Review

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Use of plant viruses as bioherbicides: the first virus-based bioherbicide and future opportunities

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Abstract

Until recently, only a few plant viruses had been studied for use as biological control agents for weeds, but none had been developed into a registered bioherbicide. This position changed in 2014, when the US Environmental Protection Agency granted an unrestricted Section 3 registration for tobacco mild green mosaic virus (TMGMV) strain U2 as a herbicide active ingredient for a commercial bioherbicide (SolviNix LC). It is approved for the control of tropical soda apple (TSA, *Solanum viarum*), an invasive 'noxious weed' in the United States. TSA is a problematic weed in cattle pastures and natural areas in Florida. The TMGMV-U2 product kills TSA consistently, completely, and within a few weeks after its application. It is part of the TSA integrated best management practice in Florida along with approved chemical herbicides and a classical biocontrol agent, *Gratiana boliviana* (Coleoptera: Chrysomelidae). TMGMV is nonpathogenic and nontoxic to humans, animals, and other fauna, environmentally safe, and as effective as chemical herbicides. Unlike the insect biocontrol agent, TMGMV kills and eliminates the weed from fields and helps recycle the dead biomass in the soil. Here the discovery, proof of concept, mode of action, risk analyses, application methods and tools, field testing, and development of the virus as the commercial product are reviewed. Also reviewed here are the data and scientific justifications advanced to answer the concerns raised about the use of the virus as a herbicide. The prospects for discovery and development of other plant-virus-based bioherbicides are discussed.

Keywords: plant virus; Tobamovirus; tobacco mild green mosaic virus; TMGMV-U2; tropical soda apple; *Solanum viarum*; invasive weed

1 INTRODUCTION

Compared to fungal pathogens, plant viruses have received meager attention as bioherbicide candidates. This is largely because of the prevalent negative views that cast viruses as unsuitable for the job. It is true that plant viruses, in general, are not host-specific to the degree that some pathogens such as the rust fungi can be. Viruses are prone to genetic variability through mutation. Typically, viruses do not kill their hosts but cause mild to moderate levels of plant damage. Hence, they are dismissed as likely to be ineffective as bioherbicides. It is difficult, perhaps impossible, to contain and control the spread of viruses that are transmitted by vectors (insects, mites, nematodes, or fungi) or by other means (e.g., pollen). There are no virus-active pesticides (virucides) that can be used to protect nontarget plants against a virus. For these reasons, vector-transmitted viruses would not be suitable weed biocontrol agents. Still, it is well known that viruses can reduce plant growth and reproduction, and thereby lessen the competitive ability of weed populations.[1] Yet, it is commonly believed that viruses cannot be used for biological weed control for some of the above-stated reasons.

Viruses do have certain advantages as weed biocontrol agents. Unlike fungi, which require suitable moisture (dew requirement) and temperature, and an ability to stick to and stay on leaf surface during inoculum germination and leaf penetration for infection to occur, viruses, being intracellular obligate parasites, are not dependent on external ambient conditions except those that slow the plant's metabolism. However, plant viruses need a healable wound or abrasion on plant surface (usually leaf surface) for entry into the plant, but once applied, they enter the plant immediately. Some plant viruses multiply to high titers in appropriate hosts so they can be mass-produced fairly inexpensively. An added benefit is that viruses typically have a far longer shelf life than fungal bioherbicides because they are, after all, chemical entities. Given these advantages, a few viruses have been studied and promoted for weed control, including two that have been used to some extent.

1.1 Pioneering efforts to use plant viruses as bioherbicides

In 1974, during a survey in Argentina for a suitable biological control agent for stranglervine, *Araujia odorata* (Syn. *Morrenia odorata*, Apocynaceae),[2] which was an important weed in citrus in Florida in the 1970s, a virus disease in four related *Araujia* species including *A. odorata*, was discovered.[3] Infected vines in the field were stunted and had systemic foliar mosaic, and young shoot tips were often infested with oleander aphid (aka milkweed aphid), *Aphis nerii* (Hemiptera, Aphididae), a vector for this virus. The virus was characterized by manual transmission to *A. odorata*, electron microscopy of virions and cytoplasmic

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inclusions, host range, and *in vitro* translation analyses.[3,4] It turned out to be a new species of Potyvirus and was named Araujia mosaic virus (ArjMV).[3,5] Its use for biological control of stranglervine was patented,[6] but because of its foreign origin and potyviruses being a problem in horticultural crop production in Florida, field release of this virus was disallowed by the state. A supplemental host range study[7] and genomic RNA sequencing[8] were done to determine the suitability of this virus as a control for moth plant, *A. hortorum*, an invasive weed of concern in New Zealand.[9] While the results reinforced the narrow host range of ArjMV, the susceptibility of three *Gomphocarpus* species (Apocynaceae) that are a food source for the monarch butterfly (*Danaus plexippus*, Nymphalidae, Lepidoptera) made its use for biocontrol an unacceptable risk. Although monarch butterfly is not native to New Zealand, it is a highly desirable species and is protected. Hence, ArjMV was not considered for biological control of moth plant in New Zealand.[7]

In 1977, a tobacco mosaic virus (TMV) strain, named the Alke strain, isolated in Czechoslovakia (now the Czech Republic or Czechia) from *Physalis alkekengi* (Solanaceae), was used as a control for *Solanum carolinense* (horsenettle, Solanaceae), a weed of American origin in the tea plantations of the Georgian SSR (now Georgia).[10] Although the weed was distributed over a small region, its occurrence in plantations made tea harvesting difficult. The Alke strain caused a 'strong systemic disease of all infected (horsenettle) plants',[10,11] and caused yellowing, mosaic, drying, and curling of leaves, and leaf drop. The shoot growth was suppressed, and after overwintering the diseased plants grew slowly, and the leaves wilted and dropped. Some shoots had symptoms of 'proliferation' and 'damp-off', and dried. Under dry conditions, infected plants wilted more quickly than uninfected plants. The virus was produced in tobacco plants and virus particles were purified and concentrated in the laboratory. The preparation of virus particles was used in tea plantations with a one-time treatment of young shoots of horsenettle plants providing 25–30% infection.[10,11] No additional information about this bioherbicide is available.

In 1986, Randles[12] experimentally inoculated *Echium plantagineum* plants (Viper's bugloss, Salvation Jane, or Patterson's curse, Boraginaceae) with tobacco mosaic virus strain U1 (TMV-U1), TMV strain U2 (tobacco mild green mosaic virus stain U2, TMGMV-U2), several isolates of alfalfa mosaic virus, tobacco ring spot virus, and tobacco necrosis virus.[12] TMV-U1 infected the plant 'locally' while the other viruses caused systemic infections. Among other viruses tested, bean yellow mosaic virus, cucumber mosaic virus, potato virus Y, red clover necrotic mosaic virus, subterranean clover mottle virus, tomato spotted wilt virus, and velvet tobacco mottle virus were not recovered from inoculated plants, presumably because they were not infective. In a pot trial, TMV-U2 (TMGMV-U2) reduced leaf production and the number of green leaves on plants, and increased leaf senescence. In the field, TMV-U2 reduced seed set in inoculated plants. All 80 *E. plantagineum* plants inoculated in pot trials and most of those inoculated in field trials were infected. A measure of reduction in the competitiveness of infected plants was evident, but the plants did not die from infection, an important difference from the tropical soda apple-TMGMV-U2 interaction discussed below. Although this work revealed the potential of TMV-U2 as a biological control agent for *E. plantagineum*, an explicit recommendation for its use was not made by the author,[12] and no further work appears to have been done on this virus-weed example.

Multiflora rose (*Rosa multiflora*, Rosaceae), native to northeastern Asia, has been introduced multiple times into North America from Japan and other northeastern Asian countries during the past 200 years as an ornamental, for use as a rootstock for commercial roses, as a living fence, and for other purposes. It is now an invasive weed in over 18 million hectares of pastures, parks, recreational areas, right-of-way, and unmanaged lands.[13] Rose rosette disease (RRD), also called the rose witches' broom, was first reported in wild roses in Manitoba, Canada, and it remained obscure until the 1970s, when its increased incidence in the United States became noticeable. It is now widely prevalent in the southeast, south-central, and north-central states, the Rocky Mountain states, California, and recently in other states.[13,14] Initially considered a disease of unknown etiology, RRD is now known to be caused by rose rosette virus (RRV) in the genus Emaravirus.[15] The disease is lethal to multiflora rose, killing the plants in the 2–5 years after infection. Infected plants express a series of symptoms characterized by intense reddening of leaves and shoots, development of witches' broom in new growth and buds, weakening of apical growth, and loss of rootlets. Infected plants are prone to winter kill. RRV is transmitted by an eriophyid mite, *Phyllocoptes fruitiphyllus* (Acari, Eriophyoidea), and can be graft-transmitted. In controlled field studies, RRD spread significantly more quickly from graft-inoculated plants than from naturally infected plants.[16] The authors claimed that the rate of RRD spread was much slower compared to the spread of viruses in commercial crops. Grafting was proposed as a feasible cost-effective means to augment RRD in the field.[16] The disease is specific to the *Rosa* genus. Commercial roses and native roses in the United States that are hosts to the mite are susceptible to RRD, and they can also be graft-inoculated with RRV. Nonetheless, Epstein and Hill[13] considered RRD to be safe for augmentative biological control on account of its lethal but slow disease spread, it being endemic to North America, and the possibility of avoiding the disease by spatial separation of infected and healthy plants. However, it has been reported that the American Rose Society and rosarians in the United States in general are opposed to using RRV as a biocontrol agent.[17]

2 THE FIRST AND ONLY EPA-REGISTERED VIRUS-BASED BIOHERBICIDE

2.1 The weed: tropical soda apple

Tropical soda apple (TSA), *Solanum viarum*, is an invasive 'noxious weed' in the United States[18] and a 'prohibited invasive weed' in Australia.[19] It is native to Brazil, Argentina, Paraguay, and Uruguay, and is now reported to occur in 30 countries as a native or an introduced plant. It is reported as invasive in nine countries around the world.[20] In the United States, it was first reported from Florida, where it continues to be a problem in cattle ranches and natural areas.[21] It is seed-dispersed by cattle that consume the fruit, and from the use of contaminated hay and commercial grass seeds.

2.2 The bioherbicide proof of concept

In an attempt to find a biological control agent for TSA, we screened in a greenhouse experiment three Solanaceae-adapted Tobamovirus species, tobacco mosaic virus (TMV, aka TMV-U1), tomato mosaic virus (ToMV), and tobacco mild green mosaic virus stain U2 (TMGMV-U2), for their effects on the weed. All three infected TSA. (*Note: throughout this paper, TMGMV is used to denote the virus species in general and TMGMV-U2 to the*

bioherbicide strain or isolate, according to the context). While TMV and ToMV caused systemic mosaic in leaves, TMGMV-U2 caused foliar local lesions in inoculated leaves followed by wilting and death of the inoculated plants. Unlike TMGMV-U2, neither TMV nor ToMV was lethal to TMV. TSA plants of all stages – seedling to mature plant – were killed, including the roots.[22] There was no variability in this sequence of response among TSA accessions from Florida and Mississippi, United States or from New South Wales, Australia.[22]

In a preliminary host range study with solanaceous plants of 32 different species, TMGMV-U2 elicited host responses ranging from asymptomatic infection (ELISA-positive) to mild systemic mosaic and local lesions. More extensive host range data are presented in Charudattan *et al.*[22] Among the susceptible species, only TSA was killed completely and in a characteristic and predictable manner as described above. Multiple greenhouse trials and two small-scale field trials in two counties of Florida confirmed the efficacy of TMGMV-U2 as a bioherbicide candidate. These findings, along with our success in producing ample inoculum on a laboratory scale, prompted us to undertake commercial development and registration of TMGMV-U2 as a bioherbicide.

2.3 The virus: TMGMV-U2

Many isolates of TMGMV have been collected by scientists from around the world. Our isolate of TMGMV was recovered from tissues of *Columnea* hybrid 'Oneidan (*C. crassifolia* × *C. allenii*, Gesneriaceae) suspected to have a latent virus infection.[23,24] The virus was propagated in tobacco (*Nicotiana tabacum)*, confirmed as TMGMV, and deposited as accession PV-0113 at the German Collection of Microorganisms and Cell Cultures.[25] TMGMV is a species, *Tobacco mild green mosaic tobamovirus*, in the genus Tobamovirus. TMGMV strain U2, previously called 'the mild TMV strain U2' or TMV-U2, is found worldwide in susceptible tobacco varieties (*N. tabacum*) and tree tobacco (*N. glauca*).[26]

Two distinct genotypes of TMGMV, named the large, TMGMV-L (strain U5), and the small, TMGMV-S (strain U2) are prevalent in *N. glauca* plants in southern California.[27] They differ in the length of the 3′ untranslated region (UTR) of the RNA genome. The difference in length is due to a repeated sequence (147–165 nucleotides) that incorporates three pseudoknots found in the 3′ UTR of TMGMV-S.[27] The two genotypes express biological differences (e.g., cross protection) when inoculated onto different hosts.[28] Morishima *et al.*[29] described a Japanese strain of TMGMV, TMGMV-J, that is distinct from TMGMV-U2. Amino acid differences between TMGMV-J and TMGMV-U2-encoded proteins were found in six regions: two in the 126-kDa protein, three in the read-through region of the 183-kDa protein, and one in the movement protein (Fig. 1).[30] These amino acid sequences were almost identical among TMGMV-J and TMV, ToMV, and pepper mild mottle virus (PMMoV) but not TMGMV-U2.[29]

Using a PCR probe analysis, we determined our bioherbicide isolate of TMGMV to be TMGMV-S (U2).[22] A satellite tobacco mosaic virus (STMV), a small spherical ssRNA virus, is associated with TMGMV-L (U5) from *N. glauca* in southern California.[30] Since satellite viruses, including STMV, can attenuate or otherwise modify the expression of the disease phenotype, at least in certain hosts,[30] we analyzed our bioherbicide isolate by using double-stranded RNA and serological reaction with anti-STMV antserum. The results confirmed that the TMGMV-U2 isolate developed as the bioherbicide did not contain STMV.[22]

2.4 Mode of action

The mode of action in a virus disease describes the functional and anatomical changes that result from infection in a susceptible host. We expected one of three possibilities, namely that TSA would be immune (I) to TMGMV-U2 with no visible changes in the inoculated leaves, resistant (R), expressing a localized hypersensitive response (HR) seen as discrete, localized necrotic spots, or susceptible, developing a systemic mosaic symptom (SS or systemic susceptibility). The killing of inoculated TSA plants by TMGMV-U2 was a surprise. Based on scientific literature and our findings, we postulate that TMGMV-U2 first elicits localized HR that turns systemic as the virus moves through the plant. We are calling this the systemic hypersensitive necrosis (SHN), defining it as the HR-elicited necrosis that spreads beyond the initial local lesions to affect the entire inoculated leaf, leaf, petiole, and stem, or the entire plant. SHN that causes total plant death is a relatively uncommon host reaction but is known in plant virology.

It has been hypothesized that HR is elicited by a virus avirulence (*avr)* gene product, possibly a protein that interacts specifically with a host resistance (*R*) gene product, a protein or other host component,[31,32] to elicit a resistance response. Normally HR results in localized necrotic leaf spots in which the virus is confined, halting its further spread.[31,32] In the TMGMV-TSA system, as has been proposed for a different virus system,[33] TMGMV provokes an inefficient defense response that fails to confine the virus to the local lesions. Under this leaky defense, the virus moves within the leaf from cell to cell through plasmodesmata and then to other parts of the plant. The latter occurs through the vasculature, namely xylem, a nonliving tissue, and phloem with all its component parts that are a living tissue. The cell-to-cell movement is slow (μm/h), while the long-distance movement through phloem tissue is relatively rapid (cm/h).[32] The state in which the virus is transported via the vasculature, as intact virions or as RNA, and whether the process is passive, namely the virus hitching a ride along the flow of photosynthates, or active, with viral proteins and/or RNA involved, is a lively area of research.[34–36] What is certain is that the vascular tissue is the essential pathway where two-way interactions involving the virus and phloem-associated genes occur[37,38] during systemic virus spread.

Our data have confirmed the above model of localized HR followed by systemic virus spread in the TMGMV-TSA system.[22] A few weeks after infection and localized HR elicitation, TMGMV-U2 spreads systemically throughout the TSA plant, including the roots, blights the phloem tissue, and causes wilting and eventual plant death (i.e., SHN).[22] The SHN is likely the result of programmed cell death and inhibition of virus multiplication.[39] The restricted or reduced virus multiplication has also been confirmed from our data.[22] Fruits on dying TSA plant may or may not rot and decay, depending on their stage of maturity when the disease started; immature fruits are killed while mature green and mature yellow fruits may survive with viable seeds inside (Charudattan, unpublished). It is therefore recommended to apply the virus bioherbicide before flowering and fruit set.

2.5 Development of TMGMV-U2 as a bioherbicide

2.5.1 Large-scale production of TMGMV-U2

Having established the proof of concept confirmed by dozens of trials in the greenhouse and the field, the first question that needed to be answered was whether it would be feasible to mass produce the virus at a realistic cost and process it into a technical-grade active ingredient. Laboratory production of tobamoviruses, particularly of TMV, is a known art.[40–42] We adapted and improved

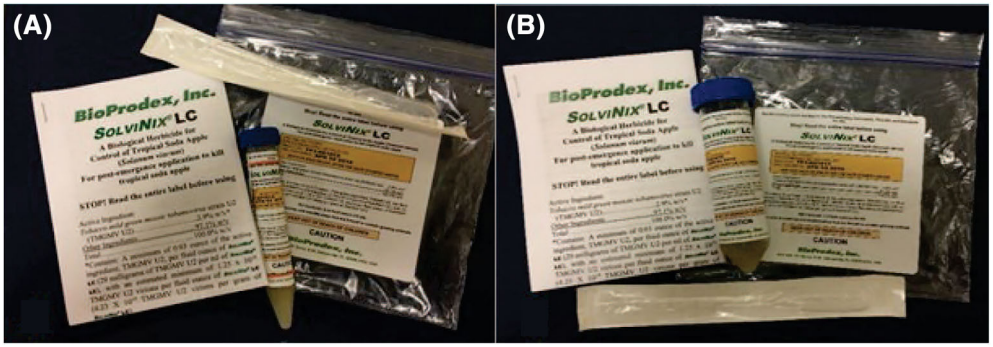

Figure 1. SolviNix LC, as sold in two sizes, 10 mL (A) and 35 mL (B), the packaging, and the contents. A folded size-specific (10- or 35-mL) SolviNix LC label (left in each picture), a 15-mL (A) or 50-mL (B) polypropylene screw-capped vial containing the product, and an outer plastic Ziploc bag with an abbreviated, size-specific label. A sterile, disposable, 1-mL plastic dropper-pipet with four 0.25-mL markings is included in each package (top in (A) and bottom in (B)).

the generally known methods to produce a standardized TMGMV-U2 inoculum in the laboratory. From greenhouse-grown *N. tabacum* cultivar 'Samsun' *nn* (hereafter Samsun nn), we produced on average 1.5 mg of TMGMV-U2 per gram of infected leaf tissue.[22] With funding from the US Department of Agriculture, National Institute of Food and Agriculture, Small Business Innovation Grant (USDA-NIFA-SBIR), we scaled up our method into a large-scale industrial manufacturing process. This involved conventional tobacco farming combined with industrial food technology wherein the virus was multiplied in Samsun nn tobacco, the harvested aerial biomass, weighing 1.8–4.5 metric tons per production batch, was transported to a food technology company for crushing, extraction, and partial purification into a crude extract, and in-laboratory purification and concentration of the virus into an aqueous liquid concentrate as the technical-grade active ingredient. The industrial process is scalable based on crop size and batch processing, as described in this equation:

$$\left[\left[\left[\mathrm{PC}=y\times u\right]^{[cy\times cs]}\right]^{>|<-ec}\right]^{>|<-dc}$$

where PC is production capacity, y is the yield (g), u is the unit (u = minimum 1.5 mg g^{-1}-), cy is crop yield (tons/ha, about 4.5 metric tons), cs is crop size (hectares), >/< indicates repeatable capacity-limited steps, ec is the extraction capacity (tons/run, equipment capacity), and dc is the downstream (finishing) capacity (volume/run, equipment capacity). From two crops of field-grown tobacco, with two harvests from each, we produced 8.14 metric tons of aerial biomass with an average virus yield of 1.5 mg g^{-1} ($n = 6$ determinations from different batches) or a virus yield of 22.7 kg per hectare. Thus, with this process, we can produce hundreds of kilograms of highly purified TMGMV-U2 virus particles to meet commercial demands. A nanomaterial, TMGMV is also useful in various nanotechnology research and applications in agriculture,[43,44] biomedicine,[45] and industrial uses.[46]

2.5.2 *EPA registration: considerations of possible risks*

From a pre-registration meeting with the US Environmental Protection Agency (EPA) Biopesticide and Pollution Prevention Division in the Office of Pesticide Programs, the division that registers biopesticides, and subsequent discussions, it was clear that empirical data and scientific analyses would need to be developed to address four possible risks from the use of TMGMV-U2 as a bioherbicide: (i) toxicology of TMGMV-U2; (ii) risks to nontarget fauna (aquatic organisms, fish, bird, animals, and human); (iii) risks to nontarget flora (crops, native plants, and Threatened and Endangered [T&E] plants); and (iv) risks from environmental persistence and spread of the virus from its use as a bioherbicide. To address these concerns, we designed experiments, gathered data, and presented them in the context of published information. Using Google and Google Scholar, we searched published, popular, and web-based data spanning 20 years (1984–2004). In this search, TMGMV and the related species TMV and ToMV, as well as their full names (tobacco mild green mosaic virus, tobacco mosaic virus, tomato mosaic virus, and Tobamovirus) were used as search terms. These terms were also modified to search for host range, epidemiology, survival, spread of these viruses, and the economic damage from TMGMV. The following is a synopsis of the data and scientific analyses (i.e., the arguments) we presented to justify registration and the outcomes.

2.5.2.1. Toxicology data at Tier I level and risks to fauna. The required studies at Tier I level include acute dermal irritation, acute dermal toxicity/pathology, acute eye irritation, acute injection toxicity/pathogenicity, acute pulmonary toxicity/pathogenicity, acute oral toxicity/pathogenicity, avian inhalation testing, avian oral testing, cell culture, estuarine and maine animal testing, freshwater aquatic invertebrate testing, freshwater fish testing, honeybee testing, hypersensitivity, immune response, nontarget insect testing, and wild mammal testing. Rather than experimentally attempt to prove the obvious, namely that TMGMV, a plant virus, has neither toxicological risks nor dangers to nontarget fauna, we provided scientific justifications to claim a full waiver from the studies. The justifications were accepted, and the studies were waived for the registration of TMGMV-U2 (EPA Memorandum, November 9, 2010, unpublished, available from the author).

We justified the waivers on the basis that TMGMV is a naturally occurring plant virus that is not toxic, infective, or pathogenic to insects, invertebrates, vertebrates, mammals, or humans and therefore no toxicology profile of TMGMV-U2 was required. Plant viruses (i.e., viruses that infect angiosperms), unlike animal viruses, do not require a specific cell-surface receptor to attach to the host and infect. Instead, they enter plant cells through injuries to the epidermal cell wall and the membrane below, and then move cell-to-cell through plasmodesmata, also without the

involvement of receptors.[47] This singular difference prevents plant viruses from attacking animals.

We searched through several virology treatises and reviews for references to adverse effects of plant viruses. However, we did not find any information that even remotely implicated TMGMV or other tobamoviruses in any adverse effects on avian, piscine, and mammalian species.[48–52] We did not find any confirmed reports of tobamoviruses infecting and multiplying in insects, other invertebrates, vertebrates, mammals, or humans; simply, there were no credible reports to the contrary.[48,49] Many organisms, such as insects, honeybees, invertebrates, vertebrates, birds, wild mammals, and humans are routinely exposed to tobamoviruses and other viruses in plants and plant products they consume as food. In fact, TMV has been reported to be a good source of protein for animal growth.[53] At 20% of the diet, TMV supplied all essential amino acids except histidine, methionine, and lysine for the growth of laboratory white rat.[53] Thus, TMV, and by extension TMGMV-U2, are unlikely to produce any adverse effects in animals. Finally, as TSA does not grow in wetland and aquatic sites, aquatic fauna, including freshwater, estuarine, and marine organisms, would not be exposed to TMGMV-U2 applied upland to TSA plants.

The cell culture study, as related to TMGMV-U2 registration, was intended to rule out (or in) that TMGMV-U2 might grow in animal cells, a potential indicator of risk to warm-blooded animals. However, a thorough literature search and Google search yielded only one report of culturing TMV in animal cell culture.[54] The report claims to show the formation of infective TMV virions in a culture of immune-suppressed monkey kidney cells. The implication that TMV can replicate in animal cell lines, however, is not strong since this was an artificial, contrived cell-culture system. Natural, intact animal cells and animal tissues do not support TMV replication. This study appears not to have been repeated by later workers. Thus, there is no scientific basis to the possibility of growing TMGMV in animal cell cultures.

TMGMV, like other plant viruses, contains proteins in the outer shell that can evoke immune response and produce specific antibodies. When properly injected, animals such as mouse, rabbit, guinea pig, chicken, goat, and horse can be immunized to produce virus-specific antiserum. Some of these animals are used routinely to produce antibodies against tobamoviruses and other plant viruses without causing them chronic adverse effects. Also, there are no reports of laboratory animals, or humans that handle and administer these viruses, developing any nasal, eye, skin, or pulmonary allergies or adverse reactions to the virus. No episodes of hypersensitivity to TMGMV-U2 were experienced by any of our coworkers during or after the 7+ years of repeated exposure from handling of the virus. Nonetheless, to address the remote likelihood of irritation and allergy incidents in applicators, the bioherbicide label states precautions and requires the use of an N95 or a similar mask while spraying the virus.[55]

2.5.2.2. Nontarget plant host-range studies at Tier I and Tier II levels. The risk to nontarget plants from the use of TMGMV-U2 as a bioherbicide was the most important concern that required considerable time and effort to address. It was addressed in a two-tiered host range study.[22] Our company has conducted the most exhaustive host range study of TMGMV-U2 to date by screening 435 species-cultivars in 183 genera and 61 families, including important crop plants, native plants, weeds, and T&E species. Species in the family Solanaceae received particular emphasis.

We categorized the susceptible species by their symptom expression as asymptomatic (AS), systemically susceptible (SS), and SHN, and analyzed their potential exposure-risk from TMGMV-U2 from their possible presence at sites where the bioherbicide is likely to be used. The species were further categorized by their economic importance as cultivated plants (as vegetable, ornamental, fruit, etc.) or biology (as native or exotic plants and as harmful weeds or nonweeds). Using this categorization, our analyses indicated that TMGMV-U2-susceptible species would not co-occur with TSA at sites where the bioherbicide will be used, namely cattle pastures and surrounding woods.

Our data indicated a 'moderately broad' host range for TMGMV-U2 (Tables 1 and 2). These data and the published host range of TMGMV confirmed that the majority of TMGMV-susceptible plants were in the Solanaceae,[22,26] and that TMGMV-U2 is undoubtedly a Solanaceae-adapted virus.[56] The SHN was expressed only in a small number of species, all in the Solanaceae. With the exception of some cultivars of pepper (*Capsicum* spp.) and tobacco (*Nicotiana* spp.), two previously known hosts, and downy groundcherry (aka tomatillo, *Physalis pubescens*), which developed SHN, the disease reaction in other economically important solanaceous crop species were AS, R, or I (Table 2), therefore only pepper and tobacco were deemed at risk. However, there are resistant cultivars of these plants that can provide defense against TMGMV in crop production.

Although there are a few recent reports of occurrence of TMGMV in pepper,[57–61] it is not considered to be the most prevalent or economically important of the pepper viruses.[62,63] In the *Compendium of Pepper Diseases*, published by the American Phytopathological Society, there is only one cursory mention of TMGMV: it is listed as a significant pathogen of pepper with no special emphasis.[64]

Tomato, according to reliable published accounts, is immune to TMGMV,[26,29,65] and our data confirmed this conclusion.[22] All 13 cultivars tested were immune and therefore tomato is not at risk. Finally, a state-by-state analysis of the occurrence of T&E species revealed that none of the listed species in states where TSA was found was susceptible to TMGMV and none occurred in the same ecological niche as TSA (i.e., cattle pastures and surrounding woodlands).[22]

There are several recent reports of TMGMV incidences in plants grown in greenhouses or nurseries by clonal propagation,

Table 1. Summary of host range studies: percentage of plant species screened against TMGMV-U2 by host reaction

Immune	Resistant	Asymptomatic	Susceptible, systemic mosaic	Systemic hypersensitive necrosis
58	11	8	15[a]	8[b]

[a] Of the 15%, 89% were in Solanaceae and 11% (seven spp.) in five other families.
[b] Species that developed systemic hypersensitive necrosis were all in Solanaceae, in four genera and eight species and cultivars: *Capsicum* spp., *Physalis pubescens*, *Nicotiana* spp., *Solanum viarum*.

Table 2. Host reaction of economically important solanaceous crop species to TMGMVV U2: number of species or cultivars by host reaction

Species and number of cultivars screened against TMGMV-U2	Host reaction and number of cultivars in brackets
Eggplant, *Solanum melongena* – 12	AS (6)[a], R (6)
Pepper, *Capsicum annuum* – 32	R (2), SS (13), SHN (17)
Pepper, *C. baccatum* – 1	R (1)
Pepper, *C. chinense* – 3	SS (1), SHN (2)
Pepper, *C. frutescens* – 1	R (1)
Pepper, *C. pubescens* – 1	I (1)
Petunia, *Petunia* × *hybrida* – 2	SS (2)
Potato, *Solanum tuberosum* – 1	R (1)
Tobacco, *Nicotiana tabacum* – 29	R (16), SS (4), SHN (9)
Tomatillo, *Physalis pubescens* – 1	SHN (1)
Other *Physalis* spp. – 4	SS (4)
Tomato, *Solanum lycopersicum* – 13	I (13)

Abbreviations: AS, asymptomatic; I, immune; SHN, systemic hypersensitive necrosis; R, resistant (local lesions); SS, systemic susceptibility (nonlethal systemic mosaic).
[a] ELISA positive.

including several ornamental species.[66–69] Nonetheless, they are unlikely to be natural hosts to TMGMV. The primary reasons for the incidence of TMGMV (and other tobamoviruses) in clonally propagated plants are the use of contaminated cutting tools, repeated handling, and the use of tobacco products by nursery workers. It has been shown that TMGMV inoculum is easily spread by workers who use cigarettes and other tobacco products in the plant propagation trade, and such contamination of propagation material plays a role in TMGMV's infection cycle in tobacco fields.[70] These ornamental species are also not likely to be present in significant numbers in TSA-infested areas where TMGMV-U2 will be used.

Lastly, the moderately broad host range of TMGMV-U2 is the result of artificial manual inoculations. In the majority of cases, the inoculations were done on seedlings, a stage when plants are most susceptible. The effect of mechanical inoculation in artificially expanding virus host range is discussed below. For species rated AS based on the ELISA test, the data did not always strongly confirm susceptibility. Nonetheless, the doubtful ratings were deliberately skewed to err on the side of caution. The potential for the spread of TMGMV-U2 from these asymptomatic plants will be remote or nil if the virus does not replicate normally to any extent, as in a systemically symptomatic plant. The data from asymptomatic plants are therefore informative but have no epidemiological significance in regard to subsequent spread.

In our arguments we stressed the biology of TMGMV-U2 and the following mitigating factors that will prevent its risk to nontarget plants: (i) it is a mechanically transmitted virus which spreads by physical force through a wound on the plant; (ii) it has no natural means of dispersal; (iii) it cannot spread from treated plants or sites without deliberate movement of inoculum via infected plant sap; (iv) it is naturally present in Florida, many other states in the United States, and many countries around the world where tobacco and pepper are grown without serious economic losses from it;[26] (v) the TMGMV-U2 bioherbicide will not be used where susceptible plants are grown as crops; (vi) published data and ours have shown that the environmental persistence and buildup of TMGMV-U2 will be inconsequential;[22] (vii) the susceptible species, even those that develop SHN, would be at risk only if they are directly sprayed with the virus; and (viii) the moderately broad host range that our data indicated is the result of intentional inoculation. TMGMV, like many other viruses, can be artificially inoculated onto plants in several unrelated families,[49] but in nature it is found only on a few solanaceous species. According to Agrios[49] and Bos,[71] with mechanically transmitted viruses the taxonomic relationship of the inoculum donor and receiving plants is unimportant, since virus from one kind of plant, whether herbaceous or a tree, may be transmitted to dozens of unrelated plants. In addition, virus incidences that would not normally occur in outdoor plants may occur when they are grown in a greenhouse.[72]

To further mitigate exposure, the following 'use restrictions' are listed in the label[55]: (i) apply the product only when tropical soda apple plants are actively growing (to promote rapid killing by TMGMV); (ii) do not apply within 100 m of greenhouse and grafting operations; (iii) do not apply within 100 m of tobacco, pepper, or other solanaceous crops; (iv) spray directly at the target plant to avoid crops and nontarget plants; (v) do not re-use the spray equipment for other uses without thoroughly cleaning as specified on the label; (vi) do not apply this product through any type of irrigation system; (vii) do not apply the bioherbicide directly to water, to areas where surface water is present, or to intertidal areas; (viii) do not contaminate water when disposing the equipment wash water or rinsate; (ix) use only in pastures and wooded areas that are fenced to contain grazing animals; and (x) apply only with a backpack sprayer set to spray at 552 kPa on impact.

Based on our data and the above-stated scientific analyses, the EPA determined that no unacceptable adverse risks are anticipated for nontarget plants from TMGMV under the labeled use. The nontarget exposure is expected to be minimal. Adverse effects are also not expected on the T&E species or their designated critical habitats.

2.5.2.3. Survival (persistence) in the environment. Whether or not the use of TMGMV-U2 as a bioherbicide may increase its background levels in fields and thereby heighten the risk of its spread, perhaps even 'widely and uncontrollably', was a concern. The latter, with the language implying wide and uncontrollable spread, came from outside the EPA, from a reviewer unconnected with the registration team. Nevertheless, having received this concern, it needed to be addressed. We addressed it with the following data and published epidemiological models of tobamoviruses and proposed that the risk of survival and spread would be minimal to nil. Our data and claim were accepted, and the virus was registered.

As noted above, TMGMV-U2 did not reach as a high titer level in TSA as it did in Samsun nn tobacco, but TMGMV-U2 is likely to persist for a few months in dead roots, soil containing dead roots, and fallen TSA leaves, as revealed from our studies in the greenhouse.[22] However, the chance of TMGMV spreading from these sources is small, as evidenced by the lack of symptom development in susceptible tobacco and pepper plants grown in the same potted soil in which TMGMV-U2-infected TSA had grown previously.[22]

A crop of a susceptible pepper (*C. annuum*) cultivar grown in a field plot where two consecutive TMGMV-U2-infected tobacco crops had been grown a year before did not become infected, and the pepper yields were comparable to or better than those from a control plot where no prior TMGMV-infected tobacco

had been grown.[22] Thus, there was no evidence of the spread of TMGMV-U2 from soil-borne inoculum in the field.

2.5.2.4. Spread in the environment. TMGMV-U2 must have been around in the North American ecosystem for a long time, although its presence was definitively known only in 1952.[73] It is also conceivable that the background levels of TMGMV in agricultural settings around the world have risen and fallen over the years with the industrial-scale cultivation of tobacco, a primary host to the virus. In the United States, tobacco has been in cultivation on a vast scale for nearly two centuries, including in states that now have TSA. A susceptible tobacco crop can therefore act as a TMGMV reservoir. Compared to tobacco, TMGMV-U2 as a bioherbicide will be used over a relatively small area (i.e., about 10 000 ha at the most) compared to the US tobacco acreage (~355 000 ha in 1974 and 174 000 ha in 2021).[74] Unlike in the TMGMV-U2-TSA interaction, where the virus does not multiply to a high titer[22] and the TSA plant dies within a few weeks after infection, a systemically infected tobacco crop can harbor TMGMV-U2 over the entire growing season and allow it to reach high titers (up to 4 mg per gram of fresh tissue in some cultivars; Charudattan *et al*., unpublished). However, long-term tobacco cultivation in the United States and around the world over vast areas has neither contributed to an increase in the incidence of TMGMV nor caused adverse effects to economically valuable crops or native nontarget plants. There are no reports (experimental or anecdotal) of a cause-and-effect relationship between tobacco cropping and incidence of TMGMV on a susceptible crop-like pepper, and no extraordinary measures have been necessary to prevent its incidence in tobacco or pepper crops. The situation would not be different if TMGMV-U2 were used on a far smaller scale compared to the US tobacco acreage.

TMGMV being a mechanically transmitted virus, the potential for its spread and environmental buildup is small compared to a vector-transmitted virus. Mechanical transmission of plant viruses in nature by direct transfer of sap between plants is considered uncommon and relatively unimportant in the epidemiology of plant virus diseases.[49] Any systemically infected susceptible plant might serve as a virus source only if there is a means of physically dispersing the virus from this plant, living or dead. It is unlikely that the proposed use of TMGMV-U2 would result in high numbers of systemically susceptible plants in the field as these plants would occur infrequently or not at all at the virus application sites.

Most importantly, being a mechanically transmitted virus, TMGMV is less likely to spread linearly or exponentially from a point source, unlike how an insect-vectored virus or a foliar fungal pathogen with wind-disseminated spores would spread. Instead, it will spread randomly from plant to plant, depending on the chance transfer of inoculum. For TMGMV-U2 to spread widely, extraordinarily numerous mechanical transmission events would need to occur simultaneously or sequentially, which is unlikely in the field. If TMGMV-U2 were spread by humans or animals to a susceptible plant, which then becomes a virus reservoir, its further spread would still be discontinuous and random.

Whether or not TMGMV-U2 can spread from weeds and native plants was addressed as follows. Broadbent,[75] in his review of the epidemiology and control of ToMV, states, 'Viruses of the TMV group occur in a wide range of cultivated plants and weeds, but it has seldom been determined if these are sources of virus for tomato crops. Apart from this there is little evidence that weeds play a part in the epidemiology of tomato mosaic'.[76] We argued that the same will be true, namely weeds (or native plants) will be unimportant to the survival and spread of TMGMV-U2 when it is used as a bioherbicide. In a review by Bos,[77] on the role of wild plants in the ecology of virus diseases, there is only one reference to a tobamovirus (to TMV in this case). Likewise, in the treatise 'Pests, Pathogens and Plant Communities' by Burdon and Leather,[78] two treatises on plant virus epidemiology edited by Thresh,[79,80] and a review by Duffus[81] on the role of weeds in the incidence of virus diseases, TMGMV receives no mention and tobamoviruses in general receive only a passing reference in the contexts of virus spread, role of native and wild plants in epidemiology, epidemiological models, or the economic impacts, whereas vector-transmitted viruses are mentioned in many of the discussions. Also, no reports of adverse environmental effects, such as high residue levels or environmental buildup of TMGMV or tobamoviruses, were found from our Google search. Thus, these findings were used to support our position that TMGMV-U2 will not pose a significant risk from its use as a bioherbicide.

Among the Florida counties where we did field trials, seven reported to have about 983 ha of pepper and three about 283 ha of tobacco, but none reported any incidences of TMGMV in these crops or other plants as determined from the disease specimens submitted to the Florida plant disease clinics at the University of Florida and the State of Florida Division of Plant Industry. The survey was from 5 to 10 years following our field trials. In addition, the South Florida Vegetable Pest and Disease Hotline from 2006 to February 2012 did not report any TMGMV incidence (Charudattan, unpublished).

In our field trials there was no indication of TMGMV-U2's dissemination from infected TSA to healthy TSA by the Colorado potato beetle (*Leptinotarsa decemlineata*, Coleoptera: Chrysomelidae), a naturally occurring pest of TSA[82] that was common at several field trial sites, or by the introduced biocontrol agent, the South American TSA tortoise beetle (*G. boliviana*) (Charudattan *et al*., unpublished). There was also no indication that TMGMV-U2 was being spread in the field by cattle, deer, wild hogs, birds, rodents, and other fauna of which some (e.g., cattle) were present in significant numbers. There was also no evidence of spread of TMGMV-U2 through shoes or clothing of applicators or by an all-terrain vehicle used to apply TMGMV-U2 in several field trials.

As stated above, TMGMV-U2 has attributes that mitigate against its potential risks. It has several inherent safety features that would preclude risks to nontarget plants in the environment. Unlike a classical biological control agent like *G. boliviana*, an exotic agent released irrevocably into the United States,[21] TMGMV is native to the United States, therefore we will not be 'releasing' anything that is not already present. If any adverse effects are found from the use of the bioherbicide, the use can be discontinued without further damage. Thus, TMGMV-U2 cannot spread 'widely and uncontrollably' from its use as a bioherbicide for the reasons stated in the above two subsections.

2.5.2.5. Field trials under an experimental use permit. Field trials are usually done to confirm the efficacy of a proposed bioherbicide agent. The efficacy of TMGMV-U2 was established beyond doubt during the proof-of-concept research. However, it was necessary to develop a tool and an application method to be able to inoculate large patches of TSA having hundreds or thousands of plants and achieve 85% or higher weed control. The tool and the method should be affordable and easy to adopt into the users' field operations. The trials were also intended to develop an effective minimal and economical virus concentration for application.

The Experimental Use Permit (EUP), 81179-EUP-R, allowed trials on 405 ha in 66 of 67 counties of Florida. One county, the Highlands county, was not included due to the occurrence in this county of *Eryngium cuneifolium* (Apiaceae), an Endangered species in Florida. While our data confirmed that *E. cuneifolium* was immune to TMGMV-U2, a few other *Eryngium* species were asymptomatic[22] or known to be susceptible[83] to TMGMV. Out of caution, no field trials were conducted in Highlands county.

We designed, fabricated, and tested 10 large-scale application devices, some based on variations of a basic design, and a CO_2-propelled backpack sprayer for efficacy comparisons. These applicators, their use in field trials, and the results have been described, illustrated, and discussed by Charudattan *et al.*[84] In brief, the efficacy of the applicators varied, ranging from negligible to high, the latter giving 85–100% weed kill, and their performance was consistent or inconsistent.[84] (Tables 1 and 2) in the end, all the large complex designs were abandoned due to unacceptable efficacy and/or consistency and doubtful user acceptance. Instead, the simple high-pressure backpack spryer was chosen. This sprayer, set to discharge the bioherbicide at 552 kPa on impact, provided consistent results (≥85% control), was easy to use and clean, and a reliable source for its purchase was on hand. Thus, the backpack spryer was approved by the EPA and is listed in the pesticide label.[55]

2.5.2.6. The timeline from discovery to registration. It took 16 years from discovery to registration of the TMGMV-U2-based bioherbicide (Table 3). The principal reason for this long time was that ours was the first application before EPA seeking to register a plant pathogenic virus as a herbicide. With no precedence, the process entailed a deliberate and careful assessment of potential risks. When questions arose, new or supplemental studies were done, which was time-consuming and paused the registration process. The EUP field trials took 8 years to complete because the window for field testing the bioherbicide in Florida is only 6 months of the year, April through September. The cooler nighttime temperatures from fall to spring slow TSA growth and consequently the virus replication and disease development.[22]

Table 3. The timeline from discovery to registration of TMGMV-U2 as the bioherbicide SolviNix LC

Number of years from discovery to registration	16
Number of years from the pre-registration meeting with the EPA to registration	10
Systemic hypersensitive necrosis interaction between TMGMV-U2 and TSA discovered	1999
Proof of concept, including field efficacy, preliminary host-range, and patents filings completed	2003
Laboratory-scale production of the virus and parameters for large-scale production developed and validated	2004
Pre-registration meeting with EPA held	2005
Registration data package submitted to the EPA and waiver sought from studies on toxicology and risks to nontarget fauna	2005
Large-scale industrial production of TMGMV and stockpiling of technical-grade active ingredient	2005–2007
Application to register TMGMV as a bioherbicide for TSA accepted by the EPA	2006[85]
Temporary exemption granted from the requirement of a tolerance for residues of TMGMV on grass and grass hay when applied/used as a bioherbicide against TSA	2007[86]
An EUP granted for field trials in Florida on up to 405 ha, using up to 1000 g of active ingredient	2007
A permanent exemption granted from the requirement of a tolerance for residues of TMGMV in or on all commodities of crop groups 17 and 18 when applied as a post-emergent herbicide	2014[87]
Amended data packages with supplemental data submitted for ongoing review	2006–2008
Field trials in working ranches in Florida to test and select effective application methods and tools, and minimal effective dose done under an EUP	2005–2011
Request to waive studies on toxicology, infectivity, and pathogenicity granted	2010
Submission withdrawn to add data and strengthen nontarget plant host-range and environmental risk sections	2011
Data package resubmitted	2013
Final labels and Safety Data Sheet developed and approved	2014
Full, unrestricted US registration granted	11 December 2014[88]

Abbreviations: EPA, US Environmental Protection Agency; EUP, Experimental Use Permit; TMGMV-U2-, tobacco mild green mosaic virus-U2; TSA, tropical soda apple.

3 SOLVINIX LC, THE PRODUCT

The name SolviNix is derived from SOLVI, the EPPO Code (BAYER Code)[89] for *Solanum viarum*, and Nix, as in nixing or preventing. As LC in the name implies, SolviNix is a liquid concentrate of purified TMGMV-U2 virions in water. SolviNix LC is offered in two quantities, 10 and 35 mL, each with an estimated 4.23×10^{14} virions per gram of product. It is sold, respectively, in 15- or 50-mL plastic screw-capped centrifuge vials with milliliter markings. Figure 1 illustrates the packages and their contents for the respective sizes. The 10-mL vial provides 286 mg of the active ingredient and the 35 mL provides 1 g to treat, respectively, about 600 and 2000 plants. The product is sold and delivered directly to the users in a frozen state, and it should be kept frozen or in a refrigerator until use. The mixing ratio is 1.5 mL of product to 3.79 L (1 gal, a unit more easily measured by Florida farmers) of potable water. It is applied with a backpack sprayer as stated above. Depending on canopy size and architecture, three to five 5-s spray hits per plant should be sufficient to initiate the disease and plant kill. In the field, plant death occurs in 4–6 weeks following application. Unused product in the vial can be stored in a refrigerator for up to 1 month and reused as needed. Use of abrasives, such as carborundum powder, which is routinely used in virology research, or adjuvants or additives, is not permitted[55] due to concerns for user safety and the potential for virus inactivation.

3.1 Registration of TMGMV-U2 and since

SolviNix LC received an unconditional registration as a bioherbicide, EPA Registration No. 81179–3, on December 11, 2014 and

a Biopesticides Registration Action Document dated March 30, 2015 was published.[88] The EPA registration is current, and the product is registered for use in Florida as a commercial herbicide. Calls or emails are received from clients during the TSA growing season. Typically, our clients are small-size ranchers and homestead ranchers who want to rid their fields of TSA. While the beetle *G. boliviana* has helped to reduce the overall TSA population in Florida, it does not kill infested plants, which continue to bear fruit, and recurring new infestations are common (Charudattan, unpublished). Admittedly, the market for SolviNix LC is small. Research into other proposed commercial uses of TMGMV[43–46] is gaining momentum, which generates additional resources for the company.

There are a few issues to note. The requirement to use a backpack sprayer, set by the EPA, is not suitable for treating large TSA infestations. It also hinders user acceptance of the product. Our clients generally do not have a high-pressure backpack sprayer capable of spraying at 552 kPa. For these clients, we provide the material and an application service. Second, the virus does not spread from infected to healthy untreated TSA in the field. While the lack of secondary spread was a desirable feature for registration from the environmental safety perspective, the lack of spread disappoints users who wish that the virus would spread from an initial application and control unsprayed TSA. The product does not have preemergence activity against season-long emergence of new seedlings (Charudattan, unpublished), therefore it is necessary to reapply the product at least twice for season-long control. Selling the product in a frozen form has not been a problem since its market is now within Florida, and it can be shipped frozen for overnight delivery.

4 UNIQUENESS OF TMGMV-U2 AS A BIOHERBICIDE

SolviNix LC is unique in the following respects:

- It is the world's first herbicide containing a plant virus as the active ingredient.
- It is the first plant-virus-containing bioherbicide.
- It is the first naturally occurring nano-herbicide.
- Unlike a chemical herbicide that is applied at a high dose to enable an effective amount to reach the site of action, the virus is applied at a minimal effective dose from which it self-replicates *in planta* and triggers the herbicidal activity.
- TMGMV is an organic material that is metabolized by organisms and broken down in time in the environment.
- TMGMV-U2, like TMV, is highly resilient[84]: it withstands industrial and laboratory processing without loss of structural integrity and infectivity.
- Its shelf-life for herbicidal activity is about 1 month at room temperature, at least 1 year in a refrigerator, and 23 years (when last assayed) in a −20 °C freezer (Charudattan, unpublished).
- TMGMV-U2 lends itself to mass production as high virus yields are possible from a systemically susceptible tobacco.[22]
- TMGMV-U2 enters sprayed leaves rapidly after application, and therefore infection and disease development are independent of external moisture and temperature conditions during and after application.
- Temperature that is conducive for normal TSA growth and reproduction, generally from April to September in Florida, before nighttime temperatures reach 10 °C and below, is conducive for TMGMV-U2 infection, replication, and disease expression.
- TMGMV-U2 is highly consistent and predictable in performance, which is an uncommon trait among microbial bioherbicides.
- TMGMV-U2 kills TSA, precluding the infected plant from being a virus reservoir in the field, and this was one of the key considerations in its registration as a bioherbicide.

5 IS IT POSSIBLE TO DEVELOP OTHER TOBAMOVIRUS-BASED BIOHERBICIDES?

Yes, it is possible. The Tobamovirus genus includes around 30 species, with its taxonomy being in a flux.[90–92] Except for a few that are seed-borne or seed-transmitted, most tobamoviruses are mechanically transmitted and no vectors are involved. Most species have moderate to wide host ranges under experimental conditions, although their natural host ranges are usually quite narrow.[91] The adaptation of Tobamovirus species to certain angiosperm families is well recognized. The known species fall into eight plant-family-related groups: those adapted to Apocynaceae, Brassicaceae, Cactaceae, Cucurbitaceae, Fabaceae, Malvaceae, Passifloraceae, and Solanaceae (with three odd species in this group adapted to Scrophulariaceae, Orchidaceae, and Gesneriaceae).[90] It is therefore possible that other host–virus interactions where the outcome is SHN could be found through a systematic screening of weed hosts against virus strains adapted to their families. Tobamoviruses have a worldwide geographic distribution, and hence it is possible that locally evolved strains, such as the Alke strain used against *S. carolinense* in the former Soviet Georgia (mentioned above) may be waiting to be discovered. Furthermore, looking ahead, registering plant-virus-based bioherbicides should not take more than 2 or 3 years as there is now a precedent in TMGMV-U2.

A gene-for-gene-based host–virus interaction resulting in SHN is key to the use of plant viruses for weed control because anything less than total weed kill may not be acceptable due to the risk of leaving virus reservoirs in the field. Vector-transmitted viruses are unlikely to be acceptable because it would be unfeasible to confine them to target sites in the field.

How common is the virus-induced SHN? Probably more common than the records in the virology literature would suggest. Reports of it are still few, but more examples are coming to light, such as clover yellow vein virus (Potyvirus) in *Vicia faba* cv Wase (broad bean),[93] soybean mosaic virus G7 (Potyvirus) in certain genotypes of *Glycine* max (soybean),[94] bean common mosaic virus (Potyvirus) in certain cultivars of *Phaseolus vulgaris* (bean) held at certain temperatures,[95] and a Japanese isolate of rehmannia mosaic virus, ReMV-J (Tobamovirus) in *Capsicum annuum* (chili pepper) and *Solanum lycopersicum* (tomato), the latter being temperature-dependent.[96]

6 EPILOGUE

It is my fervent hope that this perspective of my successful effort at registering a plant virus as a bioherbicide, and the experience gained therefrom, would be of value to others who may wish to develop similar virus-based bioherbicides. Future workers may find this article useful in developing suitable registration dossiers. Not only intact viruses but also viral genes, gene products, and their modes of action

should be studied to develop a new class of virus-based herbicides.

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CONFLICT OF INTEREST

The author, through his company BioProdex, Inc., licensed the technology underlying the bioherbicide from the University of Florida Research Foundation, a minority shareholder in the company. BioProdex is the registrant of the bioherbicide. The author has no conflict of interest as the research data on which this review is based and the bioherbicide are owned by his company.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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