**Fungicidal control efficacy of peanut smut** J.A. Paredes \*1, L.I. Cazón1,5, C. Oddino2, J.H. Monguillot1, A.M. Rago2,3, J.P. Edwards Molina4

1Instituto de Patología Vegetal; CIAP - INTA. Córdoba, Argentina.

2Facultad de Agronomía y Veterinaria, IMICO, UNRC. Córdoba. Argentina

3Centro de Investigaciones Agropecuarias - INTA. Córdoba. Argentina

4Estación Experimental Agropecuaria - INTA. Balcarce. Argentina

5Departamento de Fitopatologia. Universidade Federal de Viçosa – MG. Brazil

**\*** Corresponding author:

E-mail: [paredes.juanandres@inta.gob.ar](mailto:paredes.juanandres@inta.gob.ar)

Full postal address: Instituto de Patología Vegetal – IPAVE; Centro de Investigaciones Agropecuarias – CIAP. Av. 11 de Septiembre 4755 (X5020ICA) Córdoba – Argentina

**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 the development of strategies for peanut smut management.

**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, 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, 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; 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 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). 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).

Survival fungi structures can remain viable for new infections up to four years (Cazón et al., 2016a) which implies that a short peanut crop rotation an inefficient disease management technique in the current argentine production system. 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., 2015a; 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., 2015b). In addition, we compare the effect of time 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). 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, 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. The objectives of this work were to i) estimate 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**

A *Thecaphora frezii* isolate obtained from smutted pods in 2015 in General Deheza, (Córdoba province, Argentina) was used for the fungicide sensitivity test. A suspension of 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 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). After 7-10 days, the spores of *Thecaphora 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., 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 was represented by a 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.** **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). in which we sprayed wateractive ingredient One set of trials was conducted in pots and the other one in field plots.



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 high *T. frezii* inoculum field pressure (Paredes et al., 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 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 four nozzles (ALBUS ATR 80) delivering 120 L per ha 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.

Two experiments with peanut plants growing in pots were conducted at INTA research facilities in Córdoba city (-31°28'3.831", -64°8'50.362"). 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* isolate 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: 0 – both healthy pods; 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. 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 all the pods contained in the pods were evaluated.

For the purposes of this analysis, we used the proportion of severely damaged pods (SDP, disease class 3 and 4) as the response variable. Only peanut pods classified as lower than class 3 are considered as high-quality grain for the industry (*Morichetti S. personal communication*). SDP, representing the proportion of discarded pods, was calculated as:

SDP = (n3 + n4 ) / N (1)

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) (2)

**2.4. Data analysis**

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%.

**3.** **Results**

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

*T. frezii* isolate grown 4.76 mm per day (± 0.14) in the not fungicide - amended control plates. The effective concentration that inhibits 50% of *T. frezii* micelial growth (EC50) values ranged from 0.001 to >100. The highest inhibitions of the mycelial growth were observed with fungicides from DMIs groups, pyraclostrobin and azoxystrobin (QoI), and penthiopyrad (SDHI), with EC50 < 0.1 (Figure 1). Moderately sensitive was registered with mancozeb (EC50 = 6.28). The lowest sensitivity of *T. freezy* was observed with thiophanate-methyl (EC50 > 100 µg/ml).

**3.2.** **Control efficacy pots experiment**

Smut pressure indicated by the mean SDP at the nontreated check pots was higher in 2015 (0.75) than 2016 (0.52). Azoxystrobin presented the lowest SDP values in both years (X in 2015 and X in 2016): no significant differences in smut control were observed with Difenoconzale in 2015 and from ciproconazole or tebuconazole in 2016. In an intermediate smut control efficacy group were observed tebuconazole, picoxystrobin and ciproconazole 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).



**3.3. Control efficacy field experiments**

Mean SDP at the non-treated check plots were 0.39 and 0.43 in 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).



**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 head smut of corn (*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 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. 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 58 and 47% 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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