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Dynamics of fungal infection in duckweeds *(Lemnaceae)*

Dynamik der Pilzinfektion bei Wasserlinsen *(Lemnaceae)*

by

Eliska REJMANKOVA, Meredith BLACKWELL and Dudley D. CULLEY

1. INTRODUCTION

Few fungi have been reported as pathogens of duckweeds. Two hyphochytrids, Reesia amoeboides Fisch and R. lemnae (Fisch) Karling (KARLING 1943, WAGNER 1969), a smut, Tracya lemnae (Setchell) Syd. (DAVIS 1942, FISCHER 1953), and species of Pythium have been implicated as pathogens of Lemna and Spirodela. These papers neither describe the degree of pathogenicity nor the relationship between duckweed and fungus in much detail.

There are several reports of massive dying off of duckweeds in natural habitats (STAVES unpubl.comm., REJMANKOVA 1979). While the cause of the duckweed kills has been assumed to be a fungus, only one abstract (COLBAUGH 1981) describes the infection as a foliar blight caused by the fungus Pythium aphanidermatum (Edson) Fitzpatrick. Pythium myriotylum

Drechsler from "duckweed" is listed in the catalogue of the American Type Culture Collection (JONG and GANTT 1984).

We have observed similar infections of dense stands of duckweed in more or less closed bodies of water. Infected duckweeds were found in small ponds in South Bohemia, Czechoslovakia, where duckweeds were held at high density (about 70 g m^{-2} dry weight). The etiological agent was presumed to be a fungus, but because the infection foci did not spread, no further study was done. In contrast, in Louisiana we observed infections which increased and rapidly killed an entire duckweed stand.

In the spring of 1985 a dense stand of Lemna gibba on lagoons near the Louisiana State University Dairy Farm (for further details on the lagoon system see CULLEY et al. 1978) became infected. A fungus, Pythium myriotylum was isolated from symptomatic duckweed plants. Additional fungal isolations were made during the spring and summer months and several aspects of the duckweed - fungus interactions were investigated:

1) How fast does infection spread under natural conditions in lagoons?
2) How do the duckweeds in a small closed system (outdoor cultivation tank) react to the infection?
3) What is the difference in death rate of duckweeds under different experimental temperature conditions?
4) What is the effect of the fungus on different clones of Lemna gibba and other species of Lemnaceae?

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2. MATERIAL AND METHODS

2.1. ISOLATION OF FUNGUS

Pythium myriotylum (LSU Dairy Farm, 21 March 1985, on Lemna gibba) was isolated from dying duckweeds. Pure culture of this isolate were used to reinfect pure hypochlorite-treated cultures of duckweeds collected from

the dairy farm population. Inoculated plants showed the disease symptoms observed in the field, and P. myriotylum was isolated from these plants. Symptomatic plants were examined microscopically for presence of fungal hyphae and oospores throughout the study. Observations were made on infection in the area from which this isolate was obtained, and the isolate was used in all laboratory tests. Additional Pythium myriotylum isolates were obtained from a cultivation tank on Ben Hur Farm, June 1985, and a shallow ditch at the junction of Ben Hur and Nicholson Road. Natural infections of these isolates were observed in a tank study (see below).

2.2. DUCKWEEDS

Pure cultures of twelve clones of six species of duckweeds (see Table 1) were kindly provided by Professor E. Landolt, Geobotanical Institut ETH, Zürich. For the description of clones see LANDOLT and URBANSKA (1980). Lemna gibba G3 was obtained from Dr. Ch.F. Cleland, Smithsonian Radiation Biology Laboratory, Rockville, MD. In addition, Lemna gibba isolated from the LSU Dairy Farm (Lemna gibba LA) was used in this study.

Table 1. Symptoms of different duckweed clones infected by Pythium myriotylum and cultivated for seven days at 30°C.
Tab. 1. Symptome verschiedener Lemnaceen-Klone, die durch Pythium myriotylum infiziert und sieben Tage bei 30°C kultiviert wurden.

Infected, killed	Some fronds symptomatic, usually not killed	Non-infected
Lemna gibba G3, GLA, 7107, 8428	Lemna valdiviana 7005, 7147	L. aequinoctialis 7122, 8642
Lemna minor 6578, 7938		Spirodela punctata 7504, 7517
Spirodela polyrrhiza 7003, 7344		

2.3. LABORATORY CULTURE OF FUNGI AND DUCKWEEDS

Fungal isolates were grown aseptically on 10% V-8 juice agar with 2 mg $CaCO_3$ per liter added. Duckweeds were cultivated in 9 cm disposable Petri dishes with 20 ml of 1/2 strength Hutner nutrient solution without sucrose (POSNER 1967). For infection tests an agar plug (10 mm diameter) was placed in each Petri dish with 3 to 5 duckweed fronds. Five replicates and two uninfected controls were used for each experimental variant. The number of dead and live fronds was recorded daily during each seven days experiment. Cultures were incubated in Percival growth chambers (1-30B series) with a light intensity of 5000 Lux and a 14/10 ligh/dark regime. Growth and infection was monitored at seven different temperatures: 22, 24, 26, 28, 30, 32, and 34°C. Fungal infection of symptomatic plants was verified by microscopic examination at the end of each experiment.

In order to determine effect of distance of inoculum from duckweeds upon infection, a sterile aluminium wire net was put in the Petri dish and plants were placed 0, 1, 2, and 3 cm from inoculum.

2.4. OUTDOOR CULTIVATION

An outdoor circular metal tank with a water surface area of 6.4 m^2 and water depth of about 30 cm was used for cultivation of Lemna minor (from the ditch at the junction of Ben Hur and Nicholson Roads) and later Lemna gibba (from LSU Dairy Farm). Initial density of each species was about 50 g m^{-2} of dry weight in the tank which corresponds to a 2-3 layers cover of duckweeds.

Amount of dead duckweeds was assessed as the percentage of area of the cultivation tank. This estimation was based on percentage of dead duckweeds within at least six 30x30 cm squares randomly placed on the water surface. Each square was subdivided into four parts for a more accurate estimate.

A similar estimation of dead duckweeds at the LSU Dairy Farm lagoon was expressed as percentage of dead duckweed area per entire area of duckweed colonization.

2.5. EVALUATION

The relative growth rate (RGR) of duckweeds was calculated according to KVET et al. (1971) except that numbers of fronds were used instead of weights. The relative death rate (RDR) was calculated according to the same formula. To avoid ln of 0 (no dead fronds at the beginning of the test), data were transformed as x=x+1. This transformation results in a slightly conservative estimate of actual RDR. Although RUNECKLES (1982) has suggested that there is not a priori reason to calculate death rate as a function of the amount of dead tissue which has accumulated, there are cases of spread of disease related death in which presence of accumulated dead tissue is a factor. We consider this to be such a case.

3. RESULTS

3.1. FIELD OBSERVATIONS

The occurrence of Pythium myriotylum in lagoons at the dairy farm was first recognized by the presence of small white patches of duckweeds in early March (Fig. 1). By the end of the month over 90% of the duckweeds were dead, and rains at the end of March washed dead plants to the bottom of the lagoon. The few remaining plants propagated rapidly and overgrew the entire lagoon. The next wave of infection appeared in mid April causing massive dying again. Once more the healthy duckweeds were established from the diminished population. From late April until July when the lagoon dried out, infection foci were present but there was no substantial spread of the disease as had been observed earlier.
In the outdoor metal tank 98% of the plants of both Lemna minor (Fig. 2a) and Lemna gibba (Fig. 2b) became infected naturally with Pythium myriotylum within five days. We followed the fate of dead plants of Lemna minor. After death a dense thick mat of plants formed. Apparently anaerobic conditions developed below the surface and production of gas bubbles occured. The rising of bubbles created numerous small holes in the compact mat. Dead plants at the edges of these holes were broken and sank to the bottom.

3.2. LABORATORY INFECTION STUDIES

A typical example of results of laboratory infection of duckweed clones with Pythium myriotylum after seven days is shown in figure 3. The course of RGR of control and RGR and RDR of infected Lemna gibba G3 at 30°C is plotted.

The dependence of RGR and RDR of all four infected Lemna gibba clones on temperatures is evident (Fig. 4a-d). All clones except Lemna gibba 8428 responded in a similar way with RDR higher than RGR (except at 22°C). Lemna gibba 8428 generally showed a slower response to the infection but was affected as well, especially at higher temperatures.

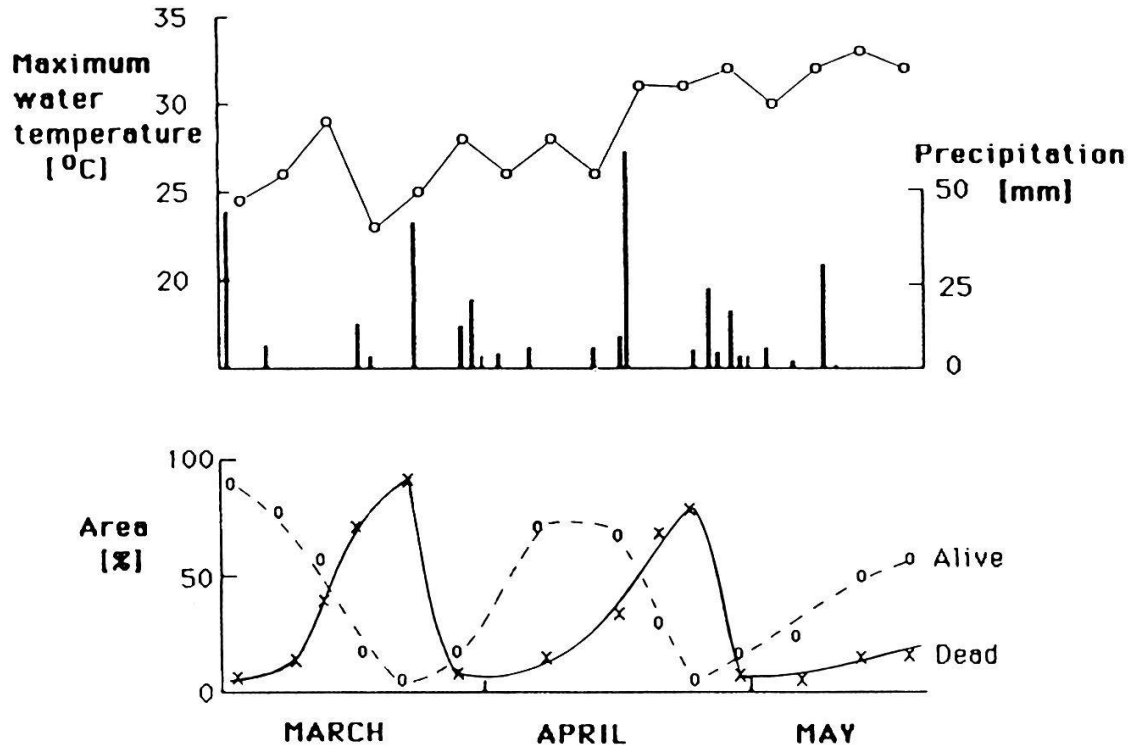

Fig. 1. Changes in the percentage of the area covered by dead (killed by the fungus Pythium myriotylum) (x——x) , and live (-o--o-) plants of Lemna gibba.
Upper part: the solid line = maximum temperature, five days averages (°C), the vertical bars = daily precipitation (mm).
LSU Dairy Lagoon, Baton Rouge, 1985.

Abb. 1. Aenderungen des Prozentanteils der bedeckten Fläche mit abgestorbenen (durch Pythium myriotylum) (x---x) und lebenden (-o--o-) Lemna gibba.
Oberer Teil: durchgezogene Linie = höchste Mitteltemperaturen während fünf Tagen in °C; senkrechte Kolonnen = Tagesniederschlag in mm. LSU Dairy Lagoon, Baton Rouge, 1985.

Ability of P. myriotylum to infect other duckweeds was tested at 30°C (Table 1). While fronds of four clones of Lemna gibba and two each of Lemna minor and Spirodela polyrrhiza were mostly dead within seven days, two clones of L. valdiviana showed some symptoms of infection, but were usually not completely killed. Microscopic examination of highly susceptible clones revealed dense mycelial growth and oospore production

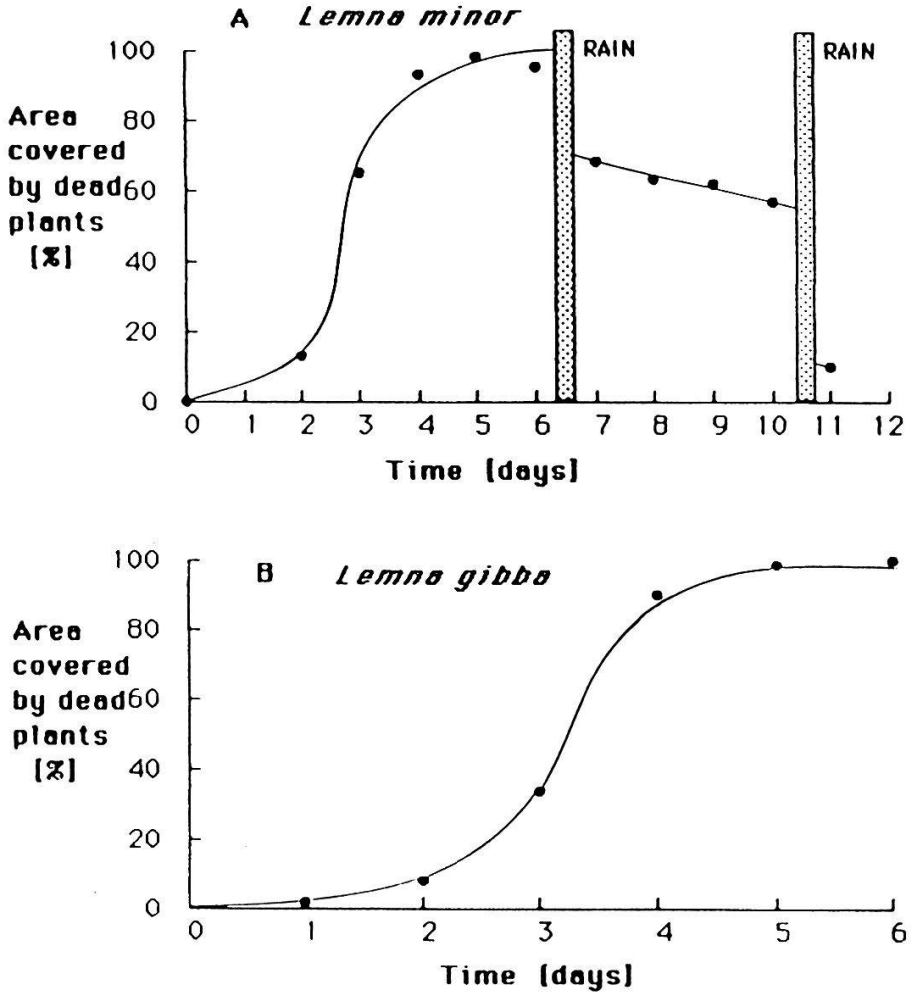

Fig. 2. Percentage of dead plants killed by Pythium myriotylum. Vertical bars show the rain that washed substantial amounts of dead plants to the bottom of the cultivation tank. LSU Ben Hur Farm, Baton Rouge.

Abb. 2. Durch Pythium myriotylum abgestorbene Pflanzen (in %). Vertikale Kolonnen zeigen die Regenmenge an, durch die ein beträchtlicher Teil der abgestorbenen Pflanzen auf den Grund des Kultivationsbeckens gedrückt wurde.
A = Lemna minor, June 1 till June 12, 1985
B = Lemna gibba, July 17 till July 23, 1885.

within root and leaf parenchymata. Vascular tissues were less often infected. Infected plants of L. valdiviana showed less mycelial invasion and few oospores within the plant tissues. Clones of L. aequinoctialis and Spirodela punctata did not become symptomatic. Microscopic examination showed that mycelial invasion of the plant tissues occured only rarely.

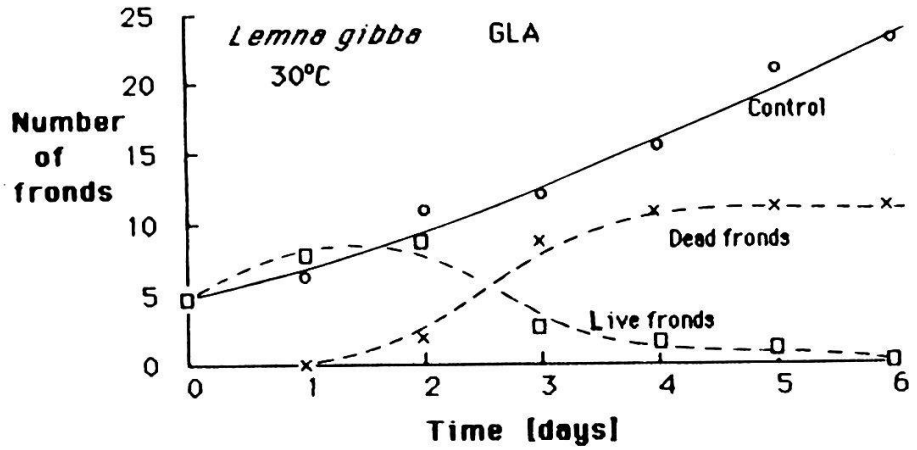

Fig. 3. Number of dead (-x--x-) and live (-□--□-) fronds of Lemna gibba GLA infected by Pythium myriotylum, and of control (-o—o-) on temperature during five days at 30°C.
Abb. 3. Anzahl toter (-x--x-), lebender (-□--□-) Lemna gibba GLA (mit Pilzbefall) und unter kontrollierten Bedingungen (-o—o-), während fünf Tagen bei 30°C.

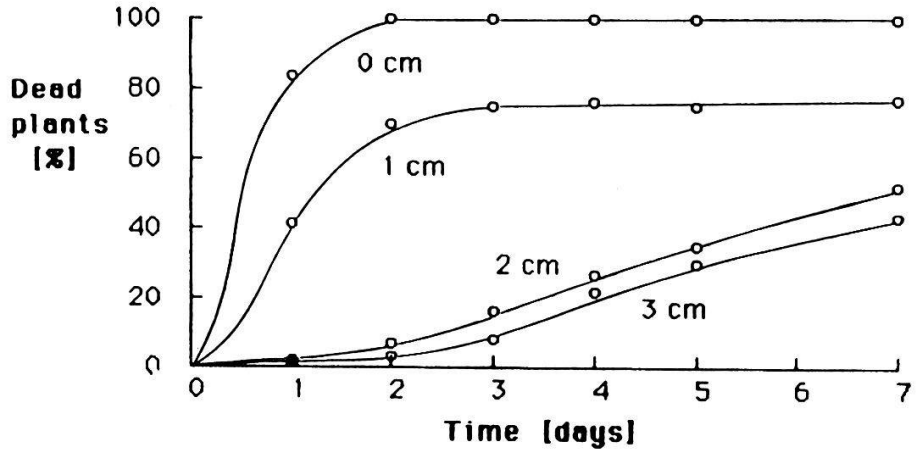

Fig. 5. Percentage of dead fronds of Lemna gibba 7107 grown in 0, 1, 2 and 3 cm distance of Pythium myriotylum inoculum at 30°C.
Abb. 5. Abgestorbene Glieder von Lemna gibba 7107 (in %), kultiviert in Abständen von 0, 1, 2 und 3 cm vom Pythium myriotylum Inoculum (bei 30°C).

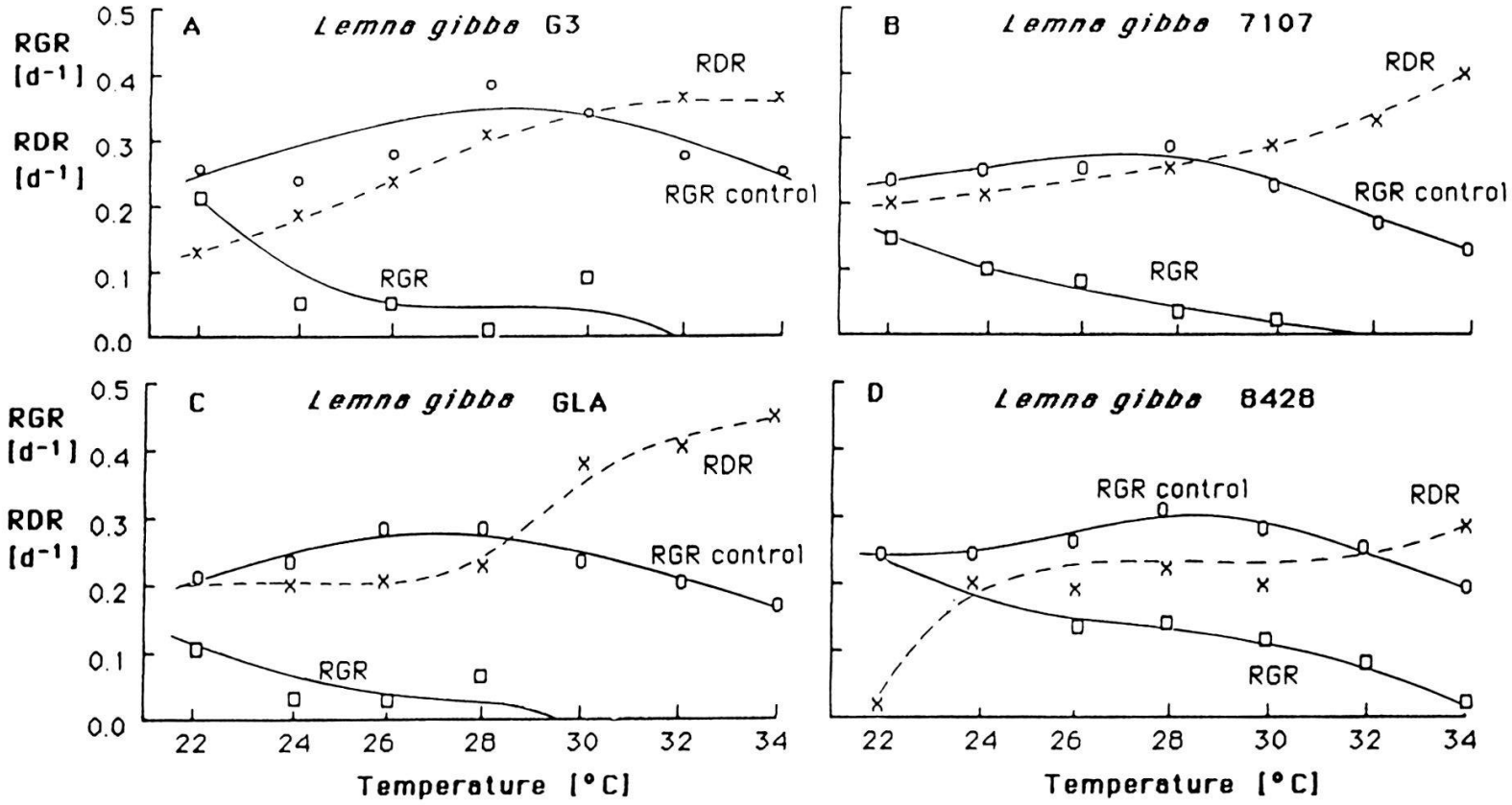

Fig. 4. The dependence of relative death rate (RDR) (-x--x-) and relative growth rate (RGR) of infected plants (-□--□-) and control (-o—o-) on temperature.

Abb. 4. Relative Sterberate (RDR) (-x--x-) und relative Wachstumsrate (RGR) der vom Pythium myriotylum befallenen Pflanzen (-□--□-) und der kontrollierten Pflanzen (-o—o-), bedingt durch die Temperatur.

A = Lemna gibba G3, B = Lemna gibba 7107, C = Lemna gibba GLA,
D = Lemna gibba 8428

Rate of death of duckweeds (Lemna gibba 7107) showed a direct relationship to the close proximity of inoculum (Fig. 5). All fronds 0 mm from the inoculum plug were dead within two days. Plants 2-3 cm away from plugs showed only 50% dead fronds in the seven days of the test.

4. DISCUSSION

The results from both the lagoon and outdoor tank show that the fungal infection can spread rapidly through a dense duckweed stand. Our laboratory studies confirm that two conditions are necessary for successful duckweed killing by Pythium myriotylum: 1) high temperature, and 2) high plant density. At temperatures below 22°C fungal growth and infection is too slow to overrun the fast growing duckweeds. Optimum temperature for infection and plant death was at the highest temperature (32°C) at which duckweed could survive. The optimum for fungal growth was even higher at 34-36°C. GAY (1969) has reported that temperatures for optimum infection of peanuts by Pythium myriotylum are lower than the optimum for growth of the fungus in the pure culture. We have been unable to determine if a similar difference in infection and growth optima occurs because the duckweeds do not survive higher temperatures.

On the basis of laboratory results of the test on dependence of RGR and RDR of infected duckweeds on temperature we can distinguish three types of plant reactions. The first, at lower temperatures (22°C) when RGR of test plants is higher than RDR, plants are able to overgrow the infection. The second, at 24-30°C, RDR is somewhat higher than RGR of test plants and RGR of the control does not decrease; RGR of the test plants is affected primarily by the fungus. In the third case, at 32-34°C, RDR is higher than RGR of the test plants and RGR of the control also decreases due to high temperature. The resulting RGR of the test plants is thus limited by both fungus and high temperature. Further tests are needed to clarify this dependence.

Climatic conditions and aquatic environments in Louisiana provide not only good conditions for fungal growth, but are also very suitable for rapid growth of duckweeds which promotes infection by Pythium myriotylum. The danger of fungal infection is apparently greater in warmer climates than in colder temperate regions.

Laboratory results showed that the fungus was able to invade tissue of all duckweed clones tested. However, pathogenicity did vary and appears to be species specific where tested against several clones of the same species. The basis for resistance is not known.

We do have preliminary results that suggest a phytotoxin may be involved in pathogenicity. Phytotoxins have been isolated from several species of Pythium, including P. myriotylum (CSINOS and HENDRIX 1978). This line of research is being continued.

We may conclude: 1) At higher temperatures (over 22°C) and with dense duckweed stands the amount of duckweeds killed by the fungus Pythium myriotylum grows exponentially and the whole stand can die in several days. 2) Pythium myriotylum seems to infect most of the duckweed species tested; very susceptible are Lemna gibba, Lemna minor, and Spirodela polyrrhiza. L. valdiviana is more resistant, L. aequinoctialis and Spirodela punctata never exhibit disease symptoms.

SUMMARY

Fungus Pythium myriotylum was isolated from dying duckweeds (Lemna gibba) growing in lagoons in Louisiana and proved to be the cause of duckweed kills. Under natural conditions in lagoons, as well as in the outdoor cultivation tanks, the amount of duckweeds killed by the fungus grew exponentially and the whole stand died in several days. From the six duckweed species tested in laboratory, the most susceptible to the fungal infection were Lemna gibba, L. minor, and Spirodela polyrrhiza. Lemna valdiviana was more resistant, L. aequinoctialis and Spirodela punctata never exhibited disease symptoms. Optimum temperature for infection was at about 32°C.

ZUSAMMENFASSUNG

Der Pythium myriotylum Pilz wurde von absterbenden Lemna gibba in Teichen in Louisiana isoliert und es konnte bewiesen werden, dass er das Absterben der Wasserlinsen (Lemna gibba) bewirkte. Unter natürlichen Bedingungen in Teichen und in Kulturbehältern im Freien nahm die Anzahl absterbender Wasserlinsen sehr schnell zu und in wenigen Tagen war der ganze Standort abgestorben. Laboruntersuchungen an sechs Wasserlinsen-Arten zeigten, dass Lemna gibba, L. minor und Spirodela polyrrhiza am anfälligsten waren für Pilzbefall. L. valdiviana war widerstandsfähiger, L. aequinoctialis und Spirodela punctata zeigten nie Infektionssymptome. Der Pilzbefall verbreitete sich am schnellsten bei 32°C.

REFERENCES

COLBAUGH P.F., 1981: Pathogenicity of Pythium aphanidermatum on duckweed and watermeal. Abstr. Phytopathology **71**, 867.

CSINOS A. and HENDRIX J.W., 1978: Parasitic and nonparasitic pathogenesis of tomato plants by Pythium myriotylum. Can.J.Bot. **56**, 2334-2339.

CULLEY D.D., J., GHOLSON J.H., CHISHOLM T.S., STANIFER L.C. and EPPS E.E., 1978: Water quality renovation on animal waste lagoon utilizing aquatic plants. EPA-600/2-78-153 report. 149 pp.

DAVIS J.J., 1942: Parasitic fungi of Wisconsin. Democr.Print.,Madison, WI. 157 pp.

FISCHER G.W., 1953: Manual of the North American smut fungi. Ronald Press, New York. 343 pp.

GAY J.D., 1969: Effects of temperature and moisture on snap bean damping off caused by three isolates of Pythium myriotylum. Pl.Dis.Reptr. **53**, 707-709.

JONG S.C. and GANTT M.J. (eds.), 1984: American type collection: Catalogue of fungi/yeasts. (16th ed.). ATCC, Rockville, MD. 441 pp.

KARLING J.S., 1943: The life history of Anisolpidium ectocarpii gen.nov. et sp.nov., and a synopsis and classification of other fungi with anteriorly uniflagellate zoospores. Amer.J.Bot. **30**, 637-648.

KVET J., ONDOK J.P., NECAS J. and JARVIS P.G., 1971: Methods of growth analysis: In: SESTAK Z., CATSKY J. and JARVIS P.G. (eds.), Plant photosynthetic production. Manual of methods. Junk, The Hague. 343-391.

LANDOLT E. and URBANSKA K., 1980: List of studied Lemnaceae samples, origin and chromosome numbers. In: LANDOLT E. (ed.), Biosystematische Untersuchungen in der Familie der Wasserlinsen (Lemnaceae). Veröff. Geobot.Inst.ETH,Stiftung Rübel,Zürich **70**, 205-247.

POSNER H.B., 1967: Aquatic vascular plants. In: WILT F. and WESSELS N. (eds.), Methods and developmental biology. Crowell, New York. 301-307.

REJMANKOVA E., 1979: The function of duckweeds in fishpond ecosystems. (In Czech.). Ph.D.Thesis, Department of Hydrobotany, Trebon, Czechoslovakia. 166 pp.

RUNECKLES V.C., 1982: Relative death rate: A dynamic parameter describing plant response to stress. J.Appl.Ecol. **19**, 295-303.

WAGNER D.T., 1969: A monocentric, holocarpic fungus in Lemna minor L. Nova Hed. **18**, 203-208.

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