In vitro susceptibility of *Pythium insidiosum* and a *Lagenidium* sp to itraconazole, posaconazole, voriconazole, terbinafine, caspofungin, and mefenoxam

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**Objective**—To evaluate the radial growth assay for use in in vitro susceptibility testing of *Pythium insidiosum* and a *Lagenidium* sp and to assess susceptibility of representative isolates to itraconazole, posaconazole, voriconazole, terbinafine, caspofungin, and mefenoxam.

**Sample Population**—6 isolates each of *P. insidiosum* and *Lagenidium* sp.

**Procedures**—Isolates were plated in triplicate onto agar supplemented with antifungal compounds at concentrations of 0.025 to 8 µg/mL. Isolates on dimethyl sulfoxide– and water-supplemented agar served as control samples. Effect of antifungal concentration on colony diameter was assessed with a mixed linear model. Assay variability was assessed with the coefficient of variation.

**Results**—Colony growth was uniform (mean intra-assay and interassay coefficients of variation were <5%). Minimal inhibition was evident with voriconazole and posaconazole at 8 µg/mL. Terbinafine at 8 µg/mL significantly reduced growth of *P. insidiosum* and at ≥1 µg/mL significantly reduced growth of the *Lagenidium* sp. Caspofungin and mefenoxam (concentrations ≥1 µg/mL and ≥0.025 µg/mL, respectively) significantly reduced growth of both pathogens. Mefenoxam at 0.1 µg/mL caused >50% growth inhibition in 11 of 12 isolates and at 1 µg/mL caused >90% inhibition in all isolates.

**Conclusions and Clinical Relevance**—Results suggested that the radial growth assay was a simple, reproducible technique for susceptibility testing of *P. insidiosum* and a *Lagenidium* sp. Azoles had limited activity, whereas terbinafine and caspofungin caused significant but minimal to moderate inhibition. Only mefenoxam had a profound effect on both pathogens at concentrations likely to be achievable in tissues. (Am J Vet Res 2008;69:1463–1468)

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Pythiosis and lagenidiosis, caused by the oomycetes *Pythium insidiosum* and *Lagenidium* sp, are devastating infections that often result in fatalities; these infections develop most often in apparently immunocompetent young adult dogs.1,2 Transmission is via direct inoculation or ingestion of motile, flagellate zoospores, which are produced in aquatic environments.3 Complete surgical resection of all infected tissues is the treatment of choice and is often effective for the treatment of dogs with pythiosis when lesions are limited to the mid-jejunum or the distal aspect of a limb. Unfortunately, many affected animals have lesions that cannot be completely resected because of the location or extent of the lesion or as a result of extension into regional lymphatics. For lagenidiosis, surgery is frequently ineffective because the disease often disseminates to distant sites in the chest or abdomen prior to the time of diagnosis.2 Medical treatment for pythiosis and lagenidiosis has historically been unrewarding, probably because ergosterol (the target for most traditional antifungal agents) is generally lacking in the cell membrane of oomycetes.4,5 Despite this fact, cures have been achieved in some human and veterinary patients following medical treatment with ergosterol-targeting drugs, such as itraconazole, terbinafine, and amphotericin B.1,6–8 Such responses, although infrequent, suggest a need for further investigation regarding the effects of these drugs on pathogenic oomycetes.

Other drugs that have the potential for efficacy against pathogenic oomycetes include the second-generation triazoles, voriconazole and posaconazole, which have a broader spectrum of action than do the older azoles.9 Although neither drug has been widely used in veterinary patients because of their expense, posacon-
azole reportedly caused improvement (although not complete resolution) of a cutaneous lesion associated with oomycosis in a single human patient. In addition, caspofungin, of the recently developed echinocandin class of β-glucan synthesis inhibitors, has the potential to be an effective drug for the treatment of animals with pythiosis and lagenidiosis because of the high content of β-glucan in the cell wall of oomycetes. However, the degree to which the echinocandins inhibit the form of β-glucan synthase found in pathogenic oomycetes has yet to be determined.

Mefenoxam, a highly effective agricultural fungicide, is a phenylamide compound that inhibits RNA polymerase and has been used extensively to control plant-pathogenic oomycetes on crops and ornamentals. Although no pharmacokinetic data for this compound are available, a mammalian safety study performed prior to approval by the Environmental Protection Agency indicated minimal toxic effects in rodents and dogs. Although this class of compounds is not currently available in a pharmacoeutical formulation, its extremely high potency for the inhibition of growth of plant-pathogenic oomycetes makes it an obvious candidate for investigations of activity against oomycetes that infect mammals.

In vitro susceptibility testing is the first step in further evaluation of the aforementioned compounds that may have efficacy for the treatment of animals with pythiosis and lagenidiosis. Unfortunately, most of the techniques used for antifungal susceptibility testing of medically important yeasts and molds (such as broth dilution) do not work well with oomycetes in large part because uniform suspensions of infectious particles needed for inocula are difficult to prepare. Alternative techniques for the evaluation of pathogenic oomycetes have been described only rarely, and those that have been described have not been widely evaluated.

The radial growth assay is a technique that has been used extensively for the in vitro evaluation of fungicide resistance in plant-pathogenic oomycetes of the genera Phytophthora and Pythium. This technique assesses growth inhibition by direct measurement of colony diameter following inoculation of isolates on agar supplemented with various concentrations of antifungal compounds, which makes it unnecessary to prepare hyphal or zoospore suspensions. The technique lends itself to evaluation of oomycetes because they tend to grow submersed in agar rather than on top of it, which keeps the antifungal compounds incorporated into the agar in contact with the organisms.

The primary purpose of the study reported here was to evaluate the radial growth assay for use as a technique for in vitro susceptibility testing of the pathogenic oomycetes Pythium insidiosum, and Lagenidium sp. Also, we evaluated the use of this technique to assess the susceptibility of representative isolates to itraconazole, posaconazole, voriconazole, terbinafine, caspofungin, and the agricultural fungicide mefenoxam.

**Materials and Methods**

**Sample population**—Six isolates each of *P. insidiosum* and of *Lagenidium* sp pathogenic to dogs were obtained from the culture collection maintained by the Pythium Laboratory at Louisiana State University. *Pythium insidiosum* and the *Lagenidium* sp were initially isolated from infected canine tissue by plating on vegetable extract agar or PYG agar, respectively, by use of a technique described elsewhere. Isolates were stored as mycelial agar plugs (3 mm²) in distilled water (for *P. insidiosum*) or on PYG slants (for the *Lagenidium* sp). Before being deposited in the culture collection, the identity of each isolate was determined on the basis of evaluation of morphologic features (including zoospore production for *P. insidiosum* isolates), growth at 37°C, and 18S and internal transcribed spacer ribosomal RNA gene sequencing. Prior to use in the study, stored isolates were inoculated onto agar plates and then subcultured intermittently as needed to provide actively growing colonies, which were then used as stock cultures throughout the study.

**Antifungal compounds**—Posaconazole, terbinafine, and caspofungin were supplied as standard powders by the manufacturers. Itraconazole formulated for IV infusion, voriconazole concentrate for IV infusion, and mefenoxam were purchased from commercial sources. Each compound was reconstituted in commercial-grade DMSO at a concentration of 10 mg/mL and stored at −20°C until use.

**Radial growth assay**—Vegetable extract agar (for *P. insidiosum* isolates) and PYG agar (for the *Lagenidium* sp isolates) were prepared as described elsewhere. After medium was autoclaved, it was allowed to cool to 30°C before antifungal compounds diluted in DMSO were added in volumes necessary to obtain the desired final concentrations. Agar and compounds were mixed and poured into plates (20 mL/plate). Water and DMSO-supplemented agar (with water and DMSO, respectively, added in volumes corresponding to the largest volume of diluted antifungals) were included as control samples in each assay.

For each assay, mycelial plugs (3 mm in diameter) were cut from the margin of actively growing colonies of 2-day-old stock cultures and placed in the center of each test plate with the mycelium in contact with the agar. Each antifungal concentration and control sample was plated in triplicate, and plates were incubated at 37°C for 48 hours. After incubation, plates were visually inspected for uniformity and symmetry of growth, and colony diameter on each plate was calculated as the mean of 2 perpendicular measurements minus 5 mm for the diameter of the plug used for inoculation.

**Experimental design**—Two experiments were conducted. Experiment 1 was designed to provide information about assay variability, ease of performance, and optimal concentrations of antifungal compounds for subsequent assessment of a larger number of isolates. Two isolates each of *P. insidiosum* and the *Lagenidium* sp were evaluated. Itraconazole, posaconazole, voriconazole, terbinafine, and caspofungin were tested at 1, 2, 4, and 8 µg/mL. Mefenoxam was tested at 0.1, 0.5, 1, and 4 µg/mL.

Experiment 2 was designed to assess in vitro susceptibility of a larger number of isolates to concentrations of antifungals that were identified on the basis of results of experiment 1. Six isolates each of *P. insidiosum*...
and the *Lagenidium* sp were evaluated (including the isolates used in experiment 1). Itraconazole, posaconazole, and voriconazole were tested at 8 µg/mL; terbinafine was tested at 1 and 2 µg/mL for the *Lagenidium* sp and at 8 µg/mL for *P. insidiosum*; caspofungin was tested at 0.025, 0.05, and 0.1 µg/mL. Percentage of growth inhibition was calculated as (100 – [mean colony diameter on test plate/mean colony diameter on water control sample] × 100).

**Statistical analysis**—Mean colony diameter was the response variable for analysis; it was found to have a normal distribution, with failure to reject the null hypothesis of normality at *P* ≤ 0.05 by use of the Shapiro-Wilk statistic. For both experiments, the fixed effect of concentration was evaluated for each compound and pathogen by use of a mixed linear model that also included isolate as a random variable. A commercially available software package was used for the analyses. When a significant (*P* ≤ 0.05) effect of concentration was detected, comparisons were made for results for water control samples by use of the Dunnett adjustment to maintain type 1 error at *α* = 0.05. Intra-assay variability was calculated as (100 – [mean colony diameter tested at 1 and 2 µg/mL; and mefenoxam was tested at 0.025, 0.05, and 0.1 µg/mL. Percentage of growth inhibition was calculated as (100 – [mean colony diameter on test plate/mean colony diameter on water control sample] × 100).

Assay variability—For all test plates in both experiments, colony growth appeared uniform and radially symmetric. Mean plate-to-plate coefficient of variation was 3.72%. For interassay variability, the coefficient of variation for mean colony diameter for isolates 1 and 2 on water and DMSO control samples was 4.60%. No significant differences in assay variability were detected between DMSO and water control samples for any isolate tested in either experiment.

**Experiment 1**—Growth of *P. insidiosum* was significantly increased at all concentrations of itraconazole tested; this effect was not evident for the *Lagenidium* sp (Tables 1 and 2). Posaconazole at all concentrations tested had no significant effect on *P. insidiosum*, but posaconazole at a concentration of 8 µg/mL significantly inhibited growth of the *Lagenidium* sp. Voriconazole at 8 µg/mL significantly inhibited growth of both pathogens. Terbinafine at all concentrations tested had no significant effect on growth of *P. insidiosum*, but all concentrations of terbinafine significantly inhibited growth of the *Lagenidium* sp. Caspofungin at concentra-
Concentration 1466 oculum for a broth dilution technique to evaluate susceptibility of pathogens from the surface of actively growing colonies as the inoculum for a broth dilution technique to evaluate susceptibility of 8 isolates of P. insidiosum to amphotericin B, 5-fluorocytosine, fluconazole, itraconazole, ketoconazole, and miconazole. Of these, only miconazole had strong activity against all 8 isolates tested. No information about the utility or reproducibility of the assay was provided.

In 1998, a broth macrodilution and checkerboard synergy technique was used to evaluate an isolate of *P. insidiosum* obtained from a periorbital lesion in a 2-year-old child who had failed to respond to treatment with amphotericin B and itraconazole. For this patient, results of susceptibility testing accurately predicted successful clinical response to a combination of itraconazole and terbinafine. Although the broth dilution technique was used effectively in this situation, the experience of the investigators at the laboratory at which the testing was performed has been that broth dilution testing often does not provide clear and reproducible results for isolates of *P. insidiosum*. This may be attributable in part to the fact that oomycetes in broth typically grow in a clumped, nonuniform manner, which makes it difficult to make a quantitative assessment of growth inhibition. In addition, the use of hyphal suspensions as inocula for broth dilution methods for susceptibility testing of molds has been discouraged because it can be difficult to create a uniform suspension for reliable dilution to standard concentrations.

Most recently, a broth macrodilution technique that used a zoospore suspension inoculum was described by investigators who used the technique to assess the susceptibility of 27 Brazilian isolates of *P. insidiosum* to caspofungin and 30 isolates of *P. insidiosum* to itraconazole.

### Discussion

One of the primary objectives of the study reported here was to develop an easily performed method for the in vitro evaluation of antifungal susceptibility of pathogenic oomycetes and to assess reproducibility of the method. To our knowledge, in vitro susceptibility testing of mammalian-pathogenic oomycetes has been described in only 3 reports. In 1992, investigators used a spectrophotometrically adjusted suspension of hyphal fragments scraped from the surface of actively growing colonies as the inoculum for a broth dilution technique to evaluate susceptibility of 8 isolates of *P. insidiosum* to amphotericin B, 5-fluorocytosine, fluconazole, itraconazole, ketoconazole, and miconazole. Of these, only miconazole had strong activity against all 8 isolates tested. No information about the utility or reproducibility of the assay was provided.

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### Table 3—Mean ± SD radial growth for 6 isolates of *P. insidiosum* cultured in agar supplemented with water, DMSO, or various concentrations of antifungal compounds in an experiment to assess assay characteristics.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Antifungal agents</th>
<th>Concentration (µg/mL)</th>
<th>Water</th>
<th>DMSO</th>
<th>1</th>
<th>2</th>
<th>8</th>
<th>P value</th>
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<tr>
<td>Antifungal agents</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Itraconazole</td>
<td>12.4 ± 1.7</td>
<td>12.3 ± 1.7</td>
<td>ND</td>
<td>ND</td>
<td>12.8 ± 1.6*</td>
<td>&lt; 0.001</td>
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<tr>
<td>Posaconazole</td>
<td>12.4 ± 1.7</td>
<td>12.3 ± 1.7</td>
<td>ND</td>
<td>ND</td>
<td>12.2 ± 1.6</td>
<td>0.228</td>
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</tr>
<tr>
<td>Voriconazole</td>
<td>12.4 ± 1.7</td>
<td>12.3 ± 1.7</td>
<td>ND</td>
<td>ND</td>
<td>11.9 ± 1.7*</td>
<td>&lt; 0.001</td>
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<tr>
<td>Terbinafine</td>
<td>12.4 ± 1.7</td>
<td>12.3 ± 1.7</td>
<td>ND</td>
<td>ND</td>
<td>11.8 ± 1.7*</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caspofungin</td>
<td>12.4 ± 1.7</td>
<td>12.3 ± 1.7</td>
<td>11.4 ± 1.5*</td>
<td>8.3 ± 1.0*</td>
<td>ND</td>
<td>&lt; 0.001</td>
<td></td>
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<tr>
<td>Agricultural fungicide</td>
<td>Water</td>
<td>DMSO</td>
<td>0.025</td>
<td>0.050</td>
<td>0.100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mefenoxam</td>
<td>12.4 ± 1.7</td>
<td>12.3 ± 1.7</td>
<td>11.5 ± 1.4*</td>
<td>8.1 ± 0.7*</td>
<td>5.7 ± 0.6*</td>
<td>&lt; 0.001</td>
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<td></td>
</tr>
</tbody>
</table>

ND = Not determined.

*See Table 1 for remainder of key.*

### Table 4—Mean ± SD radial growth for 6 isolates of a *Lagenidium* sp cultured in agar supplemented with water, DMSO, or various concentrations of antifungal compounds in an experiment to assess assay characteristics.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Antifungal agents</th>
<th>Concentration (µg/mL)</th>
<th>Water</th>
<th>DMSO</th>
<th>1</th>
<th>2</th>
<th>8</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antifungal agents</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Itraconazole</td>
<td>12.4 ± 0.6</td>
<td>12.1 ± 0.8</td>
<td>ND</td>
<td>ND</td>
<td>11.6 ± 0.7*</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posaconazole</td>
<td>12.3 ± 0.6</td>
<td>12.1 ± 0.8</td>
<td>ND</td>
<td>ND</td>
<td>11.5 ± 0.7</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Voriconazole</td>
<td>12.3 ± 0.6</td>
<td>12.1 ± 0.8</td>
<td>ND</td>
<td>ND</td>
<td>11.6 ± 0.7*</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terbinafine</td>
<td>12.3 ± 0.6</td>
<td>12.1 ± 0.8</td>
<td>11.4 ± 0.8*</td>
<td>10.6 ± 0.7*</td>
<td>ND</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caspofungin</td>
<td>12.3 ± 0.6</td>
<td>12.1 ± 0.8</td>
<td>11.4 ± 0.8*</td>
<td>10.4 ± 1.1*</td>
<td>ND</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agricultural fungicide</td>
<td>Water</td>
<td>DMSO</td>
<td>0.025</td>
<td>0.050</td>
<td>0.100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mefenoxam</td>
<td>12.3 ± 0.6</td>
<td>12.1 ± 0.8</td>
<td>10.0 ± 0.7*</td>
<td>6.5 ± 0.8*</td>
<td>2.9 ± 0.7*</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
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</tbody>
</table>

*See Tables 1 and 3 for remainder of key.*
voriconazole, and terbinafine. After a count was determined by use of a Neubauer chamber, zoospore suspensions were diluted to a standard concentration, and results were obtained by visual estimation of growth inhibition, with minimum inhibitory concentrations set at 100%, 90%, and 50% inhibition. The investigators indicated that this technique was reproducible (although data regarding assay reproducibility were not reported) and suggested that it was a more reliable technique than those based on hyphal inocula. However, in our experience, reliably standardized zoospore suspensions are difficult to create because the zoospores typically adhere to and encyst on the glass or plastic surfaces of the containers used to make, count, and dilute the suspensions. In addition, because zoospores are more difficult to produce for *Lagenidium* isolates than for *P. insidiosum*, this technique is not a good option for susceptibility testing of *Lagenidium* isolates. For these reasons, the radial growth assay was considered as an alternative method that was not dependent on use of suspensions of zoospores or hyphal fragments.

Although it has not been used to assess mammalian-pathogenic oomycetes, the radial growth assay has been used extensively to evaluate resistance of plant-pathogenic oomycetes of the genera *Pythium* and *Phytophthora* to agricultural fungicides such as metalaxyl and mefenoxam. Results are typically reported as the effective concentration of antifungal compound needed to inhibit 50% or 90% of measured colony growth, compared with results for control cultures. The uniform and radially symmetric colony growth as well as low plate-to-plate and interassay variability in the study reported here suggested that the radial growth assay is a useful and reproducible technique for in vitro evaluation of susceptibility for *P. insidiosum* and *Lagenidium* organisms. The assay is easier to perform than is the macrodilution technique based on zoospore suspension inocula, and the radial growth assay also has the advantage that it can be used to evaluate drug effects on the hyphae, rather than the zoospores, which are not present in infected tissues. It is not known which of these techniques may be superior for predicting clinical response, and future studies should focus on comparative use of both techniques for evaluation of susceptibility patterns in a larger number of isolates from veterinary and human patients in which clinical response to treatment is also determined.

Although the data generated on susceptibility patterns of *P. insidiosum* and *Lagenidium* organisms can only be considered preliminary on the basis of the small number of isolates tested, it does provide an opportunity for some insight regarding potential antiamoecytic activities of the drugs investigated. The limited in vitro activity associated with the azoles is not unexpected given the general lack of ergosterol in the cell membrane of oomycetes. The fact that some isolates of *P. insidiosum* actually had increased growth on itraconazole-supplemented agar, compared with growth on the control samples, was surprising and is difficult to explain on the basis of the known effects of this drug. However, given the small magnitude of the increase, this is unlikely to be of clinical relevance. Despite the fact that the newer triazoles, voriconazole and posaconazole, have a broader spectrum of activity against pathogenic molds, results of this study did not provide strong support for their clinical effectiveness against pathogenic oomycetes. Although voriconazole and posaconazole at 8 µg/mL significantly inhibited 1 or both pathogens, the magnitude of inhibition was small, and it is unlikely that comparable concentrations of either drug could be achieved in tissue.

Terbinafine, which acts by inhibiting squalene epoxidase in the sterol biosynthesis pathway, had limited effects on *P. insidiosum* and caused significant but minimal inhibition in some isolates when tested at a concentration of 8 µg/mL. However, it had a more substantial effect on *Lagenidium* isolates. In general, members of both genera are considered sterol auxotrophs and incorporate sterols from their environment, rather than producing them. However, the ability to use squalene and convert it to squalene oxide has been detected in 2 species of *Lagenidium* organisms, one of which (*Lagenidium giganteum*) is a close taxonomic relative of the pathogen in dogs. Because sterols are necessary for zoosporogenesis but not for growth of vegetative hyphae, it is doubtful that decreased sterol biosynthesis related to inhibition of squalene epoxidase would have a pronounced effect on the growth of oomycete hyphae in tissues. However, the effects of terbinafine on *Lagenidium* organisms may instead result from fungicidal activity related to the intracellular accumulation of squalene.

Caspofungin, which acts by inhibition of β (1,3)-D-glucan synthase, has been considered as a potential treatment for patients with pythiosis and lagenidiosis because β-glucan is an important structural component of the cell wall of oomycetes. However, little is known about the potential sensitivity of the β-glucan synthase contained in mammalian-pathogenic oomycetes to inhibition by caspofungin. Results of the study reported here revealed that concentrations as low as 1 µg/mL significantly inhibited growth of both pathogens but that the magnitude of the inhibition was only moderate, even for concentrations as high as 8 µg/mL. These results are consistent with those reported by other investigators, who found that the concentration of caspofungin required to inhibit growth by 90% was ≥ 8 µg/mL for 89% of the *P. insidiosum* isolates. As part of that same investigation, rabbits experimentally infected with *P. insidiosum* were treated with caspofungin (1 mg/kg, intraperitoneally, q 24 h) for 20 days. Although measurable progression of the disease slowed during treatment, lesions quickly resumed growth when the drug was discontinued. Considered together, these results are not encouraging regarding the potential clinical usefulness of caspofungin for the treatment of animals with pythiosis and lagenidiosis.

The most profound effect on oomycete growth in this study was associated with the agricultural fungicide mefenoxam, which is the more active enantiomer contained in the older racemic fungicide metalaxyl. This is not surprising given the potent antiamoecytic activities for these compounds against plant-pathogenic species. Unfortunately, mefenoxam is not currently available in a formulation that could be administered to veterinary patients. However, safety profiles in mammals and rodents (based on data submitted to the Environmental Protection Agency in the manufacturer’s pesticide peti-
tion) are encouraging. A 6-month dietary study of toxic effects in dogs revealed a no-observable-effect level of 8 mg/kg/d, and an acute oral administration study in rats revealed an LD50 value of 490 mg/kg. On the basis of these data, if a formulation of mefenoxam suitable for pharmaceutical use in veterinary patients were to be made available for further efficacy and tolerability investigations, it would be the most promising candidate of the compounds we evaluated.

Results of the study reported here supported the use of the radial growth assay as a simple, reproducible technique for use in vitro susceptibility testing of P. insidiosum and Lagenidium isolates. Although the technique clearly allowed for quantitation of effects of antifungal compounds at various concentrations, guidelines for interpretation of the percentage of growth inhibition and its relationship to prediction of clinical response have yet to be determined. For the technique to be clinically useful, interpretive breakpoints determined on the basis of the magnitude of inhibition of hyphal growth (such as the effective concentrations of antifungal compound needed to inhibit 50% or 90% of measured colony growth values that are used to categorize plant-pathogenic oomycetes as susceptible or resistant to mefenoxam) will need to be proposed and evaluated. As illustrated by the fact that consensus breakpoints for filamentous fungi have yet to be established, this can be a prolonged process. Inclusion of plant-pathogenic species with known susceptibility to mefenoxam as control organisms in future investigations may also help to provide additional information about the magnitude of growth inhibition needed to predict clinical relevance. In addition, future studies should focus on comparison of the radial growth assay to the zoospore broth dilution method and the evaluation of a larger number of isolates obtained from animals with a known clinical outcome.

References

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5. Lamisil, Novartis Pharmaceuticals, East Hanover, NJ.
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