**EXPLORING THE EFFICACY OF FUNGICIDAL MANAGEMENT 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 last decade turning into the main problematic crop disease. Despite this fact, growers continue producing peanut without any disease management strategy what is leading to a local accumulation of *T. frezii* inoculum. Most of the fungicide products are formulated in mixtures of active ingredients. Identifying the effect of the individual active ingredients on the control efficacy could provide information for disease management. For this reason, it is proposed to determine the effect of individual active ingredients against *T. frezii* under *in vitro* conditions and their effect against peanut smut disease. 12 active ingredients of different chemical groups were evaluated. The inhibition mycelial growth *in vitro* of *T. frezii* was analyzed. Only thiophanate-methyl is not fungitoxic to *T. frezii* (EC50 > 100), mancozeb is moderately fungitoxic (EC50 = 6.28), and the rest of the active ingredients were classified as highly fungitoxic (EC50 < 0.1). To evaluate the control efficiency, a field experiments in two growing seasons and a semi-controlled conditions experiment were performed. It was evaluated the proportion of severely damaged pods (disease severity class 3 and 4) that there are discarded for the industry as high-quality grain and represent the major epidemical impact on the increase of inoculum. Azoxystrobin show consistency in control in semi-controlled experiment, registering the highest control efficiency with a value of 58.9% and 92% in 2014/15 and 2015/16, respectively. In field experiment, cyproconazole showed greater control efficiencies in harvest 2015 and azoxystrobin in harvest 2016, registering 47.7 and 39.5% respectively. There are multiple factors that affect the disease control, so the *in vitro* inhibition effect of the active ingredients against *T. frezii* is not enough, since there is not always a direct relationship between the EC50 and disease control. The effect can be more linked to intrinsic characteristics of each active ingredient. The results obtained are an important tool for strategies development in disease management.

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

**1. Introduction**

Peanut (*Arachis hypogaea* L.) is an annual extensive crop, with its center of origin in northwestern Argentina and southeastern Bolivia (Hammons, 1982). 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, 2006; 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; Oddino et al., 2007; Rago et al, 2014). The pathogen is a biotrophic fungi that produces sorus 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 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 an inter-annual inoculum accumulation (Paredes et al., 2017). Peanut cultivar Granoleico, with known susceptibility to the smut, is planted in more than 80% of the growing region (Rago et al., 2017; Cignetti et al., 2010). 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). Currently, EC-191 RC cultivar which has partial resistance of peanut smut were registered, but at the moment is not widely used by growers.

Survival fungi structures were observed to be viable for new infections up to 4 years (Cazón et al., 2016a) which implies that a short peanut crop rotation an inefficient disease management technique. Therefore the remaining potential peanut smut management technique to be explored is chemical control. Since *T. frezii* is a soil born disease, the behavior of the fungicide molecules in the soil solution may be related to the efficacy of the disease control. Some local research has been done related to the chemical control of peanut smut reporting highly variable results (Cazón et al., 2013; Paredes et al., 2015a; Paredes et al., 2015b): doses with a higher concentration of active ingredients (a.i.) per hectare have higher peanut smut controls (Cazón et al., 2013). In some field experiments, the best results were registered when used two and a half times doses recommended of peanut leaf spot (*Passalora* *personata*) (Paredes et al., 2015a; 2015b). In previous studies, we observed that night sprayings of fungicides targeting the soil was more efficient to control peanut smut than foliar applications (Paredes et al., 2015c). 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.

Fungicide sensitiveness of local *T. frezii* populations studies is fundamental knowledge for the first screening of commercial products to control the disease and further field efficacy tests. 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) determine the fungicides efficacy against *T. frezii 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 test**

Spores were extracted from smutted pods of a *Thecaphora frezii* isolate collected in 2015 in General Deheza, province of Córdoba, Argentina (32°45′08.54″S, 63°46′06.5″W). 100 mg of teliospores were suspended in a solution of 0.63 % NaOCl and were placed for 5 minutes in continuous agitation, rinsed twice with sterile distilled water and resuspended in 2 ml sterile distilled water. Disinfected teliospores were placed in potato-dextrose agar culture medium 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 25º C ± 1º in darkness (Astiz Gassó and Wojszko, 2011). The teliospores of *Thecaphora frezii* germinate after 7-10 days. Once the spores were germinated and colonies were observed, they were transferred to a culture medium potato-dextrose agar 2% to obtain *T. frezii* purecolonies. The identities of all isolates were determined with specific primers for *T. frezii* (Cazón et al., 2016b).

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 chloronitriles (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 is represented by a Petri plate (55 mm diameter), with five repetitions per treatment.

Mycelial discs (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 the original mycelial plug 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 and by logarithmic regression the concentration that inhibits 50% of mycelial growth (EC50) was calculated.

**2.2.** **Effect of fungicides control on the disease**

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

Table 1. Chemical group of fungicides active ingredients registered for the control of peanut leaf spot and doses used in this study by treatments for peanut smut control

|  |  |  |
| --- | --- | --- |
| **Chemical group** | **Active ingredients** | **Doses g ai/ha** |
| QoI | Picoxystrobin | 120 |
| QoI | Azoxystrobin | 135 |
| QoI | Kresoxim-Methyl | 190 |
| QoI | Pyraclostrobin | 150 |
| DMI | Difenoconazole | 160 |
| DMI | Propiconazole | 200 |
| DMI | Cyproconazole | 75 |
| DMI | Tebuconazole | 300 |
| SDHI | Penthiopyrad | 120 |
| EBDC | Mancozeb | 1700 |
| MBC | Thiophanate-Methyl | 750 |
| Chloronitriles | Chlorothalonil | 1500 |

**2.2.1. Field experiment**

Field assays were conducted 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 the highest *T. frezii* inoculum incidence (Paredes et al., 2017). The inoculum concentration in the field plots area was about >4500 ± 350 teliospores/g of soil. Treatments were designated in plots via a randomized complete block design with four replications. The plot consisted of four eight-meters-long rows. 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 four nozzles (ALBUS ATR 80) delivering 120 L per ha approximately. The first application was done seven days after R2 (beginning peg) (Boote, 1982) and the second one 10 days after the first one.

For the sampling it was harvested 1 m² situated in the two central grooves of each plot, all pods produced in that area were evaluated.

**2.2.2. Pot experiments**

The two pot experiments were conducted at INTA research facilities in Córdoba city (-31°28'3.831", -64°8'50.362"). A single peanut plant was grown in each 10-liter pot 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* isolate and the soil used to fill the pots were obtained from the experimental area of the field trial. A completely randomized design, with five (2014/15) and eight (2015/16) repetitions was used for each treatment. Treatments consisted of two fungicide sprayings: at 7 to 10 days after R2 (beginning peg) (Boote, 1982) 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 the pegs.

**2.3. Disease assessment**

The evaluation was performed when the pods were mature in state R8 (Boote, 1982). Only in this phenological state, it is possible to estimate the severity of the disease (Rago et al., 2017). The disease was quantified through the parameters of incidence (proportion of infected pods of total sample) and severity (proportion of damaged pod tissue). Severity can be estimated using a five-level diagrammatic scale: 0: healthy pods; 1: normal pod with a small sorus in single kernel; 2: deformed or normal pod with half of the kernels affected; 3: deformed pod and a completely smutted kernel; and 4: deformed pod, two completely smutted kernels (Marinelli et al., 2008).

For comparison of treatments were used the proportion of severely damaged pods (disease class 3 and 4). These classes of the disease there are discarded for the industry as high-quality grain (*Morichetti S. personal communication*) and represent the major epidemical impact on the increase of inoculum. The proportion of severely damaged pods (SDP) was calculated

SDP = (n \* disease class 3 + n \* disease class 4) / N (1)

Where **n** is the number of pods corresponding to each class of severity scale of the disease (3 and 4) and **N** is the total number observed.

The reduction of disease was calculated as control efficiency (CE) using the following formula:

CE = (1 - (Treatment SDP / untreated check SDP)) \* 100) (2)

**2.4. Data statistical analysis**

All statistical analyses were performed using the statistical program R core team (2018). The comparison between treatments was analyzed considering the proportion of severely damaged pods of each treatment through ANAVA and Tuckey mean comparison test (P < 0.05) to determine the significant differences.

**3.** **Results**

**3.1.** ***In vitro* sensitivity**

In control plates, the *T. frezii* isolates grown 4.76 mm per day (± 0.14). The sensitivity of the isolates of *T. frezii* varied compared to the different a.i. fungicides. Highest inhibition of the mycelial growth were grouped in the chemical groups of DMIs, pyraclostrobin and azoxystrobin (QoI), penthiopyrad (SDHI), that achieved EC50 lower than 0.1 (Figure 1). Moderately sensitive was registered in mancozeb (EC50 = 6.28). Only thiophanate-methyl is not fungitoxic to *T. frezii* (EC50 > 100).



Figure 1. Mycelial growth inhibition curves according to fungicide concentration (μg/ml) for active ingredients evaluated. Numbers on the central point-line corresponds at EC50.

**3.2.** **Semi-controlled conditions experiment**

The proportion of severely damage pods varies in a range of 30 - 74% (azoxystrobin and chlorothalonil respectively in harvest 2015) and 4 - 52% (azoxystrobin and chlorothalonil in harvest 2016). Azoxystrobin shows consistency in control compared with the rest of the active ingredients (P > 0.05), registering the highest control efficiency with a value of 58.9% and 92% in 2014/15 and 2015/16 respectively. On the other hand, the untreated check treatment shares the same significance as chlorothalonil, penthiopyrad, thiophanate-methyl and pyraclostrobin in both harvest evaluates (P > 0.05) evidencing the absent control of peanut smut in this experiment for this a.i. (Table 2).

Table 2. Severely damaged pods, control efficiency, and corresponding statistics for the effect of differents active ingredients using for the control of peanut smut on semi-controlled condition experiment in harvest 2015 and 2016.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Harvest 2015** | | | | |  |  |  | **Harvest 2016** | | | | |
| **Active ingredients** | **SDPa** | **CLLb** | **CLUb** |  | **CEc** | **Active ingredients** | | | **SDP** | **CLL** | **CLU** |  | **CE** |
| Azoxystrobin | 0.3 | 0.23 | 0.39 | a | 58.9 | Azoxystrobin | | | 0.04 | 0.02 | 0.09 | a | 92 |
| Difenoconazole | 0.43 | 0.36 | 0.51 | ab | 41.1 | Cyproconazole | | | 0.09 | 0.06 | 0.14 | ab | 82 |
| Tebuconazole | 0.51 | 0.44 | 0.58 | bc | 30.1 | Tebuconazole | | | 0.13 | 0.08 | 0.19 | abc | 74 |
| Picoxystrobin | 0.54 | 0.46 | 0.61 | bcd | 26 | Picoxystrobin | | | 0.19 | 0.14 | 0.25 | bcd | 62 |
| Cyproconazole | 0.56 | 0.48 | 0.63 | bcde | 23.3 | Propiconazole | | | 0.27 | 0.2 | 0.35 | cde | 46 |
| Kresoxim-Methyl | 0.6 | 0.52 | 0.67 | bcdef | 17.8 | Mancozeb | | | 0.28 | 0.21 | 0.36 | cde | 44 |
| Thiophanate-Methyl | 0.65 | 0.57 | 0.72 | cdef | 11 | Difenoconazole | | | 0.29 | 0.22 | 0.36 | de | 42 |
| Mancozeb | 0.65 | 0.58 | 0.71 | cdef | 11 | Kresoxim-Methyl | | | 0.32 | 0.26 | 0.39 | de | 36 |
| Pyraclostrobin | 0.67 | 0.6 | 0.73 | cdef | 8.2 | Pyraclostrobin | | | 0.39 | 0.33 | 0.47 | ef | 22 |
| Propiconazole | 0.71 | 0.63 | 0.78 | def | 2.7 | Penthiopyrad | | | 0.43 | 0.36 | 0.5 | ef | 14 |
| Penthiopyrad | 0.72 | 0.63 | 0.79 | def | 1.4 | Check | | | 0.5 | 0.44 | 0.56 | f | 0 |
| Check | 0.73 | 0.65 | 0.8 | ef | 0 | Thiophanate-Methyl | | | 0.51 | 0.43 | 0.58 | f | 0 |
| Chlorothalonil | 0.74 | 0.68 | 0.8 | f | 0 | Chlorothalonil | | | 0.52 | 0.45 | 0.59 | f | 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.

**3.3. Field experiment**

The proportion of severely damage pods varies between 39.2 and 43.5% in check treatment for harvest 2015 and 2016 respectively. Cyproconazole and azoxystrobin in harvest 2015 were the a.i. that showed greater control efficiencies, registering 47.7 and 37.2% respectively, differing significantly from the rest of the treatments (P > 0.05). In harvest 2016, azoxystrobin showed de major CE (39.5%) differing from the rest of a.i. The untreated check shares the same significance with kresoxim-methyl, thiophanate-methyl, tebuconazole, penthiopyrad and chlorothalonil, for both years (Table 3).

Table 3. Severely damaged pods, control efficiency, and corresponding statistics for the effect of differents active ingredients using for the control of peanut smut on field experiments in harvest 2015 and 2016.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Harvest 2015** | | | | |  |  | **Harvest 2016** | | | |  |
| **Active ingredients** | | **SDPa** | **CLLb** | **CLUb** |  | **CEc** | **Active ingredients** | **SDP** | **CLL** | **CLU** |  | **CE** |
| Cyproconazole | | 0.205 | 0.185 | 0.227 | a | 47.7 | Azoxystrobin | 0.263 | 0.239 | 0.289 | a | 39.5 |
| Azoxystrobin | | 0.246 | 0.222 | 0.272 | ab | 37.2 | Propiconazole | 0.332 | 0.311 | 0.353 | b | 23.7 |
| Mancozeb | | 0.291 | 0.271 | 0.313 | bc | 25.8 | Cyproconazole | 0.344 | 0.316 | 0.373 | bc | 20.9 |
| Pyraclostrobin | | 0.311 | 0.288 | 0.335 | cd | 20.7 | Picoxystrobin | 0.375 | 0.350 | 0.401 | bcd | 13.8 |
| Picoxystrobin | | 0.318 | 0.289 | 0.348 | cde | 18.9 | Difenoconazole | 0.377 | 0.351 | 0.403 | bcd | 13.3 |
| Propiconazole | | 0.322 | 0.301 | 0.344 | cde | 17.9 | Thiophanate-Methyl | 0.381 | 0.351 | 0.412 | bcde | 12.4 |
| Chlorothalonil | | 0.326 | 0.302 | 0.351 | cdef | 16.8 | Chlorothalonil | 0.382 | 0.357 | 0.408 | bcde | 12.2 |
| Kresoxim-Methyl | | 0.327 | 0.301 | 0.354 | cdef | 16.6 | Mancozeb | 0.383 | 0.354 | 0.413 | bcde | 12.0 |
| Thiophanate-Methyl | | 0.346 | 0.323 | 0.370 | def | 11.7 | Kresoxim-Methyl | 0.392 | 0.359 | 0.426 | bcde | 9.9 |
| Difenoconazole | | 0.36 | 0.336 | 0.384 | def | 8.2 | Penthiopyrad | 0.401 | 0.379 | 0.423 | cde | 7.8 |
| Tebuconazole | | 0.363 | 0.339 | 0.387 | def | 7.4 | Pyraclostrobin | 0.421 | 0.396 | 0.447 | de | 3.2 |
| Penthiopyrad | | 0.373 | 0.347 | 0.401 | ef | 4.8 | Check | 0.435 | 0.409 | 0.461 | de | 0 |
| Check | | 0.392 | 0.363 | 0.421 | f | 0 | Tebuconazole | 0.451 | 0.419 | 0.484 | e | 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.

**4. Discussion**

In this analysis, the effect of different fungicide active ingredients on peanut smut control shows different answers regardless of the chemical group of fungicide to which it belongs. For each active ingredient, there is a limited amount of time that they are active in the soil, their localized area of effect, and large background inoculum populations present in the soil (Brantner and Windels 1998). Molecular characteristics for each actives ingredient give differences in control action. *In vitro* results of this work show the inhibition of mycelial growth. This would not necessarily reflect the infection process since for this should occur first it is necessary the germination of the spores that remain in the soil stimulated by gynophore exudates (Astiz Gasso et al., 2008; Marinelli et al., 2008), but it is difficult to obtain a uniform spores germination in culture media. QoI unlike DMIs, have the ability to inhibit spore germination (Bartlett et al., 2002). This characteristic may explain the better control efficiency by some molecules of QoI in field conditions. Kosiada et al., (2011) observed that there was not a high correlation between mycelial growth and germination of teliospores and basidiospores according to the sensitivity of some fungicides for *Sphacelotheca reiliana*. They conclude that a low inhibition of mycelial growth was observed when used azoxystrobin (QoI), but the influence on the germination of teliospores and basidiospores was significantly inhibitory. However, even though all the QoI have influences spore germination and their EC50 showed in this work prove to be fungitoxic, not all of them had a better performance in the control of the disease. The importance of complimenting laboratory experiments with field trials or under greenhouse is that the answers obtained in both studies do not always correlate (Hollowell et al, 2003). Pavez Badilla et al. (2013), showed to have 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 results *in vitro* conditions. 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. 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. 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 (Tonin et al., 2015). In both growing seasons, thiophanate-methyl did not show control of peanut smut in the field and pots experiments.

In previous results, we evaluated fungicides products containing mixtures of DMI and QoI for peanut smut control. We observed that azoxystrobin + cyproconazole and azoxystrobin + picoxystrobin showed the highest control of the disease, with efficiencies that reached 64 and 58% for each mixture fungicides (Paredes et al., 2015b; Paredes et al., 2015d). In individual a.i., cyproconazole and azoxystrobin showed better responses of peanut smut control achieving 47.7 and 39.5% of control efficiency respectively. Comparing these results, the efficiencies in individual ingredients is lower than mixtures fungicides, this could be because “potentiation” is a characteristic than showing some products that provide a better response in mixtures than each one separately (Cid, 2014).

**5. Conclusions**

There are multiple factors that affect the disease control. Therefore the *in vitro* inhibition effect of the active ingredients against *T. frezii* is not enough since there is not always a direct relationship between the EC50 and disease control. Not all active ingredients belonging to the same chemical group control peanut smut in a similar way. 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 a large part 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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