

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

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Abstract The probability and consequences of gene flow to wild relatives is typically considered in the environmental risk assessment of genetically engineered crops. This is a report from a discussion by a group of experts who used a problem formulation approach to consider existing information for risk assessment of gene flow from cassava (*Manihot esculenta*) genetically engineered for virus resistance to the ‘wild’ (naturalized) relative *M. glaziovii* in East Africa. Two environmental harms were considered in this case: (1) loss of genetic diversity in the germplasm pool, and (2) loss of valued species, ecosystem resources, or crop yield and quality due to weediness or invasiveness of wild relatives. Based on existing information, it was concluded that gene flow will occur, but it is not likely that this will reduce the genetic diversity in the germplasm pool. There is little existing information about the impact of the virus in

natural populations that could be used to inform a prediction about whether virus resistance would lead to an increase in reproduction or survival, hence abundance of *M. glaziovii*. However, an increase in the abundance of *M. glaziovii* should be manageable, and would not necessarily lead to the identified environmental harms.

Keywords Cassava · Virus resistance · Problem formulation · Environmental risk assessment

Introduction

Cassava (*Manihot esculenta* Crantz) is a major source of food and income in Africa, where its starchy

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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 (*Bemisia 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 with massive economic losses and impacts on food security that could spread across the African continent and globally (Thresh et al. 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 et al. 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 Rey 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 et 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, 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 Fig. 1).

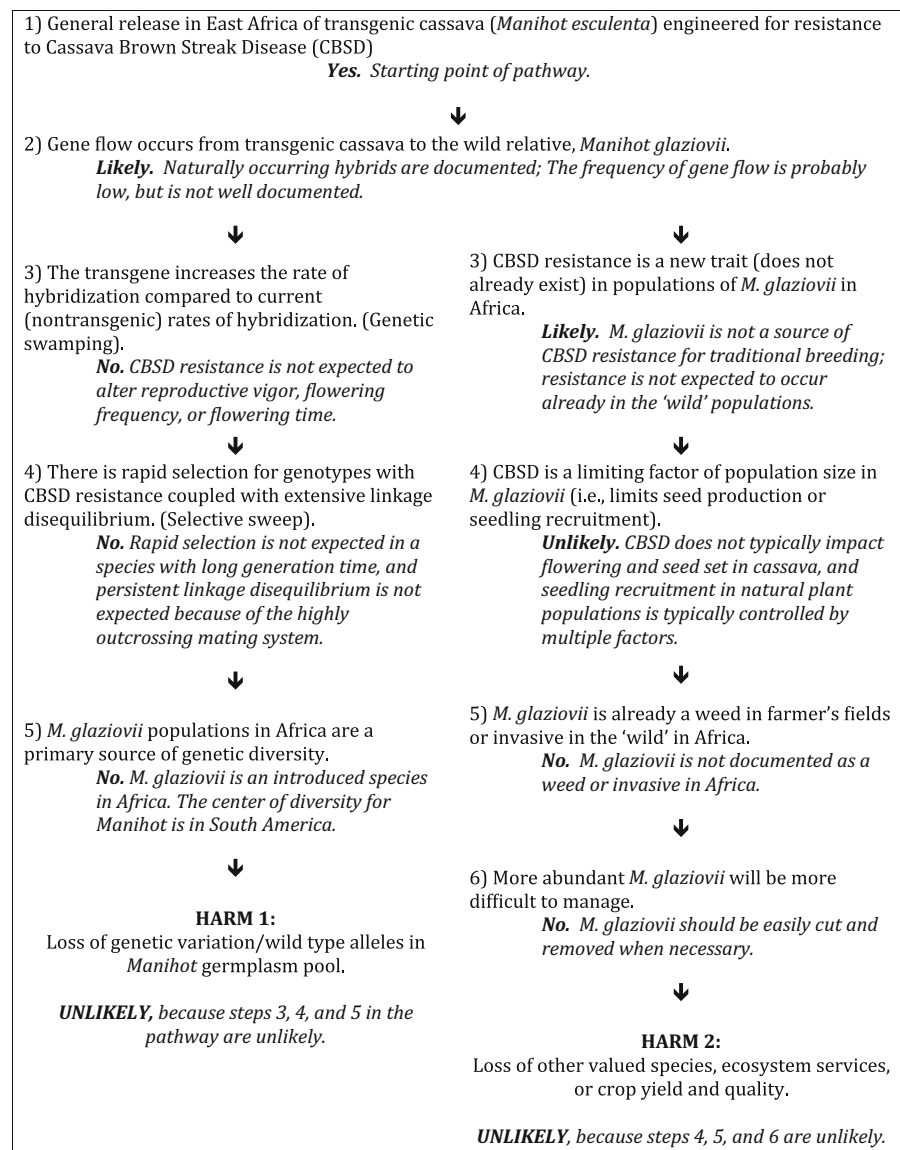
This report is the outcome of a VIRCA-sponsored workshop focused specifically on risk assessment of gene flow from transgenic cassava with resistance to Cassava Brown Streak Disease (CBSD) to wild relatives in Africa. To evaluate the situation, experts (the authors) on the subjects of gene flow, risk 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

Fig. 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



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 (*M. 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 1993). 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 1993; 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 F_1 s 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 Fig. 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 CBSB 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 CBSB 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 CBSB 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).

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