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Therapeutic and prophylactic measures for winter saprolegniosis in channel catfish

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ABSTRACT: Winter saprolegniosis in channel catfish *Ictalurus punctatus* is associated with low temperature (~12°C) induced immunosuppression and invasion by a ubiquitous, opportunistic water mold, identified as a *Saprolegnia* sp. In this study, attempts were made to identify antimicrobials/chemicals which may (1) be effective for the therapeutic treatment of winter saprolegniosis, and (2) prophylactically control water concentrations of *Saprolegnia* and hence prevent the onset of disease under laboratory challenge conditions. The antimicrobials used were amphotericin B and the cecropins A, B, and P1. While amphotericin B halted *Saprolegnia* growth, the cecropins were ineffective. The chemicals and herbicides approved for use by the U.S. Food and Drug Administration and considered in this study for the prevention of *Saprolegnia* zoospore production or cyst germination were used at concentrations less than or equal to those recommended for use in commercial catfish ponds for other purposes. Compared with malachite green (a positive control only), sodium chloride and potassium permanganate were ineffective, while copper sulphate and formalin inhibited both zoospore production and cyst germination. However, at the lowest inhibitory dosage, copper sulphate was toxic to catfish challenged under laboratory conditions; this was likely due to the low alkalinity of our tank water when compared to that of catfish ponds. Several herbicides were tested including diquat, simazine, hydrothol 191, and aquathol K. Only diquat had an inhibitory effect on *Saprolegnia* growth. In subsequent laboratory challenge experiments, both formalin and diquat were efficacious in preventing the onset of winter saprolegniosis in channel catfish.

KEY WORDS: Channel catfish · Saprolegniosis · Temperature · Immunity · Chemotherapy · Antimicrobials

INTRODUCTION

Winter saprolegniosis (colloquially termed 'winter kill') in channel catfish *Ictalurus punctatus* has been documented to result from a combination of 2 factors. The first is low-temperature-induced immunosuppression and the second is high water concentrations of *Saprolegnia* zoospores, i.e. ≥ 5 ml^{-1}. Low-temperature-induced immunosuppression can result from the occurrence of a severe cold weather front; such fronts can lower pond water temperatures by as much as 16°C in 24 h (Bly et al. 1992, 1993). Under laboratory conditions, a temperature decrease of 10°C in 24 h (from 22 to ~12°C, within the temperature ranges associated with the onset of disease in commercial ponds) induced immunosuppression in catfish for 5 to 8 wk, until the fish acclimated to the low temperature (Bly & Clem 1991, 1992). *Saprolegnia* undergoes asexual reproduction to release motile zoospores into the water. Although zoospore concentrations are normally <2 ml^{-1}, during the cold winter months, when disease is observed, levels of 5 to 10 ml^{-1} have been recorded (Bly et al. 1992). If catfish are immunosuppressed they do not appear able to inhibit *Saprolegnia* cyst germination and hyphal penetration of the skin and underlying muscle. However, if catfish are acclimated to ~11°C, and have regained immunocompetence (Bly & Clem 1991), they do not become infected (Bly et al. 1992), supporting the contention that an intact immune system protects against this disease. Further corroboration of this hypothesis came from studies wherein catfish held at the immunologically permissive temperature of 22°C were injected intramuscularly with viable *Saprolegnia* hyphae. The hyphae were rapidly destroyed in a classical foreign body response (Bly et al. 1994). Because the

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etiology of this disease is multifactorial, a vaccine would have to be effective in the face of low-temperature-induced immunosuppression or would have to be administered with an immunostimulant which would overcome low-temperature-suppressed immune mechanisms required for protection. As the development of such a vaccine is likely to be a particularly difficult undertaking, a simpler approach would be to therapeutically treat fish exhibiting signs of *Saprolegnia* associated lesions and/or to prevent the disease via chemical prophylaxis to limit the concentration of *Saprolegnia* in commercial production ponds.

In this study attempts were made in the laboratory to inhibit *Saprolegnia* hyphal growth with antimicrobials and to prevent/reduce zoospore production and/or cyst germination with chemicals and herbicides. Agents showing potential were then tested in laboratory challenge experiments to determine if they could prevent the onset of winter saprolegniosis in catfish. The antimicrobials used were amphotericin B, a clinically potent antifungal agent (Tilton & McGinnis 1987), and the cecropins A, B, and P1, naturally occurring antimicrobials produced in the intestines of insects (A and B; Boman & Hultmark 1987) and pigs (P1; Boman et al. 1993). Cecropins are potent antibacterial agents and may be inhibitory for *Saprolegnia* as it has been reported that *Achlya* and *Saprolegnia* are sensitive to some antibiotics (Olah & Farkas 1978, Beakes & Gay 1980). The chemicals used, sodium chloride, potassium permanganate, copper sulphate, and formalin, are currently approved by the U.S. Food and Drug Administration (FDA) as therapeutics for use in catfish ponds (MacMillan 1985, Schnick 1991). Malachite green which is known to kill fungal growth on catfish eggs was used as a positive control; this chemical is no longer approved for use with food fish due to its teratogenic properties (Alderman 1985, 1994). Herbicides were also used in attempting to inhibit *Saprolegnia* growth because the Oomycetes are known to have their phylogenetic origins with the chromophyte algae and are members of the Protoctista rather than true fungi (Farkas 1979, Beakes 1989, Dick 1990, Kwon-Chung & Bennett 1992).

METHODS AND MATERIALS

***Saprolegnia* culture.** Details concerning the maintenance of *Saprolegnia* isolates obtained from moribund catfish with overt signs of saprolegniosis have been described previously (Bly et al. 1992). Briefly, 6 *Saprolegnia* isolates have been maintained on corn meal agar (CMA; Difco, Detroit, MI, USA) at 17°C for over 3 yr; in this study the CF1 isolate was predominantly used. Isolates were passaged by inverting a block of agar cut from an area of dense hyphal growth onto a new CMA plate. Isolates from the American Type Culture Collection [*Saprolegnia* sp. (ATCC #36209), *S. diclina* (ATCC #42062), *S. ferax* (ATCC #26116), and *S. parasitica* (ATCC #22284)] were also used for comparative purposes and cultured as described above.

Zoospore inhibition assays. To produce zoospores, 1 cm^2 blocks of agar, covered by *Saprolegnia* (CF1) hyphal growth, were inverted onto the center of CMA plates and surrounded by autoclaved hemp seeds. Once the seeds were completely covered with *Saprolegnia* hyphae (~5 d at 22°C), blocks of agar containing hemp seeds were placed in floating mesh baskets in 1 l plastic containers containing 1 l of well water. The containers were suspended in water baths which could be chilled from ambient temperature (~22°C) to 12 ± 1°C in 24 h. Water samples were taken immediately before and 4 d after *Saprolegnia* was added to the water. Five 0.5 ml aliquots of water were plated onto CMA plates and incubated at 24 or 11°C and observed daily for hyphal growth. Hyphal colonies were counted, and the mean number ± SD of zoospores produced per 1 ml of water was calculated. In chemical inhibition assays, chemicals were added to the containers along with *Saprolegnia*, and zoospore concentrations on Day 4 were compared with those in control containers (no chemical).

Cyst germination inhibition assays. To produce cysts, 1 cm^2 blocks of CMA covered in *Saprolegnia* (CF1) hyphae were added to Petri dishes containing 30 ml of filtered, sterile pond water and maintained at ~22°C for 24 h. During the 24 h, *Saprolegnia* hyphae produced sporangia and released zoospores which quickly encysted on the bottom of the dishes. Cysts and remaining zoospores were counted using a hemocytometer, and aliquots of water were added to 6 well plates to give final concentrations of 8000 cysts per 5 ml of Leibovitz's L-15 medium (L-15; Gibco, Grand Island, NY, USA). This nutrient rich medium was used to mimic nutrient rich catfish flesh and allow cyst germination and dense hyphal growth. If sterile pond water, rather than L-15, was used as a culture medium, cyst germination resulted in very thin hyphae and a very rapid onset of asexual reproduction. Test chemicals were added as required and the cultures incubated at 11°C. Cultures were examined both macro- and microscopically daily for the presence of germinated cysts.

Chemicals. Chemicals were purchased from Sigma (St. Louis, MO, USA) and used at concentrations less than or equal to the maximum concentrations recommended for use with catfish (Alderman 1985, MacMillan 1985, Durborow et al. 1991): sodium chloride ≤100 mg l^{-1}, potassium permanganate ≤10 mg l^{-1}, copper sulphate ≤200 mg l^{-1}, formalin (37% formaldehyde solution) ≤200 mg l^{-1}, and malachite green ≤5 mg l^{-1}.

Amphotericin B (Fungizone; Bristol Myers Squibb, Princeton, NJ, USA) and cecropins A, B, and P1 (Sigma) were used at stock concentrations of 5 and 500 µg ml^{-1}, respectively. Herbicides [diquat (Chevron Chemical Company Agricultural Chemical Division, San Francisco, CA, USA), simazine (Ciba-Ceigy Corporation, Greensboro, NC, USA), hydrothol 191 and aquathol K (Pennwalt Chemical/Equipment/ Health Products, Philadelphia, PA, USA)] were donated by the Mississippi Department of Wildlife Fisheries and Parks (Fisheries Bureau, PO Box 451, Jackson, MS 39211, USA). The concentrations used were as follows: diquat, 0.125 to 1 µl l^{-1}; simazine, 0.2 to 3 mg l^{-1}; hydrothol 191, 0.625 to 1 µl l^{-1}; and aquathol K, 1.25 to 10 µl l^{-1} These were within the guidelines recommended for use with food fish (Hull et al. 1967, Johnson & Finley 1980, Durborow & Tucker 1992).

Inhibition assays with antimicrobial agents. One cm^2 blocks of CMA covered with *Saprolegnia* hyphae were inverted onto new CMA plates. Sterile filter paper disks (Difco) were placed on the agar approximately 2 cm away from the blocks. The disks were then saturated with 25 µl of antimicrobial agent solution, and the cultures incubated at 22°C and monitored for inhibition of *Saprolegnia* hyphal growth around the antimicrobial-agent-containing disks. In positive control experiments, *Escherichia coli* BB4 was streaked onto thirds of new CMA plates and filter paper disks were placed on the center of the expected growth area. An aliquot (25 µl) of antimicrobial agent solution was then added to each paper disk.

Challenge experiments. Laboratory challenge protocols have been described in detail elsewhere (Bly et al. 1992). Briefly, 8 to 12 juvenile catfish were held in 36 l tanks complete with air stone and filter. Floating mesh baskets were added to challenge tanks containing 5 agar blocks, each with a hemp seed covered with *Saprolegnia* (CF1) hyphae. The fish tanks were supported in a water jacket which could be chilled from ambient temperature (~22°C) to 12°C in 24 h. Water samples were taken before *Saprolegnia* was added (Day 0) and on Days 4 and 8 post-challenge to assess zoospore numbers. At the beginning of the challenge, chemicals were added to the water along with the addition of *Saprolegnia*, immediately before the chiller units were activated. Tank water was maintained at 12 ± 1°C for a total of 21 d, at which time the experiments were terminated. To assess the effects of chemicals, fish were monitored for mortalities and signs of saprolegniosis for 21 d post-challenge, and compared with controls: positive control = *Saprolegnia* added and negative control = no *Saprolegnia* added. Challenge protocols were repeated ≥3 times to ensure valid statistical analyses by *t*-test, in order to compare survival rates at 21 d in the treated tanks with those in the positive or negative control tanks.

RESULTS

Antimicrobials

Amphotericin B and the cecropins A, B, and P1 were tested for their abilities to inhibit the growth of *Saprolegnia* isolate CF1 hyphae (Fig 1a) Results indicated that although 0.125 µg of amphotericin B was able to

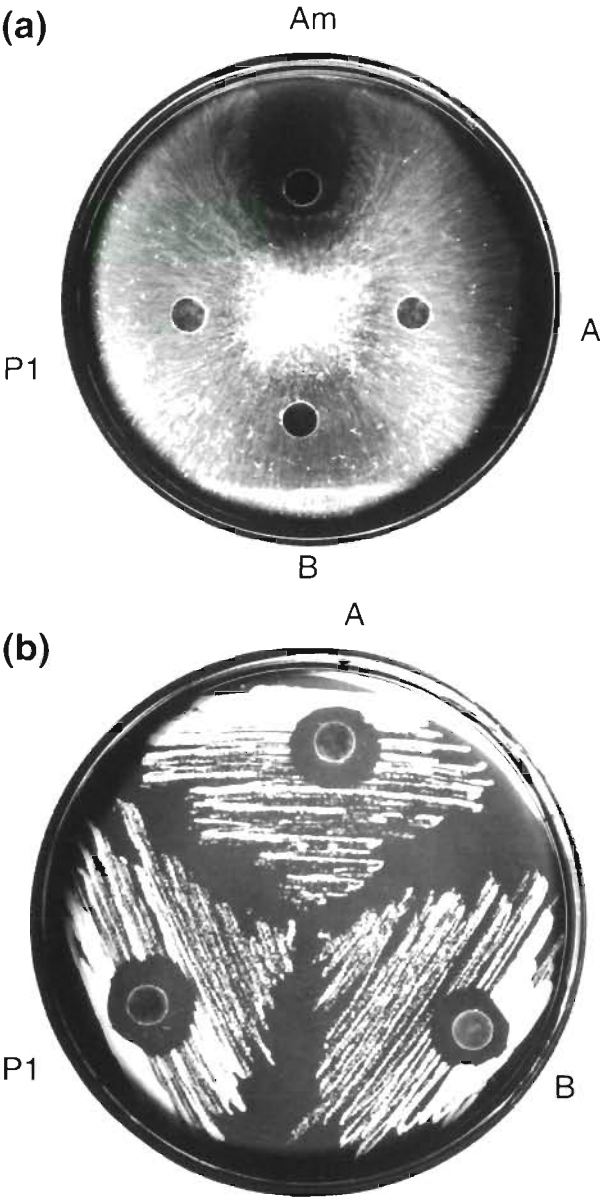

Fig. 1. Inhibition with antimicrobial agents. (a) Inhibitory effects of amphotericin B (Am; 0.125 µg $disk^{-1}$) and lack of effects with the cecropins (A, B, and P1; 12.5 µg $disk^{-1}$) on *Saprolegnia* CF1 hyphal growth. (b) Cecropin control, depicting inhibition of *Escherichia coli* BB4 growth; all cecropins were used at 12.5 µg

inhibit CF1 growth, none of the cecropins tested had any effect on *Saprolegnia* growth when used at doses ≤12.5 µg disk^{-1} In control experiments, all cecropins (A, B, and P1) were effective at inhibiting the growth of *Escherichia coli* BB4 over the same concentration range; inhibitions of *E. coli* resulting from 12.5 µg of each cecropin are shown in Fig. 1b. Amphotericin B inhibited the growth of all 6 catfish *Saprolegnia* isolates and ATCC isolates *Saprolegnia* sp., *S. diclina*, *S. ferax*, and *S. parasitica* (data not shown).

Chemical control of *Saprolegnia* zoospore production

Several chemicals were tested for inhibition or suppression of the production of *Saprolegnia* (CF1) zoospores. Results are given in Table 1 and indicate that if 1 *Saprolegnia*-covered hemp seed was added to 1 l of well water, by Day 4, ≥300 zoospores ml^{-1} water were observed. (Our well water supply contains no *Saprolegnia*.) Sodium chloride and potassium permanganate were ineffective at reducing zoospore production. Malachite green, the positive control, totally inhibited zoospore production at 2.5 mg l^{-1} and significantly suppressed zoospore production at concentrations as low as 1.0 to 0.25 mg l^{-1}. Copper sulphate inhibited zoospore production at 2.5 mg l^{-1} and suppressed production at concentrations ≥0.5 mg l^{-1}. Formalin was also inhibitory at 12.5 mg l^{-1} and was suppressive at 7.5 mg l^{-1}.

Table 1 Chemical inhibition/suppression of *Saprolegnia* (CF1) zoospore production under controlled laboratory conditions. Values are mean no. of zoospores ml^{-1} water ± SD (n = 3). –: not FDA-approved for use at this concentration; nd: not done

Culture contents	Formalin	$CuSO_4$	NaCl	$KMnO_4$	Malachite green
Saprolegnia (S)	>300	>300	>300	>300	>300
S + 200 mg l^{-1}	0	0	–	–	–
S + 150 mg l^{-1}	0	0	–	–	–
S + 100 mg l^{-1}	0	0	>300	–	–
S + 50 mg l^{-1}	0	0	>300	–	–
S + 25 mg l^{-1}	0	0	>300	–	–
S + 12.5 mg l^{-1}	0	0	>300	–	–
S + 10 mg l^{-1}	4 ± 2	0	nd	>300	–
S + 7.5 mg l^{-1}	10 ± 1	nd	>300	>300	–
S + 5 mg l^{-1}	>300	nd	>300	>300	–
S + 2.5 mg l^{-1}	>300	0	>300	>300	0
S + 1 mg l^{-1}	nd	nd	>300	>300	6 ± 4
S + 0.5 mg l^{-1}	>300	21 ± 2	nd	>300	6 ± 1
S + 0.25 mg l^{-1}	nd	>300	>300	nd	12 ± 4
S + 0.1 mg l^{-1}	nd	>300	nd	>300	>300
S + 0.05 mg l^{-1}	nd	nd	nd	nd	>300
S + 0.025 mg l^{-1}	nd	>300	nd	>300	>300

Chemical control of cyst germination

Saprolegnia isolate CF1 zoospores were placed in 6 well plates and allowed to encyst. Various concentrations of chemicals were added and the cultures were incubated at 11°C. If the chemical had no effect on cyst germination, hyphae rapidly grew and in 4 to 6 d totally covered the well, whereas if germination was inhibited the well remained clear Fig. 2 shows the results obtained with malachite green (as a positive control) and formalin on the germination of 8000 cysts contained in 5 ml of L-15 medium. All protocols were in duplicate and photographs were taken 6 d after the cultures at 11°C were initiated. There was confluent hyphal growth in the germination control, whereas growth was inhibited by 2.5 mg l^{-1} malachite green and 250 mg l^{-1} formalin. At lower doses of either chemical, growth was slowed but cyst germination was not inhibited. Potassium permanganate, copper sulphate, and sodium chloride had no effect on cyst germination at the highest doses approved by the FDA for use with catfish (data not shown). Microscopic analyses (Fig. 3) indicated that malachite green at 2.5 mg l^{-1} inhibited *Saprolegnia* germ tube growth; at 1 mg l^{-1} it slowed the growth of *Saprolegnia* and rendered many of the hyphae permeable, making them appear black due to uptake of malachite green. Formalin at 250 mg l^{-1} completely inhibited cyst germination for up to 12 d, a point exceeding the time expected for formaldehyde loss by evaporation.

Control of *Saprolegnia* growth with herbicides

Diquat, simazine, hydrothol 191, and aquathol K were tested for their effectiveness in inhibiting *Saprolegnia* (CF1) zoospore production and/or cyst germination as described above for chemical inhibition. Only diquat was effective. At concentrations of 0.5 and 0.25 µl l^{-1}, diquat significantly suppressed zoospore production ($p < 0.0001$ and $p = 0.0210$, respectively), but it had no effect at 0.12 µl l^{-1} (data not shown). Cyst germination was also slowed by diquat (1 µl l^{-1}), the treatment resulting in characteristic stunted star-like colonies after incubation at 11°C for 6 d (Fig. 4).

Laboratory challenge experiments

To determine if copper sulphate, formalin, and/or diquat could be used prophylactically to prevent the onset of winter saprolegniosis in catfish, laboratory challenge experiments were conducted. Results are given in Table 2 for formalin and diquat. In the positive control catfish challenge tanks, *Saprolegnia* was added to the water to yield zoospore concentrations of 30 to 40 ml^{-1} on Days 4 and 8 post-challenge. Fish in the positive control tanks exhibited gross *Saprolegnia*-associated lesions (denoted by * in the table) by Day 4 post-challenge, with a majority of the fish dying by Day 14. The 21 d survival rate was 3.4%. In negative control experiments, 2 fish out of 38 died without signs of winter saprolegniosis, giving a 21 d survival rate of 94.4%. Copper sulphate at 0.5 mg l^{-1} was toxic to catfish held at 11°C, with 100% mortality observed within 7 d (data not shown). Formalin used at 25 mg l^{-1} gave a survival rate of 96.6%. One fish (out of 29 challenged) died and was observed to have signs of saprolegniosis. At 12.5 mg l^{-1}, formalin was less effective at preventing saprolegniosis although the survival rate (86.7%) was still significant when compared to the positive controls. In the experiments with formalin at 12.5 mg l^{-1}, 6 fish (out of 45 challenged) died with only 4 exhibiting signs of saprolegniosis. These results suggested that concentrations of formalin lower than 12.5 mg l^{-1} would be even less effective at preventing disease and so lower concentrations of formalin were not tested. At a higher concentration of formalin (50 mg l^{-1}) all fish died in the first week post-challenge with no signs of saprolegniosis (data not shown), suggesting formalin toxicity. Diquat at ≥0.5 µl l^{-1} was toxic to all catfish in the challenge experiments, i.e. 100% mortality within 7 d (data not shown). At 0.25 µl l^{-1}, diquat toxicity was less, and although the survival rate after 21 d was only 56.2%, it was still significantly higher than the 3.4% seen in the positive challenge controls. At a diquat concentration of 0.125 µl l^{-1}, the mortality rate was only 10.7% and was not significantly different from that of the negative control, i.e. 5.6%. However, because the 3 deaths recorded using 0.125 µl l^{-1} were not associated with saprolegniosis, diquat may still be toxic at this level. In summary, formalin at 25 mg l^{-1} and diquat at 0.125 µl l^{-1} were effective at preventing winter saprolegniosis in catfish challenged under laboratory conditions.

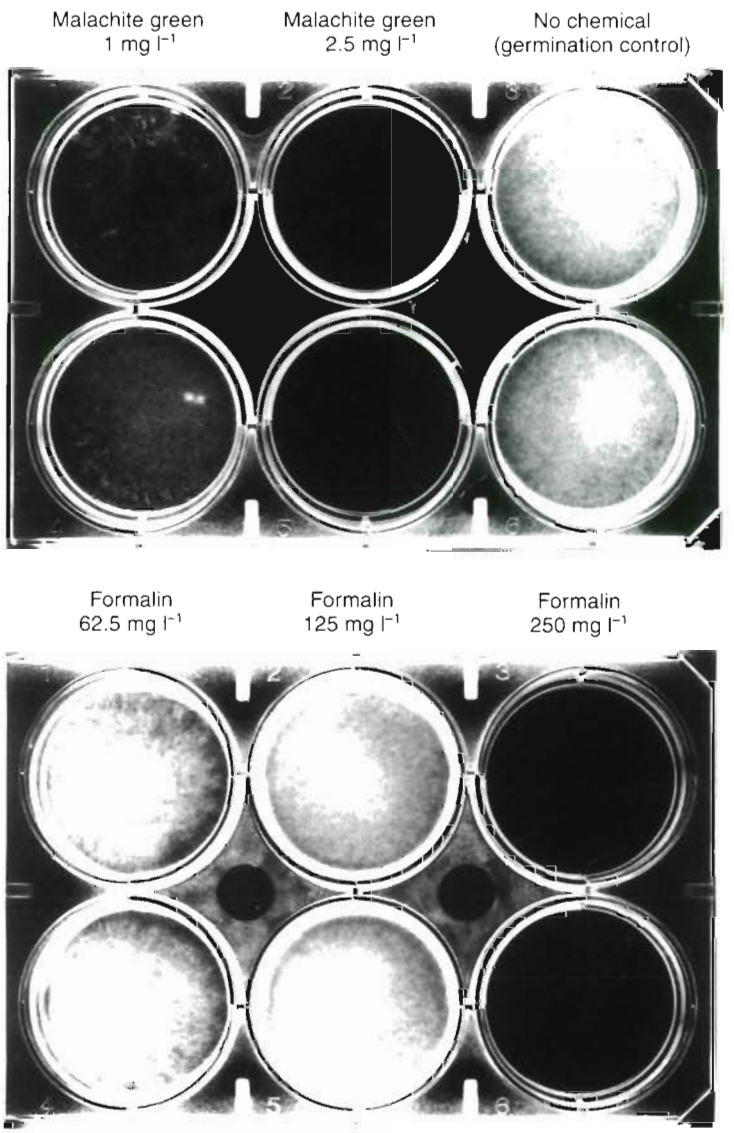

Fig. 2. Inhibition of *Saprolegnia* (CF1) cyst germination by various chemicals. Upper and lower wells on each plate are replicates. Each well contained 8000 *Saprolegnia* zoospores in 5 ml of L-15 medium and no chemical (control), or malachite green or formalin at the doses indicated. Confluent hyphal growth occurred in the germination control wells and with formalin at 62.5 and 125 mg l^{-1}; partial inhibition of hyphal growth was observed with malachite green at 1 mg l^{-1}; and what appeared to be total inhibition of cyst germination occurred with 2.5 mg l^{-1} malachite green and 250 mg l^{-1} formalin. These cultures had been incubated at 11°C for 6 d

DISCUSSION

Although the exact pathogenesis of saprolegniosis is unclear, based on information from laboratory challenge experiments (Bly

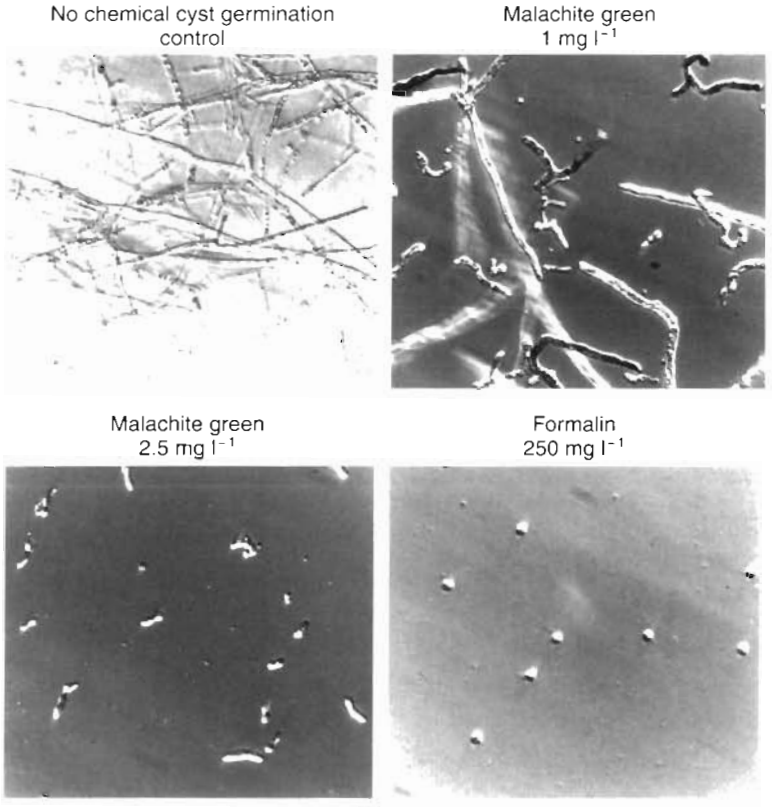

Fig. 3. Microscopic analyses of *Saprolegnia* cyst germination (200×). The control (no chemical), chemicals, and doses added are indicated. Cultures were maintained at 11°C for 6 d. Compared with the germination control, malachite green inhibited cyst germination at the germ tube stage (2.5 mg l^{-1}) or slowed hyphal growth and rendered some of the hyphae permeable, thus allowing the entry of malachite green (appearance of black hyphae; 1 mg l^{-1}). Formalin at 250 mg l^{-1} totally inhibited cyst germination

et al. 1992) and field studies (Bly et al. 1993), the following scenario is proposed. *Saprolegnia* zoospores become embedded in catfish mucus, encyst, and are sloughed as the mucus is replaced. This process is documented in trout (Wood et al. 1988). Potentially, if mucus secretion is slowed for a short period, say 2 to 4 d, cysts trapped in the mucus would have time to germinate. In order to induce winter saprolegniosis, a rapid decrease in environmental temperature is required. Although it is documented that a rapid decrease in water temperature leaves catfish immunosuppressed (Bly & Clem 1991, 1992), it is also possible that the 'temperature drop' impairs the function of mucus-secreting goblet cells, a notion which has recently been confirmed in our laboratory (unpubl. data). Once *Saprolegnia* germ tubes penetrate fish skin, it is theorized that hyphal growth meets with little immunological resistance in immunosuppressed fish, thus allowing the development of gross hyphae-associated skin lesions within a week following a rapid drop in temperature.

Saprolegniosis may thus be prevented by inhibiting sporangia development and the release of zoospores into pond water and/or by inhibiting cyst germination. Furthermore, the disease may be therapeutically treated by agents which can inhibit *Saprolegnia* hyphal growth within lesions. The simplest or most direct treatment would be to add an antimicrobial agent directly into the pond water or to incorporate it into catfish feed. The present study indicates that amphotericin B is an effective antimicrobial agent even though *Saprolegnia* is not a fungus. Amphotericin B binds ergosterol in fungal cell membranes, causing a rapid leakage of potassium which inhibits a variety of meta-

Table 2. Effects of formalin or diquat on winter saprolegniosis in channel catfish *Ictalurus punctatus* as determined by laboratory challengie experiments. D: days post-challenge; p: p-value, comparing experimental % survival with that of the positive control on D21

Treatment	No. of fish dead on:				D21, cumulative no.			% survival	p
	D4	D8	D14	D21	Dead	Alive	Total		
Positive control[a]	33•	22•	1•	1•	57	2	59	3.4	
Negative control[b]	0	2	0	0	2	34	36	94.4	<0.0001
Formalin 25 mg l^{-1}	0	1•	0	0	1	28	29	96.6	<0.0001
Formalin 12.5 mg l^{-1}	2	0	2	2•	6	39	45	86.7	<0.0002
Diquat 0.25 µl l^{-1}	1	6	6	1	14	18	32	56.2	<0.0004
Diquat 0.125 µl l^{-1}	2	0	1	0	3	25	28	89.3	<0.0019

[a]Catfish were subjected to a drop in water temperature and exposed to *Saprolegnia* (CF1) zoospores
[b]Catfish were subjected to drop in water temperature but not exposed to *Saprolegnia* (CF1) zoospores
•Overt signs of winter saprolegniosis

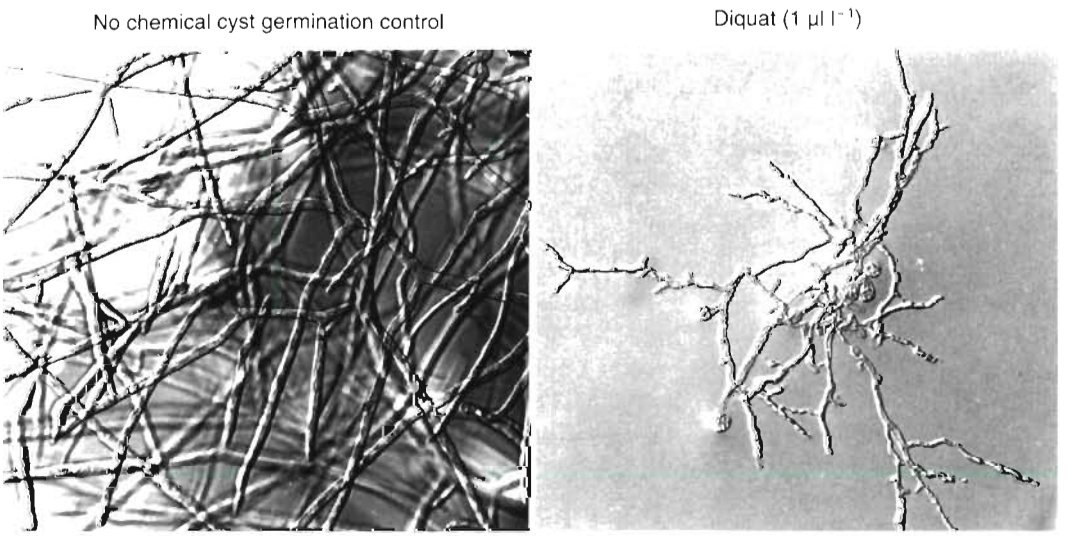

Fig. 4. Microscopic analyses of diquat suppression of *Saprolegnia* (CF1) growth from cysts (200×). Diquat at 1 µl l^{-1} suppressed the growth of hyphae once cysts had germinated to give a stunted star-shaped colony composed of apparently damaged (i.e. thin, misshapen, and granular) hyphae

bolic processes (Tilton & McGinnis 1987); the *Saprolegnia* also have sterols in the cell membrane (Berg et al. 1983). Although the use of amphotericin B with catfish is not foreseen, agents with similar actions should be investigated for their potential in the treatment of this disease.

The cecropins are potential candidates for the production of transgenic catfish, as they are natural antimicrobials secreted into the intestines of insects and pigs (Boman & Hultmark 1987, Boman et al. 1993). Several isolates of *Saprolegnia* and *Achlya* have been noted to be sensitive to certain antibiotics (Olah & Farkas 1978, Beakes & Gay 1980). However, results from the present study indicate that *Saprolegnia* is not affected by cecropins and hence transgenic catfish able to produce cecropins would probably not be able to combat *Saprolegnia* infections.

Malachite green was used in the present study as an effective positive control, preventing *Saprolegnia* zoospore production and cyst germination. However, it has been withdrawn by the FDA for use with food fish due to its teratogenic properties (Alderman 1985, 1994, Stoskopf 1993). Sodium chloride has been indicated for treatment of fungi (Stoskopf 1993) but was ineffective at preventing *Saprolegnia* growth in this study. Potassium permanganate and copper sulphate are successfully used in the catfish industry to control external parasites (MacMillan 1985), and copper sulphate is also an effective herbicide (Durborow & Tucker 1992). In this study potassium permanganate was ineffective at preventing *Saprolegnia* growth, corroborating a recent finding by Marking et al. (1994) with *S. hypogyna*. These observations are of interest because potassium permanganate is used to control fungal infections on trout eggs (Marking et al. 1994). Copper sulphate inhibited *Saprolegnia* growth, but unfortunately was toxic to catfish when used in laboratory challenge experiments. Toxicity was likely due to the well water used in our challenge experiments, which has low hardness and alkalinity, i.e. both are 20 to 40 mg l^{-1} and thus favor copper sulphate toxicity (Schwedler et al. 1985). To use copper sulphate at 0.5, 1.0, or 2.5 mg l^{-1} in commercial catfish ponds, alkalinity would have to be 50, 100, or 250 ppm, respectively; these values fall within the desired ranges for hardness and alkalinity for catfish culture, i.e. between 20 and 300 mg l^{-1} (Wellborn 1986). Formalin, which is used as an antifungal agent in catfish hatcheries, inhibited *Saprolegnia* growth and was quite effective at preventing saprolegniosis in laboratory challenge experiments at both 12.5 and 25 mg l^{-1}. However, at 50 mg l^{-1} formalin was toxic for catfish. Although the action of formalin on *Saprolegnia* is unknown, formaldehyde is a strong alkylating agent which can be used as a bactericidal agent at high concentrations but is only bacteriostatic at lower concentrations. Because formaldehyde is a gas, the activity is soon lost from water but in these studies the action on cyst germination was permanent and was sufficiently stable to prevent the onset of disease in challenge experiments. As both copper sulphate and formalin at the doses used in these challenge experiments are already FDA-approved for use in catfish ponds, they must obviously be considered as affordable methods of treatment. However, a pond cannot be screened for *Saprolegnia* zoospore levels if water temperatures are

warm, i.e. ≥17°C, because under such conditions *Saprolegnia* is outcompeted by other microorganisms (Bly et al. 1993). At lower temperatures, sampling for *Saprolegnia* takes ~4 d to yield results, thus ponds that pose a threat of saprolegniosis cannot be rapidly identified. Depending on cost effectiveness, it may still be worthwhile to treat ponds which have a history of saprolegniosis (indicating the potential for high levels of *Saprolegnia*) immediately after passage of a severe cold weather front that results in an acute decrease in pond water temperature.

Concerning the herbicides used, only diquat suppressed *Saprolegnia* growth. Diquat is approved for use in catfish ponds to control algae as well as submerged and emergent weeds. At low concentrations, 0.125 µl l^{-1}, diquat was effective at preventing saprolegniosis in laboratory challenge experiments. At higher concentrations, toxicity was a problem. However, it must again be noted that the quality of well water used in our laboratory challenge experiments was quite different from that in catfish ponds, and may have favored diquat toxicity. Unfortunately, specific information concerning diquat toxicity for catfish is apparently not available. However, diquat toxicity for other species varied considerably, e.g. the 96 h LC_{50} for rainbow trout was 10 mg l^{-1} and for bluegill was 245 mg l^{-1} (Morgan & Brunson 1989). It is also known that diquat toxicity decreases with increasing water hardness (Johnson & Finley 1980). Furthermore, diquat is inactivated by clay particles in pond water (Durborow & Tucker 1992), thus while more diquat may be required in a catfish pond to give results similar to those seen in the laboratory, it may be less toxic due to increased water hardness. Another study which used herbicides, including diquat, to inhibit *Saprolegnia* growth (Schreck et al. 1990) noted that diquat was the only herbicide effective against *Saprolegnia*-infected rainbow trout eggs.

In summary, these studies indicate the potential for the treatment and/or prevention of winter saprolegniosis in channel catfish.

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