Metabolism of Pisatin Stereoisomers by *Ascochyta rabiei* Strains Transformed with the Pisatin Demethylase Gene of *Nectria haematococca* MP VI

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The chickpea pathogen *Ascochyta rabiei* was transformed with plasmid pUCH1/PDA containing the hygromycin B resistance gene as a selectable marker and the pisatin demethylase gene of *Nectria haematococca* mating population VI, a pathogen of pea. The rate of transformation was approximately 2 transformants per µg of DNA. While untransformed *A. rabiei* is inhibited by hygromycin B at 50 µg/ml, transformants were able to grow on 300 µg of this antibiotic. Transformants demethylated the pea phytoalexin (+)-pisatin to (+)-6a-hydroxymaackiain (6a-HMK) within 2 to 4 h. The product was not further metabolized and accumulated in the medium. Untransformed strains failed to show any significant metabolism of (+)-pisatin to (+)-6a-HMK within a given interval of 24 h. One of the transformants, when tested for metabolism of (-)-pisatin, produced a demethylated product that only transiently accumulated in the medium. One subsequent degradation product was tentatively identified as 3,7,2′-trihydroxy-4′,5′-methylenedioxy isoflavone. These results support the hypothesis that the enzymes of *A. rabiei* involved in the metabolism of pterocarpan phytoalexins are specific for (-) isomers such as (-)-maackiain and (-)-medicarpin produced by the natural host of this fungus.

Additional keywords: phytopathogenic fungi, host-parasite interaction, ascomycetes.

A well-studied defense reaction of plants against a pathogenic microorganism is the accumulation of low molecular weight compounds called phytoalexins (Dixon 1986; Ebel 1986). The degradation of these substances to less toxic products has long been discussed as one means for a plant pathogen to overcome this defense barrier of the host (VanEtten et al 1989; Weltring 1992). The best studied example for the importance of phytoalexin degradation for virulence of a plant pathogen is the demethylation of the pea phytoalexin (+)-pisatin by *Nectria haematococca* Berk. & Broome mating population (MP) VI, a pathogen of pea (*Pisum sativum*) (Fig. 1; VanEtten et al. 1975). Pathogenic isolates of this fungus demethylate the phytoalexin to the less toxic product (+)-6a-hydroxymaackiain (6a-HMK) (VanEtten et al. 1980; Tegtmeyer and VanEtten 1982). The enzyme involved in this reaction has been characterized as a microsomal NADPH-dependent cytochrome P-450 monooxygenase (Matthews and VanEtten 1983; Desjardins et al. 1984; Desjardins and VanEtten 1986). Pisatin demethylase (PDA) can be independently conferred by one of at least six *PDA* genes identified so far (Kistler and VanEtten 1984; Mackintosh et al. 1989; Miao and VanEtten 1992). One *PDA* gene, designated *PDA9*, was cloned by expression in *Aspergillus nidulans* from a cosmid library of a pisatin-metabolizing isolate of *N. haematococca* MP VI (Weltring et al. 1988). The *PDA* gene was also expressed in *Cochliobolus heterostrophus* (Schäfer et al. 1989), which confirmed previous indications that the cytochrome P-450 hemoprotein encoded by *PDA9* can be reduced by a reductase present in filamentous fungi other than *N. haematococca* MP VI (Scala et al. 1988).

*Ascochyta rabiei* (Pass.) Labrousse is the causal agent of Ascochyta blight of chickpea (*Cicer arietinum*). Resistant plants accumulate high amounts of the pterocarpan phytoalexins (−)-medicarpin and (−)-maackiain (Fig. 2) in response to infection by the fungus (Jacques et al. 1987). *A. rabiei* is able to degrade the chickpea phytoalexins completely (Hölh et al. 1989; Tenhaken et al. 1991). As the first step of degradation, both pterocarpen are simultaneously converted to the corresponding 2′-OH isoflavans and the 1a-OH pterocarp dienes (Fig. 2; Kraft et al. 1987). The catalyzing enzymes, a reductase and a hydroxylase, are substrate specific and are expressed constitutively in all isolates tested to date (Hölh and Barz 1987; Tenhaken et al. 1991). Enzymatic studies on the reductase and hydroxylase indicate that these enzymes of *A. rabiei* can only convert pterocarpen with a (−) stereochemistry, as found in chickpea (Fig. 2). Another example for this type of stereospecific metabolism of phytoalexins is the 6a-hydroxylation of the (−) isomers of medicarpin and medicarpin.

![Fig. 1. Demethylation of (+)-pisatin to (+)-6a-HMK by Nectria haematococca MP VI.](image-url)
maackiain by *N. haematococca* MP VI isolate T-95, which does not metabolize the (+) isomers of these compounds (VanEtten et al. 1983).

Because of the great resemblance of (+)6α-HMK, the demethylation product of pisatin produced by *N. haematococca* MP VI (Fig. 1), to maackiain, we wanted to investigate whether the (+)6α-HMK is further metabolized by *A. rabiei*, or the proposed natural specificity of the metabolizing enzymes of this fungus for the (−) isomers prevents further degradation of this compound. To avoid direct feeding and to assure that the (+)6α-HMK, which is not a natural substrate, is available to the degrading enzymes at the correct intracellular location, we wanted to use strains of *A. rabiei* transformed with the PDAT9 gene of *N. haematococca* MP VI. For this purpose, a transformation system was developed based on hygromycin B resistance (*hygB*) as a selectable marker. In addition to the *hygB* gene, plasmid pUCH1/PDA, used for transformation, contained the *PDA* gene of *N. haematococca* MP VI (Turgeon et al. 1987; Weltring et al. 1988). Transforms were compared for metabolism of (+)pisatin, (+)6α-HMK, and the corresponding (−) isomers.

Our results show that even though transformed strains of *A. rabiei* carrying the PDAT9 gene became capable of metabolizing (+)pisatin to (+)6α-HMK, the (+)6α-HMK was not further metabolized. In contrast, (−)6α-HMK produced from (−)pisatin by one transformant accumulated only transiently in the medium. These results support the hypothesis that the enzymes of phytopathogenic fungi involved in phytalexin metabolism are adapted very closely to the stereochemistry of the phytalexins of their host plants.

**RESULTS**

**Transformation with plasmid pUCH1/PDA.**

Three isolates of *A. rabiei*, referred to as strains 8, 9, and 10, were transformed with plasmid pUCH1/PDA (Fig. 3), which contains the *hygB* gene as a selectable marker fused to a *C. heterostrophus* promoter (Turgeon et al. 1987). In addition, the plasmid includes a 3.2-kb fragment of *N. haematococca* MP VI genomic DNA containing the entire PDAT9 gene, including 5′ and 3′ regulatory sequences (Weltring et al. 1988; Maloney and VanEtten 1994).

Transformation rates of up to 2 transformants per μg of DNA were obtained. Five transformants of each strain were tested for hygromycin B resistance. All grew in the presence of 200 μg of hygromycin B per ml in the medium, and 10 transformants even tolerated 300 μg of the antibiotic per ml. In contrast, growth of the untransformed strains was completely inhibited by 50 μg/ml. The resistant phenotype was stable under nonselective conditions, although some transformants seemed to lose their hygromycin B resistance gradually when grown on nonselective medium for several passages. Nevertheless, these transformants were still able to grow on 150 μg of hygromycin B per ml.

**Southern analysis.**

Three transformants of strain 8 and two each of strains 9 and 10 were subjected to Southern analysis. DNA of transformants and untransformed strains was digested with *Bam*HI, which cuts twice in plasmid pUCH1/PDA and gives rise to a 3.2-kb fragment and a 5.6-kb fragment. In some cases, these fragments were visible on ethidium bromide stained gels within the smear of restriction fragments of the genomic DNA (data not shown). This suggested that the plasmid was integrated into the genome of these transformants in high copy numbers in a head-to-tail arrangement. Another possible explanation of a plasmid replicating autonomously was disproved by Southern analysis of uncut DNA of the transformants. In all cases, no free plasmid was detectable (data not shown).

The pattern of hybridizing fragments in the genomic DNA of the transformants (Fig. 4) showed that, with the exception of strain 8-1-1, all had integrated several copies of plasmid pUCH1/PDA into the genome. The DNA of strains 8-7-2, 9-8-3, 9-9-1, and 10-4-1 contained the 3.2-kb and 5.6-kb *Bam*HI fragments of pUCH1/PDA, indicating that these transformants integrated several copies of the plasmid in a head-to-tail order. While transformant 9-8-3 seemed to have integrated these copies at one location, the large number of additional hybridizing fragments in the other transformants suggested that integration of multiple copies of pUCH1/PDA might have taken place at several locations in the genome.

![Fig. 2. Initial steps of metabolism of (−)-maackiain and (−)-medicarpin by *Ascochyta rabiei*. Medicarpin: R₁ = H, R₂ = OCH₃, maackiain: R₁R₂ = OCH₂O.](image)

![Fig. 3. Restriction map of plasmid pUCH1/PDA. H = HindIII, B = BamHI, Pro.1 = Promoter 1 of Cochliobolus heterostrophus, hygB = hygromycin B resistance gene, PDA = pisatin demethylase gene PDAT9 of Nectria haematococca.](image)
The latter is true also for transformants 8-14-1 and 10-3-2, but in these cases integration of only one copy of the plasmid at different locations had occurred. The lack of a hybridizing 3.2-kb BamHI fragment containing the PDA gene could indicate that this fragment was involved in the recombination event leading to integration of the plasmid into the genome. In this case, the site of recombination must be located outside of the coding region of the PDA gene, because both transformants are able to demethylate pisatin (see below). Transformant 8-1-1 integrated only one copy of the plasmid, with no BamHI site left. A possible explanation for this loss of the restriction sites is the deletion of the PDA fragment of pUCH1/PDA, which is flanked by the BamHI sites. This would also explain why this transformant was not able to metabolize pisatin (see below).

The number of hybridizing fragments and their intensity in the DNA of the transformants was not correlated with hygromycin B tolerance. For example, transformants 8-1-1 and 9-9-1 were equally tolerant to the antibiotic, although the latter transformant contained many more copies of the plasmid (Fig. 4).

**Pisatin demethylation.**

*A. rabiei* strains 8, 9, and 10 and their transformants were analyzed for their ability to demethylate (+)pisatin. For this purpose, mycelium of each strain was incubated with $5 \times 10^5$ M (+)pisatin as substrate, and the metabolism was measured by high-pressure liquid chromatography (HPLC) using extracts of aliquots taken at different time intervals. As shown in Figure 5, pisatin disappeared from the medium of untransformed strains within 10 to 24 h. As sole metabolite, (+)-6a-HMK, the demethylation product of pisatin, was detectable in trace amounts. This metabolite was identified by its UV spectrum and by comparison of the HPLC retention time with authentic (+)-6a-HMK.

In contrast to the wild-type strains, all tested transformants with the exception of strain 8-1-1 rapidly converted (+)pisatin to the demethylation product (+)-6a-HMK, which was not further metabolized over the test period of 24 h. The conversion usually occurred within approximately 2 to 4 h with no detectable lag phase (Fig. 5, e.g., transformant 8-14-1) except for transformant 10-3-2, which needed around 12 h (Fig. 5). In any case, the demethylation rate of the transformants was much faster than the rate of disappearance of (+)pisatin from the medium of untransformed strains.

No difference was found between mycelia preincubated with (+)pisatin for 18 to 20 h and untreated mycelia (data not shown). This indicates that the PDA gene is constitutively expressed in *A. rabiei* essentially as in *C. heterostrophus* and *A. nidulans* (Schäfer et al. 1989; Weltrig et al. 1988). This constitutive expression is in contrast to *N. haematococca* MP VI, in which expression is induced by the substrate (VanEtten and Matthews 1984).

As mentioned above, transformant 8-1-1 was an exception insofar as it showed the same demethylation phenotype as untransformed strain 8. This transformant was not able to metabolize pisatin within 24 h, and it accumulated only trace amounts of (+)-6a-HMK. This suggests that 8-1-1 does not contain a functional PDA gene. Transformant 10-3-2 also seemed to have a reduced demethylation activity, but in this case the accumulation of (+)-6a-HMK proves that it contained a functional PDA gene.

A possible explanation for the quantitative accumulation of (+)-6a-HMK by the transformants and of only trace amounts of this compound by the untransformed strains is the (+)-configuration of this stercolipan, which is the opposite stereoisomer of the configuration found in the phytoalexins maackiaain and medicarpin of chickpea, the natural host of *A. rabiei* (Jacques et al. 1987). This implies that the enzymes of *A. rabiei* for maackiaain and medicarpin degradation are specific for the (+) configuration (Tenhaken et al. 1991). To obtain further evidence for this hypothesis, (-)pisatin was used as substrate. Since pisatin demethylase of *N. haematococca* was shown to be nonstereospecific, this stereoisomer of pisatin is readily converted to (-)-6a-HMK by the enzyme (VanEtten et al. 1989).

As shown in Figure 6, transformant 9-9-1 was able to further metabolize the demethylation product, (+)-6a-HMK. This proves that the enzymes of *A. rabiei* are indeed specific for the (+)-configuration of chickpea stercolipans (Tenhaken et al. 1991). A second product, which accumulated transiently at the expense of (+)-6a-HMK, was tentatively identified by its UV spectrum and its retention time during HPLC as 3,7,2'-trihydroxy-4',5'-methylenedioxy isoflavon. In a parallel experiment with transformant 9-9-1 using (+)pisatin as substrate, this isoflavon was not detectable and (+)-6a-HMK accumulated in the medium (Fig. 6). Therefore, the slow metabolism of (+)pisatin by *A. rabiei* remains to be elucidated.

**Fig. 4.** Southern blot analysis of DNA of strains 8, 9, and 10 and transformants 8-1-1, 8-7-2, 8-14-1, 9-8-3, 9-9-1, 10-3-2, and 10-4-1. DNA was cut with BamHI and hybridized to radioactively labeled pUCH1/PDA. The arrows indicate the positions of the 3.2-kb and 5.6-kb BamHI fragments of plasmid pUCH1/PDA (Fig. 3).
DISCUSSION

This paper reports the successful transformation of the chickpea pathogen *A. rabiei*. We chose the hygB gene as a selection marker because *A. rabiei* is very sensitive to the antibiotic and the vector containing the PDA gene carried the corresponding resistance gene. The tolerance of all transformants to large amounts of hygromycin B proves that the promoter from *C. heterostrophus* fused to the hygB gene functions in *A. rabiei* essentially as shown for other filamentous

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**Fig. 5.** Time course of (+)pisatin demethylation by *Ascochyta rabiei* strains 8, 9, and 10, and some representative transformants. —— (+)pisatin, —— (+)-HMK
fungi (Turgeon et al. 1987). On the other hand, this heterolo-
gous promoter is possibly one explanation for the low trans-
formation efficiency of up to 2 transformants per µg of DNA.
The use of a strong homologous promoter, for example of the
Gpd gene, or the use of the niaD selection system would
probably improve the transformation rate, as shown for As-
pergillus (Punt et al. 1987; Campbell et al. 1989; Unkles et al.
1989).

With one exception, the plasmid pUCH1/PDA was inte-
grated into the genome of the transformants in several copies,
with no obvious effect on the degree of resistance to hygro-
mycin B. For example, transformant 8-1-1, which contains
only one copy of the hygB gene (Fig. 4), is as resistant to
the antibiotic as transformant 9-9-1, which contains many copies.
This result has also been found with other fungi (Rodriguez
and Yoder 1987).

The ability of the transformants to demethylate (+)-pisatin
indicates that the PDA gene is functional in A. rabiei. This
suggests that the transcription signals and the splicing of
the four introns of this gene of N. haematococca MP VI
(Maloney and VanEtten 1994 are recognized and correctly
processed in A. rabiei. Since this gene is also active in A.
nidulans and C. heterostrophus (Schäfer et al. 1989; Weltring
et al. 1988), our experiment provides an additional demon-
stration that genes of filamentous fungi are interchangeable
among these organisms.

The results obtained with the PDA transformants support
the hypothesis that the enzymes involved in maackiai
metabolism are specific for the stereo-isomers (Tenhaken et al.
1991; VanEtten et al. 1983). This is the best explanation for the
result that the (+)-6a-HMK produced by the transformants
is not further metabolized. The detection of 3,7,2′,4′,5′-methyleneoxy
isoflavan shows that (−)-6a-HMK is
mainly metabolized via the reductive cleavage of the dihy-
drofuran ring (Fig. 2). These results are supported by enzy-
matic studies on the reductase and 1a-hydroxy-lase involved in
the metabolism of maackiai and medicarpin (Fig. 2; Höhl
and Barz 1987; Tenhaken et al. 1991) and by recent experi-
ments by R. Tenhaken and W. Barz (unpublished), who
showed that A. rabiei is able to metabolize (−)-6a-HMK,
which was obtained by biocconversion of (−)-maackiai by
N. haematococca MP VI isolate T-200 (Lucy et al. 1988).

Similar results with regard to stereospecific detoxification
of (+) and (−) isomers of maackiai and differential sensitiv-
ity to these isomers were obtained with N. haematococca MP
VI (VanEtten et al. 1983) and pathogens of red clover
(Delsereone et al. 1992), respectively. In the first case, N. hae-
matococca MP VI isolate T-95 6a-hydroxylated the (−) iso-
mer of maackiai but failed to metabolize the (+) isomer. In
the second study, five out of seven fungal pathogens of red
clover were inhibited by (−)-maackiai, which is the isomer
produced by this plant. These results and the data presented
in this paper provide the basis for experiments suggested by
VanEtten et al. (1989). Plants like pea or chickpea are to be
transformed with genes coding for biosynthetic enzymes that
control the stereochernistry of pterocarpan phytoalexins.
The result would be transgenic plants that produce phytoalexins
with the opposite stereochernistry. These plants should be
more resistant to pathogens that depend on the ability to me-
tabolize the phytoalexins of their host plant in order to be
pathogenic.

MATERIALS AND METHODS

Strains and plasmids.

The A. rabiei strains 8, 9, and 10 were supplied by M. C.
Saxena, ICARDA, Syria, and have been described previously
(Höhl et al. 1989). The fungi were grown on solid chickpea
extract medium (Kraft et al. 1987) and in liquid modified
Richard’s medium (Tenhaken et al. 1991).

Escherichia coli strain HB101 was used for propagation
of pUCH1/PDA. This plasmid was constructed by ligating a 3.2-
kH fragment of N. haematococca MP VI genomic DNA
(Weltring et al. 1988) containing the PDA gene into plasmid
UCH1 containing promoter 1 of C. heterostrophus in front of
the hygB gene (Turgeon et al. 1987).

Chemicals.

Hygromycin B, ampicillin, restriction endonucleases, and
random primed labeling kit were purchased from Boehringer,
Mannheim, Germany; Novozym 234 from Novo Nordisk A/S,
Copenhagen, Denmark; β-glucuronidase from Sigma Chemical
Co., St. Louis, MO; CaCl2, polyethylene glycol 4000, and
sodium dodecyl sulfate (SDS) from Merck Darmstadt, Germany;
Biodyne A membrane from Pall, Dreieich, Germany; and 32P
dATP from Amersham Buchrve, Braunshweig, Germany.

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Fig. 6. Demethylation of (+) and (−)-pisatin by Ascochya rabiei transformant 9-9-1. —— pisatin, —— 6a-HMK

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A small amount of (−)-pistarin was a gift of H. D. VanEtten. (+)-Pistatin was isolated from etiolated pea germings as described by VanEtten et al. (1980). (+)-6a-HMK was obtained by incubating transformants of A. rabiei with (+)-pistarin for 10 h, followed by an ether extraction of the product from the incubation medium (10 mM potassium phosphate buffer, pH 7.5). The ether was evaporated, and the residue was dissolved in methanol and submitted to HPLC (RP 18 column, Merck) for further purification. HPLC pure 6a-HMK was characterized by co-chromatography with authentic material on HPLC and thin-layer chromatography (TLC) in different solvent systems, and by using diagnostic reagents (Mabry et al. 1970).

DNA preparation.
Plasmid DNA was isolated according to Maniatis et al. (1982). Fungal DNA was prepared from lyophilized mycelium grown for 6 to 16 days in modified Richard's medium as described by Yelton et al. 1984.

Transformation.
A total of 5 x 10^8 spores was inoculated in 250 ml of modified Richard's medium for at least 24 h. Protoplasting of the germinated spores and transformation were carried out as described by Yelton et al. (1984) with the following modifications. Germinated spores were incubated in 10 ml of osmotic medium per g (fresh weight) containing 5 mg of Nonozym 234 per ml and 0.4 mg of β-glucuronidase for 3 h. After transformation, protoplasts were resuspended in 200 μl of STC (1.2 M sorbitol/10 mM Tris-HCl, pH 7.5/10 mM CaCl_2) divided into four aliquots. Each aliquot was embedded in 20 ml of chickpea medium containing molten agar and 1 M sucrose as osmoticum. After 16 h, the plates were overlaid with 10 ml of top agar containing 2.25 mg of hygromycin B (final concentration 75 μg/ml). Transformants were visible after 1 to 2 weeks. For further analysis, all tested transformants were purified by single sporing.

Southern analysis.
DNA was separated on 0.6% agarose gels, transferred to Pall Biodyne B membrane by capillary blotting, and hybridized under stringent conditions according to the supplier's manual. Plasmid DNA was labeled with a random primed labeling and detection kit (Boehringer) according to the manufacturer's instructions.

Metabolism assay.
Mycelium (5 g fresh weight [FW]) was incubated in 100 ml of potassium phosphate buffer (10 mM, pH 7.5) on a rotary shaker at 22°C. 150 rpm with 5 x 10^3 M (+)-pistarin or 2 x 10^3 M (+)-6a-HMK as substrate. At different times, 5-ml aliquots were taken and extracted twice with 3 ml of diethyl ether. The organic phase was evaporated. The residue was dissolved in 150 μl of methanol and analyzed by HPLC (Waters, Eschberg, Germany) on an RP 18 column (Merck) in a gradient of 40 to 90% acetonitrile in water within 15 min. Substrates and products of the extracts were detected by the Photo Diode Array-System 990 (Waters), which allowed the UV spectral analysis of each compound in the extracts. Concentrations of pistatin and 6a-HMK were calculated from the HPLC signal measured at 309 nm.

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LITERATURE CITED
Rodriguez, R. J., and Yoder, O. C. 1987. Selectable genes for transformation of the fungal plant pathogen Glomerella cingulata f. sp.
chemical relatedness of fungal NADPH-cytochrome P-450 reducta-
enzyme makes a fungal pathogen, but not a saprophyte, virulent on a
Tegtmeier, K. J., and VanEtten, H. D. 1982. The role of pisatin tolerance
and degradation in the virulence of Nectria haematococca on peas: A
Tenhaken, R., Salmen, H. C., and Barz, W. 1991. Purification and char-
acterization of pterocarpan hydroxylase, a flavoprotein monooxyge-
Turgeon, B. G., Garber, R. C., and Yoder, O. C. 1987. Development of a
fungal transformation system based on selection of sequences with
Unkles, S. E., Campbell, E. I., de Ruiter-Jacobs, Y. M. J. T., Broekhui-
jsen, M., Macro, J. A., Carrez, D., Contreras, R., van den Hondel, C.
gous transformation system for Aspergillus oryzae based on the nitrate
assimilation pathway: A convenient and general selection system for
detoxification: Importance for pathogenicity and practical implica-
in the inducibility of pisatin demethylating activity in Nectria haemat-
VanEtten, H. D., Matthews, P. S., Mercer, E. H. 1983. (+)Maackiaiin and
(+)-medicarpin as phytoalexins in Sophora japonica L. and identifica-
tion of the (-) isomers by biotransformation. Phytochemistry 22:2291-
2295.
VanEtten, H. D., Matthews, P. S., Tegtmeier, K. J., Dietert, M. F., and
Stein, J. I. 1980. The association of pisatin tolerance and demethyla-
tion with virulence on pea in Nectria haematococca. Physiol. Plant
Pathol. 16:257-268.
VanEtten, H. D., Puepke, S. G., and Kelsey, T. C. 1975. 3,6a-Dihy-
droxy-8,9-methylenedioxypterocarp as a metabolite of pisatin pro-
duced by Fusarium solani f. sp. pisi. Phytochemistry 14:1103-1105.
Weltring, K.-M. 1992. Phytoalexins in the relation between plants and
their fungal pathogens. Pages 111-124 in: Molecular Biology of Fil-
amentous Fungi. U. Stahl and P. Tuzdinsky, eds. VCH, Weinheim,
Germany.
Weltring, K.-M., Turgeon, B. G., Yoder, O. C., and VanEtten, H. D.
1988. Isolation of a phytoalexin-detoxification gene from the plant
pathogenic fungus Nectria haematococca by detecting its expression