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T.C. : REINHARDSBRUNN SYMPOSIUM COLLECTION

Sensitivity of 40 *Pythium* species to the new fungicide Picarbutrazox

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Abstract

Picarbutrazox is the active ingredient of Vayantis®, a novel seed treatment fungicide developed by Syngenta and discovered by Nippon Soda. Vayantis® received registration from the US EPA in 2021. Picarbutrazox is a systemic fungicide and belongs to the tetrazolyloximes chemical group of fungicides, and its mode of action is still unknown (FRAC group U17). Picarbutrazox targets oomycete plant pathogens and controls diseases caused by *Pythium*, *Phytophthora*, and downy mildews. Picarbutrazox is in a new mode-of-action group with no known cross-resistance to other oomycete compounds. The sensitivity to Picarbutrazox of 528 isolates belonging to 40 different *Pythium* species was determined in vitro. *Pythium* isolates were collected from infected plants including corn, soybean, cotton, and other crops from 17 different states. *Pythium* isolates were also obtained from collections kept at universities. Isolates were identified at the species level using morphological and molecular tools. Mycelial growth inhibition was quantified using the following Picarbutrazox concentrations: 0, 0.00001, 0.0001, 0.001, 0.01, and 0.1 mg/L. All the species of *Pythium* tested were sensitive to Picarbutrazox. The sensitivity distribution (EC50 values) across all the *Pythium* species tested had a mean sensitivity of 0.000376 mg ai/L. The Picarbutrazox sensitivity of 40 species of *Pythium* species has been documented, and results demonstrate the high in vitro intrinsic activity of this fungicide to control *Pythium* species. The high potency of Picarbutrazox to control *Pythium* diseases in corn, soybeans, cotton, and other crops has also been demonstrated under field conditions when used as a seed treatment. Picarbutrazox, with its novel mode of action, will contribute to the management of potential fungicide resistance issues developed by other fungicide products controlling *Pythium*, including mefenoxam and ethaboxam.

Keywords Picarbutrazox · Ethaboxam · Mefenoxam · Pythium species · Vayantis · FRAC group U17 · Seed treatment · Corn · Soybean · Cotton · Wheat

Introduction

Pythium species are among the most common and important plant pathogens causing diseases in different crops including damping-off, seedling blight, seed rot, and root rot diseases. *Pythium* diseases are limiting factors that reduce plant stand and yield. The increase in *Pythium* diseases is due to shifts toward early planting dates (cooler conditions) and reduced tillage systems, poorly drained soils, longer emergence time, and longer exposure to soil-borne pathogens. *Pythium* diseases in corn are responsible for about 100 million bushels in losses annually (Bickel and Koehler 2021).

Picarbutrazox is the active ingredient of Vayantis®, a novel seed treatment fungicide developed by Syngenta and discovered by Nippon Soda. Vayantis® received registration from the US EPA in 2021. Picarbutrazox is a systemic fungicide and belongs to the tetrazolyloximes chemical group of fungicides, and its mode of action is still unknown (FRAC group U17). Picarbutrazox targets oomycete plant pathogens and controls diseases caused by *Pythium*, *Phytophthora*, and downy mildews. Picarbutrazox is in a new mode-of-action group with no known cross-resistance to other oomycete compounds (e.g., Phenylamides, Carboxylic Acid Amides, Quinone Outside Inhibitors, OSBPIs, or Thiazole Carboxamide) (Brandl et al. 2018; Olaya et al. 2018).

Field and glasshouse studies have demonstrated a strong and reliable activity of picarbutrazox against pathogenic

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Pythium species causing diseases in several crops including corn, soybeans, canola, cereals, sugarbeets sorghum, and vegetables at very low use rates (Fig. 1). No crop tolerance issues have been reported, and its compatibility with other seed treatment products is good.

After treatment with picarbutrazox mycelia of *Pythium aphanidermatum* and *Phytophthora capsici* have abnormal swelling and excessive branching compared to the untreated mycelia (Nippon Soda, *unpublished data*). Picarbutrazox is very effective in key life cycles of *Pythium* and *Phytophthora*. The activity of picarbutrazox is very high against the mycelia of *Pythium* and *Phytophthora* (Table 1). Picarbutrazox is ineffective on the zoospore motility of *P. aphanidermatum* and on the zoospore motility and encystment of *Phytophthora sojae* (Nippon Soda, *unpublished data*; Blum, M., Syngenta, *unpublished data*).

Picarbutrazox has low water solubility and a high log P value of 4.16, allowing rapid movement into the lipid membranes (Table 1). The solubility value of picarbutrazox in water is 0.333 (mg/L), and the soil adsorption coefficient value is 3471 Koc g/Kg. In sandy soils, picarbutrazox distributes laterally, and in loam soils, it percolates downward from the application site. The percent of organic matter in the soil appeared to have less effect on the distribution of picarbutrazox (Syngenta, *unpublished data*). The movement of picarbutrazox in plants when applied as a seed treatment has been studied in seeds of soybeans and corn. In soybeans, picarbutrazox rapidly accumulates in the treated seed and upper root system and remains in the cotyledons with little upward movement. In corn, picarbutrazox concentrates in the treated corn seed, root system, and stem, with limited movement to the leaves. Due to the high potency of picarbutrazox, the small amount taken up by the roots still

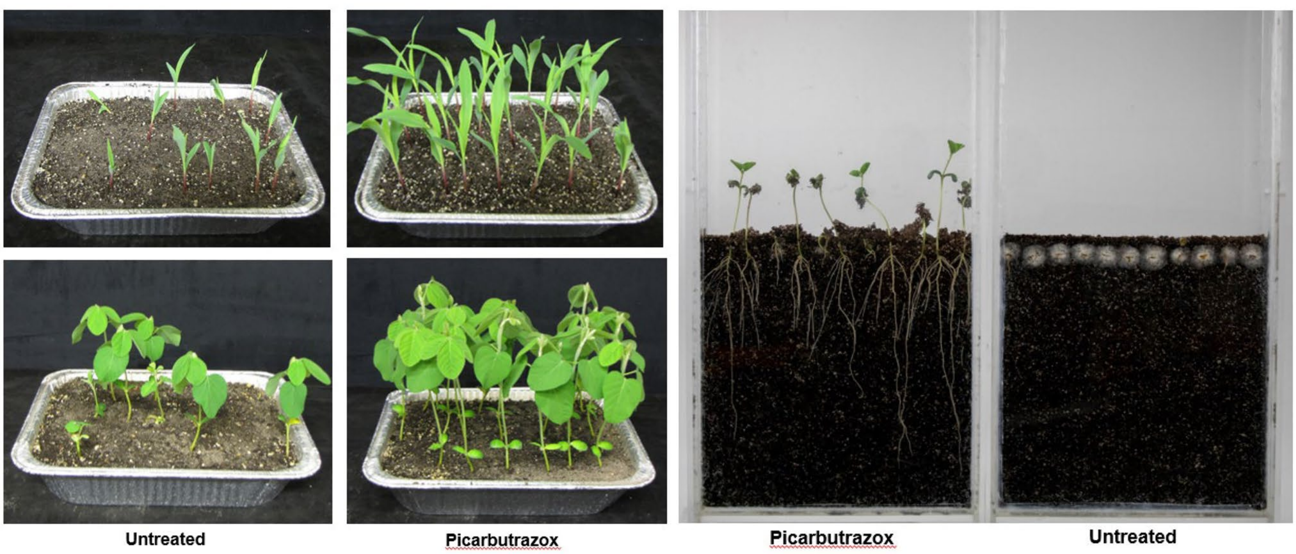

Fig. 1 Picarbutrazox seed treatment at a rate of 0.003 mg.ai/seed for the control of *Pythium ultimum* on corn (upper left) and *P. Sylvaticum* on soybeans (lower left). Rhizotron test of picarbutrazox against *P. ultimum* (right)

Table 1 Picarbutrazox effects on life cycles of *P. aphanidematum* and *P. sojae*

Life cycle stage	*Pythium aphanidermatum* EC_{50} values (mg ai/L)	*Phytophthora sojae* EC_{50} values (mg ai/L)
Mycelial growth	0.0009	0.0008
Zoosporangium formation	–	–
Zoospore formation	< 0.0001	–
Zoospore motility	No effect (> 10)	No effect (> 10)
Zoospore encystment	0.013	No effect (> 10)
Cytospore germination	0.1	0.004
Germtube growth	–	0.002
Oogonium formation	0.014	–
Oospore formation	–	–
Oospore germination	0.01	–

provides excellent protection against *Pythium* spp. (Syngenta, *unpublished data*).

The main objective of this study was to determine the sensitivity of 528 *Pythium* isolates belonging to 40 *Pythium* species to picarbutrazox under in vitro conditions and to determine the sensitivity of the same isolates to mefenoxam and ethaboxam. Intrinsically, picarbutrazox has a strong activity against *Pythium* species, and its activity needed to be confirmed across a good number of *Pythium* species. *Pythium* causes complex diseases that are difficult to control, with a high diversity of species, with specific or multiple crop targets, and diseases caused by single or multiple *Pythium* species.

Materials and methods

Isolates collection

Pythium isolates were recovered from soils and symptomatic plants including corn, soybean, wheat, and other crops from 17 different states (Fig. 2). Most of the *Pythium* isolates were obtained from plant samples collected from untreated plots from field trials where picarbutrazox was under evaluation. Other isolates were obtained from Syngenta in-house collections and from collections at Iowa State University and Ohio State University (Fig. 1). Isolates were identified at the species level using morphological and molecular tools (Broders et al. 2007; Rojas et al. 2017; Olaya et al. 2018). All the *Pythium* isolates were kept in sterile water and stored at room temperature (about 20 °C).

Sensitivity test

The sensitivity to picarbutrazox of 528 isolates belonging to 40 species of *Pythium* was determined in vitro. The sensitivity of each *Pythium* isolate was determined by comparing the colony radial growth on ½ strength Potato Dextrose Agar media plates amended or not with picarbutrazox. The in vitro mycelial growth inhibition was quantified using the following Picarbutrazox concentrations: 0, 0.00001, 0.0001, 0.001, 0.01, and 0.1 mg ai/L. The plates were incubated at room temperature (about 22 °C), and the diameter of *Pythium* colonies was measured before the colony growth reached the border of the plates. The sensitivity tests were set in a completely randomized design with two replications for each concentration of picarbutrazox. Data obtained for each isolate were transformed using an arcsine transformation. Effective concentrations at 50% growth reduction (EC50 values) were calculated by regressing the transformed radial growth data against the log of the fungicide concentration. The sensitivity of the *Pythium* isolates was also determined in vitro for mefenoxam (0, 0.01, 0.1, 1, 10, and 100 mg ai/L) and ethaboxam (0, 0.03, 0.1, 0.3, 1, and 10 mg ai/L).

Results

Pythium isolates were retrieved from samples collected from 18 different crops and from 17 states across the USA (Fig. 2). The majority of the isolates were collected from soybeans (262 isolates) and corn (162 isolates). The geographical origin of the isolates was from the states of Iowa (281 isolates), Ohio (129 isolates), Washington (41 isolates), and Indiana (23 isolates). *Pythium* isolates were identified to belong to 40 species. *Pythium sylvaticum* was the most common species isolated (118 isolates), followed by *P. ultimum* (81 isolates), *Pythium oopapillum* (53 isolates), *P. torulosum* (37 isolates), and *P. irregulare* (32 isolates). The frequency of the isolates belonging to the rest of the *Pythium* species was lower (Fig. 3).

All the 40 *Pythium* species tested were sensitive to picarbutrazox. The sensitivity distribution (EC50 values) across all the 528 *Pythium* isolates tested had a mean sensitivity of

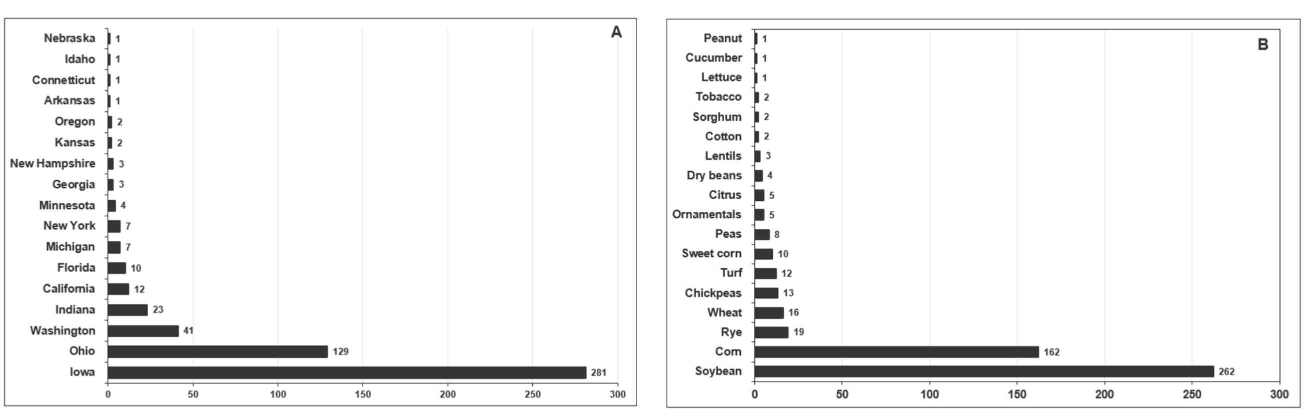

Fig. 2 Origin of the *Pythium* isolates collected in the USA: **a** geographical; **b** crop host

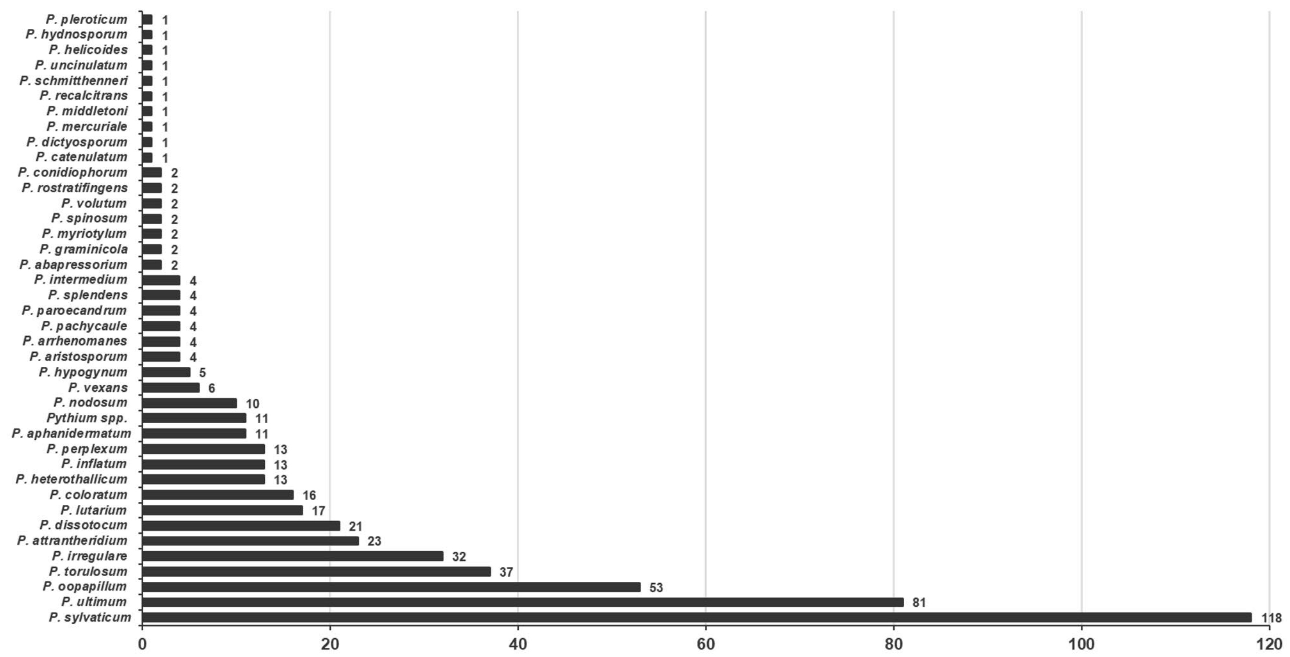

Fig. 3 *Pythium* species identified from a collection of 528 isolates retrieved from different crops and geographical regions in the USA

0.000376 mg ai/L. The Picarbutrazox sensitivity of more than 40 species of *Pythium* has been documented, and the results demonstrated the high in vitro intrinsic activity of the fungicide picarbutrazox (Fig. 4).

The most sensitive *Pythium* species to picarbutrazox based on the mean sensitivity were *P. arrhenomanes*, *P. mercuriale*, *P. helicoides*, *P. inflatum*, and *P. aphanidermatum*. The mean sensitivity values of *P. myriotylum*, *P. dissotocum, P. volutum, and P. lutarium* were about 10–20-fold higher compared to the values of one of the most sensitive *Pythium* species (Fig. 5).

Among the 15 most common *Pythium* species affecting corn and soybeans, *P. inflatum* was the most sensitive species to picarbutrazox. The sensitivities to picarbutrazox of *P. dissotocum* and *P. lutarium* were higher compared to the sensitivities of *P. inflatum* about four to sixfold. The response variability to picarbutrazox of the isolates in each species was in general low. The highest variability in the

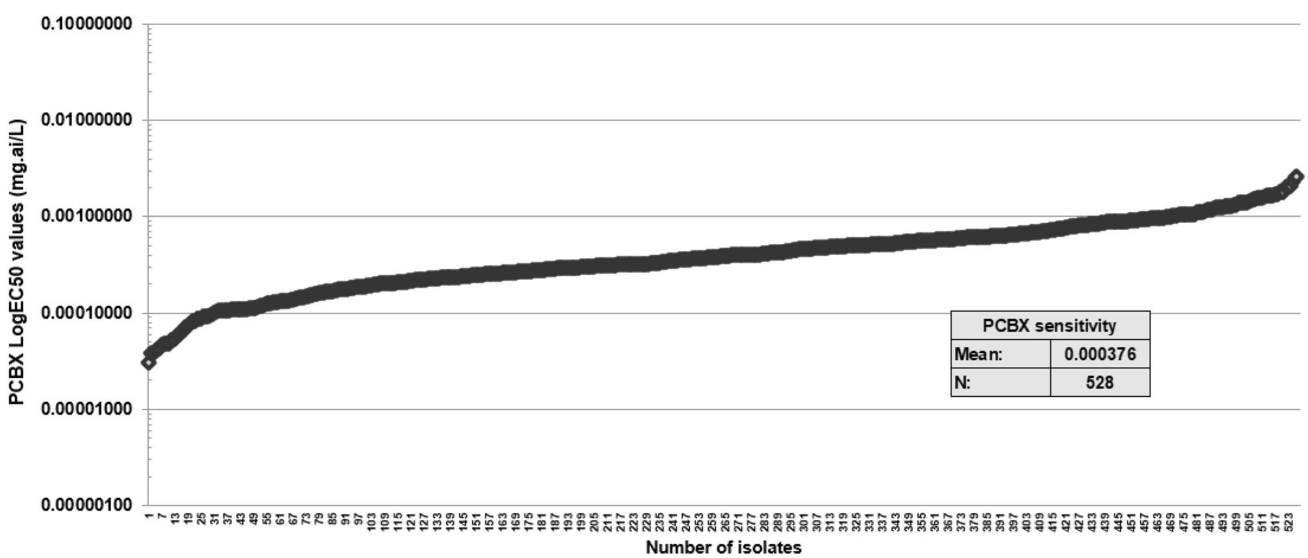

Fig. 4 Picarbutrazox sensitivity response (EC_{50} values) of 528 *Pythium* isolates collected from 17 states and 18 crops in the USA

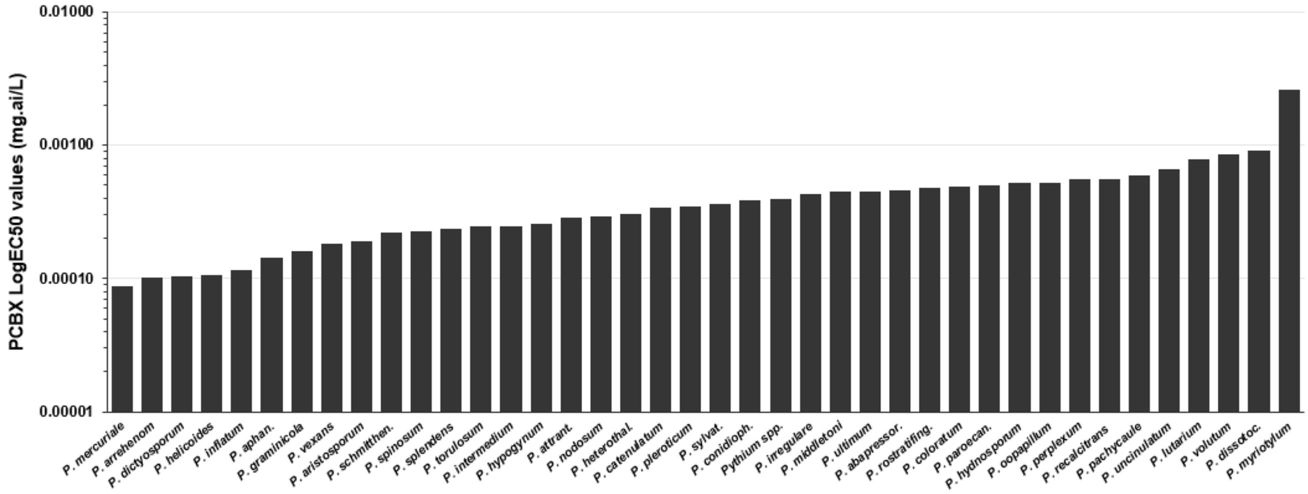

Fig. 5 Picarbutrazox mean sensitivity response of 40 *Pythium* species retrieved from different states and crops in the USA

sensitivity response was obtained across 118 isolates of *P. sylvaticum* (53-fold), followed by the response of 81 *P. ultimum* isolates (31-fold) (Fig. 6).

The sensitivity of the *Pythium* isolates to ethaboxam and mefenoxam was lower compared to picarbutrazox. Resistance development to mefenoxam and the lack of activity of ethaboxam to certain *Pythium* species affected the sensitivity profile to these fungicides (Fig. 7). *Pythium ultimum* was the species with the highest number of isolates resistant to mefenoxam (37%). Several species of *Pythium* showed no response to ethaboxam including *P. aphanidermatum*, *P. tolurosum*, *P. hypogynum*, *P. catenulatum*, *P. schmitthenneri*, and *P. rostratifingens*.

Discussion

The picarbutrazox sensitivity of 528 isolates belonging to 40 *Pythium* species was tested to assess the inherent activity of picarbutrazox across this high number of *Pythium* species. These laboratory results complement an array of studies that were and have been conducted when picarbutrazox is used as a seed treatment for the control of *Pythium* diseases caused by a diverse number of *Pythium* species on different crops under greenhouse and field conditions.

Pythium seedling diseases have a high impact on crop agronomy and yield production causing more damage than

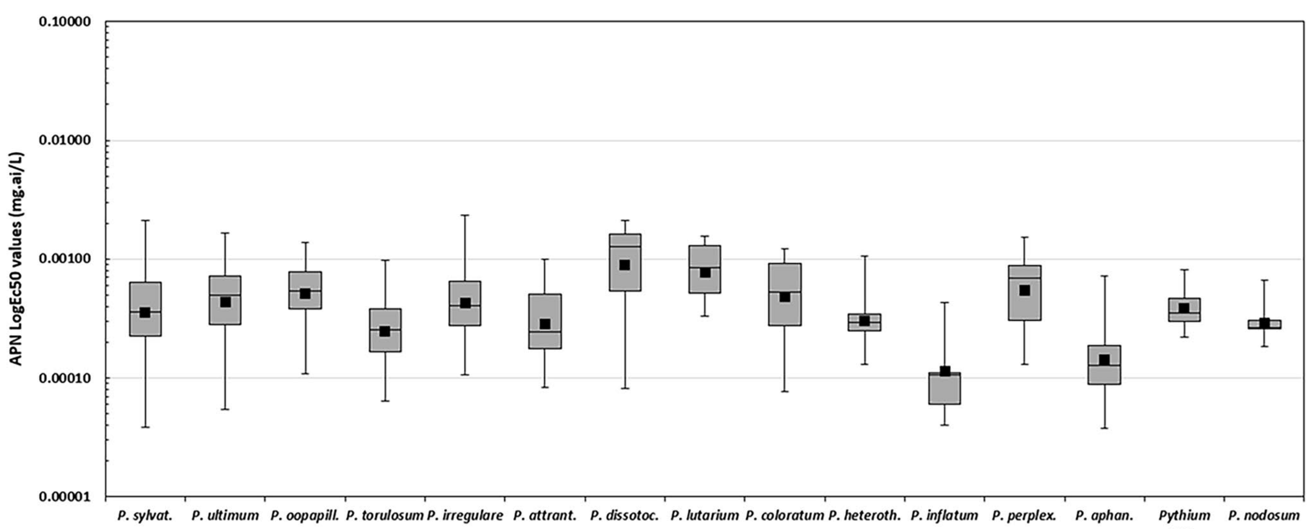

Fig. 6 Box plots of picarbutrazox sensitivities of *Pythium* isolates belonging to the 15 most common species affecting corn and soybean in the USA

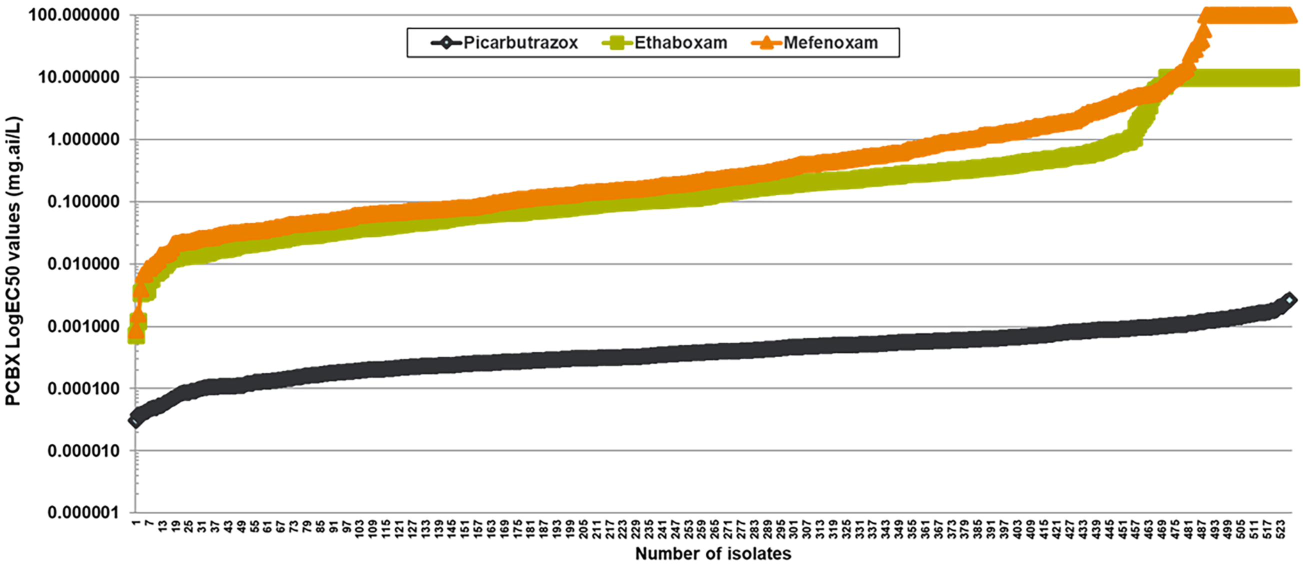

Fig. 7 Picarbutrazox, ethaboxam, and mefenoxam sensitivity response (EC_{50} values) of 528 *Pythium* isolates collected from 17 states and 18 crops in the USA

Fusarium and *Rhizoctonia* seedling diseases combined. Picarbutrazox (Vayantis®) fungicide seed treatment has a unique mode of action that offers a new solution to control *Pythium* diseases in multiple crops. The high intrinsic activity of picarbutrazox to a high diversity of *Pythium* species protects the genetic potential of high-value crop varieties. Picarbutrazox increased seed germination and emergence and improved plant stand uniformity across variable soil types and environmental conditions, resulting in crops with high yields and excellent quality. The high activity of picarbutrazox also allows the use of the fungicide at lower rates maintaining a high disease control.

Picarbutrazox has no known cross-resistance to other oomycete compounds (e.g., Phenylamides, Carboxylic Acid Amides, Quinone Outside Inhibitors, OSBPIs, or Thiazole Carboxamide), making it a great alternative to resistance management to *Pythium* fungicides by overlapping different modes of actions. Picarbutrazox could prevent or delay resistance development to other single-site mode-of-action fungicides keeping resistance frequencies to a level that allows good disease control.

It was demonstrated that picarbutrazox has excellent control of *Pythium* isolates that have developed resistance or are naturally insensitive to other different classes of fungicides, including mefenoxam and ethaboxam. Mefenoxam (FRAC group 4) was introduced in the seed treatment market in several crops between 1999 and 2002. Resistance has been reported in several *Pythium* species isolates affecting different crops. The frequency of mefenoxam resistance in *Pythium* isolates has remained low compared to that of other oomycetes causing foliar diseases, making it a key fungicide for the management of *Pythium* diseases (Hermann et al. 2019). *Pythium* species with natural resistance or insensitivity to ethaboxam (FRAC group 22) have been reported. The resistance mechanism has been associated with a mutation in the *B*-tubulin gene, the amino acid Cysteine is substituted for Serine at position 239 (C239S), and it was determined that the insensitivity of certain *Pythium* species to ethaboxam is not due to the process of selection from ethaboxam exposure; it is a process of evolution in the Peronosporales (Noel et al. 2019).

Picarbutrazox is reliable with a high-performing technology that controls the huge variability in pathogenicity of the *Pythium* species. The compound has excellent seed safety and mixture compatibility with additional fungicides, insecticides, and nematicides (Brandl et al. 2018). Resistance management recommendations for picarbutrazox include the use of good agronomical practices to minimize the risk of pathogen infections, e.g., avoid planting in wet and cool soils and ensure good soil drainage. It is recommended to always apply the label rate and ensure good and uniform coverage of treated seeds. When picarbutrazox is used in a mixture with a fungicide with a different mode of action, use the label rate of picarbutrazox and make sure that the mixing partner also provides sufficient disease control.

Overall, the seed treatments with picarbutrazox have a stand and yield protection benefit against a wide range of *Pythium* species causing diseases in many different crops. Picarbutrazox is also a valuable tool to include in fungicide resistance management with other high-value *Pythium* fungicides. The seed treatment use of picarbutrazox is also labeled for the control of *Phytophthora* species causing *Phytophthora* root and stem rot of soybeans.

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