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Dry Rot Caused by the Complex *Colletotrichum falcatum* and *Thielaviopsis paradoxa* Emerges as a Key Stalk Disorder in Newly Expanded Sugarcane Plantations from Northwestern São Paulo, Brazil

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Abstract: Sugarcane dry rot emerged as an important stalk disorder in newly expanded plantations in northwestern São Paulo, Brazil, under the current no-burning fully mechanical harvest policy gradually implemented in the past 20 years. This emergence was probably due to a considerable increase in both pathogen inocula and insect pest populations in sugarcane crop residues kept in the field. In this study, we surveyed the incidence of three stalk-related disorders in commercial sugarcane fields in six municipalities in northwestern São Paulo and the corresponding yield losses. The three stalk-related disorders surveyed were as follows: the red rot disease caused by the fungal pathogen *Colletotricum falcatum*, the spittlebug-induced shoot stunting, and the stem dry rot, which is associated with the simultaneous infection of *C. falcatum* and *Thielaviopsis paradoxa*, the pineapple set rot pathogen. Red rot disease was detected in 88.2% of the fields surveyed, while the spittlebug-induced shoot stunting disorder and the internal stem dry rot were found in 97.1% of the fields. Stem dry rot had the highest incidence and resulted in the highest yield losses. Total sugarcane yield losses were estimated at 20.1%, with an average of 14.2 (±3.8) t·ha^{-1} per field. The multiple regression model constructed to determine which of the three stem-related disorders contributed the most to total yield losses was not significant. Subsequently, the performance analyses of single-variable polynomial regression models indicated that the simple linear model was the best fit in terms of independently predicting sugarcane yield losses based on each stem-related disorder. Positive and significant correlations were only detected between sugarcane yield losses in t·ha^{-1} and the incidence of red rot disease or leafhopper-induced shoot stunting. We concluded that the stalk's internal dry rot, as a disease complex associated with both *C. falcatum* and *T. paradoxa*, was the most important disorder in sugarcane fields in the northwest region of São Paulo state. A sustainable pest management program is needed to reduce the impact of all three stalk-associated disorders on regional sugarcane production.

Keywords: emerging disease; red rot complex; yield loss; ineffective control

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1. Introduction

Brazil is the leading player in agriculture, being a major exporter and the world's largest sugarcane (*Saccharum officinarum*)-producing country, and the crop is also the most important driver of Brazilian agribusiness [1,2]. In 2021/2022, Brazil produced 585.2 million t of sugarcane in approximately 8.317 million hectares of planted area [1]. Within the country, São Paulo state alone represented more than 51% of all sugarcane, sugar, and ethanol produced [3]. In São Paulo, the sugarcane cropping areas have undergone constant expansion, mainly due to the growing global demand for biofuels as a sustainable replacement for fossil fuels and to enable the reduction in greenhouse gases emissions [4–6]. Statewide, the northwest of São Paulo is considered today to be the newest frontier for the expansion of the sugarcane cropping industry [3,7], although historical reports from the Expansion Program for Sugarcane Production in the State of São Paulo (PROCANA) indicate that the first initiatives for expansion occurred as early as 1975/76 and 1985/86 [4].

In terms of figures, the total sugarcane cropping areas in Brazil expanded by 46% from 5.2 million ha in 2001/2002 to 8.3 million ha in 2021/2022 [1]. This enormous expansion in cropping areas resulted in increased environmental and social impacts associated with sugarcane pre-harvest field burning [8–11]. This century-old practice has been used to both facilitate manual sugarcane cutting and harvesting and reduce harvesting costs [2,10]. However, sugarcane pre-harvest burning raises the levels of pollutants in the air, contributes to global warming by increasing the release of greenhouse gases, increases the risk of accidents on roads near sugarcane fields, pollutes cities with soot, and increases hospital admissions due to respiratory problems, with a consequent increase in public health expenses in counties near sugarcane plantations [8–11].

Considering the serious environmental, social, and public well-being/public health impacts, a definite policy passed by lawmakers from São Paulo in 2003 enforced the prohibition of sugarcane field burning [12]. These new policies outlined a long-term plan for increasing legal restrictions on field burning beginning in 2003 until total prohibition in 2021 in areas with feasible total harvest mechanization, or until 2031 for the remaining areas [12]. In the last decade, around 85% of São Paulo state's sugarcane cropping area was already being mechanically green harvested [13].

The legal prohibition of sugarcane field pre-harvest burning [12] resulted in a major change in the sugarcane cropping system, having a direct negative impact on plant health by impeding the physical management of important stalk-associated fungal diseases and insect pests [14–19], such as the stalk red rot, caused by *Colletotrichum falcatum* (syn. *Glomerella tucumanensis*) [20–23] (Figure 1A,B), and pineapple sett rot, caused by *Thielaviopsis paradoxa* (formerly *Ceratocystis paradoxa;* syn. *T. ethacetica*) [24–28]. Contrastingly, while pre-harvest burning resulted in a substantial decrease in the survival of soil-borne insect pests populations and fungal plant pathogens' inocula, with no burning, mechanical harvesting maintains sugarcane straw as abundant crop residues in the areas, favoring the survival of both insect pests and plant pathogens [18,20,29–33]. In fact, in areas where harvest was carried out without prior burning, there was a 1.5% decrease in sugar levels due to damage caused by *Mahanarva fimbriolata* on stalks [34].

Concerning sugarcane red rot disease (Figure 1A,B), there is evidence of increased incidence of *C. falcatum* at levels that have never been reported before the prohibition of field burning [16,20]. A change in the biological cycle of the pathogen *C. falcatum* has also been reported [16,20,22,29]. With a considerable increase in the pathogens' inocula on crop residues, foliar symptoms of the disease on sugarcane leaf veins (Figure 1A) became common, with no sign of pre-infection wounds on the stalk, suggesting infection through natural openings or via direct penetration of *C. falcatum* into plant tissues [20,22]. It is likely that the cropping of more susceptible sugarcane varieties also contributed to this change in the biological cycle of the pathogen [31,35].

Similarly, since the prohibition of pre-harvest crop burning, the incidence of pineapple sett rot associated with *T. paradoxa* has also significantly increased in Brazilian sugarcane fields, which led to high yield losses [25,36,37]. This is because the pathogen rots and destroys the infected cuttings or setts (i.e., sugarcane segments that sprout to form new stalks) (Figure 1C), which are prevented from sprouting or undergoing further development, impacting the final plants' stand per area [27,32,37]. Controlling pineapple root rot has become a growing concern for local sugarcane growers and plantation extension personnel [20,23,25,30,37].

Figure 1. Symptoms of red rot on sugarcane leaf midribs and stalks caused by *Colletotrichum falcatum* (**A**,**B**). Advanced symptoms of sugarcane pineapple sett rot caused by *Thielaviopsis paradoxa*, both external and internally, with abundant signs of the pathogen's sporulation (**C**). Whole sugarcane stalk (**D**) and cross-section of a dry rot symptomatic sett (**E**). Pathogen isolations derived from dry rot symptomatic stalks (**E**) indicate simultaneous infection by *C. falcatum* and *T. paradoxa*. Contrast between diseased or injured (d) and healthy sugarcane stalks (h) (**F**). Pre-sprouted sugarcane seedlings infested with the spittlebug *Mahanarva fimbriolata*, with a soap-foam-like exudation on the roots, which is typically produced by insect pest nymphs (**G**). Damaged sugarcane stalks are thinner, presenting cracks and deterioration due to the spittlebug attack (**H**). The straw left in the field, as a result of the mechanized harvesting of green sugarcane, harbors *M. fimbriolata* and keeps the populations of the spittlebug high (**I**). Adult-stage spittlebug close to a massive foam exudation (**J**) and on a sugarcane leaf (**K**). Sugarcane straw accumulated in a sugarcane plantation as a source of inocula for the fungal pathogens (**L**). [a,b]; [a] Sugarcane leaf infected by *C. falcatum* presents red lesions that are prominent on the central vein. As the disease progresses, these lesions develop a discolored center. Notably, in older leaves, the lesions can span the entire length of the vein (Figure 1A). In the nodal region, necrotic areas stand out with their defined edges and can quickly lead to the complete necrosis of the internode, which takes on a dark brown color, showcasing the fungus' acervuli. Within the stalks, the red discoloration indicates progressive degradation caused by the pathogen (Figure 1B). [b] Sugarcane stalks infected by *T. paradoxa* present a gradual change in tissue color, noted as the rot advances. Initially gray, the tissues become dark brown and, eventually, black. At this advanced stage, only the outer husk of the sett remains intact, while the internal fibrovascular bundles are overwhelmed by a black mass of pathogen spores (Figure 1C). Additionally, the setts emit a pleasant and unique pineapple essence aroma, a result of fermentation induced by the pathogen.

The simultaneous infection of sugarcane stalks by *C. falcatum* and *T. paradoxa*, resulting in the stalk internal dry rot disorder (Figure 1D,E), has been a particular concern in plantations in northwestern São Paulo, where the occurrence of pineapple sett rot disease alone seemed rare (Usina Santa Adélia Sugarcane Mills, personal information).

With regard to the spittlebug (*M. fimbriolata*)-associated disorder, the most significant damage impacting sugarcane yield is excessive budding resulting from the deaths of young shoots due to the injection of toxic substances from the insect's saliva. The insect also induces hydric stress and shoot stunting, as well as decreasing the plants' sucrose content [38,39].

An important factor that contributed to the increased incidence of stalk-associated disorders was the planting of smaller stalk cuts as propagating materials, including mini-stems, billets, and seedlings, which became the current standards for sugarcane planting [14,40–42]. Using smaller stalk cuts favored the plant tissue exposure to the attack of insect pests and the infection of plant pathogens, leading to failures in planting stands, ultimately resulting in up to five consecutive years of decreasing yields [16,19,20,31,37,43]. Another factor that contributed to the higher incidence of sugarcane-stalk-associated insect pests and pathogens is the predominantly staggered planting process, which favors continuous insect infestation and the maintenance of fungal pathogens' inocula in the field [15,16,31,44,45].

Despite the current perception that sugarcane-stalk-associated disorders have emerging importance in recently established sugarcane plantations in northwestern São Paulo, there is no information in the recent literature describing field levels, prevalence, and the relative importance of the three stalk disorders, their geographical distribution in the region, and the corresponding yield losses.

Therefore, the objectives of this study were to survey the spontaneous incidences of spittlebug-induced shoot stunting, the *C. falcatum* red rot disease, and stalk internal dry rot disorder caused by the simultaneous infection of *C. falcatum* and *T. paradoxa*; their relative importance; and the corresponding yield losses in 34 commercial sugarcane fields in northwestern São Paulo, installed during the last decade's sugarcane expansion initiative, followed by the establishment of sugar and alcohol industry plants in the region.

We hypothesized that stalk red rot disease is the major stalk-related disorder occurring in recently established sugarcane commercial fields in northeastern São Paulo due to its higher incidence, widespread distribution, and resultantly high yield losses.

2. Materials and Methods

2.1. Survey of Sugarcane Stalk-Related Disorders

A survey of the spontaneous incidence of the red rot disease for determining its relative importance in contrast to two other stalk-related disorders commonly found in the region (i.e., the spittlebug-induced budding and stalk internal dry rot) was carried out during the 2017/2018 cropping season in 34 commercial sugarcane fields from six counties in northwestern São Paulo: Castilho, Ilha Solteira, Itapura, Pereira Barreto, Santo Antônio do Aracanguá, and Sud Menucci (Figure 2). The fields were established with the two most commonly planted cultivars in the region: SP81-3250 and CTC4 (Tables 1 and 2).

The region's predominant climate, according to Köppen's classification, is Aw, which is characterized as tropical humid, with a rainy season during the summer and drought during the winter. The region's existing vegetation is very fragmented and impoverished, forming part of an original interior Atlantic Forest (seasonal semideciduous forest) in transition to Cerrado (savanna) lowlands. The average altitude is 347 m. Sugarcane plantations currently occupy 31% of the area, while natural vegetation represents only 5% (according to MapBiomas, https://brasil.mapbiomas.org/estatisticas/, accessed on 25 October 2023).

Table 1. Yield losses associated with the incidences of stalk-related disorders in 34 commercial sugarcane fields with the varieties CTC4 and SP81-3250 from six counties in northwestern São Paulo in 2017/2018.

County and Sugarcane Variety	Cropped Area Surveyed (ha)	Stalk-Related Disorder Incidence (%)			Total Losses in the Areas (t)	Yield Losses (TL) ($t \cdot ha^{-1}$)	Relative Yield Losses (RL) (%)	Yield Losses Associated with the Stalk Disorders ($t \cdot ha^{-1}$)			Relative Yield Losses Associated with the Stalk Disorders (%)		
		Red Rot (I.rr)	Spittlebug (I.sib)	Dry Rot (I.dr)				Red Rot (rrTL)	Spittlebug (sbiTL)	Dry Rot (drTL)	Red Rot (rrRL)	Spittlebug (sbiRL)	Dry Rot (drRL)
Castilho, SP81-3250	151.32	12.68	11.00	25.00	1938.62	12.81	19.71	3.34	2.89	6.58	5.14	4.45	10.12
	258.26	30.16	12.34	0.00	8427.94	32.63	42.04	23.16	9.47	0.00	29.83	12.21	0.00
	333.79	3.20	4.93	30.55	7499.61	22.47	34.57	1.86	2.87	17.74	2.86	4.41	27.30
Ilha Solteira, CTC4	78.42	0.00	12.00	39.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	95.27	16.00	24.00	32.00	1875.56	19.69	23.27	4.37	6.56	8.75	5.17	7.76	10.34
Ilha Solteira, SP81-3250	216.10	2.42	1.41	18.39	6438.41	29.79	49.66	3.25	1.89	24.66	5.41	3.15	41.10
	420.46	30.91	7.50	2.94	7988.70	19.00	25.33	14.20	3.44	1.35	18.94	4.59	1.80
Itapura, SP81-3250	47.95	0.39	2.54	13.00	792.72	16.53	15.03	0.41	2.64	13.49	0.37	2.40	12.26
	104.93	2.51	4.57	20.78	753.62	7.18	11.97	0.65	1.18	5.36	1.08	1.96	8.93
	157.28	0.00	4.48	27.00	2332.28	14.83	19.77	0.00	2.11	12.72	0.00	2.81	16.96
	268.32	2.06	7.02	16.00	7394.32	27.56	22.96	2.26	7.71	17.58	1.89	6.42	14.65
	325.07	4.51	2.17	15.72	8025.85	24.69	32.92	4.97	2.39	17.33	6.63	3.19	23.10
	405.36	5.46	13.29	8.36	7821.90	19.30	25.73	3.88	9.46	5.95	5.18	12.61	7.94
Pereira Barreto, SP81-3250	15.20	6.32	4.79	42.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	21.00	4.22	0.00	17.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	66.00	7.03	31.62	3.99	999.78	15.15	21.64	2.50	11.23	1.42	3.57	16.05	2.03
	127.79	10.25	15.47	24.17	2599.85	20.34	29.06	4.18	6.31	9.86	5.97	9.01	14.08
	131.56	5.79	18.47	2.97	2912.99	22.14	29.52	4.71	15.02	2.42	6.28	20.02	3.22
	117.51	10.83	2.45	22.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S.A.Aracanguá, SP81-3250	25.07	0.66	2.96	15.46	104.87	4.18	7.61	0.14	0.65	3.39	0.26	1.18	6.16
	33.90	1.34	0.81	16.94	141.96	4.19	7.61	0.29	0.18	3.72	0.54	0.32	6.76
	75.03	0.00	2.36	21.89	1033.95	13.78	25.06	0.00	1.34	12.44	0.00	2.44	22.62
	123.88	0.33	1.95	17.21	2449.37	19.77	32.95	0.33	1.98	17.47	0.55	3.29	29.11
	139.26	1.55	8.11	32.69	4419.05	31.73	45.33	1.16	6.07	24.50	1.66	8.68	35.00
	169.20	4.98	4.76	22.60	2574.46	15.22	23.41	2.34	2.24	10.64	3.60	3.44	16.36
	195.33	5.09	10.81	38.87	2058.17	10.54	14.05	0.98	2.08	7.48	1.30	2.77	9.97
	221.59	5.12	17.33	38.29	9862.19	44.51	59.34	3.75	12.70	28.05	5.00	16.93	37.41
Sud Mennucci, SP81-3250	12.07	2.10	0.00	13.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	32.21	0.23	4.63	17.36	133.34	4.14	6.37	0.04	0.86	3.23	0.07	1.33	4.98
	44.43	5.65	1.00	15.95	961.54	21.64	36.07	5.41	0.96	15.27	9.02	1.60	25.46
	46.67	5.30	8.52	13.33	417.58	8.95	14.91	1.75	2.81	4.39	2.91	4.68	7.32
	47.77	5.78	0.00	10.87	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	66.45	0.00	2.38	2.98	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	278.39	5.26	6.31	25.10	311.85	1.12	10.05	0.16	0.19	0.77	1.44	1.73	6.88

Table 1. *Cont.*

County and Sugarcane Variety	Cropped Area Surveyed (ha)	Stalk-Related Disorder Incidence (%) Red Rot (I.rr)	Spittlebug (I.sib)	Dry Rot (I.dr)	Total Losses in the Areas (t)	Yield Losses (TL) (t·ha^{-1})	Relative Yield Losses (RL) (%)	Yield Losses Associated with the Stalk Disorders (t·ha^{-1}) Red Rot (rrTL)	Spittlebug (sbiTL)	Dry Rot (drTL)	Relative Yield Losses Associated with the Stalk Disorders (%) Red Rot (rrRL)	Spittlebug (sbiRL)	Dry Rot (drRL)
Mean	**142.73**	**5.83**	**7.41**	**19.54**	**2713.84**	**14.23**	**20.17**	**2.65**	**3.45**	**8.13**	**3.67**	**4.69**	**11.82**
95% CI	37.68	2.43	2.42	3.68	1044.87	3.83	5.22	1.51	1.34	2.72	1.98	1.76	3.93
Total	**4852.84**	-	-	-	**92,270.48**	**483.88**	-	**90.09**	**117.23**	**276.56**	-	-	-

Table 2. Yield losses associated with the incidences of stalk-related disorders in commercial sugarcane fields with the varieties CTC4 and SP81-3250 grouped by county in northwestern São Paulo in 2017/2018.

County, Sample Size (= *N* Fields)	Mean Cropped Area Surveyed (ha)	Stalk-Related Disorder Incidence (%) Red Rot (I.rr) *		Spittlebug (I.sib)	Dry Rot (I.dr)	Total Losses in the Areas (t)	Yield Losses (TL) (t·ha^{-1})	Relative Yield Losses (RL) (%)	Yield Losses Associated with the Disorders (t·ha^{-1}) Red Rot–rrTL *		Spittlebug (sbiTL)	Dry Rot (drTL)	Relative Yield Losses Associated with the Disorders (%) Red Rot (rrRL) *		Spittlebug (sbiRL)	Dry Rot (drRL)
Castilho, *N* = 3	247.79	15.35	**a**	9.42	18.52	5955.39	22.64	32.11	9.45	**a**	5.08	8.11	12.61	**a**	7.02	12.47
Ilha Solteira, *N* = 4	202.56	12.33	**a**	11.23	23.08	4075.67	17.12	24.57	5.46	**a**	2.97	8.69	7.38	**a**	3.87	13.31
Itapura, *N* = 6	218.15	2.49	**b**	5.68	16.81	4520.12	18.35	21.40	2.03	**b**	4.25	12.07	2.52	**b**	4.90	13.97
Pereira Barreto, *N* = 6	79.84	7.41	**b**	12.13	18.82	1085.44	9.61	13.37	1.90	**b**	5.43	2.28	2.64	**b**	7.51	3.22
S.A.Aracanguá, *N* = 8	122.91	2.38	**b**	6.13	25.49	2830.50	17.99	26.92	1.12	**b**	3.41	13.46	1.61	**b**	4.88	20.42
Sud Mennucci, *N* = 7	75.43	3.47	**b**	3.26	14.10	260.62	5.12	9.63	1.05	**b**	0.69	3.38	1.92	**b**	1.33	6.38
Mean	142.73	5.83		7.98	19.54	2713.84	14.23	20.17	2.65		3.45	8.13	3.67		4.69	11.82
95% CI	37.68	2.43		2.55	3.68	1044.87	3.83	5.22	1.51		1.34	2.72	1.98		1.76	3.93

* Means followed by the same letter are not significantly different based on the Scott–Knott test ($p \leq 0.05$).

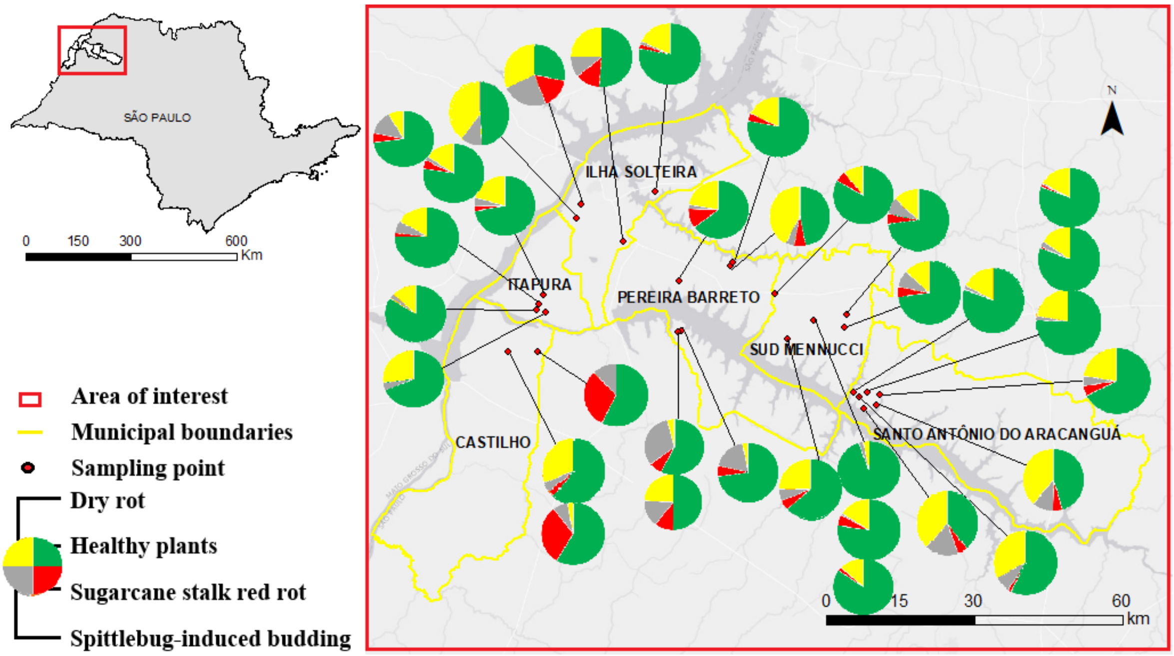

Figure 2. Relative frequencies of the occurrence and distribution of the sugarcane stalk red rot disease caused by *Colletotrichum falcatum* (in red), the spittlebug-induced budding caused by the insect pest *Mahanarva fimbriolata* (in gray), stalk internal dry rot associated with the simultaneous occurrence of *C. falcatum* and *T. paradoxa* (in yellow), and healthy plants (in green) from 34 sugarcane cropping fields sampled from six counties in northwestern São Paulo in 2017/2018. [a]; [a] The data were derived from a large-scale survey run by Usina Santa Adélia Mills. A total of 100 plants·ha^{-1} were sampled from each field. The average field area surveyed was 142.7 ha (95% CI = 37.8 ha), with a minimum area of 12.1 ha and a maximum of 420.5 ha. The total area surveyed spanned 4852.8 ha of sugarcane cropping in the region.

A total of 100 plants·ha^{-1} were randomly sampled from each sampling field. Sampling was performed between 9 May and 11 December 2018 during the sugarcane maturation and ripening physiological phase. To assess the incidence of the red rot disease, the plant stalks sampled were split in half along their longitudinal axes to detect the typical internal colonization symptoms of the red rot disease (Figure 1A,B). These stalks were simply categorized as either healthy or diseased. The incidences of the two other stalk-related disorders (stalk internal dry rot (Figure 1D,E) and the spittlebug-induced shoot stunting), when detected, were also cataloged.

2.2. Mapping and Estimates Related to the Field Prevalence and Distribution of Three Stalk-Associated Disorders in Sugarcane Plantations

Mechanical harvesting was conducted in order to determine the total yield from each commercial field surveyed, using the available technical resources and infrastructure from Usina Santa Adélia Sugarcane Mills.

2.2.1. Mapping the Frequency Field Distribution of Sugarcane Stalk-Associated Disorders in Northwestern São Paulo

With the help of ArcGIS 10.2.2 software [40], a thematic map was generated to graphically represent the frequency distribution of the incidence of red rot disease in relation to healthy stalks, spittlebug-induced budding, and the stalk internal dry rot due to unknown causes.

2.2.2. Estimating the Prevalence and Distribution of Sugarcane Stalk-Associated Disorders

Based on estimated and actual yield data, as well as on data regarding the relative incidence of the sugarcane red rot and the two other stalk-related disorders detected in the fields, the total and the relative yield losses caused by each of the three disorders were estimated as follows:

(1) TL = (Estimated yield–Actual yield)/total area sampled, where TL is total yield loss in the area in t·ha^{-1};
(2) RL = [(Estimated yield–Actual yield)/Estimated yield], where RL is the relative yield loss in each area as a percentage;
(3) rrTL = TL × [(I.rr)/(I.rr + I.sib + I.dr)], where rrTL is the yield loss associated with the incidence of the red rot disease (t·ha^{-1}), I.rr = incidence of the red rot disease (%), I.sib = incidence of the spittlebug-induced budding (%), and I.dr = incidence of the stalk internal dry rot (%);
(4) rrRL = RL × [(I.rr)/(I.rr + I.sib + I.dr)], where rrRL is the relative yield loss associated with the red rot disease (%);
(5) sbiTL and (6) drTL (the yield losses associated with spittlebug-induced budding or stalk internal dry rot) were calculated in a similar manner to (3), while (7) sbiRL and (8) drRL (the corresponding relative yield losses) were calculated in a similar manner to (4).

The medians of the percentage incidences of red rot disease (I.rr), spittlebug-induced budding (I.sib), and stalk internal dry rot (I.dr) disorders, as well as the relative yield loss and the total yield loss (in percentage and t·ha^{-1}) estimates, were depicted on a boxplot distribution constructed using the R software version 4.2.3 [46] (Figure 3). The Scott–Knott test (at 5% probability) was performed for means comparison in the R environment using the statistical packages *agricolae* and *ScottKnott* [46].

2.3. Predicting the Yield Losses in Northwestern São Paulo Sugarcane Fields on the Basis of Each Independent Stalk-Related Disorder

To determine the contributions of the three stalk-related disorders to the total yield losses detected in the sugarcane fields, we started by building the multiple regression model lm (TL~rrTL + sbiTL + drTL) and calculated the estimates for the model's coefficients, their standard errors, their t values and the corresponding $p(>|t|)$, the multiple and adjusted R^2, and the $F_{statistic}$. Using the R software, we also used the non-parametric random bootstrap approach with N = 5000 resampling iterations to determine the 95% confidence interval for the model's coefficients estimates [47] using the R code:

```
BootstrapRandomX <- function(dat=mydata, mod.formula=formula
    (TL~rrTL + sbiTL + drTL))
    {dat.boot <- dat[sample(x = nrow(dat), size = nrow(dat), replace=T),]
    boot.lm <- lm(mod.formula, data=dat.boot)
    coef(boot.lm)}
    N = 5000
    vector.boot <- t(replicate(N, BootstrapRandomX()))
    apply(vector.boot, MARGIN = 2, sd)
    t(apply(vector.boot, MARGIN = 2, quantile, probs=c(0.025, 0.975)))
```

We continued by building the simple linear and polynomial (quadric and cubic) regression models to predict the sugarcane yield losses on the basis of each independent stalk-related disorder variable. The data were randomly split into a training set (77.5% of the data, used for building a predictive model) and a test set (22.5%, used for evaluating the model), and a random seed for reproducibility was set [47].

Next, the R function *predict()* (specifying the model, the data set, and the option *interval = "prediction"*) was used for predicting outcome values and the corresponding 95% confidence interval (95% CI), reflecting the uncertainty around the mean predictions for each regression model. The comparisons between regression models performance were performed by analyzing the *RMSE* and R^2 metrics [47]. The *RMSE* represents the model prediction error, which is the average difference between the observed outcome values and the predicted outcome values. The R^2 represents the squared correlation between the observed and predicted outcome values for the model. The best model fit to the data was the one with the lowest *RMSE* and the highest R^2. For depicting a scatter plot containing a regression line and the confidence interval band for the linear model chosen [47] (Figure 4), we used the *R* library *ggplot2* and the *R* code:

```
# Building the model–Linear model:
    model <- lm(y~x, data = train.data)
# For a quadric or cubic polynomial, the model would be:
        model <- lm(y~poly(x, 2, raw = TRUE), data = train.data) # for quadratic
        model <- lm(y~poly(x, 3, raw = TRUE), data = train.data) # for cubic
# Making predictions:
    predictions <- model %>% predict(test.data)
# Model performance
    data.frame(RMSE = RMSE(predictions, test.data$y), +
    R2 = R2(predictions, test.data$y))
    # Visualizing the data:
    p1<-ggplot(train.data, aes(x, y)) +
    geom_point() + stat_smooth(method = lm, formula = y~x)
    p1
# For a quadric or cubic polynomial, the code would be:
    stat_smooth(method = lm, formula = y~poly(x, 2, raw = TRUE)) # for quadratic
    stat_smooth(method = lm, formula = y~poly(x, 3, raw = TRUE)) # for cubic
    # Adding predictions:
    pred.int <- predict(model, interval = "prediction")
    datalwrupr <- cbind(train.data, pred.int)
    # Regression line plus confidence intervals:
    p2 <- ggplot(datalwrupr, aes(x, y)) +
    geom_point() +
    coord_cartesian(xlim = c(0, 45), ylim = c(-10,45))+
    stat_smooth(method = lm, formula = y~x) +
# For a quadric or cubic polynomial, the code would be:
    stat_smooth(method = lm, formula = y~poly(x, 2, raw = TRUE)) + # for quadratic
    stat_smooth(method = lm, formula = y~poly(x, 3, raw = TRUE)) + # for cubic
# Adding prediction intervals to the regression line:
    geom_line(aes(y = lwr), color = "red", linetype = "dashed")+
    geom_line(aes(y = upr), color = "red", linetype = "dashed")+
    theme (panel.background = element_rect(fill = "white", color = "black"),
    panel.grid.minor = element_line(color = "gray50"),
    panel.spacing = unit(5, "lines"))
p2
```

2.4. Detection of Pathogens Associated with Sugarcane Stalk Internal Dry Rot

Based on information shared by the regional sugar mills, the stalk internal dry rot disorder has been associated with simultaneous infection with *C. falcatum* and *T. paradoxa* (Usina Santa Adélia Sugarcane Mills, personal information). To confirm this a priori assumption, we aimed to assert the etiology of the stalk internal dry rot disorder via field surveys of symptomatic stalks.

One hundred samples of sugarcane stalks either showing or not showing symptoms of internal dry rot were washed with water and soap to remove coarse dirt. Stalk fragments were taken from the boundary area between healthy and diseased tissue and subjected to indirect fungal isolation. Prior to isolation, the stalk fragments were surface disinfected in 70% alcohol for 30 s and 2% sodium hypochlorite for one minute, washed in sterile distilled water, dried on a sterile filter paper, and platted on Potato-Dextrose-Agar (PDA) culture medium (24 $g \cdot L^{-1}$ potato-dextrose (Kasvi, India), 20 $g \cdot L^{-1}$ agar), amended with tetracycline at 0.05 $g \cdot L^{-1}$. The plates were incubated at 25 °C for seven days.

Once pure cultures of the fungal isolates were obtained, sterilized filter paper disks were put on top of the cultures for a 5-day colonization period, after which they were transferred to cryotubes containing sterilized silica gel for long-term storage at −20 °C.

The micro-morphological characterization of *C. falcatum* and *T. paradoxa* strains was performed using pure cultures growing on low-strength PDA (5 $g \cdot L^{-1}$ potato-dextrose (Kasvi, India), 20 $g \cdot L^{-1}$ agar) for 10 days at 25 °C. After this incubation period, the fungal structures were observed via bright field microscopy (BFM) and scanning electron microscopy (SEM).

Representative strains from each pathogen were chosen for the micro-morphological characterization, taking 20 to 30 measurements per structure [19,21,28,43]. The specimens for BFM analysis were mounted with slides and coverslip using lactoglycerol (1:1:1 lactic acid, glycerol and water), and the images and biometric measures were acquired via Zeiss AxioVert.A1 microscopy using the AxioVision version 4.5 software.

For SEM, 5 mm diameter samples of plug colonies with fungal sporulation were collected, fixed in 70% formalin acetic alcohol (FAA) [48], and stored under refrigeration. The fixed samples were dehydrated in ethanol series treatment (at 70, 80, 90, and 99.5%), dried at critical points, and metallized with gold [49]. The images were acquired using a Zeiss EVO/LS15 Scanning Electron Microscope at the Chemistry–Physics Department (UNESP, Ilha Solteira Campus).

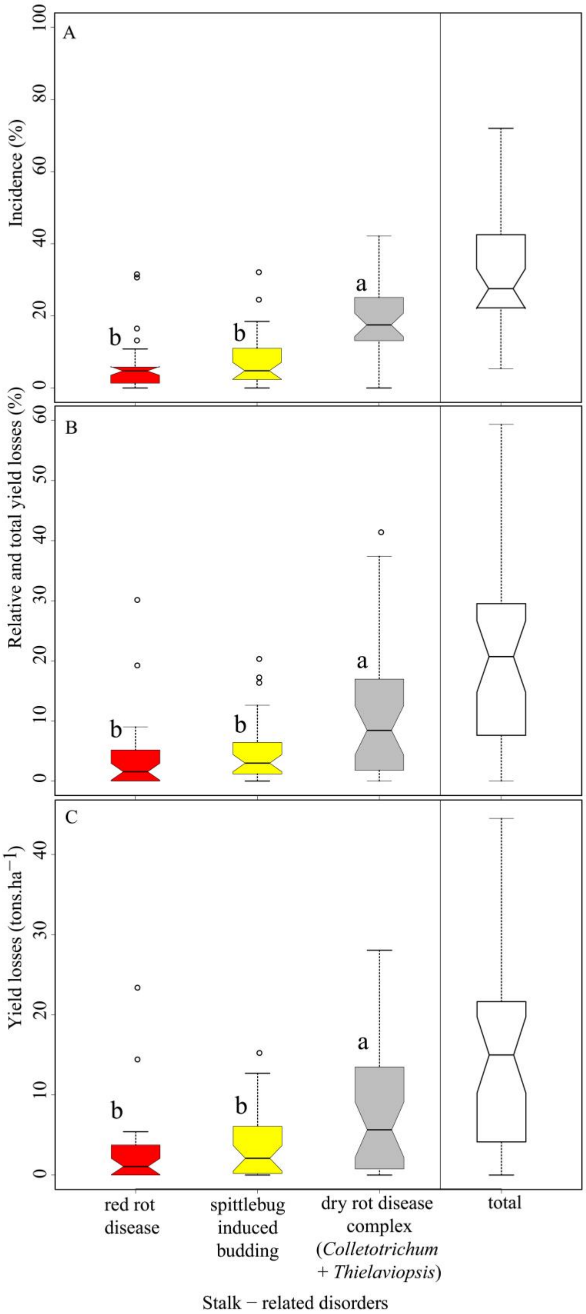

Figure 3. Boxplots depicting the distribution of percentage incidence (**A**), percentage of relative and total yield losses (**B**) and yield losses in t·ha^{-1} (**C**) associated with stalk red rot disease (red), spittlebug-induced budding (yellow), stalk internal dry rot (gray), and the overall total (white) in 34 cropping areas from counties in northwestern São Paulo in 2017/2018. [a]; [a] Means followed by the same letter are not significantly different when assessed via the Scott–Knott test at $p \leq 0.05$.

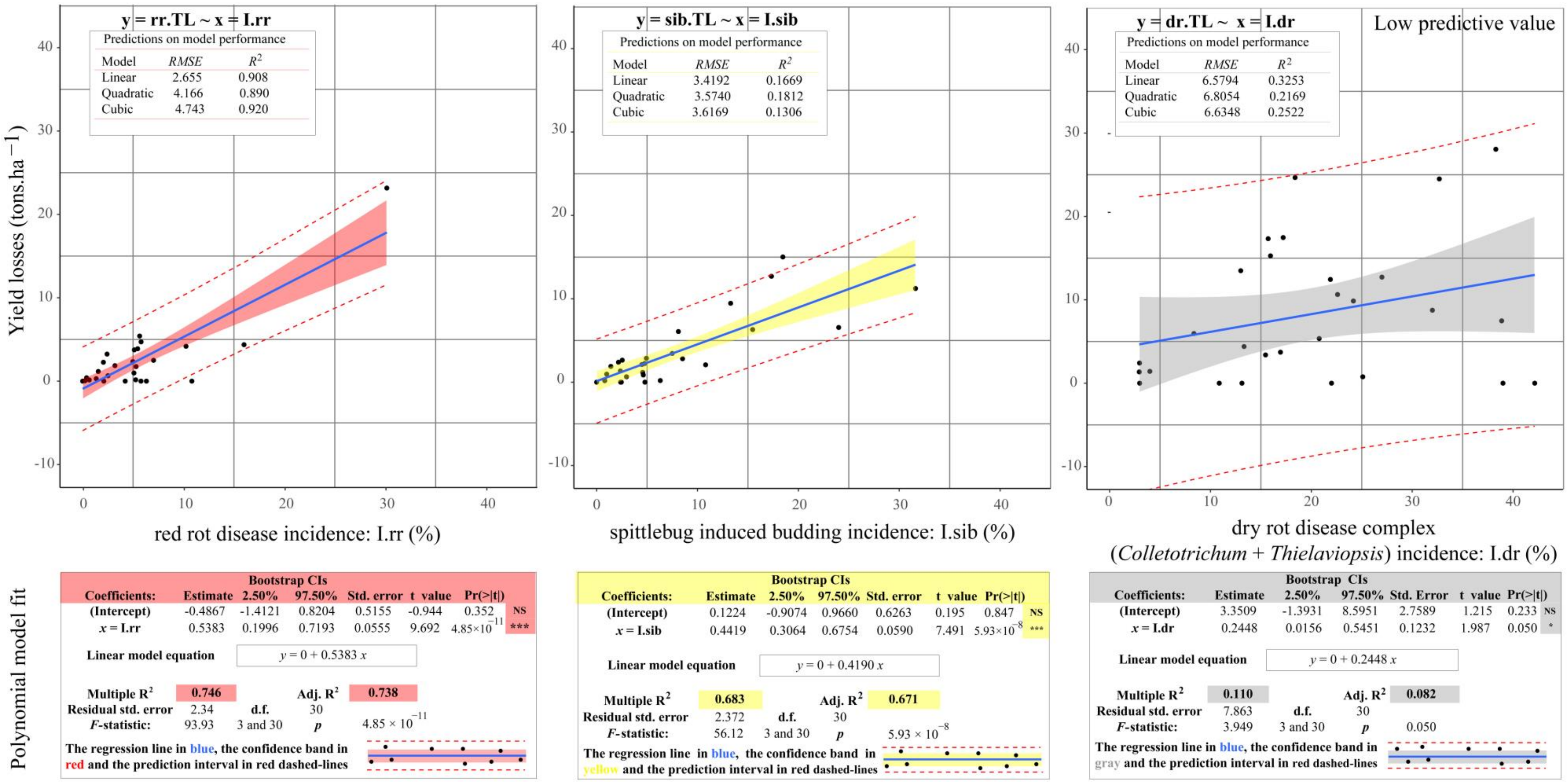

Figure 4. Simple linear regression models used to describe and predict the yield losses in sugarcane fields in northwestern São Paulo on the basis of each independent stalk-related disorder, as well as the corresponding model fit coefficients and parameters. [a]; [a] The data were obtained by sampling 34 commercial fields in the 2017/2018 cropping season. [NS] Non-significant, * Significant at $p \leq 0.05$; *** Significant at $p \leq 0.001$.

3. Results

3.1. Mapping the Frequency Field Distribution of Sugarcane Stalk-Associated Disorders in Northwestern São Paulo

The survey of the incidences of stalk-related disorders in sugarcane commercial fields in six counties in northwestern São Paulo indicated a widespread occurrence of all three disorders and a significant impact on sugarcane yield (Table 1 and Figure 2).

3.2. Estimating the Prevalence and Distribution of Sugarcane Stalk-Associated Disorders

The red rot disease caused by *C. falcatum* was detected in 88.2% of the fields surveyed (30 in 34 fields total). The average incidence of the red rot disease was 5.8% (95% CI = 2.4%), ranging from a minimum of 0% to a maximum of 30.9% (Table 2). Fields in Castilho and Ilha Solteira counties had the highest incidences of red rot disease (15.4 and 12.3%, respectively), which were significantly different to the averages of all the remaining counties, with incidences ranging from 2.4 to 7.4% (Table 2).

Both spittlebug-induced budding and the stalk internal dry rot disorder were detected in 97.1% of the fields surveyed (33 in 34 fields). The average incidence of spittlebug-induced budding was 7.4% (95% CI = 2.4%), while the maximum incidence was 31.6%.

The disorder with the highest level of incidence was stalk internal dry rot, with an average of 19.5% (95% CI = 3.7%) and a maximum of 42.2% (Table 2). In fact, the incidence of the internal dry rot was significantly higher than those of the other two stalk disorders (Figure 3A). The incidences of red rot and spittlebug budding were similar across all the six counties surveyed (Table 2).

In general, the total yield losses associated with the stalk disorders detected in all 34 sugarcane fields surveyed reached 92,270 t, with an average of 2714 t (95% CI = 1045 t) per field. The total yield losses per hectare (TL·ha^{-1}) were 483.9 t over the entire area surveyed, with an average of 14.2 ($\pm$3.8) and a maximum of 44.5 t·ha^{-1} (Table 2) This represented an average yield loss of 20.1 ($\pm$5.2)% of the total estimated sugarcane yield for the area. However, there was a single field in Santo Antonio do Aracanguá with a maximum yield loss of 59.3% (Table 1).

Individually, the stalk internal dry rot was also the disorder associated with the highest yield losses, with an average of 8.1 t·ha^{-1} (95% CI = 2.7) per field (Table 2) and a maximum of 28.5 t·ha^{-1} in the same field in Santo Antonio do Aracanguá (Table 1). The yield losses associated with the internal dry rot were significantly higher than the losses caused by the other two stalk disorders (Figure 3B,C), adding up to 276.6 t·ha^{-1} in all fields surveyed (Table 1), representing 57.2% of the total yield losses detected.

In comparison, the red rot and the spittlebug-induced budding total yield losses were 90.1 and 117.2 t·ha^{-1} (Table 1), representing 18.6 and 24.2% relative yield losses, respectively.

Castilho and Ilha Solteira counties had the higher yield losses associated with red rot disease (9.5 and 5.5 t·ha^{-1}, respectively), which were significantly different to the averages of all the other counties, with yield losses ranging from 1.1 to 2.0 t·ha^{-1} (Table 2). The amount of yield losses associated with spittlebug-induced budding and the dry rot disorder were similar across all the six counties surveyed (Table 2).

3.3. Predicting Yield Losses in Northwestern São Paulo Sugarcane Fields on the Basis of Each Independent Stalk-Related Disorder

The multiple regression model built to determine which of the three stalk-related disorders contributed most to the total yield losses detected in sugarcane fields (i.e., the total yield losses (TL) as a function of *rrTL*, *sbiTL*, and *drTL*) was not significant (F_{model} = 1.754, p = 0.177), and the multiple R^2 was considerably low (0.1493). The *t*-test's *p*-values and the bootstrap confidence intervals for each one of the equation's coefficients indicated that all the estimates (the equation intercept and the stalk-related disorders variables *rrTL*, *sbiTL*, and *drTL*) were not significantly different to zero. This indicates that the multiple regression model that was built to join together all three variables could not explain the sugarcane yield losses detected.

Subsequently, single-variable analyses of polynomial (linear, quadratic, and cubic) regression models' performances indicated that, for all three variables, the simple linear was the best fit to independently predict sugarcane yield losses on the basis of each stalk-related disorder (Figure 4). The simple linear model had both the lowest model prediction error *RMSE*, as well as either similar or higher R^2 metrics, which is the squared correlation between the observed and predicted outcome values for the model (Figure 4). However, positive and predictively significant correlations were only detected between sugarcane yield losses in $t{\cdot}ha^{-1}$ and the incidences of stalk red rot disease (x = *I.rr*) and spittlebug-induced budding (x = *I.sib*). The multiple and the adjusted R^2 values for these yield loss linear models were 0.68 for *I.sib* and 0.75 for *I.rr*, and the F values for the model were at $p \leq 0.001$, indicating a significant predictive value for these variables, supported by a narrower predictive interval (red dashed line). In contrast, the incidence of the dry rot disorder (*I.dr*) had low predictive value for the yield losses detected (Adj. R^2 = 0.084, F value for the model at the cutting p = 0.05), spanning a much wider or uncertain predictive interval (Figure 4).

3.4. Detection of Pathogens Associated with Sugarcane Stalk Internal Dry Rot

Using the indirect isolation technique, we detected the simultaneous infection of the pathogens *C. falcatum* and *T. paradoxa* in 99% of sugarcane stalks with dry rot symptoms, which confirmed the a priori assumption based on information shared by Usina Santa Adélia Sugarcane Mills.

Regarding the micro-morphological characterization of *C. falcatum* isolated from the infected stalks (Figure 5A–C), the fungal strains had setae that were septate, dark brown in color, and rounded at the apex, measuring between 75.5 and 110 µm (Figure 5B). The conidia were either sickle-shaped or spindle-shaped, hyaline, and aseptate, with 15.5–25 × 5.0 µm (Figure 5A,C). Appressoria and perithecia were not observed.

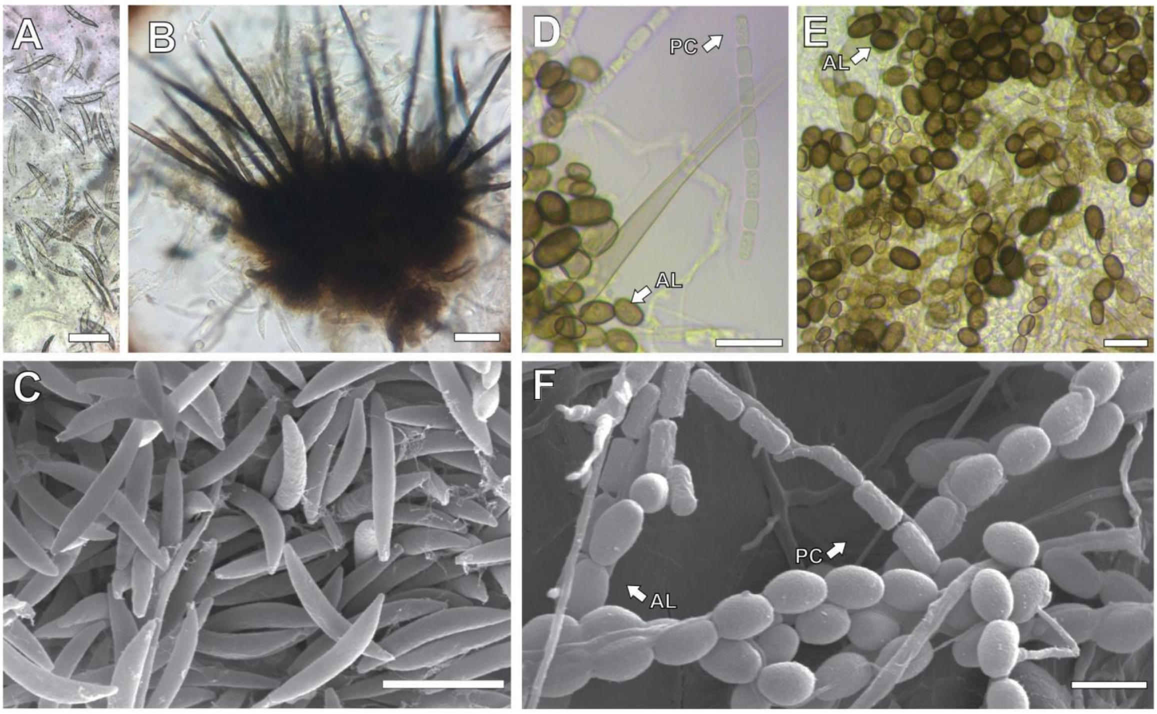

Figure 5. Photomicrographs (**A**,**B**) and scanning electron micrograph (**C**) showing *Colletotrichum falcatum* structures: hyaline falcate and aseptate conidia (**A**,**C**) and acervulus structure with septate and dark brown setae (S, arrow) (**B**). Scale bars: A, B = 10 µm, C = 20 µm. Photomicrographs (**D**,**E**) and scanning electron micrograph (**F**) showing *Thielaviopsis paradoxa* structures: (**D**,**E**)—hyaline, cylindrical, and unicellular primary conidia (PC, arrows); aleuroconidia produced in chains with a subglobose shape and brownish color when mature (AL, arrows). (**F**): primary conidia (PC, arrow) and aleuroconidia (AL, arrow) produced in chains. Scale bars: (**D**,**E**) = 100 µm; (**F**) = 10 µm.

Concerning the micro-morphological characterization of *T. paradoxa* isolated from dry rot symptomatic stalks, the fungal strains produced long and unbranched phialides, measuring 73.0–180.5 × 6.0–9.0 µm (Figure 5D). Two distinct types of conidia were identified: the primary conidia, which was hyaline, produced in chains, cylindrical, and unicellular, measuring 2.0–4.0 × 6.0–10.0 µm (Figure 5D,F), and the aleurioconidia, which turned brown upon maturation, was produced holoblastically, was either singly distributed or in short chains, and ovoid to subglobose in shape, measuring 3.0–4.0 × 8.0–10 µm (Figures 4E,F and 5D). Perithecia were not observed.

The morphological characteristics observed for *C. falcatum* strains aligned with those formerly described by Costa et al. [22] and Hossain et al. [50]. Similarly, the specific characteristics of *T. paradoxa* strains resembled those in the description of the pathogen in the CABI Compendium [28].

4. Discussion

Major changes in the sugarcane cropping system implemented in the last decade to promote more sustainable agriculture [2], as well as the fast expansion to new agricultural frontiers in northeastern São Paulo, Brazil [51], have unintentionally increased the incidence of biotic stresses on the crop. These major changes included the prohibition of pre-harvest burning [10,12], associated with the generalized adoption of mechanical harvesting [11,14]. Together, they resulted in the buildup of large amounts of crop residues and favored the survival of both insect pests and plant pathogens' inocula in sugarcane straw [17,19,22–26]. Besides the quantitative yield losses associated with these disorders [16,29,31,52], damaged sugarcane stalks arrive at the industry dead and dry, reducing their milling capacity, as well as microbiologically contaminated due to cracks and deterioration, thus decreasing sugar production and inhibiting fermentation required for alcohol production [33].

Our study aimed to reveal the relative importance of three major stalk-associated disorders by surveying their spontaneous incidences and corresponding yield losses in 34 commercial sugarcane fields in northwestern São Paulo, installed during the last decade's sugarcane expansion. This extensive survey was conducted in 2017/2018. These three disorders included the spittlebug-induced shoot stunting (*M. fimbriolata*), the stalk red rot disease caused by *C. falcatum*, and the stalk internal dry rot disorder associated with simultaneous infection with *C. falcatum* and *T. paradoxa*, i.e., the pineapple sett rot pathogen. Until now, there has been no such large-scale study to reveal the relative importance of major stalk disorders in newly expanded sugarcane areas in São Paulo, Brazil, to support an appropriate and sustainable pest management program that could reduce their impact.

We tested the hypothesis that the stalk red rot disease was the major stalk-related disorder in the recently established sugarcane commercial fields in northeastern São Paulo, considering previous studies conducted elsewhere in Brazil [20,22]. The red rot disease caused by *C. falcatum* was widely distributed, being detected in 88% of the 34 fields surveyed, with an average incidence of 5.8% (95% CI = 2.4%), causing yield losses of 90.1 t·ha^{-1} (Table 1), equivalent to 18.6% of the total yield losses. Pearson's correlation coefficient between the incidence of the red rot disease (I.rr) and yield losses was high (r = 0.87), indicating that the higher the incidence of the red rot disease (*I.rr*), the higher the yield losses [45]. Supporting this observation, the incidence of the red rot disease in varietal crosses of sugarcane from India negatively correlated with soluble solid content, brix, sucrose accumulation, and yield [53].

Subsequently, in order of importance, the spittlebug-induced budding was detected in 92% of fields, with an average incidence of 7.4% (95% CI = 2.4%), causing yield losses of 117.2 t·ha^{-1} on average (Table 1), representing 24.2% of the total yield losses. However, the disorder with the highest level of incidence was the stalk internal dry rot, with an average incidence of 19.5% (95% CI = 3.7%). The average yield losses associated with the internal dry rot were 276.6 t·ha^{-1} in all fields surveyed (Table 1), representing 57.2% of the total yield losses detected. Therefore, we concluded that the stalk internal dry rot, as a disease complex associated with both *C. falcatum* and *T. paradoxa*, was the most important disorder in sugarcane fields in the northwestern São Paulo region.

With the goal of proposing a sustainable pest management program to ensure good practices [54] that reduce the impacts of all three stalk-associated disorders on regional sugarcane production, the most effective strategy to implement is the improvement in the general health quality of the seedlings produced in northwestern São Paulo. Nevertheless, no policy exists that enforces minimum plant health standards for sugarcane nurseries, in which locations healthy seedlings can be produced, marketed, and distributed in attendance to the demands of these vast new-frontier cropping areas [2,7,55]. Such nurseries ought to be established by planting thermally, chemically or biologically treated sugarcane cuts for managing, in particular, the stalk-borne inocula from *C. falcatum* and *T. paradoxa*, as well as spittlebug infestation [2,17,31,54]. However, no such high-plant-health-quality nurseries have been established either historically or recently in these new sugarcane frontier [7,44]. Consequently, a large number of sugarcane varieties from nurseries with low or inadequate plant health quality statuses were introduced into the newly expanded cropping areas, resulting in the general spread of the insect pests and stalk-associated fungal pathogens [15,19,20,22,24,32,37,40,42].

The second key sustainable strategy for managing the stalk-related disorders is the removal of sugarcane residues from the fields to substantially decrease *C. falcatum* and *T. paradoxa* inocula [16,20,23,27,29,32] and the spittlebug *M. fimbriolata* field population [14,18,19]. This strategy is considered implementable and fully sustainable, since the industrial use of sugarcane residues in boilers to cogenerate electricity is now feasible, and the biorefinery production of second-generation ethanol from sugarcane residues is a current reality [56,57]. Residues from sugarcane crop may represent a total of 10 to 30 t ha^{1} year^{1} dry biomass [58,59].

The third key sustainable strategy is the choice of resistant sugarcane varieties. However, the most planted sugarcane varieties in São Paulo state are susceptible or highly susceptible to stalk red rot and/or pineapple sett rot. Resistance, when detected, seemed not to be durable [20,29,31,44,60]. This is possibly due to the high genetic variability in *C. falcatum* and *T. paradoxa* for virulence [20,25,32,50]. The populations of pathogens with high genetic variability for virulence may overcome the resistance of new genetic materials under high selection pressure via the large-scale cultivation of clonal sugarcane genotypes, putting the durability of the cultivation of new varieties at risk [35,44,61,62]. Since the levels of genetic and phenotypic variation in the virulence of *C. falcatum* and *T. paradoxa* in sugarcane in São Paulo are not yet fully known, future studies involving their characterization are extremely relevant for predicting the durability of the genetic resistance of cultivars and designing management strategies.

The fourth key sustainable strategy proposed is biological control with microbial fungicides and insecticides via the mass introduction of antagonist or entomopathogenic microorganisms against the pathogens and insect pests associated with the stalk disorders [27,34,38,39,43,45,63]. There are currently 12 commercial microbial fungicide formulations labeled for the management of stalk diseases caused by *C. falcatum* (N = 1) or *T. paradoxa* (N = 11), composed of mixtures of the antagonistic *Bacillus* species *Bacillus subtilis*, *B. pumilus*, *Bacillus velezensis*, *Bacillus amyloliquefaciens*, and/or *Bacillus thuringiensis;* a mixture of *B. velezensis* and *Trichoderma harzianum*; a mixture of *B. amyloliquefaciens*, *T. harzianum* and *T. asperellum;* and *T. harzianum* alone [64]. These diverse microbial fungicides can act directly as antagonists against the pathogens via parasitism, antibiosis, and competition, as well as by indirectly inducing systemic resistance in the plants [65]. In comparison, there are 72 commercial microbial insecticide formulations labeled for the biocontrol of the spittlebug *M. fimbriolata*. These insecticide formulations are basically composed of the entomopathogenic fungus species *Metarhizium anisopliae* strain IBCB425 (N = 70 commercial formulations), the *M. anisopliae* strain E9 (N = 1), or the *M. anisopliae* strain IBCB348 (N = 1). The use of the entomopathogenic fungus *Metarhizium* against sugarcane spittlebugs in Brazil is considered one of the most successful and long lasting biocontrol initiatives in the world [66].

A broader pest management program against sugarcane stalk-associated disorders could also integrate the application of synthetic pesticides [2,67]. There are currently 51 synthetic fungicide formulations labeled for the management of the stalk rot diseases caused by *C. falcatum* or *T. paradoxa* [64]. These formulations include mixtures of the (i) single-site systemic fungicides quinone outside inhibitors (QoI) (azoxystrobin, picoxystrobin and pyraclostrobin), demethylation inhibitors (DMI) (cyproconazole, difenoconazole, flutriafol, mefentrifluconazole and tebuconazole), succinate dehydrogenase inhibitors (SDHI) (carboxin, fluxapyroxad), methyl benzimidazole carbamates (MBC) (carbendazim, thiabendazole, thiophanate-methyl), phenylpyrrole (fludioxonil), and 2,6-dinitro-anilines (fluazinam) and (ii) the multisite protectant fungicides dithiocarbamates (mancozeb, metiram, thiram), inorganics (copper oxychloride), and phthalimides (captan) [64]. In addition, there are 34 synthetic insecticide formulations labeled for the management of the *M. fimbriolata* [64], containing the following active ingredients from the following chemical groups: neonicotinoid (acetamiprid, dinotefuran, imidacloprid, thiacloprid, and thiamethoxam); pyrethroid (alpha-cypermethrin, bifenthrin, and lambda-cyhalothrin); benzofuranyl methylcarbamate (carbosulfan); phenylpyrazole (ethiprole); pyridyloxypropyl ether (pyriproxyfen); and sulfoximines (sulfoxaflor).

However, the application of synthetic fungicides and insecticides for the management of the sugarcane stalk-related disorders is of great concern, since the common in-furrow applications result in the accumulation of high amounts of pesticide residues in the soil environment [30,68–70]. The use of synthetic fungicides is also discouraged by the potential onset of fungicide-resistant populations of fungal pathogens if large-scale applications of high-risk systemic single-site fungicides are adopted in extensive sugarcane plantations [65,71,72]. In particular, for synthetic insecticides, their use is also considered problematic because of their low efficacy in terms of controlling nymphs of *M. fimbriolata* and their probable impact on crops' natural enemies' populations and biological structures [70]. Therefore, the application of synthetic fungicides or insecticides, with proven efficacy, should be restricted to sugarcane cuttings and pre-sprouted seedlings prior to planting to reduce the environmental impacts of these pesticides. Improved and appropriate application technologies are still needed for the large-scale pre-planting treatment of sugarcane cutting and seedling [40–42,55]. The comparative efficacy of all the distinct microbial and synthetic fungicides or insecticides labeled for the management of *C. falcatum*, *T. paradoxa*, or *M. fimbriolata* should also be consistently experimentally checked, cataloged, and publicly released so as to properly guide pest management decisions [72].

Finally, for the more effective management of sugarcane red rot, we should highlight the need for additional studies to reveal the more complex etiology and species diversity of the pathogens associated with both red rot and pineapple rot in a broader survey of traditional and newly expanded sugarcane cropping areas in São Paulo state. Three species of *Colletotrichum* (*C. falcatum*, *C. plurivorum*, and *C. siamense*) [20,22] and three species of *Fusarium* (*F. sacchari*, *F. proliferatum*, and *F. madaense*) [22] have already been associated with the sugarcane red rot disease in plantations from Alagoas (Northeastern Brazil), Minas Gerais (Southeastern Brazil), or Paraná state (Southern Brazil). Though *C. falcatum* has been considered the major species and the most aggressive on sugarcane stalks and leaves [20,22], *Fusarium* can also cause typical red rot symptoms, either alone or in co-infection with *Colletotrichum* [22]. In contrast, the *Fusarium* species also induced symptoms of pokkah boeng, which was not caused by any of the *Colletotrichum* species [22]. In addition, *C. plurivorum*, whose sexual stage was observed on the surfaces of sugarcane stems, did not induce stalk rot or leaf symptoms [22]. Concerning the etiology of the pineapple sett rot pathogens, based on phylogenetic analyses of three strains of the fungus from São Paulo, *T. ethacetica* has been indicated as the species associated with the disease on sugarcane, excluding the involvement of *T. paradoxa* as the pathogen [24]. A more thorough study, with a much higher sample size spanning the diversity of sugarcane cropping areas in São Paulo, is warranted to clarify this assertion.

5. Conclusions

The red rot disease caused by *C. falcatum*, the internal dry rot disease complex associated with both *C. falcatum* and *T. paradoxa*, and spittlebug-induced budding were major stalk disorders with widespread distribution in sugarcane fields in northwestern São Paulo, occurring in 88 to 92% of the 34 fields surveyed, causing significant yield losses. On average, the yield loss associated with these three stalk disorders was 14.2 (±3.8) t·ha^{-1}, which represented 20.1 (±5.2)% of the total estimated sugarcane yield. The disorder with the highest level of incidence was stalk internal dry rot, with an average of 19.5% (95% CI = 3.7%), followed by spittlebug-induced budding [7.4% (95% CI = 2.4%)] and the red rot disease [5.8% (95% CI = 2.4%)], which had similar incidences. A sustainable pest management program is needed to reduce the impacts of all three stalk-associated disorders on regional sugarcane production.

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