Perspective

Received: 28 June 2023 Revised: 31 August 2023 Accepted article published: 8 September 2023 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/ps.7761

The Toothpick Project: commercialization of a virulence-selected fungal bioherbicide for *Striga hermonthica* (witchweed) biocontrol in Kenya

Claire S. Baker,[a*] David C. Sands[b] and Henry Sila Nzioki[c]

Abstract

The high-level view of global food systems identifies three all-encompassing barriers to the adoption of food systems solutions: knowledge, policy, and finance. These barriers, and the siloed characteristics of each of these, have hindered the development and adoption of microbial herbicides. How knowledge, policy, and finance are related to the Toothpick Project's path of commercializing a new bioherbicide, early in the scope of the industry, is discussed here. The Toothpick Project's innovation, developed over four decades and commercialized in 2021, uses strains of *Fusarium oxysporum* f.sp. *strigae* selected for overproduction and excretion of specific amino acids, killing the parasitic weed *Striga hermonthica* (Striga or witchweed), Africa's worst pest threat to food security. Historically, bioherbicides have not been a sufficient alternative to the dominant use of synthetic chemical herbicides. To be used safely as bioherbicides, plant pathogens need to be host specific, non-toxic, and yet sufficiently virulent to control a specific weed. For commercialization, bioherbicides must be affordable and require a sufficient shelf life for distribution. Given the current triple storm encountered by the chemical herbicide industry (herbicide-resistant weeds, lawsuits, and consumer pushback), there exists an opportunity to use certain plant pathogens as bioherbicides by enhancing their virulence. By discussing barriers in the scope of knowledge, policy, and finance in the development of the Toothpick Project's new microbial bioherbicide, we hope to help others to anticipate the challenges and provide change-leaders, particularly in policy and finance, a ground level perspective of bioherbicide development.

Keywords: biocontrol; biological herbicidefood securityKenya*Striga hermonthica*witchweed*Fusarium oxysporum*amino acid inhibition

1 INTRODUCTION

In a long search for effective bioherbicides to control weeds in agricultural lands, there have been relatively few successes in both the biocontrol and the commercialization.[1,2] This has been despite considerable effort by weed scientists, entomologists, plant pathologists and agronomists. One of several exceptions in the quest for a commercialized bioherbicide would be the successful biocontrol of strangler weed (*Morrenia odorata*) in citrus by the application of the fungal plant pathogen, *Phytophthora palmivora*.[3] This treatment worked so well that farmers only needed it for 1 year, resulting in an unsustainable business model. Weed control, an important and yield-limiting aspect of agriculture, is predominantly done with chemical herbicides, selected to be effective against a broad spectrum of weeds. The successful development of synthetic herbicides by the commercial agrichemical industry over the past 75 years has contributed to considerable increases in crop yield. Research into alternate methods have been attempted, but the agrichemical industry's synthetic herbicides dominate with little financial incentive to develop new methods.[4]

As expected with industries of scale, the chemical herbicide industry prioritizes research funding on major crops and large-scale production of chemical herbicides. This industry has concentrated on development of site-specific inhibitors that inhibit specific plant enzymes, as these compounds may not affect animal enzyme systems. Nonetheless, the perfect storm of rapidly evolving and spreading herbicide resistance, large and numerous lawsuits involving herbicide toxicity and off target effects, increasing pressure to reduce greenhouse gas emissions attributed to weed management techniques such as tillage and synthetic chemical pesticides, and consumer and governmental backlash has challenged the agricultural chemical industry. This presents an opportunity to provide innovative alternatives to chemical herbicides. Many plant pathologists have observed and reported weeds with serious disease symptoms, and often these researchers have been

* Correspondence to: CS Baker, Toothpick Company Ltd, P.O. Box 2138 – 50100, Kakamega, Kenya, E-mail: claire@toothpickproject.org

a Toothpick Company Ltd., Kakamega, Kenya

b Department of Plant Pathology, Montana State University, Bozeman, MT, USA

c KALRO-AMRI, Machakos, Kenya

involved in attempts to use fungi, bacteria, and viruses to control weeds. There are some fundamental economic and biological reasons why so few bioherbicides have been developed.[4,5] Gressel describes Four Pillars required to support a biocontrol fungus: selection for virulence; inexpensive production; agent establishment and shelf life; and biosafety.[6]

With the current and escalating opportunity, we see that bringing an agricultural biocontrol product from laboratory to customer is not possible without the aforementioned pillars in addition to an interconnected web of knowledge, policy, and finance. Knowledge is defined by the research – both successful and unsuccessful – in the laboratory, glasshouse, field, and marketplace, as well as the comprehension of this research by stakeholders, spanning farmers to scientists to policymakers. Policy defines the regulatory protocol required to enter the commercial market. Policy is also related to goals for reducing greenhouse gas emissions, increasing nutrition and/or yield, and improving soil and water health. Finance includes funding for the research, as well as funding for intellectual property, regulatory trials and registration, company establishment, extension services, marketing, and actual commercial scale up. There is also a grand conversation happening about economic transformation, which often links policy to regenerative agriculture practices.

As we reflect on knowledge, policy, and finance, we can see how influential their intersections have been throughout the path of commercialization and adoption of a bioherbicide innovation in Africa called the Toothpick Project, as discussed throughout this perspective. The Toothpick Project targets *Striga hermonthica* (Striga or witchweed), a weed considered the worst pest threat to food security in Africa. Fifty million hectares of croplands in Africa have Striga infestation, causing $US 9+ billion in crop loss annually.[7–9] A pilot project, developed through a social enterprise named the Toothpick Company Ltd, was launched in 2018 in western Kenya, where approximately 340 000 ha are infected with Striga, causing annual crop losses of $US 53 million.[10,11] This area, considered the breadbasket of the country, is heavily infested with Striga. A survey of 83 farms indicated that 73% are infected with Striga.[12] An informal survey of three counties in western Kenya indicated that all farms had some Striga impact. The loss represents 12.3% of maize production in Kenya which translates to about 39 kg of maize loss per capita (20% of typical person's annual food requirement).[13]

Our Toothpick Project pilot has navigated knowledge, policy, and finance to now stand as a model for expansion of the Striga bioherbicide across sub-Saharan Africa as well for other bioherbicide developments globally. We view knowledge as an area that has been a historical void of success while policy and finance have been misaligned with the needs of farmers, industry, governments, and researchers. The Toothpick Project's strength has been its knowledge development, hence its emphasis in this perspective, but the Project's overall success is related to the ability to pair this knowledge with the policies and finances required to gain commercial use. As an innovative organization, we have an influential role in these intersections moving forward and hope our perspective of each area can help expedite the future progress of bioherbicide development.

2 KNOWLEDGE

Is there adequate knowledge about alternatives to synthetic-chemical herbicides to start making an impact? Since the green revolution, agricultural research has been dominated by plant breeding for yield, herbicide-resistant crops, and postharvest shelf life. The advancements have been tremendous – accounting for increased food production and attributed to saving over a billion lives. However, as even Norman Borlaug attested, these advancements only delayed the problems of food insecurity.[14] Herbicide-resistant weeds are winning their battle against synthetic herbicides with most modes of action, and there have been few or no significant herbicides with new modes of action for decades.[15] The paired seed/herbicide systems have reduced biodiversity, resulting in soil deficiencies, and increased fertilizer use. As discussed in 'The Breeder's Dilemma' in breeding crops for an increase in pest resistance, we have selected for lower nutritional quality (the insects are cleverly selective).[16] This is supported by the market which has been almost entirely measured by yield, not nutrients.[17] Also, of course, pesticide-related toxins are impacting our flora and fauna.

Since Norman Borlaug, we have had three generations dedicated to the continued increase in yield through crop breeding and supportive pesticides.[18] Recognizing the potential of biological solutions, we have seen advances in biofungicides and bioinsecticides. There have been bioherbicide efforts related to phytotoxic allelochemicals (e.g., strigolactones, plant extracts) and plant pathogens (e.g., *Fusarium oxysporum*). Yet, there are fewer than two dozen registered bioherbicides in the world and most have not seen success commercially.[1,19,20]

One of the attempts to use *F. oxysporum* as a bioherbicide was for Striga, using a wild-type strain called Foxy2 from Ghana for bioherbicide development in Kenya.[21] This product did not have adequate efficacy in the field and therefore, despite high level development for over a decade, Foxy2 was not commercialized.[22] During our development of *Fusarium oxysporum* f.sp. *strigae* for control of Striga, we were told several times that Foxy2 had already been tried and failed, and, therefore, our efforts were discredited. What people did not comprehend is that the Foxy2 wild-type strains of *F. oxysporum* f.sp. *strigae* were not virulent enough to allow for an efficacious, affordable commercial product.[23] Others failed when trying to use a fungal pathogen that was not host-specific enough to ensure safe deployment. There have been multiple research projects on strigolactones to inhibit Striga through hormone interference. However, this research is still at least 5 years from having a minimal viable product to put through regulatory approvals.[24,25]

The novel virulence enhancement technology developed by Sands and coworkers and described later in this perspective combines the potential for inhibition of Striga by certain amino acids with the ability for *F. oxysporum* to be host specific, vascular in the host, and soil borne.[26,27] Given the need for adequate virulence of bioherbicides, it is important to build the platform. We have a growing list of ways to broaden the concept over the next decade: a long list of target weeds; methods of enhancing virulence; application rates; application systems; cocktail approaches for a broader spectrum treatment; continued validation of host specificity; and molecular characterization and build-up of the *F. oxysporum* gene database.

Bioherbicides are chosen for their host specificity,[2,5,6] as use of multi-host pathogens would lead to escapes from the area of application, and severe regulatory and agronomic sanctions would result. Unfortunately, host specificity is a two-edged sword, in that no pathogen could last for long if it seriously damages its only host.[28] Unless a bioherbicide is hypervirulent, it will not cause sufficient mortality of the target weed(s) to be viewed as a herbicide relative to competing chemical herbicides. Given this situation, it is not surprising that there are so few commercially available bioherbicides based on plant pathogens. Nonetheless, there is a reason for optimism in that biochemical/genetic approaches, not available until recently, that could be used to develop highly virulent plant pathogens. The several modern genetic approaches that could be attempted are: (1) host-specific pathogens could be made more virulent[5]; (2) the specific host range requirements of a bioherbicide could be altered by use of host range-altering genetic cassettes (not yet defined), enabling multi-host pathogens to attack multiple weed species; and (3) pathogens could be programmed to die after having their effects on target weeds. In each case, a unique business model that would enable commercialization will be desired. We report here just one example of this biochemical/genetic approach. This involves the case of development, in country registration and commercialization of an endemic plant pathogen of Striga, in Kenya.

2.1 The need to enhance virulence

A problem with bioherbicidal development of most plant pathogenic fungi, with few exceptions, is that wild-type pathogens tend to be insufficiently virulent to function as alternatives to chemical herbicides. We estimate that most plant pathogens damage weeds in the level of 5–20% in weed biomass reduction, clearly an obstacle if they are to be used as bioherbicides in comparison with the far more efficacious chemical herbicides, especially as the latter have a much broader spectrum of target weeds. Some of the plant pathogens developed for bioherbicidal use are effective, but are toxigenic, and registration is a problematic because of precautionary environmental and public safety concerns. It is probably not in the public interest to replace a synthesized herbicide with a bioherbicide that is potentially toxigenic. We proceeded to find a way to enhance virulence with a host-specific plant pathogen without producing toxins. An early paper by Steinberg[29] from the University of Chicago, IL, USA gave us a hint as to a solution of this problem. In working with the frenching disease of tobacco, he determined that the symptoms in tobacco were elicited by two common soil bacteria excreting isoleucine, a common and essential amino acid. Isoleucine is an inhibitor of a certain regulatory plant isozymes (acetolactate synthase) at the start of the pathway for biosynthesis of the branched amino acids (isoleucine, valine, and leucine). Prompted by this report, we proceeded to develop a selection of amino acid excretion of pathogens to inhibit plants without producing toxins. The method involves the fact that in plants, biosynthesis of at least nine essential amino acids is regulated by production of isozymes at the start of three biosynthetic pathways. As plants are self-contained multicellular systems, there apparently has been degeneracy and loss of some of the isozymes in plants. Our conjecture is that many, if not most, plants display such misregulation of amino acid biosynthesis and are inhibited by at least one amino acid. This is the case of hound's tongue (*Cyanoglossum officinale*), *Cannabis sativa*,[30] water hyacinth (*Eichornia crassipes*), Canada thistle (*Cirsium arvense)*, field bindweed (*Convovulus arvensis*), and many other weeds. Generally, external applications of 5 mM amounts of selected amino acids are sufficient to inhibit plant growth.[5]

Given these findings, it was a matter of finding which amino acids inhibit Striga and then selecting the host specific endemic fungi (*F. oxysporum*) to overproduce these amino acids as they infect their single host. Pot experiments determined that leucine and tyrosine were inhibitory to Striga but not the host maize. Since these fungi grow on a minimal medium (without amino acids supplied), it became a relatively straightforward matter of searching for inhibitory amino acid analogs of leucine and tyrosine that can be used to inhibit the wild-type fungi but not their analog resistant mutants. As a high number of resistant mutants are excreters of amino acids, we then followed with development of an assay to detect excretion of these amino acids. For Striga bioherbicide development, multiple selections with the amino acid analogs norleucine and methyltyrosine resulted in three sister strains of *F. oxysporum* that overproduced these amino acids.[31] Also, a selection was then made for methionine excretion with seleno-methionine, not because it is inhibited Striga, but rather it was determined by Primrose[32] to be converted by soil microbes to ethylene. Ethylene, by causing Striga seed to germinate in the soil, could reduce the soil bank of weed seed. As the soil seed bank of Striga is problematic, with enormous numbers of dormant seeds added every cropping season, the seed bank was an additional target that required consideration. The hypothesis is that the known conversion of methionine to ethylene by selected pathogens may assist in the decline of the soil bank of Striga seeds, given that *F. oxysporum* chlamydospores are reported to survive in the soil for long periods.[24] The Striga bioherbicide development experiments are described in detail by Nzioki *et al*.[31]

2.2 Delivery of fresh secondary inoculum

The traditional biocontrol industry produces stabilized biocontrol agents of insects, nematodes, bacteria, and fungi for augmentative release, mostly for high value crops grown in glasshouses. Alternatively, our project in western Kenya, was designed to provide bioherbicidal Striga control for small hand-planted acreage, low-income subsistence farmers where the production of maize was primarily to feed the farmer's family. The primary inoculum of the bioherbicidal fungus was produced without contaminants in a modern microbiological laboratory, but there was a need to develop a way to use our selected fungal strains to produce secondary fresh microbes for on-farm inoculum for use at the time of planting.

Increased maize yields in the long and short seasons on 500 paired plots comparing toothpick fungal treatment with controls in western Kenya in 2014–2015, were reported by Nzioki *et al*.[31] Our initial successful solution involved distribution of singly-packaged, dried toothpicks coated with the fungus. The shelf life of the fungus in these toothpicks was determined to be several years, allowing their efficient distribution to subsistence farmers, along with training as to how to deploy this technology. At 3 days prior to planting, the farmer or group of farmers could transfer the toothpicks to freshly cooked and cooled rice. If the container containing the rice after cooking was swabbed with ethanol, the resulting rice concoction could be kept clean to avoid aerial contaminants. This done, the farmer had a supply of fresh secondary rice-borne inoculum with which to plant with maize seeds after the first or second rainfall. In 500 paired plot field trials this 'toothpick method' resulted in an average of 42–56% increase in yields.[31]

While giving farmers the opportunity to inoculate their own product from the toothpicks, we found that quality control, particularly regarding sanitation, was inconsistent. To better manage the inoculum production, we trained Village Inoculum Producers (VIPs) to manufacture the secondary inoculum product, commercialized as Kichawi Kill™ in 2021, and sell it to their farmer networks. This product is currently available for purchase in seven counties in Kenya. Although it is affordable and effective, there are limitations. Most importantly, the 10- to 14-day shelf life of the live inoculum presents challenges to VIPs and farmers who are timing their planting according to the rains. Fortunately, we found that a dried inoculum can act quickly enough to protect the crop from the parasite. Using the same *F. oxysporum* strains, our new seed coating product received expedited regulatory approval in June 2023.[33] This product has an increased shelf life, giving farmers more planting flexibility. Farmers can coat seeds themselves, streamlining the distribution process and giving farmers their own choice of seeds. Preliminary projections indicate the price will be about 60% less because the rice substrate in the fresh inoculum product is relatively expensive, not to mention that rice is a food source during a time of famine.

The developments of the biocontrol project in Kenya were specifically designed by Kenyan agronomists for small-scale farmers who hand plant their maize seeds. This human-centered design approach has been a key point to the success of the product. How does our product differ from that of other Striga management products on the market in Kenya? StrigAway (a product with imazapyr coating on imazapyr-resistant maize),[34] while effective, has not been well adopted, tried by only about 4% of the market according to a survey conducted by Boston Consulting Group in November 2017, and in our survey of 316 farmers in November 2022, none had tried it. The product is distributed with gloves for application and, anecdotally, farmers do not like the gloves and are concerned about toxins damaging other crops. This product is an example of something not designed for hand planting, but rather the concept is adapted from large-scale production farming. Knowledge is most effective when it spans throughout research, development, and adoption – from the scientists understanding the needs of the producers, the funders understanding the needs of the scientists and the producers, and the policymakers understanding these priorities.

3 POLICY

When looking at global impacts related to food security, agriculture, and climate, recent goal-setting has been made by state-leaders through international events such as COP27 (the Conference of the Parties at its twenty-seventh session), the Paris Agreement (an international treaty on climate change), the 2021 United Nations Food Systems Summit, and the Bonn Climate Change Conference (a climate summit focused on sustainable food systems). Examples include the 2022 draft decision entitled 'Joint work on implementation of climate action on agriculture and food security' from COP27,[35] which implies that there is an action plan with multiple stakeholders.

The top-level discussions at the global summits are now focused on moving from pledges and intentions to actual implementation. The use of the word 'implementation' at COP27 in 2022 still feels like a hopeful tagline. Within a month of COP27, critics highlighted the failure to put the goals into action, calling out the lack of dedicated funding to match the research and development required to fulfil the policy goals.[36,37] Food, water and agriculture were absent at the previous COP26. COP27 corrected the omission with an emphasis on food security, leaving out the fact that food systems contribute about a third of global greenhouse gas emissions. These conversations seemed more productive the year earlier at the first United Nations Food Systems Summit in 2021, where we saw significant financial pledges as well as representation from farmers and on-the-ground implementers.[38,39] We are yet to see if these pledges represent the changes that need to take place at the ground level (including both research laboratories and farms).

We launched our project in Kenya in 2007. Often, we are asked why we picked that location when there are nightmare weeds in our own backyard in the United States. Primarily, the decision was humanitarian – one weed is causing a crisis across a continent struggling with food insecurity that is often related to erratic climate conditions and civil unrest. Across Africa, a weed on 40 million farms had been mostly ignored, probably because selling products to low-income (poverty level) farmers 1 ha at a time was not economically attractive for the agrochemical companies.[13] In hindsight, we can see how our path was also directed by our key assumptions: first, we were not big enough to make it through the United States' regulatory processes, and second, we were not big enough to protect our innovation from getting buried by the big agrochemical companies. There was some truth in those assumptions. But, in 2018, major lawsuits regarding glyphosate toxicity began, and, soon after, complaints and lawsuits over dicamba drift from dicamba-resistant crops were filed. The organic industry was seeing 12–17% growth rates each year. Herbicide-resistance in weeds was increasing annually. And, tied to policymakers' goals to reduce greenhouse gases and sequester carbon through agriculture, regenerative agriculture gained traction as the newest movement. It quickly became apparent that a new solution was needed by all stakeholders. From when our project started in 2007 to today, it has finally become clear that the innovation would not be buried as competition but rather elevated as a new place to launch.

Regulations are being implemented to curb perceived health risks, both accompanied and provoked by lawsuits. There are now at least 18 countries banning glyphosate, seemingly for the purpose of managing its perceived toxicity rather than to prevent the longer-term crisis of the evolution and spread of herbicide resistance. In April 2021, Sri Lanka's President Rajapaksa banned the import of agrochemicals, with the goal of boosting the country's status for organic production and to save on the import costs of fertilizer ($400 million).[40] It appeared to be a sound goal but, between a gaping hole in bioherbicide development and the lack of agronomical knowledge by the farmers, the experiment failed, exacerbating food insecurity in the county. The ban was lifted 7 months later and, with rioting farmers across the country, the president fled the country in April 2022. Sri Lanka had banned glyphosate in 2015, but lifted that ban in September 2022, citing no alternative for weed management as the reason. This is a noted example of how inextricably linked knowledge, policy, and finance are. One cannot move forward without the others.

Regulatory protocols for bioherbicides are less defined than those for chemical herbicides. Bioherbicides are also less defined than other biological inputs such as seeds. With proof-of-concept trials in 2014–2015, our regulatory processes started in 2015 with toxicology and ecotoxicology testing at the University of Nairobi, Kenya[31] and with concomitant testing at Virginia Tech University, Blacksburg, VA, USA.[41] This was followed by a dossier including details for the proposed labeling and the designs for trials conducted by third party implementers. The trials were to take place over three seasons and were co-designed by the Kenya Pest Control Products Board (PCPB). The process seemed straight forward. However, what started as three trials turned into seven. The first implementer failed to put fertilizer on the control. The next season, with a new implementer, the plots were devastated by the first appearance of fall army worm (*Spodopetera frugiperda*), which we learned how to treat in subsequent seasons. We had a season hit by severe drought. And, we had an implementer who failed to submit their report to the PCPB for over a year. Finally, the three trials were completed, and the complete dossier was submitted for approval in November 2019. Then COVID-19 arrived, and the quarterly review cycle was put on hold for almost a year, at which time a molecular characterization report was requested. Molecular characterization was obtained from Kansas State University, Manhattan, KS, USA. However, our timeline was pressured due to the upcoming long-rains planting season (starting around March). We had been sustaining our staff throughout the pandemic shutdowns and could not afford to miss another season. A provisional commercial approval was granted in March 2021, just in time to sell the product to 750 eager farmers and conduct demonstration plots such as one with World Food Program's Farm to Market Alliance (Fig. 1). We received the official certificate of registration for the product (tradename Kichawi Kill™) in the fourth quarter of 2021, a full 6 years after starting the registration process.

How do we make product improvements in a timely fashion? We identified a better strain in 2016. However, because our regulatory trials had already started, we could not veer from our course. We believe updating strains every 5 to 10 years is an important safeguard against evolved herbicide-resistance. At this point, it is not feasible, due to the time and resources required, to go through the required regulatory process again. Similarly, as previously mentioned, we have developed a seed coating from our approved fungal strains.[33] The early estimate from the PCPB was that it would take approximately 2 years (two growing seasons of field trials plus paperwork) to get this new iteration approved. Through further discussions with the PCPB, they expedited the process with the recognition that we are using the already-approved *F. oxysporum* strains with simply a formulation of the primary inoculum on wood powder rather than toothpicks. This seed coating product was approved for commercial use in June 2023, and the company is rapidly expanding laboratory operations to accommodate the new spore powder production for the immediate growing season.

Now that we have commercial approval in Kenya, it is time to expand to other countries (Striga is on 40 million farms across Africa).[13] Each country has its own regulatory process. This is currently the greatest barrier for our product, and we are not alone in our concerns. ICGEB (International Center for Genetic Engineering and Biotechnology), through a grant from STDF (Standards and Trade Development Facility), is currently researching, evaluating, and designing sample harmonized biocontrol regulatory guidelines for the SADC (Southern Africa Development Committee). The Toothpick Project will then go through a simulation with the proposed guidelines, showing how the new concept would impact our ability to advance our innovation to countries that need it. While we remain hopeful for harmonization, it is not likely to happen quickly enough for our development. Therefore, we are researching trade agreements between countries.

Figure 1. Farm in Busia County, Kenya, with significant crop failure due to Striga infestation (the plant with the purple flowers). This is an informal demonstration plot conducted by the United Nations World Food Program Farm to Market Alliance in 2021. The same maize seed was used throughout. The plot on the right was treated with the bioherbicide containing virulence-selected strains of *Fusarium oxysporum*. Photograph: Geoffrey Wanjala.

As a biological agent, there are potential benefits to using a locally-sourced fungal strain, rather than moving the Kenya strain across the continent. However, this means that scientists in each country (we have researchers in 13 countries) need to have the knowledge, equipment, supplies, and team in place to run the selection processes. Alternatively, if the Kenya strain is deemed suitable for rapid registration in other countries, the Nagoya Protocol is in place to protect against the exploitation of genetic resources. This policy is put in place for good reason, but, it is a critical barrier nonetheless. Biologicals should be monitored against the possibility of evolution of resistance, as exemplified by evolution and spread of synthetic chemical herbicide-resistance. This will demand attention and planning for resistance management, which could include – or even require – new strains to stay ahead of herbicide resistance in weeds.

4 FINANCE

If we need transformational research and development, we need transformational financial visions. Gap-filling approaches are necessary, especially related to the food security crises that have arisen due to COVID-19 and the conflict in Ukraine. However, these stopgaps are not transformational.

Innovative and game-changing research is often considered too unconventional and high risk for standard funding tracks.[42] This is where there appears to be a gap between the needs of the farmers, the needs of the researchers, and the calls for proposals. For example, as we scour funding opportunities for a fit for our bioherbicide research, we see a surge of interest in digital solutions and relatively little support for biological innovations that fall in between proof of concept and commercial scale up. Non-biological solutions such as digital tech, solar applications, and drones are serving a purpose and delivering a product quickly and for a low cost. For a funder, these solutions give a suitable fast return. But are these requests for proposals a response to the needs of farmers? Are they deterring funding for longer-term and more transformational concepts? For the Toothpick Project, securing funding for the required regulatory protocol has been denied many times, often with the funders' responses stating that they would prefer to help scale the project once the commercial registration has been approved. We have found ourselves in an unfortunate funding gap that prevents expansion to other countries.

Funders, including governments and multinational non-governmental organizations, are emphasizing long-term financial sustainability – often with the end goal being a commercial product. We have been the grateful beneficiary of these funds, based on our enterprise structure. However, the very nature of soil-borne fungi is that they can stay effective in the soil for years. This long-term aspect may require some adjustment in how the bioherbicides are viewed from traditional agricultural and agrichemical industry strategies (essentially nature provides a commercial obsolescence).

In review, funding streams need these components if we want to get beyond incremental change: (1) longevity that extends over multiple growing seasons; (2) flexibility in the call for proposals; (3) enough funding that the team is not constantly looking for more funding rather than focusing on the project; (4) willingness to fund the regulatory process, which has been by far the most challenging funding segment; (5) open to non-profit, for profit, and government; (6) trust that we are doing the work, without the burden of bureaucratic paperwork; (7) attention to smaller organizations that exist outside major research organizations [such as CGIAR (Group for International Agricultural Research) units, large universities, etc.] and (8) the initiatives should be farmer driven, and reflect the needs of the farmers, not the needs of the policymakers or funders.

5 CONCLUSIONS

We have built up significant knowledge to develop and commercialize a microbial bioherbicide. An innovative use of an endemic fungus, enhanced for virulence by virtue of selection for excretion of host-inhibiting amino acids, was produced in-country, and distributed for secondary inoculation for on-farm fermentation. By selecting for virulence enhancement using amino acid analogs of leucine, tyrosine, and methionine, we were able to obtain sufficient excretion of these amino acids to inhibit Striga and, to reduce the soil seed bank of Striga because methionine is converted by soil microbes to ethylene, a seed germination stimulant.[30,31] Through this virulence selection method of host-specific fungi, we were able to produce a more effective bioherbicide. This enhanced bioherbicide, distributed to the village-level in the form of fungi embedded in toothpicks and later with wooden dowels, for secondary inoculation of boiled rice, was found to meet the needs of impoverished subsistence farmers, mostly women with on-farm families, in bringing this weed under control on their farms in western Kenya. We describe the genetic selection-based innovations of virulence enhancement of host specific fungi (by their enhanced excretion of certain amino acids). As other weeds are also inhibited by certain amino acids, the techniques involving virulence enhancement can also be used to improve the efficacy of other host specific weed pathogens. In short, the arguments against bioherbicides involve their required specificity, their few successes, and they would be entering a market dominated by the chemical industry with patent protection of limited value. We see bioherbicides in a positive and more sustainable light. They are host-specific, safe, non-toxic, effective if enhanced for virulence, specifically selected for multiple sites of inhibition of a certain weed, lowered chance of weed resistance, and with lower local development and manufacturing costs.[4,6,13] They appear to persist in the soil for many years, providing farmers with a long-term solution. The combined developments of virulence enhancement and the cost-cutting, secondary inoculum distribution systems were key to success of this project. Further development of similar approaches to weed control could have a positive impact on bioherbicidal development in many agricultural regions.

The Toothpick Project's research, development, and adoption of a commercialized microbial herbicide is an optimistic story. The big question is: can we see finances linked with supportive policies to build knowledge for bioherbicide commercialization in the future? Even though we had several obstacles, and we anticipate many updates to our early edition product over the next few decades (particularly in the delivery methodologies), we stand as a positive example of a small team successfully discovering, developing and commercializing a novel bioherbicide. We know government leaders have agricultural and regulatory policies and goals, yet these are often crafted without tangible solutions. Through events such as the United Nations Food Systems Summit, and even through increased mobile phone connectivity in rural agricultural communities, farmers are becoming more heard in the conversations about priority setting. Now that our product has achieved a base-level commercial success, we believe we will see a larger flow of investments into bioherbicide research, development, and ventures.

William Shakespeare (*Julius Caesar* – Brutus):
There is a tide in the affairs of men.
Which, taken at the flood, leads on to fortune;
Omitted, all the voyage of their life
Is bound in shallows and in miseries.
On such a full sea are we now afloat;
And we must take the current when it serves,
Or lose our ventures.

ACKNOWLEDGEMENTS

Funding and support were provided by The Starfish Foundation (San Diego, CA, USA), The Bill and Melinda Gates Foundation, The Kenya Agriculture and Livestock Organization, Welthungerhilfe, DBU (German Environmental Foundation), Dr Peter Lüth, Biotech Investments LLC, United Nations World Food Program, the Ohrstrom Foundation, the Roddenberry Foundation, Milken-Motsepe Prize for Agritech, and the USDA National Institute of Food and Agriculture Hatch Project.

This article was presented orally at the workshop 'Overcoming the barriers to adoption of microbial bioherbicides', which took place in Bari, Italy on 25–29 September 2022, which was sponsored by the OECD Co-operative Research Program: Biological Resource Management for Sustainable Agricultural Systems. The opinions expressed and arguments employed in this article is the sole responsibility of the authors and do not necessarily reflect those of the OECD or of the governments of its member countries.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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