第 18 回

殺菌剤耐性菌研究会シンポジウム
講 演 要 旨 集

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第18回殺菌剤耐性菌研究会シンポジウム

プ ロ グ ラ ム

2008年4月29日（火）

＜午前の部＞

（昼　食）

＜午後の部＞

16:00　閉　会

島根県における耐性菌の発生事例

Occurrence of Fungicide-resistant Pathogens in Shimane Prefecture

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Abstract

In the isolates of *Fusarium* spp. collected from Fusarium head blight of barley and wheat in Shimane Prefecture from 2002 to 2004, the frequency distribution of minimum inhibitory concentrations of triflumizole against mycelial growth showed three peaks at 1.56ppm, 12.5ppm and over 800ppm, respectively. In inoculation tests on wheat, the efficacy of triflumizole against the strains (MIC values of 12.5ppm and over 800ppm) was found to be poor. On the other hand, since thiophanate-methyl showed high control efficacy against all of these strains and no strains showing reduced sensitivity to this fungicide were detected in our monitoring for three years, thiophanate-methyl was considered to be a useful fungicide for Fusarium head blight control in Shimane Prefecture.

In *Botrytis* spp. frequently affecting flower bulbs, the occurrence of various multiple fungicide-resistant strains have been confirmed in Shimane Prefecture. The heavy occurrence of tulip fire (*Botrytis tulipae*) experienced in Hakuta Town and Hikawa Town in 1998 was presumed to be partly due to the extraordinary high ratios of occurrence of strains which were resistant to both dicarboximide and benzimidazole fungicides in these areas. The strains resistant to benzimidazoles as well as to diethofencarb were found on lily infected with *B. elliptica* in 1993. Assuming MIC values of 0.05~0.2ppm for benomyl to be the baseline sensitivity, these strains with MIC values of 0.78~6.25ppm (with the peak of 3.13ppm) were considered to be weakly-resistant strains. These resistant strains were found in ascospore-derived strains as well. On neck rot of narcissus caused by *B.narcissicola* and gray mold of gladiolus caused by *B.gladiolorum*, strains multiple resistant to benzimidazoles and diethofencarb were also detected in 2004. EC_{50} values of thiophanate-methyl to these resistant strains for mycelial growth ranged 9.6ppm~15.9ppm and 14.6ppm~23.3ppm, respectively, and were considered to be moderately-resistant.

In part of the isolates of *Colletotrichum* sp. collected from *Sarcandra glaber* in 2007, moderately benzimidazole-resistant strains to similar with *C. gloeosporioides* isolates affecting Japanese pear in Kochi Prefecture were confirmed.

1．はじめに

島根県は日本海に臨む中国地方の北側に位置し、東西の市町村を結ぶ距離（安来市ー津和野町間）が約230キロと東西に細長い県土を有する人口74万人（2005年10月、全国46位）の県である。県の中央部を北緯35°の緯度が通っており、京都、名古屋あたりと同じ緯度上にある。年平均気温は15.8℃（全国28位）、日照時間は年間1,856時間（全国36位）、年間雪日数は41日（全国13位）である。県土面積は6,708km²（全国16位）であるが、大半を森林が占める。農業産出額は648億円（2005年、全国42位）で、その中

ジャガイモ疫病菌のフルオピコリドに対する感受性検定法とモニタリング

Assay method for sensitivity of *Phytophthora infestans* to fluopicolide and the first monitoring results

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Abstract

Fluopicolide, 2,6-dichloro-*N*-{[3-chloro-5-(trifluoromethyl)-2-pyridinyl]-methyl}benzamide, is a novel fungicide which belongs to a new chemical class, the acylpicolides. It has an excellent efficacy against oomycete phytopathogens in various crops such as grapevine (downy mildew), potato, tomato (late blight). Several studies revealed that fluopicolide induces delocalization of spectrin-like proteins, representing a unique mode of action that is quite different from that of competitor molecules. Sensitivity of *Phytophthora infestans* from potatoes to fluopicolide could be determined by the leaf disk assay. The first monitoring by means of the assay with 37 field isolates in Japan (33 and 4 isolates from Hokkaido and Nagasaki prefecture, respectively) showed unimodal distribution of sensitivity. The EC_{50} values for these field isolates were in the range of 0.12-3.5ppm with a mean value of 1.02ppm.

1. はじめに

フルオピコリドは、1997年に旧アグレボ社によって発見され、バイエルクロップサイエンス社によって開発されたアシルピコライド系に属する新規殺菌剤である。本剤は、疫病およびべと病を代表とする卵菌類に属する植物病原菌によって引き起こされる病害に対して優れた防除効果を示す。本剤は、国内において2008年1月24日にプロパモカルブ塩酸塩との混合剤であるリライアブル®フロアブル（フルオピコリド5.5%とプロパモカルブ塩酸塩55.5%の混合剤）として農薬登録を取得した。海外では、英国をはじめ2006年から、同じ混合剤がInfinito®の商品名で上市されている。現在、フルオピコリドを含む混合剤の国内外での登録取得に向けて、順次申請作業を進めている。

フルオピコリドは新規骨格を有し、これまでに得られた作用機構に関する知見から、新規の作用点を有する殺菌剤であると考えられる。一方、本剤の対象病害であるジャガイモ疫病では、メタラキシルに対する耐性菌の発達事例が報告されており、FRAC（Fungicide Resistance Action Committee）において、本菌は耐性菌の発生リスクが中程度に分類されている[1)]。このことから、フルオピコリドに関しても、感受性検定法を確立するとともに、ジャガイモ疫病菌について感受性ベースラインデータを取得する必要がある。

本報告では、フルオピコリドの物理化学的性状、作用特性および作用機構について紹介するとともに、ジャガイモ疫病菌における感受性検定法と日本およびヨーロッパでの感受性検定結果について、現在までに得られている知見を紹介する。

Resistance Risk Assessment for Mandipropamid and other CAA Fungicides

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ABSTRACT

The new carboxylic acid amide (CAA) fungicide mandipropamid (MPD) is very effective against downy mildews and potato late blight. Resistance against CAA fungicides such as mandipropamid, dimethomorph, flumorph, iprovalicarb and benthiavalicarb was reported in *Plasmopara viticola* but not in *Phytophthora infestans*. Sensitivity to MPD was investigated in isolates of *P. infestans* collected between 1989 and 2007 from commercial fields and from over 40 trial sites in ten European countries and in isolates of *P. viticola* collected since 2001 from major grape growing regions in Europe. All *P. infestans* isolates were sensitive to MPD with ED_{50} values ranging between 0.03-5.0 mg/L. In contrast, some resistant isolates of *P. viticola* were detected in certain regions of France, Germany and Italy. CAA resistance in *P. viticola* segregated in a recessive manner and declined under field conditions when CAA treatments were stopped. Plastic tunnel, shade house and field experiments conducted during 2001 to 2005 showed that enforced selection pressure imposed by repeated sub-lethal (5 mg/L) or excessive (500-1000 mg/L) doses of MPD on mixed field populations of *P. infestans* resulted in an effective control of late blight on potato or tomato crops and produced no resistant isolates against the compound. When artificial mutagenesis was applied to sporangia of *P. infestans*, resistant mutants developed at generation 0. They all showed instable resistance *in planta*, diminishing after 1-7 asexual infection cycles and failed to grow on CAA-amended medium. A1 and A2 mutants were crossed and the F1 and F2 progeny isolates were tested for resistance. All progeny isolates failed to show stable resistance to CAAs *in planta* or *in vitro*. The results indicate that the probability of a build up of resistant sub-populations of *P. infestans* to mandipropamid in the field is low. Resistance risk for the entire class of CAA fungicides is estimated to be low to medium.

INTRODUCTION

The probability for the development of resistant sub-populations depends largely on the mode of action of a fungicide. Compounds with a site-specific action are more vulnerable than multi-site compounds. Resistance against metalaxyl, an rRNA polymerase inhibitor, has developed in

Mode of action, biological performance and latest monitoring results of boscalid sensitivity

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Summary

Boscalid is a new broad-spectrum fungicide belonging to the class of carboxamides (succinate dehydrogenase inhibitors). It is effective against different stages of fungal development, mainly against spore germination, germ tube elongation but inhibits also other stages such as appressoria formation or mycelial growth. After leaf uptake, boscalid is transported translaminarily and acropetally. Boscalid is very safe to the plants and covers a wide disease spectrum. It controls a broad range of fungal pathogens in arable and speciality crops including ornamentals. Sensitivity studies with isolates of *Botrytis cinerea* showed that different mutations in the ubiquinone binding site of the target gene can affect the sensitivity towards carboxamides. The founding of a FRAC Carboxamide Working Group is initiated.

1. Introduction

Boscalid is the common name of the new broad-spectrum fungicide discovered and developed by BASF. It belongs to the carboxamide class of fungicides. Different commercialized carboxamides are shown in Figure 1. The structural alignment of these carboxamides (Figure 2) demonstrates their common structural features.

The target enzyme of carboxamides is succinate dehydrogenase (SDH), which is a functional part of the tricarboxylic acid cycle and of the mitochondrial electron transport chain (Keon *et al.* 1991, Matsson and Hederstedt 2001). SDH consists of four subunits, the hydrophilic flavoprotein (A) and iron sulphur protein (B) and two lipophilic transmembrane C- and D-subunits which anchor the protein to the inner mitochondrial membrane (Figure 3). The chemical, physical, and biological properties of boscalid as a fungicide as well as monitoring data and specific studies on amino acid substitutions in the binding site affecting the sensitivity towards carboxamides are described in this contribution.

茨城県におけるボスカリド剤耐性キュウリ褐斑病菌の発生

Occurrence of *Corynespora cassiicola* isolates resistant to boscalid on cucumber in Ibaraki Prefecture, Japan

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Abstract

Corynespora leaf spot, caused by *Corynespora cassiicola*, is one of the most important diseases in cucumber production in Japan. To obtain the data on baseline sensitivity to boscalid, *in vitro* tests have been carried out using *C. cassiicola* isolates obtained from cucumber, tomato, eggplant and cowpea in different locations in Japan before boscalid registration in January 2005. Minimum inhibitory concentration (MIC) and 50% effective concentration (EC_{50}) values for the 115 isolates ranged from 0.5 to 7.5μg ml^{-1} and from 0.05 to 0.44μg ml^{-1}, respectively. As a result of monitoring, 450 out of 936 isolates obtained from 28 cucumber greenhouses with a history of boscalid uses from August 2005 to November 2007 in Ibaraki Prefecture, Japan, were resistant to boscalid with MIC values higher than 30μg ml^{-1}. Resistant isolates were detected from 26 out of 28 greenhouses. Detection ratio of resistant isolates exceeded 50% in 13 greenhouses. Moreover, resistant isolates were divided into two groups: moderately resistant (MR) isolates with EC_{50} values ranging from 1.1 to 6.3μg ml^{-1}, and highly resistant (HR) isolates with EC_{50} values ranging from 24.8 to 48.9μg ml^{-1}, respectively. In the inoculation tests which used potted cucumber plants, control failures of boscalid against resistant isolates were observed. Efficacy of boscalid was remarkably low against HR isolates in particular. Moreover, resistant isolates were also detected from two out of five greenhouses in October 2007 without a history of boscalid use. To our knowledge, this is the first report of boscalid resistant isolates in Japan and differential levels of resistance to carboxamide group of fungicides.

1．はじめに

茨城県のキュウリ栽培は、2003 年産で作付面積 720ha、生産量 37,600t といずれも全国 5 位であり、県内農産物の主要品目の一つである。作型としては、促成、半促成、トンネル、露地、抑制など様々であるが、主要産地である常総市や筑西市などでは、促成と抑制栽培により年間二作の作型で栽培を行っている場合が多い。

これらキュウリ産地では近年、主要病害の一つである褐斑病（病原菌：*Corynespora cassiicola*）が収穫開始直後から多発生し、その被害が大きな問題となっている。本病は古くから知られている病害であるが、長い間、問題となることはなかった。しかし、1980 年代ころから、ブルームレス台木の普及や多肥栽培、周年栽培による菌の常在化（挟間，1993；宮本ら，2007）により被害が増加し、さらに、近年のキュウリ品種の変遷（宮本ら，2006）により被害が深刻化するようになった。現在、本病の防除は主に化学的防除に頼っており、茨城県では本病の防除のためだけにほぼ毎週のように薬剤を散布している

MBI-D剤耐性イネいもち病菌の発生推移

—栃木県の事例—

The occurrence and annual change of MBI-D-fungicide-resistant strains of *Pyricularia oryzae*

—In Tochigi prefecture—

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ABSTRACT

In recent years, nursery box treatment with MBI-D fungicides was a common practice to control rice blast in Tochigi prefecture. However, in 2004, MBI-D-resistant strains of rice blast fungus, *Pyricularia oryzae,* occurred in Nasu area. Therefore, rice blast fungus was collected from some more areas in Tochigi prefecture, and their sensitivity against MBI-D was monitored in 2005. As a result, resistant strains were also found from other areas. However, even in the paddy fields where MBI-D resistant strains were detected, leaf blast was effectively controlled by nursery box treatment with MBI-D fungicides, except in Nasu area. After the monitoring, the use of MBI-D fungicides was discontinued in Nasu area. Furthermore, to avoid the increase in the density of resistant isolates, MBI-D formulations were recommended for nursery box treatment in a rotational application with other fungicides every year, and in a combination with the use of sanitized seeds and appropriate control of panicle blast. Thereafter, the nursery box treatment with MBI-D fungicides is continued in Tochigi prefecture, and contributes to control rice blast.

1．はじめに

栃木県は広大な平野を持ち、2007年産水稲の作付面積は67,200ha、収量は534kg/10aであり、作付面積、収量ともに、全国８位に位置づけられる米産地である。また、米は本県農業産出額の約３割を占めており、主要な農産物の一つである。本県における水稲の品種構成はコシヒカリが全体の８割以上を占め、特に県北・県中部ではコシヒカリの作付割合が高い。このような生産状況にあって、イネいもち病は、本県の稲作において最も重要な病害の一つとなっている。

近年、良質米生産の気運の高まりから、JA米としてのブランドを確立するため、種子更新率がほぼ100%まで達するとともに、薬剤処理済みの購入種子の導入により、殆どの農家が種子消毒を実施していることになる。このため、農業環境指導センター(病害虫防除所)の巡回調査においては、苗いもちの発生は殆ど確認できない状況である。本田防除では、箱施用剤や粒剤の水面施用等による初期防除と無人ヘリコプターによる出穂期防除が基幹的な防除法となっている。特に、箱施用剤は省力的であり、安定した効果が長期間持続するため、2003年以降は県北部を中心に普及面積が急増した(図1)。

しかしながら、MBI-D剤については2001年の佐賀県をはじめとして九州、西日本等において薬剤耐性イネいもち病菌の発生が確認された(宗ら，2002；山口ら，2002)。

このため、2004年に県内で発生したいもち病菌のMBI-D剤に対する感受性検定を行ったところ、県内

MBI-D剤耐性イネいもち病菌の発生推移 ー佐賀県の事例

Fluctuation of MBI-D-resistant Populations of *Magnaporthe grisea* after Withdrawal of the Fungicide Selection Pressure－ in Saga Prefecture

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Abstract

MBI-D-resistant isolates of *Magnaporthe grisea* occurred in Saga prefecture in 2001, and the use discontinuance of MBI-D-fungicide was guided in 2003. In the resistance monitoring in Saga prefecture, rate of resistant isolates with 70-55% in 2002 decreased to 51-30% in 2003, and they were several % in 2006, 2007. At the paddy field in Karatsu city, rate of resistant isolate with 77 % in 2002 decreased to 16% in 2003, and they weren't detected with a low fungal population density in 2004-2006. The control efficacy of MBI-D-fungicide was shown to be high in these years when the population density was very low. Then, it was thought that reuse of the MBI-D might be possible. The rate of resistant isolates on the rice seed influence the rate of resistant isolates of leaves at the field. The seed was very important as the primary infection source of rice blast, therefore, seed disinfectants and optimal timing of fungicide applications for panicle blast control were examined.

はじめに

いもち病に対し高い防除効果と持続効果を持つカルプロパミド箱粒剤は、1998 年頃から佐賀県内のいもち病常発地帯を中心として広く普及していた。ところが、2001 年に県西北部地帯を中心として本箱粒剤を施用したにもかかわらず、葉いもちが多発生し、中にはずりこみ症状を呈する圃場もみられるなど、その効果が著しく低下する現象がみられた。そこで病原菌の薬剤感受性、気象要因、作付け品種、発病苗の持ち込み等各種要因について解析を行った結果、当該地区における多発生は MBI-D 剤（シタロン脱水酵素阻害型メラニン合成阻害剤）耐性菌の出現が原因であることが明らかとなった（山口ら，2002)。02 年には、県内のいたる所や九州各県で耐性菌が認められ（荒井，2004)、その後西日本の各地、東北でも確認されるなど発生は全国的に拡大している。

本耐性菌に対し、各県において耐性菌のモニタリングが実施され、発生が認められたところで、代替薬剤や防除体系の組み直し等緊急的な対応が速やかに行われたため、耐性菌による実質的な被害は最小限に抑えられている。

一方で、いもち病の防除薬剤については、長期残効型 QoI 箱剤の上市による耐性菌リスクマネージメントや本田散布剤の主力であるフサライド剤の生産中止による散布剤不足への対応など、新たな局面が展開しつつある。

耐性菌の発生以来、耐性菌についての菌学的、遺伝学的、生態学的な解析が進み多くの知見が得られ、当シンポジウムにおいても多くの成果が取り上げられてきた。本講演においては、耐性菌発生以来、九州沖縄農研センターと取り組んできた県内および現地での耐性菌の発生推移と耐性菌対策のために実施した試験について紹介するとともに、今後の MBI-D 剤の使用再開について述べたい。

イネいもち病菌における殺菌剤耐性菌マネジメント

－MBI-D剤及びQoI剤に関して－

Management of MBI-D and QoI fungicide resistance on rice blast caused by *Magnaporthe grisea*

殺菌剤耐性菌研究会
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Abstract

Rice blast caused by *Magnaporthe grisea* is the most important disease for rice cultivation in Japan and has caused serious damage in the past. Until today, many kinds of fungicides have been developed to prevent this disease and have enabled stable rice production. However, like kasugamycin in 1971, organophosphorus fungicides in 1976 and MBI-Ds (inhibitors of scytalone dehydratase in melanin biosynthesis) in 2001, the effectiveness of fungicides, which had maintained high control performance, drastically decreased at once and harmed the quality and harvest of rice. The main cause of this incident was the occurrence of MBI-D-resistant isolates of *M. grisea*. Resistant isolates caused significant damage to the rice production especially because MBI-D-fungicides were used widely.

Because resistant isolates have potentials to cause serious damage, it is necessary to manage the risk for resistance development in fungal pathogens in order not to experience the case such as MBI-D-fingicide resistance. However, in the recent years, the reduction of pesticide applications and change to the less-drifting pesticides are further required after the establishment of 'Positive List System'. It prompted rice growers to use highly active and long-lasting pesticides for rice blast control, granular formulation of pesticides in nursery box treatment in particular. The pesticides which meet such requirements are strobilurin fungicides (QoIs), e.g. including azoxystrobin, metominostrobin and orysastrobin and they are more in use year by year. However, as QoIs are generally considered to have high risk for resistance development in fungi, the management of its use should be required in order to prevent the occurrence of similar case with MBI-D-fungicides.

The related institutions have already proposed some considerations on the use of QoIs. For example, Nara Plant Protection Center (2007) proposed that "use QoIs one time for one cropping, no use or at most every other year use QoIs in the area of consistent occurrence of rice blast and prohibit to use QoIs in the propagation farms". In addition to this, Tottori Plant Protection Office