Decay of Pondweed and Hydrilla Hibernacula by Fungi

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ABSTRACT

Asexual propagules of curlyleaf pondweed (Potamogeton crispus L.) and American pondweed (P. nodosus L.) were frequently rotted when collected during the winter from soil in drained irrigation canals. Three of the fungi isolated from decayed overwintering propagules, Fusarium crookwellense Burgess, Nelson & Toussoun, Papulaspora aspera Bern. & Dun. and Geotrichum sp., colonized healthy propagules of pondweeds and healthy tubers of Hydrilla verticillata (L.F.) Royle when inoculated with mycelium and incubated under laboratory conditions. Propagules of curlyleaf pondweed incubated under field conditions with debris colonized by any one of the three fungi were significantly more decayed than noninoculated propagules.

Key words: biological control, plant pathogens, Potamogeton nodosus, Potamogeton crispus, Hydrilla verticillata.

INTRODUCTION

Plants are an integral part of aquatic ecosystems, providing other organisms with food, shelter, and attachment sites. However, in irrigation systems the growth of aquatic plants may reach such proportions that water delivery is impeded and canal carrying capacity is reduced (14, 20, 23). Submerged aquatic plants cause some of the most serious weed problems in irrigation systems (14, 20, 23). Although they can be controlled to some extent by mechanical harvesting and herbicide applications, neither method is entirely satisfactory. Both methods are expensive and effective only until weed regrowth occurs, and herbicides may have toxic effects on nontarget organisms (3, 23, 25).

Efforts to find methods to control aquatic weeds have broadened to include biological agents such as plant pathogens (10, 13, 23, 25). However, only a few diseases of submerged weeds are known. Several fungi have been identified which attack hydrlle (9) and Eurasian watermilfoil (Myriophyllum spicatum L.) (1), but it is not yet possible to control any submerged aquatic weed with plant pathogens (8). The search for pathogens of submerged weeds has focused mainly on those which attack foliage (1, 13).

The possibility of using pathogens of asexual overwintering propagules as control agents has not been investigated, although asexual overwintering propagules are essential to the survival of many important submerged aquatic weeds (12, 16, 17, 18, 22). Northern California offers a unique opportunity to study diseases of asexual overwintering propagules. Many irrigation canals are not used during the winter months and contain only small amounts of water from seepage and rainfall, allowing easy access to overwintering propagules. Two submerged weeds common in California irrigation systems, curlyleaf and American pondweed were chosen for study. American pondweed forms subterranean tubers borne singly or in clusters on specialized branches at a soil depth of 15-20 cm (24). Curlyleaf pondweed forms turions in the leaf axils of growing plants which collect in the hydrosol when the plants senesce in autumn (18). The goals of the research reported here were to determine a) the extent to which tubers and turions decayed under field conditions, b) the identity of pathogens causing decay, and c) the potential for pathogens of tubers and turions to be used for biological control of American and curlyleaf pondweeds. In addition, pathogens of tubers and turions of the pondweeds were tested for pathogenicity to hydrlle. A preliminary report of the results has been presented (5).

MATERIALS AND METHODS

Overwintering tubers of American pondweed and turions of curlyleaf pondweed were collected from 2 unlined canals in the Richvale Irrigation System in Butte County, California. At site 1, located 3 km east of Richvale, the canal was approximately 3m deep and 6m wide, and supported high populations of American pondweed during the growing season. At site 2, located 8 km north of Gridley, the canal was 3m deep and 10m wide, and supported mixed populations of American and curlyleaf pondweeds, as well as elodea (Elodea canadensis Michx.) and Eurasian watermilfoil. Six hundred tubers and turions, collected in January and February of 1981 from the mud of the canal floor, were stored in plastic bags at 4 C and examined within 1 or 2 days.

Tubers and turions were washed and sorted for decay. Decayed areas of turions were black in color and soft, while decayed portions of tubers were soft and either black or

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dark reddish brown. Isolations were made from decayed portions of tubers and turions after treatment with 0.5% sodium hypochlorite for 0.5 to 5 min. Tissue pieces were transferred onto petri dishes containing water agar and potato dextrose agar (PDA) acidified with lactic acid (21) or streaked over the surface of 523 (15) or yeast dextrose carbonate (YDC) agar (21). Plates were incubated at 19-21°C on a laboratory bench. Isolations were also made from tubers of American pondweed which decayed after 6 months of storage in plastic bags at 4°C.

The microorganisms isolated by the above procedures and a binucleate Rhizoctonia sp. isolated from vegetative shoots of sago pondweed (Potamogeton pectinatus L.) (4) were screened for pathogenicity to non-stere, apparently healthy and unblemished tubers and turions which had been collected from the field and stored at 4°C for 2-3 wks. Fungal inoculations were performed by placing a 6-mm diameter agar plug, taken from an actively growing fungal colony on PDA or cornmeal agar, in contact with each tuber or turion. Bacterial inoculations were performed by dipping tubers and turions into turbid cell suspensions made from two 2-day-old YDC slants. Tubers and turions were incubated on moist filter paper in petri dishes in the dark at 6 and 9°C for up to 4 months, and evaluated at 9 and 15 weeks for symptoms of decay. Pathogenicity was evaluated as follows: + = all tubers and turions completely decayed and nongerminated, − = none of the tubers or turions decayed, or +/- = some of the tubers or turions completely decayed or all of them partially decayed but germinating.

Fungi which decayed tubers of American pondweed under laboratory conditions were tested individually for pathogenicity to tubers under field conditions from 11 November 1981 to 3 March 1982 at site 1. Fungi which decayed turions of curlyleaf pondweed were tested on turions at site 2 from 18 November 1981 to 3 March 1982. Two inoculation methods were used in separate plots at both sites and after inoculation, tubers and turions were buried in the hydrosol at depths of 15-20 cm and 5-10 cm, respectively. Treatments were replicated 10 times in a randomized complete block design. Each replication consisted of 6 freshly collected, apparently healthy tubers or turions, enclosed within an 8-cm-square envelope of plastic mesh (1 mm openings). Results were analyzed by a 2-way analysis of variance.

In the first method of inoculation, the inoculum consisted of fungus-infested debris (12 mg dry weight) that was placed inside each mesh envelope. Autoclaved shoots of American pondweed which had been colonized for 1 or 2 weeks by individual fungal isolates served as the infected debris. Tubers and turions incubated in envelopes with noninfested sterile debris or without debris served as controls.

In the second method of inoculation, spore suspensions from 1- to 2-week-old agar cultures, mycelium on infected cellophane, or sclerotia were used. Tubers or turions were dipped into suspensions containing 10-100 spores/ml of Actinospora aerinum (Hart) New., F. crookwelless or Geotrichum sp. for about 10 sec. One piece of cellophane carrying a colony of either P. aspera, Rhizoctonia sp. or Pythium carolinianum Matt. (21) was placed in each mesh envelope. Sclerotia of Botrytis cinerea Pers. ex. Fr. were collected from American pondweed tubers that decayed while stored at 4°C, and were used as inoculum at the rate of 10 sclerotia per envelope. Noninoculated tubers or turions served as controls in these plots.

Mesh envelopes containing tubers or turions were recovered from 4 blocks in each plot on 21 January 1982, and the envelopes in the remaining 6 blocks in each plot were removed on 3 March 1982. Each individual tuber and turion was rated for decay on the following scale; 0 = 0 to 15% of the tuber or turion decayed, 1 = >15 to 50% of the tuber or turion decayed, 2 = >50 to 85% decay, and 3 = >85 to 100% decay. The individual decay ratings for the 6 tubers or turions in each replicate were averaged to give a decay value for that replicate. The tubers or turions were then planted in 266-ml plastic cups containing nonsterile Yolo fine sandy loam and water to give a depth of 5 cm. The cups were placed in a growth chamber at 20/25°C night/day temperatures and a 14-hr photoperiod. Tubers and turions were allowed to germinate and grow for 2 wks, after which shoot dry wts were measured. Isolations were made from those tubers and turions that failed to germinate.

Soil physical characteristics were monitored during the field experiment (4). Soil samples collected from each plot in January had electrical conductivities of 0.2-0.7 dS/m and pH values of 6.8-8.0. The site soil texture at site 1 ranged from a sandy loam to a sandy clay loam, while at site 2 was a sandy loam. At the time the field plots were inoculated the soil temperature was 12.5°C and it decreased to a low of 6°C by mid-January; no differences were observed in soil temperatures between the two sites. During the course of the experiment 71.7 cm of rainfall was measured at the Oroville Dam (2). The matric potential of the soil at both sites, as measured periodically with tensiometers, was zero.

Fungi pathogenic to turions of curlyleaf pondweed under field conditions were tested for their pathogenicity to tubers of hydrilla. Nonsterile tubers of hydrilla capable of nearly 100% germination (Lars Anderson, personal communication) were inoculated with either P. aspera, Geotrichum sp., or F. crookwelless. Six-mm diameter plugs, taken from 16-day-old fungal colonies grown on potato dextrose agar in petri dishes, were placed in contact with tubers (1 plug/tuber). Tubers inoculated with uncolonized agar plugs served as controls. Twelve or 14 petri dishes, each containing 5 tubers on a piece of moistened filter paper, were used per treatment. Tubers were incubated in the dark at 11°C following inoculation. After 5 wks, half the petri dishes in each treatment were uncovered, placed individually in 2L plastic beakers and filled to 1-cm depth with Yolo fine sandy loam. Each beaker was filled with distilled water and placed in a growth chamber with a 16 hr photoperiod and 30/20°C night/day temperatures. The water in the beakers was changed weekly and after 8 wks germination was evaluated. At 9.5 weeks after inoculation, the tubers which had remained at 11°C were assessed for decay by gently squeezing with forceps; those which yielded to pressure were considered decayed. The decayed tubers were allowed to germinate an additional 4 wks. Differences in the proportions of decay and germination between control and inoculated tubers were tested.
using the normal approximation to the binomial distribution (19).

RESULTS

Isolations. Nearly 25% of the tubers or turions collected from canals during the winter of 1981 were found to be totally or partially decayed at the time of collection. Sixteen different fungi were isolated from decayed tubers and turions (4). Among the fungi most frequently isolated from freshly collected and decayed tubers of American pondweed were Cylindrocarpon sp., binucleate Rhizoctonia spp., and a newly described hypomycelite, P. aspera (6). In addition Heliscus bugdunensis Sacc. & Ther., B. cinerea and C. acerina were isolated from American pondweed tubers which rotted after 6 months in cold storage at 4 C. The fungi most frequently isolated from decayed turions of curlyleaf pondweed collected from the field were P. aspera, P. carolinianum, and a slow growing Geotrichum sp. (previously called Fusidium sp. [5]) which formed dry, powdery, white colonies on PDA (4).Six bacterial isolates were cultured from curlyleaf pondweed turions and seven bacterial isolates were recovered from American pondweed tubers.

Among the fungi and bacteria isolated from field-collected tubers and turions, only the fungi listed in Table 1 were pathogenic. Pathogenic fungi were able to decay healthy tubers or turions, preventing germination (Figure 1). Frequently mycelium and spores of the pathogenic isolates were visible on the surface of the decayed tubers or turions. Nonpathogenic fungi and bacteria were unable to cause decay and did not impede germination (Figure 1).

Only F. crookwellense and P. aspera caused decay of both tubers and turions; however, both fungi required longer times and warmer temperatures to decay tubers than to decay turions.

Turions of curlyleaf pondweed collected after 9 weeks of incubation in the field with debris colonized by either P. aspera or F. crookwellense were significantly more decayed than turions in noninoculated controls (Table 2). After 15

Table 1. Fungi which decayed tubers of American pondweed and turions of curlyleaf pondweed after incubation at 6 and 9 C, where 0 represents no decay, 0/+ represents some decay, and + represents decay causing 100% mortality.

<table>
<thead>
<tr>
<th>Host</th>
<th>Fungus</th>
<th>Amount of decay after incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6C,4wks</td>
<td>9C,4wks</td>
</tr>
<tr>
<td>Tubers</td>
<td>Pythium carolinianum</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Botrytis cinerea</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Centrospora acerina</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Fusarium crookwellense</td>
<td>0/+</td>
</tr>
<tr>
<td></td>
<td>Papulaspora aspera</td>
<td>0</td>
</tr>
<tr>
<td>Turions</td>
<td>Fusarium crookwellense</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Geotrichum sp. 'F10-81-3'</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Geotrichum sp. 'F11-81-8a'</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Papulaspora aspera</td>
<td>0/+</td>
</tr>
<tr>
<td></td>
<td>Binucleate Rhizoctonia sp.</td>
<td>0</td>
</tr>
</tbody>
</table>

Six tubers of American pondweed or four turions of curlyleaf pondweed were used in each experiment and decay ratings represent composite results from several experiments.

Isolate identification number. Isolates ‘F10-81-3’ and ‘F11-81-8a’ appeared to be the same species.

weeks, turions incubated with debris colonized by either isolate of a Geotrichum sp. were also decayed significantly more than control turions (Table 2). However, after 9 or 15 weeks of incubation in the field, only debris colonized by P. aspera significantly reduced the growth of new shoots from turions (Table 2). No significant decay or reduction in shoot production was evident among turions inoculated with spores or mycelium and incubated in the field (data not shown).

Reisolations from decayed turions were most successful for those fungi which grew rapidly in culture. For example, F. crookwellense and a binucleate Rhizoctonia sp. were frequently reisolated, but the slowly growing P. aspera and Geotrichum sp. were reisolated infrequently. Fusarium crookwellense was also isolated from turions inoculated with debris colonized by both isolates of the Geotrichum sp., Rhizoctonia sp., and P. aspera.

There was essentially no decay of American pondweed tubers recovered from field plots 9 weeks after inoculation (data not shown). Although there was more decay among tubers collected 15 weeks after inoculation, none of the treatments caused significantly more decay than occurred in the controls (data not shown).

Hydriella inoculations. Germination of hydriella tubers removed from 11 C after 5 weeks was low in all treatments (Table 3). However, there was significantly less germination among tubers inoculated with P. aspera and F. crookwel-

Figure 1. Results of laboratory experiments showing differences in the ability of fungi inoculated as mycelium on agar plugs to rot tubers of American pondweed and turions of curlyleaf pondweed after 2 months at 9 C. (A) Tubers inoculated with a binucleate Rhizoctonia sp. (left) and Papulaspora aspera (right). (B) Turions inoculated with Doctyliella aquatic (left) and Fusarium crookwellense (right). In both (A) and (B) pathogenicity was evaluated as '0' on the left and '+' on the right.

TABLE 2. AMOUNT OF DECAY AND SHOOT PRODUCTION OF CURLYLEAF PONDWEED TURIONS RECOVERED FROM THE FIELD AFTER 9 AND 15 WEEKS OF INCUBATION WITH DEBRIS OF AMERICAN PONDWEED INFESTED WITH VARIOUS FUNGI*. SHOOT GROWTH WAS MEASURED AS DRY WEIGHT AFTER TURIONS HAD BEEN ALLOWED TO GERMINATE FOR 2.5 WEEKS.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Decay*</th>
<th>Shoot dry wt (gm)</th>
<th>Decay*</th>
<th>Shoot dry wt (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>0.3</td>
<td>0.12</td>
<td>0.4</td>
<td>0.11</td>
</tr>
<tr>
<td>Sterile debris</td>
<td>0.3</td>
<td>0.11</td>
<td>0.5</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Papulaspora aspera    | 1.5**  | 0.04*             | 1.6**  | 0.05**            |
| Fusarium crookwellense| 1.5**  | 0.05              | 1.3*   | 0.07              |
| Geotrichum sp. 'F10-81-3' | 0.8   | 0.13              | 1.4*   | 0.08              |
| Geotrichum sp. 'F11-81-8a' | 1.0   | 0.07              | 1.7*   | 0.06              |
| Binucleate Rhizoctonia sp. | 0.8   | 0.07              | 1.2    | 0.06              |

*According to Dunnett's procedure: ** = significantly different from controls at P = 0.01; * = significantly different from controls at P = 0.05; + = significantly different only from untreated control at P = 0.05. At 9 weeks each value is the mean of 4 replications; at 15 weeks n=6.
*Turions within each replicate were rated for decay on a scale from 0 to 3 where 3 = 85% decay.
*Isolate identification number. Isolates 'F10-81-3' and 'F11-81-8a' appeared to be the same species.

TABLE 3. DECAY AND SUBSEQUENT GERMINATION OF HYDRILLA TUBERS INOCULATED WITH 5 FUNGI AND INCUBATED 5 OR 9 1/2 WEEKS AT 11°C. TUBERS INCUBATED ON MOIST FILTER PAPER IN PETRI DISHES (5/DISH) WERE INOCULATED INDIVIDUALLY WITH A 6-MM-DIAMETER PLUG CUT FROM AN ACTIVELY GROWING FUNGAL COLONY ON AGAR.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5 wks</th>
<th>9 1/2 wks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Germinated</td>
<td>% Decayed</td>
</tr>
<tr>
<td>Control</td>
<td>36</td>
<td>20</td>
</tr>
<tr>
<td>Geotrichum sp.</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Papulaspora aspera</td>
<td>10*</td>
<td>50*</td>
</tr>
<tr>
<td>Fusarium crookwellense</td>
<td>0*</td>
<td>100*</td>
</tr>
</tbody>
</table>

*Significantly different from controls at P < 0.05; for each value n=30, except the control at 9 1/2 weeks, where n=40.

DISCUSSION

Among the fungi pathogenic to tubers and turions, only Rhizoctonia spp. have been previously reported to be pathogenic to aquatic plants. Rhizoctonia solani is reported to be a pathogen of sago pondweed (7) and a Rhizoctonia sp. with the perfect state of Aquathanetaphorus pendulus Tu and Kimbrough causes a blight of the floating weed waterhyacinth (Eichhornia crassipes [Mart.] Solms.) (11). The isolates of Rhizoctonia obtained from decayed tubers and turions of pondweeds did not appear to be pathogenic to tubers and turions in the laboratory tests. However a binucleate isolate of Rhizoctonia cultured from necrotic sago pondweed stems was capable of decaying turions of curlyleaf pondweed (Table 1).

One measure of the potential efficacy of the fungi as biological control agents is the extent to which they decrease shoot production of inoculated tubers and turions. Only turions incubated with debris infested by P. aspera tended to have both a higher level of decay and lower shoot dry weights than noninoculated control turions (Table 2). The length of the field experiment was approximately 2 months shorter than the time for which canals are drained in winter. As incubation time may be a limiting factor in the ability of the inoculated fungi to decay tubers and turions, it is possible that further reductions in shoot production could be achieved during the winter season through longer incubation periods.

The results presented here indicate that overwintering propagules of curlyleaf and American pondweeds are subject to decay. Several fungi were isolated which appear to be primary determinants of decay, and were shown to produce decay when inoculated onto turions of curlyleaf pondweed in natural soils. The same fungi were also able to decay tubers of hydrida in laboratory tests (Table 3). It is not presently possible to control submerged aquatic weeds with plant pathogens which attack vegetative shoots (8). The results indicate that plant pathogens of overwintering propagules should be studied for their potential to control aquatic weeds.

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