Resistance management recommendations and proposals for Fungicides not included in current working groups as requested by manufacturers

Compounds	Dodine
GROUP NAME	Guanidines
FRAC MoA Code	U12
TARGET SITE AND CODE	Unknown - cell membrane disruption (proposed)
Uses	Used mainly to control diseases in perennial crops including scab on pome fruit and pecan, scab, shot hole and leaf curl on peach, leaf spot on cherry and olive and black sigatoka in banana
Resistance Status	• The first failures of apple scab control with guanidine were detected in orchards of New York State, where guanidine had been applied as the exclusive scab fungicide for more than 10 years (Köller and Wilcox, 1998). • In subsequent years, resistance of apple scab to the guanidine class also became apparent in other states of North-East USA and also in Canada (McKay and McNeill, 1979; Ross and Newberry, 1977; Sholberg et al., 1989). • Until today, resistance to guanidine has occurred in the apple scab fungus *Venturia inaequalis*, but not in other fungi. Besides North-Eastern USA and Canada, resistance screening programs carried out in Poland (Nowacka, 1991; Meszka and Bielenin, 2001; Meszka et al., 2008) and New Zealand (Beresford et al., 2013) identified some level of resistance in apple orchards in various apple regions of the country.
Resistance Mechanism	• The exact mechanism of resistance to the guanidine class is not yet understood. • See paragraphs on next pages for further details
Recommendations	**Recommendations for *Venturia inaequalis*:** • Not more than two applications early in the season (first two applications in a fungicide program) • Use preferably in a preventative mode • Avoid rescue applications to burn out established scab lesions • In North-Eastern USA and Canada where historically, resistance occurred, it is recommended to tank-mix guanidine with a contact product (both products at full dose rates).
Requested by / date	Agreed with UPL December 2020
FRAC SC approval / date	February 12th 2021

Relationship between guanidine (U12) and bis-guanidines (M07):

Introduction

Bis-guanidines and guanidine comprise a small chemical family of three fungicides: guazatine, iminoctadine and dodine. Guazatine (based on iminoctadine) and iminoctadine are "bis-guanidines", while dodine is chemically a single chained molecule, therefore called "guanidine". This document will only talk about guanidine. However, as it is known that bis-guanidines and guanidine are cross-resistant, reference to the bis-guanidines will be made where relevant.

The guanidine class was first reported as a fungicide in 1957 (Tomlin, 1997). The only member of this group dodine is a foliar fungicide used mainly to control diseases in perennial crops including scab on pome fruit and pecan, scab, shot hole and leaf curl on peach, leaf spot on cherry and olive and black sigatoka in banana (Tomlin, 1997).

Currently, the guanidine class is commercially available in a wide range of countries around the globe.

Resistance status

The guanidine class was the first on the market that provided post-infection activity on fungi. It was also the first fungicide class for which field failures due to resistance occurred after 10 to 12 years of heavy use for control of apple scab. The first failures of apple scab control with guanidine were detected in orchards of New York State, where guanidine had been applied as the exclusive scab fungicide for more than 10 years (Köller and Wilcox, 1998). In subsequent years, resistance of apple scab to the guanidine class also became apparent in other states of North-East USA and also in Canada (McKay and McNeill, 1979; Ross and Newberry, 1977; Sholberg et al., 1989). In the 1970s, guanadine was largely replaced by new classes of scab-controlling fungicides (Köller and Wilcox, 1998).

Until today, resistance to guanidine has occurred in the apple scab fungus *Venturia inaequalis,* but not in other fungi. Besides North-Eastern USA and Canada, resistance screening programs carried out in Poland (Nowacka, 1991; Meszka and Bielenin, 2001; Meszka et al., 2008) and New Zealand (Beresford et al., 2013) identified some level of resistance in apple orchards in various apple regions of the country. In New Zealand, guanidine sensitivity has not increased since the 1990s and may have actually decreased (Beresford et al., 2013). Field failures due to *Venturia inaequalis* resistance to guanidine have not been described in these countries.

In North-Eastern USA, annual resistance monitoring programs for apple scab by Cornell University since 2004 (Cox et al., 2010; Cox, 2011; Cox et al., 2012), show that there has been a sharp decline of resistance to guanidine. Also in Canada, a resistance monitoring project was performed by the Ontario Ministry of Agriculture in which the guanidine class was included (Craig, 2013). Results were very comparable to those from the USA.

In Poland, the guanidine class is very often used to burn out established scab lesions (Meszka et al., 2008). Indeed, it seems to be these panic or rescue applications in apple orchards that cause development of apple scab resistance to the guanidine class. In order to safeguard this

class of fungicides, it is very important to avoid this type of eradicant use. According to Beresford et al. (2013), the relatively conservative resistance management guideline in New Zealand of three applications per season is helping to reduce guanidine resistance development. Guidelines for appropriate use of guanidine will be outlined at the end of this page.

Mode of action

Shortly after discovery of the guanidine class in 1957, a lot of research studies on various fungi were performed to elucidate the mode of action of this new fungicide class (Brown and Sisler, 1960; Somers and Pring, 1966; Bartz and Mitchell, 1969). In many of these studies, it was observed that high fungicide concentrations led to damage of cellular membranes, loss of cellular content and consequently acute death. At lower concentrations, germination was still inhibited but no loss of cellular content could be observed. Cabral (Cabral, 1991; Cabral and Smith, 1991; Cabral, 1993) confirmed these observations in his research on *Pseudomonas syringae* cells. Based on these studies and more recent, unpublished work by Wong (2009) and Biotransfer (2011), it is hypothesized that the guanidine class plays a role in the disruption of fungal cell membranes.

The guanidine class was traditionally classified in the FRAC Code list as M7 (multi-site contact activity). As resistance to guanidine was reported in *Venturia inaequalis*, it was suggested that this class might not be multi-site inhibitors. In 2009, guanidine was reclassified under the FRAC code U12 (Unknown mode of action, cell membrane disruption as proposed mode of action). The bis-guanidines guazatine and iminoctadine remained classified as M7.

Today, it remains unknown how guanidine affects exactly the functions of the fungal cell membrane. Köller (1992) hypothesized that guanidine might inhibit the oxidosqualene cyclase (OSC) enzyme (ERG7; full name is 2,3-oxidosqualene-lanosterol cyclase) which catalyzes a specific step in the ergosterol biosynthesis. This step is obviously a different site of action than the other fungicide classes which also inhibit the ergosterol synthesis (mode of action G in table of FRAC codes) as no cross resistance with these other classes exists. Some preliminary evidence for this hypothesis was given by Henry and Sisler (1979) who described that upon treatment with dodecylimidazole (which also contains a dodecyl residue), high levels of lanosterol and 2,3-oxidosqualene, the substrate of ERG7, were detected in *Ustilago maydis* in comparison with the untreated control.

Further research work is ongoing in order to verify this hypothesis and to better understand the mode of action of the guanidine class.

Mechanism of resistance

Resistance to guanidine is typically of a quantitative nature. In those areas where it developed in the past, it developed slowly with gradual declines in sensitivity of the apple scab pathogen as demonstrated by monitoring tests (Köller and Wilcox, 1998; Cox, 2011).

The exact mechanism of resistance to the guanidine class is not yet understood. Genetic analysis of guanidine resistance in lab mutants of *Fusarium solani* revealed four unlinked loci and additional modifiers linked to guanidine resistance (Kappas and Georgopoulos, 1970). Other studies show that the acquired tolerance of *Venturia inaequalis* to guanidine is genetically controlled and that at least two major independent genes are involved (Polach,

1973; Yoder and Klos, 1976). There is also an indication of the existence of minor genes that affect the level of tolerance to guanidine (Yoder and Klos, 1976).

Based on the hypothesis by Köller (1992) – see previous paragraph – potential candidate genes could be involved in ergosterol biosynthesis and in particular, the gene coding for the cyclase enzyme could be an interesting candidate gene. Further research is ongoing to verify this hypothesis.

More information on the mode of action of guanidine and possible resistance mechanisms could help to improve recommendation guidelines for this class of fungicides.

Cross resistance with other fungicides and multiple resistance

Positive cross resistance of the guanidine class (FRAC group U12) exists with the bis-guanidines (FRAC group M7). However, bis-guanidines are commercially available in a few countries only today. Therefore, this cross resistance is not relevant for practical applications in most countries.

Furthermore, there is no positive cross resistance with commercial fungicides from other chemical classes. The unusual phenomenon of negative cross resistance has not been reported for guanidine.

Persistence of resistant isolates

The only case of widespread, practical resistance to guanidine was for apple scab in North-Eastern USA and Canada where field failures due to resistance to guanidine occurred after 10 to 12 years of heavy (mis)use of this fungicide class. By the mid-1970s, guanidine use had strongly diminished in these regions (Cox, 2011). Twenty years later, by the 1990s, the stability of guanidine resistance was re-assessed by investigating several orchards in New York and Michigan State. It was found that although the frequencies of resistant isolates had declined, they had not returned to baseline sensitivity levels. More recent annual evaluations of guanidine resistance in New York State started in 2004 and have shown that the resistance level to guanidine has declined sharply (Cox, 2011; Cox et al., 2012).

This case shows that development of practical field resistance to guanidine is not easily achievable, but once established, it seems that resistance to guanidine can be quite persistent over time. However, it has to be noted that although guanidine use diminished strongly by the mid-1970s, it was still used on a regular basis in so-called rescue applications (eradicant applications) to eradicate visible apple scab lesions. This continued, wrong use might have helped to maintain resistance levels of apple scab to the guanidine class. The sharp decline in resistance observed in this apple growing region from 2007 on coincided with the implementation of specific recommendation guidelines for guanidine use in apple (see following paragraph). However, there is no direct proof of an actual causal relationship between these two events.

Use recommendations

The only pathogen with (historical) resistance issues to guanidine in some areas is *Venturia inaequalis* (apple scab). Therefore, specific recommendation guidelines are needed for this use:

- Not more than two applications early in the season (first two applications in a fungicide program)

- Use preferably in a preventative mode
- Avoid rescue applications to burn out established scab lesions
- In North-Eastern USA and Canada where historically, resistance occurred, it is recommended to tank-mix guanidine with a contact product (both products at full dose rates).

A standard fungicide program including the guanidine class following these recommendations, has been applied for several consecutive years on an apple orchard with historical guanidine resistance at Cornell University (New York State, USA). After more than 5 consecutive years of guanidine use following these recommendations, annual sensitivity monitoring has shown that there is no shift of the apple scab population towards resistance (Cox, 2011; Cox et al., 2012).

Since 2008, the guanidine class is also used in banana to control *Mycosphaerella fijiensis*. As this is a high risk pathogen for resistance development, specific recommendation guidelines for use of guanidine in banana can be found on the banana working group webpage of FRAC.

In all other crops on which the guanidine class is used, no resistance problems have occurred up to now. Nevertheless, it is recommended to limit the number of applications per year to maximum four applications (6 in banana) and not more than 33% of the total number of applications for the target disease. It is not necessary to tank-mix with a contact product if the above recommendations are respected.

The above recommendations must be integrated in an overall disease management program combining appropriate methods of cultural, biological as well as chemical disease control. Implementation of the above strategies must take into account the particular characteristics of the crop, pest and geographic area in which the guanidine product is to be applied.

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