Elsevier Editorial System(tm) for Crop Protection

Manuscript Draft

Manuscript Number: CROPRO-D-20-00707R2

Title: EFFICACY OF FUNGICIDES AGAINST PEANUT SMUT IN ARGENTINA

Article Type: Full Length Article

Keywords: Key Words: Thecaphora frezii; Chemical control; in vitro sensitivity; peanut disease; active ingredients

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Abstract: Peanut smut, caused by Thecaphora frezii, increased its incidence and prevalence in the main growing region of Argentina in the last decade becoming the main peanut disease. Despite this fact, growers continue producing peanut without any disease management strategy what is leading to a local accumulation of T. frezii inoculum. The goal of the present study was to assess the efficacy of 12 fungicides for controlling T. frezii: in vitro assays, pots and field experiments were performed during 2014/15 and 2015/16. In vitro assays classified the fungicides upon their capacity to inhibit T. freezy mycelium growth as: thiophanate-methyl - not fungitoxic (EC50 > 100); mancozeb - moderately fungitoxic (EC50 = 6.28); difenoconazole, propiconazole, cyproconazole, tebuconazole, picoxystrobin, azoxystrobin, kresoxim-methyl, pyraclostrobin, penthiopyrad and chlorothalonil - highly fungitoxic (EC50 < 0.1). Azoxystrobin showed the highest control levels in pots experiments: 58.9% (2014/15) and 92% (2015/16). The greatest control efficiency in field experiments was observed for cyproconazole in 2015 and azoxystrobin in 2016, reducing peanut smut by 47.7 and 39.5% respectively. Based on our results, chemical control can be considered as a moderately efficient technique which may complement the cultivar genetic resistance and cultural practices in an integrated approach for managing peanut smut.

Research Data Related to this Submission

Title: Data for: EXPLORING THE EFFICACY OF FUNGICIDES IN CONTROL OF PEANUT SMUT
Repository: Mendeley Data
https://data.mendeley.com/datasets/9vbfc3h39g/draft?a=2f4d72e7-1939-4260-8660-d76b89867b55

Cover Letter

Peanut (*Arachis hypogaea* L.) is a high-value crop in Argentina with a significant contribution to the local economy. Peanut smut, caused by the soil-borne fungi *Thecaphora frezii* Carranza and Lindquist, is an endemic and yield reducing disease for the argentine main growing region. The pathogen is a biotrophic fungi that produces sorus consisting of a powdery mass of spores which colonize seed tissue partially or totally, with potential total damage of the plant pods. Peanut smut increased its incidence and prevalence in the main growing region of Argentina in the last decade becoming the main peanut disease. Survival fungi structures can remain viable for new infections up to four years which implies that a short peanut crop rotation is an inefficient disease management technique in the current argentine production system. In addition, no management strategies for reducing smut infections are adopted by argentine growers, what is leading to a local accumulation of *T. frezii* inoculum. Local preliminary research concerning chemical control of peanut smut reported a wide range of control efficacy: higher doses of DMI+QoI mixture fungicides presented the highest control efficacy. However, no studies of fungicide sensitiveness of local *T. frezii* populations are registered in Argentina, which is cornerstone knowledge for the setting of a chemical management strategy. The lack of studies examining a wide set of fungicides to control peanut smut led us to perform this work. In addition, it allows us to determine the effect of a large number of the active principles registered for the peanut culture in Argentina, being an important tool for the development of strategies for disease management. Results from this research show azoxystrobin and cyproconazole were the active ingredients that showed best responses, belonging to the QoI and DMI groups respectively. This report can be considered as an effective technique for the integrative smut management strategy, in addition to peanut genetic resistance and cultural practices

***Declaration of Interest Statement**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Data in Brief

Click here to download Data in Brief: efficacy_experiments.xlsx

Credit Author Statement

J.A. Paredes: Conceptualization, Investigation **L.I. Cazón:** Writing - Original Draft, Methodology **C. Oddino:** Project administration **J.H. Monguillot:** Data Curation, **A.M. Rago:** Supervision **J.P. Edwards Molina:** Validation, Formal analysis, Visualization, Writing - Review & Editing

EFFICACY OF FUNGICIDES AGAINST PEANUT SMUT IN ARGENTINA

~~EXPLORING THE EFFICACY OF FUNGICIDES IN CONTROL OF PEANUT SMUT~~

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Abstract

Peanut smut, caused by *Thecaphora frezii*, increased its incidence and prevalence in the main growing region of Argentina in the last decade becoming the main peanut disease. Despite this fact, growers continue producing peanut without any disease management strategy what is leading to a local accumulation of *T. frezii* inoculum. The goal of the present study was to assess the efficacy of 12 fungicides for controlling *T. frezii*; *in vitro* assays, pots and field experiments were performed during 2014/15 and 2015/16.~~The goal of the present study is to assess the efficacy of fungicide active ingredients for controlling *T. frezii*. We tested 12 fungicides from different chemical groups in agar plates assays, pots and field experiments during two growing seasons (2014/15 - 2015/16).~~ In vitro assays classified the fungicides upon their capacity to inhibit *T. freezy* mycelium growth as: thiophanate-methyl - not fungitoxic (EC50 > 100); mancozeb - moderately fungitoxic (EC50 = 6.28); difenoconazole, propiconazole, cyproconazole, tebuconazole, picoxystrobin, azoxystrobin, kresoxim-methyl,

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pyraclostrobin, penthiopyrad and chlorothalonil - highly fungitoxic (EC50 < 0.1). Azoxystrobin showed the highest control levels in pots experiments: 58.9% (2014/15) and 92% (2015/16). The greatest control efficiency in field experiments was observed for cyproconazole in 2015 and azoxystrobin in 2016, reducing peanut smut by 47.7 and 39.5% respectively. Based on our results, chemical control can be considered as a moderately efficient technique which may complement the cultivar genetic resistance and cultural practices in an integrated approach for managing peanut smut. ~~Thiophanate-methyl resulted not fungitoxic to *T. frezii* (EC50 > 100), mancozeb was moderately fungitoxic (EC50 = 6.28), and the rest of the active ingredients were classified as highly fungitoxic (EC50 < 0.1). We observed variability in the control efficacy however azoxystrobin showed the highest control levels in pots experiment 58.9% (2014/15) and 92% (2015/16). The greatest control efficiency in field experiment was observed for cyproconazole in 2015 and azoxystrobin in 2016, reducing peanut smut by 47.7 and 39.5% respectively. Based on our results, chemical control can be considered as a moderately efficient technique which may complement the cultivar genetic resistance and cultural practices in an integrated approach for managing peanut smut.~~

Key Words: *Thecaphora frezii*; Chemical control; *in vitro* sensitivity; peanut disease; active ingredients

1. Introduction

Peanut (*Arachis hypogaea* L.) is an annual extensive crop, with its center of origin in northwestern Argentina and southeastern Bolivia (Hammons et al., 2016). Argentina produces a high-quality peanut in an area of 350,000 has, concentrating more than 90% in the province of Córdoba located at the center region of the country. More than 95% of the peanut production is

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exported, previously processed in situ by the industry established in the same growing region (Agüero, 2017; Calzada and Rozadilla, 2018).

Peanut smut, caused by the soil-borne fungi *Thecaphora frezii* Carranza and Lindquist, is an endemic and yield reducing disease for the argentine main growing region (Marinelli et al., 2008, 2010; Rago et al., 2014). The pathogen is a biotrophic fungus that produces sori consisting of a powdery mass of spores which colonize the seed tissue partially or totally, with potential total damage of the plant pods ~~The pathogen is a biotrophic fungus that produces sori consisting of a powdery mass of spores which colonize seed tissue partially or totally, with potential total damage of the plant pods~~ (Astiz Gasso et al., 2008). The infection process occurs during the crop pegging ~~time~~stage: the stage that flower gynophore penetrates the soil surface, releasing an exudate that stimulate the spore germination~~the process in which the flower gynophore penetrates the soil surface, releasing an exudate that stimulate the spore germination~~ (Marinelli et al., 2008).

Currently, no management strategies for reducing *T. frezii* infections are adopted by argentine growers, which is leading to inter-annual inoculum accumulation (Paredes et al., 2017). Granoleico peanut cultivar (known susceptibility to the smut) is planted in more than 80% of the growing region (Cazón et al., 2018; Cignetti et al., 2010; Rago et al., 2017). Breeding programs with the aim of incorporating wild resistance genes have initiated recently, but new genotypes are not expected to be adopted in the short term (Bressano et al., 2019).

Survival structure can remain viable for new infections up to four years (Cazón et al., 2016b) which implies that a short peanut crop rotation is an inefficient disease management technique in the current argentine production system. Preliminary researches suggested certain levels of fungicide efficiency, applications with higher doses of DMI + QoI mixture fungicides reported to provide best control~~Local preliminary research concerning chemical control of peanut smut reported a wide range of control efficacy higher doses of DMI+QoI mixture~~

~~fungicides presented the highest control efficacy better (Cazón et al., 2013; Paredes et al., 2015b). In previous studies,. we observed~~ 58% smut control were observed by spraying a mixture of azoxystrobin and ~~+~~cyproconazole at a rate of 1000 cm³/ha, which is twice and half the recommended dose for peanut late leaf spot management caused by *Nothopassalora personata* (syn. *Cercosporidium personatum*) (~~(~~Cazón et al., 2013; Paredes et al., 2015b~~)~~; Paredes et al., 2015a). In addition, ~~we compare~~ the effect of timing of the day on the fungicide smut control efficacy was compared: fungicide applications during the night had higher control efficacy than sprayings during the day (Paredes et al., 2015c). The application timing ~~This time of the application~~ effect can be due to the fact that peanut plants fold their leaves at night and open them in the daytime (a process known as nyctinasty), allowing for more spraying drops to reach the soil surface.

No studies of fungicide sensitiveness of local *T. frezii* populations are registered in Argentina, and this information is essential for setting up of a chemical management strategy. The lack of studies examining a wide set of fungicides to control peanut smut led us to perform this ~~work~~study. The objectives of this work were ~~to~~ i) to assess the fungicides efficacy against *T. frezii* in in vitro conditions and ii) to test the field performance of fungicides against peanut smut.

2. Materials and Methods

2.1. In vitro sensitivity of fungicides based on mycelial growth inhibition test

To measure the *in vitro* sensitivity of fungicides, a mycelial growth test was carried out. A mixture of 10 *T. frezii* isolates obtained from smutted pods in 2015 in General Deheza (Córdoba province, Argentina) were used for the fungicide sensitivity test. Smut teliospores (100 mg) were suspended in aqueous solution of 0.63% NaOCl, agitated during 5 minutes, rinsed twice with sterile distilled water and resuspended in 2 ml sterile distilled water.

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Disinfected teliospores were placed in potato dextrose agar made up of broth extract of healthy grains at a rate of 50 g/l (Potato Peanut Dextrose Agar, PPDA). ~~The p~~Plates were incubated at 25±1º C in darkness (Astiz Gasso and Wojszko, 2011). After 7-10 days, ~~the~~ spores of *T. frezii* were germinated and colonies were transferred to a culture medium potato-dextrose agar 2% to obtain *T. frezii* pure colonies. The identities of all isolates were confirmed with specific primers for *T. frezii* (Cazón et al., 2016a).

A total of twelve fungicides products (isolated active ingredient and adjuvant) varying in their mode of action were included in the study (Table 1). Five concentrations of each active ingredient were analyzed: 0.01, 0.10, 1.00, 10.00 and 100.00 ~~To measure the *in vitro* sensitivity of fungicides, a mycelial growth test was carried out. Fungicide amended agar medium for the characterization of fungal mycelial growth inhibition is one of the most common methods to determine fungicide sensitivity (Liang et al., 2015; Saville et al., 2015). Twelve fungicides products were used: four belong to the group of triazoles (DMIs) (difenoconazole, propiconazole, cyproconazole, tebuconazole), four to strobilurins (QoI) group (picoxystrobin, azoxystrobin, kresoxim methyl, pyraclostrobin), one to carboxamide (SDHI) (penthiopyrad), one to dithiocarbamates (EBDC) (mancozeb), one to methyl benzimidazole carbamates (MBC) (thiophanate-methyl) and one to chloronitrile (chlorothalonil). Five concentrations of each active ingredient were analyzed: 100.00; 10.00; 1.00; 0.10; 0.01~~ µg a.i./ml medium. Petri plates without fungicides were used as experimental control. Each experimental unit was represented by a plate (55 mm diameter), with five repetitions per treatment.

Mycelial discs ~~plug~~ (6 mm in diameter) were extracted from actively growing ~~*T. frezii* isolates 8-day old~~ culture isolates *T. frezii* (8-day-old) and~~. The plugs were~~ transferred to~~in~~ the center of ~~the~~ Petri plates ~~with~~ containing PDA amended medium~~with the concentrations of the fungicides of each treatment~~. Five replicated plates were used for each fungicide concentration~~.~~ ~~Plates were~~ incubated at 25 ºC in darkness ~~conditions~~.

The radial growth (colony diameter) of each isolate was measured with a digital caliper in two perpendicular directions, and it was subtracted to the original mycelial disc diameter (6 mm). The measurements were taken at the moment in which mycelium of control plates reached their plates margins~~when the control plates mycelium reached the plate edge~~. The inhibition of mycelial growth relative to the treatment without fungicide was calculated as:

~~The inhibition of mycelial growth relative to the treatment without fungicide was calculated and by logarithmic regression the concentration that inhibits 50% of mycelial growth (EC_{50}) was calculated~~.

$$GI_i = (CD_{check} - CD_i)/CD_{check} \times 100 \quad (1)$$

w~~W~~here GI_i is the inhibition of i-th colony growth; CD is the mean colony diameter for the control without fungicide (check) or for the *i*-th fungicide. Linear model regression was fitted to the GI obtained along the exponential - transformed doses~~logarithmic doses~~, and the EC50 was estimated by replacing components of the fitted equations.

The fungitoxicicity of fungicides was classified according to Tonin et al. (2015): EC50<1 µg/ml ~~is considered as~~ highly fungitoxic; between 1 to 50 µg/ml ~~as~~ moderately fungitoxic; EC50 > 50 µg/ml ~~as~~ non-toxic.

2.2. Fungicides efficacy to control peanut smut trials

Two sets of replicated experiments were conducted during growing seasons 2014/2015 (from now on "2015") and 2015/2016 (from now on "2016"). Peanut cultivar Granoleico, was used in all experiments due to the known susceptibility to smut (Oddino et al., 2013). Twelve fungicides labeled for peanut leaf spot were tested (Table 1). A non-treated control was included in each experiment ~~in~~ by spraying only ~~which we sprayed~~ water. We used one and a half fold the active ingredient dose recommended for peanut leaf spot control ~~(Table 1)~~. One set of trials was conducted in pots and the other one in field plots.

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Table 1. Fungicides included in the study: chemical group, commercial name, formulation type, doses (for peanut leaf spot and peanut smut)~~Chemical group of fungicides active ingredients registered for the control of peanut leaf spot and rates used in this study by treatments for peanut smut control~~

Chemical group	Active ingredient~~s~~	Trading name® (Company)	Formulation	Commercial rate for leaf spot (g ai/ha)	Rate ~~using~~ used for peanut smut (g ai/ha)
QoI	Picoxystrobin	*	SC 20%	80	120
QoI	Azoxystrobin	Amistar (Syngenta)	SC 25%	90	135
QoI	Kresoxim-Methyl	*	SC 18%	125	190
QoI	Pyraclostrobin	*	SC 25%	100	150
DMI	Difenoconazole	*	SC 16%	106	160
DMI	Propiconazole	*	SC 25%	135	200
DMI	Cyproconazole	*	SC 15%	50	75
DMI	Tebuconazole	Tebuco 25 (NOVA)	SC 25%	200	300
SDHI	Penthiopyrad	*	SC 10%	80	120
EBDC	Mancozeb	Mancozeb 75 (Nufarm)	WG 75%	1100	1700
MBC	Thiophanate-Methyl	Abrigo (NOVA)	SC 50%	500	750
Chloronitriles	Chlorothalonil	Funda (Gleba)	SC 50%	1000	1500

* Experimental compounds provided by company.

2.2.1. Field experiment

Field trials were conducted at Aceitera General Deheza (AGD) experimental station in General Deheza ~~AGD experimental station in General Deheza~~ (province of Córdoba, Argentina - 32° 45′ 20.53″S, 63° 46′ 56.5″W), located in the main peanut producing region with high *T. frezii* inoculum field pressure (Paredes, 2017). The inoculum concentration in the experimental area was higher than 4500 smut teliospores/g of soil. Treatments were distributed upon a randomized complete block design with four replications. Plots consisted of four rows of peanut seedlings at 0.7~~-~~ m spacing and 8 m long. Fungicides were applied at night (between 9 - 11 pm) considering the soil as spraying target, with a CO_2~~CO^2~~ pressurized backpack and handheld boom equipped with 4-nozzles (hollow cone, ALBUS ATR 80) spaced 50-cm, delivering 120 L/ha at a pressure of 310 kPa and 0.9 m/s spray walking speed~~delivering 120 L per ha~~

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~~approximately~~. The first application was done seven days after R2 stage (beginning peg) (Boote, 1982) and the second one 10 days after the first one.

2.2.2. Pots experiments

Two experiments with peanut plants growing in pots were conducted at INTA research facilities in Córdoba city (-31° 28' 3.8"S, -64° 8' 50.4"W). Single peanut plants were grown in 10 L pots containing a substrate composed of a 3:1 mixture of sterilized soil-vermiculite and *T. frezii* teliospores. ~~The i~~Inoculum was added at a rate of 10,000 teliospores/g of substrate by drenching of a *T. frezii* spore suspension. ~~Both, the~~ *T. frezii* spores and ~~the~~ soil used to fill the pots were obtained from the experimental area of the field trials. A completely randomized design, with five (2014/15, from now on 2015) and eight (2015/16, from now on 2016) repetitions was used for each treatment. Treatments consisted of two fungicide sprayings: at 7 to 10 days after R2 (beginning peg) and 10 days after the first one. Each fungicide application consisted of three manual sprays (approximately 2.5 ml), directed to the plant base and pegs.

2.3. Disease assessment

Smut intensity assessment was performed at physiological mature crop stage (R8, Boote, 1982) (Rago et al., 2017). Severity was visually estimated using a five-class scale (Astiz Gasso et al., 2008) which takes into account both grains disease status within the pod (Figure 1). Disease assessment in the field plots experiment was performed by collecting 1 m² from the two central grooves of each plot (and all pods produced in that area were evaluated) and in the pots experiment all the pods contained in the pots were evaluated.

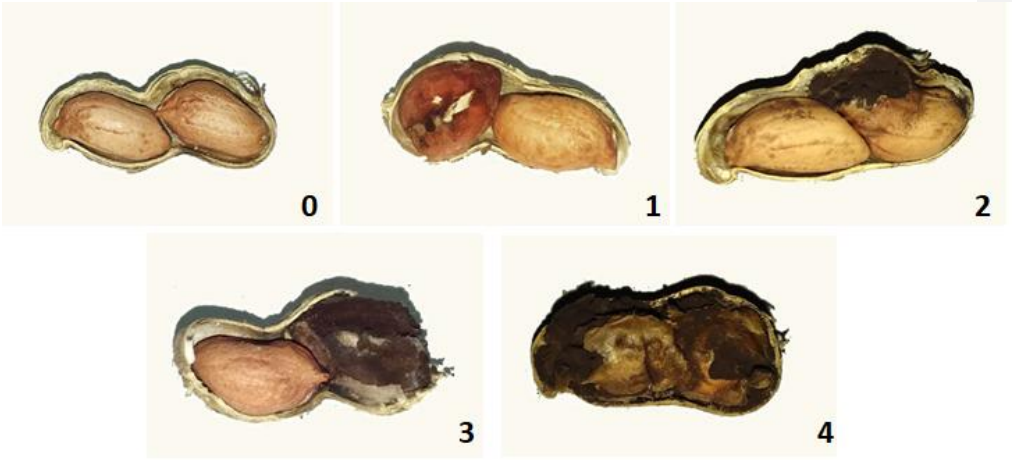

Figure 1. Five-class severity scale for peanut smut, where: 0 = both healthy kernels;~~.~~ 1 = presence of a small sorus in single kernel;~~.~~ 2 = at least one kernel with half area affected;~~.~~ 3 = deformed pod, with one single kernel completely smutted;~~.~~ 4 = deformed pod with two kernels completely smutted

For the purposes of this analysis, we used the proportion of severely damaged pods (SDP, disease class 3 and 4) as the response variable. Disease levels for these classes are considered to have impact in inoculum increase as they are lost at harvest and produces are discarded by the industry because of quality issues (Morichetti S. personal communication)~~These disease classes are frequently lost at harvest or discarded by the industry (Morichetti S. personal communication) and represent a high impact in inoculum increase~~. SDP was calculated as:

$SDP = (n_3 + n_4) / N$ (~~1~~2)

where n_3 and n_4 are the number of pods corresponding to disease severity class 3 and 4 respectively and N is the plot sample size.

Then, for graphical displaying of the results, smut control efficiency (CE) was calculated by using the following formula:

$CE = (1 - (\text{Treatment SDP} / \text{untreated check SDP})) * 100$ (~~2~~3)

2.4. Data statistical analysis

Univariate generalized linear models (GLMs) with binomial variance and logit link function were used to analyze SDP. The full model included the year - treatment interaction, which resulted significant ($P < 0.01$), then a separated model was fitted to each year. Treatments marginal means were estimated with the 'emmeans' function from the emmeans R package (Lenth et al., 2018) and post-hoc comparisons among them were done with Tukey test at a significance level of 5%.

~~Univariate generalized linear models (GLMs) were used to analyze SDP. Treatments marginal means were estimated with the "emmeans" function from the emmeans R package (Lenth 2018) and post hoc comparisons among them were done with Tukey test at a significance level of 5%.~~

Multivariate analysis was performed to integrate all the efficacy tests (Deepak et al., 2006): principal component analysis (PCA) was conducted to the scaled variables, as: (x - mean(x)) / sd(x), where x is each variable, and the scaling is done by dividing the (centered) vectors of x by their standard deviations. CE% were obtained from the field plots and peanut plants grown in pots trials (2015 and 2016). We obtained the correlation coefficients for each variable with the first and second principal components to explain the variability explained by the first and second principal component. Each fungicide (individuals, represented by points) and their performance in each trial (variables, represented by vectors) are displayed together on a biplot.

3. Results

3.1. *In vitro* sensitivity

The growth of *T. frezii* isolates was 4.76 (± 0.14) mm per day ~~*T. frezii* isolates grown 4.76 mm per day (± 0.14)~~ in ~~untreated~~ non-treated control plates. A wide range of sensitivity of

T. frezii isolates was observed to the evaluated fungicides~~:~~, EC50 value ranging from 0.001 to >100. The highest mycelial growth inhibitions ~~(lowest EC50 values)~~ was observed with the DMI's fungicides, QoI's pyraclostrobin and azoxystrobin, and penthiopyrad, EC50<0.1 (Figure 2). Moderate~~ly~~ sensitivity was observed with mancozeb (EC50 = 6.28). The lowest sensitivity of *T. frezii* was observed with thiophanate-methyl (EC50 > 100 µg/ml). According to the EC50, only thiophanate-methyl was classified as non-toxic, and mancozeb as moderately fungitoxic, the rest of the a.i. were classified as highly fungitoxic.

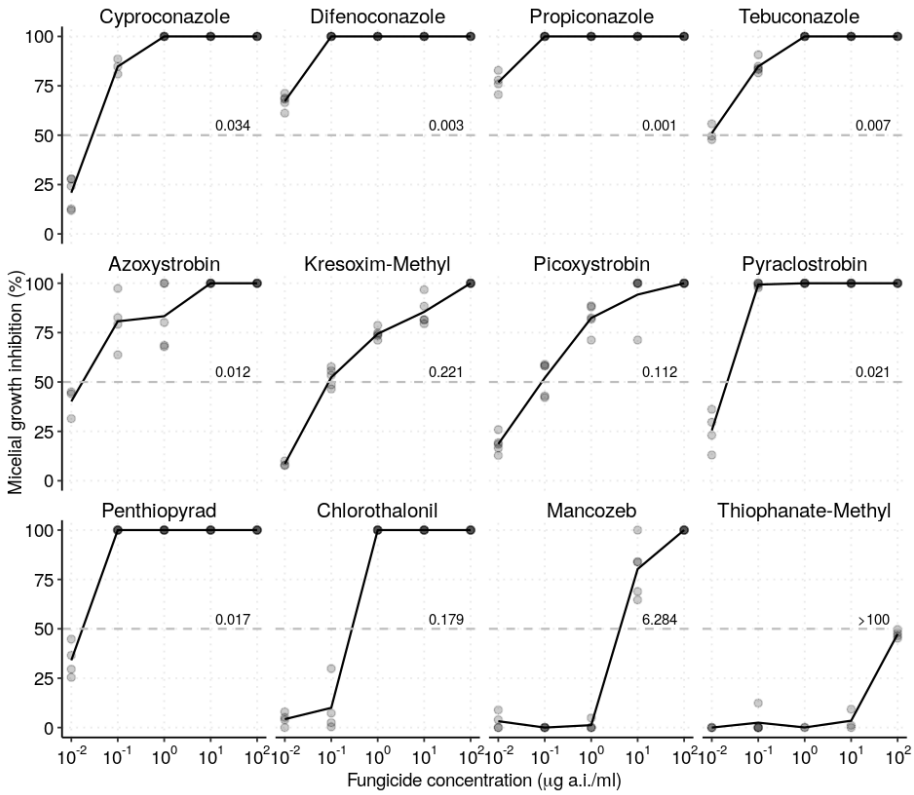

Figure 2. Mycelial growth inhibition curves according to fungicide concentration (µg a.i./ml) for the twelve active ingredients evaluated. Numbers above ~~on~~ the central dashed line corresponds to EC50 value.

3.2. Control efficacy experiments

Accumulated precipitations and mean temperature registered in General Deheza during the peanut cropping season (December to April) in 2014/15, 2015/16 are presented in Table S1. A normal crop development (without severe hydric stress) was observed in both field experiments. A total precipitation of 465 and 417 mm was recorded during the pegging phenological stage (January to March) in 2015 and 2016 respectively.

Smut pressure in pots experiments, indicated by the mean SDP at the non-treated check plants, was higher in 2015 (0.73) than 2016 (0.52). Azoxystrobin ~~presented~~ resulted the lowest SDP values in both years: 0.3 and 0.04 in 2015 and 2016, respectively. The latter values represented 59% and 92% control efficacy relative to their corresponding non-treated control. No significant differences in smut control were observed between azoxystrobin and ~~with~~ difenoconazole (0.43 SDP, 41% control efficiency) in 2015 or from cyproconazole (0.09 SPD, 82% control efficiency) or tebuconazole (0.13 SPD, 74% control efficiency) in 2016. Intermediate smut control ~~were~~was observed ~~with~~ for tebuconazole, picoxystrobin and cyproconazole in 2015, and picoxystrobin, propiconazole, mancozeb, difenoconazole and kresoxim-methyl in 2016. On the other hand, the mean SDP for chlorothalonil, penthiopyrad, thiophanate-methyl and pyraclostrobin did not differ from the nontreated check in both years (Table 2; Figure 3).

Table 2. Severely damaged pods, control efficiency, and corresponding statistics for the 12 fungicides ~~the effect of different active ingredients used~~evaluated for the control of peanut smut on pots experiments in 2015 and 2016.

	2015					2016			
Active ingredients	**SDP[a]**	**CL_L[b]**	**CL_U[b]**	**CE[c]**	**Active ingredients**	**SDP**	**CL_L**	**CL_U**	**CE**
Azoxystrobin	0.30	0.23	0.39	58.9	Azoxystrobin	0.04	0.02	0.09	92
Difenoconazole	0.43	0.36	0.51	41.1	Cyproconazole	0.09	0.06	0.14	82
Tebuconazole	0.51	0.44	0.58	30.1	Tebuconazole	0.13	0.08	0.19	74

Picoxystrobin	0.54	0.46	0.61	26	Picoxystrobin	0.19	0.14	0.25	62
Cyproconazole	0.56	0.48	0.63	23.3	Propiconazole	0.27	0.2	0.35	46
Kresoxim-Methyl	0.60	0.52	0.67	17.8	Mancozeb	0.28	0.21	0.36	44
Thiophanate-Methyl	0.65	0.57	0.72	11	Difenoconazole	0.29	0.22	0.36	42
Mancozeb	0.65	0.58	0.71	11	Kresoxim-Methyl	0.32	0.26	0.39	36
Pyraclostrobin	0.67	0.60	0.73	8.2	Pyraclostrobin	0.39	0.33	0.47	22
Propiconazole	0.71	0.63	0.78	2.7	Penthiopyrad	0.43	0.36	0.5	14
Penthiopyrad	0.72	0.63	0.79	1.4	Check	0.5	0.44	0.56	0
Check	0.73	0.65	0.80	0	Thiophanate-Methyl	0.51	0.43	0.58	0
Chlorothalonil	0.74	0.68	0.80	0	Chlorothalonil	0.52	0.45	0.59	0

[a] Proportion of severely damaged pods (disease classes: 3 - deformed pod, with one single kernel completely smutted and 4 - deformed pod with two kernels completely smutted).

[b] Lower (CI_L) and upper (CI_U) limits of 95% confidence interval around SDP estimated mean.

[c] Percentages of control efficiency of active ingredients calculated in relation to the non-treated check.

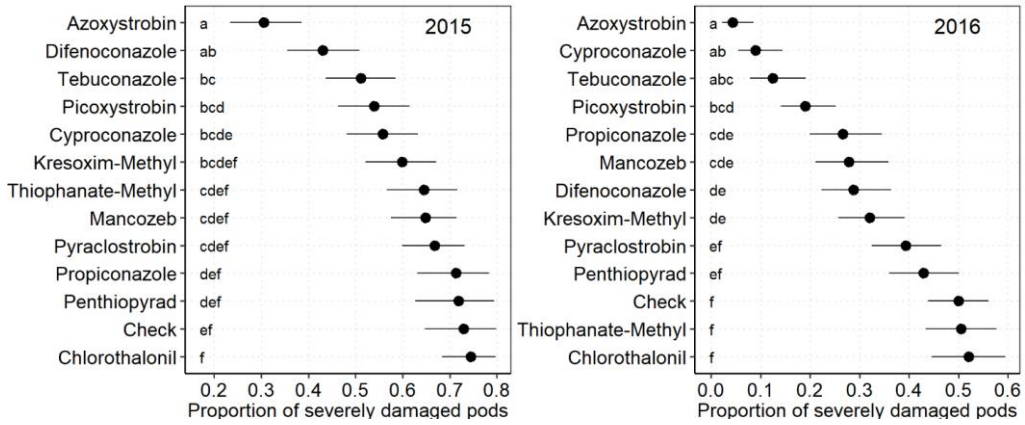

Figure 3. Proportion of severely damaged pods for different active ingredients used for the control of peanut smut on pots experiment in 2015 and 2016.

In the field experiments, mean SDP at the non-treated control was 0.39 (2015) and 0.43 (2016). Cyproconazole and azoxystrobin in 2015 presented the lowest SDP values: 0.21 and 0.25 (control efficacy of 47% and 37%, respectively) ($P<0.05$). Azoxystrobin was the most

efficient fungicide in 2016 to control smut, with a lowest SPD: 0.26. No significant differences of SPD with the non-treated control ~~in smut control~~ was observed ~~with~~ for kresoxim-methyl, thiophanate-methyl, tebuconazole, penthiopyrad and chlorothalonil for both years (Table 3; Figure 4).

Table 3. Severely damaged pods, control efficiency, and corresponding statistics for the 12 fungicides evaluated for the control of peanut smut on field experiments in harvest 2015 and 2016.~~Severely damaged pods, control efficiency, and corresponding statistics for the effect of different active ingredients used for the control of peanut smut on field experiments in harvest 2015 and 2016.~~

	2015					2016			
Active ingredients	**SDP[a]**	**CL_L[b]**	**CL_U[b]**	**CE[c]**	**Active ingredients**	**SDP**	**CL_L**	**CL_U**	**CE**
Cyproconazole	0.21	0.19	0.23	47.7	Azoxystrobin	0.26	0.24	0.29	39.5
Azoxystrobin	0.25	0.22	0.27	37.2	Propiconazole	0.33	0.31	0.35	23.7
Mancozeb	0.29	0.27	0.31	25.8	Cyproconazole	0.34	0.32	0.37	20.9
Pyraclostrobin	0.31	0.29	0.34	20.7	Picoxystrobin	0.38	0.35	0.40	13.8
Picoxystrobin	0.32	0.29	0.35	18.9	Difenoconazole	0.38	0.35	0.40	13.3
Propiconazole	0.32	0.30	0.34	17.9	Thiophanate-Methyl	0.38	0.35	0.41	12.4
Chlorothalonil	0.33	0.30	0.35	16.8	Chlorothalonil	0.38	0.36	0.41	12.2
Kresoxim-Methyl	0.33	0.30	0.35	16.6	Mancozeb	0.38	0.35	0.41	12.0
Thiophanate-Methyl	0.35	0.32	0.37	11.7	Kresoxim-Methyl	0.39	0.36	0.43	9.9
Difenoconazole	0.36	0.34	0.38	8.2	Penthiopyrad	0.40	0.38	0.42	7.8
Tebuconazole	0.36	0.34	0.39	7.4	Pyraclostrobin	0.42	0.40	0.45	3.2
Penthiopyrad	0.37	0.35	0.40	4.8	Check	0.44	0.41	0.46	-
Check	0.39	0.36	0.42	-	Tebuconazole	0.45	0.42	0.48	0

[a] Mean of the proportion of severely damaged pods (disease class 3 and 4)

[b] Lower (CL_L) and upper (CL_U) limits of 95% confidence interval around SDP

[c] Percentages of control efficiency of active ingredients calculated in relation to the untreated check treatment.

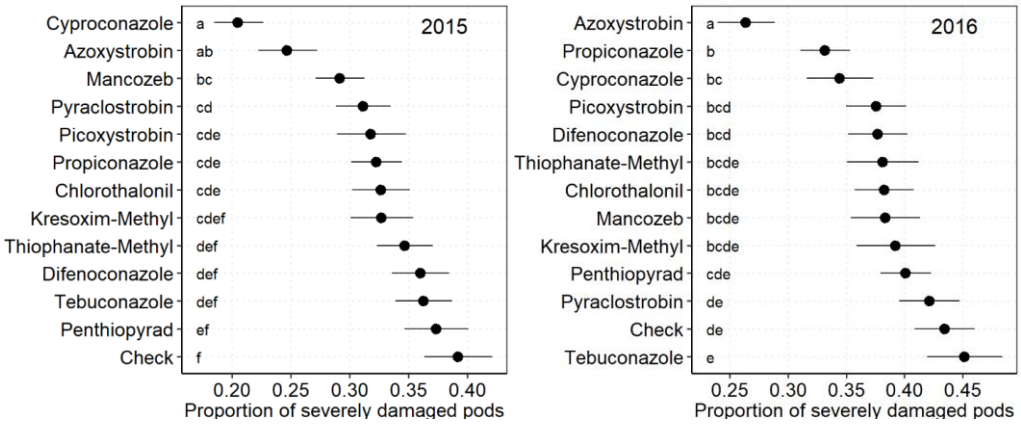

Figure 4. Proportion of severely damaged pods for different active ingredients used for the control of peanut smut on field experiments in 2015 and 2016.

3.3. Multivariate analysis

The multivariate analysis allowed us to integrate all the trials results: two independent principal components (PC) accounted for 76.5% of the total variation (56.2% and 20.3% for the PC1 and PC2 respectively). PC1 (horizontal axe) represented the control efficacy values in the field plots trials (with correlation coefficients of 0.86 and 0.77, in 2015 and 2016 respectively) and pots experiments (~~with~~ correlation coefficients ~~of~~ were 0.83 and 0.73, in 2015 and 2016 respectively). The further right on the PC1-axis, the higher the efficacy control of the fungicide (Figure 5). PC2 on the y-axis represented the EC50 values: the upper in the axis, the lower the sensitivity of the smut to the fungicide. Considering this coordinate meaning, we may select as best fungicides those ones located in the right quadrants, and the lower as possible: azoxystrobin and picoxystrobin (QoI group) and cyproconazole and difenoconazole (DMI group).

We observed that control efficacy in 2015 followed a similar trend in both types of experiments (plants grown in pots and field plots), but a weak correlation among both experimental types was observed in 2016.

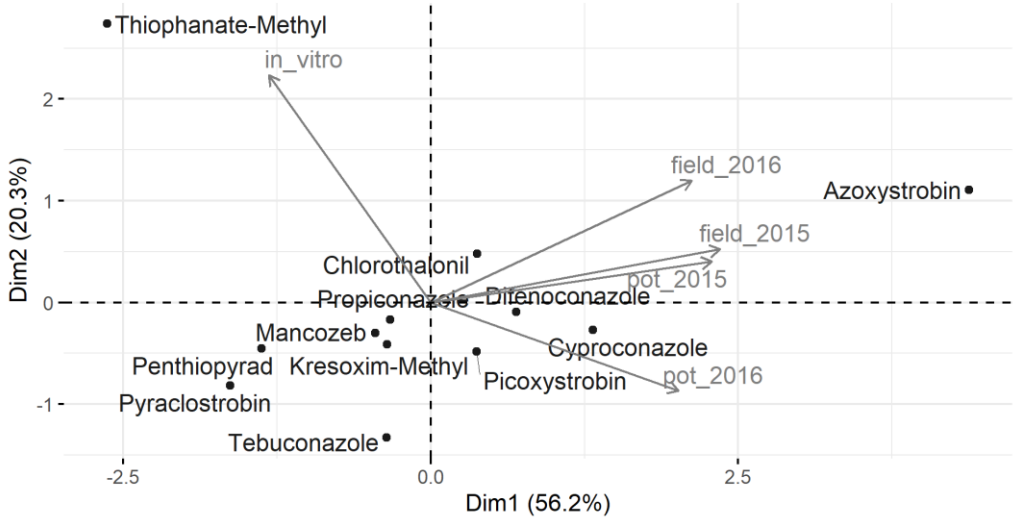

Figure 5. Biplot model showing the relationship among fungicides (individuals, represented by points) and their performance in each trial (variables, represented by vectors): in vitro experiments and pot and field experiment in 2015 and 2016.

4. Discussion

Since its re-emergence in 2006, peanut smut has continueds increasing its prevalence and incidence in the main Argentine peanut-growing region, without good perspectives for the future due to the current absence of use adoption of management practices for controlling smut (Rago et al., 2017). By means of the present two-year fungicide-screening study we observed variability in the available set of commercial fungicides, including highly efficacious active ingredients to non-effective ones. To the best of our knowledge this is a first study exploring assessing the chemical management as a technique to maintain peanut smut in low intensity levels.

In vitro mycelial growth inhibition tests showed a general good performance of the DMI´s group, QoI´s azoxystrobin and pyraclostrobin and SDHI´s penthiopyrad. The two last

chemical groups are powerful inhibitors of spore germination (Fungicide Resistance Action Committee [FRAC] group 11 and 7), a process that was not evaluated in the *in vitro* tests. This fact could have a potential sub-estimation effect of the evaluated active ingredients since the smut spore germination is a fundamental first step on epidemic ~~triggering~~ onset after the gynophore exudates stimulation (Astiz Gasso et al., 2008; Marinelli et al., 2008). In relation to this both fungi cycle processes, Kosiada~~,~~ (2011) observed a weak correlation between mycelial growth and germination of teliospores and basidiospores according to the sensitivity of some fungicides for head smut of corn (*Sphacelotheca reiliana).*

Another weak correlation was reported between results of laboratory and field conditions experiments (Hollowell et al., 2003), which led us to perform pots and field trials for assessing the fungicide efficacy to control peanut smut. However, i~~I~~n both years of the study we observed that azoxystrobin was the fungicide with highest smut control efficacy levels in pot or field experiments.

Pavez Badilla et al. (2013), showed different levels of sensitivity *in vitro* for pyraclostrobin and kresoxim-methyl (QoI) in front of the *Venturia inaequalis*, nevertheless they assure that it is very difficult to extrapolate this type of investigation to the field conditions due to the action mode. Differences in control levels for peanut smut were observed compared to *in vitro* results. The DMIs a.i. showed EC50 lower than the rest of the chemical groups. Cyproconazole has the highest EC50 comparatively with other DMIs, however it is one of the a.i. that shows the highest control efficiency in field and pots experiments. For its part, chlorothalonil and penthiopyrad (SDHI) have an EC50 like QoI, however did not demonstrate disease control efficiency. Azoxystrobin, picoxystrobin, cyproconazole and tebuconazole were the a.i. best responses demonstrated in the assessment; however, they are not the lowest EC50 within the chemical groups to which they belong.

The lack of consistency of the observed results between pot and field experiments can be due to differences in soil properties in both experimental conditions: soil humidity content may lead to different fungicide degradation rates or particles absorption. Further studies may be done for a better understanding of the latter process and consequently a more efficient use of fungicides against *T. frezii* infections. Paredes et al. (data not published) observed in growing chambers experiments that the lower the soil humidity, the higher the incidence of smut in peanut plants.

QoI, SDHI and DMI are the most common fungicides used to control peanut diseases in crops. These fungicides groups performed less than 40-50% effective to peanut smut control in field, even when used at higher doses comparatively leaf spot (Rago et al., 2017). Mutations in genes encoding fungicide targets of pathogenic fungi are often the cause of developed resistance to QoI, SDHI and DMI fungicides (Sierotzki and Scalliet, 2013). Nevertheless, research on the molecular basis of *T. frezii* fungicide resistance has not been possible due to the lack of genetic information. Recently, Arias et al., (2019) report the complete mitogenome of *T. frezii* and provide molecular tools to study fungicide target genes and suggests potential resistance to strobilurin and carboxamide fungicides.

Individual performances of cyproconazole and azoxystrobin had the highest control efficacy in the present study. However, higher performances were observed as mixture formulations of the fungicides in previous field experiments under the same disease pressure (4400 teliospores/g of soil): azoxystrobin + cyproconazole and azoxystrobin + picoxystrobin reduced smut severity by 58 and 47% respectively relative to non-treated control (Paredes et al., 2015b, 2015a). This higher performance of the fungicides as mixtures could be due to a "potentiation" effect (Cid, 2014).

~~5. Conclusions~~

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~~There are multiple factors that affect the disease control. Therefore *in vitro* inhibition effect of the active ingredients against *T. frezii* is not enough since there is not a direct relationship between the EC50 and disease control. Active ingredients belonging to the same chemical group do not predict the same peanut smut control. The effect can be more linked to the intrinsic characteristics of the formulation or the active ingredient and not to the chemical group. Azoxystrobin and cyproconazole were the active ingredients that showed best responses, belonging to the QoI and DMI groups respectively. Results reported here allow us to determine the effect of large number of the active principles registered for the peanut culture in Argentina, being an important tool for the development of strategies for disease management.~~

Funding

This work was supported by INTA [Programa Nacional de Cultivos Industriales, PNIND-1108072] and Fundación Maní Argentino.

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Supplementary tables

Table S1: Data were extracted from AGD local station data: Rainfall (monthly precipitation), average monthly maximum and minimum temperatures in the experimental peanut area during 2014/15; 2015/16.

	Rainfall (mm)		Temperature (°C)					
			Mean		Average max.		Average min.	
Month	2015/16	2016/17	2015	2016	2015	2016	2015	2016
December	38	122	22	24	32	31	14	16
January	89	123	24	23	32	30	18	18
February	255	249	21	24	28	30	16	18
March	121	45	21	19	28	26	16	13
April	57	143	20	15	28	21	13	11
Acumulated	560	682						

EFFICACY OF FUNGICIDES AGAINST PEANUT SMUT IN ARGENTINA

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Abstract

Peanut smut, caused by *Thecaphora frezii*, increased its incidence and prevalence in the main growing region of Argentina in the last decade becoming the main peanut disease. Despite this fact, growers continue producing peanut without any disease management strategy what is leading to a local accumulation of *T. frezii* inoculum. The goal of the present study was to assess the efficacy of 12 fungicides for controlling *T. frezii*: *in vitro* assays, pots and field experiments were performed during 2014/15 and 2015/16. In vitro assays classified the fungicides upon their capacity to inhibit *T. freezy* mycelium growth as: thiophanate-methyl - not fungitoxic (EC50 > 100); mancozeb - moderately fungitoxic (EC50 = 6.28); difenoconazole, propiconazole, cyproconazole, tebuconazole, picoxystrobin, azoxystrobin, kresoxim-methyl, pyraclostrobin, penthiopyrad and chlorothalonil - highly fungitoxic (EC50 < 0.1). Azoxystrobin showed the highest control levels in pots experiments: 58.9% (2014/15) and 92% (2015/16). The greatest control efficiency in field experiments was observed for cyproconazole in 2015 and azoxystrobin in 2016, reducing peanut smut by 47.7 and 39.5%

respectively. Based on our results, chemical control can be considered as a moderately efficient technique which may complement the cultivar genetic resistance and cultural practices in an integrated approach for managing peanut smut.

Key Words: *Thecaphora frezii*; Chemical control; *in vitro* sensitivity; peanut disease; active ingredients

1. Introduction

Peanut (*Arachis hypogaea* L.) is an annual extensive crop, with its center of origin in northwestern Argentina and southeastern Bolivia (Hammons et al., 2016). Argentina produces a high-quality peanut in an area of 350,000 has, concentrating more than 90% in the province of Córdoba located at the center region of the country. More than 95% of the peanut production is exported, previously processed in situ by the industry established in the same growing region (Agüero, 2017; Calzada and Rozadilla, 2018).

Peanut smut, caused by the soil-borne fungi *Thecaphora frezii* Carranza and Lindquist, is an endemic and yield reducing disease for the argentine main growing region (Marinelli et al., 2008, 2010; Rago et al., 2014). The pathogen is a biotrophic fungus that produces sori consisting of a powdery mass of spores which colonize the seed tissue partially or totally, with potential total damage of the plant pods (Astiz Gasso et al., 2008). The infection process occurs during the crop pegging stage: the stage that flower gynophore penetrates the soil surface, releasing an exudate that stimulate the spore germination (Marinelli et al., 2008).

Currently, no management strategies for reducing *T. frezii* infections are adopted by argentine growers, which is leading to inter-annual inoculum accumulation (Paredes et al., 2017). Granoleico peanut cultivar (known susceptibility to the smut) is planted in more than 80% of the growing region (Cazón et al., 2018; Cignetti et al., 2010; Rago et al., 2017).

Breeding programs with the aim of incorporating wild resistance genes have initiated recently, but new genotypes are not expected to be adopted in the short term (Bressano et al., 2019).

Survival structure can remain viable for new infections up to four years (Cazón et al., 2016b) which implies that a short peanut crop rotation is an inefficient disease management technique in the current argentine production system. Preliminary research suggested a wide range of control efficacies with fungicide applications while higher doses of DMI + QoI mixture fungicides reported to provide best control. 58% smut control were observed by spraying a mixture of azoxystrobin and cyproconazole at a rate of 1000 cm^3/ha, which is twice and half the recommended dose for peanut late leaf spot management caused by *Nothopassalora personata* (syn. *Cercosporidium personatum*) (Cazón et al., 2013; Paredes et al., 2015b; Paredes et al., 2015a). In addition, the effect of timing of the day on the fungicide smut control efficacy was compared: fungicide applications during the night had higher control efficacy than sprayings during the day (Paredes et al., 2015c). The application timing effect can be due to the fact that peanut plants fold their leaves at night and open them in the daytime (a process known as nyctinasty), allowing for more spraying drops to reach the soil surface.

No studies of fungicide sensitiveness of local *T. frezii* populations are registered in Argentina, and this information is essential for setting up of a chemical management strategy. The lack of studies examining a wide set of fungicides to control peanut smut led us to perform this study. The objectives of this work were i) to assess the fungicides efficacy against *T. frezii* in in vitro conditions and ii) to test the field performance of fungicides against peanut smut.

2. Materials and Methods

2.1. In vitro sensitivity of fungicides based on mycelial growth inhibition test

To measure the *in vitro* sensitivity of fungicides, a mycelial growth test was carried out. A mixture of 10 *T. frezii* isolates obtained from smutted pods in 2015 in General Deheza

(Córdoba province, Argentina) were used for the fungicide sensitivity test. Smut teliospores (100 mg) were suspended in aqueous solution of 0.63% NaOCl, agitated during 5 minutes, rinsed twice with sterile distilled water and resuspended in 2 ml sterile distilled water. Disinfected teliospores were placed in potato dextrose agar made up of broth extract of healthy grains at a rate of 50 g/l (Potato Peanut Dextrose Agar, PPDA). Plates were incubated at 25±1º C in darkness (Astiz Gasso and Wojszko, 2011). After 7-10 days, spores of *T. frezii* were germinated and colonies were transferred to a culture medium potato-dextrose agar 2% to obtain *T. frezii* pure colonies. The identities of all isolates were confirmed with specific primers for *T. frezii* (Cazón et al., 2016a).

A total of twelve fungicides products (isolated active ingredient and adjuvant) varying in their mode of action were included in the study (Table 1). Five concentrations of each active ingredient were analyzed: 0.01, 0.10, 1.00, 10.00 and 100.00 μg a.i./ml medium. Petri plates without fungicides were used as experimental control. Each experimental unit was represented by a plate (55 mm diameter), with five repetitions per treatment.

Mycelial discs (6 mm in diameter) were extracted from actively growing culture isolates *T. frezii* (8-day-old) and transferred to the center of Petri plates containing PDA amended medium. Five replicated plates were used for each fungicide concentration incubated at 25 ºC in darkness.

The radial growth (colony diameter) of each isolate was measured with a digital caliper in two perpendicular directions, and it was subtracted to the original mycelial disc diameter (6 mm). The measurements were taken at the moment in which mycelium of control plates reached their plates margins. The inhibition of mycelial growth relative to the treatment without fungicide was calculated as:

$$GI_i = (CD_{check} - CD_i)/CD_{check} \times 100 \quad (1)$$

where GI_i is the inhibition of i-th colony growth; CD is the mean colony diameter for the control without fungicide (check) or for the *i*-th fungicide. Linear model regression was fitted to the GI obtained along the exponential - transformed doses, and the EC50 was estimated by replacing components of the fitted equations.

The fungitoxicicity of fungicides was classified according to Tonin et al. (2015): EC50<1 µg/ml - highly fungitoxic; between 1 to 50 µg/ml - moderately fungitoxic; EC50 > 50 µg/ml - non-toxic.

2.2. Fungicides efficacy to control peanut smut trials

Two sets of replicated experiments were conducted during growing seasons 2014/2015 (from now on "2015") and 2015/2016 (from now on "2016"). Peanut cultivar Granoleico, was used in all experiments due to the known susceptibility to smut (Oddino et al., 2013). Twelve fungicides labeled for peanut leaf spot were tested (Table 1). A non-treated control was included in each experiment by spraying only water. We used one and a half fold the active ingredient dose recommended for peanut leaf spot control. One set of trials was conducted in pots and the other one in field plots.

Table 1. Fungicides included in the study: chemical group, commercial name, formulation type, doses (for peanut leaf spot and peanut smut)

Chemical group	Active ingredient	Trading name® (Company)	Formulation	Commercial rate for leaf spot ** (g ai/ha)	Rate used for peanut smut (g ai/ha)
QoI	Picoxystrobin	*	SC 20%	80	120
QoI	Azoxystrobin	Amistar (Syngenta)	SC 25%	90	135
QoI	Kresoxim-Methyl	*	SC 18%	125	190
QoI	Pyraclostrobin	*	SC 25%	100	150
DMI	Difenoconazole	*	SC 16%	106	160
DMI	Propiconazole	*	SC 25%	135	200
DMI	Cyproconazole	*	SC 15%	50	75
DMI	Tebuconazole	Tebuco 25 (NOVA)	SC 25%	200	300
SDHI	Penthiopyrad	*	SC 10%	80	120
EBDC	Mancozeb	Mancozeb 75 (Nufarm)	WG 75%	1100	1700
MBC	Thiophanate-Methyl	Abrigo (NOVA)	SC 50%	500	750

Chloronitriles	Chlorothalonil	Funda (Gleba)	SC 50%	1000	1500

* Experimental compounds provided by company.

** Rates were calculated according to labeled products

2.2.1. Field experiment

Field trials were conducted at Aceitera General Deheza (AGD) experimental station in General Deheza (province of Córdoba, Argentina - 32° 45′ 20.53″S, 63° 46′ 56.5″W), located in the main peanut producing region with high *T. frezii* inoculum field pressure (Paredes, 2017). The inoculum concentration in the experimental area was higher than 4500 smut teliospores/g of soil. Treatments were distributed upon a randomized complete block design with four replications. Plots consisted of four rows of peanut seedlings at 0.7 m spacing and 8 m long. Fungicides were applied at night (between 9 - 11 pm) considering the soil as spraying target, with a CO_2 pressurized backpack and handheld boom equipped with 4-nozzles (hollow cone, ALBUS ATR 80) spaced 50-cm, delivering 120 L/ha at a pressure of 310 kPa and 0.9 m/s spray walking speed. The first application was done seven days after R2 stage (beginning peg) (Boote, 1982) and the second one 10 days after the first one.

2.2.2. Pots experiments

Two experiments with peanut plants growing in pots were conducted at INTA research facilities in Córdoba city (-31° 28' 3.8"S, -64° 8' 50.4"W). Single peanut plants were grown in 10 L pots containing a substrate composed of a 3:1 mixture of sterilized soil-vermiculite and *T. frezii* teliospores. Inoculum was added at a rate of 10,000 teliospores/g of substrate by drenching of a *T. frezii* spore suspension. *T. frezii* spores and soil used to fill the pots were obtained from the experimental area of the field trials. A completely randomized design, with five (2014/15, from now on 2015) and eight (2015/16, from now on 2016) repetitions was used for each treatment. Treatments consisted of two fungicide sprayings: at 7 to 10 days after R2

(beginning peg) and 10 days after the first one. Each fungicide application consisted of three manual sprays (approximately 2.5 ml), directed to the plant base and pegs.

2.3. Disease assessment

Smut intensity assessment was performed at physiological mature crop stage (R8, Boote, 1982) (Rago et al., 2017). Severity was visually estimated using a five-class scale (Astiz Gasso et al., 2008) which takes into account both grains disease status within the pod (Figure 1). Disease assessment in the field plots experiment was performed by collecting 1 m² from the two central grooves of each plot (and all pods produced in that area were evaluated) and in the pots experiment all the pods contained in the pots were evaluated.

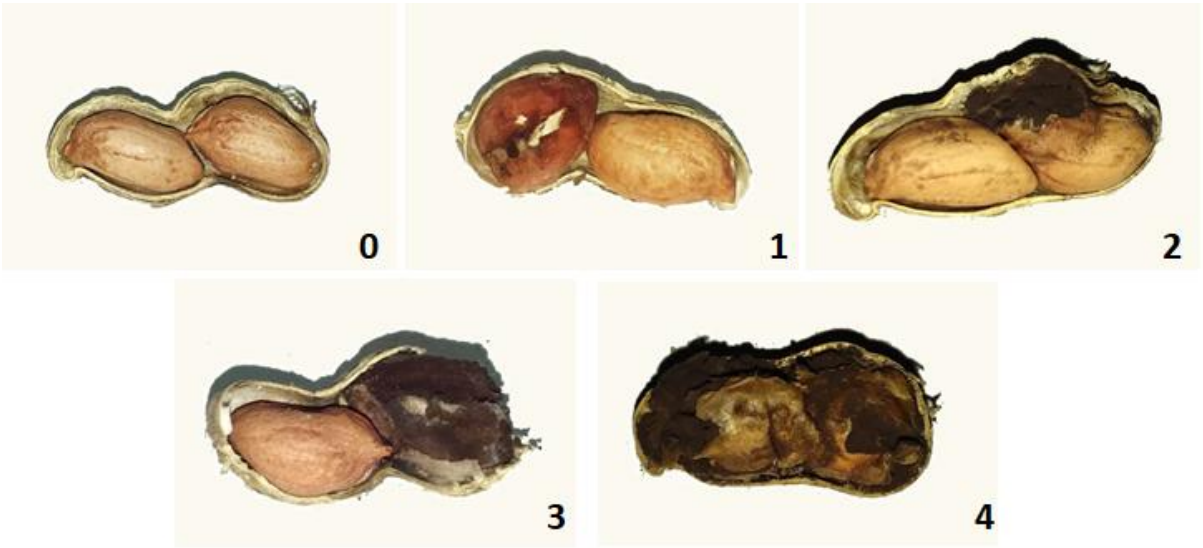

Figure 1. Five-class severity scale for peanut smut, where: 0 = both healthy kernels; 1 = presence of a small sorus in single kernel; 2 = at least one kernel with half area affected; 3 = deformed pod, with one single kernel completely smutted; 4 = deformed pod with two kernels completely smutted

For the purposes of this analysis, we used the proportion of severely damaged pods (SDP, disease class 3 and 4) as the response variable. Disease in these classes is considered to have impact in inoculum increase as they are lost at harvest and produces are discarded by the

164 industry because of quality issues (Morichetti S. personal communication). SDP was calculated
165 as:
166 $SDP = (n_3 + n_4) / N$ (2)
167 where n_3 and n_4 are the number of pods corresponding to disease severity class 3 and 4
168 respectively and N is the plot sample size.
169 Then, for graphical displaying of the results, smut control efficiency (CE) was
170 calculated by using the following formula:
171 CE = (1 - (Treatment SDP / untreated check SDP)) * 100) (3)
172
173 **2.4. Data statistical analysis**
174 Univariate generalized linear models (GLMs) with binomial variance and logit link
175 function were used to analyze SDP. The full model included the year - treatment interaction,
176 which resulted significant ($P < 0.01$), then a separated model was fitted to each year. Treatment
177 marginal means were estimated with the 'emmeans' function from the emmeans R package
178 (Lenth et al., 2018) and post-hoc comparisons among them were done with Tukey test at a
179 significance level of 5%.
180 Multivariate analysis was performed to integrate all the efficacy tests (Deepak et al.,
181 2006): principal component analysis (PCA) was conducted to the scaled variables, as: (x -
182 mean(x)) / sd(x), where x is each variable, and the scaling is done by dividing the (centered)
183 vectors of x by their standard deviations. CE% were obtained from the field plots and peanut
184 plants grown in pots trials (2015 and 2016). We obtained the correlation coefficients for each
185 variable with the first and second principal components to explain the variability explained by
186 the first and second principal component. Each fungicide (individuals, represented by points)
187 and their performance in each trial (variables, represented by vectors) are displayed together on
188 a biplot.

3. Results

3.1. *In vitro* sensitivity

The growth of *T. frezii* isolates was 4.76 (± 0.14) mm per day in non-treated control plates. A wide range of sensitivity of *T. frezii* isolates was observed to the evaluated fungicides: EC50 value ranging from 0.001 to >100. The highest mycelial growth inhibitions was observed with the DMI's fungicides, QoI's pyraclostrobin and azoxystrobin, and penthiopyrad, EC50<0.1 (Figure 2). Moderate sensitivity was observed with mancozeb (EC50 = 6.28). The lowest sensitivity of *T. frezii* was observed with thiophanate-methyl (EC50 > 100 µg/ml). According to the EC50, only thiophanate-methyl was classified as non-toxic, and mancozeb as moderately fungitoxic, the rest of the a.i. were classified as highly fungitoxic.

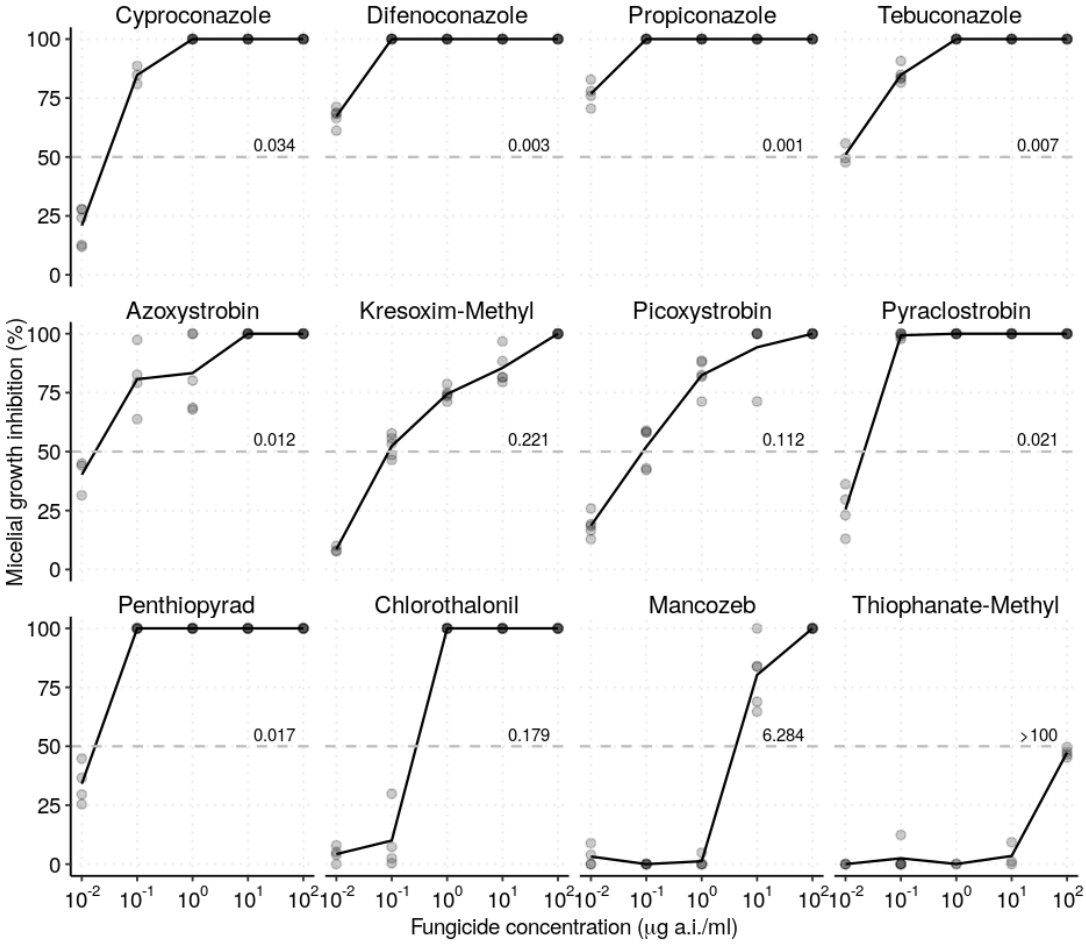

Figure 2. Mycelial growth inhibition curves according to fungicide concentration (µg a.i./ml) for the twelve active ingredients evaluated. Numbers above the central dashed line corresponds to EC50 value.

3.2. Control efficacy experiments

Accumulated precipitations and mean temperature registered in General Deheza during the peanut cropping season (December to April) in 2014/15, 2015/16 are presented in Table S1. A normal crop development (without severe hydric stress) was observed in both field experiments. A total precipitation of 465 and 417 mm was recorded during the pegging phenological stage (January to March) in 2015 and 2016 respectively.

Smut pressure in pots experiments, indicated by the mean SDP at the non-treated check plants, was higher in 2015 (0.73) than 2016 (0.52). Azoxystrobin resulted the lowest SDP values in both years: 0.3 and 0.04 in 2015 and 2016, respectively. The latter values represented 59% and 92% control efficacy relative to their corresponding non-treated control. No significant differences in smut control were observed between azoxystrobin and difenoconazole (0.43 SDP, 41% control efficiency) in 2015 or from cyproconazole (0.09 SPD, 82% control efficiency) or tebuconazole (0.13 SPD, 74% control efficiency) in 2016. Intermediate smut control were observed with tebuconazole, picoxystrobin and cyproconazole in 2015, and picoxystrobin, propiconazole, mancozeb, difenoconazole and kresoxim-methyl in 2016. On the other hand, the mean SDP for chlorothalonil, penthiopyrad, thiophanate-methyl and pyraclostrobin did not differ from the nontreated check in both years (Table 2; Figure 3).

Table 2. Severely damaged pods, control efficiency, and corresponding statistics for the 12 fungicides evaluated for the control of peanut smut on pots experiments in 2015 and 2016.

	2015					2016			
Active ingredients	**SDP[a]**	**CL_L[b]**	**CL_U[b]**	**CE[c]**	**Active ingredients**	**SDP**	**CL_L**	**CL_U**	**CE**
Azoxystrobin	0.30	0.23	0.39	58.9	Azoxystrobin	0.04	0.02	0.09	92
Difenoconazole	0.43	0.36	0.51	41.1	Cyproconazole	0.09	0.06	0.14	82
Tebuconazole	0.51	0.44	0.58	30.1	Tebuconazole	0.13	0.08	0.19	74

Picoxystrobin	0.54	0.46	0.61	26	Picoxystrobin	0.19	0.14	0.25	62
Cyproconazole	0.56	0.48	0.63	23.3	Propiconazole	0.27	0.2	0.35	46
Kresoxim-Methyl	0.60	0.52	0.67	17.8	Mancozeb	0.28	0.21	0.36	44
Thiophanate-Methyl	0.65	0.57	0.72	11	Difenoconazole	0.29	0.22	0.36	42
Mancozeb	0.65	0.58	0.71	11	Kresoxim-Methyl	0.32	0.26	0.39	36
Pyraclostrobin	0.67	0.60	0.73	8.2	Pyraclostrobin	0.39	0.33	0.47	22
Propiconazole	0.71	0.63	0.78	2.7	Penthiopyrad	0.43	0.36	0.5	14
Penthiopyrad	0.72	0.63	0.79	1.4	Check	0.5	0.44	0.56	0
Check	0.73	0.65	0.80	0	Thiophanate-Methyl	0.51	0.43	0.58	0
Chlorothalonil	0.74	0.68	0.80	0	Chlorothalonil	0.52	0.45	0.59	0

[a] Proportion of severely damaged pods (disease classes: 3 - deformed pod, with one single kernel completely smutted and 4 - deformed pod with two kernels completely smutted).

[b] Lower (CI_L) and upper (CI_U) limits of 95% confidence interval around SDP estimated mean.

[c] Percentages of control efficiency of active ingredients calculated in relation to the non-treated check.

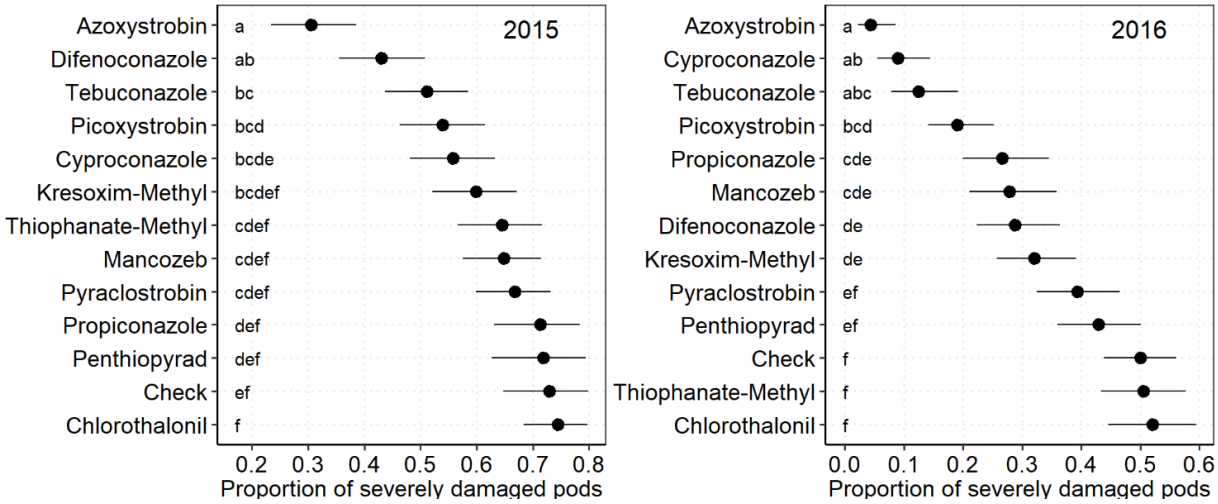

Figure 3. Proportion of severely damaged pods for different active ingredients used for the control of peanut smut on pots experiment in 2015 and 2016.

In the field experiments, mean SDP at the non-treated control was 0.39 (2015) and 0.43 (2016). Cyproconazole and azoxystrobin in 2015 presented the lowest SDP values: 0.21 and 0.25 (control efficacy of 47% and 37%, respectively) ($P<0.05$). Azoxystrobin was the most

efficient fungicide in 2016 to control smut, with a lowest SPD: 0.26. No significant differences of SPD with the non-treated control was observed for kresoxim-methyl, thiophanate-methyl, tebuconazole, penthiopyrad and chlorothalonil for both years (Table 3; Figure 4).

Table 3. Severely damaged pods, control efficiency, and corresponding statistics for the 12 fungicides evaluated for the control of peanut smut on field experiments in harvest 2015 and 2016.

	2015					2016			
Active ingredients	**SDP[a]**	**CL_L[b]**	**CL_U[b]**	**CE[c]**	**Active ingredients**	**SDP**	**CL_L**	**CL_U**	**CE**
Cyproconazole	0.21	0.19	0.23	47.7	Azoxystrobin	0.26	0.24	0.29	39.5
Azoxystrobin	0.25	0.22	0.27	37.2	Propiconazole	0.33	0.31	0.35	23.7
Mancozeb	0.29	0.27	0.31	25.8	Cyproconazole	0.34	0.32	0.37	20.9
Pyraclostrobin	0.31	0.29	0.34	20.7	Picoxystrobin	0.38	0.35	0.40	13.8
Picoxystrobin	0.32	0.29	0.35	18.9	Difenoconazole	0.38	0.35	0.40	13.3
Propiconazole	0.32	0.30	0.34	17.9	Thiophanate-Methyl	0.38	0.35	0.41	12.4
Chlorothalonil	0.33	0.30	0.35	16.8	Chlorothalonil	0.38	0.36	0.41	12.2
Kresoxim-Methyl	0.33	0.30	0.35	16.6	Mancozeb	0.38	0.35	0.41	12.0
Thiophanate-Methyl	0.35	0.32	0.37	11.7	Kresoxim-Methyl	0.39	0.36	0.43	9.9
Difenoconazole	0.36	0.34	0.38	8.2	Penthiopyrad	0.40	0.38	0.42	7.8
Tebuconazole	0.36	0.34	0.39	7.4	Pyraclostrobin	0.42	0.40	0.45	3.2
Penthiopyrad	0.37	0.35	0.40	4.8	Check	0.44	0.41	0.46	-
Check	0.39	0.36	0.42	-	Tebuconazole	0.45	0.42	0.48	0

[a] Mean of the proportion of severely damaged pods (disease class 3 and 4)

[b] Lower (CL_L) and upper (CL_U) limits of 95% confidence interval around SDP

[c] Percentages of control efficiency of active ingredients calculated in relation to the untreated check treatment.

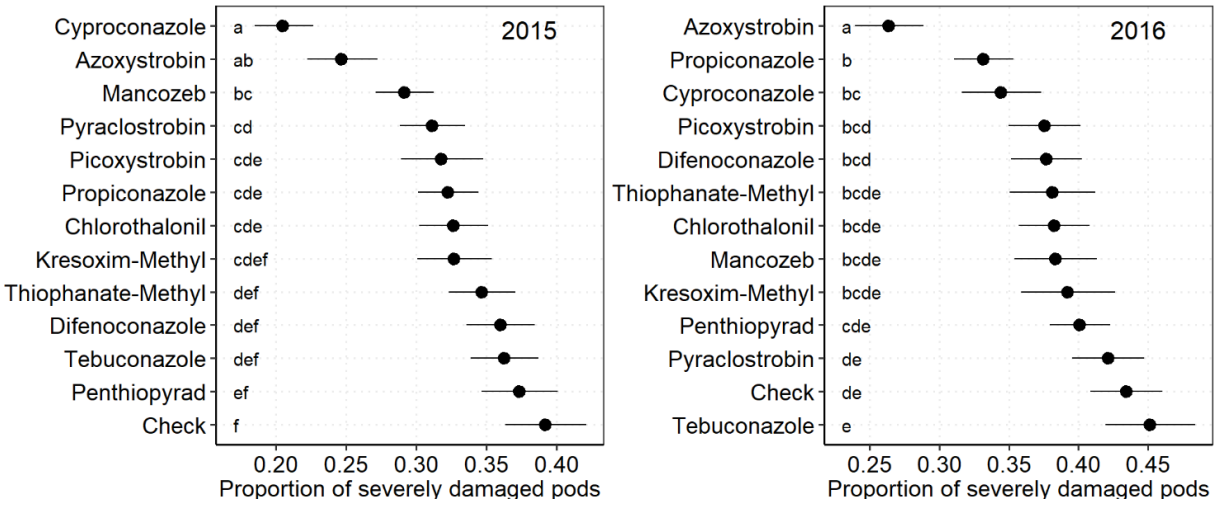

Figure 4. Proportion of severely damaged pods for different active ingredients used for the control of peanut smut on field experiments in 2015 and 2016.

3.3. Multivariate analysis

The multivariate analysis allowed us to integrate all the trials results: two independent principal components (PC) accounted for 76.5% of the total variation (56.2% and 20.3% for the PC1 and PC2 respectively). PC1 (horizontal axe) represented the control efficacy values in the field plots trials (with correlation coefficients of 0.86 and 0.77, in 2015 and 2016 respectively) and pots experiments (correlation coefficients were 0.83 and 0.73, in 2015 and 2016 respectively). The further right on the PC1-axis, the higher the efficacy control of the fungicide (Figure 5). PC2 on the y-axis represented the EC50 values: the upper in the axis, the lower the sensitivity of the smut to the fungicide. Considering this coordinate meaning, we may select as best fungicides those ones located in the right quadrants, and the lower as possible: azoxystrobin and picoxystrobin (QoI group) and cyproconazole and difenoconazole (DMI group).

We observed that control efficacy in 2015 followed a similar trend in both types of experiments (plants grown in pots and field plots), but a weak correlation among both experimental types was observed in 2016.

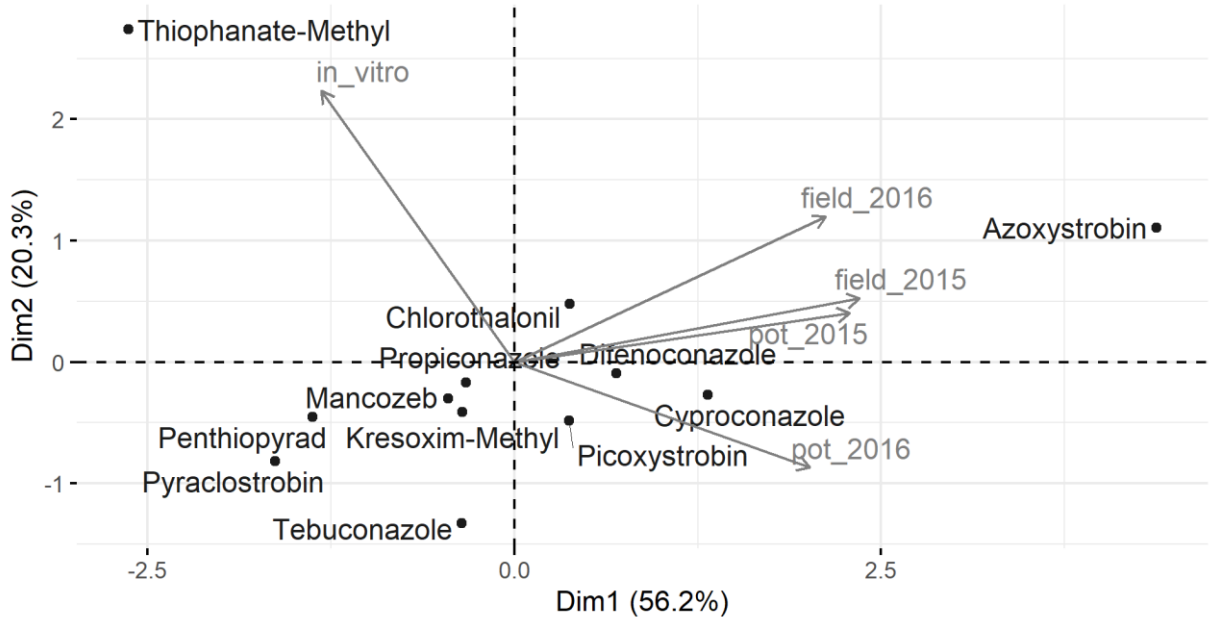

Figure 5. Biplot model showing the relationship among fungicides (individuals, represented by points) and their performance in each trial (variables, represented by vectors): in vitro experiments and pot and field experiment in 2015 and 2016.

4. Discussion

Since its re-emergence in 2006, peanut smut has continued increasing its prevalence and incidence in the main Argentine peanut-growing region, without good perspectives for the future due to the current absence of adoption of management practices for controlling smut (Rago et al., 2017). By means of the present two-year fungicide-screening study we observed variability in the available set of commercial fungicides, including highly efficacious active ingredients to non-effective ones. To the best of our knowledge this is a first study assessing the chemical management as a technique to maintain peanut smut in low intensity levels.

In vitro mycelial growth inhibition tests showed a general good performance of the DMI´s group, QoI´s azoxystrobin and pyraclostrobin and SDHI´s penthiopyrad. The two last chemical groups are powerful inhibitors of spore germination (Fungicide Resistance Action

Committee [FRAC] group 11 and 7), a process that was not evaluated in the *in vitro* tests. This fact could have a potential sub-estimation effect of the evaluated active ingredients since the smut spore germination is a fundamental first step on epidemic onset after the gynophore exudates stimulation (Astiz Gasso et al., 2008; Marinelli et al., 2008). In relation to this both fungi cycle processes, Kosiada (2011) observed a weak correlation between mycelial growth and germination of teliospores and basidiospores according to the sensitivity of some fungicides for head smut of corn (*Sphacelotheca reiliana).*

Another weak correlation was reported between results of laboratory and field conditions experiments (Hollowell et al., 2003), which led us to perform pots and field trials for assessing the fungicide efficacy to control peanut smut. However, in both years of the study we observed that azoxystrobin was the fungicide with highest smut control efficacy levels in pot or field experiments.

Pavez Badilla et al., (2013) showed different levels of sensitivity *in vitro* for pyraclostrobin and kresoxim-methyl (QoI) in front of the *Venturia inaequalis*, nevertheless they assure that it is very difficult to extrapolate this type of investigation to the field conditions due to the action mode. Differences in control levels for peanut smut were observed compared to *in vitro* results. The DMIs a.i. showed EC50 lower than the rest of the chemical groups. Cyproconazole has the highest EC50 comparatively with other DMIs, however it is one of the a.i. that shows the highest control efficiency in field and pots experiments. For its part, chlorothalonil and penthiopyrad (SDHI) have an EC50 like QoI, however did not demonstrate disease control efficiency. Azoxystrobin, picoxystrobin, cyproconazole and tebuconazole were the a.i. best responses demonstrated in the assessment; however, they are not the lowest EC50 within the chemical groups to which they belong.

The lack of consistency of the observed results between pot and field experiments can be due to differences in soil properties in both experimental conditions: soil humidity content

may lead to different fungicide degradation rates or particles absorption. Further studies may be done for a better understanding of the latter process and consequently a more efficient use of fungicides against *T. frezii* infections. Paredes et al. (data not published) observed in growing chambers experiments that the lower the soil humidity, the higher the incidence of smut in peanut plants.

QoI, SDHI and DMI are the most common fungicides used to control peanut diseases in crops. These fungicides groups performed less than 40-50% effective to peanut smut control in field, even when used at higher doses comparatively leaf spot (Rago et al., 2017). Mutations in genes encoding fungicide targets of pathogenic fungi are often the cause of developed resistance to QoI, SDHI and DMI fungicides (Sierotzki and Scalliet, 2013). Nevertheless, research on the molecular basis of *T. frezii* fungicide resistance has not been possible due to the lack of genetic information. Recently, Arias et al., (2019) report the complete mitogenome of *T. frezii* and provide molecular tools to study fungicide target genes and suggests potential resistance to strobilurin and carboxamide fungicides.

Individual performances of cyproconazole and azoxystrobin had the highest control efficacy in the present study. However, higher performances were observed as mixture formulations of the fungicides in previous field experiments under the same disease pressure (4400 teliospores/g of soil): azoxystrobin + cyproconazole and azoxystrobin + picoxystrobin reduced smut severity by 58 and 47% respectively relative to non-treated control (Paredes et al., 2015b, 2015a). This higher performance of the fungicides as mixtures could be due to a "potentiation" effect (Cid, 2014).

Funding

This work was supported by INTA [Programa Nacional de Cultivos Industriales, PNIND-1108072] and Fundación Maní Argentino.

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Supplementary tables

Table S1: Data were extracted from AGD local station data: Rainfall (monthly precipitation), average monthly maximum and minimum temperatures in the experimental peanut area during 2014/15; 2015/16.

	Rainfall (mm)		Temperature (°C)					
			Mean		Average max.		Average min.	
Month	2015/16	2016/17	2015	2016	2015	2016	2015	2016
December	38	122	22	24	32	31	14	16
January	89	123	24	23	32	30	18	18
February	255	249	21	24	28	30	16	18
March	121	45	21	19	28	26	16	13
April	57	143	20	15	28	21	13	11
Acumulated	560	682						

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Receiving Editor
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We want to thank you and the reviewers for providing excellent comment and suggestions on manuscript Ms. Ref. No.: **CROPRO-D-20-00707**. Below we provide responses to all comments and questions made/raised by the reviewers, making changes to the manuscript where appropriate.

REVIEWER #1

A reviewer #1 highlights, the title needs to be modified, the validity of your experiments needs to be authenticated, and the references need to be cross-checked.

Response: The title was changed to "EFFICACY OF FUNGICIDES AGAINST PEANUT SMUT IN ARGENTINA" The rest of highlights was explained in the comment below.

Reviewer #1: Comments are posted in the body of the text itself and can be found in the journal's system.

Comment P1 Line 1:
1. The manuscript is of routine type work.
Response 1: We are conscious of it, however to the best of our knowledge it is the first paper evaluating fungicides to control peanut smut. We consider our work should be discussed in the peanut protection community and CP journal can be an excellent medium. Items as operational feasibility or environmental impact of chemical management of peanut smut needs to be discussed.

2. References are not properly listed as per text
Response 2: The entire manuscript was corrected in accordance with citation in text.

3. Experiment is too old, needs re-validation.
Response 3: We partially agree with this issue because the argentine peanut system production remains exactly as it was during the experimental years of the present work.

- Granoleico peanut cultivar, which is susceptible to smut, is currently planted in more than 80% of the peanut area (Rago et al., 2017, Cazón et al., 2018)
- *T. frezii* inoculum pressure remains at the same level in the growing region (Asinari et al. 2019)
- Fungicide actives included in our study are still commercially available, and any new compounds have been released after our study (Rago et al., 2017, Cazón et al., 2018)

Moreover, we continued working on chemical control of peanut smut and our results are practically the same (Paredes et al. data not published)

4. Fungicides showed efficacy differently in different conditions.
Response 4: We added a paragraph in the discussion section about this topic.

“The lack of consistency of the observed results between pot and field experiments can be due to differences in soil properties in both experimental conditions: soil humidity content may lead to different fungicide degradation rates or particles absorption. Further studies may be done for a better understanding of the latter process and consequently a more efficient use of fungicides against *T. frezii* infections. Paredes et al. (data not published) observed in growing chambers experiments that the lower the soil humidity, the higher the incidence of smut in peanut plants.” (Line 300)

Comments 3 and 4 are also pointed out in lines 25-29: “fungicides showed efficacy differently in different environment, how will you justify? Moreover, experiments were undertaken during 2014-15 & 2015-16, which is too old? Need to re-validate those trials?”

5. In vitro studies need to be more focused before testing in the field?
Response 5: The observation is appreciated. *T. frezii* is a fungus that presents difficulties to obtain as in vitro pure culture, particularly spore germination. For this reason, EC50 estimates were performed by assessing mycelium growth inhibition. This fact could benefit DMI's fungicides in contrast to QoI's active ingredients since the latter act by inhibiting spore germination.

Comment P1 Line 22: Year of experiment?
Response: The suggestion was accepted “experiments were performed during 2014/15 and 2015/16” were incorporated. (Line 20-21)

Comment P2 Line 45: change “fungi” to “fungus”
Response: “fungi” was replaced by “fungus”

Comment P2 Line 47: change “sorus” to “sori”
Response: “sorus” was replaced by “sori”

Comment P2 Line 49: (Marinelli et al., 2008)
Response: Change made

Comment P2 Line 51-52: there are fungicides reported effective against this disease across the globe, so, you need to check this statement?
Response: *T. frezii* has been only reported infecting peanut in Argentina, and as stated in the discussion section (lines 267-275), local growers do not apply any management practices for controlling peanut smut. To the best of our knowledge there is only one fungicide recently labeled for controlling *T. frezii* (Iridium: Triadimenol 30% + Myclobutanil 20% p/v SC – rate 1500 cc/ha). However, this fungicide is not frequently adopted (Morichetti personal communication)

Comment P2 Line 57: change “Survival fungi structures” to “Survival structure”
Response: Sentence was corrected (Line 55)

Comment P3 Line 59-62: This is contradictory to your own statement that there is no management strategy?
Response: The suggestion was accepted. To clarify, the sentence was replaced by “Preliminary research suggested a wide range of control efficacies with fungicide applications while higher doses of DMI + QoI mixture fungicides reported to provide best control” (line 58)

Comment P4 Line 85: change “25º C ± 1º” to “25±1º C”

Response: Change made

Comment P4 Line 86: change "*Thecaphora frezii*" to "*T. frezii*"
Response: Change made

Comment P4 Line 100: disc (6 mm in diameter), is it a standard?
Response: "Plug" was replaced by "disc". In this pathogen is normally used 6mm diameter mycelial disc to growing plate (Rago et al., 2017; Cazón et al., 2018).

Comment P4 Line 101: discs, not plugs, and also replace plug(s) with disc(s) in the entire manuscript
Response: "plug(s)" was replaced by "disc(s)" throughout the manuscript as reviewer suggested

Comment P5 Line 109-110: Formula needs to be mentioned here
Response: Suggestion was accepted, and the formulas were re-enumerated (line 164, 169).
$GI_i = (CD_{check} – CD_i)/CD_{check} \times 100$
where GIi is the inhibition of i-th colony growth; CD is the mean colony diameter for the control without fungicide (check) or for the i-th fungicide. Linear model regression was fitted to the GI obtained along the exponential - transformed doses, and the EC50 was estimated by replacing components of the fitted equations.

Comment P6 Line 133: is it right?
Response: The observation is appreciated, and "CO^2" was replaced by "CO_2"

Comment P9 Line 191: is it appropriate?
Response: The observation is appreciated. The sentence "(lowest EC50 values)" was removed (Line 191). We explain that "The highest mycelial growth inhibitions" correspond to low EC50, we considered it is duplicate information.

Comment P11 Table1: There is a need to elaborate first time as staric below table
Response: The observation is appreciated, and supra and sub-indices were corrected following references.

Comment Line 325: References: Need to check thoroughly in accordance with citations in the text.
Response: The observation is appreciated. The entire manuscript was corrected in accordance with citation in text.

REVIEWER #2

In this paper, Paredes et al. assessed the efficacy of different fungicides of strobilurin, triazole and carboxamide group using invitro assays and field experiments for the management of peanut smut. Overall, this study will provide a good information to peanut growers in the event of smut infection. Most of the work seems to be suitably done, however, results should be discussed in detail with information on mode of action of the fungicides studied, the information on pathogen resistance to the fungicides and also in the event of using more than double of the recommended dose from the manufacturer. Some minor comments, specific suggestions, and questions are presented below.

Comment Line 59-62. I would suggest rephrasing like this, "Preliminary research suggested a wide range of control efficacies with fungicide applications while higher doses of DMI + QoI mixture fungicides reported to provide best control".
Response: The sentence was replaced.

Comment Line 66: change 'time' to 'timing'.
Response: "time" was replaced by "timing"**.**

Comment: Line 68: change 'the time of the application effect' to 'The application timing effect'.
Response: The sentence was replaced.

Comment: Line 72: change 'which is cornerstone knowledge' to 'and this information is essential for setting up of a chemical management strategy".
Response: The sentence was replaced.

Comment: Line 92-100: Were the actual active ingredient used in this experiment or a.i. concentrations contained within the formulated products? Were actual active ingredients obtained from the industry and used at different dilutions in the experiment? This needs to be clearly stated for those used in pot and field experiments.
Response: We used the active ingredient separately provided by the companies as experimental compounds (not as technical drugs, the single active ingredients + adjuvants).

Comment: Line 106: Add 'with' after subtracted.
Response: Change made

Comment: Line 112: remove 'it' from 'it is considered as highly fungitoxic'.
Response: "it" was removed

Comment: Line 118-119: If authors have used the formulated products, I would suggest updating the table 1 with their trade name, manufacturer, and their addresses. Also include the recommended dose as the dose used in the study already appears in the table.
Response:
As we explained in the comment above, fungicides were provided by companies as experimental compounds (active ingredient + adjuvants). To clarify, products and company, formulation and commercial rate for leaf spot, of each product were incorporated in Table 1.

Comment: Line number 120-121: Is there any specific reason to use two and a half fold higher a.i. dose than recommended? Why not the recommended dose? Is the decision made based on preliminary studies utilizing all fungicides? How would it impact the fungicide resistance development? Needs to be clarified and discussed in detail.
Response: The observation is appreciated. "one and a half fold the active ingredient dose recommended for peanut leaf spot control" were corrected (line 115). The commonly commercial fungicides are no registered for this pathogen. In this work, we use active ingredients labeled to peanut leaf spot. Based on our experience and obtained from preliminary studies, the recommended dose for leaf spot have low or no effect to peanut smut, but when we increased doses the control improved. This is explained in the introduction section (lines 57-62), to clarify "Preliminary research suggested a wide range of control efficacies" was incorporated at the sentence (line 57). For that reason, we decided to use the rate

(higher comparatively dose than recommended to leaf spot) in those experiments. However, the high doses and their impacts on fungicide resistance development is not clarified yet. Information about this species is very limited. Recently study (Arias et al., 2019) provided molecular tools to study fungicide target genes. To clarify this, we incorporate a paragraph in the discussion section (Line 308)
"QoI, SDHI and DMI are the most common fungicides used to control peanut diseases in crops. These fungicides groups performed less than 40-50% effective to peanut smut control in field, even when used at higher doses comparatively leaf spot (Rago et al., 2017). Mutations in genes encoding fungicide targets of pathogenic fungi are often the cause of developed resistance to QoI, SDHI and DMI fungicides (Sierotzki and Scalliet, 2013). Nevertheless, research on the molecular basis of *T. frezii* fungicide resistance has not been possible due to the lack of genetic information. Recently, Arias et al., (2019) report the complete mitogenome of *T. frezii* and provide molecular tools to study fungicide target genes and suggests potential resistance to strobilurin and carboxamide fungicides."

Comment: Line 132: Plots consisted of four rows 'of peanut seedlings'?
Response: Yes, and to clarify the field plot design "of peanut seedlings" was incorporate after "four rows"

Comment: Line 133-134: Better detail of the spraying process: nozzle type, nozzle spacing, pressure, and spray velocity, etc.
Response: The suggestion was accepted. To explain the spraying process "4-nozzles (hollow cone, ALBUS ATR 80) spaced 50-cm, delivering 120 L/ha at a pressure of 310 kPa and 0.9 m/s spray walking speed." was incorporated.

Comment: Line 164: It's difficult to understand, I would suggest wording like this or similar: 'Disease in these classes is considered to have impact in inoculum increase as they are lost at harvest and produces are discarded by the industry because of quality issues' (Morichetti S. personal communication).
Response: The suggestion was appreciated. The sentence was incorporate as reviewer indicated.

Comment Line 174-177: I would suggest including the link function with associated error type of the univariate generalized linear models used in statistical analysis. Any preliminary tests done to see year or factor x year interaction effect before making decision to analyze the results separately for each year?
Response: The suggestion was accepted. The paragraph was reformulated in the manuscript. Error type was incorporated. (Line 172-177)

Comment Line 189: Rephrase like this, 'The growth of T. frezii isolates was 4.76 (± 0.14) mm per day in untreated control plates. A wide range of sensitivity of T. frezii isolates was observed to the evaluated fungicides, EC50 value ranging from …'.
Response: The sentence was incorporate as suggested.

Comment Line 193: Rephrase like 'Moderate sensitivity was observed with mancozeb…'.
Response: Change made

Comment: Line 208: Rephrase like 'Azoxystrobin resulted the lowest SDP….'.
Response: Change made

Comment Line 213-214: Rephrase like 'Intermediate smut control were observed with tebuconazole, picoxystrobin and cyproconazole in 2015, and picoxystrobin, propiconazole, mancozeb, difenoconazole and kresoxim-methyl in 2016'.

Response: The sentence was rephrased as reviewer suggestion

Comment Line 277-279: This is confusing and needs rephrasing, "The two last chemical groups have as mode of action the inhibition of spore germination, process that was not evaluated in the in vitro tests." Is inhibition of spore germination is defined as mode of action of the fungicides? Mode of action for these two chemicals are defined by their ability to inhibit respiration which utilizes separate bio-chemical pathways.

Response:

Line 278: The authors agree with the explanation raised by reviewer. The sentence was replaced by “are powerful inhibitors of spore germination (Fungicide Resistance Action Committee [FRAC] group 11 and 7)” (Line 276)

Authors thank suggestion made by the reviewers which significantly improve the quality of the manuscript.

Highlights:

We evaluated the performance of 12 fungicides against peanut smut in Argentina

Cyproconazole and azoxystrobin had the highest control

In vitro mycelial growth inhibition and disease control presented low correlation

The results can be considered in an integrated approach for managing peanut smut.