## トマト褐色輪紋病菌(Corynespora cassiicola)及びキュウリ褐斑病菌(Corynespora cassiicola)の薬剤感受性につい

**T**/Sensitivity of tomato target spot fungus (Corynespora cassiicola) and cucumber target leaf spot (Corynespora cassiicola) to thiophanate-methyl, diethofencarb and azoxystrobin

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第 13 回殺菌剤脈性菌研究会シンポジウム講演要旨(2003, P1-8) / Abstracts of the 13th Symposium of Research Committee on Fungicide Resistance

トマト褐色輪紋病は 1991 年に我が国で初めて岡山県で確認された。本病の防除対策確立の一環として、2000~2001 年にチオファネートメチル水和剤及びジエトフェンカルブ水和剤に対する本病原菌の感受性を検定した結果、多くの菌株がチオファネートメチルに高度耐性で、一部にジエトフェンカルブとチオファネートメチルの両剤に耐性を示す菌株が確認された。

キュウリ褐斑病は 1980 年代以降、関西以西の夏秋栽培を中心に重要病害として位置付けられ、発生生態や防除対策について検討された。岡山県でも 1976 年に本病が初めて確認され、主要作型の夏秋栽培で特に被害が大きい病害であった。本病の防除対策確立の一環として、2000~2001 年にチオファネートメチル剤、ジェトフェンカルブ剤及びアゾキシストロビン剤に対する本病原菌の感受性を検定した結果、多くの菌株がチオファネートメチル剤に高度耐性で、一部にジェトフェンカルブとチオファネートメチルの両剤及びアゾキシストロビン剤に耐性を示す菌株が確認された。

#### ダイズ紫斑病の防除対策とベンゾイミダゾール系薬剤耐性

/Chemical control of benzimidazole-resistant strains of Cercospora kikuchii

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Occurrence of benzimidazole-resistant strains of *Cercospora kikuchii*, the causal fungus of purple stain on soybean seed, was investigated during 1991 to 2001 in Tottori Prefecture. The effective methods for disease control by chemicals were also examined. Benzimidazole-resistant strains were detected in a high ratio. Strains resistant to thiophanate-methyl showed cross-resistance to benomyl and thiabendazole. Treatment with thiuram that has been used as the main disinfectant of seeds in Tottori Prefecture was effective for controlling the disease development on cotyledons caused by benzimidazole-resistant strains. A new fungicide, azoxystrobin was highly effective against the disease caused by benzimidazole-resistant strains. A single application of azoxystrobin between 20 and 35 days after flowering significantly suppressed disease incidence on seeds up to harvest stage.

#### 灰色かび病菌のシカルボキシイミド系薬剤耐性の分子機構

/ Molecular mechanism of dicarboximide resistance in Botrytis cinerea

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灰色かび病はブドウや様々な野菜・花卉の重要な病害であり、その防除剤としてジカルボキシイミド剤が使用されてきたが、1980 年代の初頭から耐性菌が出現し、世界中で問題になっている。ジカルボキシイミド剤の作用機構や耐性メカニズムについては、これまで多くの研究がなされてきたにもかかわらず、多くの部分が未解明で残されていた。

我々は以前に、アカパンカビをモデル菌として植物病原菌のベンズイミダゾール耐性変異を推定した。この手法をジカルボキシイミド剤に適用し、アカパンカビを用いてジカルボキシイミド剤の作用機構を解析し、その結果を応用して、灰色かび病菌のジカルボキシイミド圃場耐性変異を同定した。さらに、この圃場耐性変異を検出する遺伝子診断法を構築し、それを用いて日本各地の圃場分離株のモニタリングを行い、耐性変異分布を調査した。

#### イネいもち病菌のシタロン脱水酵素阻害型メラニン合成阻害剤(MBI-D)耐性 1. 佐賀県における耐性菌の発生経過

Resistance of Magnaporthe grisea to melanin biosynthesis inhibitors targeting scytalone dehydratase (MBI-D) 1. The occurrence of MBI-D-fungicide-resistant strains of Magnaporthe grisea in Saga Prefecture

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第 13 回殺菌剤脈性菌研究会シンポジウム講演要旨(2003, P29-36) / Abstracts of the 13th Symposium of Research Committee on Fungicide Resistance

カルプロパミド箱粒剤は、いもち病に対する高い効果が持続することから、1998 年頃より佐賀県内のいもち病多発地帯を中心として普及していた。ところが、2001 年に県西北部地帯を中心としてカルプロパミド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込み症状を呈してい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、当該地区の多発生は MBI-D 系統薬剤(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。さらに 2002 年には、2001 年に問題化した地域のみならず佐賀県内のいたる所で効果低下の事例がみられ、耐性菌が確認された。また

他の九州各県や西日本においても耐性菌が新たに確認されるなど、今後とも発生が拡大していく様相を呈している。今回の耐性菌の発生は、生産現場においてカルプロパミド箱粒剤がいもち病防除の柱として依存度が高かっただけに大きな問題となり、代替薬剤の選定や防除体系の緊急的な組み直し等対応に追われている。

佐賀県における耐性菌の発生経過とその対応を紹介し、今後の本耐性菌の発生拡大防止策並びに発生後の対応の参考になればと考える。

### イネいもち病菌のシタロン脱水酵素阻害型メラニン合成阻害剤(MBI-D)耐性 2. 薬剤感受性検定と防除対策 /

Resistance of Magnaporthe grisea to melanin biosynthesis inhibitors targeting scytalone dehydratase (MBI-D) 2. Methods for testing the sensitivity to MBI-D and countermeasure for disease control

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In the northwest area of Saga Prefecture, less carpropamid-sensitive rice blast fungus appeared in July, 2001. The study was done by Saga Prefecture and the agrochemicals companies concerned, and it was found that suitable conditions for the rice blast occurrence stimulated the disease development. But, existence of the fungal isolates in which sensitivity toward carpropamid decreas was also confirmed by the investigation after wards Carpropamid-resistance in rice blast fungus was then determined. Those isolates also showed resistance to other melanin biosynthesis inhibitors which target scytalone Dehydratase (MBI-D). Control efficacy of various fungicides other than MBI-D was high against MBI-D -resistant isolates of rice blast fungus.

## イネいもち病菌のシタロン脱水酵素阻害型メラニン合成阻害剤(MBI-D)耐性 3. 耐性機構と遺伝子診断法 /

Resistance of Magnaporthe grisea to melanin biosynthesis inhibitors targeting scytalone dehydratase (MBI-D) 3. Mechanism of resistance and gene diagnostic method

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第 13 回殺菌剤脈性菌研究会シンポジウム講演要旨(2003, P49-57) / Abstracts of the 13th Symposium of Research Committee on Fungicide Resistance

The inhibitory activity of carpropamid on scytalone dehydratase (SDH) extracted from the MBI-D-resistant strain of *Magnaporthe grisea* was obviously reduced to 1/20 and 1/100 with its I50 value and I90 value, compared with that extracted from the sensitive strain. A single-point mutation (G to A), which locates at the upstream region (233 bp downstream from the ATG codon) leading to change of one amino acid (Valine 75 to Methionine: V75M) was found in the resistant strain. To examine if the V75M mutation is the primary reason for decreasing the sensitivity of SDH to the MBI-D, the SDH cDNAs of both the sensitive and the resistant strain were cloned into the GST-fused protein expression vector. The GST-fused SDHs of both strains to carpropamid exhibited the same sensitivities as those extracted from mycelia. These data clearly revealed that the V75M mutation causes the low sensitivities of SDH of the MBI-D-resistant strains, and strongly suggested that the V75M mutation confers resistance of these strains to the MBI-D. Furthermore, we established the simple diagnosis method of the resistant strain to the MBI-D by combination of direct preparation of PCR template from lesion and the primer-introduced restriction enzyme analysis PCR (PIRA-PCR).

# イネいもち病菌のシタロン脱水酵素阻害型メラニン合成阻害剤(MBI-D)耐性 4. 耐性菌の性質および拡大防止策

Resistance of Magnaporthe grisea to melanin biosynthesis inhibitors targeting scytalone dehydratase (MBI-D) 4. Biological properties of resistant isolates and general countermeasure against their predomination

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第 13 回殺菌剤耐性菌研究会シンポジウム講演要旨(2003, P59-68) / Abstracts of the 13th Symposium of Research Committee on Fungicide Resistance

In 2001, field resistance against carpropamid {WINR}, one of the scytalone dehydratase (SDH) inhibitors (dehydratase inhibitors in melanin biosynthesis: MBI-D) was reported in the Matsuura River area of Saga Prefecture in Japan. In this case, MBI-D was treated by nursery box application. Rice blast fungus, *Magnaporthe grisea*, was collected in around Saga, and their sensitivity against MBI-D was investigated. This study revealed that there were resistant strains against MBI-D in Saga. A single point mutation in SDH gene sdh1 causing substitution of one amino acid in SDH (Valine 75 to Methionine: V75M) was found in the resistant isolates. DNA fingerprinting with MGR 586 suggested that sensitive and resistant strains belonged to a single lineage. Fitness of resistant isolates under cold or hot conditions was not inferior to sensitive strains. The resistance did not change during at least one year of maintenance in the laboratory. In 2001, the resistant isolates were dominantly distributed in Matsuura River area of Saga where control failure of blast was reported. In 2002, they were detected in all prefectures of Kyushu. Considering the distribution and these properties, resistant

isolates may occur and survive under various conditions. However, they could be controlled with commercial rice seed disinfectants, as well as sensitive strains. Therefore, to avoid the prevalence of resistant isolates, MBI-D formulations are recommendable for nursery box application in combination with thoroughly sanitized seed and appropriate control of panicle blast.