岡山県における野菜病害虫と耐性菌 ／Plant Disease in vegetables and fungicide resistance in Okayama Prefecture

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第 10 回殺菌剤耐性菌研究会シンポジウム講演要旨(2000, P1-6) ／ Abstracts of the 10th Symposium of Research Committee on Fungicide Resistance

岡山県で栽培される農作物は栽培面積が取り立てて多いものはないが、種類は多い。主なものは水稲 37,600ha、大豆 3,310ha、モモ 780ha、ブドウ 1,194ha、トマト 144ha、ナス 225ha、キュウリ 183ha、ダイコン 610ha、ハクサイ 468ha、キャベツ 415ha、タマネギ 240ha、レタス 137ha、イチゴ 75ha、キク 47ha である。これらの作物には多種類の病害が発生しており、その防除の主体は薬剤防除となっている。そして薬剤防除の宿命として、病害の種類によっては薬剤耐性の発達が問題化している。

ここでは、野菜を中心に薬剤耐性菌の発達実態と対応を紹介したい。

マメ類灰色かび病菌のフルアジナム耐性と対策

／Fluazinam resistance in leguminous Botrytis cinerea and resistance management

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第 10 回殺菌剤耐性菌研究会シンポジウム講演要旨(2000, P7-16) ／Abstracts of the 10th Symposium of Research Committee on Fungicide Resistance

Botrytis cinerea Persoon :Fries によるマメ類の灰色かび病は、北海道においてしばしば多発し、大きな被害をもたらす。

本病の防除には当初ジクロゾリン剤が用いられたが、本剤は残留毒性の問題で 1973 年に製造中止となった。その後チオファネートメチル剤、ジカルボキシイミド系剤が次々と登場し、菌核病防除と兼ねて幅広く用いられてきた。しかし、チオファネートメチル剤については 1978 年青田らによって、ジカルボキシイミド系剤については 1991 年堀田、谷井らによって耐性菌の出現が報告され、使用が制限されてきた。このようなことから 1991 年から代替薬剤としてフルアジナム剤が登場し（長浜ら、1994）、集中的に使用され、マメ類生産の安定化がはかられた。ところが、フルアジナム剤も 1996 年ころから防除効果の低下が農家から報告されるようになった。

このことから本剤に対する灰色かび病菌の感受性検定を行ったところ、耐性菌の出現が明らかとなったので、インゲンマメの灰色かび病及び菌核病の防除体系についても併せて検討した。

シモキサニルの作用機構と耐性菌対策 ／Mode of action of cymoxanil and resistance management

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第 10 回殺菌剤耐性菌研究会シンポジウム講演要旨(2000, P17-26) ／ Abstracts of the 10th Symposium of Research Committee on Fungicide Resistance

シモキサニル（cymoxanil, 商品名 CurzateR）は、1972 年に米国デュポン社（E. I. Du Pont de Nemours and Company）によって開発された卵菌類による植物病害に対して卓効を示す殺菌剤で、1976 年にフランスで登録・上市されて以来、現在ではその混合剤は 50 ヶ国以上において 15 種類以上の作物の病害に登録されている。 シモキサニルは、ブドウや蔬菜類のべと病、ジャガイモ疫病等に対して卓効を示し、強い治療効果を持つが、植物体内における迅速な代謝に起因する比較的短い残効性が特徴としてあげられる（Douchet et al., 1977; Klopping & Delp, 1980; Clerjeau et al.,1981）。

この特性と、耐性菌対策の観点から、シモキサニルはほぼ全ての場合において、他の保護殺菌剤もしくはシステミックな作用を持つ殺菌剤との混合剤として使用されてきた。フェニルアマイド耐性菌の出現（Clerjeau & Simone,1982）以降は、とくに多くの混合剤において主要な殺菌成分として使用されてきている。混合剤においては、相手剤との高い相乗効果が認められ、それぞれの単剤使用において期待されるよりも高い防除効果を示すとともに（Grabski and Gisi,1987; Samoucha & Gisi, 1987; Bugaret et al.,1996）、混合相手剤を単剤で使用する場合に比較して、散布間隔を長くとることが可能となる。例えばマンゼブとの混合の場合、ブドウべと病防除場面で、マンゼブ単剤使用は 7～8 日間隔の散布が標準であるのに対し、混合剤は通常 10～12 日間隔の散布が推奨されている。 これまで、シモキサニルは耐性菌の発現しない薬剤という認識が強かったが、近年イタリアから低感受性菌の報告例もあるので、ここでは本剤の作用機構・作用特性にあわせて、シモキサニル耐性菌検定において当社の推奨する試験方法と、それに関連する種々の試験例ならびに当社の見解を紹介する。

灰色かび病菌のフルジオキソニルに対する感受性検定法

／Methods to determine the sensitivity to fludioxonil in Botrytis cinerea

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第 10 回殺菌剤耐性菌研究会シンポジウム講演要旨(2000, P27-34) ／ Abstracts of the 10th Symposium of Research Committee on Fungicide Resistance

In order to know the baseline sensitivity to fludioxonil, 106 isolates of Botrytis cinerea were collected from vegetable fields, where fludioxonil had not been applied before, in 6 prefectures during 1996 - 1997. The tests were conducted by dilution plate technique using PDA (potato dextrose agar) medium. The EC50 value of each isolate was determined based on the mycelial growth. The EC50 values varied from 0.001 to 0.011 mg/l with a median of 0.004 mg/l. About 95% of the tested isolates showed EC50 values below 0.008 mg/l. All the isolates which showed EC50 value above 0.008 mg/l were confirmed to be sensitive to fludioxonil *in vivo*. Since the isolates varied in resistance to benzimidazoles, dicarboximides and N-phenylcarbamates, but were all sensitive to fludioxonil, the results demonstrate the absence of cross-resistance between fludioxonil and the other botryticides. No cross-resistance between fludioxonil and anilinopyrimidines was also indicated *in vivo*.

ストロビルリン系薬剤耐性キュウリうどんこ病菌およびべと病菌の生物学的特性

／Biological properties of strobilurin-resistant isolates of Sphaerotheca fuliginea and Pseudoperonospora cubensis

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第 10 回殺菌剤耐性菌研究会シンポジウム講演要旨(2000, P35-42) ／ Abstracts of the 10th Symposium of Research Committee on Fungicide Resistance

ストロビルリン系殺菌剤は、園芸用として日本ではストロビー剤が 1997 年 12 月、アミスター剤が 1998 年 4 月に登録を取得し上市された。両剤とも、幅広い抗菌スペクトラムと高い効果を併せ持つことから広く使用されている。しかし、1999 年 10 月の日本植物病理学会関東部会および関西部会で、キュウリうどんこ病およびキュウリべと病で耐性菌の発生が報告された。薬剤耐性菌が発生した場合、その後の防除対策を検討する上で、薬剤耐性菌の適応性が薬剤感受性菌に比べて劣っているかの検討が重要と考えられる。ここでは、キュウリうどんこ病とべと病で、耐性菌と感受性菌の競合力を検討し、若干の知見が得られたので紹介する。

なお、ここで紹介する試験は、全農農薬研究部殺菌剤研究室の試験結果と、宮崎県立総合農業試験場および農業環境技術研究所と共同で実施した試験の結果である。

植物病原菌のストロビルリン系薬剤耐性菌と耐性機構に関する考察 ／Strobilurin resistance in phytopathogenic fungi and supposed mechanism of resistance

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第 10 回殺菌剤耐性菌研究会シンポジウム講演要旨(2000, P43-52) ／Abstracts of the 10th Symposium of Research Committee on Fungicide Resistance

In Japan, the strobilurin fungicides kresoxim-methyl and azoxystrobin were registered between late 1997 and early 1998. However, soon after the commercialization, control failure of powdery mildew by these fungicides has been reported frequently in cucumber growing areas. Bioassay data clearly showed the wide range of distribution of fungal isolates resistant to strobilurins. Subsequently, resistant isolates have also been detected in the population of downy mildew pathogen against which control efficacy of these fungicides was inadequate. Fragments of mitochondrial cytochrome b gene were PCR-amplified from fungal DNA and their sequences were analyzed to know the molecular mechanism of strobilurin resistance. A single point mutation, I.e. one base change at codon 143, was found in the gene from two resistant isolates of *Pseudoperonospora cubensis*. Substitution of glycine at codon 143 in cytochrome b by alanine seemed to result in high resistance to strobilurins. The same mutation was found in one resistant isolate of *Sphaerotheca fuliginea*. In this fungus, however, mutations in other sites of cytochrome b gene might also be related with resistance. The background involving in the rapid development of strobilurin resistance is discussed.

Strobilurin resistance, recent observations in cereal and cucurbit pathogens

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第 10 回殺菌剤耐性菌研究会シンポジウム講演要旨(2000, P53-60) ／Abstracts of the 10th Symposium of Research Committee on Fungicide Resistance

The strobilurin fungicides were first used, commercially, in Western Europe for the control of cereal diseases in 1996. Subsequently, this new group of fungicides (classified by FRAC as STAR's: Strobilurin Type Action and Resistance) have been developed globally on a wide range of crops. The STAR fungicides have a novel single site mode of action, exerting their effect on fungi by blocking the ubiquinol: cytochrome c oxidoreductase complex (cytochrome bc1) thus reducing the generation of energy rich ATP in the fungal cell, and constitute a new cross-resistance group. Resistance to single site inhibitors is well known in fungicides (Brent and Hollomon 1998) and is often characterised by changes to the target protein of the fungicide (Hollomon et al. 1997). Resistance to strobilurin fungicides was first reported in strains of *Erysiphe graminis* tritici from northern Germany, in 1998. We report here on the detection of resistance by our laboratory and on recent attempts to detect strobilurin resistance in two other important cereal pathogens, *Mycosphaerella graminicola (Septoria tritici*) and *Pyrenophora teres*. We also report on a more recent case of strobilurin resistance in *Sphaerotheca fuliginea*. Sensitivity and survey data are presented with a molecular analysis of resistant strains which has helped to indicate the mechanism of resistance determining strobilurin resistance in these pathogens in the field.

Resistance to strobilurins and management strategies

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第 10 回殺菌剤耐性菌研究会シンポジウム講演要旨(2000, P61-71) ／Abstracts of the 10th Symposium of Research Committee on Fungicide Resistance

Fungicide resistance as a phenomenon already known since the late 1960's, but has become an increasing concern during the seventies and the eighties. Table I shows an updated list of different fungicide groups, their market introduction and first appearance of resistance. Isolated cases of resistance under trial conditions are not listed.Factors influencing the risk of resistance development or selection stem from two sides. On the one hand there are the properties of the fungicide (active ingredient) itself. Higher risk has to be assumed for single site inhibitors, chemicals with high intrinsic activity, systemic compounds and products with curative properties. On the other hand there are target pathogens that are more prone to develop resistance than others. Higher resistance risk lies with pathogens with an abundant production of spores and means of widespread spore dispersal, short generation times, pathogens against which applications are commonly curative or even eradicant, fungi that can infect all crop growth stages, have a high spontaneous mutation rate and diseases which need to be sprayed many times per season or where other control measures are not available (Brent, K; Hollomon, D.; 1998).Generally there are two main types of fungicide resistance, one being the quantitative or stepwise selection of less sensitive strains, the other being the qualitative or disruptive type, with a clear separation of sensitive and resistant strains within a population. In Table 1, both types are included; I.e. with the benzimidazoles the qualitative type, where efficacy is insufficient to control for example grey mould in many regions. Sometimes qualitative pathogen resistance is closely linked to a more a less severe competitive disadvantage for the pathogen, an example being phenylamide resistance of potato late blight (Staub, T; 1994).

An example for quantitative selection are the prominent DMI fungicides against powdery mildews. Although there is a widespread shifting of populations to more insensitive strains, efficacy can still be sufficient to control the disease. In the case of the strobilurin fungicides, failure to control powdery mildew of wheat, *Blumeria graminis* var. *tritici* occurred only two years after market introduction in North Germany. A different species, cucumber powdery mildew (*Sphaerotheca fuliginea*), showed strobilurin resistance in Taiwan only one year after market introduction and in some Japanese regions after only two applications with strobilurin fungicides no disease control was achieved. To date only found in Japan were resistant strains of cucurbit downy mildew (*Pseudoperonospora cubensis*) (Ishii et al.; 1999). For three further pathogens isolated cases of insensitivity are reported. These .are barley powdery mildew *(Blumeria graminis* f. sp. *Hordei*) grape downy mildew (*Plasmopara viticola*) and Black Sigatoka on bananas *(Mycosphaerella fljiensis*) (STAR-FRAC WebPage, 1998 & 1999). Resistant barley powdery mildew was found under practical conditions, whereas grape downy mildew and Black Sigatoka where reported from trial sites. Tests showed for the above mentioned diseases a clear cross-resistance with all fungicides exhibiting the same biochemical mode of action of inhibition of mitochondrial respiration resulting from a blockage of the electron transport from ubihydroquinone to cytochrome c by means of a binding to the ubihydroquinone oxidation centre (Qo) of the cytochrome bc1 complex (Complex III) (Ammermann et al., 1992). Sequencing showed a point mutation in the cytochrome bc1 complex to be the cause of resistance (not yet confirmed for cucurbit downy mildew and barley powdery mildew). Fitness and stability tests for the mutants are in progress. Results for wheat powdery mildew and cucumber powdery mildew indicate only slight disadvantages in pathogenicity for the resistant strains. Spore mixture tests of 50% sensitive and 50% resistant spores do not show a practically exploitable reduction of resistant strains under conditions without selection pressure [Cronshaw, 1999 personal communication]. Other pathogens like *Plasmopara viticola* show less stability without selection pressure in some tests, in other test no significant reduction in vitality could be found. In field conditions on the isolated trial area where the resistance was found in 1998, only very few resistant isolates could be found after one strobilurin free season; this could indicate some competitive disadvantage.Monitoring activities of all three companies presently involved in the STAR working group of FRAC in 1999 have yielded no further evidence for resistant strains of any other pathogen than the ones stated. Monitoring though cannot practically cover all regions, where STAR products are used and also not every pathogen which is a target.