and 2) Post-F1 reproduction is absent in natural populations, therefore the CBSD resistance gene will not persist in *M. glaziovii* populations.

The panel agreed that time-consuming studies to characterize gene flow from cassava to *M. glaziovii* might only provide weak tests of these hypotheses of ‘no harm’, and would therefore be unconvincing or not particularly meaningful for the purposes of risk assessment. They concluded that it is better to assume that gene flow will occur and consider the consequences, than to pursue studies to test these hypotheses. It is difficult to define the criteria for ‘how rare’ is ‘rare enough’ for ‘no harm’ regarding gene flow.

**Question 2: What are the potential adverse effects that might result from gene flow of the VR gene into *M. glaziovii*?**

The harms to the environment that were identified for discussion by the panel fall into two categories:

1. *Loss of genetic variation, including potential adaptive alleles, in the wild M. glaziovii germplasm.*
2. *Loss of other valued species, ecosystem services, or crop yield and quality*

The panel acknowledged that the two categories are those typically considered in risk assessments of gene flow from crops to wild relatives (Raybould and Cooper 2005; Hokanson et al. 2010; Huesing et al. 2011). The harms to consider in a risk assessment, should depend upon the protection goals defined by the legal instruments requiring the risk assessment (Garcia-Alonso and Raybould 2014), and therefore might be the same or different from those considered here.

**Question 3: What information can be used to effectively predict whether these potential adverse environmental consequences following gene flow from VR cassava will or will not occur? Are additional studies needed to address these questions?**

***Loss of wild type alleles or genetic variation in the germplasm pool***

Gene flow from CBSD-resistant cassava to *M. glaziovii* poses no more of a threat to genetic diversity in the cassava germplasm pool (*M. glaziovii* in Africa used for breeding) than does gene flow from other cassava varieties to *M. glaziovii* in Africa. Harmful loss of genetic diversity is only possible if the frequency of hybridization were to increase to a level where the cultivated cassava and *M. glaziovii* became genetically indistinguishable (genetic swamping), or if there were strong, rapid selection for genotypes with the CBSD-resistance transgene coupled with extensive linkage disequilibrium, such that large portions of the genome were being selected along with the virus resistance gene (selective sweep) (Ellstrand 2003.)Although there is evidence for gene flow between cassava and *M. glaziovii* in Africa, what is known regarding the frequency of naturally occurring hybrids (Nichols 1947; Jennings 1957; Lefevre 1988; Beeching et al. 1993; Wanyera 1993; Wanyera et al. 1994) suggests that genetic swamping is not occurring currently.

It is unlikely that the hybridization rate will increase owing to the introduction of transgenic plants because they are not expected to flower more than non-transgenic cassava. CBSD does not typically have a significant impact on the reproductive vigor (flowering and seed set) of cassava, unless the infection is severe enough to cause dieback from the vegetative tips. Although dieback may occur in some susceptible varieties before harvest, cassava is usually harvested before the symptoms reach this point. Moreover, extensive linkage disequilibrium associated with selective sweeps is not expected to persist over multiple generations because of the predominantly outcrossing mating system in cassava and in *M. glaziovii*; thus, a selective sweep for CBSD resistance would not be expected to cause extensive loss of alleles for other useful traits even if there is selection for genotypes with virus resistance.

Furthermore, the entire genus of *Manihot* is not native to Africa, and Africa is not a center of origin or diversity for either *M. glaziovii* or cultivated cassava. Primary sources of cassava genetic diversity are in the *Manihot* center of diversity in South America and to a lesser extent Mexico (Andersson and de Vicente 2010; OECD 2014). *M. glaziovii* is a genetic resource used for breeding, including a source of resistance to Cassava Mosaic Disease and other cassava viruses (OECD 2014). Although populations of *M. glaziovii* in Africa are not a primary source of genetic diversity for cassava, breeders in Africa are currently screening African populations of *M. glaziovii* and other wild related species of cassava for a source of resistance to CBSD. To date no source of resistance has been identified. Gene flow between cultivated cassava and *M. glaziovii* is more likely to increase the genetic diversity of the free-living populations, at least in the short-term (Ellstrand 2003). In fact, virus resistance in *M. glaziovii* could help to preserve the African diversity of this wild relative if it protects *M. glaziovii* from the virus.

The panel concluded that no additional studies were needed to assess the potential for loss of wild genetic diversity in the germplasm pool.

***Loss of a valued species, loss of ‘ecosystem services’, loss of crop yield and quality***

The panel discussed scenarios by which CBSD resistance could cause *M. glaziovii* to become weedy or invasive, which would lead to the other harms to the environment identified by the panel: loss of a valued species, loss of ‘ecosystem services’, or loss of crop yield and quality. A loss of a valued species might be possible if *M. glaziovii* dramatically increased in abundance to a level so that it would outcompete native plant species, that is, if it became an invasive in unmanaged ecosystems (Ellstrand 2003; Ellstrand et al. 2010). A loss of ecosystem services might be possible if *M. glaziovii* increased in abundance to a level where it disrupted availability of resources for the health of the unmanaged ecosystem (light, soil nutrients, water) (Pimentel et al. 2001; Raybould and Cooper 2005; Keese et al. 2013). A loss of crop yield or quality might be possible if *M. glaziovii* increased in abundance to a level where it competed with cassava (or other crops) for resources or disrupted the resources essential for the health of the managed ecosystem, that is, if it became an agronomic weed. To address the potential for these harms, the panel mainly considered different questions to be answered (hypotheses to be tested) in order to determine whether CBSD currently limits populations of *M. glaziovii*, and therefore whether the acquisition of resistance is likely to make *M. glaziovii* more abundant.

A review of the scientific literature via appropriate databases/search engines (e.g., Google Scholar, Web of Knowledge, etc.) revealed no evidence that *M. glaziovii* is currently considered a weed or invasive anywhere in Africa. It is cited as a “weed” or “invasive” primarily in the Pacific Islands (See Pacific Island Ecosystems at Risk: http://www.hear.org/pier/species/manihot\_glaziovii.htm; and Global Compendium of Weeds: <http://www.hear.org/gcw/species/manihot_glaziovii/>). But these appear only to be reports of an introduced species that has become ‘naturalized’. The panel could not find any mention of *M. glaziovii* being problematic either in its native range or anywhere it has naturalized. There is no evidence that it is a serious weed or serious invasive plant.

While *M. glaziovii* is not uncommon in parts of Africa, it is not found in abundance (Wanyera 1993; Wanyera et al. 1994). In theory, some introduced species will become invasive when they are released from the ecological constraints that limit them in their native range (e.g., Liu and Stiling 2006). Although the ecological limits on natural populations of all but a very few plant species are not well understood, natural populations are not typically limited by a single disease or pest (Sasu et al. 2010; Catford et al. 2011), and given its minimal impacts on the reproductive biology of cassava, it does not seem likely that CBSD is currently limiting the *M. glaziovii* populations. However, little information is available about the incidence of CBSD in *M. glaziovii* or the impact of the virus on *M. glaziovii* plants or populations.

It is known that CBSD is capable of infecting *M. glaziovii* (Mbanzibwa et al. 2010). Some earlier cassava breeding work by Nichols (1947) and Jennings (1957, 1960, 1975) suggested *M. glaziovii* as a possible source of CBSD resistance, although resistance to CBSD in *M. glaziovii* has not been found by breeders who are searching currently. If CBSD resistance were already present in some *M. glaziovii* populations, as there is for CMD (OECD 2014), it would suggest that pre-existing virus resistance has not released *M. glaziovii* from an ecological constraint that in its absence would be sufficient to allow invasiveness or weediness. *Manihot glaziovii* was present in Africa for many years before the CBSD incidence became prevalent, and there are no reports of *M. glaziovii* changing abundance subsequent to the increased prevalence of CBSD, suggesting that the recent increased incidence of CBSD has not reduced the frequency of *M. glaziovii*. However, the data are scant; what is known of the relative abundance of *M. glaziovii* before or after the occurrence of CBSD in Africa is anecdotal and not documented. The period of time in which *M. glaziovii* and the virus have coexisted in Africa is relatively short in ecological time, so it is difficult to know whether the populations of *M. glaziovii* are expanding or contracting relative to the prevalence of the virus.

Little is known about the factors that currently limit *M. glaziovii* populations in Africa, or what affects seed production, seed viability, or seedling survivorship, life history stages in plants that would most directly impact population growth (Harper 1977). Reports of naturally occurring hybrid populations, found outside of agricultural fields, between cassava and *M. glaziovii*, indicate that *M. glaziovii* in Africa does flower and produce seed on occasion, and that seeds do occasionally germinate and survive to the next generation. *M. glaziovii* in Africa apparently does not flower frequently or produce large amounts of seed (Wanyera 1993, Wanyera et al. 1994), but it is not known whether populations are limited by seed production or instead by viability at another life stage. The expectation is that CBSD resistance will not increase the seed production in *M. glaziovii* because the virus, at least in cassava, only reduces flowering and seed set when it is severe enough to cause dieback. If *M. glaziovii* populations are limited by seed production, it does not seem likely that CBSD is what is limiting the amount of seed produced.

Seedling recruitment may be a more important factor than seed production in limiting populations of *M. glaziovii* (and more straight-forward to evaluate). Most mortality occurs at the seedling life stage in most plants (Harper 1977); there is no reason to think that *M. glaziovii* should be different, although there are no relevant demographic data for this species. If seedling recruitment is rare in *M. glaziovii* populations, and the CBSD virus does not negatively impact seedling survival, such data would corroborate a hypothesis that some other factors besides CBSD must be limiting seedling survival and hence population size in *M. glaziovii*. There is reason to believe that the virus may not reduce seedling survival because the virus symptoms are usually not expressed in cassava plants until long after the seedling stage.

There are two issues that could be answered empirically to investigate this scenario involving seedling recruitment, i.e., CBSD resistance could increase the abundance of *M. glaziovii* if (1) populations are limited by low levels of seedling recruitment, and (2) seedling recruitment is limited by the virus. One study would be to survey natural populations of *M. glaziovii* to determine the frequency at which seedlings are found, to test the hypothesis that seedling recruitment is rare. This approach presents a challenge because it would be difficult to sample sufficiently over both time and space to have confidence in the conclusions from observations. From an ecological perspective, it could be assumed that seedling recruitment is rare, because this is true in most plant species in nature. Therefore, the more useful question to answer experimentally would be to test whether CBSD infection does or does not limit seedling recruitment. One test would be to artificially infect seedlings of *M. glaziovii* in a common garden experiment to determine whether infected seedlings survive at rates significantly different from non-infected seedlings, even under a high dose exposure to the virus, to test the hypothesis that virus infection does not reduce seedling survival (e.g., Maskell et al. 1999). The panel agreed that the aforementioned experiment would be the most straight-forward and useful, if it is necessary to collect additional information for this assessment. Corroboration of the hypothesis that CBSD does not limit seedling recruitment would provide evidence that *M. glaziovii* with CBSD resistance will not become more abundant, and that if it does not become more abundant, it will not become weedy or invasive.

However, the panel recognized that even if *M. glaziovii* were to become more abundant, this does not necessarily lead to the conclusion that it will be weedy or invasive and therefore cause the identified harms to the environment, i.e., loss of a valued species, loss of ecosystem services, or loss of crop yield and quality. Increased abundance is a necessary, but not sufficient, component of whether a species becomes problematic (Keese et al. 2013). *Manihot glaziovii* in Africa can currently be easily managed by cutting and removing it from where it is not wanted. Within agricultural fields, the few hybrids should be obvious, and quite likely removed by cutting as farmers remove other unwanted plants. Outside of agricultural fields, increases in reproduction and survival, if any, of the transgenic hybrids are expected to be so small relative to already infrequent F1s that the hybrids should not necessitate special removal efforts. A better idea about changes in terms of reproduction and survival could be obtained through experimentation. However, if *M. glaziovii* were to become more abundant, it is difficult to define the threshold at which it would become ‘harmful’. Ultimately, regulators will have to consider whether the increased abundance of *M. glaziovii*, even should it occur, would outweigh the benefits of deploying the virus resistant cassava that will potentially improve cassava yields and improve the livelihood of Africa’s farmers.

CONCLUSIONS

The expert panel considered existing information to assess the potential for and consequences of gene flow from cultivated cassava resistant to Cassava Brown Streak Disease (CBSD) to the compatible naturalized relative *Manihot glaziovii* in Africa, and focused on identifying information to determine whether a ‘pathway to harm’ is likely or not. The panel identified two harms to consider in this case: 1) loss of genetic diversity in the germplasm pool due to genetic swamping or a selective sweep, and 2) loss of valued species ecosystem resources, or crop yield and quality due to weediness or invasiveness of wild relatives. The steps that could lead to each of these harms, and the information considered to evaluate the likelihood of each step are summarized in Figure 1. From this discussion, there are a number of conclusions: 1) There is likely to be hybridization between cultivated CBSD resistant cassava and *M. glaziovii*, and although gene flow will be at a low level, it is likely that the virus resistance transgene will persist in naturalized populations; 2) Gene flow from CBSD resistant cassava to *M. glaziovii* will not reduce the genetic diversity in the germplasm pool; 3) *M. glaziovii* is not weedy or invasive in Africa and it is not likely that *M. glaziovii* will become weedy or invasive if there is gene flow from CBSD resistant cassava, although more information, particularly about the impact of the virus in natural populations, would allow a better prediction; 4) If existing information is not considered sufficient to conclude with confidence that the level of risk is acceptable, a study of the impact of CBSD on seedling recruitment would be most informative to determine whether the virus limits the abundance of *M. glaziovii*; 5) An increase in the abundance of *M. glaziovii* should be manageable, and would not necessarily lead to environmental harm (loss of valued species, loss of ecosystem services, loss of crop yield or quality).

REFERENCES

Akano A, Dixon A, Mba C, Barrera E, Fregene M (2002) Genetic mapping of a dominant gene conferring resistance to cassava mosaic disease. Theor Appl Genet 105(4):521-525.

Alicai T, Omongo CA, Maruthi MN, Hillocks RJ, Baguma Y, Kawuki R, Bua A, Otim-Nape GW, Colvin J (2007) Reemergence of cassava brown streak disease in Uganda. Plant Dis 91:24–29.

Andersson, MS, de Vicente, MC (2010) Gene flow between crops and their wild relatives. Johns Hopkins University Press, Baltimore, MD.

Beeching, JR, Marmey P, Gavalda MC, Noirot M, Hayson HR, Hughes MA, Charrier A (1993) An assessment of genetic diversity within a collection of cassava germplasm using molecular markers. Ann Bot 72:515–520.

Catford JA, Daehler CC, Murphy HT, Sheppard AW, Hardesty BA et al. (2011) The intermediate disturbance hypothesis and plant invasions: Implications for species richness and management. Perspect Plant Ecol 14(3) 231-241.

Craig W, Tepfer M, Degrassi G, Ripandelli D (2008) An overview of general features of risk assessments of genetically modified crops. Euphytica 164: 853-880.

Ellstrand NC (2003) Dangerous liaisons? When cultivated plants mate with their wild relatives. Johns Hopkins University Press, Baltimore, MD.

Ellstrand NC, Heredia SM, Leak-Garcia JA, Heraty JM, Burger JC, Yao L, Nohzadeh-Malakshah S, Ridley CE (2010) Crops gone wild: evolution of weeds and invasives from domesticated ancestors. Evol Appl3:494–504.

Ellstrand NC, Meirmans P, Rong J, Bartsch D, Ghosh A, de Jong T, Haccou P et al. (2013) Introgression of crop alleles into wild or weedy populations. Annu Rev Ecol Evol S 44: 325-334.

Fermont AM, Babirye A, Obiero HM, Abele S, Giller KE (2010) False beliefs on the socio-economic drivers of cassava cropping. Agron Sustain Dev 30:433-444.

Fregene M, Bernal A, Duque M, Dixon A, Tohme J (2000) AFLP analysis of African cassava (Manihot esculenta Crantz) germplasm resistant to the cassava mosaic disease (CMD). Theor Appl Genet 100(5):678-685.

Garcia-Alonso M, Raybould A (2014) Protection goals in environmental risk assessment: a practical approach. Transgenic Res 23: 945-956.

Gbadegesin MA, Olaiya CO, Beeching, JR (2013) African cassava: Biotechnology and molecular breeding to the rescue. Brit Biotechnol J 3:305-317.

Gray A (2012) Problem formulation in environmental risk assessment for GM crops: A practitioner’s approach. Collection of Biosafety Reviews 6:14-65.

Halsey ME, Olsen K, Taylor NJ, Chavarriaga-Aguirre P (2008) Reproductive biology of Cassava (*Manihot esculenta* Crantz) and isolation of experimental field trials. Crop Sci 48:49-58.

Harper JL (1977) Population biology of plants. Academic Press, London, U.K.

Hillocks RJ, Raya M, Mtunda K, Kiozia H (2001) Effects of brown streak virus disease on yield and quality of cassava in Tanzania. J Phytopathol 149:1–6.

Hokanson, KE, Ellstrand NC, Ouedraogo, JT, Olweny, PA, Schaal, BA, Raybould, AF (2010) Biofortified sorghum in Africa: using problem formulation to inform risk assessment. Nat Biotechnol 28(9):900-903.

Huesing JE, Romeis J, Ellstrand NC, Raybould, A, Hellmich RL et al. (2011) Regulatory considerations surrounding the deployment of Bt-expressing cowpea in Africa: report of the deliberations of an expert panel. GM Crops 2(3):211-224.

Jennings DL (1957) Further studies in breeding cassava for virus resistance. East African Agricultural Journal 22: 213-219.

Jennings DL (1960) Observations on virus disease of cassava in resistant and susceptible varieties. II. Brown streak disease. Empire Journal of Experimental Agriculture 28: 261-270.

Jennings DL (1975) An evaluation of some sources of resistance to two virus diseases of cassava. Journal of Root Crops 1:19-23.

Jennings D (2003) Historical perspective on breeding for resistance to cassava brown streak virus disease. In: Legg, JP and Hillocks, RJ (eds), Cassava brown streak virus disease: Past, present, and future. Proceedings of an international workshop, Mombasa, Kenya 27-30 October. Aylesford, UK: Natural Resources International Limited. pp. 55-57.

Kanju E, Mahungu N, Dixon A, Whyte J (2003) Is resistance/tolerance to cassava brown streak disease associated with the zigzag stem trait? Roots 8: 15-19.

Kanju E, Masumba E, Masawe M, Tollano S, Muli B et al. (2007) Breeding cassava for brown streak resistance: regional cassava variety development strategy based on farmers and consumer preferences. In: Kapinga R, Kingamkono R, Msabaha M, Ndunguru J, Lenmaga B, Tusiime G (eds) Tropical root and tuber crops: Opportunities for poverty alleviation and sustainable livelihoods in developing countries. Proceedings of the thirteenth triennial symposium of the international society for tropical root crops (ISTRC) held at AICC, Arusha, Tanzania 10-14 November, 2003,pp. 95-101.

Keese PK, Robold, AV, Myers, RC, Weisman S, Smith J (2013) Applying a weed risk assessment approach to GM crops. Transgenic Res DOI 10.1007/s11248-013-9745-0.

Lefevre F (1988) Resources genetique et amelioration du manioc (*Manihot esculenta* Crantz) en Afrique. Dissertation, Institut National Agronomique Paris-Grignon.

Légère A (2005) Risks and consequences of gene flow from herbicide-resistant crops: canola (*Brassica napus* L) as a case study. Pest Manag Sci 61: 292-300.

Legg J, Attiogbevi Somado E, Barker I, Beach L, Ceballos H et al. (2014) A global alliance declaring war on cassava viruses in Africa. Food Security 6:231-248.

Legg JP, Jeremiah SC, Obiero HM, Maruthi MN, Ndyetabula I, Okao-Okuja G, Bouwmeester H, Bigirimana S, Tata-Hangy W, and Gashaka G (2011) Comparing the regional epidemiology of the cassava mosaic and cassava brown streak virus pandemics in Africa. Virus Res 159:161-170.

Liu H, Stiling P. (2006) Testing the enemy release hypothesis: a review and meta-analysis. Biol Invasions 8:1535–1545.

Maruthi M, Bouvaine S, Tufan HA, Mohammed IU, Hillocks RJ (2014) Transcriptional Response of Virus-Infected Cassava and Identification of Putative Sources of Resistance for Cassava Brown Streak Disease. PLoSOne 9(5): e96642.

Maskell LC, Raybould AF, Cooper JI, Edwards M-L, Gray AJ (1999) Effects of turnip mosaic virus and turnip yellow mosaic virus on the survival, growth and reproduction of wild cabbage (*Brassica oleracea*). Ann Appl Biol 135: 401-407

Mbanzibwa DR, Tian YP, Tugume AK, Mukasa SB, Tairo F, Kyamanywa S, Kullaya A, Valkonen JPT (2010) Simultaneous virus-specific detection of the two cassava brown streak-associated viruses by RT-PCR reveals wide distribution in East Africa, mixed infections, and infections in *Manihot glaziovii*. J Virol Methods 171:394-400.

National Research Council (NRC) (2002) Environmental Effects of Transgenic Plants. Washington, DC: National Academy Press.

Nichols RFJ (1947) Breeding cassava for virus resistance. East African Agricultural Journal 12:184-194.

Odipio J, Ogwok E, Taylor NJ, Halsey M, Bua A, Fauquet CM, Alicai T (2014) RNAi-derived field resistance to Cassava brown streak disease persists across the vegetative cropping cycle. GM Crops and Food: Biotechnology in Agriculture and the Food Chain 5(1):1-4.

Ogwok E, Alicai T, Rey M, Beyene G, Taylor N (2014) Distribution and accumulation of cassava brown streak viruses within infected cassava (Manihot esculenta Crantz) plants. Plant Pathol 64(5): 1235.

Okogbenin E, Egesi CN, Oasanmi B, Ogundapo O, Kahya S, Hurtado P, Marin J, Akinbo O, Mba C, Gomez H, de Vicente C, Baiyeri S, Uguru M, Ewa F, Fregene M (2012) Molecular marker analysis and validation of resistance to cassava mosaic disease in elite cassava genotypes in Nigeria. Crop Sci 52(6): 2576-2586.

Organization for Economic Cooperation and Development (OECD) (1993) Safety Considerations for Biotechnology: Scale Up of Crop Plants. Publications Service, OECD, Paris.

Organization for Economic Cooperation and Development (OECD) (2009) Consensus Document on Compositional Considerations for New Varieties of Cassava (*Manihot esculenta* Crantz): Key Food and Feed Nutrients, Anti-nutrients, Toxicants and Allergens. Series on the Safety of Novel Foods and Feeds 2009, 18.

Organization for Economic Cooperation and Development (OECD) (2014) Consensus document on the biology of cassava (*Manihot esculenta* Crantz). Series on Harmonization of Regulatory Oversight in Biotechnology No. 57. ENV/JM/MONO(2014)13.

Paterson RT, Karanja GM, Nyaata OZ, Kariuki IW, Roothaert RL(1998) A review of tree fodder production and utilization within smallholder agroforestry systems in Kenya. Agroforestry Systems 41:181–199.

Pennisi E (2010) Armed and dangerous. Science 327:804-805.

Pimentel D, McNair S, Janecka J, Wightman J, Simmonds C, O’Connell C, Wong E, Russel L, Zern J, Aquino T, Tsomondo T (2001) Environmental and economic threats of alien plant, animal, and microbe invasions. Agr Ecosyst Environ 84:1-20.

Rabbi IY, Hamblin MT, Kumar PL, Gedil MA, Ikpan AS, Jannink JL, and Kulakow PA (2014) High-resolution mapping of resistance to cassava mosaic geminiviruses in cassava using genotyping-by-sequencing and its implications for breeding. Virus Res 186:87-96.

Raybould A (2006) Problem formulation and hypothesis testing for environmental risk assessments of genetically modified crops. Environ Biosafety Res 5(3):119-125.

Raybould A (2010) Reducing uncertainty in regulatory decision-making for transgenic crops: more ecological research or clearer environmental risk assessment? GM Crops 1: 25-31.

Raybould AF, Cooper JI (2005) Tiered tests to assess the environmental risk of

fitness changes in hybrids between transgenic crops and wild relatives: the example

of virus resistant *Brassica napus*. Environ Biosafety Res 4: 127-140.

Romeis J, Lawo NC, Raybould A (2009) Making effective use of existing data for case-by-case risk assessments of genetically engineered crops. J Appl Entomol 133:571-583.

Rwegasira GM, and M.E. Re C (2012) Response of selected cassava varieties to the incidence

and severity of cassava brown streak disease in Tanzania. J Agr Sci 4(7):237-245.

Sasu MA, Ferarri MJ, Stephenson AG (2010) Interrelationships among a virus-resistance transgene, herbivory, and a bacterial disease in a wild *cucurbita*. Int J Plant Sci 171(9):1048–1058.

Taylor NJ, Halsey M, Gaitan-Slois E, Anderson P, Gichuki S, Miano D, Bua A, Alicai T, Fauquet C (2012) The VIRCA Project: Virus resistant cassava for Africa. *GM Crops and Food: Biotechnology in agriculture and the Food Chain* 3(2):93-103.

Tepfer M, Jacquemond M, Garcia-Arenal F (2015) A critical evaluation of whether recombination in virus-resistant transgenic plants will lead to the emergence of novel viral diseases. New Phytol doi:10.1111/nph.13358.

Tepfer M, Racovita M, Craig W (2013) Putting problem formulation at the forefront of GMO risk analysis. GM Crops and Food: Biotechnology in Agriculture and the Food Chain 4(1):10-15.

Thresh JM, Otim-Nape GW, Legg JP, Fargette D (1997). African cassava mosaic virus disease: The magnitude of the problem. African Journal of Root and Tuber Crops 2:13–19.

Wanyera, NWM (1993) Phylogenetic relationships among cultivated cassava (*Manihot esculenta* Crantz) and two wild Manihot species. Dissertation. University of Ibadan, Ibadan Nigeria. 193 pp.

Wanyera NMW, Hahn, SK, Aken’ova, ME (1994) Introgression of Ceara rubber (*Manihot glaziovii* Muell-Arg) into cassava (*M. esculenta* Crantz): A morphological and electrophoretic evidence. In Akoroda, MO (ed) Root crops for food security in Africa. Proc. of the Fifth Triennial Symp. of the Int. Soc. for Tropical Root Crops–Africa Branch, Kampala, Uganda. 22–28 Nov. 1992. p. 125–130.

Winter S, Koerbler M, Stein B, Pietruszka A, Paape M, and Butgereitt A (2010) Analysis of

cassava brown streak viruses reveals the presence of distinct virus species causing cassava brown

streak disease in East Africa. J Gen Virol91:1365-1372.

Wolt JD, Keese P, Raybould A, Fitzpatrick JW, Burachik M, Gray A, Olin SS, Schiemann J, Sears M, Wu F (2010) Problem formulation in the environmental risk assessment for genetically modified plants. Transgenic Res 19(3):425-436.

Figure 1. The necessary steps in the ‘pathway to harm’, starting with the general release in East Africa of a transgenic virus resistant cassava and leading to two different ‘harms’ if there is gene flow to a wild relative. Each step is followed by an assessment of whether it will occur, based on existing information.

|  |  |
| --- | --- |
| 1) General release in East Africa of transgenic cassava (*Manihot esculenta*) engineered for resistance to Cassava Brown Streak Disease (CBSD)  ***Yes.*** *Starting point of pathway.* | |
|  | |
| 2) Gene flow occurs from transgenic cassava to the wild relative, *Manihot glaziovii*.  ***Likely.*** *Naturally occurring hybrids are documented; The frequency of gene flow is probably low, but is not well documented.* | |
|  |  |
| 3) The transgene increases the rate of hybridization compared to current (nontransgenic) rates of hybridization. (Genetic swamping).  ***No.*** *CBSD resistance is not expected to alter reproductive vigor, flowering frequency, or flowering time.* | 3) CBSD resistance is a new trait (does not already exist) in populations of *M. glaziovii* in Africa.  ***Likely.*** *M. glaziovii is not a source of CBSD resistance for traditional breeding; resistance is not expected to occur already in the ‘wild’ populations.* |
|  |  |
| 4) There is rapid selection for genotypes with CBSD resistance coupled with extensive linkage disequilibrium. (Selective sweep).  ***No.*** *Rapid selection is not expected in a species with long generation time, and persistent linkage disequilibrium is not expected because of the highly outcrossing mating system.*    5) *M. glaziovii* populations in Africa are a primary source of genetic diversity.  ***No.*** *M. glaziovii is an introduced species in Africa. The center of diversity for Manihot is in South America.* | 4) CBSD is a limiting factor of population size in *M. glaziovii* (i.e., limits seed production or seedling recruitment).  ***Unlikely.*** *CBSD does not typically impact flowering and seed set in cassava, and seedling recruitment in natural plant populations is typically controlled by multiple factors.* |
|  |
| 5) *M. glaziovii* is already a weed in farmer’s fields or invasive in the ‘wild’ in Africa.  ***No.*** *M. glaziovii is not documented as a weed or invasive in Africa.*    6) More abundant *M. glaziovii* will be more difficult to manage.  ***No.*** *M. glaziovii should be easily cut and removed when necessary.*    **HARM 2:**  Loss of other valued species, ecosystem services, or crop yield and quality.  ***UNLIKELY****, because steps 4, 5, and 6 are unlikely.* |
|  |
| **HARM 1:**  Loss of genetic variation/wild type alleles in *Manihot* germplasm pool.  ***UNLIKELY,*** *because steps 3, 4, and 5 in the pathway are unlikely.* |
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