Characterization and Genetic Analysis of Laboratory Mutants of *Ustilago maydis* Resistant to Dicarboximide and Aromatic Hydrocarbon Fungicides

A. B. Orth, A. Sfarra, E. J. Pell, and M. Tien

First and fourth authors: Department of Molecular and Cell Biology; second and third authors: Department of Plant Pathology, The Pennsylvania State University, University Park 16802.

Current address A. B. Orth: Dow Elanco Discovery Research, Indianapolis, IN 46268.

Supported in part by a Pennsylvania State University Intercollege Research Grant (Project 3138). A. Orth is a postdoctoral Fellow supported by National Research Service Award 1-F32-ES05503-01 from the National Institute of Environmental Health. M. Tien is the recipient of Presidential Young Investigator Award DCB-8657853 from the National Science Foundation.

We thank S. A. Leong and A. D. Budde for their valuable assistance on this project.

Accepted for publication 22 July 1994.

ABSTRACT

Orth, A. B., Sfarra, A., Pell, E. J., and Tien, M. 1994. Characterization and genetic analysis of laboratory mutants of *Ustilago maydis* resistant to dicarboximide and aromatic hydrocarbon fungicides. Phytopathology 84:1210-1214.

Mutants of *Ustilago maydis* resistant to dicarboximide (DCOF) and aromatic hydrocarbon fungicides (AHF) were isolated after chemical mutagenesis or by selection of spontaneous mutants on fungicide-amended agar medium. A wide range of mutants, with low to high levels of resistance, were isolated. The resistance factor compared to the wild type was greater than 150 for three mutants isolated on tolclophos-methyl and greater than 50 for three mutants isolated on vinclozolin. Growth rates for these six most tolerant isolates were similar to that of the wild type. The phenomenon of cross-resistance between these two groups was confirmed. None of the mutants were resistant to the ergosterol biosynthesis inhibitor propiconazole. Two mutants, VR43 and VR44, demonstrated the same osmotic sensitivity when grown in the presence of NaCl (1–8%) as the wild type under these conditions. One mutant, TR02, showed a small but significant difference in osmotic sensitivity. Genetic analysis revealed that a single chromosomal gene codes for resistance in four of these isolates. Further analysis demonstrated the presence of at least three resistance genes (*adr-1, adr-2,* and *adr-3*). These studies have laid the groundwork for further research to elucidate the mechanism of resistance to AHF and DCOF and possibly the mode of action as well.

Resistance of plant pathogenic fungi to selective fungicides has become increasingly important as a higher incidence of resistance appears in the field (3,6,28). The dicarboximide fungicides (DCOF) are a very important group, with activity against *Botryotinia fuckeliana* (de Bary) Whetzel (anamorph: *Botrytis cinerea* Pers.:Fr.), the causal agent of gray mold of grapes, but resistance to these antimycotics is widespread among field isolates (21,26,29). Because resistance to benzimidazoles also has become a problem in recent years, the choices for control of this and other important diseases could become severely limited. Detailed biochemical and genetic study of laboratory isolates resistant to these compounds will aid in the development of antiresistance strategies that could avoid further resistance problems and extend the usefulness of these fungicides.

Laboratory and field resistance of a number of fungi to these compounds has been characterized. Resistance to DCOF has been investigated in field isolates of *Monilinia laxa* (18) and *Sclerotinia homoeocarpa* (7). In *B. fuckeliana*, the genetics of resistance to

DCOF also has been studied in some detail (10). The phenotype of high levels of resistance co-segregates with higher osmotic sensitivity in meiotic crosses of *B. fuckeliana*. Field isolates of *B. fuckeliana* generally display a lower level of resistance than the laboratory mutants. The most complete studies have been done on *Neurospora crassa*, in which mutation of any of seven major genes confers resistance and is often associated with increased osmotic sensitivity (15). That these two phenotypes are controlled by the same gene in some mutants is of great interest, because this may shed light on the nature of resistance to these compounds.

In this study, *Ustilago maydis* (DC.) Corda was used as a model to help further our understanding of the nature and genetic basis for resistance to the DCOF. Because previous studies have suggested that the aromatic hydrocarbon fungicides (AHF) may have a mode of action similar to the DCOF, as indicated by cross-resistance between the two groups (20,22), we have included representative compounds from each. *U. maydis* is an ideal organism for this investigation because it is quite sensitive to these compounds (24), it grows in culture in a yeastlike form that allows convenient biochemical study, it can easily be genetically characterized by established techniques, and techniques of molecular manipulation in this organism are becoming fairly straightforward. Once resistant isolates are well understood, they then can be used to clone the genes for resistance, which will be a powerful tool to increase our understanding of the mode of action and the mechanisms of resistance. This work describes the isolation, characterization, and genetic analysis of laboratory mutants of *U. maydis* resistant to these fungicides.

MATERIALS AND METHODS

Culture methods. *U. maydis* wild-type strains 518 (*a2b2*) and 521 (*a1b1*) were a gift from S. Leong (University of Wisconsin, Madison). The wild type and all resistant isolates of this organism were cultured in a defined liquid nutrient medium that has been described previously by Coursen and Sisler (4) supplemented with yeast extract at 2 g/L (medium 63). Cultures were incubated at 30 C with agitation at 230 rpm on a rotary shaker overnight.

Inhibitors. Inhibitors were added to cultures as methanolic solutions with appropriate solvent controls. The solvent did not exceed 0.5% of the culture medium in treated or control cultures. The chemicals used were as follows: tolclophos-methyl (Sumitomo Chemical Company, Ltd., Takarazuka, Japan), iprodione (Rhone-Poulenc Agrochimie, Aubervilliers, France), chloroneb (DuPont Company, Wilmington, DE), vinclozolin (BASF Aktiengesellschaft, Limburgerhof, Germany), and propiconazole (Ciba-Geigy Corp., Greensboro, NC).

Isolation of resistant mutants. Mutants were derived from wild-type strain 521 (*a1b1*) by treatment with *N*-methyl-*N*-nitro-*n*-nitrosoguanidine (MNNG) at 5 μg/ml (30). Cells were incubated for 1.5 h in growth medium containing the mutagen, washed once, and resuspended in a defined liquid medium previously described by Ragsdale and Sisler (27; medium 72) for 4 h. A concentrated suspension of treated sporidia was spread over the surface of agar medium (medium 72 with agar added at 20 g/L; approximately 250,000 viable cells per plate) containing vinclozolin at 64 μg/ml or tolclophos-methyl at 6.4 μg/ml. Resistant isolates were maintained on medium 63 containing tolclophos-methyl at 5 μg/ml or vinclozolin at 20μg/ml.

Toxicity measurements. Growth rates and resistance levels of six isolates were determined in liquid shaking culture. Inoculum of each strain grown in liquid culture was standardized to 2.0 $\times 10^7$ cells per milliliter and diluted 50-fold with defined nutrient medium 72 (27) containing various concentrations of vinclozolin or tolclophos-methyl. Cultures were incubated at 30 C with agitation at 230 rpm on a rotary shaker, and absorbance at 450 nm was measured at 3 and 6 h. Values obtained represent the mean of triplicate flasks plus or minus standard deviation. Preliminary determinations of levels of resistance of 88 isolates were made by the same method based on growth in fungicide-amended medium 72 after 6 h.

Osmotic sensitivity. The sensitivity of selected isolates to high osmotic pressure as compared to that of the wild type was determined as described above for toxicity measurements with liquid culture medium 72 (27) amended with 0–8% (w/v) NaCl. Growth in agitated liquid cultures was determined by measuring the change in absorbance (A_{450nm}) after 6 h. Values are means of triplicate flasks plus or minus standard deviation, expressed as percent growth of untreated control.

Cross-resistance. Cross-resistance of vinclozolin- and tolclophos-methyl–resistant isolates of *U. maydis* to other inhibitors was determined by plating approximately 100 sporidia per plate on agar medium 72 (27) amended with various concentrations of the fungicides. Plates were evaluated 4 days after inoculation for the ability of the isolate to form colonies.

Genetic techniques. Crosses were carried out on maize cultivar Golden Bantam by previously described procedures (17). Five-day-old corn seedlings were injected at the soil line with 500 μl of fungal cell suspensions with a 1-ml syringe and 26-gauge needle. Plants were maintained in a greenhouse until formation of mature galls, whereupon teliospores were germinated and haploid basidiospores were plated on medium 72 then replica plated onto medium 72 containing vinclozolin at 50 μg/ml. At least 200 meiotic products (sporidia) per cross were analyzed.

RESULTS

Toxicity measurements. An important indication that potential for field resistance exists with a particular fungicide is the frequency of mutation resulting in fungicide-resistant isolates in the laboratory (19). Vinclozolin- and tolclophos-methyl–resistant isolates of *U. maydis* were readily obtained by treatment with MNNG or merely by selection of spontaneous mutants on high concentrations of fungicide. A wide range of resistance levels were apparent when isolates were screened in fungicide-containing liquid medium (Table 1). Isolates showed from 3–62% inhibition by tolclophos-methyl at 6.4 μg/ml and 3–43% inhibition by vinclozolin at 32 μg/ml after 6 h of growth. These concentrations inhibited growth of the wild-type isolate by 100%, indicating a range of resistance from low to high levels for these mutants. From this initial screening, the most highly resistant isolates were further characterized. Three vinclozolin-resistant isolates (VR43, VR44, and VR02) and three tolclophos-methyl–resistant isolates (TR02, TR04, and TR08) were resistant to the limits of solubility of vinclozolin (64 μg/ml) or tolclophos-methyl (50 μg/ml) after growth in liquid shaking culture for 6 h (Table 2).

TABLE 1. Levels of resistance of *Ustilago maydis* laboratory isolates selected on tolclophos-methyl (TM)- or vinclozolin (VN)-amended media[a]

Isolates on TM	Inhibition at 6.4 μg/ml TM[b] (%)	Isolates on VN	Inhibition at 32 μg/ml VN[b] (%)
TR02	3	VR43	3
TR08	5	VR44	3
TR04	6	VR02	5
TR05	6	VR33	8
TR30	6	VR28	13
TR16	7	VR06	14
TR21	9	VR20	15
TR28	10	VR08	16
TR27	12	VR21	18
TR33	18	VR23	18
TR37	27	VR29	21
TR40	31	VR09	22
TR38	32	VR22	22
TR35	34	VR24	22
TR41	40	VR03	23
TR18	43	VR01	24
TR34	43	VR04	25
TR32	62	VR05	43

[a]Levels of resistance were determined by spectrophotometrically evaluating growth of resistant isolates in liquid shaking cultures after 6 h.
[b]This fungicide concentration inhibits growth of the wild-type isolate by 100% under these conditions.

Growth rates of the resistant mutants is one parameter to assess their fitness relative to the wild type. Figure 1 shows that the growth rates of the six isolates described above were all similar to that of the wild type. The wild-type doubling time was approximately 3 h, whereas two resistant isolates had faster doubling times of 2.6 h (VR43) and 2.2 h (VR02). Two other isolates, TR08 and VR44, showed doubling times nearly identical to that of the wild type (3.1 h), whereas the remaining two grew slightly slower. The high level of resistance found with these mutants does not appear to be associated with a reduced growth rate by the cells.

Cross-resistance. It was determined previously that the AHF and DCOF exhibit cross-resistance (20,22). We tested this using our resistant isolates of *U. maydis* (Table 3). Mutants isolated with tolclophos-methyl for selection showed at least a fourfold greater tolerance to the AHF chloroneb and a two- to eightfold greater tolerance to the DCOF vinclozolin and iprodione than did the wild-type isolate. Similar results were obtained with mutants isolated on vinclozolin-amended medium. Mutants isolated with vinclozolin for selection showed a greater than eight- to 64-fold increase in tolerance to chloroneb and tolclophos-methyl. None of the isolates showed cross-resistance to the ergosterol biosynthesis inhibitor (EBI) propiconazole.

Osmotic sensitivity. In *B. fuckeliana* and *N. crassa*, resistance to these fungicides is often associated with an increase in osmotic sensitivity (5,14). We tested three of the *U. maydis* mutants for this phenotype by growing them for 6 h with varying concentrations of NaCl (Fig. 2). VR43 and VR44 both had the same sensitivity to NaCl (1–8%) as the wild type. TR02 showed a small but significant difference in sensitivity to all concentrations of NaCl, with approximately a 10–12% greater inhibition of growth than the wild type.

Genetic analysis of resistance. The resistant isolates showing the highest levels of resistance and growth rates were evaluated for the genetics of their resistance. This was accomplished by crossing the resistant mutants derived from wild-type strain 521 (*a1b1*) with the sensitive wild-type strain 518 of the opposite mating type (*a2b2*) and evaluating the phenotype of resulting haploid progeny on fungicide-containing medium by random spore analysis. With four mutants, a 1:1 ratio of sensitive/resistant progeny indicated that a single chromosomal gene was responsible for resistance (Table 4).

TABLE 2. Fungicide concentration giving 50% inhibition of growth (I_{50}) in highly resistant isolates of *Ustilago maydis*[a]

Isolate	Tolclophos-methyl I_{50} (μg/ml)	Isolate	Vinclozolin I_{50} (μg/ml)
Wild type	0.3	Wild type	1.3
TR02	>50.0	VR43	>64.0
TR04	>50.0	VR44	>64.0
TR08	>50.0	VR02	>64.0

[a]The I_{50} was determined by spectrophotometrically evaluating growth of resistant isolates in liquid shaking cultures during early log phase growth.

Further crosses were performed to determine the number of complementation groups. Opposite mating types of each strain were isolated and crossed. If the gene for resistance was the same from different isolates, one would expect all the haploid progeny to be resistant. However, if different genes code for resistance, independent assortment would dictate that approximately 75% would be resistant and 25% would be sensitive. The data in Table 5 demonstrate that two isolates, VR33 and VR43, harbored the same gene for resistance (*adr-1*), because crossing these two mutants produced 100% resistant progeny. However, VR44 had a resistance gene different from *adr-1* (*adr-2*), giving 68% resistant progeny when crossed with VR33 and 67% resistant progeny when crossed with VR43. TR02 showed an independent assortment of a third gene (*adr-3*) from the first two genes, producing 72, 70, and 71% resistant progeny when crossed with VR33, VR43, or VR44, respectively. Thus, at least three different complementation groups are possible to confer resistance to these fungicides in *U. maydis*.

DISCUSSION

Tolclophos-methyl- and vinclozolin-resistant mutants of *U. maydis* were readily obtained by chemical mutagenesis with MNNG or by selecting for spontaneous mutations. Several highly resistant mutants exhibited a tolerance level that exceeded the solubility of the compounds in culture medium. Each of these isolates also showed normal morphology and a growth rate similar to that of the wild-type isolates. These growth characteristics are important indicators as to the ability of these mutants to survive under natural conditions. In addition, when these strains were

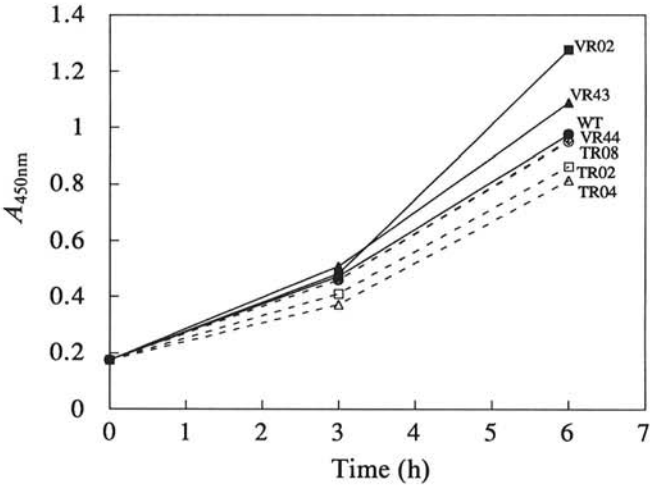

Fig. 1. Growth rates of sensitive *Ustilago maydis* sporidia and mutants highly resistant to tolclophos-methyl (TR08, TR02, TR04) or vinclozolin (VR02, VR43, VR44). Growth was determined by measuring the change in optical density after 3 and 6 h of incubation in agitated liquid cultures. Mean of triplicate flasks plus or minus standard deviation. Error bars are smaller than symbols. Initial $A_{450nm} = 0.18$.

TABLE 3. Cross-resistance of tolclophos-methyl- and vinclozolin-resistant isolates of *Ustilago maydis* to various fungicides

Fungicide	Isolate[a,b] Wild-type 518	TR02	TR04	TR08	VR43	VR02	VR44
Tolclophos-methyl	0.5	>32 (>64)	>32 (>64)	>32 (>64)	>32 (>64)	>32 (>64)	>32 (>64)
Chloroneb	8	>64 (>8)	>64 (>8)	32 (4)	>64 (>8)	>64 (>8)	>64 (>8)
Vinclozolin	8	16 (2)	>64 (>8)	>64 (>8)	>64 (>8)	>64 (>8)	>64 (>8)
Iprodione	8	16 (2)	>64 (>8)	16 (2)	16 (2)	16 (2)	16 (2)
Propiconazole	<2	<2 (1)	<2 (1)	<2 (1)	<2 (1)	<2 (1)	<2 (1)

[a]Evaluated after 4 days on fungicide-containing agar medium based on 100% colony formation. Maximum concentration (micrograms per milliliter) permitting colony formation was used. Values with a > symbol indicate that colonies were formed at fungicide concentrations at the limits of solubility in agar medium.

[b]Figures in parentheses are resistance factors (maximum concentration permitting colony formation by mutant/maximum concentration permitting colony formation by the wild-type isolate).

crossed and inoculated on corn to complete their life cycle, they produced galls as with the wild-type 518 × wild-type 521 cross, indicating no loss of pathogenicity. From our studies, it appears that the mechanism of fungicide resistance in these mutant fungal strains does not compromise their growth or pathogenicity, suggesting that their potential to compete with the wild-type isolates may not be compromised.

Previous researchers have found results in other organisms that contrast those presented here with *U. maydis*. For example, resistant strains of *B. fuckeliana* were markedly less aggressive in infection of carrot roots, were relatively slow growing, and had a markedly lower level of sporulation than did the sensitive wild type (5). Similar results for *B. fuckeliana* were obtained by other workers, who speculated that a decrease in fitness of the resistant strains could be accounted for by the correlation between resistance and sensitivity to high osmotic pressure (2). Beever (1) found only a low level of resistance in vitro in strains that were not osmotically sensitive. Grindle and Temple (16) showed that, in general, resistant mutants of *N. crassa* are less fit than DCOF-sensitive strains, based on growth, sporulation, and osmotic sensitivity.

A correlation between osmotic sensitivity and DCOF resistance has been found in many studies of laboratory and field isolates of *B. fuckeliana* (1,2,10), *Aspergillus nidulans* (1), *Penicillium expansum* (1), and *N. crassa* (12,14–16). In *B. fuckeliana*, the relationship between osmotic sensitivity and fungicide resistance is variable both in field and laboratory isolates (26). Although an increase in osmotic sensitivity was found in field isolates containing *Daf*LR (low resistance) alleles, it was so small that it was difficult to interpret among other changes in phenotype (10). Laboratory isolates with the *Daf*HR (high resistance) alleles showed a much larger increase in osmotic sensitivity that co-segregated in meiotic crosses, although one highly resistant exception that was not osmotically sensitive also was found.

Studies have ruled out the possibility that mutants of *N. crassa* lack the ability to synthesize and accumulate polyols during osmotic adjustment but may differ in their ability to retain these solutes because of a change in membrane permeability (9). It has been shown that resistance to these fungicides in *N. crassa* can result from mutations in *os-1, os-2, os-4, os-5* (14), *smco-2, smco-8,* and *smco-9* (15), which may be associated with a change in cell wall composition. One *N. crassa* isolate that combined the traits of high fungicide resistance, low osmotic sensitivity and excellent sporulation, and that was identified as carrying the *os-5* gene, also carried a modifier gene, *mod* (13). This research with *N. crassa* supports our observation with *U. maydis* that mutants

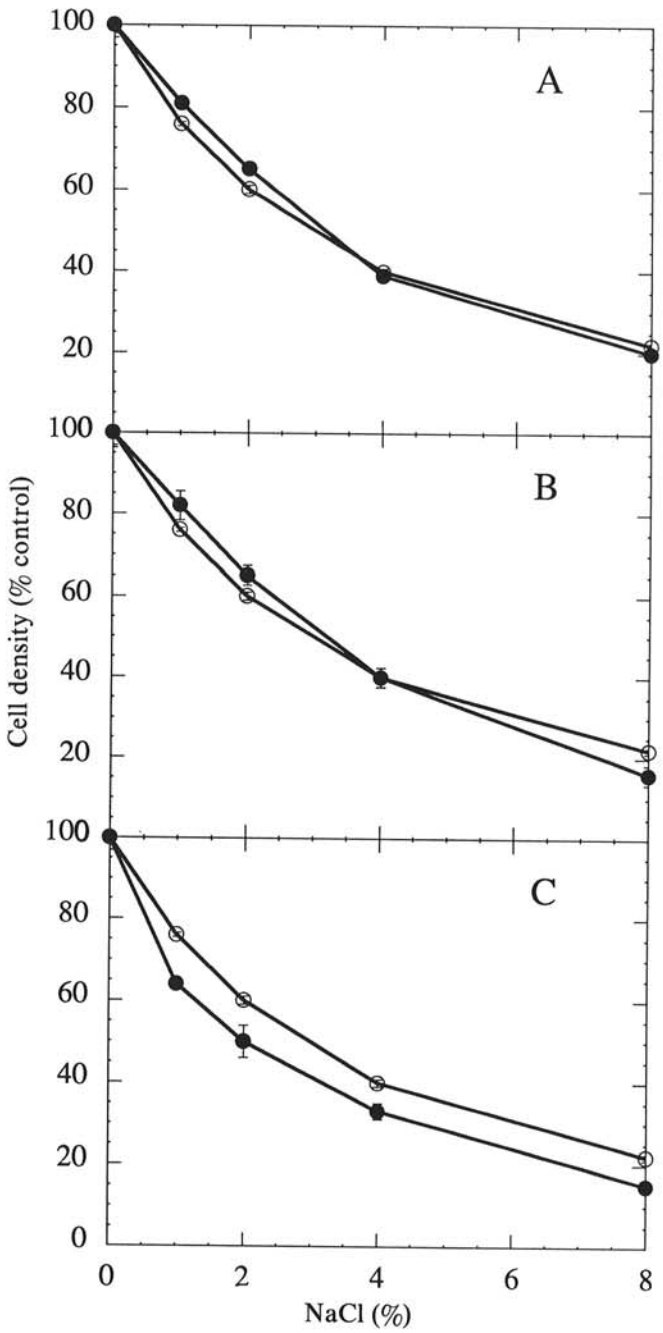

Fig. 2. Osmotic sensitivity of *Ustilago maydis* wild-type isolate (●) and mutant (○) isolates highly resistant to vinclozolin (**A,** VR43 and **B,** VR44) or tolclophos-methyl (**C,** TR02). Growth in agitated liquid cultures in the presence of increasing concentrations of NaCl was determined by measuring the change in absorbance (A_{450nm}) after 6 h. Values are means of triplicate flasks plus or minus standard deviation, expressed as percent growth of untreated control.

TABLE 4. Random spore analysis of crosses between vinclozolin- or tolclophos-methyl–resistant mutants and sensitive strains of *Ustilago maydis*[a]

Parents	Mating type[b]	Resistance phenotype[c]	Resistant F_1 progeny[d] (%)
Wild type × VR43	*a2b2* × *a1b1*	S × R	47
Wild type × VR44	*a2b2* × *a1b1*	S × R	48
Wild type × VR33	*a2b2* × *a1b1*	S × R	57
Wild type × TR02	*a2b2* × *a1b1*	S × R	55

[a]Sporidia were analyzed for colony forming ability on media amended with tolclophos-methyl at 32 μg/ml or vinclozolin at 64 μg/ml after 4 days.
[b]Wild-type strain 518 (*a2b2*) was crossed with mutants derived from wild-type strain 521 (*a1b1*). Mating type was determined by assessing ability to back-cross with wild-type strain 518 (*a2b2*) or 521 (*a1b1*).
[c]S = sensitive; R = resistant.
[d]Two hundred meiotic products (sporidia) per cross were analyzed.

TABLE 5. Identification of resistance classes among single-gene resistant isolates of *Ustilago maydis*[a]

F_1 progeny cross[b]	Mating type[c]	Resistance phenotype[d]	Resistant F_2 progeny[e] (%)
VR33-A × VR44-D	*a1b1* × *a2b2*	R × R	68
VR33-A × VR43-B	*a1b1* × *a2b2*	R × R	100
VR33-A × TR02-B	*a1b1* × *a2b2*	R × R	72
VR43-A × VR44-A	*a1b1* × *a2b2*	R × R	67
VR43-A × TR02-B	*a1b1* × *a2b2*	R × R	70
VR44-A × TR02-A	*a2b2* × *a1b1*	R × R	71

[a]Sporidia were analyzed for colony forming ability on media amended with vinclozolin at 64 μg/ml after 4 days.
[b]F_1 generation strains were derived from previous crosses described in Table 4.
[c]Mating type was determined by assessing ability to back-cross with wild-type strain 518 (*a2b2*) or 521 (*a1b1*).
[d]R = Resistant to vinclozolin.
[e]Three hundred meiotic products (sporidia) per cross were analyzed.

may possess high resistance to these compounds while retaining a high degree of fitness according to the laboratory criteria of normal growth and sporulation and low osmotic sensitivity.

The results presented here show that resistance to the DCOF or AHF in four isolates of *U. maydis* is conferred by a single chromosomal gene and that at least three complementation groups are possible. Three isolates carrying different genes for resistance were tested for osmotic sensitivity, and only one showed a small difference in growth from the wild-type isolates. It appears that *U. maydis* can become resistant without the presence of a modifier gene to mediate fitness. The types of mutants obtained in this organism, with their high growth rates, pathogenicity, and lack of osmotic sensitivity, provide a strong contrast to those found in other organisms. We speculate that our isolates of *U. maydis*, particularly mutants carrying the genes *adr-1* and *adr-2*, likely contain a mutation in the target site of these compounds. A target site mutation may not involve changes in other phenotypes, as is supported by research conducted with terbinafine-resistant isolates of *U. maydis* (23).

Previous workers have established that cross-resistance occurs between resistance to DCOF and AHF (20,22). Our confirmation of these reports in the present study indicates that the target site may be the same for these two groups of compounds. It has been speculated that inhibition of sterol synthesis is the primary target of DCOF (25). If this is the case, one would expect at least some level of cross-resistance of DCOF- or AHF-resistant isolates to some members of the EBI. Although our studies do indicate that a high level of cross-resistance exists in our isolates between DCOF and AHF, no increased tolerance was observed to the EBI propiconazole by any of the five isolates tested. Other researchers have not found strong cross-resistance among EBI-resistant isolates of *A. nidulans* and *P. italicum* to iprodione, vinclozolin, or procymidone (6). Only two strains of *Cladosporium cucumerinum* showed simultaneous resistance to both EBIs and DCOF, but here the authors speculated that, lacking genetic evidence, this could reflect a nonspecific induction of the efflux mechanism (11).

The mode of action of DCOF and AHF has been under investigation for many years, using biochemical and genetic tools. Evidence for an oxygen-free radical mechanism exists for *B. cinerea* (8), but not for *U. maydis* (24). With the isolation of single-gene resistant mutants described in this work, we can now begin the process of cloning and identifying the resistance gene product by molecular methods. In this way, we may be able to elucidate the basis for resistance and the mode of action of these compounds.

LITERATURE CITED

1. Beever, R. E. 1983. Osmotic sensitivity of fungal variants resistant to dicarboximide fungicides. Trans. Br. Mycol. Soc. 80:327-331.
2. Beever, R. E., and Brien, H. M. R. 1983. A survey of resistance to the dicarboximide fungicides in *Botrytis cinerea*. N.Z. J. Agric. Res. 26:391-400.
3. Beever, R. E., and Byrde, R. J. W. 1982 Resistance to the dicarboximide fungicides. Pages 101-117 in: Fungicide resistance in Crop Protection. J. Dekker and S. G. Georgopoulos, eds. PUDOC, Wageningen, the Netherlands.
4. Coursen, B. W., and Sisler, H. D. 1960. Effect of the antibiotic, cycloheximide, on the metabolism and growth of *Saccharomyces pastorianus*. Am. J. Bot. 47:541-545.
5. Davis, R. P., and Dennis, C. 1981. Properties of dicarboximide-resistant strains of *Botrytis cinerea*. Pestic. Sci. 12:521-535.
6. Dekker, J., and Georgopoulos, S. G. 1982. Fungicide Resistance in Crop Protection. PUDOC, Wageningen, the Netherlands.
7. Detweiler, A. R., Vargas, J. M., Jr., and Danneberger, T. K. 1983. Resistance of *Sclerotinia homoeocarpa* to iprodione and benomyl. Plant Dis. 67:627-630.
8. Edlich, W., and Lyr, H. 1992. Target sites of fungicides with primary effects on lipid peroxidation. Pages 53-68 in: Target Sites of Fungicide Action. W. Koller, ed. CRC Press, Inc., Boca Raton, FL.
9. Ellis, S. W., Grindle, M., and Lewis, D. H. 1991. Effect of osmotic stress on yield and polyol content of dicarboximide-sensitive and -resistant strains of *Neurospora crassa*. Mycol. Res. 95:457-464.
10. Faretra, F., and Pollastro, S. 1991. Genetic basis of resistance to benzimidazole and dicarboximide fungicides in *Botryotinia fuckeliana* (*Botrytis cinerea*). Mycol. Res. 95:943-951.
11. Fuchs, A., de Vries, F. W., and deWaard, M. A. 1984. Simultaneous resistance in fungi to ergosterol biosynthesis inhibitors and dicarboximides. Neth. J. Plant Pathol. 90:3-11.
12. Grindle, M. 1984. Isolation and characterization of vinclozolin-resistant mutants of *Neurospora crassa*. Trans. Br. Mycol. Soc. 82:635-643.
13. Grindle, M., and Dolderson, G. H. 1986. Effects of a modifier gene on the phenotype of a dicarboximide-resistant mutant of *Neurospora crassa*. Trans. Br. Mycol. Soc. 87:457-459.
14. Grindle, M., and Temple, W. 1982. Fungicide resistance of *os* mutants of *Neurospora crassa*. Neurospora Newsl. 29:16-17.
15. Grindle, M., and Temple, W. 1983. Fungicide resistance of *smco* mutants of *Neurospora crassa*. Neurospora Newsl. 30:7-8.
16. Grindle, M., and Temple, W. 1985. Sporulation and osmotic sensitivity of dicarboximide-resistant mutants of *Neurospora crassa*. Trans. Br. Mycol. Soc. 84:369-372.
17. Holliday, R. 1979. *Ustilago maydis*. Pages 575-594 in: Handbook of Genetics, vol. 1. R. C. King, ed. Plenum Publishing Corp., New York.
18. Katan, T., and Shabi, E. 1982. Characterization of a dicarboximide fungicide-resistant laboratory isolate of *Monilinia laxa*. Phytoparasitica 10:241-245.
19. Köller, W., and Scheinpflug, H. 1987. Fungal resistance to sterol biosynthesis inhibitors: A new challenge. Plant Dis. 71:1066-1074.
20. Leroux, P., Fritz, R., and Gredt, M. 1981. Cross resistance between 3.5-dichlorophenyl cyclic imide fungicides (dichlozolin, iprodione, procymidone, vinclozolin) and various aromatic compounds. Pages 79-88 in: Systemic Fungicides and Antifungal Compounds. H. Lyr and C. Polter, eds. Akademie-Verlag, Berlin.
21. Lorenz, G. 1988. Dicarboximide fungicides: History of resistance development and monitoring methods. Pages 45-51 in: Fungicide Resistance in North America. C. J. Delp, ed. American Phytopathological Society, St. Paul, MN.
22. Lyr, H., and Casperson, G. 1982. On the mechanism of action and the phenomenon of cross resistance of aromatic hydrocarbon fungicides and dicarboximide fungicides. Acta Phytopathol. Acad. Sci. Hung. 17:317-326.
23. Orth, A. B., Henry, M. J., and Sisler, H. D. 1990. Mechanism of resistance to terbinafine in two isolates of *Ustilago maydis*. Pestic. Biochem. Physiol. 37:182-191.
24. Orth, A. B., Sfarra, A., Pell, E. J., and Tien, M. 1992. An investigation into the role of lipid peroxidation in the mode of action of aromatic hydrocarbon and dicarboximide fungicides. Pestic. Biochem. Physiol. 44:91-100.
25. Pappas, A. C., and Fisher, D. J. 1979. A comparison of the mechanism of action of vinclozolin, procymidone, iprodione, and prochloraz against *Botrytis cinerea*. Pestic. Sci. 10:239-246.
26. Pommer, E.-H., and Lorenz, G. 1982. Resistance of *Botrytis cinerea* Pers. to dicarboximide fungicides—A literature review. Crop Prot. 1:221-230.
27. Ragsdale, N. N., and Sisler, H. D. 1972. Inhibition of ergosterol synthesis in *Ustilago maydis* by the fungicide triarimol. Biochem. Biophys. Res. Commun. 46:2048-2052.
28. Sanders, P. L. 1991. Fungicide resistance in the United States. Report by the USDA/States NAPIAP Fungicide Assessment Project. USDA, Washington, D.C.
29. Sisler, H. D. 1988. Dicarboximide fungicides: Mechanisms of action and resistance. Page 52 in: Fungicide Resistance in North America. C. J. Delp, ed. American Phytopathological Society, St. Paul, MN.
30. Walsh, R. C., and Sisler, H. D. 1982. A mutant of *Ustilago maydis* deficient in C-14 demethylation: Characteristics and sensitivity to inhibitors of ergosterol biosynthesis. Pestic. Biochem. Physiol. 18:122-126.