Journal of Agricultural Science; Vol. 13, No. 11; 2021
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Fungi Resistance to Multissite Fungicides

Erlei Melo Reis[1], Wanderlei Dias Guerra[2], Andrea Camargo Reis[3], Mateus Zanatta[3], Marcelo Carmona[1] & Francisco Sautura[1]

[1] Universidad de Buenos Aires, Facultad de Agronomía, Escuela para Graduados, Buenos Aires, Argentina

[2] Aprosoja MT, Associação de Produtores de Soja E Milho de Mato Grosso, Brazil

[3] Instituto Agris, Passo Fundo, RS, Brazil

Correspondence: Erlei Melo Reis, Facultad de Agronomía, Escuela para Graduados, Buenos Aires, Argentina. E-mail: erleireis@upf.br

Received: August 31, 2021 Accepted: September 27, 2021 Online Published: October 15, 2021

doi:10.5539/jas.v13n11p141 URL: https://doi.org/10.5539/jas.v13n11p141

Abstract

Multisite fungicides have been used for many years in fruit and vegetable crops worldwide. Cases of the fungi resistance development to these fungicides have been rare. From the 2002 season onwards, with the outbreak of Asian soybean rust in Brazil, caused by *Phakopsora pachyrhizi*, site-specific fungicides became the main weapon for its control. From 2002 to 2011, penetrant mobile site-specific fungicides were used and until today in double (DMI + QoI) or triple (DMI + QoI + SDHI) co-formulatoons in an area of more than 30 million hectares and with three sprays per area. This resulted, as expected, in the fungus sensitivity reduction, today with cross and multiple resistance to those site-specific fungicides. From the 2011 season in an attempt to recover control that for some chemicals and mixtures reached < 30%, research was started with site-specific + multi-site mixtures, taking as example *Phytophthora infestans* resistance development to metalaxyl in Europe showinig long-lasting solution found by the addition of multisite mancozeb. It is expected that the effective life of site-specific + multi-site mixtures may be as long in controlling soybean rust as it has been for potato, tomato and grape downy mildews. This review presents the concepts involved in the sensitivity reduction to fungicides. Some fungal species and fungicides involved are listed. Considering the *P. pachyrhizi* sporulation potential, the great soybean area sprayed and the number of sprays per area mainly with site-specific co-formulations and the reduced area sprayed with multisites, we discuss the need for annual monitoring of *P. pachyrhizi* sensitivity to the these chemicals.

Keywords: arylaminopyridine, chloronitriles, dithiocarbamates, phthalimides, reduced fungal sensitivity

1. Introduction

Between 1940 and 1970, fungicidal organic compounds with a broad spectrum of activity were developed by the plant protection chemical industry. Most of them were multisite inhibitors, *i.e.*, captan, chlorothalonil, folpet, mancozeb whose fungicidal action is attributed to their reaction with proteins containing sulphydryl groups (*i.e.*, glutathione, glyceraldehyde 3-phosphoate dehydrogenase or alcohol dehydrogenase) or proteins not containing thiols (*e.g.*, α-chymotrypsin or lysozyme) in the cell (Klitthich, 2008).

Mobile-penetrating fungicides necessarily have an intimate association with plant biochemistry and physiology and their modes of action are specific and usually involve only one biochemical mode of action (Hollomon, 2015). The market introduction of these fungicides over 50 years ago (*i.e.*, benomyl in the 1970s) revolutionized the chemical plant protection, showing high efficacy and low toxicology for the control of diseases caused by fungi. However, it was soon found that plant pathogenic fungi can easily and quickly adapt to these fungicides through mutations (among other resistance mechanisms) that lead to reduced efficacy (Klittich, 2008; Mosbach et al., 2017).

Reducing sensitivity to fungicides involves a fundamental property of fungi, the ability to adapt to different environmental conditions, usually adverse, and thus to survive. Frequent use of fungicides creates an adverse environment for a fungus that was previously sensitive to a particular compound, leading to its adaptation and resistance to the new situation (Bardas et al., 2008; Kretschmer, et al., 2009).

From the 1970s, the resistance of phytopathogenic fungi became a problem with the predominant use of mobile-penetrating fungicides that were site-specific (Klittich, 2008).

On the other hand, the resistance of fungi to multisite fungicides (arylaminopyridine, chloronitriles, dithiocarbamates, copper, tin and mercury derivatives, phthalimides, sulfur, etc.) is still a rare event. The difficulty is due to the low probability of the occurrence of the minimum necessary number of mutations at different loci in the same fungus. On the contrary, with the introduction and repeated use of site-specific, acquired resistance has become common but incomparable to multisite (van den Boch & Gilligan, 2008).

2. Basic Concepts

2.1 Fungicides

Fungicides are synthetic or natural chemical compounds, or biological organisms capable of killing or inhibiting fungi, or the germination of fungi and oomycete spores (Mueller et al., 2013).

2.2 Fungitoxicity

Fungitoxicity is the property that a chemical substance has of being toxic to fungi and stramenopylae (pseudo fungi or chromists) in low concentration. This property is a molecule attribute.

2.3 Mode of Action, Mechanism of Action or Biochemical Mechanism of Action

The chemical structure of the fungicde active ingredient (a.i.) defines its mode of action by determining its uptake, movement in the plant, and its ability to reach and bind to the site of action—the physical location where the fungicide acts (Delp & Dekker, 1985). Mode of action is the process by which a chemically active substance produces an effect on a living organism or on a biochemical system. Or, the mechanism refers to the biochemical interaction through which the substance produces its toxic effect (Hewtt, 1988; Latin, 2017; Mueller et al., 2013).

2.4 Site of Action

Site of action, or target site, are specific enzymes in cellular processes to which the fungicide binds (Hewitt, 1988).

2.5 Sensitivity (of Sensitive, That Feels)

Property of the fungus to receive changes from the environment and to react to them. Sensitivity is an attribute of the fungal species (Reis et al., 2019).

2.6 Insensitivity

Not all fungi are sensitive to all fungicides (spectrum of action); some are always insensitive to certain molecules. For example, fungi of the genera *Alternaria*, *Bipolaris*, *Curvularia*, *Drechslera*, *Exserohilum* are insensitive to benzimidazole fungicides; on the other hand, benzimidazoles are not fungitoxic to these genera. Another example is the insensitivity of oomycetes, which cause mildews, to triazoles and benzimidazoles (Reis et al., 2019).

A fungus sensitive to a fungicidal molecule may have altered sensitivity, which is why it is said to have developed resistance. However, an insensitive fungus will never become sensitive.

2.7 Control Failure

The resistance of plant pathogenic fungi to fungicides is observed as a control failure or as a reduction in the performance of the fungicide; in this situation, farmers often react by increasing the dose and/or by reducing the interval between sprayings. In the next step, field experiments confirm the control failure. Situation in which the farmer observes that, when compared to previous crops, the fungicide efficiency was reduced. He says that there was a “failure of control” and starts to complain and seeks explanations for the fact (Reis et al., 2019).

2.8 Loss of Sensitivity

The word loss implies total insensitivity, which is not always true. Nevertheless, the concept of loss can be delimited following the Edgingnton & Klew (Edgington & Kew, 1971) criterium. Thus, it can be considered as sensitivity loss, or non-toxic, when the fungus presents inhibitory concentration, IC_{50}, > 50 mg/L to a fungicida, and when lower than 50%, sensitivity reduction.

2.9 Sensitivity Reduction

Reduction is a slow process, requiring the application of a site-specific fungicide for many seasons and over a large area such as *P. pachyrhizi* and the DMIs (FRAC group 3, demethylase inhibitors), QoIs (Group 11, quinone

outside inhibitors) and SDHIs (Group 7, succinate dehydrogenase inhibitors) fungicides. The reduction is present when the inhibitory concentration (IC_{50}) increase over time for the mycelial growth, spore germination or disease control. Therefore, in most cases, what is happening is a slow reduction instead of sensitivy loss.

Molecular techniques are useful in proving the presence of reduced sensitivity after resistance has been quantified in laboratory bioassays (Hollomon, 2015).

2.10 Erosion of the Fungicide

Expression taken as a synonym for reduced sensitivity of a fungus to a given fungicide (Hahn, 2014).

2.11 Resistance

Fungicide resistance is the result of the adaptation of a fungus to a fungicide due to its stable hereditary genetic alteration leading to the emergence and spread of mutants with reduced sensitivity to the fungicide (Delp & Dekker, 1985). The term proposed by FRAC (2019) refers to a stable and hereditary adjustment of a fungus to a fungicide, resulting in a reduction in the pathogen sensitivity. This adjustment results in a 'considerable' reduction in the sensitivity of the pathogen to the chemical compound, which can be partial or total, always with an increase in the IC_{50} [sensitivity reduction factor (SRF)] > 1.0. This ability is gained through evolutionary processes (Mueller el al., 2013).

2.12 Cross-Resistance

Fungicides of the same chemical group, for example tebuconazole and cyproconazole, have different chemical structures. However, both have the same toxicity to fungi. Therefore, both are considered demethylation inhibitor fungicides (DMI), a name that expresses the same-shared mode of action. This fact means that even if you rotate two within the same fungicide group, the fungus detects them as being the same fungicide. It also means that if resistance develops for one member of the group, it will be present for all other members of that family. The resistance is called crossed reaching all group members (EPPO, 1998).

2.13 Multiple Resistance

When fungicides of different chemical groups, *i.e.*, carboxamides, strobilurins, triazoles) and with different mechanisms of action (DMI, QoI, SDHI) the reduction in sensitivity affects everyone equally (EPPO, 1988).

2.14 Multiple Drug Resistance (MDR)

It is the sensitivity reduction to various fungicides with different modes of action shown by a fungus specie. MDR is defined as the acquired sensitivity reduction of at least one fungus to at least three fungicides with a distinct mechanism of action. The main resistance mechanism involved here is the overexpression of the efflux transporter genes present in the plasma membrane. It results in increased cellular expulsion of the fungicide reducing the fungus sensitivity to several unrelated fungicides (Alekshun & Levy, 2007; Chapman et al., 2011; Chen et al., 2017; Hahn & Leroch, 2015; Leroux et al., 2002).

2.15 Acquired Resistance

It refers to a fungus that in the wild state was sensitive to the fungicide and that developed resistance after exposure to the chemical (Hollomon, 2015). This is what happens with fungicides applied to control diseases in the field.

2.16 Site-Specific, Monosite or Unisite Fungicide

Of the millions of biochemical reactions that take place in the fungus cell, the site-specific fungicide (monogenic resistance) interferes with only one biochemical site (an enzyme). This is a vital enzyme for the fngus physiology, so if it is blocked, the fungus will die. Fungicides with a site-specific mode of action are at high risk for the development of resistance compared to multiple-site fungicides (Mueller et al., 2013).

2.17 Multisite Fungicide

It refers to the fungicide that paralyzes at least five metabolic processes of the fungus (Mueller et al., 2013). For this reason, the development of resistance to them has not yet been frequently reported.

The main multisite fungicides in use in Brazil are: captan, chlorothalonil, cuprics (copper hydroxide, copper oxychloride, cuprous oxide, basic copper sulfate), dithianon, famoxadone, fluazinam, fludioxonil, mancozeb, pencicuron, pyrimethanil and thiram.

2.18 Site-Specific and Penetrant Mobile

Many use these two terms considering that all site-specific are penetrant mobile, however iprodione is a site-specific, signal transduction inhibitor, non-penetrating with protectant and some eradicant activity (PPDB, 2021).

2.19 Fungicide Effective Life

The effective life of a fungicide is the time from its introduction on the market for use in the control of a given fungus, until the moment when efficient control is no longer obtained due to the development of resistance of the target fungus (Hobbelen et al., 2011).

2.20 Mechanisms of Fungi Resistance to Fungicides

How do fungi defend theselves against fungicides? There are four main mechanisms by which fungi become resistant to fungicides.

For a better understanding of the mechanisms, the functions of cell organelles involved in the defense mechanisms of fungi are briefly reviewed.

2.21 Plasma Membrane

The cell, or cytoplasmic, membrane is a biological membrane that has selective permeability to organic molecules and ions. Controls the movement of substances into and out of the cell. This membrane is made up of a double layer of phospholipids and interspersed with proteins embedded in it. The membrane is said to be semi-permeable in that it can let a substance (molecule or ion) pass freely, pass through a limited form, or pass at all. Membranes also contain receptor proteins that allow cells to detect external signaling molecules such as hormones (Ishii & Hollomon, 2015).

The main mechanisms of fungi resistance to fungicides are:

(a) Substance transport across the plasma membrane: To reach the intracellular organelles, the fungicide has to cross the plasma membrane with a complex constitution.

There are three forms of transport across the cell membrane (Alekshun & Levy, 2007; Ishii & Hollomon, 2015; Ward et al., 2006). The movement of substances across the membrane can be passive by simple diffusion, or by diffusion facilitated by the transport proteins channel following a positive concentration gradient and active, with energy consumption (ATP) against a concentration gradient.

(b) Change in target site reducing sensitivity to fungicide: The most common mechanism of resistance is a change in target site (enzyme) in the fungus and occurs only with site-specific fungicides, which dominated the market after 1970. Multisite fungicides, most of those developed since 1969, are not prone to the development of resistance at the target or action site. As the fungus grows, its DNA is replicated when new cells are created. This replication process is imperfect and errors can occur. Such errors are known as mutations. DNA is the code used to produce enzymes in the cell, and some mutations result in a change in the target site's amino acid sequence which in turn alters the shape of the receptor site (lock) of the fungicide. Thus the (key) fungicide may not fit into the site (lock) resulting in a partial or total reduction of the fungus' sensitivity to the fungicide. Therefore, an alteration by mutation in the fungicide target of action reduces the drug fitness through this target site, resulting in reduced sensitivity (Ishii & Hollomon, 2015).

(c) Gene overexpression: Gene overexpression is the abnormal production of large amounts of a substance which is encoded by one or more genes. In the case of overexpression, the target enzyme does not undergo any change (mutation). Instead, the pathogen produces it in large quantities (Cools et al., 2012).

For example the overexpression of the Cyp51 gene. Azole fungicides (DMIs) inhibit the Cyp51 gene encoding the demethylase enzyme involved in the ergosterol biosynthesis process. The fungus to defend itself from the effect of the fungicide increases the production of the enzyme in order to produce much more enzyme (demethylase) so that ergosterol is still produced even in the presence of the fungicide. Due to the increased production of the enzyme, the amount of fungicide present in the cell is not enough to couple with all the available enzyme, completely blocking the production of ergosterol. This leaves an amount of free enzyme without the coupling of the fungicide, producing enough of the ergosterol to keep the cell alive. In this case, the amount of fungicide is not enough to completely inhibit ergosterol production. Gene overexpression results in greater production of demethylase beyond normal. Therefore, even though triazole inhibits part of its synthesis, there is still an amount of enzyme remaining, maintaining the cell's functional activity (Alekshun & Levy, 2007; Hahn & Leroch, 2015; Leroux et al., 2001; Price et al., 2015; Ward et al., 2006).

(d) Exclusion of the fungicide from the cell: The cell's efflux is the elimination of a certain substance from its interior to the outside. Active efflux is a condition where pathogen cells pump the fungicide out of the cell faster than it accumulates to a toxic concentration. However, the target site remains unchanged. Active efflux prevents the accumulation of sufficient concentration to stop cell function and fungus growth.

Unlike influx, entry to the interior of the cell, efflux pumps occur naturally in cells that exclude or expel foreign substances or import substances useful to their metabolism. In fungi, the most common efflux pumps are protein transport pumps. Occasionally, these transporters succeed in expelling sufficient amounts of the fungicide from within the cell. Transport or carrier proteins in the plasma membrane are responsible for the active efflux of foreign material, including fungicides (Price et al., 2015).

There are two types of efflux: (i) Passive efflux is the expulsion of a certain substance to the outside of a cell (movement, or passive diffusion). (ii) There is also the participation of an efflux pump, which consists of the active pumping of the fungicide from the intracellular to the extracellular environment, that is, the active efflux. Efflux pumps are transmembrane proteins that can act to expel fungicides against a concentration gradient. There may also be an oveexpression of efflux pumps consisting of an increase in the concentration of their number (Hahn & Leroch, 2015). Multiple drug resistance is related to the overexpression of transport proteins.

(e) Detoxification or molecule inactivation by thiols overproduction: Substances that inactivate molecules of fungicides, has been suggested as the most likely mechanism that confers fungal resistance to mancozeb (Barak & Edgingtom, 1984; Gilpatrick, 1982; Yang et al., 2019). However, the mechanisms that confer resistance to this fungicide are questionable and very complex to be clarified. The main genomic and molecular study was carried out with the yeast *Saccharomyces cerevisiae*, having determined 286 genes that would be involved with resistance to a xenobiotic (Dias et al., 2009).

Many papers have been published on the fungi resistance to multisite fungicides, some were selected as an example (Table 1).

Table 1. Reports of fungi resistance to multissite fungicides

Year	Plant specie	Fungus	Fungicide	Resistance mechanism	Reference
1965	-	*Aspergillus niger*	Mercury	-	Ashworth & Amin (1964)
1966	Oat	*Drechslera avenae*	Mercury	Inactivation by a group of thiols	Nobel et al. (1966); Ross & Old (1973)
1968	Oat	*D. avenae*	Mercury	Inactivation by a group of thiols	Malone (1966)
1971	Oat	*D. avenae*	Phenylmercury acetate	Mercury chelation by the production of red pigments	Greenaway (1971)
1973	Apple	*Venturia inaequalis*	Dodine	Unknown	Szkolnik & Gilpaterick (1973)
1976	Apple	*V. inaequais*	Dodine	Unknown	Jones & Walker (1976)
1976	Apple	*V. inaequalis*	Dodine	Unknown	Yoder & Kloss (1976)
1977	Apple	*V. inaequalis*	Dodine	Unknown	Ross & Newberry (61)
1978	Beet	*Cercospora beticola*	Triphenyl tin acetate and triphenyl tin hydroxide	Unknown	Giannopolitis (1977)
1980	Pimentão Pepper	*Colletotrichum capsici*	Mancozeb	Inactivation of the fungicide molecule by overproduction of thiols	Thind & Jhooty (1980)
1980	-	*Rhizopus stolonifer*	Copper	Physiological adaptation to Cu	García-Toledo et al. (1980)
1980	Beet	*Cercospora beticola*	triphenyltin	-	Giannopolis (1978)
1984	-	*Botrytis cinerea*	Chlorothalonil, captafol, folpet, thiram	Inactivation of the fungicide molecule by overproduction of thiols	Barak & Edgington (1984)
1982	Apple	*V. inaequalis*	Dodine	Unknown	Gilpatrick (1982)
1989		*Botrytis cinerea*	Dichlofluanid	Unknown	Malathakis (2006)
1989	Apple	*V. inaequalis*	Dodine	Unknown	Sholberg et al. (1089)
1989	-	*Exserohilum rostrata*	Mancozeb	Inactivation of the fungicide molecule by overproduction of thiols	Reddy & Anilkumar (1989)
1993	Pesseguiro	*Taphrina deformans*	Copper hydroxide	Unknown	Cheah et al. (1993)
1994	-	*Trichoderma viridae*	Cupric	Metallothione in chelation mechanism	Cervantes & Gutierez-Corona (1994)
1995	-	*Phytophthora infestans*	Chlorothalonil	Inactivation of the fungicide molecule by overproduction of thiols	Sujkowsky et al. (1995)

1998	Potato	*P. infestans*	Chlorohtalonil	Inactivation of the fungicide molecule by overproduction of thiols	Cooke et al. (1998)
1998	Beet	*Cercospora beticola*	Triphenyl tin hydroxide	Unknown	Campbell, et al. (1998)
1999	Apple	*V. inaequalis*	Dodine	Unknown	Köller et al. (1999)
2002	-	*Botryotinia fuckeliana* (*Botrytis cinerea*)	Fludioxonil	-	Vignutelli et al. (2006)
2003	-	*B. cinerea*	Fenilpirrole	-	Baraffio et al. (2003)
2003	Potato	*Alternaria solani*	Chlorothalonil	Inactivation of the fungicide molecule by overproduction of thiols	Holm et al. (2003)
2005		*A. brassicae*	Fenilpirrole	Unknown	Avenot et al. (2005
2007	Mango	*C. gloeosporiodes*	Mancozeb	Inactivation of the fungicide molecule by overproduction of thiols	Kumar et al. (2007)
2006	Greenhouse vegtables	*Botrytis cynerea*	Dichlofluanid	-	Malathrakis (2006)
2008	-	*Penicillium digitatum*	Fludioxonil	Unknown	Kanetis et al. (2008)
2008	Rubber tree	*C. gloeosporiodes*	Mancozeb	Inactivation of the fungicide molecule by overproduction of thiols	Cai et al. (2008)
2008	Rice	*Helminthosporium oryzae*	Maneb	Inactivation of the fungicide molecule by overproduction of thiols	Gupta & Kaiser (2008)
2008	Apple	*C. gloeosporiodes*	Mancozeb	Inactivation of the fungicide molecule by overproduction of thiols	Tam et al. (2008)
2009	Banana	*Mycosphaerella fijensis*	Mancozeb	Inactivation of the fungicide molecule by overproduction of thiols	Aguilar-Barragan et al. (2014)
2009	-	*C. gloeosporiodes*	Mancozeb	Inactivation of the fungicide molecule by overproduction of thiols	Ferreira et al. (2009)
2010	Apple	*V. inaequalis*	Dodine	Unknown	Carisse & Jobin (2010)
2010	-	*C. acutatum*	Mancozeb	Inactivation of the fungicide molecule by overproduction of thiols	Gullino et al. (2010)
2010	Beet	*C. beticola*	Triphenyl tin hydroxide	Unknown	Secor et al. (2010)
2011	Wheat	*Septoria tritici* (= *Zymoseptoria tritici*)	Chlorothalonil & folpet	Overexpression of thiols that inactivate the fungicide	Beyer et al. (2011)
2011	-	*B. cinerea*	Fludioxonil	Unknown	Webber (2011)
2013	-	*B. cinerea*	Fludioxonil	Unknown	Leroux & Walker (46)
2013	Strawberry	*B. cinerea*	Fludioxonil	Unknown	Fernandez-ortunõ et al. (2013)
2013	-	*B. cinerea*	Fludioxonil	Unknown	Leroch et al. (45)
2013	Potato	*A. solani*	Chlorothalonil	Inactivation of the fungicide molecule by overexpression of thiols	Fairchild et al. (2013)
2014	-	*B. cinerea*	Fludioxonil	Unknown	Hahn (2014)
2015	-	*A. alternata*	Mancozeb	Inactivation of the fungicide molecule by overexpression of thiols	Malandrakis et al. (2015)
2016	-	*B. cinerea*	Fludioxonil	Unknown	Ren et al. (2016)
2017	Apple	*Colletotrichum* spp.	Mancozeb	Inactivation of the fungicide molecule by overexpression of thiols	Moreira et al. (2019)
2018	Potato	*P. infestans*	Fluazinam	Inactivation by conjugation of fluaziam with glutathione	Schepers et al. (2018)
2019	Apple	*C. acutatum*	Mancozeb	Inactivation of the fungicide molecule by overexpression of thiols	Moreira et al. (2019)
2019	-	*A. alternata*	Mancozeb	Inactivation of the fungicide molecule by overexpression of thiols	Yang et al. (2019)

3. Final Remmarks

Although, fungal resistance to iprodione, a nonpenetrant fungicide, has been reported, it was not included in this review due to its site-specific mode of action.

The number of site-specific fungicide molecules marketed is considerably higher than multisite. From the 1970s onwards, site-specifics dominate the world market, being used to control diseases in a greater number of plant species, in a larger area and with a greater number of sprayings per season, in addition to their high risk to resistance development. This has resulted in the largest number of citations of site-specific resistance.

It is likely that for all commercialized, both multisite and site-specific fungicides, regardless of their active principle and resistance mechanism, at least one fungus resistant to them has already been reported. However, as site-specifics dominate the market, the large volume published focuses on this group.

Based on the consulted literature, even new site-specific mechanisms of action developed in the future will have the potential to select, in a few seasons, fungi resistant to them, shortening their effective life.

Although, Bordeaux mixture is considered the oldest foliage protectant fungicide, developed in 1885, no resistance of *Phytophthora infestans* (Mont.) de Bary to this fungicide was found in the consulted literature. Perhaps it is the fungicide with the longest effective life in the history of downy mildew chemical control on potatoes, tomatoes and grape. Are the cuprics the hardest to be defeated by fungi? In this sense, in the consulted literature only two reports of reduced sensitivity of fungi to cupric fungicides were found, but to a large number of species of phytopathogenic bacteria (Lamichane et al., 2018).

In the available literature, no reports were found on rust fungi resistant to mutissites.

It would also be important to determine the time required since it first use to the emergence of resistance or the duration of their effective life. According to the FRAC (2019), fungal resistance to pencycuron (phenylurea; recommended for the control of *Rhizoctonia solani* in the treatment of potato tubers) and to tricyclazole (triazolobenzothiazole) penetrant-mobile for the control of *Pyricularia oryzae* Cav in rice has not yet been reported.

Should the chemical industry continue to synthesize site-specific fungicides, as it has been doing intensively, even with a relatively short effective life as is happening with the new carboxamides towards *Phakopsora pachyrhizi* Sydow & Sydow.

In Brazil, the greatest use of multi-site fungicides (chlorothalonil, mancozeb and copper oxychloride) has been in soybean crop, to control *P. pachyrhizi*, the causal agent of Asian rust. Its use began in 2010/11, therefore beeing used in the last 10 seasons. To give an idea of the selection pressure that multi-sites are subject in soybean crop, in the 2020/21 season, the area cultivated with soybeans was > 38 million hectares, with 2.6 sprayings/ha, but with multi-site in an area of only 12%. What can happen with these multi-sites in the control of soybean rust under this situation? Should multissítes be used alone for ASR control?

At the moment, in Brazil, the use of multisite is the main weapon to face the development of *P. pachyrhizi* resistance to mobile penetrant site-specicif fungicides.

Multiple resistance is present in *P. pachyrhizi* to DMIs, QoIs and SDHIs and even so, these fungicides are applied in the largest area of soybean, without the multisite mixture, and thus, their efficacy has been reduced season after season. If the efficacy, which is already low, but has been reduced season after season, reaches < 30%, could the addition of multi-site revert the situation? Would multi-sites be used solo because they have superior control than site-specific?

Considering the chemical control of ASR in Brazil, with the well-defined presence of cross and multiple resistance to site-specific, reflected in a constant sensitivity reduction evolution of *P. pachyrhizi*, season after season, we will reach a situation in which the most efficient control would be achieved with multisites solo? Therefore, would multisites withstand the enormous selection pressure for resistance?

Let us remmember the development of *P. infestans* resistance to metalaxyl (in the 1977) and the solution given by the ready-made commercial mixture with mancozeb (in the 1980) would not be an indication that this would be the practice to be pursued in Brazil for economically sustainable control and fungicide with long effective life in controlling soybean rust? What has been the effective life of the metalaxyl + mancozeb mixture in controlling mildews and whether cases of mildew resistance to this mixture or similar ones has been reported? In the same direction, and similarly to ensure a long effective life in the control of *P. pachyrhizi,* the use of ready-made, liquid commercial mixture, containing IDM (prothioconazole and/or tebuconazole) and IQe (picoxystrobin and/or trifloxystrobin) + multisite (chlorothalonil), or mancozeb, or copper oxychloride) would be a solution?

The exposure time of *P. pachyrhizi* to multi-site fungicide is still too short to make a judgment about their effective life.

References

Aguilar-Barragan, A., García-Torres, A. E., Odriozola-Casas, O., Macedo-Raygoza, G., Ogura, T., Manzo-Sánchez, G., Beltrán-García, M. J. (2014). Chemical management in fungicide sensitivity of *Mycosphaerella fijiensis* collected from banana fields in México. *Brazilian Journal of Microbiology, 45*(1), 359-364. https://doi.org/10.1590/S1517-83822014000100051

Alekshun, M. N., & Levy, S. B. (2007). Molecular mechanisms of antibacterial multidrug resistance. *Cell, 128*, 1037-1050. https://doi.org/10.1016/j.cell.2007.03.004

Ashworth, L. J. Jr., & Amin, J. V. (1964). A mechanism for mercury tolerance in fungi. *Phytopathology, 54*, 1459-1463.

Avenot, H., Simoneau, P., Iacomi-Vasilescu, B., & Bataille-Simoneau, N. (2005). Characterization of mutations in the two-component histidine kinase gene AbNIK1 from Alternaria brassicicola that confer high dicarboximide and phenylpyrrole resistance. *Current Genetics, 47*, 234-243. https://doi.org/10.1007/s00294-005-0568-2

Barak, E., & Edgington, L. V. (1984). Cross-resistance of *Botrytis cinerea* to captan, thiram, chlorothalonil, and related fungicides. *Canadian Journal of Plant Pathololgy, 6*, 318-320. https://doi.org/10.1080/07060668409501536

Bardas, G. A., Myresiotis, C. K., & Karaoglanidis, G. S. (2008). Stability and fitness of anilinopyrimidine-resistant strains of Botrytis cinerea. *Phytopathology, 98*, 443-450. https://doi.org/10.1094/PHYTO-98-4-0443

Baroffio, C. A., Siegfried, W., & Hilber, U. W. (2003). Long-Term monitoring for resistance of *Botryotinia fuckeliana* to anilinopyrimidine, phenylpyrrole, and hydroxyanilide fungicides in Switzerland. *Plant Disease, 87*(6), 662-666. https://doi.org/10.1094/PDIS.2003.87.6.662

Beyer, M., Kiesner, F., Verreet, J. A., & Klink, H. (2011). Fungicide sensitivity of *Septoria tritici* field isolates is affected by an interaction between fungicidal mode of action and time. *Journal of Plant Pathology, 93*(1), 7-13.

Cai, Z., Li, J., Wang, J., Zange, C., He, M., & Hed, L. (2008). Sensitivity test of *Colletotrichum gloeosporioides* and *Colletotrichum acutatum* isolate from rubber to fungidides. *Yunnan Nong Ye Da Xue Bao, 23*, 787-790.

Campbell, L. G., Smith, G. A., Lamey, H. A., & Cattanach, A. W. (1998). *Cercospora beticola* tolerant to triphenyltin hydroxide and resistant to thiophanate methyl in North Dakota and Minnesota. *Journal of Sugar Beet Research, 35*(1/2), 29-41. https://doi.org/10.5274/jsbr.35.1.29

Carisse, O., & Jobin, T. (2010). Resistance to dodine in populations of *Venturia inaequalis* in Quebec, Canada. Online. *Plant Health Progress, 11*(1). https://doi.org/10.1094/PHP-2010-0614-01-RS

Cervantes, C., & Gutierrez-Corona, F. (1994). Copper resistance mechanisms in bacteria and fungi. *FEMS Microbiological Review, 14*(2), 121-37. https://doi.org/10.1111/j.1574-6976.1994.tb00083.x

Chapman, K. S., Sundin, G. W., & Beckerman, J. L. (2011). Identification of resistance to multiple fungicides in field populations of *Venturia inaequalis*. *Plant Disease, 95*, 921-926. https://doi.org/10.1094/PDIS-12-10-0899

Cheah, L. H., Tate, K. G., Hunt, A. W., & Silva de, N. (1993). Decreased sensitivity of *Taphrina deformans* (peach leaf curl) to copper fungicides in Hawkes Bay. *Proceedings of the Forty Sixth New Zealand Plant Protection Conference, Hotel Russley, Christchurch*. New Zealand Plant Protection Society, Rotorua, New Zealand. https://doi.org/10.30843/nzpp.1993.46.11190

Chen, L.-H., Tsai, H.-C., Yu, P.-L., & Chung, K.-R. (2017). A major facilitator superfamily transporter mediated resistance to oxidative stress and fungicides requires Yap1, Skn7, and MAP kinases in the citrus fungal pathogen *Alternaria alternata*. *PLoS ONE, 12*, e0169103. https://doi.org/10.1371/journal.pone.0169103

Cooke, L. R., Little, G., & Wilson, D. G. (1998). Sensitivity of Phytophthora infestans to fluazinam and its use in potato blight control in Northern Ireland. *Proceedings Brighton Crop Protection Conference, Pests and Diseases* (pp. 517-522).

Cools, H. J., Bayon, C., Atkins, S., Lucas, J. A., & Fraaije, B. A. (2012). Overexpression of the sterol 14α-demethylase gene (MgCYP51) in *Mycosphaerella graminicola* isolates confers a novel azole fungicide sensitivity phenotype. *Pest Management Science, 68*(7), 1034-1040. https://doi.org/10.1002/ps.3263

Delp, C. J., & Dekker, J. (1985). Fungicide resistance: Definitions and use of terms. *Bulletin OEPP/EPPO Bulletin, 15*, 333-335. https://doi.org/10.1111/j.1365-2338.1985.tb00237.x

Dias, P. J., Teixeira, M. C., Telo, J. P., & Sá-Correia, I. (2009). Insights into the mechanisms of toxicity and tolerance to the agricultural fungicide mancozeb in yeast, as suggested by a chemogenomic approach. *OMICS: A Journal of Integrative Biology, 14*(2), 211-27. https://doi.org/10.1089/omi.2009.0134

Edgington, L. V., & Klew, K. L. (1971). Fungitoxic spectrum of benzimidazole compounds. *Phytopathology, 61*, 42-44. https://doi.org/10.1094/Phyto-61-42

EMPPO (European and Mediterranean Plant Protection Organization). (1988). Fungicide resistance: Definitions and use of terms. *Bulletin OEP/EPPO Bulletin, 8*(4), 569-571. https://doi.org/10.1111/j.1365-2338.1988.tb00090.x

Fairchild, K. L., Miles, T. D., & Wharton, P. S. (2013). Assessing fungicide resistance in populations of Alternaria in Idaho potato fields. *Crop Protection, 49*, 31-39. https://doi.org/10.1016/j.cropro.2013.03.003

Fernandez-Ortuño, D., Chen, F. P., & Schnabel, G. (2013). Resistance to cyprodinil and lack of fludioxonil resistance in *Botrytis cinerea* isolates from strawberry in North and South Carolina. *Plant Disease, 97*, 81-85. https://doi.org/10.1094/PDIS-06-12-0539-RE

Ferreira, J. B., Abreu, M. S., Pereira, I. S., Fernandes, K. D., & Pereira, R. B. (2009). Sensibilidade de *Colletotrichum gloeosporioides* (Mancha manteigosa do cafeeiro) a diferentes concentrações de fungicidas. *Ciência Agrotécnica, 33*, 2052-258. https://doi.org/10.1590/S1413-70542009000700059

FRAC (Fungicide Resistance Action Committee). (2019). *Definition of fungicide resistance*. Retrieved December 12, 2019, from https://www.frac.info/resistance-overview

Garcia-Toledo, A., Babich, H., & Stotzky, G. (1980). Training of *Rhizopus stolonifer* and *Cunninghamella blakesleeana* to copper: cotolerance to cadmium, cobalt, nickel, and lead. *Canadian Journal of Microbiology, 31*(5), 485-492. https://doi.org/10.1139/m85-090

Giannopolitis, C. N. (1978). Occurrence of strains of *Cercospora beticola* resistant to triphenyltin fungicides in Greece. *Plant Disease Reporter, 62*, 205-208.

Gilpatrick, J. D. (1982). Case study 2: Venturia of pome fruits and Monilinia of stone fruits. In J. Dekker, & S. G. Georgopoulos (Eds.), *Fungicide Resistance in Crop Protection* (pp. 195-206). Pudoc, Wageningen.

Greenaway, W. (1971). Relationship between mercury resistance and pigment production in *Pyrenophora avenae*. *Transactions of the British Mycological Society, 56*, 37-44. https://doi.org/10.1016/S0007-1536(71)80105-5

Gullino, M. L., Tinivella, F., Garibaldi, A., Kemmitt, G. M., Bacci, L., & Sheppard, B. (2010). Mancozeb, past, present and future. *Plant Disease, 94*(9), 1076-1087. https://doi.org/10.1094/PDIS-94-9-1076

Gupta, P. K., & Kaiser, A. K. M. S. (2008). Adaptation of *Helminthosporium oryzae* to Maneb. *Journal of Phytopathology, 72*(4), 315-320. https://doi.org/10.1111/j.1439-0434.1971.tb03203.x

Hahn, M. (2014). The rising threat of fungicide resistance in plant pathogenic fungi: Botrytis as a case study. *Journal of Chem. Biol., 7*(4), 133-141. https://doi.org/10.1007/s12154-014-0113-1

Hahn, M., & Leroch, M. (2015). Multidrug efflux transporter. In H. Ishii, & W. Hollomon (Eds.), *Fungicide resistance in plant pathology* (Chapter 15, pp. 233-248). Japan: National Institute for Agricultural Environmental Sciences. https://doi.org/10.1007/978-4-431-55642-8_15

Hewitt, H. G. (1988). *Fungicides in Crop Protection* (p. 221). Wallingford, UK: CAB International.

Hobbelen, P. H. F., Fraainje, B., Lucas, J. A., Pavely, N. D., & van den Bosch, F. (2011). Derivation in and testing for a model to predict selectin for fungicide resistance. *Plant Pathology, 60*, 304-313. https://doi.org/10.1111/j.1365-3059.2010.02380.x

Hollomon, D. W. (2015). Fungicide resistance: 40 years and sitill a major problem. In H. Ishii, & W. Hollomon (Eds.), *Fungicide resistance in plant pathology* (Chapter 1, pp. 3-11). Japan: National Institute for Agricultural Environmental Sciences. https://doi.org/10.1007/978-4-431-55642-8_1

Holm, A. L., Rivera, V. V., Secor, G. A., & Gudmestad, N. C. (2003). Temporal sensitivity of *Alternaria solani* to foliar fungicides. *American Journal Potato Research, 80*, 33. https://doi.org/10.1007/BF02854554

Ishii, H., & Hollomon, W. (2015). *Fungicide resistance in plant pathology* (p. 490). Japan: National Institute for Agricultural Environmental Sciences. https://doi.org/10.1007/978-4-431-55642-8

Jones, A., & Walker, R. J. (1976). Tolerance of *Venturia inaequalis* to dodine and benzimidazole fungicides in Michigan. *Plant Disease, 60*, 40-44.

Kanetis, L., Forster, H., Jones, C. A., Borkovich, K. A., & Adaskaveg, J. E. (2008). Characterization of genetic and biochemical mechanisms of fludioxonil and pyrimethanil resistance in field isolates of Penicillium digitatum. *Phytopathology, 98*, 205-214. https://doi.org/10.1094/PHYTO-98-2-020

Klittich, C. J. (2008). Milestones in fungicide discovery: Chemistry that changed agriculture. *Plant Health Progress, 9*(1). https://doi.org/10.1094/PHP-2008-0418-01-RV

Köller, W., Wilcox, W. F., & Jones, A. L. (1999). Quantification, persistence, and status of dodine resistance in New York and Michigan orchard populations of *Venturia inaequalis*. *Plant Disease, 83*, 66-70. https://doi.org/10.1094/PDIS.1999.83.1.66

Kretschmer, M., Leroch, M., Mosbach, A., Walker, A.-S., Fillinger, S., Mernke, D., … Hahn, M. (2009). Fungicide-driven evolution and molecular basis of multidrug resistance in field populations of the grey mould fungus *Botrytis cinerea*. *PLoS Pathog., 5*, e1000696. https://doi.org/10.1371/journal.ppat.1000696

Kumar, A. S., Reddy, N. P. E., Reddy, K., & Devi, M. C. (2007). Evaluation of fungicidal resistance among *Colletotrichum gloeosporioides* isolates causing mango anthracnose in Agri Export Zone of Andhra Pradesh, India. *Plant Pathology Bulletin, 16*, 157-160.

Lamichhane, J. R., Osdaghi, E., Behlau, F., Köhl, J., & Jones, J. B. (2018). Thirteen decades of antimicrobial copper compounds applied in agriculture: A review. *Agronomy for Sustainable Development, 38*(3), 28. https://doi.org/10.1007/s13593-018-0503-9

Latin, R. (2017). Chapter 2: Modes of action of fungicides. *A Practical Guide to Turfgrass Fungicides* (pp. 27-47). APS Publications, MN, USA. https://doi.org/10.1094/9780890544976.002

Leroch, M., Plesken, C., Weber, R. W., Kauff, F., Scalliet, G., & Hahn, M. (2013). Gray mold populations in german strawberry fields are resistant to multiple fungicides and dominated by a novel clade closely related to *Botrytis cinerea*. *Applied Environmental Microbiology, 79*, 159-167. https://doi.org/10.1128/AEM.02655-12

Leroux, P., & Walker, A. S. (2013). Activity of fungicides and modulators of membrane drug transporters in field strains of *Botrytis cinerea* displaying multidrug resistance. *European Journal of Plant Pathology, 135*, 683-93. https://doi.org/10.1007/s10658-012-0105-3

Leroux, P., Fritz, R., Albertini, C., Lanen, C., Bach, J., Gredt, M., & Chapeland, F. (2002). Mechanisms of resistance to fungicides in field strains of *Botrytis cinerea*. *Pest Management Sciences, 58*, 876-888. https://doi.org/10.1002/ps.566

Malandrakis, A. A., Apostolidou, Z. A., Markoglou, A., & Flouri, F. (2015). Fitness and cross-resistance of *Alternaria alternata* field isolates with specific or multiple resistance to single site inhibitors and mancozeb. *European Journal of Plant Pathology, 42*, 489-499. https://doi.org/10.1007/s10658-015-0628-5

Malathrakis, N. E. (2006). Resistance of *Botrytis cinerea* to dichlofluanid in greenhouse vegetables. *Plant Disease, 73*, 138-141. https://doi.org/10.1094/PD-73-0138

Malone, P. (1968). Mercury resistant *Pyrenophora avenae* in North Ireland seed oats. *Plant Pathology, 17*, 41-45. https://doi.org/10.1111/j.1365-3059.1968.tb00414.x

Moreira, R. R., Hamada, N. A., Peres, N. A., & De May de Mio, L. L. (2019). Sensitivity of the *Colletotrichum acutatum* species complex from apple trees in Brazil to dithiocarbamates, methyl benzimidazole carbamates, and quinone outsite inhibitor fungicides. *Plant Disease, 103*(10), 2569-2576. https://doi.org/10.1094/PDIS-07-18-1144-RE

Mosbach, A., Edel, D., Farmer, A. D., Widdison, S., Barchietto, T., Dietrich, R. A., … Scalliet, G. (2017). Anilinopyrimidine resistance in *Botrytis cinerea* is linked to mitochondrial function. *Front Microbiol., 8*, 2361. https://doi.org/10.3389/fmicb.2017.02361

Mueller, D. S., Wise, K. A., Dufault, N. S., Bradley, C. A., & Chilvers, M. I. (2013). *Fungicides for field Crops*. American Phytopathological Society, St. Paul.

Noble, M., McGarvie, Q. D., Hams, A. F., & Leafe, E. L. (1996). Resistance to mercury of *Pyrenophora avenae* in Scottish seed oats. *Plant Pathology, 15*, 23-28. https://doi.org/10.1111/j.1365-3059.1966.tb00316.x

PPDB (Pesticide Properties Data Base). (2021). Retrieved July 2, 2021, from https://scholar.google.com.br/scholar?q=PPDB.+Pesticide+Properties+Database.+Agriculture+%26+Environment+Research+Unit,+University+of+Hertfordshire.&hl=pt-BR&as_sdt=0&as_vis=1&oi=scholart

Price, C. L., Parker, J. E., Warrilow, A. G., Kelly, D. E., & Kelly, S. L. (2015). Azole fungicides-understanding resistance mechanisms in agricultural fungal pathogens. *Pest Management Sciences, 71*, 1054-1058. https://doi.org/10.1002/ps.4029

Reddy, K. P., & Anilkumar, T. B. (1989). Resistance development to mancozeb in *Helminthosporium halodes*. *Mysore Journal of Agricultural Sciences, 23*, 184-188.

Reis, E. M., Reis, A. C., & Carmona, M. A. (2019). *Manual de fungicidas: Guia para o controle químico de doenças de plantas* (8th ed., p. 259). Passo Fundo: Editora Berthier.

Ren, W., Shao, W., Han, X., Zhou, M., & Chen, C. (2016). Molecular and biochemical characterization of laboratory and field mutants of Botrytis cinerea resistant to fludioxonil. *Plant Disease, 100*, 1414-1423. https://doi.org/10.1094/PDIS-11-15-1290-RE

Ross, I. S., & Old, K. M. (1973). Thiol compounds and resistance of *Pyrenophora avenae* to mercury. *Trans British Mycological Society, 60*, 301-310. https://doi.org/10.1016/S0007-1536(73)80013-0

Ross, R. G., & Newbery, R. J. (1977). Tolerance of *Venturia inaequalis* to dodine in Nova Scotia. *Canadian Plant Disease Survey, 57*, 57-60.

Schepers, H. T. A. M., Kessel, G. J. T., Lucca, F., Förch, M. G., van den Bosch, G. B. M., Topper, C. G., & Evenhuis, A. (2018). Reduced efficacy of fluazinam against Phytophthora infestans in the Netherlands. European Journal of Plant Pathology, 151, 947. https://doi.org/10.1007/s10658-018-1430-y

Secor, G. A., Rivera, V. V., Khan, M. F. R., & Gudmestad, N. C. (2010). Monitoring fungicide sensitivity of *Cercospora beticola* of sugar beet for disease management decisions. *Plant Disease, 94*(11), 1272-1281. https://doi.org/10.1094/PDIS-07-09-0471

Sholberg, P. L., Yorston, J. M., & Warnock, D. (1989). Resistance of *Venturia inaequalis* to benomyl and dodine in British Columbia, Canada. *Plant Disease Reporter, 73*, 667-669. https://doi.org/10.1094/PD-73-0667

Sujkowski, L. S., Fry, B. A., Power, R. J., Goodwin, S. B., Peever, T. L., Hamlen, R. A., & Fry, W. E. (1995). Sensitivities of Mexican isolates of *Phytophthora infestans* to chlorothalonil, cymoxanil, and metalaxyl. *Plant Disease, 79*, 1117-1120. https://doi.org/10.1094/PD-79-1117

Szkolnik, M., & Gilpaterick, J. D. (1973). Tolerance of *Venturia inaequalis* to dodine in relation to the history of dodine usage in apple orchards. *Planta Disease Reporter, 57*, 817-821.

Thind, T. S., & Jhooty, J. S. (1980). Adaptability of *Colletotrichum capsici* to Dithane M-45 and Blitox. *Indian Phytopathology, 33*, 570-573.

van den Bosch, F., & Gilligan, C. A. (2008). Models of fungicide resistance dynamics. *Annual Review of Phytopathology, 46*, 123-47. https://doi.org/10.1146/annurev.phyto.011108.135838

Vignutelli, A., Hilber-Bodmer, M., & Hilber, U. W. (2006). Genetic analysis of resistance to the phenylpyrrole fludioxonil and the dicarboximide vinclozolin in *Botryotinia fuckeliana* (*Botrytis cinerea*). *Mycol. Res., 106*, 329-335. https://doi.org/10.1017/S0953756202005683

Ward, M. A., Andrade, A. C., Hayashi, K., Schoonbeek, R. H., Stergiopoulos, I., & Zwiers, L.-H. (2006). Impact of fungal drug transporters on fungicide sensitivity, multidrug resistance and virulence. *Pest Management Science, 62*, 195-207. https://doi.org/10.1002/ps.1150

Weber, R. W. S. (2011). Resistance of *Botrytis cinerea* to multiple fungicides in Northern German small-fruit production. *Plant Disease, 95*, 1263-1269. https://doi.org/10.1094/PDIS-03-11-0209

Yang, L., He, M., Ouyang, H., Zhu, W., Pan, Z.-C., Sui, Q.-J., Shang, L.-S., & Zan, J. (2019). Cross-resistance of the pathogenic fungus *Alternaria alternata* to fungicides with different modes of action. *BMC Microbiology, 19*, 205. https://doi.org/10.1186/s12866-019-1574-8

Yoder, K. S., & Klos, E. J. (1976). Tolerance to dodine in *Venturia inaequalis*. *Phytopathology, 66*, 918-923. https://doi.org/10.1094/Phyto-66-918