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SCIENTIFIC ARTICLE
Phytopathology

Effect of satellite-emitted frequency sequences on *Phakopsora pachyrhizi* control, on soybean nutrition and yield

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ABSTRACT
The obstacles in *Phakopsora pachyrhizi* management result especially from susceptible soybean genotypes and resistant fungal strains. The objective of the current study was to evaluate the applicability of the emission of extremely low and specific frequencies by Effatha technology in the soybean Asian rust control, nutrition, and its impact on yield. The *in-vivo* test followed the detached leaves method, with six treatments: frequencies 1 and 2 individually and in association; the conventional chemical treatment (fungicide azoxystrobin + benzovindiflupyr); and witnesses in presence and absence of the fungus. Frequency 1 relates to inhibition of the enzyme succinate dehydrogenase and 2 to ubiquinone oxidase. In the field, frequencies 1 and 2 associated (with the same fungicidal action of the *in-vivo* study); nutritional frequency; application of azoxystrobin + benzovindiflupyr + mancozeb, and control without application were evaluated. *In vivo*, the fungicide provided 85% control of the disease symptoms, against 65% of frequencies 1 and 2 in association, which showed a higher efficiency compared to the isolated frequencies. In the field, the rate of increase of symptoms were reduced by all treatments compared to the control. At the end of the soybean cycle, the conventional fungicide resulted in 33% severity against 56% of frequencies 1 and 2 associated, and 69.2% of the control. The emission of the frequency for increased nutrient efficiency stood out positively on yield in relation to all the other ones. The conventional application provided the highest weight of 1,000 grains, possibly a direct reflection of the better control of the disease.

Keywords: Effatha technology; nutrients; fungicides; field conditions; Asian rust.

INTRODUCTION

Increasingly, the exploration of the use of digital tools can contribute to the achievement of higher plant yields in the context of modern and sustainable agriculture. Asian rust (*Phakopsora pachyrhizi*) is one of the main diseases in soybean cultivation, causing severe damage under favorable conditions. The obstacles in Asian rust management result especially from susceptible soybean genotypes and the presence of pathogen strains resistant to the main fungicide molecules, which act in specific sites. Currently, the occurrence of cross and multiple resistance reduces the efficiency provided by the chemical groups.

The advance of soy cultivation in Brazil has brought great benefits to our economy. However, the challenges faced in solving problems and seeking greater profitability grow continuously. Then, digital technology, among other modern tools, represents an opportunity to innovate with quality and competitive advantages.

Effatha technology is at its core, composed of an algorithm capable of creating sequences of extremely low frequencies (ELF) (below 100 Hz), harmless to humans and the environment, typically found in nature, to increase or decrease the inter-atomic space of any element or substance, chemical or biological. The frequency sequences created by Effatha technology are extremely specific and applied in agriculture via satellites, directed to areas delimited by georeferencing.

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In addition, they can be also applied by contact through RFID cards (with proprietary Effatha system) that are used in laboratory and greenhouse tests, among other applications. These cards receive information sent through Effatha's proprietary system, i.e., they can be activated and/or deactivated remotely, besides having the specific programming replaced when necessary (personal communication)[1]. For example, they can provide a better nutritional use of the plants and, consequently, a greater productive potential, or they can be effective against a certain pathogen or a complex of them.

Thus, some advantages are evident in the use of this "clean" technique, which does not generate harmful impact on the environment and that can be employed in conjunction with other conventional tools available. Therefore, it can add efficiency and profitability to the production chain (personal communication)[1].

In view of the technical advances coming from digital tools, such as artificial intelligence and automation, there is a huge challenge to visualize the ways of exploring the existing resources, in face of an extremely competitive environment (DILLON, 2021). In this context, for large-scale crop management in Brazil, this digital transformation can considerably favor national production and, thus, partially supply the needs or deficiencies of traditional models, at an increasing speed.

The efficiency of Effatha technology has not been demonstrated before in any research publication in the field of agriculture. However, in human health, the effects of electromagnetic fields of frequency below 300 Hz began to be researched several years ago and are intensified by the benefits obtained, such as in use in clinical therapy for bone treatment against osteoporosis and other disorders (HADDAD et al., 2007; PASI et al., 2016), treatment against cancer cells (TANG et al., 2019), tissue repair (BERTOLINO et al., 2006), among others. Also, it is known that the beneficial effects of ELF can vary according to cell types and distinct conditions (PASI et al., 2016), by being able to affect various cellular activities, including the expression of specific genes through mRNA responses (SANIE-JAHROMI et al., 2016), also observed more recently in a study with *Trichophyton rubrum*, the major causative agent of superficial skin infections, when gene (CSF2) expression was reduced by up to 10-fold compared to no frequency (PETRUCELLI, 2021).

Asian rust (*P. pachyrhizi* H. Sydow & Sydow), for being a very severe disease in soybean culture in several countries, with recorded losses of up to 90% of yield (YANG et al., 1991; BONDE et al., 2006; HARTMAN et al., 2015), requires a set of preventive measures. The use of digital tools can minimize the production costs attributed to the traditional management against this pathogen, whose chemical control according to REIS et al. (2017) can be inefficient due to the resistance to the main molecules of site-specific action.

It is known that the main tool to reduce the damage caused by Asian rust has been the application of fungicides in aerial part, because the disease has gained relevance in Brazil since 2002. Although there is currently genetic material with some level of resistance to the disease, its cultivation is done on a smaller scale, and the use of fungicides is still necessary and has proven to be viable (GODOY et al., 2016; FURLAN et al., 2018). However, with the occurrence of cross and multiple resistance of *P. pachyrhizi* to systemic fungicides, new control methods must be developed.

The objective of the present study was to evaluate the *in-vivo* and field control of Asian rust symptoms and soybean yield with the use of Effatha technology, which employs the emission, by satellite, of a sequence of ELF to two distinct targets, in this case the fungus *P. pachyrhizi* and the availability of nutrients in the soil, compared to the conventional use of fungicides and uniformly fertilized area.

MATERIAL AND METHODS

Laboratory conditions

The *in-vivo* experiment was conducted in the Laboratory of Phytopathology at Centro Avançado de Pesquisa e Desenvolvimento em Sanidade Agropecuária (CAPSA) of the Instituto Biológico, in Campinas, São Paulo state (SP), Brazil. The fungus inoculum (*P. pachyrhizi*) was obtained from soybean leaves with Asian rust symptoms from a cultivated area in Conchal, SP, Brazil, cv. BMX Valencia. The methodology used in the in-vivo experiment was detached soybean leaves (FRAC, 2006; MEHL, 2009), whose plants of the same variety were grown in pots until the age of 21 days after sowing. The unifoliolated leaflets were removed and placed in Petri dishes containing three moistened filter paper discs, after being treated and inoculated. The treatments used were:

- Soybean leaves inoculated with *P. pachyrhizi* + frequency 1;

[1] Information (email) received by Dr. Silvânia H. Furlan from Luzo Dantas Junior and Rodrigo Lemos Lovato, Effatha's researchers, in June 9, 2020.

- Soybean leaves inoculated with *P. pachyrhizi* + frequency 2;
- Soybean leaves inoculated with *P. pachyrhizi* + frequencies 1 and 2;
- Soybean leaves inoculated with *P. pachyrhizi* + conventional fungicide;
- Soybean leaves inoculated with *P. pachyrhizi* + water - positive control;
- Soybean leaves without the pathogen + water - negative control.

Frequencies 1 and 2 refer respectively to the inhibition of the enzyme succinate dehydrogenase (action of SDHIs) and the inhibition of the enzyme ubiquinone oxidase (action of QIos). The inoculum concentration was measured in a Neubauer chamber, adjusted to 1×10^5 spores·mL^{-1}, and then sprayed on the abaxial side of the leaflets.

In the conventional chemical treatment, the leaves were immersed in azoxystrobin + benzovindiflupyr fungicide solution at the concentration of 1 g i.a.·L^{-1}, for 3 seconds, and then placed on filter paper saturated with distilled water. After drying in the environment, fungal inoculation was performed.

In the treatments of frequency emission (Effatha technology), after closing the Petri plates with parafilm, a card responsible for the emission was stuck to the bottom of each plate. The plates were incubated in a growth chamber for 14 days at 24°C with a 12-hour photoperiod.

The percentage of Asiatic rust symptoms severity was evaluated based on the diagrammatic scale of GODOY et al. (2006), at 14 days of inoculation, besides the percentage of phytotoxicity.

The experimental design used with magnetic cards was entirely randomized, with six treatments and three repetitions. The repetition was represented by a 15-cm diameter Petri dish containing four leaflets each. The experiment was conducted twice in its entirety.

Field conditions

The field experiment was conducted in the municipality of Conchal, at the Promip Experimental Station, altitude 530 m, latitude 22°22'18.5"S and longitude 47°10'51.8W". The experimental area was fallow between the 2019 and 2020 crop, and in February 2021 the BMX Potência soybean was sown. Soil fertility conditions were uniform throughout the experimental area, per the macro and micronutrient analysis performed in 2019. All techniques recommended for soybean cultivation were used, including phytosanitary treatments for pest control.

The experiment consisted of four treatments with five repetitions each. Each treatment was arranged side by side. The blocks refer to the application strips, in the direction of the sowing lines, with the total area of 150 m long × 30 m wide each, where treatments T1 and T2 were located, in order to avoid overlapping frequencies. Treatment 3, which refers to conventional application (CO_2-based sprayer), received three applications (R2, R4 and R5.2 stages) of the systemic + multisite fungicide mixture (benzovindiflupyr + azoxystrobin) at 0.2 L·ha^{-1} + mancozeb at 1.5 Kg·ha^{-1} + mineral adjuvant at 0.25 L·ha^{-1}, considered the standard treatment (ALVES; JULIATTI, 2018). This treatment and the control one without application (T4) covered an area of 20 × 30 m each and were also arranged side by side, close to T1 and T2, but far enough apart not to receive the effect of frequencies. The treatments T1 and T2 received the specific frequency sequences via satellite (TECNOLOGIA EFFATHA, 2021), from the beginning of plant emergence until the end of the cycle. T1 refers to fungicide actions responsible for inhibiting mitochondrial respiration in the cytochrome bc1 complex (complexes II and III of the respiratory chain), and T2 to nutritional action, a frequency that favors the availability of nutrients to plants.

Fungicide applications were performed with the aid of a knapsack sprayer at a constant pressure of 2 bar, equipped with XR 11002 fan nozzles. The spraying equipment was regulated to provide perfect coverage of the target, with a volume of 150 L·ha^{-1}.

Five useful plots of 20 m^2 (4 m wide × 5 m long) were demarcated for each treatment, in which five sub-plots were sampled in each, at least 2 m apart. The total of 10 plants was randomly evaluated in each sub-plot, stratified in three thirds, called bottom, middle, and top, totaling 50 plants per treatment.

The evaluation of the symptoms of Asiatic rust was made on the day of the first application of fungicides (previous), through the percentage of severity, based on the infected leaf area (diagrammatic scale from 0 to 100%, according to GODOY et al. (2006)). These evaluations, performed in six phenological stages, were made while there was no significant defoliation of the plants, between the R1 and R6 stages. We also evaluated the height of plants measured from ground level to the last node, in R5.1, the percentage of defoliation in R6 and the apparent symptoms of phytotoxicity (scores from 1 to 5 with 1 = 0 and 5 = more than 50% severity of symptoms), which, however, did not occur. Rainfall (mm) and temperatures were monitored during the trial.

The yield evaluations were obtained from the manual harvest of the plants to obtain the total weight, in an area of two lines of 5-m length, within the sub-plots, and the weight of 1,000 grains.

Statistical analysis

The percent of disease severity and of defoliation data were transformed into arc sine $\sqrt{(x/100)}$, and the data of area under the disease progress curve (AUDPC) were statistically analyzed by Tukey's test at 5% significance level. The data of plant weight (yield and 1,000 grains) were analyzed according to variance analysis, and Duncan's test was applied at 5% significance level for comparison of means.

RESULTS

In vivo, the most efficient treatment in controlling the disease, was the fungicide benzovindiflupyr + azoxystrobin, followed by the use of the associated frequencies 1 and 2, while frequencies 1 and 2 issued separately were less efficient. The fungicide provided 85.2% control, the associated frequencies 65.3%, and when isolated they were similar to each other, with 55.2 and 54.9% (Fig. 1).

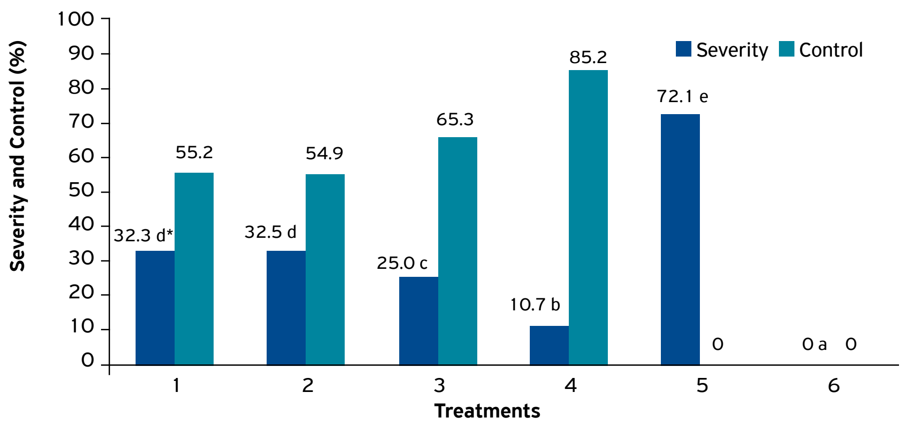

Figure 1. *In-vivo* trial: percentage of symptom severity and control of Asiatic rust (Phakopsora pachyrhizi) on detached soybean leaves in the different treatments. Average of two trials (six repetitions)*.

CV (%): 1.9 [original data were transformed into arc sine $\sqrt{(x/100)}$]; *averages followed by the same letter do not differ by Tukey's test at 5% significance level; 1: frequency 1, succinate dehydrogenase; 2: frequency 2, ubiquinone oxidase; 3: associated frequencies 1 + 2; 4: fungicide benzovindiflupyr + azoxystrobin; 5: positive control (inoculated leaflets); 6: negative control (no inoculation). Source: Elaborated by the authors.

In both the fungicide treatment and those receiving the frequencies, no phytotoxicity was observed on the detached soybean leaves.

In the field, the Asian rust appeared naturally on the plants at the R2 (flowering) stage. Local weather conditions, especially lower rainfall during the grain filling period, negatively influenced symptom severity and plant productivity. However, it was possible to differentiate the effect of treatments. Towards the end of the crop cycle, the severity of the disease in the control plants (T4) reached 39.1% at the R5.1 stage and almost 70% at R6 (beginning of plant maturation), which was higher than the other treatments (T1, T2 and T3).

The most efficient treatments in controlling Asian rust (based on AUDPC) were fungicide (T3), followed by emission of nutritional frequency (T2), with significant variations between them regarding disease severity, AUDPC and control percentages, in the three parts of the plant (Fig. 2).

At the beginning of the disease development, T1 (associated frequencies with fungicidal action) stood out by obtaining a higher percentage of control, but, as the symptoms advanced, T3 (standard fungicide) became more efficient than the other ones, followed by T1 (Fig. 2).

There were differences among treatments for plant height at the R5.1 stage (beginning of granulation). The treatment related to frequency emission to make nutrients available to the plants stood out by the higher plant height. This indicates that there was a significant availability of nutrients by Effatha technology, possibly due to the greater absorption and utilization of nutrients. Next comes the standard treatment with fungicides (Fig. 2).

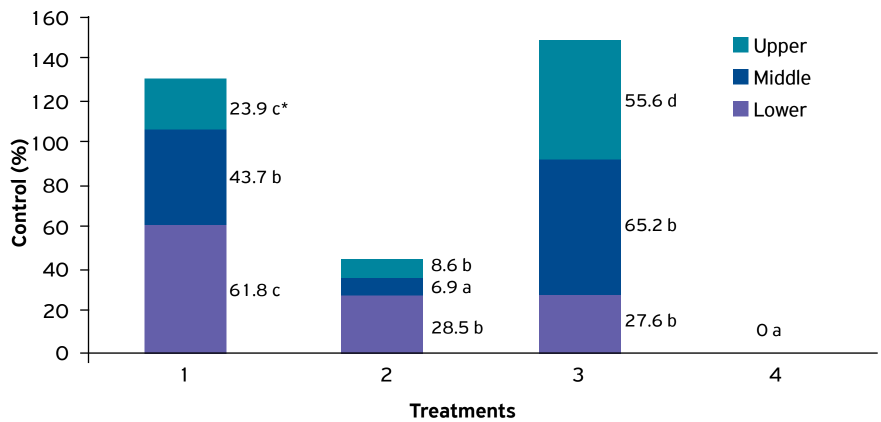

Figure 2. Treatments effect on the percentage of Asian rust in the lower (bottom), middle (middle) and upper (top) thirds of soy plants, based on the area under the disease progress curve, obtained from six evaluations of the percentage of severity of the disease symptoms. Conchal, SP, Brazil, 2021.
CV (%): 11 (bottom), 7 (middle) and 5.5 (top); *averages followed by the same letters between the columns, for each third of the plant, did notŸiffer by Tukey's test at 5% significance level; 1: fungicide frequencies with action on the enzymes succinitate dehydrogenase + ubiquinone oxidase; 2: nutritional frequency; 3: application of the fungicides (benzovindiflupyr + azoxystrobin) + mancozeb + adjuvant; 4: control.
Source: Elaborated by the authors.

The percentage of plant defoliation was significantly reduced by the treatments tested, when compared to the control (T4), which was 60.4%. Among the other ones, T1 (associated frequencies with fungicidal action) showed the lowest percentage of defoliation (32.6%), lower than the standard fungicide treatment (T3), with 38.6%, lower than T2 (emission of nutritional frequency) with 49% (Fig. 3). There were no phytotoxicity symptoms on plants in any of the treatments employed, as in the *in-vivo* trial.

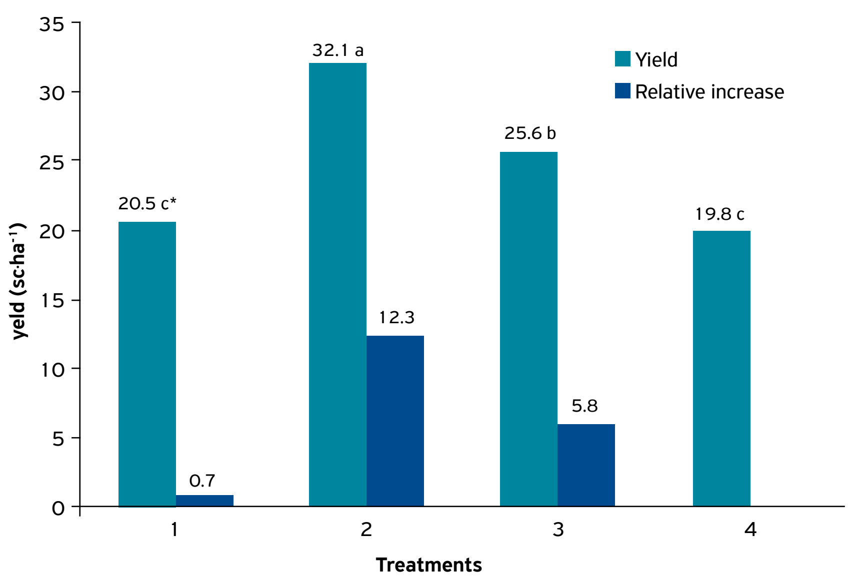

Figure 3. Treatment effect on yield in sc·ha^{-1} and relative increase in sc.ha-1 based on the control. Conchal, SP, Brazil, 2021.
CV (%): 14.8; *averages followed by the same letters do not differ by Duncan's test at 5% significance level; T1: fungicide frequencies with action on the enzymes succinitate dehydrogenase + ubiquinone oxidase; T2: nutritional frequency; T3: application of the fungicides (benzovindiflupyr + azoxystrobin) + mancozeb + adjuvant; T4: control. Source: Elaborated by the authors.

Due to the late sowing season and local climatic conditions, the low productivity of soy plants in all treatments is justified. Even so, it was possible to observe differences among them, highlighting the nutritional frequency treatment (T2) as the one that provided the highest yield among the other ones, in accordance with the results of plant growth.

The treatment of associated fungicide frequencies (T1) showed a yield similar to the control treatment, with only 0.7 sc·ha^{-1} more, although it showed some efficiency in controlling the disease. This result may be justified by the occurrence of powdery mildew with high severity in all treatments (data not shown), except for the conventional fungicide treatment (T3), which provided significant control. This result suggests the need for a better adjustment and expansion of frequencies, with action on different pathogens and possible multisite action. The highest yield was obtained by the nutritional frequency treatment (T2), which provided 12.3 sc.ha more than the control treatment, while the chemical resulted 5.8 sc.ha more (Fig. 3).

Although the nutritional treatment (T2) had the highest yield among the other ones, the gain in 1,000 grain weight was not significant compared to the control, while the conventional application resulted in a higher specific weight of grains, which is most likely due to the better control of the Asian rust and powdery mildew (Fig. 4).

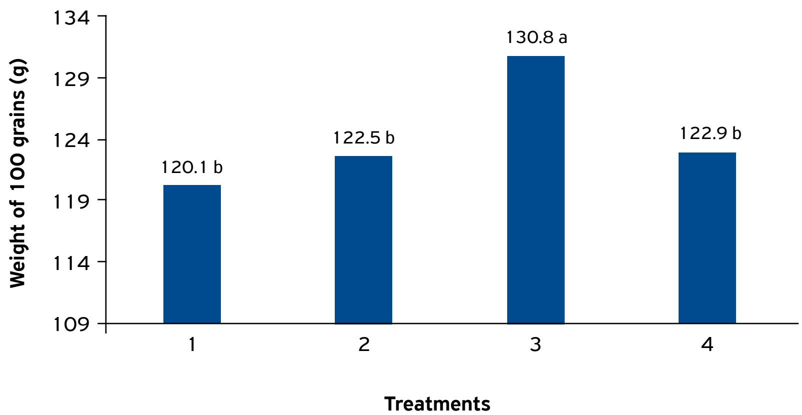

Figure 4. Treatment effect on the weight of 1,000 grains (g) of soybeans. Conchal, SP, Brazil, 2021.
CV %: 4.4; *averages followed by the same letters do not differ by Duncan's test at 5% significance level; T1: fungicide frequencies with action on the enzymes succinate dehydrogenase + ubiquinone oxidase; T2: nutritional frequency; T3: application of the fungicides (benzovindiflupyr + azoxystrobin) + mancozeb + adjuvant; T4: control. Source: elaborated by the authors.

DISCUSSION

One of the benefits of using frequencies in relation to conventional application is that it does not suffer influence of climate, it is not affected by the pH of the mixture, types of formulations, drift, size, and volume of drops, among others. In the traditional technique, there is not always satisfactory plant coverage, especially in low-volume spraying, with low reach of the lower parts of the plants, in which the first fungal infections usually start (CUNHA; RUAS, 2006; OLIVEIRA; ANTUNIASSI, 2012; FURLAN et al., 2018).

The frequencies evaluated targeted the key processes of mitochondrial respiration of the fungi, as occurred in the performance of the active ingredients of the QoI group, azoxystrobin, and SDHIs, benzovindiflupyr. The former is an inhibitor of quinone oxidase at the Qo site binding of the cytochrome bc1 complex (complex III), preventing electron transfer and ATP synthesis, culminating in energy deficiency and fungus death (FURLAN, 2016; KLOSOWSKI et al., 2016). The target protein of Qol is cytochrome bc 1 ubiquol oxidase (BRENT; HOLLOMON, 2007). The second one is an inhibitor of succinate dehydrogenase, an enzyme that plays a key role in two key processes of cellular respiration in eukaryotic organisms: the citric acid cycle, and the electron transport in complex II (SIEROTZKI; SCALIETT, 2013), inhibiting ATP formation for cells (KEON et al., 1991). For these two groups of fungicides, there are cases of gene mutation for resistance, besides for the group of demethylation inhibitors (DMIs) (FRAC, 2006; REIS et al., 2017; FURLAN et al., 2018). In the field trial, the multisite mancozeb, from the dithiocarbamate chemical group, which interferes in many cellular functions (FRAC, 2006), was added to the systemics in order to use a more complete chemical standard and promote the increment of control efficiency, as indeed was obtained and, thus, met the technical recommendations and strategies against pathogen resistance.

Therefore, in the present study, the conventional treatments with application of fungicides, in double (*in vivo*) or triple (field conditions) mixtures, provided the best control of Asiatic rust, followed by the use of the sequence of associated fungicide frequencies, that is, related to the two actives of the chemical standard. TOLOTI et al. (2016) found the efficiency of the active ingredients benzovindiflupyr + azoxystrobin in the control of soybean Asian rust under field conditions, in two applications, one at the V5 stage and the other one at R1, which increased grain productivity compared to the control treatment without application.

The frequency to increase nutritional efficiency, used in the field where no chemical fungicide was applied or by fungicide frequency, provided the best plant productivity among the treatments, although the treatment with fungicide (benzovindiflupyr + azoxystrobin) + mancozeb + adjuvant provided the highest weight of 1,000 grains. This may be related to the direct impact of the disease on this factor of production, and also the occurrence of powdery mildew, which was efficiently controlled only by the conventional chemical treatment, which very possibly influenced the final result. On the other hand, the treatments with the use of frequencies were not programmed to control powdery mildew.

At the beginning of the disease development, the treatment associated frequencies with fungicidal action stood out by obtaining a higher percentage of control, but, as the symptoms advanced, the treatment standard fungicide became more efficient than the other ones. Therefore, new studies with the frequencies should be tested in high inoculum pressure of the pathogen or application programs aimed at greater control effectiveness.

Also under field trial conditions, the levels of control were lower than those obtained in the *in-vivo* trial, which was expected due to the absence of environmental adversities in the laboratory.

In the lower part of the plants, the frequencies stood out positively in controlling the disease, while in the upper parts (middle and top) the positive highlight was the conventional chemical treatment. Maybe it is possible to create sequences of frequencies that have as specific target other mechanisms of action, similar to the multisites, which could increase the gains related to the control of pathogens through the use of Effatha technology.

Fungicides belonging to some recommended chemical groups, while they are employed to efficiently control diseases, can generate some benefits to plants such as increasing photosynthetic capacity, reducing respiration, delaying senescence, increasing protein concentration, among others (RAMOS et al., 2013). At the same time, it presents the disadvantage that applications can select resistant mutants, especially when in isolated use of site-specific active, while the use of frequencies may not present this risk, and, if it does, the frequency change is a faster process to obtain than the development of a product (personal communication)[2], besides the costs involved.

In addition, the development time for the specific frequency sequences for a biological target with Effatha technology is extremely reduced (about 48 hours) compared to traditional chemical methods, which provides a rapid defense against field mutations, because, in just 48 hours after identifying the resistance-generating mutation, all fields that are being treated with the technology can be treated with the new frequency sequences that target the mutant biological sequence. In a computational analogy, it would be something similar to the operation of computer anti-virus software. Thus, this tool can be extremely relevant for phytosanitary control, whether the causal agents already exist or not.

The frequencies can also stimulate or inhibit gene expression (PETRUCELLI, 2021), which can bring several benefits beyond the fungicidal effect, especially by not depending on climate neither on other adverse factors.

Thus, frequencies can provide, like fungicides and nutrients, several functions such as promoting the reduction of damage caused by pathogens and favoring the availability of nutrients to plants. They can be also employed in conjunction with traditional tools that aim for productive potential and thus achieve greater benefits, especially by applying frequency relative to the availability of nutrients and consequently greater productivity, as evidenced in this work.

Another great advantage of Effatha technology compared to conventional applications of products is the absence of the impact to the environment and the applicator (personal communication)[2], which already justify the importance of studies in agriculture.

This work on the applicability of frequency use is pioneering in agriculture, and, as soybean crops are of wide occupation in Brazilian states (CONAB, 2018), the benefits may extend to all locations, as well as to other crops of less expression. Studies should be enhanced to better understand the potential of the technique and the generation of distinct frequency sequences for the control of the pathogen complex, for the nutritional utilization of the plants and the water resistance capacity, among others, especially in the face of different producing regions and climatic adversities. Emission frequencies alone or combined with conventional treatment is a possibility in the soybean Asian rust control.

CONCLUSION

Conventional treatments with application of fungicides, in double (*in vivo*) or triple (field conditions) mixtures, provided the best control of Asiatic rust and weight of grains. However, the use of the sequence of nutritional and associated fungicide frequencies showed promise for use. Studies should be enhanced to better understand the potential use and how to apply the technique.

[2] Information (email) received by Dr. Silvânia H. Furlan from Luzo Dantas Junior and Rodrigo Lemos Lovato, Effatha's researchers, in March 2, 2021.

AUTHORS' CONTRIBUTIONS

Conceptualization: Bueno, C.J.; Furlan, S.H.; Dantas Junior, L.; Lovato, R.L. **Data curation:** Bueno, C.J.; Furlan, S.H.; Oliveira, G.F.F.M.; Leite, J.A.B.P. **Formal analysis:** Bueno, C.J.; Furlan, S.H.; Oliveira, G.F.F.M.; Leite, J.A.B.P. **Funding acquisition:** Bueno, C.J.; Furlan, S.H. **Investigation:** Bueno, C.J.; Furlan, S.H.; Oliveira, G.F.F.M.; Leite, J.A.B.P. **Methodology:** Bueno, C.J.; Furlan, S.H.; Oliveira, G.F.F.M.; Leite, J.A.B.P. **Project administration:** Bueno, C.J.; Furlan, S.H. **Resources:** Bueno, C.J.; Furlan, S.H. **Supervision:** Bueno, C.J.; Furlan, S.H. **Validation:** Bueno, C.J.; Furlan, S.H.; Oliveira, G.F.F.M.; Leite, J.A.B.P.; Dantas Junior, L.; Lovato, R.L. **Visualization:** Bueno, C.J.; Furlan, S.H.; Oliveira, G.F.F.M.; Leite, J.A.B.P. **Writing – original draft:** Bueno, C.J.; Furlan, S.H. **Writing – review & editing:** Bueno, C.J.; Furlan, S.H.; Dantas Junior, L.; Lovato, R.L.

AVAILABILITY OF DATA AND MATERIAL

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest.

ETHICAL APPROVAL

Not applicable.

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