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Current recommendations and novel strategies for sustainable management of soybean sudden death syndrome

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ABSTRACT

The increase in food production requires reduction of the damage caused by plant pathogens, minimizing the environmental impact of management practices. Soil-borne pathogens are among the most relevant pathogens that affect soybean crop yield. Soybean sudden death syndrome (SDS), caused by several distinct species of *Fusarium*, produces significant yield losses in the leading soybean producing countries in North and South America. Current management strategies for SDS are scarce since there are no highly resistant cultivars, and only a few fungicide seed treatments are available. Because of this, innovative approaches for SDS management need to be developed. Here, we summarize recently explored strategies based on plant nutrition, biological control, priming of plant defenses, host-induced gene silencing, and the development of new SDS-resistance cultivars using precision breeding techniques. Finally, sustainable management of SDS should also consider cultural control practices with minimal environmental impact.

Keywords: Sudden death syndrome, *Fusarium virguliforme*, *Fusarium tucumaniae*, disease control, integrated disease management, *Glycine max*.

INTRODUCTION

Soybean sudden death syndrome (SDS) is one of the most yield-limiting soybean diseases in North and South America. Multiple variables influence SDS development, such as edaphic properties of each field plot, the interaction with other soil-borne pathogens like nematodes, the agronomic practices carried out by each producer (rotations, tillage system, fertilization), and the associations with certain field-specific environmental factors of each particular year. These variables interact in many ways, explaining why numerous field experiments have obtained contradictory results in the last 50 years.

The disease is caused by several species of the soil-borne fungus *Fusarium*: *F. virguliforme* (*F.v.*), *F. tucumaniae* (*F.t.*), *F. brasiliense* (*F.b.*), *F. crassistipitatum* (*F.cr.*), and a novel undescribed *Fusarium*

spp. (**Table 1**) □.[1-5] *F. cuneirostrum* was reported to cause SDS in earlier literature, but it was recharacterized and removed from the list in recent classification[6]. These species are classified into clade 2 of the *Fusarium solani* species complex (FSSC).[7] In North America, *F.v.* is widely prevalent, but *F.b.* and *F.cu.* have also been detected in some regions of the USA.[8] In South America, *F.t.* and *F.b.* are the most prevalent species in Argentina and Brazil, respectively, but other *Fusarium* species have also been isolated.[1-4]□ Members of the FSSC are identified based on phenotypic analyses of macro- and microscopic characters and phylogenetic analyses of multilocus DNA sequence data.[1,3-]□[4] Additionally, the comparison of whole-genome sequences has allowed a better characterization of the FSSC, establishing differences in their life cycle,[9,10]□ and in their sensitivity to chemical compounds.[11] Finally, differences in the reproduction between members of FSSC in clade 2 have been described based on the structural organization of the mating-type locus, indicating that some species could be sexually propagated. However, only *F.t.* has been shown to exhibit this type of propagation to date.[10,12-13]

F. tucumaniae and *F. virguliforme* overwinter primarily as chlamydospores both in infested crop residues and free in the soil. These resistance structures can survive in the soil for several years. Sexual recombination of *F. tucumaniae* was confirmed to have occurred. In contrast, *F. virguliforme* never produced perithecia[12]. Therefore, *F. tucumaniae* life cycle in S. America includes a sexual reproductive mode while the *F. virguliforme* population in the U.S. may be exclusively asexual. Other sources of primary inoculum are residues from other crops (such as corn) and alternative hosts. Germinating soybeans plus warming temperatures lead to the germination of the chlamydospores allowing for the infection cycle to begin (Figure1). After planting, the roots of the newly emerging seedlings are particularly susceptible to *Fusarium* and root infection in soybean can occur within days of planting. Primary infection is favored by high soil moisture. Initially, symptoms generated by SDS-causing pathogens of the FSSC include root discoloration, necrosis, and dieback. Later, the pathogen colonizes the vascular tissue in the basal area of the plant, a few centimeters above the soil line. Once established in the xylem, the pathogen produces toxins which are systemically transferred throughout the plant leading to the foliar symptoms. Thus, foliar symptoms are not typically seen until later in the season around the flowering stage. Various phytotoxins were isolated from soybean xylem sap.[14-][16] Among them, a 13.5 kDa protein named Fvtox1 causes interveinal chlorosis and necrosis in leaves of susceptible soybean varieties.[14,17] Interestingly, foliar SDS-like symptoms development requires light, which suggests that FvTox1 alters the photosynthetic process.[14,□18] Besides, toxin-associated symptoms are affected by other environmental conditions such as temperature and media substrate[19-][21] and, consequently, environmental changes over the years can explain the appearance of various degrees of foliar symptoms in the field.[22-23] In addition to this, some reports have shown inconsistencies between SDS foliar symptom severity and yield reduction,[23-24]□ making the screening for SDS-resistant germplasm and evaluation of other management tools more complex. Recently, Kandel *et al.* (2020) found a mean yield decline of 0.51% for each unit increase in a foliar disease index that combines foliar disease incidence and disease severity, providing an estimated yield reduction based on foliar symptoms.[25]

Yield losses caused by severe SDS infection are the result of premature defoliation of soybean plants and decreased seed size.[23,26] Flower and pod abortion can also be observed when severe SDS occurs during flowering and pod-filling.[23,27]□ However, yield losses due to SDS are variable and dependent on the interaction of soybean genotype, *Fusarium* pathotypes present in the field plots, weather and

edaphic conditions such as relative abundance of multiple microbial taxa in the soil, macro- and micronutrients availability, soil pH, macroporosity and cultural practices, among the main factors.[28-30] In terms of its economic impact, SDS is considered to be one of the top ten soybean diseases of North America[31] with average yield losses of 0.82 million metric tons per year between 1996 to 2009[32] and 321,5 million USD per year between 1996 to 2016[31]. Additionally, SDS generates significant yield losses in Argentina and Brazil, two other leading soybean-producing countries[33-34] (Table 1).

Particularly in the USA, the synergism between *F.v.* and *Heterodera glycines*, the soybean cyst nematode (SCN) has been reported to cause a decrease in soybean yield when both pathogens are present in a given field plot. [35-36] According to a three-year research study, Westphal *et al.* (2014) suggested that *F.v.* depended on infections by *H. glycines* to cause highly severe damage.[37] Similarly, Roth et al. (2019) reported that the additional presence of SCN can increase the risk of severe SDS epidemics.[38] In Argentina, the presence of *H. glycines* and other nematode species such as *Meloidogyne incognita*, *M. javanica,* and *Helicotylenchus* spp. has been reported,[39] but their association with SDS is not common (Scandiani M, pers. comm.). In Brazil, *H. glycines* has been reported in at least 10 states,[40-41] but there are no reports of an association between these two pathogens.

Due to the increase in human population and dietary changes, food demand is expected to grow by 60 percent by the year 2050.[42] In parallel, soil depletion, disease risk associated with monoculture, and increasing costs due to fungicide resistance, negatively impact food production and affordability.[43-44] Therefore, improvement in the efficiency of management practices is needed to reduce the environmental impact of agriculture and to increase food production. In the last decades, new practices, such as reduced tillage strategies that use less fuel and preserve organic carbon closer to the surface of the soil, have been implemented in soybean production. However, no-till or reduced tillage can cause an increase in disease pressure, especially of those diseases caused by soil-borne fungal pathogens.[45] Also, the demand for sustainable agriculture calls for the development of alternative strategies for the control of fungal diseases. In particular, the management of SDS is limited due to the lack of highly resistant cultivars and the availability of only a few fungicides.[25,46] Therefore, the development of new tools for the sustainable management of SDS is required. Here, we discuss different strategies currently being tested for SDS management, together with some recent approaches developed for other fungal pathogens based on the use of gene-editing techniques and RNA silencing mechanisms. Finally, we evaluate the efficacy of these tools for the management of SDS (Table 2).

CHEMICAL CONTROL

Infection of soybean root at early seedling stages allows the invasion of xylem and phloem tissues by SDS-causing *Fusarium* species and the translocation of the toxins that cause the aboveground symptoms.[47] Thus, delaying the pathogen infection by seed-applied fungicides can be an effective way to reduce the appearance of foliar symptoms. Recently, fluopyram (ILEVO™, a succinate dehydrogenase inhibitor developed by BASF, Ludwigshafen, Germany) was registered for the treatment of soybean seed to manage SDS. This fungicide reduces the initial establishment of the disease by inhibiting the early stages of the infection process and has proved to be effective against *F.v., F.b.,* and *F.t.*.[11,48-49] In particular, Sjarpe et al.[48] observed that fluopyram seed or in-furrow

treatment reduced SDS and increased yield relative to the control. Moreover, these authors evaluated the effect of fluopyram and other seed treatment and foliar products that have been registered for management of SDS and found that fluopyram provided the highest level of control of root and foliar symptoms of SDS among all the treatments.[49] Although phytotoxicity has been observed in cotyledons when fluopyram was applied as a seed treatment, likely causing a small reduction in plant population, fluopyram seed treatment has an overall positive impact on soybean yield under SDS disease pressure[50]. A second active ingredient, pydiflumetofen (Saltro®, another succinate dehydrogenase inhibitor developed by Syngenta), was also recently introduced to the market. A preliminary report has indicated promising results.[51] A third active ingredient, cyclobutrifluram (TYMIRIUM™), presumed to be an inhibitor of the mitochondrial electron transport chain complex II based on its similarity in chemical structure to fluopyram,[52] has shown to be effective for the control of SDS,[53] but it is not yet commercially available.[54]

Since the only two active ingredients currently available against SDS to date have the same single-site mode of action, the risk of fungicide resistance is high.[55] Based on this, their use should be carefully evaluated to avoid the appearance of resistance. In this sense, sustainable management should consider and quantify the disease risk to bypass the unnecessary use of fungicide seed-treatment.[56] Additionally, fluopyram and pydiflumetofen fungicide sensitivities should be monitored to evaluate and detect changes in the sensitivity to these compounds.[57]

CULTURAL CONTROL

Cold temperatures and wet conditions during the early reproductive stages of soybean increase SDS foliar symptoms.[58-59] Agricultural practices that reduce the exposure to wet and cool weather, such as late planting, have been suggested to lower disease severity.[60-61] However, late-planted soybean produces less yield because of the shorter daylight hours during flowering and seed filling and, hence, it is not recommended as an effective management practice.[61-62]

Another factor influencing SDS development is the degree of soil compaction affecting the root system of soybean plants. Compacted soils tend to promote SDS development[63-65]. Therefore, any measure that allows for more aeration, porosity, and less soil compaction will be beneficial. This will reduce soil moisture and will avoid low soil temperatures, making the disease less intense.[65] In this regard, conventional tillage may be effective to lower the incidence and severity of SDS foliar symptoms.[65-66] However, other authors reported little or no effect of tillage on the intensity of SDS.[67] No-till systems usually depend on herbicide applications, particularly glyphosate, the most widely-used herbicide worldwide since 2001.[68] The effect of glyphosate on SDS development is still unclear. A report indicated that glyphosate can increase the incidence and severity of SDS foliar symptoms,[69] while others claimed that it has no effect.[70-72] Finally, it seems that when assessing the impact of tillage, crop rotation should also be taken into consideration.[73-74]

Fusarium species have several survival strategies such as resting conidia or chlamydospores in soil, debris infestation, and infecting a wide range of host plant species.[23,75] *Fusarium* chlamydospores can survive in the soil for many years.[76] Crop rotation has been proposed as an environment-friendly approach for soil-borne pathogen management.[77] Experiments testing short-term rotations have obtained mixed results, without a clear trend. For example, Pérez-Brandán *et al.*[78] found that SDS incidence was significantly higher under soybean monoculture than when soybean was grown in rotation with maize. Other studies reported that the current corn-soybean rotation is insufficient to

reduce the risk of damage by SDS,[79,80] probably because *F.v.* can remain asymptomatic in maize tissues.[81] These authors suggested that soil suppressiveness is an important component that can impact the SDS-pathogens independently of short-term crop rotations. The impact of long-term crop rotation practices on SDS severity has also been evaluated.[82-83] In particular, SDS incidence and severity were evaluated in a 6-year study including a 3 year-cropping system that incorporated corn-soybean-oat + red clover and a 4-year cropping system that included corn-soybean-oat + alfalfa-alfalfa rotation compared with a 2-year cropping system consisting of corn-soybean rotation.[82] Interestingly, the diversification of the cropping system reduced SDS incidence and severity and increased soybean yield in 5 of 6 years compared with the 2-year system. In this study, the crop rotations were confounded with fertility regime, and may have been affected by the crop rotation as well as the fertility regime used. Thus, the factors involved in the reduction of SDS severity need further characterization. Long-term rotations may help the development of suppressiveness and improvement of soil health which is a key factor to reducing the onset of SDS.[30,78,84] The succession of crops also influences the outcome of the rotation, since not all non-soybean crops are effective in reducing the soil population densities of *Fusarium* species causing SDS. For example, when fescue was incorporated in the rotation, *F.v.* density in soil was higher than when wheat and sorghum were included.[83] In connection with this, other cultural practices that can impact SDS development are growing cover crops and green manures. Cover crops are increasingly being used as a soil conservation practice when planted to improve soil structure, suppress the growth of weeds, and protect the soil from erosion caused by wind and water. However, while several species of legumes used as cover crops, such as *Trifolium* spp, *Medicago sativa*, and *Pisum sativum*, are considered hosts of *F.v.*, other legumes, grasses, cereals, and Brassicaceae cover crops, such as *Vicia villosa*, *Camelina sativa*, *Brassica juncea*, *Pennisetum glaucum*, *Secale cereale*, *Lolium multiflorum*, *Triticale hexaploide,* and *Triticum aestivum*, are nonhosts or poor hosts.[85] Therefore, knowledge of the susceptibility of cover crops to SDS-causing *Fusarium* species can help farmers choose which species should be planted in field plots with a history of SDS epidemics. Similarly, recent research reported promising results when green manure amendments were tested for SDS suppression in experimental plots in the greenhouse.[86] Amendments of oat and rye reduced root rot severity of soybean by 85% and 67%, respectively. However, these experiments involved a short period, until the soybean plants reached growth stage V3 (approximately 40 days after plating), and thus long-term field studies, involving rotations with different crops, are necessary to evaluate the real impact of this practice.

Along with these agronomic measures, precision farming practices, such as the quantification of inoculum level and the determination of the disease risk, are being implemented in some countries.[38,56, 87-91] For example, Roth *et al.*[38] showed that the quantification of *F.v.* abundance in soil at-planting provides valuable information that can be used to develop SDS risk prediction models. This tool may allow farmers to minimize the risk of yield loss, reducing treatment costs and the environmental impact associated with chemical treatments by applying these treatments only where they are required.[56] Additionally, detecting SDS-diseased patches using remote sensing methods is a promising tool that would allow to identify infected fields and adapt the management strategy for the following growing seasons in specific field-patches.[87,89-91]

PLANT NUTRITION

Soil-borne pathogens cause root rot symptoms that affect nutrient uptake. Additionally, fungal toxins and enzymes with hydrolytic and catalytic activities can alter nutrient translocation and utilization[92]. Consequently, nutrition can influence crop diseases and can help to reduce infection symptoms in plants.[93] For example, high calcium (Ca) concentration in plant tissues is correlated with resistance to root diseases caused by *Fusarium solani* (*F.s.*).[94] Besides, potassium fertilization has been linked to a significant reduction in the incidence of many infectious diseases.[93] Nevertheless, while the addition of KCl to the soil notably reduced the SDS severity, different potassium salts had different effects on the growth of *F.v.*[28]. Therefore, the role of nutrients such as K in disease increase or suppression must be examined in conjunction with other mineral elements.

Additionally, sulfur (S) and micronutrients (MNs) such as Mn, copper (Cu), boron (B), zinc (Zn), and molybdenum (Mo) have been suggested as capable of priming systemic defense responses.[93,95] In particular, it was observed that treatment of soybean plants with nanomaterials (NMs) of CuO, B, MoO3 or ZnO significantly decreases the impact of SDS, causing a reduction of 17-25% in root rot severity.[96] The direct effect of NPs over the pathogen was tested *in vitro*, showing no effect. Therefore, the authors suggest that the NPs may act through the increase of plant defenses. Another recent investigation confirmed that foliar applications of Cu-based NMs at the seedling stage significantly reduce SDS symptoms.[97] In addition, the authors demonstrated that Cu treatment alleviates the increased expression of antioxidant enzymes together with the changes in the fatty acid profile, and induces the expression of a set of defense genes. These investigations show that the use of Cu, Zn, B and Bo-based NMs have the potential to be used for management of SDS. Although these responses have been known for many years, it is not yet clear how specifically these ions can trigger defense responses in plants. Even though many studies discuss the impact of macro and micronutrients on host-pathogen relationships, very little research examines the role of nutrition in the control of SDS.

BIOLOGICAL CONTROL

Biological pesticides employ a wide range of beneficial microorganisms (biological control agents, BCAs) that control or suppress populations of plant pathogens, reducing plant disease incidence.[98] In recent years, biological pesticides have gained increased interest due to their eco-friendly properties compared to the use of chemical pesticides. Biopesticides are less prone to generate tolerance in the target pathogens, are less harmful to other beneficial microorganisms, and show an extended persistence due to their natural reproduction capacity.[99] Because of these properties, various investigations have evaluated the use of BCAs and their mode of action for the control of SDS. For example, *Trichoderma harzianum* and two arbuscular mycorrhizal fungus (AMF), *Rhizophagus irregularis* and *R. intraradices*, reduced SDS severity caused by *F.v.*[100-102]. On the other hand, plant growth-promoting bacteria (PGPB) also proved to be effective in controlling SDS-causing *Fusarium* species. Pin viso et al.[103] reported a significant *F.t.* and *F.v.* mycelial growth inhibition caused by *Bacillus subtilis*, *Pseudomonas fluorescens* and *Chryseobacterium vietnamense in vitro*. In the case of *Lysobacter enzymogenes* strain C3, inoculated plants show increased biomass compared to non-inoculated plants.[104] Regarding the mode of action, both *T. harzianum* and *Lysobacter enzymogenes* strain C3 directly suppress *F.v.* mycelial growth.[100,104] Additionally, *T. harzianum* induces the activation of defense responses in soybean plants, increasing the expression of defense-related genes associated with the salicylic acid (SA) and jasmonic acid/ethylene (JA/ET) pathways.

The up-regulation of SA and JA defense-related genes was also detected in the soybean/AMF/*F.v.* interaction,[105] highlighting the importance of these defense pathways in the control of SDS. For the *R. intraradices*/soybean interaction, it was proposed that AMF-mediated root growth may alleviate SDS symptoms.[101] Altogether, these studies show that biological control may be an effective tool for the management of SDS, although additional studies should be performed to evaluate the efficacy of these treatments in field conditions.

PRIMING OF PLANT DEFENSES

Priming is an adaptation mechanism that increases plant defense responses after exposure to abiotic or biotic stimuli. Following the stimulus perception, induced defense mechanisms are activated. [106-107] In general, after the pathogen perception by plant cells systemic acquired resistance (SAR), a long-lasting broad-spectrum systemic defense response, is induced.[108] Generally, priming is triggered by molecules produced by pathogens (PAMPs, from pathogen-associated molecular patterns) that are perceived by plants through appropriate protein receptors. Typical fungal PAMPs include chitosan, a natural biopolymer composed of D-glucosamine and N-acetyl-D-glucosamine that is found in fungal cell walls[109] and generates an immune response in plants called chitosan triggered immunity (CTI). [110] It has been shown that chitosan delayed SDS symptom expression when applied preventively on soybean leaves. Interestingly, chitosan treatment increased the level of chitinase activity in fungal infected leaves, suggesting that it can induce defense responses through CTI against SDS-causing *Fusarium* species in soybean plants. Additionally, chitosan effectively inhibited the *in vitro* growth of *Fusarium.*[111] Priming can also be induced by treatment with defense hormones or some natural secondary metabolites. The effect of salicylic acid (SA) treatment in the induced resistance against *F.s.* has been characterized. Pre-treatment of soybean seedlings with 200 µM of SA reduced the disease symptoms caused by *F.s.* and the relative levels of fungal biomass in soybean seedlings after 3 days post-inoculation. Moreover, SA treatment significantly increased the activity of antioxidant enzymes and reduced the levels of malondialdehyde and hydrogen peroxide. Additionally, the level of SA-responsive genes in fungal infected seedlings was increased compared to non-treated infected seedlings. These results indicate that SA plays a role in the defense against *F.s.*, alleviating SDS symptoms.[112] Similarly, the efficacy of ethephon, an ethylene inducer, was evaluated by Abdelsamad *et al.*[113]. This compound was able to lower SDS foliar symptoms compared to control treatments in both susceptible and SDS-resistant cultivars. Moreover, ethephon treatment induced the ethylene biosynthesis genes, ethylene synthase and ethylene oxidase, and defense response genes like pathogenesis-related proteins, basic peroxidase (*IPER*), chalcone synthase, and defense-associated transcription factors. Particularly, *IPER* was highly upregulated 24 hours after ethephon application compared to the control treatment. Although the precise role of this enzyme was not characterized, additional studies may shed light on its role during *F.v.* infection. Finally, levels of pipecolic acid (Pip), a non-proteinaceous product of lysine catabolism that can induce SAR,[114] were increased in the xylem sap of leaves of *F.v.* infected plants.[115] Thus, this compound may be implicated in the defense response against SDS. Further studies evaluating the L-Pip exogenous effect are needed to clarify the role of this compound in the defense response against *F.v.* Altogether, these results indicate that treatments with natural compounds such as plant hormones and PAMPs could be a promising sustainable approach for partial control of SDS.

Other defense inductors like phosphites (PO_3^{3-}) may be effective for the priming of plant defenses against SDS. Phosphites induce transcriptional changes that primes plants to defend themselves against several fungal pathogens, as was demonstrated in the case of *Phakopsora pachyrhizi* in soybean. Particularly, phosphites highly induced the expression of the enzyme phenylalanine ammonia-lyase in soybean plants[116] which was also up-regulated in a SDS-partially resistant soybean cultivar,[117] and thus, they could be promising compounds for the activation of defense responses against FSSC. Additionally, manganese phosphite inhibited *F.v.* and *F.t.* mycelial growth *in vitro.*[118]

HOST-INDUCED GENE SILENCING

Host-induced gene silencing (HIGS) refers to the strategy that employs plant produced sRNAs (small RNAs) to silence specific genes in a non-viral pathogen to generate protection against pathogens. Hairpin RNA structures can be introduced into the plant genome by transgenesis to trigger the silencing mechanism. Alternatively, a non-transgenic strategy, named spray-induced gene silencing (SIGS), involves the exogenous application of dsRNAs (double-stranded RNAs) or siRNAs (small interfering RNAs) to plants. In recent years, HIGS and SIGS have been proposed as an emerging approach to control filamentous fungi such as *F. graminearum*, *F. oxysporum*, *F. verticilloides*, and *F. culmorum.* [119-120] However, this approach has not yet been exploited against SDS-causing *Fusarium* species. Considering the potential of this technique against filamentous fungi, here we briefly emphasize some relevant aspects for the selection of putative targets that could confer resistance against members of the FSSC. Also, we discuss the advantages of this technique in the light of sustainable management.

To design an effective strategy for SDS control by HIGS, the selection of a suitable pathogen gene that will be targeted by sRNAs must be very well established. Its role in pathogenesis must be considered to avoid those target genes that are redundant for the infection process. Also, it is important to prevent the off-target silencing of host plant or beneficial microorganism genes. [119] Genes involved in protein transport, cell differentiation, conidiation, regulation of primary metabolism, and modulation of plant-hormone pathways are generally essential for pathogenicity making them good target for silencing. Moreover, cell wall-degrading enzymes (CWDEs) are induced during the *F.v.* late infection phase[92] and are required for the invasion of plant tissues,[121] and hence, could be interesting targets for HIGS. Despite the impact of SDS on soybean yield, only a few *F.v.* genes involved in the pathogenesis process have been characterized to date. *FvSTR1*, a striatin orthologue in *F.v.*, plays a role in asexual development and virulence.[122] Interestingly, the disruption of *FvSTR1* resulted in complete loss of virulence in *F.v.* in a greenhouse experiment, showing that this gene could be a promising target for the HIGS strategy against SDS-causing *Fusarium* species. The sucrose non-fermenting protein kinase 1 gene (*FvSNF1*) could also be a target for HIGS against SDS. This gene codifies for a key component of the glucose de-repression pathway and its disruption abolishes the expression of the galactose oxidase gene. Moreover, *Fvsnf1* mutants exhibit a reduced expression of CWDE-coding genes in contrast to the wild-type strain and are severely impaired in their ability to cause SDS on challenged soybean.[123] Additional identification of new fungal genes involved in pathogenesis will increase the repertoire of HIGS targets, and therefore allow the successful implementation of this strategy against SDS.

HIGS has some advantages over the use of fungicides for SDS management since it can avoid the off-target effects against beneficial microorganisms through the careful selection and design of the

fungal targets. Furthermore, the scarce availability of fungicide target molecules increases the risk of selecting resistant strains. The HIGS strategy is advantageous since it is highly versatile in terms of the availability of target RNA molecules in the pathogen, hence, the appearance of HIGS-resistant strains could be easily overcome. [119] Finally, the use of SIGS can replace the employment of transgenic crops and, in this way, bypass the regulatory process required for their approval.

BREEDING FOR SDS RESISTANCE

Tolerant cultivars are the most effective way of managing SDS. Despite the efforts of breeding programs, to date, only moderately resistant genotypes have been identified which can exhibit SDS foliar symptoms under favorable environmental conditions. In recent years, several *loci* and SNPs involved in tolerance against SDS have been identified through quantitative trait loci (QTL) and genotyping-by-sequencing (GBS) analysis, contributing to marker-assisted breeding programs.[26] These findings have been extensively summarized in a previously published review.[124] Considering that the development of improved cultivars by conventional breeding methods takes several years, new breeding techniques (NBTs) have recently appeared as a faster strategy that allows breeders to generate improved varieties in a few generations. NBTs comprise a wide range of techniques that include genome editing, cisgenesis, and intragenesis. Occasionally, changes to DNA sequences introduced by these techniques can be indistinguishable from those that can occur by conventional breeding and, therefore, the regulatory process may be short compared to the time required for the approval of transgene breeding cultivars.[125] The use of NBTs involves the identification of single major resistance genes or the discovery of disease-susceptibility *loci.* Here, we review some recent works that characterize soybean genes whose modification may enable the development of novel SDS resistance cultivars through NBTs.

Two single major genes conferring SDS resistance have been characterized.[126-127] These authors found a set of soybean genes whose expression is downregulated following *F.v.* infection and hypothesized that *F.v.* suppresses the transcription of these genes to induce host susceptibility.[126] In agreement with this, the overexpression of two of these genes, ankyrin repeat-containing protein (GmARP1) and a plasma membrane protein named *Glycine max* disease resistance 1 (GmDR1), were shown to enhance resistance against *F.v.* in transgenic soybean lines. Additionally, the overexpression of GmDR1 confers resistance against a broad spectrum of pathogens such as the soybean cyst nematode, spider mites, and soybean aphids, presumably through the recognition of PAMPs.[127] Interestingly, both defense genes, GmARP1 and GmDR1, were overexpressed under root-specific and infection inducible soybean promoters and, thus, these cultivars could be considered intragenic since soybean genetic elements were employed for the design of expression constructs. Therefore, these findings show that the overexpression of single major genes under endogenous promoters can be an effective strategy to provide resistance against SDS. Nevertheless, more research is needed at the field level to evaluate the durability and applicability of resistant-modified plants.

In recent years, the appearance of genome editing (GE) techniques such as the CRISPR-Cas system has made it possible to improve plant tolerance to biotic stress. This system is more adaptable and cheaper than other GE techniques and enables either DNA mutation or DNA base editing, expanding its uses.[121] CRISPR/Cas can be employed to reduce the susceptibility to fungal pathogens by disrupting the expression of susceptible genes (*S* genes) that are involved in plant sensitivity to

phytotoxins.[128,129] Based on this, the study of S *loci* against SDS has gained relevance in the last years. In the searching for *S loci*, two soybean STAY-GREEN genes (GmSGR1 and GmSGR2) were found to be associated with the resistance to SDS foliar chlorosis.[130] However, soybean plants with a double mutation of GmSGR1 and GmSGR2 stayed green but displayed necrosis and reduced photosynthesis in response to *F.v.* phytotoxins. These detrimental agronomic traits make it impractical to use these S loci for the improvement of soybean tolerance against SDS. Further characterization of not deleterious *S loci* may be an effective strategy in the control of SDS. In this regard, the analysis of FvTox1-induced transcriptional changes in soybean leaves may help characterize additional S *loci* involved in FvTox1 recognition and to develop new SDS tolerant cultivars.[131] Future research on this topic, combined with the development of precise and reproducible SDS phenotyping methods,[124] will favor the generation of new tools for the management of SDS.

CONCLUSIONS AND FUTURE OUTLOOK

Innovative technologies that emerged in recent years, such as gene editing and HIGS, together with a better understanding of the FSSC/soybean interaction, can be exploited to develop new sustainable management strategies for the control of SDS. The adoption of these tools is driven by the lack of efficient management options for the control of this disease. In particular, the current management options for SDS are limited because no completely resistant cultivars are available. Additionally, only two fungicide seed treatments, fluopyram and pydiflumetofen, are commercially available for the control of SDS. There is also an increased risk of fungicide resistance as these two molecules have the same specific target site. Moreover, fungicides generally impact both pathogenic and nonpathogenic fungi, and consequently, tend to reduce the population of beneficial microorganisms that help to avoid soil-borne pathogen epidemics. While efforts to develop resistant and tolerant soybean varieties continue, all available management measures should be applied in an integrated approach to control the incidence of SDS. In the present review, we discuss currently used strategies for SDS control. Besides, we propose new potential approaches that can be implemented. It is expected that in the coming years, new strategies based on recent technologies (HIGS, CRISPR, the use of nano micronutrients) will expand the repertoire of effective tools for the management of SDS. Finally, the implementation of each of these tools should be part of an integrated management strategy for the control of SDS.

DISCLOSURE

All authors declare that they have no competing interests.

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Table 1. Differential aspects of SDS in the three main soybean producing countries

Feature	Argentina	Brazil	USA	Reference
Soybean area in 2020 (million hectares)	16.7	36.9	30.33	Global Market Analysis, FAS, USDA, November 2020
Soybean production in 2020 (million metric tons)	49	126	96.67	
Yield losses caused by SDS	22% (4-46)[34] and up to 90%[136]	38% (30-40)[135,137]	5 to 15% on average, 20 to 80% according to cultivar reaction, weather conditions and growth stage at the time of infection[23,132-134]	23, 34,☐☐ 132-134, 136☐
SDS and cyst nematode association importance	not important / unknown	not important / unknown	Important	37☐
Prevalent *Fusarium* species causing SDS	*F. tucumaniae*[1,2]	*F. brasiliense*[2]	*F. virguliforme*[1]	
Other *Fusarium* species reported to cause SDS[a]	*F. virguliforme*[3] *F. brasiliense*[2] *F. crassistipitatum*[4]	*F. tucumaniae*[1,2] *F. crassistipitatum*[4]	*F. brasiliense*[8]	1-4,8
Other *Fusarium* species associated with SDS[b]	-	*F. paranaense* sp. nov.[137]	*F. phaseoli*[8] *Fusarium* sp.[8(c)]	
Fusarium sexual stage	Present	not reported	not reported	3,12

[a] In South Africa, a novel undescribed *Fusarium* sp. was isolated from soybean and caused root-rot and foliar SDS symptoms on soybean.[5]☐ In Japan, *Fusarium azukicola* sp. nov. was isolated from azuki bean (*Vigna angularis*) and caused root-rot and typical SDS foliar symptoms when inoculated on soybean[138]☐.

[b] *Fusarium* species isolated from soybean and other Fabaceae causing only root rot or root rot and foliar chlorosis, but not typical SDS symptoms on soybean.

[c] undescribed *Fusarium* sp. from FSSC clade 2 and strains in FSSC clade 5 and FSSC clade 11.

Table 2. Efficacy of the management tools reported for SDS control

SDS management practices	Efficacy [a]	Availability [b]	Reference
Fungicide seed treatments			
-fluopyram	+	USA	11, 48-50, 57, 62, 139
-pydiflumetofen	+	USA	51
-cyclobutrifluram	?	(c)	53-54
Tillage	?	ARG, BRA, USA	65-66
Late-planting [d]	+	ARG, BRA, USA	60
Short-term crop rotations	-	ARG, BRA, USA	78-80
Long-term crop rotations	+	ARG, BRA, USA	82- 83
Soil suppressiveness development	+	ARG, BRA, USA	30
Legume cover crops	-	ARG, BRA, USA	85
Other cover crops (grasses, Brassicaceae)	+	ARG, BRA, USA	85
Green manures	+	ARG, BRA, USA	86
Remote sensing methods	+	USA	84, 86-88
Disease risk prediction models	+	USA	38, 88
Plant nutrition	+	ARG, BRA, USA	28, 96, 97
Biological control	+	ARG, USA	100-105
Plant defense induction	+	under research	111-113
Host-induced gene silencing	*	under research	119-120
Genetic resistance	+	ARG, BRA, USA	124, 126-127

[a] − = not effective , + = effective, ? = mixed results, * = expected results in the coming years.

[b] Management practices that are being used or are available.

[c] Registration and commercial release are planned for Latin America in 2021 / 2022.

[d] There is no conclusive evidence to recommend growers to sacrifice yield at late planting to combat SDS regarding early planting.

Figure 1. Soybean sudden death syndrome disease cycle. *Fusarium* overwinters in soybean and corn residues as macroconidia (stained with cotton blue) in sporodochia on root surface (A), early symptoms of root infection (B), late symptoms of root infection (C), leaf and pod symptoms caused by fungal toxins (D), reddish pigmentation is observed in basal stems at the end of the disease cycle (E), *F. tucumaniae* can overwinter as perithecia (F).

Soybean SDS management strategies

Chemical control

Resistant cultivars — Conventional breeding, NBTs

Plant nutrition

Host-induced gene silencing (HIGS, SIGS)

Biological control/ plant defense priming

Cultural control

B

Early root infection of seedlings

C

Brown to grey discoloration of the vascular system

D

Leaf symptoms (fungal toxins travel upward from roots)

Leaf and pod may drop (petioles remain on stem)

Fusarium overwinters in soybean and corn residues, alternative hosts and soil (chlamydospore)

***F. tucumaniae* overwinters as perithecia (sexual stage)**

F

A

Sporulation of macroconidia (left) in sporodochia (middle) on root surface (right)

E

Basal stem with reddish pigmentation