Relationship between IC_{50} determined *in vitro/in vivo* and the fungicide rate used in the field

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RESUMO

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Analisou-se trabalhos publicados contendo valores da concentração de um fungicida que controla 50% (CI_{50}) um dado fungo. Na análise considerou-se: (i) a CI_{50} determinada *in vitro* e *in vivo* para um dado fungicida e para um fungo específico; (ii) a concentração (g/ha) de ingrediente ativo do fungicida indicada para o controle da doença alvo no campo; (iii) volume de água de 120/L usado na pulverização; (iv) a concentração (mg/L) de i.a. dos fungicidas nesse volume (120 L/ha); (v) e determinou-se a relação da concentração usada no campo com a determinada no laboratório. As análises foram feitas com dados da CI_{50} de fungicidas IDMs, IQes, um carbamato e um benzimidazol e com os seguintes fungos: *Bipolaris sorokiniana, Drechslera tritici-repentis, D. siccans, Fusarium graminearum* e *Puccinia triticina*, *Exserohilum turcicum, Phakopsora pachyrhizi* e *Corynespora cassiicola*. A concentração do fungicida na calda pulverizada no campo variou de 33,9 (*D. siccans* e trifloxistrobina) a 500.000,0 (*E. turcicum* e iprodiona) vezes superior a determinada em laboratório. Concluiu-se que a CI_{50} não teve relação com a concentração usada no campo e por isso deve ser usada na comparação da potência entre fungicidas e no monitoramento da sensibilidade de fungos.

Palavras-chave adicionais: Concentração inibitória, fungitoxicidade, sensibilidade fungicida.

ABSTRACT

Reis, E. M.; Zanatta, M.; Carmona, M.; Menten, J. O. M. **Relationship between IC_{50} determined *in vitro/in vivo* and the fungicide rate used in the field. *Summa Phytopathologica,*** v.41, n.1, p.49-53, 2015.

Published data containing fungicide concentrations that control 50% (IC_{50}) of a given fungus were analyzed. In the analysis we considered: (i) the IC_{50} determined *in vitro* and *in vivo* for a given fungicide and for a specific fungus; (ii) the concentration (g/ha) of active ingredient for the fungicide indicated to control a specific disease in the field; (iii) water volume of 120/L used in the spray; (iv) the fungicide a.i. concentration (mg/L) in 120 L volume; (v) and the ratio of the concentration used in the field with that determined in the laboratory. The analysis were performed by using IC_{50} data for DMIs, QoIs, a carbamate and a benzimidazol against the following fungi *Bipolaris sorokiniana, Drechslera tritici-repentis, D. siccans, Fusarium graminearum, Puccinia triticina, Exserohilum turcicum, Phakopsora pachyrhizi* and *Corynespora cassiicola*. The fungicide concentrations sprayed in the field were 33.9 (*D. siccans* and trifloxystrobin) to 500,000.0 (*E. turcicum* and iprodione) times higher than that determined in the laboratory. It was concluded that the IC_{50} was not related to the concentration used in the field and therefore should be used to compare the power among fungicides and to monitor the fungal sensitivity shift towards fungicides.

Additional keywords: Inhibitory concentration, fungitoxicity, fungicide sensitivity.

Fungicides are chemical agents that inhibit or eliminate the growth of fungi or fungal spores (8). The chemical, physical and biological characteristics of a fungicide determine its suitability to control a determined disease. Fungicides effectively control diseases caused by fungi and for some crops and diseases are the most efficient control measure (7, 8).

A large number of fungi have the ability to adapt and become less sensitive to the fungicides used to control them, especially if they are frequently applied over a long period of time. For this reason, it is very important to establish sensitivity monitoring programs for each fungicide/pathogen combination (6). This would allow observation and/or confirmation of possible and/or future sensitivity shift and help improve our understanding of epidemiological and biological aspects of the target fungi. The determination of the inhibitory concentration IC_{50} (the concentration that controls 50% of the pathogen population) is essential to develop sensitivity studies (10).

The IC_{50} is determined *in vitro* for a particular necrotrophic fungus and specific fungicide, estimating the values of mycelial growth and/or spore germination inhibition. Studies are conducted with the fungal development on leaf discs, detached leaves and seedlings to calculate the IC_{50} (1, 2, 3, 10, 11, 12).

According to Russell (10), the baseline is a profile of the sensitivity of the target fungus to the fungicide, constructed by using biological or molecular techniques to assess the response of previously unexposed fungal individuals or populations to the fungicide. The primary use of baseline is as a tool for the establishment and subsequent the monitoring of fungicide resistance management strategies.

The baseline (IC_{50}) for a fungicide should be determined before its application to control a target fungus in the field. This value expresses the highest sensitivity degree of a fungus to a fungicide prior to exposure and therefore in the absence of selection pressure towards strains adapted to the fungicide. After several years of use, a fungal sensitivity to a fungicide can be reduced. In this case, the shift can be measured by the increase in the actual IC_{50} compared to the IC_{50} reference value. It is also used to compare the power of fungicides in the control of a specific fungus by identifying the most efficient one (6). The lower the IC_{50}, the greater the toxicity of the chemical.

Different studies have been carried out on the *in vitro* and *in vivo* sensitivity of fungi to fungicides (1, 2, 3, 10, 11, 12). The generated data often raise doubts about their usefulness and the value of the relationship between the inhibitory concentration of a fungicide, obtained in the laboratory, and the recommended and used rate to control diseases in the field.

We hypothesized that there is a relationship between the IC_{50} and the fungicide rate used to control a disease in the field.

The aim of this study was to analyze and discuss the relationship between the IC_{50} of a fungicide for a given fungus, obtained in the laboratory, with the same fungicide rate used in the field.

MATERIAL AND METHODS

The IC_{50} values determined and published for various fungi and fungicides were used in our analysis (1, 2, 3, 10, 11, 12). A large number of them show the reduction/loss of a fungus sensitivity to fungicides after a long time of use. Field rate calculated by the IC was not found in the available literature. In our calculations, the volume of water sprayed per ha was fixed to 120 liters and the active ingredient (mg/L a.i.) concentration as function of the recommended rate was calculated. The a.i. concentration used in the field (recommended rate) divided by the IC_{50} determined in the laboratory was obtained to calculate the ratio between the recommended rate and the IC_{50} generated in the laboratory.

In the case of co-formulations of DMI + QoI, the sum of each fungicide concentration expressed as a.i. g/ha. was considered. For example, tebuconazole (200g) + trifloxystrobin (125g) ha a.i. was considered 325 a.i. g/ha.

The analyses were performed for DMIs, QoIs, a carbamate and a benzimidazole for the following fungi *Bipolaris sorokiniana* (Sacc.) Shoem., *Drechslera tritici-repentis* (Died.) Shoem., *D. siccans, Fusarium graminearum* Schwabe, and *Puccinia triticina* Eriks., pathogenic to wheat, *Exserohilum turcicum* Leonard & Suggs, causal agent of corn southern leaf blight, *Phakopsora pachyrhizi* Sydow, and *Corynespora cassiicola* (Berk. & Curtis) Wei, causal agents of Asian soybean rust and target spot.

RESULTS AND DISCUSSION

The *in vitro* IC_{50} of nine fungicides for the mycelium growth of *B. sorokiniana* was determined. The IC_{50} ranged from 0.1 (metconazole) to 5.3 a.i. g/L (prochloraz). The recommended concentration for the control of wheat leaf blotch in the field ranged from 90 (metconazole) to a.i. 450/ha (prochloraz), according to the fungicide. The fungicide concentration (mg/L) in 120 L/ha water ranged from 166.6 (tebuconazole) to 3,750.0 a.i. mg/L (metconazole). Therefore, the used field concentration was 373.1 (triadimenol) to 7,500.0 (metconazole) times greater than the IC_{50} (Table 1).

Regarding the *in vitro* IC_{50} of five fungicides for *D. tritici-repentis* mycelial growth inhibition, values ranged from 0.13 (azoxystrobin) to 36.6 a.i. mg/L (triadimenol). The recommended concentration for yellow spot control in wheat in the field ranged from 100 (cyproconazole and tebuconazole) to 250 a.i. g/ha (azoxystrobin). The concentration (mg/L) of fungicides in 120 L/ha of water ranged from 833.3 (cyproconazole and tebuconazole) to 1,250.0 a.i. mg/L (triadimenol). In field application, the concentrations were 34.0 (triadimenol) to 13,023.1 (azoxystrobin) times greater than the IC_{50} determined *in vitro* (Table 2).

Table 1. Relationship between the IC_{50} of fungicides and the recommended/used rate in the field to control *Bipolaris sorokiniana* in wheat

Fungicide	Trade name/ concentration/formulation	IC_{50} i. mg/L)	Recommended rate i. g/ha)	Field concentration i. mg/L water in 120 L/ha)	Ratio (Field concentration/IC_{50})
QoI					
Azoxystrobin	Priori 250 SC	1.09	250	2,083.0	1,911.0
DMI					
Prochloraz	Jade 450 CE	5.03	450	3,750.0	745.5
Cyproconazole	Alto 100 SC	0.75	100	833.33	1,111.0
Epoxiconazole	Opus 125 SC	1.05	125	1,041.6	992.0
Flutriafol	Impact 125 SC	0.22	125	1,041.6	4,734.8
Metconazole	Caramba 90 CS	0.10	90	750.0	7,500.0
Propiconazole	Tilt 250 CE	0.42	250	2,083.0	4,959.0
Tebuconazole	Folicur 200 CE	0.13	200	1,666.7	12,820.5
Triadimenol	Baytan 150 CE	3.35	150	1,250.0	373.13

Data from Stolte (11), Tonin (12).

Table 2. Relationship between the IC_{50} of fungicides and the recommended/used rate in the field to control *Drechslera tritici-repentis* in wheat

Fungicide	Trade name/ concentration/formulation	IC_{50} i. mg/)	Recommended rate i. g/ha)	Field concentration (a .i. mg/L water in 120 L/ha)	Ratio (Field concentration/IC_{50})
QoI					
Azoxystrobin	Priori 250 SC	0.13	250	2,083.0	16,025.6
DMI					
Cyproconazole	Alto 100 SC	0.97	100	833.3	859.0
Epoxiconazole	Opus 125 SC	0.33	125	1,041.6	3,156.3
Flutriafol	Impact 125 SC	4.80	125	1,041.6	217.0
Tebuconazole	Folicur 200 EC	0.32	100	833.3	2,604.0
Triadimenol	Baytan 125 SC	36.7	150	1,250.0	34.0

Data from Tonin (12).

Table 3. Relationship between the IC_{50} of fungicides and the recommended/used rate in the field to control *Drechslera siccans* in wheat

Fungicide	Trade name/ concentration/formulation	IC_{50} i.mg/L)	Recommended rate i. g/ha)	Field concentration i. mg/L water in 120 L/ha)	Ratio (Field concentration/IC_{50})
QoI					
Pyraclostrobin	Comet 250 EC	1.34	250	2,083.0	1,554.4
Trifloxystrobin	Twist 125 EC	30.72	125	1,041.6	33.9
DMI					
Cyproconazole	Alto 100 SC	0.37	100	833.3	2,252.1
Epoxiconazole	Opus 125 SC	0.33	125	1,041.6	3,156.3
Propiconazole	Tilt 250 EC	0.34	125	1,041.6	3,063.5
Tebuconazole	Folicur 200 EC	0.57	100	833.3	1,461.9
Prothioconazole	Proline	0.21	250	2,083.0	9,919.0

Data from Tonin (12).

Table 4. Relationship between the IC_{50} of fungicides and the recommended/used rate in the field to control *Fusarium graminearum* causing head blight of wheat

Fungicide	Trade name/concentration/ formulation	IC_{50} i. mg/L)	g/ha i.)	Concentration (a i. mg/L water in120L/ha)	Ratio (Field concentration/IC_{50})
Metconazole	Caramba 90 CS	0.07	90	750.0	10,714.3
Prothioconazole	Proline 200 SC	0.10	250	2,083.0	20,833.3
Tebuconazole	Folicur 200 EC	0.19	100	833.3	4,385.9
Metconazole + Trifloxystrobin	Caramba 90 + Twist CE/125	0.14	215	1,791.6	12,797.6
Prothioconazole + Trifloxystrobin	Proline 200 SC + Twist CE/125	0.08	375	3,125.0	39,062.5
Tebuconazole + Trifloxystrobin	Folicur 200 EC + Twist CE/125	0.12	225	1,875.0	15,625.0

Data from Avozan1 et al. (2, 3).

The *in vitro* IC_{50} of seven fungicides for the mycelial growth of *D. siccans* ranged from 0.21 (prothioconazole) to 30.72 a.i. mg/L (trifloxystrobin). The recommended concentration for wheat yellow spot control in the field ranged from 100 (cyproconazole and tebuconazole) to 250 (pyraclostrobin) a.i. g/ha. The fungicide concentration (mg/L) in 120 l/ha water ranged from 833.3 (cyproconazole and tebuconazole) to 2,083 (pyraclostrobin, prothioconazole). Therefore, in the field, the concentration was 33.9 (trifloxystrobin) to 9,919.02 (prothioconazole) times greater than the IC_{50} (Table 3).

The *in vitro* IC_{50} of six fungicides for *F. graminearum* mycelial growth ranged from 0.07 (metconazole) to 0.19 a.i. mg/L (tebuconazole). The recommended concentration for the control of head blight of wheat ranged from 90 (metconazole) to 375 g a.i./ha (prothioconazole + trifloxystrobin). The fungicide concentration (mg/L), in 120 L/ha water, ranged from 750.0 (metconazole) to 3,125.0 mg/L (prothioconazole + trifloxystrobin). Thus, the field concentrations were 4,385.9 (tebuconazole) to 39,062.5 (prothioconazole + trifloxystrobin) times greater than the IC_{50} determined in the laboratory (Table 4).

For *P. triticina,* the *in vitro* IC_{50} of seven fungicides for uredia density reduction ranged from 0.005 (pyraclostrobin) to 0.82 a.i. mg/L (tebuconazole). The recommended concentration for wheat leaf rust control in the field ranged from 90 (metconazole) to 250 a.i. g/ha

Table 5. Relationship between the IC_{50} of fungicides and the recommended/used rate in the field to control *Puccinia triticina* in wheat

Fungicide	Trade name/ concentration/formulation	IC_{50} i. mg/L)	Recommended rate i. g/ha)	Field concentration i. mg/L water in 120 L/ha)	Ratio (Field concentration/IC_{50})
QoI					
Azoxystrobin	Priori 250 SC	0.007	250	2,083.0	297,619.0
Pyraclostrobin	Comet 250 EC	0.005	250	2,083.0	416,666.6
Trifloxystrobin	Twist 125 EC	0.025	125	1,041.6	41,666.6
DMI					
Cyproconazole	Alto 100 SC	0.6	100	833.3	1,388.8
Epoxiconazole	Opus 125 SC	0.67	125	1,041.6	1,554.7
Metconazole	Caramba 90 CS	0.66	90	750.0	1,136.3
Tebuconazole	Folicur 200 EC	0.82	100	833.3	1,016.2

Data from Arduim el al, (1)

Table 6. Relationship between the IC_{50} of fungicides and the recommended/used rate in the field to control *Exserohilum turcicum* in corn seeds

Fungicide	Trade name/ concentration/formulation	IC_{50} i. mg/L)	Recommended rate i. mg/100kg seeds)	Field concentration (a. i. mg/100/Kg seeds)	Ratio (Field concentrations: IC_{50})n
Fludioxonil	Maxim 250 F	0.31	25	250.0	806.4
Thiram	Mayran 700 WP	1.37	700	5,833.3	5,1019.4
Iprodione	Rovral 500 SC	0.01	500	4,166.6	500,000.0

Datas from De Rossi et al. (7)

(azoxystrobin and pyraclostrobin). The fungicide concentration (mg/L), in 120 L/ha water, ranged from 750.0 (trifloxystrobin) to 2.083 a.i. mg/L (azoxystrobin and pyraclostrobin). The field concentrations were 1,016.2 (tebuconazole) to 416,666.6 (pyraclostrobin) times greater than the IC_{50} determined in the laboratory (Table 5).

Considering *E. turcicum,* the *in vitro* IC_{50} of three fungicides for the mycelial growth ranged from 0.01 (iprodione) to 1.37 a. i. mg/L (thiram). For *E. turcicum* control in seed treatment, the concentration is recommended to range from 25 (fludioxonil) to 700 mg a.i./100 kg seeds (thiram). The fungicide concentration, in 100 kg seed, ranged from 250.0 mg (fludioxonil) to 5,833.3 a.i mg/100 kg seed (thiram). Therefore, the concentration for seed treatment was 806.4 (fludioxonil) to 500,000 (iprodione) times greater than the IC_{50} determined in the laboratory (Table 5).

Regarding *P. pachyrhizi,* the *in vivo* IC_{50} of eleven fungicides for uredia density ranged from 0.11 (azoxystrobin and prothioconazole) to 5.61 a.i. mg/L (flutriafol). The recommended concentration for rust control in the field ranged from 90 (metconazole) to 250 g a.i./ha (azoxystrobin, picoxystrobin, pyraclostrobin, myclobutanil, prothioconazole). The fungicide concentration (mg/L), in 120 L water, ranged from 750.0 (metconazole) to 2,083.0 mg/L (azoxystrobin, picoxystrobin, pyraclostrobin, myclobutanil and prothioconazole). Therefore, the concentration for field spray was 659.2 (myclobutanil) to 18,939.3 (azoxystrobin) times greater than the IC_{50} determined *in vivo* in the laboratory (Table 7).

The *in vitro* IC_{50} of six fungicides for *C. cassiicola* mycelial growth ranged from 0.047 (prothioconazole) to 24.09 a.i. mg/L (carbendazim). The recommended concentration for *C. cassiicola* control in soybean farms ranged from 100 (cyproconazole and tebuconazole) to 500 g a.i./ha (carbendazim). The fungicide concentration, in 120 L water, ranged from 833.3 (cyproconazole and tebuconazole) to 4.167 a.i. mg/L (carbendazim). Therefore, the concentration for field spray was 56.5 (cyproconazole) to 44,362.2 (prothioconazole) times greater than the IC_{50} determined *in vitro* in the laboratory (Table 8).

Some factors should be considered to explain the higher concentration used in the field, compared to that determined in the laboratory. All analyzed fungicides are penetrant-mobile chemicals in the leaf tissues: (i) deficient field deposition of the fungicide can result in many leaves in the middle and in the lowest part of the canopy showing lower coverage quality, compared to those in the top; (ii) derived spray in application does not reach the target which are the leaves; (iii) once the fungicide has undergone uptake, it suffers dilution in leaf tissues, resulting in lower concentration, compared to the IC_{50} determined in the laboratory; (iv) a further fungicide amount, which remains unabsorbed on the leaf surface can suffer removal, photolysis, hydrolysis and sublimation; (v) the IC_{50} is determined for a particular fungus, while many others occur in the crop, requiring a concentration higher than the IC_{50} to be controlled; (vi) the host leaf tissues have detoxification activity to eliminate the xenobiotic, which does not occur in laboratory tests; (vii) the fungicide concentration in laboratory tests is stable throughout the assay and would decrease in the field; (viii) the metabolic activity of a plant in the field is higher than in *in vivo* tests conducted under optimal watering, temperature, relative humidity and photoperiod.

Therefore, it has been shown that the a.i. concentration (mg/L) in the water volume sprayed in the field is many times higher than that determined in the laboratory. Thus, we may infer that there is no relationship between the IC_{50} determined under controlled laboratory conditions and the concentration recommended for field applications. Thus, the IC_{50} has not been used for this purpose.

Table 7. Relationship between the IC_{50} of fungicides and the recommended/used rate in the field to control *Phakopsora pachyrhizi* in soybean

Fungicide	Trade name/ concentration/ formulation	IC_{50} i. mg/L)	Recommen ded rate (a. i. g/ha)	Field concentration i. mg/L water in 120 L/ha)	Ratio (Field concentration/IC_{50})
QoI					
Azoxystrobin	Priori 250 SC	0.11	250	2,083.0	18,939.3
Picoxystrobin	Acapela 250 F	0.13	250	2,083.0	16,025.6
Pyraclostrobin	Comet 250 EC	0.19	250	2,083.0	10,964.9
Trifloxystrobin	Twist 125 CE	0.12	125	1,041.6	8,680.5
DMI					
Cyproconazole	Alto 100 SC	0.89	100	833.3	936.3
Epoxiconazole	Opus 125 F	0.54	125	1,041.6	1,929.0
Flutriafol	Impact 125 F	5.61	125	1,041.6	157.5
Metconazole	Caramba 90 CS	0.44	90	750.0	1,704.5
Myclobutanil	Systhane 250 EC	3.16	250	2,083.0	659.2
Prothioconazole	Proline 200 F	0.11	250	2,083.0	1,893.9
Tebuconazole	Folicur 200 EC	0.33	100	833.3	2,525.2

Blum (4), Blum & Reis (5, 9).

Table 8. Relationship between the IC_{50} of fungicides and the recommended/used rate in the field to control *Corynespora cassiicola* in soybean

Fungicide	**Trade name/ Concentration/ Formulation**	**IC_{50} i. mg/L)**	**Recommended rate (a. i. g/ha)**	**Field concentration i. mg/L water in 120 L/ha)**	**Ratio (Field concentration/IC_{50})**
Carbendazim	Derosal 500 F	24.09	500	4.167	172.9
Cyproconazole	Alto 100 EC	14.73	100	833.3	56.5
Epoxiconazole	Opus 125 F	2.63	125	1.042	396.0
Flutriafol	Impact 125 F	1.49	125	1.042	699.1
Tebuconazole	Folicur 200 EC	2.35	100	833.3	354.5
*Prothioconazole	Proline 200 F	0.047	250	2,083.0	44,326.2

Data from Avozani et al. (2, 3), (*) Xavier et al. (13).

The procedure used to determine the fungicide rate in the field is performed through other known methodologies. The rate for the maximum economic efficiency of a fungicide should be determined in field experiments by testing an increasing concentration, followed by economic analysis. The maximum technical efficiency concentration is greater than the maximum economic one.

To compare the power of fungicides and to monitor the sensitivity shift of fungi to fungicides, the best tool is IC_{50} (10).

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