**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 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). 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). In field experiment, the greatest control efficiency was observed for cyproconazole in 2015 and azoxystrobin in 2016, reducing peanut smut by 47.7 and 39.5% respectively. The results obtained in this work can be considered as an effective technique for the integrative smut management strategy, in addition to peanut genetic resistance and cultural practices.

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 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: 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 research suggested certain levels of fungicide efficiency, applications with higher doses of DMI + QoI mixture fungicides reported to provide best control (Cazón et al., 2013; Paredes et al., 2015b). In previous studies, we observed 58% smut control with fungicides azoxystrobin+cyproconazole at doses of 1000 cm³/ha, which is twice and half the recommended dose for peanut late leaf spot management caused by *Passalora personata* (syn. *Cercosporidium personatum*) (Paredes et al., 2015a). In addition, we compare the effect of timing of the day on the fungicide smut control efficacy: 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 work. The objectives of this work were to i) estimate the fungicides efficacy against *T. frezii in vitro* conditions and ii) test the field performance of fungicides against peanut smut.

**2. Materials and Methods**

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

A mixture of 10 *Thecaphora 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). The 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* purecolonies. The identities of all isolates were confirmed with specific primers for *T. frezii* (Cazón et al., 2016a).

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 disc (6 mm in diameter) were extracted from actively growing *T. frezii* isolates 8-day-old culture. The discs were transferred in the center of the Petri plates with PDA amended with the concentrations of the fungicides of each treatment. Five replicate 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 with the original mycelial disc diameter (6 mm). The measurements were taken when the control plates mycelium reached the plate edge. The inhibition of mycelial growth relative to the treatment without fungicide was calculated:

GIi = (CDcheck - CDi)/CDcheck × 100 (1)

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

The fungitoxicicity of fungicides was classified according 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. A non-treated control was included in each experiment in 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.

Table 1. 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

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Active ingredients** | **Products® (Company)** | **Formulation** | **Commercial rate for leaf spot (g ai/ha)** | **Rate using for peanut smut (g ai/ha)** | **Chemical group** |
| 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 | QoI |
| 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 | DMI |
| Penthiopyrad | \* | SC 10% | 80 | 120 | SDHI |
| Mancozeb | Mancozeb 75 (Nufarm) | WG 75% | 1100 | 1700 | EBDC |
| Thiophanate-Methyl | Abrigo (NOVA) | SC 50% | 500 | 750 | MBC |
| Chlorothalonil | Funda (Gleba) | SC 50% | 1000 | 1500 | Chloronitriles |

\* Experimental compounds provide by company. Rate were calculated comparatively as mixture in commercial products

2.2.1. Field experiment

Field assays were conducted at 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 CO2 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 velocity. 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 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 (Marinelli et al., 2008) which take into account both grains 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.

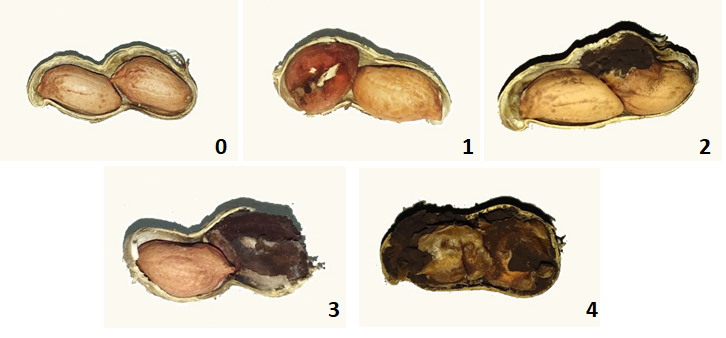


Figure 1. Severity five-class scale for peanut smut, where: 0 = both healthy kernels. 1 = presence of a small sorus in single kernel. 2 = at least one kernel with half area affected. 3 = deformed pod, with one single kernel completely smutted. 4 = deformed pod with two kernels completely smutted

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 industry because of quality issues (Morichetti S. personal communication). SDP was calculated as:

SDP = (n3 + n4 ) / N           (2)

where n3 and n4 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 - (Treatment SDP / untreated check SDP)) \* 100)         (3)

**2.4. Data statistical analysis**

Univariate generalized linear models (GLMs) with binomial as 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%.

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 in untreated control plates. A wide range of sensitivity of *T. frezii* isolates was observed to the evaluated fungicides, EC50 value ranging from0.001 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 active ingredients evaluated. Numbers on the central point-line corresponds at EC50.

**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 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 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 effect of different active ingredients using for the control of peanut smut on pots experiment in 2015 and 2016.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **2015** | | | | |  |  | **2016** | | | | |
| **Active ingredients** | **SDPa** | **CLLb** | **CLUb** | **CEc** |  | **Active ingredients** | | | **SDP** | **CLL** | **CLU** | **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 (CIL) and upper (CIU) 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 with the non-treated control in smut control was observed with 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 effect of different active ingredients using for the control of peanut smut on field experiments in harvest 2015 and 2016.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **2015** | | | | |  |  | **2016** | | | |
| **Active ingredients** | | **SDPa** | **CLLb** | **CLUb** | **CEc** |  | **Active ingredients** | **SDP** | **CLL** | **CLU** | **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 (CLL) and upper (CLU) 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 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. 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 type of experiments (plant grown in pots and field plots), but a weak correlation among both experimental types was observed in 2016.

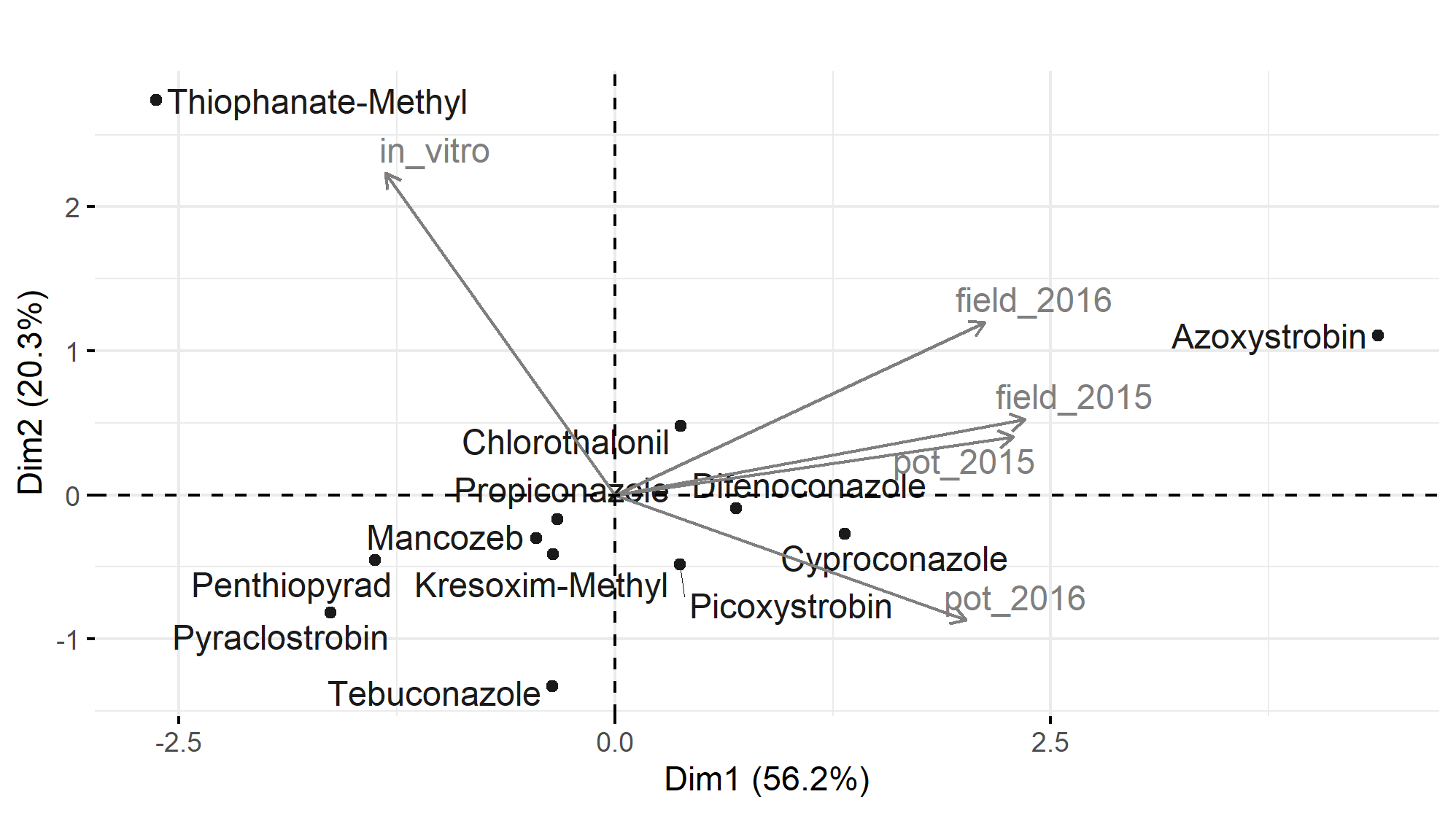


Figure 5. Biplot model showing relationship among fungicides (individuals, represented by points) and their performance in each trial (variables, represented by vectors): in vitro experiments and pot and field experiment in 2015 and 2016.

**4. Discussion**

Since its re-emergence in 2006, peanut smut continues 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 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 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), 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 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. In both years of the study we observed that azoxystrobin was the fungicide with highest smut control efficacy levels.

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 EC50within the chemical groups to which they belong.

The lack of consistency of the observed results between pots 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 particle absorption. This fact should needs further studies for a better understanding of the use of fungicides against *T. frezii* infections. Paredes et al. (data not published) observed that the lower the soil humidity, the higher the incidence of smut in peanut plants growing in chambers.

QoI, SDHI and DMI are the most common fungicides used to control peanut diseases in Argentinian crops. These fungicides groups performed less than 40-50% effective to peanut smut control in field, even when used at high 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 nontreated 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**

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.

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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 |  |  |  |  |  |  |