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Integrated Pathogen Management in Stevia Using Anaerobic Soil Disinfestation Combined with Different Fungicide Programs in USA, Mexico, and Paraguay

Andres D. Sanabria-Velazquez [1,*], Guillermo A. Enciso-Maldonado [2], Marco Maidana-Ojeda [2], Jose F. Diaz-Najera [3], Sergio Ayvar-Serna [3], Lindsey D. Thiessen [1] and H. David Shew [1]

[1] Department of Entomology and Plant Pathology, North Carolina State University (NCSU), Raleigh, NC 27695, USA; ldthiess@ncsu.edu (L.D.T.); shew@ncsu.edu (H.D.S.)
[2] Centro de Desarrollo e Innovación Tecnológica (CEDIT), Ruta 6 Km 38, Hohenau 6290, Itapúa, Paraguay; guillermo.enciso@cedit.org.py (G.A.E.-M.); marco.maidana@cedit.org.py (M.M.-O.)
[3] Departamento de Fitotecnia, Colegio Superior Agropecuario del Estado de Guerrero (CSAEGRO), Iguala CP 40000, Guerrero, Mexico; sergio.ayvar@csaegro.edu.mx (S.A.-S.)
* Correspondence: adsanabr@ncsu.edu

Abstract: Stevia is a semi-perennial crop grown to obtain the diterpene glycosides in its leaves, which are processed to manufacture non-caloric sweeteners. Anaerobic soil disinfestation (ASD) and fungicide application were evaluated for the management of stevia stem rot (SSR) and Septoria leaf spot (SLS) in lab and field experiments. In 2019 and 2021, experiments using carbon sources for ASD were carried out in microplots at NCSU (Clayton, NC, USA). In 2020/21 and 2021/22 seasons, field experiments were conducted at CSAEGRO, Mexico (MX) and CEDIT, Paraguay (PY) using a 2 × 3 factorial design with two ASD treatments and three fungicide treatments. ASD treatments included soil amended with cornmeal (MX) or wheat bran (PY) at a rate of 20.2 Mg ha^{-1}, molasses at 10.1 Mg ha^{-1}, and non-amended controls. Fungicide applications included chemical (azoxystrobin), organic (pyroligneous acid, PA), and a non-treated control. ASD was effective in reducing sclerotia viability of *Sclerotium rolfsii* in laboratory assays ($p < 0.0001$) and microplot trials ($p < 0.0001$) in NC. During field trials, the viability of sclerotia was significantly reduced ($p < 0.0001$) in soils amended with cornmeal + molasses or wheat bran + molasses as carbon sources for ASD. While there was no significant effectiveness of ASD in reducing SLS in 2020 and 2021 or SSR in MX 2020 field trials ($p = 0.83$), it did exhibit efficacy on SSR in 2021 ($p < 0.001$). The application of fungicides was significantly effective in reducing SSR ($p = 0.01$) and SLS ($p = 0.001$), with azoxystrobin being the most consistent and PA not being statistically different from the control or azoxystrobin. The effects of ASD on fresh yield were inconsistent, exhibiting significant effects in Mexican fields in 2020 but not in 2021. During Paraguayan field trials, ASD only significantly interacted with fungicide applications in the dry yield in 2022. In the 2020/21 MX and 2020 PY field trials, fungicides were significantly effective in enhancing dry leaf yields, with azoxystrobin showing the highest consistency among treatments and PA variable control. In conclusion, utilizing ASD alongside organic fungicides can be a valuable tool for stevia farmers when the use of chemical fungicides is limited. Further research is required to enhance consistency and reduce the costs associated with these treatments under diverse edaphoclimatic conditions.

Keywords: *Stevia rebaudiana* [Bertoni]; sweetener; organic agriculture; pyroligneous acid; soil disinfestation; azoxystrobin; *Sclerotium rolfsii*; *Septoria steviae*

Citation: Sanabria-Velazquez, A.D.; Enciso-Maldonado, G.A.; Maidana-Ojeda, M.; Diaz-Najera, J.F.; Ayvar-Serna, S.; Thiessen, L.D.; Shew, H.D. Integrated Pathogen Management in Stevia Using Anaerobic Soil Disinfestation Combined with Different Fungicide Programs in USA, Mexico, and Paraguay. *Agronomy* **2023**, *13*, 1358. https://doi.org/10.3390/agronomy13051358

Academic Editors: Joji Muramoto, Carol Shennan, Erin Rosskopf and Noriaki Momma

Received: 17 March 2023
Revised: 1 May 2023
Accepted: 6 May 2023
Published: 12 May 2023

1. Introduction

Stevia (*Stevia rebaudiana* [Bertoni]) is a semi-perennial herbaceous plant grown to obtain the diterpene glycosides in its leaves, stevioside and rebaudioside, processed by the food industry for the manufacture of non-caloric sweeteners [1]. Paraguay cultivates approximately 800 ha of commercial stevia [2]. Stevia was introduced to Mexico in 2010

through the Instituto Nacional de Investigaciones Forestales y Agropecuarias (INIFAP) to investigate if this crop could be adapted to the region [3]. Subsequently, stevia production expanded to 57 ha under irrigation by 2014 and continues to increase. In North Carolina (NC) and the southeastern United States of America (USA), cultivation of stevia began as an experimental rotation crop in 2011 [4]. Many agronomical variables, such as crop nutrition, spacing, and pest management, are still being adjusted for the commercial development of stevia in NC [5,6].

The increase in stevia production areas brought a corresponding increase in plant diseases, limiting the commercial potential of the crop. The most significant soilborne pathogen of cultivated stevia is *Sclerotium rolfsii* Sacc. 1911 (teleomorph *Athelia rolfsii* [Curzi] C.C. Tu & Kimbr. 1978), the causal agent of stevia stem rot (SSR) [7]. This disease can significantly reduce the plant stand and drastically reduce yield; moreover, it is difficult to manage because of its broad host range and production of sclerotia that overwinter for several seasons in the soil [8]. Another significant disease is the foliar pathogen, *Septoria steviae* (teleomorph unknown Ishiba, T. Yokoy. & Tani. 1982) [9], a significantly yield-limiting foliar pathogen that causes Septoria leaf spot (SLS) [10]. Ishiba et al. [9] observed this fungus causing necrotic leaf spots across stevia production areas and described the causal agent as *S. steviae*. The fungus can overwinter in leaf debris buried in soil, serving as primary inoculum during periods of precipitation [6,10]. Water drops disperse the spores from the ground, causing new lesions on healthy plants [10]. The disease is more intense in areas with conditions of high temperature and humidity for prolonged periods [11].

Farmers can produce stevia under organic or conventional production systems depending on the market demands. Conventional stevia is produced with small amounts of mineral fertilizers and chemical pesticides [6,12]. In contrast, organic stevia production relies on organic matter fertilization and biological or mechanical pest controls [13,14]. Therefore, disease management strategies for both production systems are necessary to control SSR and SSL, which can significantly reduce yields [6,8].

Stevia is a new specialty crop in the USA, so no fungicides are labeled to manage pathogens in this crop, though multiple fungicides are still in IR4 testing [8]. Some alternatives used in organic agriculture are biocontrol agents, copper, and sulfur-based fungicides. However, organically approved products tested, such as Serenade Opti (*Bacillus subtilis* QST 713; Bayer Crop Science, Research Triangle Park, NC, USA) and Kocide 3000 (copper fungicide, Dupont, Wilmington, DE, USA), did not significantly reduce the severity of the disease compared to non-treated controls [12]. Therefore, there is a need to continue investigating disease controls under conventional and organic agriculture systems.

A new alternative for soilborne pathogen management is anaerobic soil disinfestation (ASD). This technique requires incorporating carbon sources into the soil that microorganisms can metabolize under anaerobic conditions [13]. During the process, compounds unfavorable to pathogen survival are released into the soil [14,15]. The incorporation of organic matter into the soil is followed by irrigation until the soil reaches field capacity. Once the soil is saturated with water, plastic mulch is placed for 3–6 weeks [16], which creates anaerobic conditions in the soil [15]. In the context of integrated management, after conducting ASD, it is possible to apply organic fungicide programs targeted at both the base and leaves of the stevia plants for further disease management [17]. One of these fungicides, used in Japanese traditional agriculture, is pyroligneous acid (PA), a byproduct of the pyrolysis process of plant material [18]. The application of PA induces plant growth and has direct effects against plant pathogens; previous research has shown inhibition of plant pathogenic fungi and bacteria when treated with PA [19]. Soil treated with PA may also increase biological activity that stimulates the suppression of multiple soilborne pathogens [19,20]. Therefore, applying soil disinfestation combined with fungicides such as PA is compatible with conventional and organic production systems and can be an important tool for integrated pathogen management (IPM) in stevia.

Using organic amendments and botanical fungicides to manage plant pathogens could be a promising alternative for enhancing the productivity of stevia smallholder farmers when

the use of chemical fungicides is limited. We hypothesize that soil organic amendments can induce conditions unfavorable to pathogen survival when used for ASD and coupled with the application of fungicide programs targeted at stevia plants, satisfactory yields can be obtained in stevia production systems. The objectives of this study were to (i) evaluate the efficacy of ASD with different carbon sources, separately and combined, for the management of stevia pathogens in USA, Mexico, and Paraguay; (ii) evaluate the efficacy of organic fungicide applications in reducing stevia diseases; and (iii) evaluate the efficacy of the combined use of ASD and the application of fungicide programs on stevia yield in Mexico, and Paraguay.

2. Materials and Methods

2.1. Fungal Isolates

For in vitro and microplot trials, one isolate of *S. rolfsii* was obtained from stevia plants with stem rot symptoms in NC [8]. For field trials in Paraguay, one isolate of *S. rolfsii* was previously isolated from stevia cv. "Eirete" grown in the experimental field of the Instituto Paraguayo de Tecnologia Agraria in Cordillera, Paraguay [2]. For field trials in Mexico, one isolate of *S. rolfsii*, previously isolated from tomato cv. "DRD 8551" at Colegio Superior Agropecuario del Estado de Guerrero, Mexico, was used after the pathogenicity to stevia was confirmed. All fungal isolates were kept on potato-dextrose-agar (PDA) at 4 °C in slant tubes and were transferred to fresh PDA before experiments. To obtain sclerotia of *S. rolfsii*, 4-week-old stevia plants were inoculated with the mycelia of *S. rolfsii* in the greenhouse. After three weeks, the sclerotia were collected from the dead plants and utilized in field experiments. Additionally, the plant debris colonized by *S. rolfsii* was utilized to infest microplots. For SLS evaluation, the disease developed naturally in all fields.

2.2. Fungicides

For field trials, organic and chemical fungicide programs were considered:

Organic fungicide: For all the trials in Mexico and Paraguay, PA was included due to its use in stevia production in Paraguay, Brazil, and Japan as an alternative pesticide [18,21–24]. The PA was comprised of 75% three- to five-year-old Eucalyptus (*Eucalyptus grandis* [Hill ex Maiden]) and 25% five- to seven-year-old Neem (*Azadirachta indica* [Juss.]) wood [25] with a rate of 30 mL L^{-1} of water [26]. The PA was prepared following the methodology described by Campos [27].

Chemical fungicide: Fungicides containing azoxystrobin as the active ingredient (a.i.) were used in the trials with a rate of active ingredient 230 g ha^{-1} [6,8,26,27]. In Paraguay, Quadris™ (22.9% a.i., Syngenta, Greensboro, NC, USA) was used, and in Mexico, the fungicide Amistar™ (25% a.i., Syngenta, Greensboro, NC, USA) was used.

2.3. Anaerobic Soil Disinfestation Laboratory Simulation

The sandy loam soil utilized in this assay was obtained from the experimental stevia field in the Upper Coastal Plain Research Station, Rocky Mount, NC, USA. Soil disinfestation simulations were arranged using a completely randomized design with five replicates and seven treatments: cornmeal, molasses, wheat bran, cornmeal + molasses, and wheat bran + molasses amended soils; in addition, two treatments served as controls, an aerobic control without a carbon source and saturation and an anaerobic control saturated with water but without a carbon source. Sixty-five grams of soil per Petri plate were mixed with various potential carbon sources for ASD (molasses, cornmeal, wheat bran, plus their combinations) to a rate of 2 kg of carbon source m^{-2} of soil and placed in a Petri plate of 9 cm diameter (Figure S1). Subsequently, water was added to the mixture until soil saturation. A 9 cm diameter Petri plate with acidified PDA medium and a mycelial plug of *S. rolfsii* was placed facing the bottom of the Petri plate with soil. The Petri plates were sealed using electrical tape to minimize gaseous exchange with the external environment (Figure S1). Plates were incubated in darkness for four weeks (28 days) at room temperature (28 ± 2 °C). The same experiment was replicated once more.

The mycelial growth of *S. rolfsii* was measured as the area of the colony in cm^2 using the ImageJ program [28], and the number of sclerotia produced was counted (Figure S1). Mycelia from all the colonies were transferred to a fresh PDA medium to check regrowth, incubating in darkness for four weeks at room temperature. Soil oxidation-reduction potential (ORP) was measured using a pH meter (Accumet AB 15/15z bench-top meter, Fisher Scientific, NJ) combined with an ORP electrode, and readings were recorded in millivolts (mV).

2.4. Effect of Carbon Sources Incorporated into the Soil on Stevia Stem Rot Incidence

In 2019 and 2021, experiments using carbon sources for ASD were carried out in 1 m^2 microplots at the Central Crops Research Station-NCSU, Clayton, NC, USA (Figure S2). The combined effect of plastic mulching and the incorporation of molasses or cornmeal into the soil was evaluated using a $2 \times 2 \times 2$ factorial design for a total of eight treatments: plastic mulch + cornmeal, plastic mulch + molasses, plastic mulch + cornmeal plus molasses, plastic mulch + no carbon sources, no plastic mulch + cornmeal, no plastic mulch + molasses, no plastic mulch + cornmeal plus molasses, and no plastic mulch + no carbon sources. Experiments were established using a completely randomized block design with five replications, and each microplot was considered an experimental unit (EU). Each EU was infested with 350 g of stevia debris colonized by *S. rolfsii* just before the application of carbon sources to evaluate disease progression after ASD was completed. Debris and carbon sources (rate of 2 kg m^{-2}) were incorporated manually into the soil to a depth of 15 cm using a hoe. Before saturation of the microplots, mesh packets containing ten sclerotia of *S. rolfsii* were interred in the center of each microplot to monitor the effect of the treatments on sclerotia germination. Immediately after the incorporation of carbon sources, microplots were flooded with water to field capacity and covered with black plastic mulch (polyethylene selective reflecting mulch 1.0 mm. thick; Berry Global©, Charlotte, NC, USA), ensuring that the borders were sealed entirely while the rest of the plots were left uncovered. The microplots were kept sealed for four weeks. Once the four weeks were completed, the mesh packets with sclerotia were recovered, the inoculum's viability was assessed, and the soil oxidation-reduction potential was measured. Holes were made in the plastic mulch to allow the dissipation of phytotoxic gases. After one week, 6-week-old stevia-rooted cuttings line "G3" were transplanted into microplots to evaluate the percentage of plants with stem rot symptoms (Figure S2).

Sclerotia of *S. rolfsii* in the mesh packets were recovered after soil disinfestation, cleaned, surface-disinfested, and plated on Bromophenol-blue PDA medium to evaluate their viability [29]. Sclerotia viability was evaluated in percentage; samples were considered 100% viable if all sclerotia germinated. In addition, stem rot incidence progression was evaluated by assessing the percentage of plants with wilting symptoms in each plot. Disease incidence was recorded weekly, starting 15 days after planting (DAP) stevia seedlings until the control plots were dead. The progression of the incidence of the disease was expressed as the standardized area under the disease progress curve (sAUDPC) [8]. The weed density was evaluated by counting the number of weeds in the total area of the microplot and expressing them as the number of weeds per m^2, as previously described in other studies [30,31] (Figure S2).

2.5. Effectiveness of Integrated Pathogen Management under Field Conditions

Trials were conducted to assess the effectiveness of ASD combined with the application of fungicides for managing SRR and SLS under field conditions. Field experiments were carried out in 2020 and 2021 at CSAEGRO, Guerrero, Mexico, and in 2019/2020 and 2022 at CEDIT, Itapua, Paraguay (Tables 1 and S1). For all trials, fertilization with 0.5 kg m^{-2} of compost (2N-2P-2K), plastic mulch, drip irrigation, and management practices were employed as commonly performed by smallholder farmers [2]. No other chemical treatments were added to the test plots during the experiments.

Table 1. Description of each location's characteristics and conditions during the field experiments combining anaerobic soil disinfestation and application of fungicides to manage stevia diseases.

Location	Season	Soil Type	Previous Crops	ASD [s]	Tarp. [t]	Carb. Source [u]	Plas. [v]	Plant Density [w]		Fun. [x]	Cult. [y]	Harvest [z]
CEDIT, Itapua, Paraguay	2019/2020	Clay loam	Tomato, Soybean	19 December	3	W + M	Black/white	100,000	30 × 20 × 70	4	Eirete	20 April
	2022	Clay loam	Tomato, Carrot	9 January	4	W + M	Black/white	55,000	50 × 30 × 70	3	Eirete	30 April
CSAEGRO, Guerrero, Mexico	2020	Clay soil	Pepper	20 June	4	C + M	Black	100,000	30 × 20 × 70	4	Morita II	20 October
	2021	Clay soil	Stevia	16 June	4	C + M	Black	100,000	30 × 20 × 70	4	Morita II	21 October

[s] Date that the anaerobic soil disinfestation (ASD) was performed. [t] Tarping period in weeks after ASD. [u] Carbon sources used for ASD. M = molasses at a rate of 10.1 Mg ha^{-1}; C = cornmeal at a rate of 20.2 Mg ha^{-1}; W = wheat bran at 20.2 Mg ha^{-1}. [v] Type of plastic used for ASD. Black/white = white on black plastic mulch 1.0 mm. thick. Black = black plastic mulch 1.0 mm. thick. [w] Plant density and spacing in cm between plants in double row beds. [x] In Paraguay 2019/2020, 4 applications were done in (1) 20 January (2) 10 February 22 (3) 3 March (4) 24 March; and in 2022, 3 applications were done in (1) 2 March 22 (2) 19 March 22, and (3) 2 May 22. In Mexico 2020, 4 applications were done in (1) 27 July (2) 18 August (3) 9 September (4) 6 October; and in 2021 (1) 19 July (2) 9 August (3) 2 September (4) 27 September. [y] Stevia cultivars used in field experiments. [z] Date that the plants were harvested.

Treatments consisted of 2 × 3 factorial arrangements where factor A was the soil disinfestation treatment and factor B was the fungicide application. Treatment combinations were: ASD + no fungicide application, ASD treated + chemical fungicide application (azoxystrobin, Table 1), ASD soil + organic fungicide application (PA, Table 1), non-amended soil + no fungicide application, non-amended soil + chemical fungicide application, and non-amended soil + organic fungicide application. The trials were arranged in a randomized complete block experimental design with five replications. Before the experiments, the soil was tilled and leveled and plot beds were prepared with 1 m width × 3 m length with two spaced plant rows per bed (Table 1).

For all the ASD treatments, molasses (10.1 Mg ha^{-1}) + cornmeal or wheat bran (20.2 Mg ha^{-1}, Table 1) were incorporated 15 cm deep into the soil beds using hoes (Figure S3). Ten sclerotia of *S. rolfsii* contained inside mesh packets together with Hobo temperature loggers (Onset Computer, Bourne, MA, USA) that recorded the temperature every hour were buried in the center of the plots to a depth of 6 cm. Immediately, plots were saturated with water using double drip irrigation, and the plots were covered with plastic film, sealing the edges with soil to entrap volatile compounds generated by the degradation of carbon sources (Figure S3). The controls were not treated with carbon sources nor saturated but kept the plastic mulch. The soil treatment lasted four weeks. Mesh packets with sclerotia were recovered, and the viability of the inoculum was assessed. Holes for planting were made in the plastic mulch, and after one week, six-week-old stevia seedlings were transplanted into plots (Table 1). Beginning at 15 DAP, SSR disease incidence and SLS severity were evaluated weekly (Figure S3). Similarly, fungicide applications began 15 DAP with treatments indicated in Table 1. Fungicides were applied with calibrated sprayers to the base of the plants and on the upper canopy of the plants.

Sclerotia of *S. rolfsii* in the mesh packets were recovered after soil disinfestation, cleaned, surface-disinfested, and plated on Bromophenol-blue PDA medium to evaluate their viability. Sclerotia viability was evaluated in percentage; samples were considered 100% viable if all sclerotia germinated. In addition, stem rot incidence progression was evaluated by assessing the percentage of plants with wilting symptoms in each plot. The progression of the incidence of the disease was expressed as the sAUDPC. Disease incidence was recorded weekly, starting 15 DAP stevia seedlings until they were harvested.

SLS severity progression and plant yield were evaluated only for the field trials. Severity was evaluated through visual assessment of the percentage of necrotic leaf area of stevia with symptoms only from the center rows of each plot [32]. Disease severity was recorded weekly, starting 15 DAP stevia until plants were harvested. The progression of the severity of the disease was expressed as the sAUDPC. Ninety DAP, the fresh and dry yields of all plants from each plot were measured in kg ha^{-1}, harvesting manually using

pruning shears by cutting 8 cm above the base of the plant's main stem. Fresh leaf weight was measured in the field with a portable scale by separating leaves from stems, and dry weight was measured after 48 h of drying and then weighed using an electronic scale. In addition, the cost of disease management was estimated for each treatment combination in terms of annual stevia production with plastic mulch and irrigation based on prices of each region as reported in previous studies [33–37] (Tables S2–S4).

2.6. Statistical Analyses

All statistical analyses were done in SAS (Ver. 9.4, SAS Institute Inc., Cary, NC, USA). Data from ASD simulation and microplots from two experiments were pooled and analyzed using ANOVA, and treatment means were compared using Tukey's test with a family-wise error rate of 5%. Field trial data were analyzed and presented separately for each trial and location after checking for significant interaction between the experiment run and treatments. Data were analyzed using a mixed-model ANOVA where treatments were fixed effects, while blocks and experimental error were random effects. Treatment means were compared using the LSMEANS with the SLICE statement when there was a significant interaction between the treatments with an error rate of 5%.

3. Results

3.1. ASD Simulation Using Various Carbon Sources

In soils amended with carbon sources, there was a significant reduction in mycelial growth of *S. rolfsii* compared to the covered and uncovered controls ($p < 0.0001$; Table 2). The highest growth reduction was observed for the wheat bran + molasses combination with a colony area of 2.33 (±1.92) cm^2. However, cornmeal, molasses, wheat bran, and cornmeal + molasses were not significantly different in inhibiting mycelial growth (Table 2, Figure S1).

Table 2. Effect of anaerobic soil disinfestation (ASD) simulations utilizing various carbon sources on mycelial growth of *Sclerotium rolfsii*.

Treatment [y]	Mycelial Growth (cm^2)	Number of Sclerotia	Soil Redox Potential (mV)
Cornmeal	3.61 ± 2.11 a [z]	1 ± 1.29 a	−282.6 ± 112.53 a
Molasses	9.04 ± 10.93 a	7 ± 11.32 a	−274.7 ± 93.62 a
Wheat bran	2.42 ± 1.79 a	0 ± 0.63 a	−288.9 ± 80.95 a
Cornmeal + molasses	3.16 ± 2.70 a	0 ± 0 a	−317.4 ± 87.20 a
Wheat bran + molasses	2.33 ± 1.92 a	0 ± 0 a	−250.6 ± 40.08 a
Covered	52.31 ± 8.80 b	201 ± 32.05 b	286.2 ± 99.03 b
Uncovered	57.55 ± 4.66 b	209 ± 21.99 b	417.1 ± 62.65 c
p-value	<0.0001	<0.0001	<0.0001

[y] Uncovered = non-amended non-saturated control; Covered = non-amended saturated plastic covered control; Molasses = molasses at 20.2 Mg ha^{-1}; Cornmeal = cornmeal at of 20.2 Mg ha^{-1}; Wheat bran = wheat bran at of 20.2 Mg ha^{-1}. [z] Values in a column indicated by different letters are significantly different at $p < 0.05$ according to Tukey's test. Values are means ± standard deviation with five replications. Data represent the combined results of two trials.

The number of sclerotia produced by *S. rolfsii* colonies was significantly lower in the plates that were treated with carbon sources compared to the covered and uncovered controls, which had a higher number of sclerotia ($p < 0.0001$; Table 2, Figure S1). No sclerotia were observed in Petri plates treated with cornmeal + molasses and wheat bran + molasses. However, these treatments were not significantly different from wheat bran, cornmeal, and molasses (Table 2). All the sclerotia collected from these experiments germinated when transferred to fresh PDA regardless of the treatment, confirming the fungistatic effect of the treatments.

The oxidation-reduction potential of the soil was significantly lower in the plates treated with carbon sources compared to the covered and uncovered controls, which had a higher soil redox potential ($p < 0.0001$; Table 2). The lowest soil redox potential was

observed for cornmeal + molasses (−317.4 ± 87.20 mV). However, these treatments were not significantly different from wheat bran + molasses, cornmeal, molasses, and wheat bran (Table 2).

3.2. Effect of Carbon Sources Incorporated into the Soil on Stevia Stem Rot Incidence

The germination of *S. rolfsii* sclerotia was significantly reduced when plots were carbon amended and covered with plastic ($p < 0.0001$; Table 3). Sclerotia placed in ASD cornmeal-amended plots had 11% viability, while those amended with molasses had 20%, and those with a combination of cornmeal and molasses had 4%.

Table 3. Effect of plastic mulch coverture and different carbon sources for anaerobic soil disinfestation (ASD) during four weeks in microplots, Clayton, NC, USA.

Plastic Mulch [x]	Carbon Sources [y]		Sclerotia	sAUDPC	Temperature	Redox	Weeds Count
	Cornmeal	Molasses	(%) [w]		(°C)	(mV)	(weed m^2)
Plastic mulch	Yes	No	11 ± 14 a [z]	12.87 ± 9.09 a	30.50 ± 0.43 b	−342.20 ± 46.69 a	3.00 ± 5.10 a
	No	Yes	20 ± 14 a	12.13 ± 12.26 a	30.00 ± 0.26 b	−312.00 ± 27.75 a	6.00 ± 9.59 a
	Yes	Yes	04 ± 06 a	9.90 ± 10.53 a	30.42 ± 0.42 b	−390.40 ± 72.13 a	7.80 ± 15.30 a
	No	No	71 ± 19 b	25.90 ± 4.22 ab	29.58 ± 0.26 b	397.40 ± 61.47 b	1.60 ± 2.30 a
No plastic mulch	Yes	No	93 ± 06 b	45.78 ± 7.96 c	29.94 ± 0.27 b	288.40 ± 45.98 b	37.60 ± 42.51 ab
	No	Yes	69 ± 24 b	19.37 ± 11.01 ab	29.22 ± 0.56 ab	326.00 ± 74.22 b	23.40 ± 18.23 ab
	Yes	Yes	73 ± 29 b	48.08 ± 8.39 c	30.36 ± 1.03 b	235.40 ± 341.44 b	33.80 ± 31.63 ab
	No	No	91 ± 12 b	35.93 ± 9.52 bc	27.92 ± 1.71 a	346.40 ± 47.40 b	81.40 ± 60.76 b
p-value							
Main effects	A = Plastic mulch		<0.0001	<0.0001	0.0018	<0.0001	0.0002
	B = Cornmeal		0.0044	0.0475	<0.0001	<0.0001	0.4217
	C = Molasses		0.0001	0.0101	0.028	<0.0001	0.1665
Interaction	A× B		0.0009	<0.0001	0.0501	0.0004	0.3314
	A × C		0.4903	0.8279	0.1318	0.0003	0.0655
	B × C		0.0451	0.0134	0.1318	0.0008	0.1515
	A × B × C		0.0681	0.479	0.6723	0.0003	0.1574

[w] Percentage of *S. rolfsii* sclerotia germinated. [x] No Plastic mulch = non-saturated control non-covered; Plastic mulch = saturated plastic covered. [y] Molasses = molasses at 20.2 Mg ha^{-1}; Cornmeal = cornmeal at 20.2 Mg ha^{-1}. [z] Values in a column indicated by different letters are significantly different at $p < 0.05$ according to Tukey's test. Values are means ± standard deviation with five replications.

Only flooding and covering with plastic mulch was non-significant on sclerotia viability (Table 3). No significant reduction in sclerotia viability was observed when amendments were incorporated into the plots without covering them with plastic mulch.

The disease progression of stem rot was significantly lower in ASD plots than in control plots ($p < 0.0001$). The lowest sAUDPC values were registered on ASD plots amended with cornmeal + molasses; however, these were not statistically different from plots amended with molasses or only cornmeal (Table 3). Non-amended ASD plots had sAUDPC values of 25.90 (±4.22), being not statistically different from carbon amended ASD plots or the control. The incorporation of molasses without ASD significantly reduced the progression of stem rot (19.37 ± 11.01) compared to only applying cornmeal or cornmeal + molasses (Table 3). The average soil temperature was significantly higher in ASD plots compared to the control plots ($p < 0.0001$; Table 3). The non-amended ASD plots showed an average temperature of 29.58°C, which was not significantly different from the temperatures observed in plots where only carbon sources were added.

A significant decrease in the ORP was observed in ASD plots ($p < 0.0001$). Soils from carbon amended ASD had statistically lower ORP values compared to control plots (Table 3). In ASD plots without carbon sources added, no significant decrease in the soil reduction potential was observed (397.40 ± 61.47 mV).

The number of weeds per m^2 was significantly lower in plots covered with plastic mulch compared to non-treated plots without plastic coverture ($p < 0.0001$). In plots without plastic but with carbon sources incorporated, the number of weeds per m^2 was not statistically different from the non-treated control plots (Table 3, Figure S2).

3.3. Effectiveness of Integrated Pathogen Management under Field Conditions

3.3.1. Soil Temperatures

Based on the data collected in Mexico and Paraguay, soil temperatures were significantly affected by ASD ($p < 0.001$). In Mexico, the mean soil temperatures in 2020 were notably higher in plots treated with ASD (38.04 ± 4.46 °C) compared to non-treated plots (33.62 ± 3.12 °C; Figure 1A). This trend continued in 2021, with ASD plots experiencing higher temperatures than non-treated plots. Meanwhile, in Paraguay, soil temperatures were significantly higher in plots treated with carbon sources in both 2020 and 2022 compared to non-treated plots (Figure 1B).

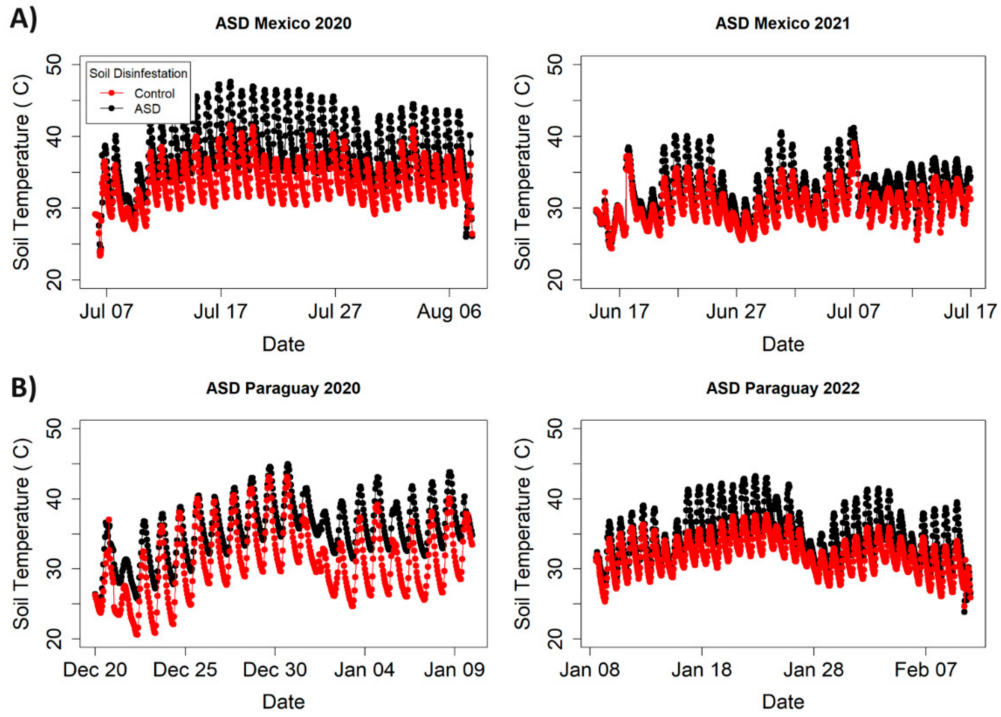

Figure 1. Mean soil temperatures (°C) were recorded under plastic mulch at 6 cm depth during anaerobic soil disinfestation (ASD). (**A**) 2020 and 2021 field trials in Guerrero, Mexico. (**B**) 2020 and 2022 field trials in Itapúa, Paraguay. Treatments were: Control = non-amended non-saturated plastic covered control; ASD = plastic covered saturated amended with molasses at 10.1 Mg ha^{-1} and cornmeal at 20.2 Mg ha^{-1} (in Mexico) or wheat bran in a rate of 20.2 Mg ha^{-1} (in Paraguay).

3.3.2. Sclerotia Viability

In the field trials conducted in Mexico and Paraguay, it was observed that the viability of sclerotia buried in ASD plots was significantly lower than those placed in control plots ($p < 0.0001$; see Table 4).

Table 4. Effect of the combination of plastic mulch coverture and different carbon sources for anaerobic soil disinfestation (ASD) during four weeks on *Sclerotium rolfsii* sclerotia viability (%) during field trials in Guerrero, Mexico and Itapúa, Paraguay.

	Mexico		**Paraguay**	
Treatment [y]	**2020**	**2021**	**2020**	**2022**
ASD	15.0 ± 17.6 a [z]	28.4 ± 17.7 a	14.6 ± 10.6 a	21.2 ± 17.2 a
Control	45.8 ± 23.9 b	59.5 ± 28.6 b	58.2 ± 20.8 b	79.7 ± 13.1 b
p-value	<0.0001	<0.0001	<0.0001	<0.0001

[y] Control = non-amended non-saturated plastic covered control; ASD = plastic covered saturated amended with molasses at 10.1 Mg ha^{-1} and cornmeal at 20.2 Mg ha^{-1} (in Mexico) or wheat bran in a rate of 20.2 Mg ha^{-1} (in Paraguay). [z] Values in a column indicated by different letters are significantly different at $p < 0.05$ according to Tukey's test. Values are means ± standard deviation with five replications.

3.3.3. Disease Progression

The effect of soil disinfestation on the progression of SSR was not significant for field trials in Mexico in 2020 (p = 0.83). Additionally, the interaction between soil disinfestation and fungicide application was also not significant (p = 0.18). The fungicides were significantly effective in reducing the disease progression (p = 0.01). Plots treated with azoxystrobin had the lowest standard AUDPC (6.38 ± 5.25) compared to non-treated controls, while PA (6.48 ± 10.05) was not significantly different from azoxystrobin or the non-treated control (14.85 ± 10.08; Figure 2A). In 2021, ASD (p < 0.001) and fungicides were significant in reducing SSR (p < 0.001). There was no interaction between these treatments (p = 0.59). The combination of ASD + azoxystrobin significantly reduced SSR with an sAUDPC of 5.65, not statistically different from ASD + PA (sAUDPC = 19.23) or the application of azoxystrobin alone (sAUDPC = 24.64; Figure 2A).

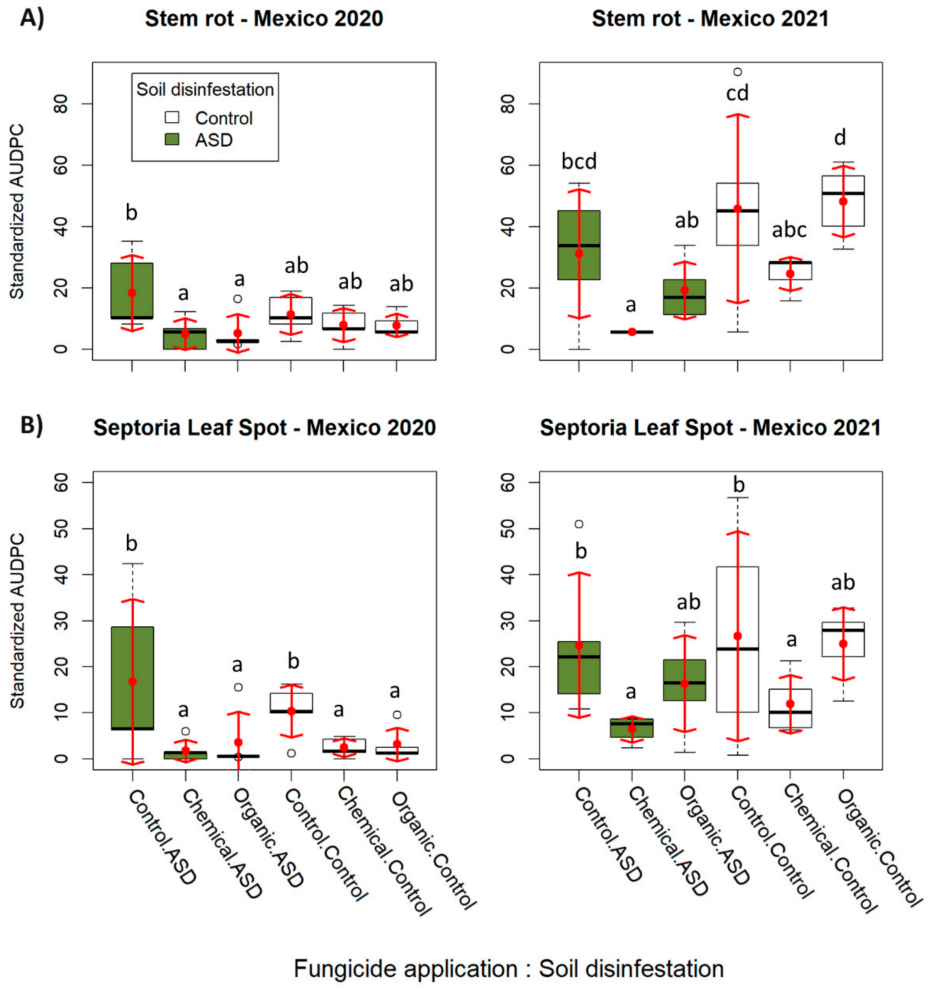

Figure 2. Effect of anaerobic soil disinfestation (ASD) and fungicide application on stevia disease progression during 2020 and 2021 field trials in Guerrero, Mexico. (**A**) Stevia stem rot caused by *Sclerotium rolfsii*. (**B**) Septoria leaf spot caused by *Septoria steviae*. Treatments were: Control = non-amended non-saturated plastic covered control; ASD = plastic covered saturated amended with molasses at 10.1 Mg ha^{-1} and cornmeal at 20.2 Mg ha^{-1}; Chemical fungicide = azoxystrobin as the active ingredient (a.i.) with a rate of 230 a.i. g ha^{-1}; Organic fungicide = pyroligneous acid with a rate of 30 mL L^{-1}; Control fungicide = non-treated control. Gray boxes represent the quartile distribution with the black dash as the median, the whiskers as the maximum and minimum values, and white dots as outliers. Red dots represent the means, and red arrows show the standard deviation of standardized AUDPC. Points represent the mean data of five replicates. Values indicated by different letters are significantly different at p < 0.05 according to Tukey's test.

The progression of SLS symptoms did not reduce significantly due to ASD treatment ($p = 0.51$) and there was no interaction between ASD and fungicide application ($p = 0.59$). However, the application of fungicides significantly reduced SLS ($p < 0.01$; Figure 2B). During 2020 and 2021 trials conducted in Mexico, azoxystrobin application consistently reduced SLS compared to the non-treated control. In contrast, the application of PA showed more variability, with significant SLS reduction observed in 2020 but not in 2021 (Figure 2B). Data on disease progression was not obtained for field trials in Paraguay due to the sudden COVID-19 pandemic in 2020.

3.3.4. Fresh Yield

For field trials in Mexico in 2020, ASD ($p = 0.04$) and fungicides ($p < 0.001$) had significant a significant effect on fresh yield, but there was not a significant interaction between these treatments ($p = 0.1$). The yield observed in ASD + chemical fungicide applications was significantly higher (3257 $\pm$ 851 kg ha^{-1}) than the untreated control (1322 $\pm$ 298 kg ha^{-1}), while the rest of the treatments were not significantly different from the control (Figure 3A). However, in 2021, only the application of fungicides significantly affected fresh yield ($p = 0.01$), with chemical fungicides-treated plots yielding at least 350 kg ha^{-1} more than those plots treated with organic fungicide or untreated (Figure 3A).

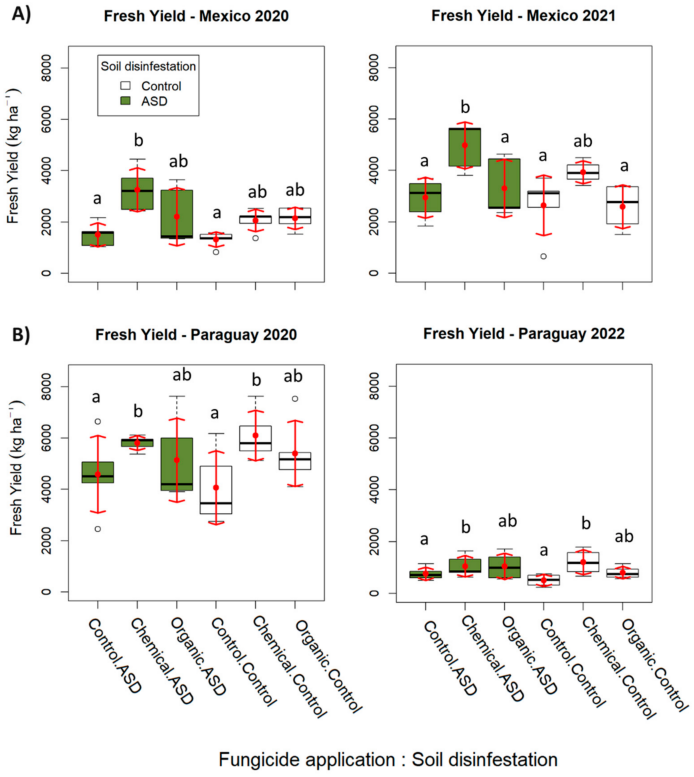

Figure 3. Effect of anaerobic soil disinfestation (ASD) and fungicide application on stevia yield during field trials. (**A**) 2020/2021 Field trials in Guerrero, Mexico. (**B**) 2020/2022 Field trials in Itapúa, Paraguay. Treatments were: Control = non-amended non-saturated plastic covered control; ASD = plastic covered saturated amended with molasses at 10.1 Mg ha^{-1} and cornmeal at 20.2 Mg ha^{-1} (in Mexico) or wheat bran at 20.2 Mg ha^{-1} (in Paraguay); Chemical fungicide = azoxystrobin as the active ingredient (a.i.) with a rate of 230 a.i. g ha^{-1}; Organic fungicide = pyroligneous acid with a rate of 30 mL L^{-1}; Control fungicide = non-treated control. Gray boxes represent the quartile distribution with the black dash as the median, the whiskers as the maximum and minimum values, and white dots as outliers. Red dots represent the means, and red arrows show the standard deviation of fresh yield. Points represent the mean data of five replicates. Values indicated by different letters are significantly different at $p < 0.05$ according to Tukey's test.

In 2020 Paraguay field trials, the ASD did not significantly affect fresh yield ($p = 0.1$), and only the application of fungicides was significantly effective in increasing the yield ($p = 0.03$). Azoxystrobin application had the highest fresh yield (5952 ± 701 kg ha^{-1}), while non-treated control plots had a significantly lower yield (4325 ± 1417) kg ha^{-1}. The application of PA (5270 ± 1397 kg ha^{-1}) was not significantly different from the chemical treatment or the control plots (Figure 3B).

Similarly, in 2022, only the application of the chemical fungicide was significant ($p = 0.02$), with treated plots yielding 1119 (±422) kg ha^{-1} while the organic treated and control plots yielded 930 (±394) kg ha^{-1} and 650 (±265) kg ha^{-1}, respectively (Figure 3B).

3.3.5. Dry Yield

For field trials in Mexico in 2020, dry yield did not increase significantly due to the ASD treatment ($p = 0.6$). No interaction was observed between the ASD and fungicide application ($p = 0.9$). However, applying chemical fungicides increased dry yield ($p = 0.001$). Plots treated with azoxystrobin had the highest yield (1058 ± 269 kg ha^{-1}), while control plots had a significantly lower dry yield (488 ± 172 kg ha^{-1}). The application of PA, with yields of 756 (±343) kg ha^{-1}, was not significantly different from the azoxystrobin treatment or the control plots. In 2021, a similar pattern was observed: only the chemical fungicide was significant ($p = 0.01$), with treated plots yielding 1315 ± 235 kg ha^{-1} while the control plots only yielded 941 ± 315 kg ha^{-1} (Figure 4A).

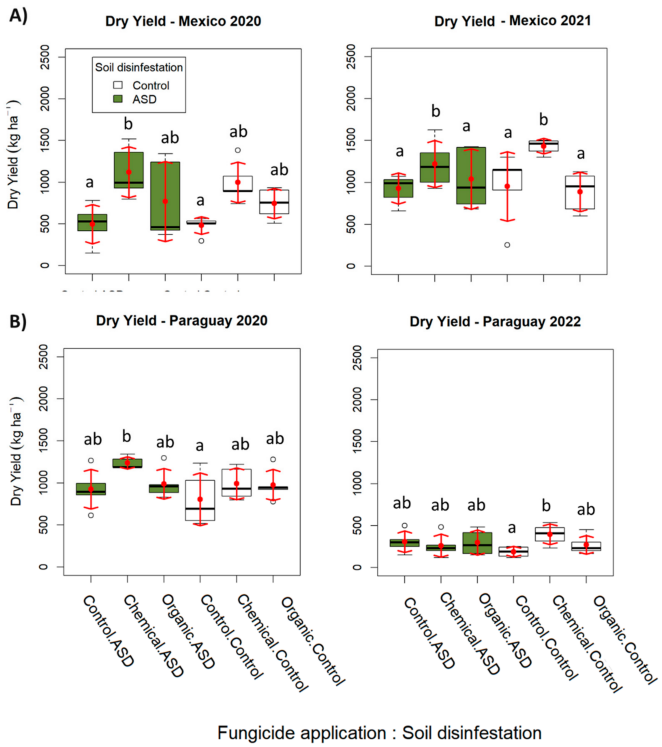

Figure 4. Effect of anaerobic soil disinfestation (ASD) and fungicide application on stevia yield during field trials. (**A**) 2020/2021 Field trials in Guerrero, Mexico. (**B**) 2020/2022 Field trials in Itapúa, Paraguay. Treatments were: Control = non-amended non-saturated plastic covered control; ASD = plastic covered saturated amended with molasses at 10.1 Mg ha^{-1} and cornmeal at 20.2 Mg ha^{-1} (in Mexico) or wheat bran at 20.2 Mg ha^{-1} (in Paraguay); Chemical fungicide = azoxystrobin as the active ingredient (a.i.) with a rate of 230 a.i. g ha^{-1}; Organic fungicide = pyroligneous acid with a rate of 30 mL L^{-1}; Control fungicide = non-treated control. Gray boxes represent the quartile distribution with the black dash as the median, the whiskers as the maximum and minimum values, and white dots as outliers. Red dots represent the means, and red arrows show the standard deviation of dry yield. Points represent the mean data of five replicates. Values indicated by different letters are significantly different at $p < 0.05$ according to Tukey's test.

In 2020 Paraguayan field trials, the ASD did not significantly affect dry yield ($p = 0.1$), and only the application of fungicides significantly increased the yield ($p = 0.04$). Azoxystrobin application had the highest dry yield (1114 ± 188 kg ha^{-1}), while non-treated control plots had a significantly lower yield (865 ± 270) kg ha^{-1}. The application of PA (982 ± 173 kg ha^{-1}) was not significantly different from the chemical treatment or the control plots (Figure 4B). In 2022, yields were significantly lower than in 2020. Moreover, a significant interaction between ASD and fungicide application was observed ($p = 0.01$), where plots only treated with chemical fungicide had a significantly higher dry yield (396 ± 124 kg ha^{-1}) than the untreated control plots (188 ± 64 kg ha^{-1}). In contrast, ASD treated plots were not significantly different from the control or azoxystrobin (Figure 4B).

3.3.6. Cost Disease Management Approaches

Costs of stevia production were higher in Mexico (Table S2) compared to Paraguay (Tables S3 and S4). In both countries, the highest cost was for the combination of ASD + chemical fungicide, while the lowest cost was for the application of PA. The incorporation of carbon sources for ASD increased production costs by at least 20% compared to only applying fungicides for the management of diseases (Tables S2–S4). The application of fungicides costs at least 3% more than the untreated control.

4. Discussion

Stevia is a crop originally from Paraguay that is rapidly expanding to new production areas in North America and Europe [5,38,39]. Because of this, only a limited number of pesticides have been approved for use in USA stevia fields [8]. Moreover, stevia is marketed as an all-natural non-caloric sweetener, and many markets are willing to pay extra value for pesticide-free products, especially in European markets [40]. This work explored alternative pesticides for their use in stevia disease management, such as PA, which can be applied in combination with ASD as part of an IPM program. In this study, the use of PA in stevia cultivation was explored for the first time, and it was observed to have a varying impact on disease reduction, unlike chemical fungicides such as azoxystrobin, which showed more consistency. Similarly, ASD had variable effects on disease and yield improvement during field trials. However, it was effective in small areas, suggesting potential for soilborne pathogen management in stevia, but more research on carbon source type and mechanisms of action is needed [41].

4.1. Inhibitory Effect of Fungistatic Volatile Compounds Produced during ASD

During the in vitro screening of carbon sources, ASD simulated in Petri plates significantly reduced the mycelial growth and the sclerotia formation of *S. rolfsii* through the production of volatile compounds, given that the colonies were not in direct contact with the soil. We suspect the aromatics resulted from the production of volatile fatty acids produced during anaerobic conditions, which coincided with significantly lower oxidation-reduction potential values of the soil [14,42–46]. Additionally, fewer sclerotia formed in the treated plates with organic amendments than in the controls. However, this effect was likely only fungistatic, given that sclerotia resumed their growth when transferred to fresh PDA, similar to previous research [47]. Compounds like organic acids, phenolic compounds, and volatile organic compounds (VOCs) produced during ASD have been found to have fungistatic properties. Organic acids, such as acetic, butyric, and propionic acid, can reduce the pH of the soil, making it less conducive to fungal growth and germination of *Sclerotinia* spp. [48], while phenolic compounds produced by anaerobic bacteria have been shown to inhibit the growth of some soilborne pathogens [49]. VOCs, such as dimethyl disulfide, were significantly higher in ASD treatments compared to the control [41]. The fungistatic properties of the compounds produced during ASD are a promising way of screening for carbon sources; however, further research is needed to better understand their mode of action and how they can be optimized for disease management.

4.2. Impact of ASD on Sclerotia, Disease Reduction, and Weed Control in Microplots

Sclerotia buried in the soil were exposed directly to anaerobic conditions and the action of antagonist microorganisms, which reduced their viability. Wheat bran as a carbon source for ASD has been previously reported to significantly reduce the viability of sclerotia of *S. rolfsii* while stimulating colonization by antagonist microorganisms such as *Trichoderma* that directly affect sclerotia [50]. Additionally, we observed an increase in temperature of 4 °C during the tarping period in plots treated with carbon sources, which can be linked to higher microbial metabolic activity in the soil [51,52]. Similarly, Testen and Miller [53] observed temperatures increased by 1.5 to 6.3 °C in plots where ASD was conducted compared with non-treated control plots. This was correlated to soil microorganisms breaking down carbon sources and inducing anaerobic conditions. The production of fungistatic and fungicidal compounds produced by the microbial community has also been implicated in reducing other populations of plant pathogens [42,54–57].

Stem rot progression on stevia and the number of weeds per m^2 were significantly lower in plots where ASD was performed using cornmeal (20.2 Mg ha^{-1}) + molasses (10.1 Mg ha^{-1}). The impact of anaerobic conditions on disease and weed reduction is evidenced by the low oxidation-reduction potential values detected in ASD plots. Therefore, the development of anaerobic conditions is beneficial to generate a fumigant effect against pathogens and weeds [58–62]. Previous research applied corn gluten meal for ASD, significantly reducing the soilborne disease intensity on tomatoes grown in treated plots [63]. However, the same authors reported a phytotoxic effect of corn gluten meal on the germination and growth of tomatoes [63]. Corn gluten meal has been reported as an herbicide or a cause of phytotoxicity in plants [64]. Therefore, the herbicide characteristics of corn gluten meal prevent its use for perennial systems due to the potential long-term toxic effect on plants [63,65]. In contrast, the cornmeal treatment in this study did not have phytotoxic effects on stevia, and the fumigant effect was only observed under anaerobic conditions during our trials.

We observed a significantly higher stem rot incidence in plots where only cornmeal was added without the water saturation or plastic cover. This higher incidence could be because cornmeal serves as a source of nutrients for the pathogen, and no antifungal compounds are produced in the absence of anaerobic conditions. Mayo-Prieto et al. [66] reported that bentonite and/or cornmeal in substrates (vermiculite or peat) favored *Rhizoctonia solani* growth, causing higher disease incidence in beans (*Phaseolus vulgaris*). Furthermore, cornmeal could also improve colonization by biocontrol fungi such as *Trichoderma harzianum* [67]. Therefore, given that cornmeal is a rich carbon source, its use without anaerobic disinfestation can lead to the colonization of opportunistic fungi.

Molasses was also evaluated as a carbon source, significantly reducing stem rot's progression in microplot experiments. This result is similar to research on other pathogens, including soilborne pathogens such as *Fusarium oxysporum* [55], *Rhizoctonia solani* [68], *Sclerotinia sclerotiorum*, and *S. minor* [69], and *S. rolfsii* [50]. A similar effect has been observed in reducing nematode populations, translating into lower disease and higher yields in perennial crops [70]. The advantage of liquid molasses over other carbon sources is the potential to be applied through drip irrigation or spray, which is ideal for some farm operations [71,72]. Additionally, the application of molasses may also reduce the requirements of fertilizer necessary for crop nutrition [36,73].

In this work, a higher rate (20 to 40 Mg ha^{-1}) of carbon sources for ASD was incorporated into the soil compared to previous works [33,74,75]. The addition of these high amounts of carbon to soil may have a significant impact on soil health and fertility. Greater carbon input can provide more food for microbes, which may lead to increased microbial activity and production of CO_2 [29]. In addition, excessive carbon input can lead to imbalances in soil nutrients and can negatively impact soil health, such as nitrogen immobilization [76], soil acidity [77], and higher N_2O emissions [73,78]. To avoid these adverse effects, it is important to carefully manage the amount, C:N ratio, and type of

carbon added to the soil [50]. Further research is needed to understand the impact of these higher rates of carbon when used for ASD in different edaphoclimatic conditions.

4.3. Effectiveness of Integrating ASD and Fungicide Programs under Field Conditions

In all field studies, plots treated with ASD showed a constant rise in soil temperature. During ASD, adding organic matter creates a substrate for microbial growth, which leads to the production of carbon dioxide and other gases, and an increase in soil temperature [29]. The temperature increase is due to the exothermic reactions that occur during microbial respiration and fermentation, which release heat as a byproduct [51]. The extent of the temperature increase during ASD depends on several factors, including the type and quantity of carbon source added, the soil, and climate type [29,53].

A consistent reduction in the viability of *S. rolfsii* sclerotia buried in the soil was observed across all field trials. Sclerotia are responsible for the persistence and spread of the pathogen in stevia fields, making them a key target for disease control strategies [8]. ASD can reduce the viability of sclerotia by creating an anaerobic environment, physically damaging sclerotia, releasing toxic compounds, and producing organic acids that lower the soil pH and disrupt the internal metabolism of sclerotia [50,79].

There was a variable effect of ASD on disease progression. There was no effect on SSR in 2020, but there was a significant effect in 2021. Similarly, the effects on fresh yield were inconsistent, exhibiting significant effects in Mexican fields in 2020 but not in 2021. Likewise, during Paraguayan field trials, ASD had a significant interaction with fungicide applications only in terms of dry yield in 2022. These results agree with previous research where chemical fumigation provided variable results in stevia, with a reduction in disease but no effect on yield in the first harvest [80]. Given that stevia has the potential to be grown as a perennial crop, soil disinfestation might have a more noticeable effect on the reduction in disease progression over several years than on the first year of production. This particular study did not study the effect of soil disinfestation over several years of production. Therefore, long-term studies are needed to evaluate the year-to-year impacts of ASD in stevia [81].

During field experiments, applying the chemical fungicide azoxystrobin reduced the progression of SSR and SLS and increased yield compared to non-treated control plots. These results are congruent with previous studies that established the efficacy of QoI fungicides in reducing SSR intensity [8]. In another study, three applications of triazoles and strobilurins reduced SLS severity by 80% and increased stevia yield by 50% during field trials in NC [6]. The same authors reported that during the second harvest, yields of fungicide-treated plots were significantly greater than the non-treated control ($p < 0.003$), with the azoxystrobin + difenoconazole treatment having the highest yield [6].

In this study, the application of PA did not lead to significantly lower disease progression and higher yield compared to azoxystrobin or the non-treated control, likely due to high variability between plots. Inconsistency in disease reduction might also be related to the environmental degradation of antifungal compounds [25]. In contrast, applications of PA have been successful in other pathosystems, significantly reducing the incidence of *Peronospora parasitica* and *S. sclerotiorum* by 12.14% and 17.33%, respectively [82]. Similarly, bamboo PA in a concentration of 30 mL L^{-1} significantly inhibited *Botrytis cinerea* and prevented gray mold disease in harvested apples [83]. While our current study did not show significant disease control using PA, it may still serve as an alternative fungicide for stevia production; however, more research focused on timing, mode of application, and formulation is necessary to help to improve its efficacy [19].

Soil disinfestation increased stevia's production cost by at least 23% compared to not conducting the ASD for the first year of stevia production. This higher cost of conducting ASD is similar to those reported in tomato production in Florida, where ASD accounted for 32% of the production cost [33] and 22% in strawberry production using rice bran as a carbon source compared to the non-treated fields [35]. In this study, much of the cost of ASD was related to carbon source type and the labor necessary for application. The

use of cover crops such as cowpea, annual ryegrass, oat, rye, and mustard, among others, may reduce the costs of ASD [36] and has shown to be as effective as soil fumigation in other perennial systems such as apple [84]. However, further research is needed to explore this approach to ensure consistent control across different environments. The use of ASD and organic fungicides in stevia may be justified if the stevia dry leaves have a differential value in the organic market and disease pressure is high. Soil disinfestation may not be beneficial in all stevia fields given that soilborne bacterial diseases and nematodes are not significant threats to stevia production and fungal pathogens such as *S. rolfsii* can be effectively managed with the applications of fungicides [6,8].

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13051358/s1, Figure S1: Anaerobic soil disinfestation (ASD) laboratory simulation; Figure S2: Effect of carbon sources incorporated into the soil on stevia stem rot incidence; Figure S3: Effectiveness of integrated pathogen management under field conditions at Colegio Superior Agropecuario del Estado de Guerrero (CSAEGRO), Guerrero, Mexico; Table S1: Description of soil characteristics of soils from field experiments combining anaerobic soil disinfestation and application of fungicides to manage stevia diseases; Table S2: The cost of stevia production in Mexico with a plant density of 100,000 (30 × 20 × 70 cm) for different combinations of disease management approaches for stevia production; Table S3: Cost of stevia production in Paraguay with a plant density of 100,000 (30 × 20 × 70 cm) for different combinations of management approaches for stevia production; Table S4: The cost of stevia production in Paraguay with a plant density of 55,000 (50 × 30 × 70 cm) for different combinations of disease management approaches for stevia production.

Author Contributions: Conceptualization, A.D.S.-V. and H.D.S.; methodology, A.D.S.-V., G.A.E.-M., J.F.D.-N. and H.D.S.; Software, A.D.S.-V., L.D.T. and H.D.S.; validation, A.D.S.-V., G.A.E.-M., M.M.-O., S.A.-S. and J.F.D.-N.; formal analysis, A.D.S.-V.; investigation, A.D.S.-V., G.A.E.-M., M.M.-O., S.A.-S., J.F.D.-N., L.D.T. and H.D.S.; resources, A.D.S.-V., L.D.T. and H.D.S.; data curation, A.D.S.-V., G.A.E.-M. and J.F.D.-N.; writing—original draft preparation, A.D.S.-V.; writing—review and editing, L.D.T. and H.D.S.; visualization, A.D.S.-V.; supervision, L.D.T. and H.D.S.; project administration, A.D.S.-V., G.A.E.-M., J.F.D.-N. and H.D.S.; funding acquisition, A.D.S.-V., G.A.E.-M., J.F.D.-N., S.A.-S., L.D.T. and H.D.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the United States Department of Agriculture Grant 2017-51181-26828 and the Storkan-Hanes-McCaslin Foundation Awards 2021 of the American Phytopathological Society.

Data Availability Statement: The data presented in this study are available from the corresponding author upon reasonable request.

Acknowledgments: We would like to recognize the invaluable help of the staff at the Central Crops Research Station, Clayton, NC; at Colegio Superior Agropecuario del Estado de Guerrero, Mexico; and Centro de Desarrollo e Innovación Tecnológica, Paraguay. We also thank the Instituto Paraguayo de Tecnologia Agraria for their help with fungal isolates and stevia plants.

Conflicts of Interest: The authors declare no conflict of interest. Commercial names are provided to ensure the reproducibility of the methods and results reported in this paper. The authors do not endorse any products, services, or companies mentioned in this paper.

Abbreviations

NCSU: North Carolina State University, CEDIT: Centro de Desarrollo e Innovación Tecnológica, CSAEGRO: Colegio Superior Agropecuario del Estado de Guerrero, ASD: Anaerobic Soil Disinfestation, SSR: Stevia Stem Rot, SLS: Septoria Leaf Spot, MX: Mexico, PY: Paraguay, PA: Pyroligneous Acid, NC: North Carolina; USA: United States of America, a.i.: active ingredient, PDA: Potato-Dextrose-Agar, ORP: Oxidation-Reduction Potential, Mg ha^{-1}: Megagram per hectare, EU: Experimental Unit, DAP: Days After Planting, sAUDPC: Standardized Area Under The Disease Progress Curve, N-P-K: Nitrogen, Phosphorus and Potassium, SAS: Statistical Analysis System, ANOVA: Analysis of Variance, VOC: Volatile Organic Compounds.

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