Development of a novel fungicide, pyribencarb#

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Pyribencarb is a novel benzylcarbamate-type fungicide against a wide range of plant pathogenic fungi, especially gray mold caused by Botrytis cinerea and stem rot caused by Sclerotinia sclerotiorum. The target site of pyribencarb is cytochrome b of complex III in the electron transport system of the respiratory chain. On the other hand, the inhibitory potency of pyribencarb to the succinate-cytochrome c reductase activities of plants, rats and carp was relatively weak compared with those of strobilurin fungicides, indicating that pyribencarb is a novel Qo inhibitor of cytochrome b, whose properties are superior to the well-known Qo inhibitor fungicides in terms of selectivity and selective toxicity in the target. The inhibitory effects of pyribencarb to the cytochrome b enzyme on the transfer system of a QoI-resistant strain of B. cinerea are stronger than those of other QoI fungicides. Pyribencarb exhibited adequate control in the field experiments where QoI-resistant strains existed. This indicated that, although QoI-resistant strains develop cross-resistance against pyribencarb, as well, the extent of sensitivity reduction in QoI-resistant strains to pyribencarb was clearly lower than to other QoI fungicides. Among the QoI fungicides, pyribencarb is assumed to have unique properties. Pyribencarb belongs to the benzylcarbamate-analogue class, not the strobilurin-analogue class. Therefore, pyribencarb is thought to be a new type of QoI fungicide (BC-QoI). © Pesticide Science Society of Japan

Keywords: benzylcarbamate fungicide, pyribencarb, Botrytis cinerea, QoI.

Introduction

Pesticides are required to have efficacy and selectivity and must be safe for humans, animals, and aquatic organisms. Currently, pesticides are also required to have benefits for the environment and must also be safe for useful organisms such as natural enemies. Agricultural fields have an increasing demand for pesticides with enhanced efficiency that lower pest-control costs, solve problems such as drift onto surrounding crops, and are registered for a wide range of crops. In such circumstances, strobilurin (ST) has been the worldwide mainstay among fungicides. However, the commercial ST fungicides pose problems such as phytoxicity and decreasing effect due to resistant pathogens.

Our study aimed to introduce into the market a useful and safe fungicide that resolves the above demands and problems.

From our study results, we developed pyribencarb, a novel fungicide with a low risk of phytoxicity and practical control in the field where ST-resistant strains are dominant.

This paper describes the history of the discovery, synthesis, structure-activity relationships, mode of action, and biological activities of pyribencarb.

Generation of lead compound

When we started to study ST fungicides having a widespread fungicidal spectrum, the patent competition was intense. Therefore, to find a novel chemical class of ST fungicides, we tried to use a relatively classical method, as follows. First, we started to place existing ST fungicides such as kresoxim-methyl and naphthalene derivatives reported to have fungicidal activities on a two-dimensional hexagonal grid and compared them with each other. They are supposed to be divided into “carrier moiety,” which contributes to biological mobility, and “toxophore moiety,” which is an essential part of showing fungicidal activities. In the case of most ST fungicides, toxophore lies at the ortho position of the carrier moiety via the phenyl ring. However, from the above two-dimensional hexagonal grid studies, we found that methyl ([1,1’-biphenyl]-3-ylmethyl)carbamate (I),
which has a carbamate group as a toxophore at the meta position of the carrier moiety via the phenyl ring, also shows fungicidal activities. Then we started the optimization from (I) as a lead compound.

**Discovery of pyribencarb**

The structure-activity relationships for the lead compound (I) were investigated. In the toxophore moiety, only methyl carbamate showed good fungicidal activities; no substituent on the nitrogen atom was more favorable. In addition, a substitution of the methylene-bridge exhibited lower activities.

Although the biphenyl derivatives showed good fungicidal activities, we worried that they might not always show stable activity in various field conditions because of their high lipophobicity and low water solubility. To solve the problem, we restarted an optimization of the carrier moiety. When we introduced a 1-(2-chlorobenzoxoimino) ethyl group instead of a phenyl group, although the physicochemical properties of the corresponding compound were not improved, it showed fungicidal activities as good as the biphenyl derivative. After various modifications, the compound having picoline instead of chlorobenzene, namely, pyribencarb, had better fungicidal activities and showed favorable physicochemical properties that could be mobile in plants.

**Physical and chemical properties**

Common name: pyribencarb
Chemical name (IUPAC): methyl [2-chloro-5-[[E]-1-(6-methyl-2-pyridylmethoxyimino)ethyl][benzyl] carbamate
CAS registry number: 799247-52-2
Molecular formula: C₁₈H₂₀CIN₃O₃
Molecular weight: 361.82
Appearance: white powder
Melting point: 95.0°C
Vapor pressure: <1.0×10⁻⁵ Pa (20°C)
Solubility in water: 6.76×10⁻³ µg/L (20°C)
Partition coefficient: Log 𝑃_{ow}=3.77 (25°C, pH 6.9)

**Mode of action**

The inhibitory effects of this fungicide on the electron transport system of fungi, plants, rats, and carp were examined to elucidate its mode of action and selectivity. Pyribencarb potently inhibited the succinate-cytochrome c reductase (SCR) activities of cucumber gray mold (*Botrytis cinerea*), leaf spot (*Corynespora cassiicola*), and the decylubiquinol-cytochrome c reductase (UCR) activity of *B. cinerea*. Pyribencarb inhibited the UCR of *B. cinerea* in an uncompetitive manner with respect to decylubiquinol, as with ST fungicides. These results suggested that the target site of pyribencarb is cytochrome *b* of complex III in the electron transport system of the respiratory chain. On the other hand, the inhibitory potency of pyribencarb to the SCR activities of plants, rats and carp was relatively weak compared with those of ST fungicides, indicating that pyribencarb is a novel Qo inhibitor of cytochrome *b*, whose properties are superior to the well-known Qo inhibitor fungicides in terms of the selectivity and selective toxicity in the target. The binding site of pyribencarb on cytochrome *b* was assumed to be a little different from those of ST fungicides through comparison of amino acid sequences of plants, rats, and fungi, including *B. cinerea*.

**Biological properties**

Pyribencarb exhibited not only a preventive effect but also a curative effect. When spraying was performed 48 hr after inoculation (after visible symptoms appeared), pyribencarb also showed strong inhibitory activity against lesion development by cucumber gray mold that was significantly superior to its preventive activity. Experiments in the greenhouse demonstrated good translaminar and residual activities, resulting in pyribencarb's high performance in the field. To conclude, the fungicidal and disease-controlling activities of pyribencarb are characterized by its preventive, curative, translaminar, and residual activities; its inhibitory activity toward lesion development; and its rain fastness. The most striking feature of pyribencarb was thought to be its stronger disease-controlling activity after the appearance of visible symptoms compared with its preventive effects. These results suggest that the biological properties of pyribencarb contribute to its excellent ability to control several diseases in the field.

In *in vitro* tests, pyribencarb significantly inhibited spore germination, germ tube elongation, mycelial growth, secondary appressorium formation, and sporulation of *B. cinerea*. Pyribencarb inhibited all stages in the life cycle of *B. cinerea*, and its inhibitory activities against all tested fungal life stages were significantly superior to those of several anti-*Botrytis* fungicides.

Pyribencarb demonstrated good effectiveness toward strains resistant to benzimidazole fungicides, dicarboximide fungicides, both benzimidazole and *N*-phenylcarbamate fungicides (diethofencarb), anilinopyrimidine fungicides, and fluazinam. Therefore, this finding suggests that pyribencarb is a proper candidate to replace these fungicides in areas where resistant strains are dominant. The strain of *B. cinerea* resistant to QoI fungicides have already been reported in Japan. The inhibitory effects of pyribencarb on the electron transfer system and mycelial growth of a QoI-resistant strain of *B. cinerea* (G143A) are stronger than those of other QoI fungicides. Furthermore, pyribencarb exhibited adequate control in the greenhouse trial and field trial where resistant strains are dominant.