大阪府における薬剤耐性菌への取り組み ／Research activities on fungicide resistance in Osaka Prefecture

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卓効を示す農薬の恩恵により作物生産が飛躍的に向上した反面、過度の農薬依存により薬剤耐性菌の出現を招き、防除効果の低下に至るという、いわゆる薬剤耐性菌の発生と農薬との悪循環が繰り返されている。本府においても、薬剤耐性菌の発生により防除効果の低下を招き、生産上の大きな障害となっている例は少なくない。ここでは灰色かび病薬剤耐性菌を中心に、その発生状況と耐性菌への取り組みについて紹介したい。

タマネギ灰色腐敗病菌のベンゾイミダゾール系薬剤耐性

／Benzimidazole resistant strains of Botrytis allii (onion gray-mold neck rot)

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タマネギ灰色腐敗病は淡路島におけるタマネギの最重要病害で、本格的な発生が始まったのは 1971 年産タマネギからである。そこで、本病の伝染環など発生生態の究明や防除法の確立について試験研究に取り組んだ結果、冷蔵庫や屑タマネギの加工場が建設され、これらから病原菌の分生子が圃場に飛散侵入するという、人為的な伝染環が明らかになった。

ベンゾイミダゾール系薬剤は 1973 年以来、年数回散布されてきたにもかかわらず、耐性菌は全く検出されなかった。しかし 1999 年、少数菌株ではあったが耐性を示したため、2000 年春以降は防除体系を変更し、ベンゾイミダゾール系薬剤の使用を中止して代替薬剤の散布を行うとともに、実態調査、防除対策に取り組んだ。

シフルフェナミド（NF-149，パンチョ R）感受性検定法とベースラインデータ

／Monitoring methods for testing sensitivity of some pathogens to cyflufenamid (NF-149), and its baseline data

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A novel fungicide, cyflufenamid, (Z)-N-[α-cyclopropylmethoxyimino-2,3-difluoro-6-(trifluoromethyl)benzyl]-2-phenylacetamide, has excellent control activity against powdery mildew of various crops and brown rot of stone fruits. Fungicidal activity of cyflufenamid is characterized by preventive, curative, and long residual activity. It has also vapor action. Cross-resistance between cyflufenamid and other commercial fungicides has not been observed in powdery mildew on wheat, barley and cucumber. Several methods were used in order to obtain the baseline data of sensitivity to cyflufenamid. Both pot and sprayed leaf segment assay were carried out on wheat powdery mildew.

The peak of EC50 values is at 0.03 ppm in pot assay, at 0.01 ppm in sprayed leaf segment assay, respectively. Sprayed leaf segment assay was carried out on barley powdery mildew, and the peak of EC50 values is at 0.03 ppm. Leaf disc assay was carried out on cucumber powdery mildew, and the peak of EC50 value is at 0.001 ppm. No significant change of sensitivity to cyflufenamid was observed after sprayed with the chemical on wheat powdery mildew under greenhouse condition at sixteen times at 6.25 a.I./ha. When cyflufenamid was sprayed on wheat powdery mildew in the field for three years (on the condition of two times application per year at 25g a.I./ha every time), no significant changes of sensitivity to cyflufenamid was observed. Cyflufenamid shows neither effect on electrolyte leakage from plasmamembrane, biosynthesis of lipids and chitin nor mitochondrial respiration. The mode of action of cyflufenamid has not been clarified.

ナスすすかび病菌の薬剤耐性－DMI

／DMI-fungicide-resistant isolates of Mycovellosiella nattrassii Deighton, causal fungus of leaf mold of eggplant

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Isolates of *M. nattrassii* isolated from infected eggplants in greenhouses were tested for sensitivity to triflumizole in June 1998 because the effectiveness of triflumizole for control of leaf mold of eggplant in Saga prefecture appeared to have declined in recent years. All of 100 isolates collected from the fields were resistant to triflumizole with EC50 values ranging from 0.0651 to 24.1 μg/ml. Both the preventive and curative effects of triflumizole for control of leaf mold decreased in eggplants infected with higher resistnat isolates for triflumizole. The resistant isolates for triflumizole also exhibited resistance to the DMI fungicides such as fenarimol and mycrobutanil. The resistant isolates for triflumizole also increased by successive applications of fenarimol or mycrobutanil in greehouse. The resistant isolates had similar properties in mycelial growth and pathogenicity to the sensitive isolates, whereas tolerance of the conidiospores of DMI-resistant isolates to heat treatment at 35-40℃ for 10-20 days was lower than that of the

sensitive isolates. Even in the fields where DMI-resistant isolates occurred, leaf mold was effectively controlled by systematical application of DMIs combined with iminoctadinealbesilate and azoxystrobin just after appearance of the first lesions.

ストロビルリン系薬剤耐性ナスすすかび病菌の発生と防除対策

／Occurrence of resistant strains to strobilurins in leaf mold caused by Mycovellosiella nattrassii on eggplant and its control

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ナスうどんこ病に対して登録されたアゾキシストロビン及びクレソキシムメチルは、ナスすすかび病に対しても卓効を示し、本病に対する登録も取得した。しかし、1998 年に高知県で実施された日本植物防疫協会の委託試験において、既に耐性菌の発生が予見される事例があった。これを実証するかのように、使用開始後間もない 1999～2000 年にかけて、ナスすすかび病に対してストロビルリン系薬剤の防除効果が低いという現地農家の声が相次いだ。

そこで、本病の耐性菌調査を実施したところ、ストロビルリン系薬剤耐性ナスすすかび病菌の発生を確認した。

遺伝子診断法による黒あし病菌の識別とジャガイモ塊茎からの検出

／Detection of Erwinia chrysanthemi in infected potato tubers by tuber incubation method and PCR

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Potato blackleg, a seed-borne bacterial disease affecting potato plants, is one of the serious diseases found in Hokkaido. Three species, *Erwinia carotovola* subsp. *Carotovora*, *E. carotovora* subsp. *Atroseptica*, *E. chrysanthemi* are known as pathogens of potato blackleg. Within a decade, the main causal pathogen of potato blakleg in Hokkaido has been *E. chrysanthemi*. The seed tubers contaminated by this pathogen cause rot of the aerial parts of the potato plants and progeny tubers. In a recent study, an effective method to detect blakleg pathogen *E. chrysanthemi* in potato tubers was established by Tuber Incubation Method and polymerase chain reaction (PCR) using specific primers. Therefore, by using this method the occurrence of potato blackleg would be able to be forecasted before the next planting season.

薬剤耐性菌の遺伝子診断 ／Gene diagnosis of fungicide-resistant isolates

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When considering supervised disease management systems, it is important to detect and quantify fungicide-resistant strains precisely and rapidly. Classical methods used for testing fungicide resistance include mycelial growth and spore germination tests in vitro, and inoculation tests on plants. These methods are time-consuming and laborious in general although they are still widely used. Recently, however, the DNA-based techniques for diagnosis of fungicide resistance have been developed and quantification of pathogen isolates resistant to particular fungicides is effectively achieved. In this paper, the author will summarize the recent advance of research related with gene diagnosis of fungicide resistance.

カンキツ緑かび病菌におけるステロール脱メチル化酵素遺伝子の大量発現と、DMI 剤耐性の遺伝子診断 ／Molecular mechanism of overexpression of the sterol 14-demethylase gene, encoding target enzyme of DMIs, and DNA diagnosis of DMI resistance in Penicillium digitatum

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We investigated the mechanism of demethylation inhibitor (DMI) fungicide-resistance in Penicillium digitatum, by isolating the CYP51 gene, which encodes the target enzyme (P45014DM) of DMI, from three DMI-resistant and three DMI-sensitive strains. The structural genes of all six strains were identical, but in the promoter region, a unique 126 bp sequence was tandemly repeated five times in the DMI-resistant strains, while present only once in the DMI-sensitive strains. Constitutive expression of CYP51 in the resistant strains was about 100-fold higher than that in the sensitive strains. We introduced CYP51, including the promoter region, from a DMI-resistant strain into a -sensitive strain, which rendered the transformants DMI-resistant and increased CYP51 expression. These results indicate that the 126 bp unit acts as a transcriptional enhancer and that a tandem repeat of the unit enhances CYP51 expression, resulting in DMI-resistance. Based on this finding, we developed a simple method for detecting DMI-resistant strains of this fungus.