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Technical Report A-96-9
September 1996

**US Army Corps
of Engineers**

Waterways Experiment
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Aquatic Plant Control Research Program

Specific Association of Plant Pathogens with Submersed Aquatic Plants

*by Edwin A. Theriot, Stewart L. Kees, WES
Haim B. Gunner, University of Massachusetts*

WES

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by Edwin A. Theriot, Stewart L. Kees

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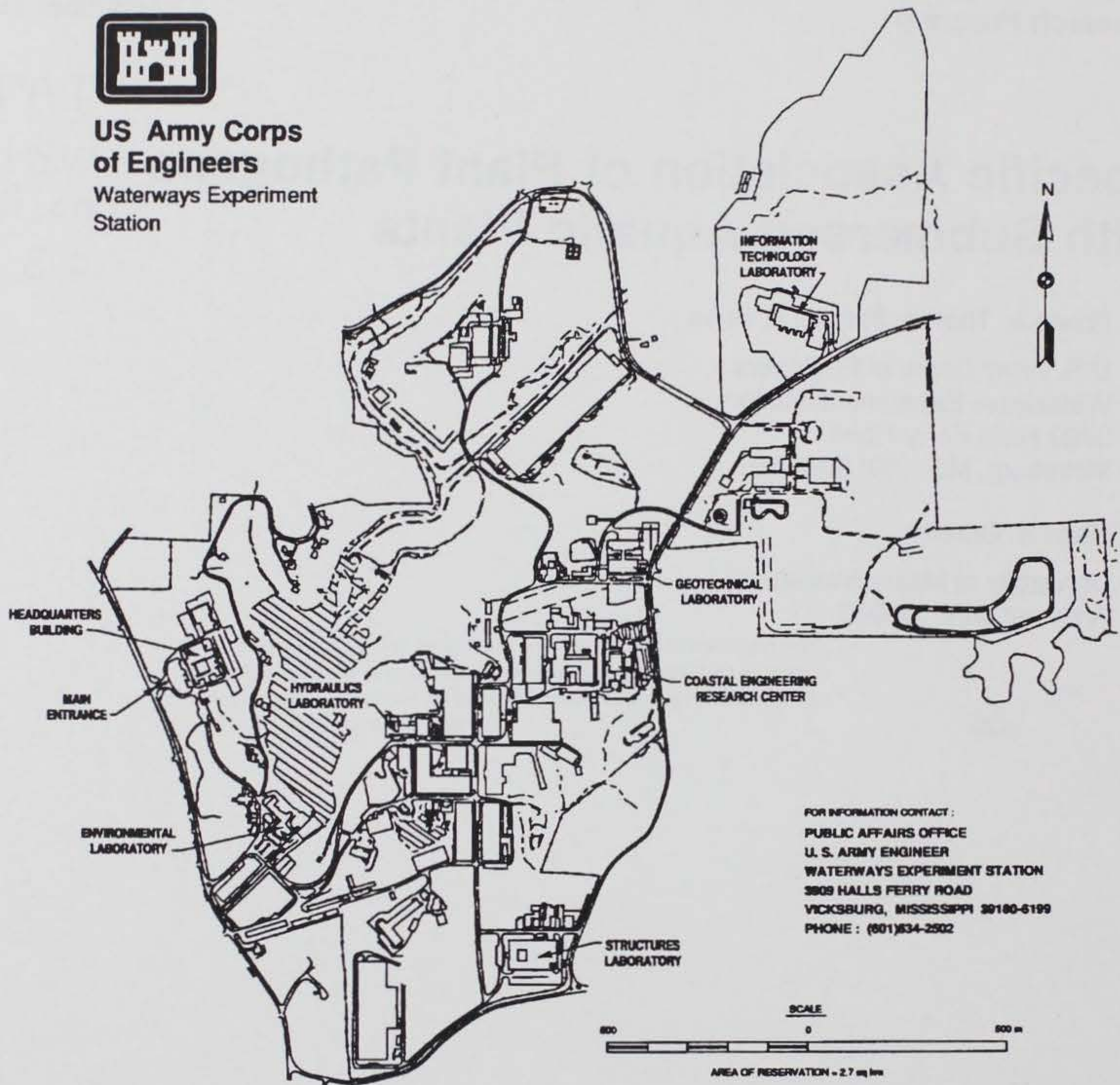
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of Engineers**
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Preface

The work reported herein was conducted as part of the Aquatic Plant Control Research Program (APCRP), Work Unit 32388. The APCRP is sponsored by the Headquarters, U.S. Army Corps of Engineers (HQUSACE), and is assigned to the U.S. Army Engineer Waterways Experiment Station (WES) under the purview of the Environmental Laboratory (EL). Funding was provided under Department of the Army Appropriation No. 96X3122, Construction General. The APCRP is managed under the Center for Aquatic Plant Research and Technology (CAPRT), Dr. John W. Barko, Director. Mr. Robert C. Gunkel was Assistant Director for the CAPRT. Program Monitor during this study was Ms. Denise White, HQUSACE.

The report was prepared by Dr. Edwin A. Theriot and Mr. Stewart L. Kees of the Aquatic Ecology Branch (AEB), EL, and Mr. Haim B. Gunner of the University of Massachusetts at Amherst. The research and data analyses were performed by the authors. Dr. Theriot was Principal Investigator. Appreciation is expressed to Mr. Dick Kasul for his statistical advice, to Ms. Jean Johnston for her assistance in typing the draft manuscript, and to Dr. Alfred F. Cofrancesco, Jr., Team Leader, Biomanagement Team, and Ms. Linda Winfield, Ms. Ramona Warren, Mr. Harvey Jones, Dr. Gary Joye, and Dr. Jan Hoover for their support and technical assistance.

The study was conducted under the direct supervision of Dr. Edwin A. Theriot, Assistant Director, EL, and under the general supervision of Dr. Conrad J. Kirby, Chief, Ecological Research Division, and Dr. John W. Keeley, Director, EL.

At the time of publication of this report, Director of WES was Dr. Robert W. Whalin. Commander was COL Bruce K. Howard, EN.

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1 Introduction

Background

Control of nuisance aquatic plants in our Nation's waterways is of major concern to Federal, State, and local resource agencies. Millions of dollars are spent yearly on chemical, mechanical, and physical control methods that are costly and often detrimental to the aquatic ecosystem (McGehee 1979). Even when successful, such stop-gap measures are temporary and require repeated treatment throughout the growing season.

Biological control offers a long-term management approach that is more desirable in many respects than mechanical and chemical control methods. Eradication is not the aim of biological control. Seldom will a parasite destroy the entire host population (Huffaker, Simmons, and Laing 1976). Once the biocontrol agent has established an association with the plant, continual stress is exerted on the plant population. Thus, plant population decline is normally gradual, preventing nutrient loading of the system and allowing it to cope with minimal imbalance.

Microbial control of aquatic and wetland plants is a viable approach. It is a proven method of control for emergent and floating aquatic plants, including waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) (Sanders and Theriot 1986), winged waterprimrose (*Ludwigia decurrens* (Walt.) DC.) (Boyette, Templeton, and Smith 1979), and northern jointvetch (*Aeschynomene virginica* (L.) B.S.P.) (Daniel, Templeton, Smith, and Fox 1973; Templeton, TeBeest, and Smith 1984).

Host specificity is a major prerequisite for biocontrol agents. Interactions between microorganisms and plants range from beneficial symbiosis to pathogenic and saprophytic relationships. In searching for host-specific pathogens for hydrilla and Eurasian watermilfoil, it has become necessary to understand the mechanisms involved in the association of plant pathogens with submersed aquatic plants. There are four distinct phases of the infection process at which specific association may occur between the host plant and the pathogen (Ingram, Sargent, and Tommerup 1975).

Foliar plant pathogens begin to interact with the host plant by colonizing the plant surface. Attachment represents the first phase of the infection process. A pathogen must attach and begin to grow if it is to appreciably influence the growth of the plant or to promote a defensive response (Smith, Chand, Harris, and Andrews 1989). Adhesion of the microbe to the plant cell surface is not only a prerequisite to successful colonization but may also determine the type of relationship that will occur between the two. In some cases, selective adhesion of the microbe to the host cell wall is an early determinant of host specificity (Dazzo and Hubbell 1975). Understanding the recognition and adhesion process occurring between plant pathogens and aquatic plants is therefore important in determining compatibility, host specificity and, possibly, pathogenicity.

Once the pathogen reaches the host, various activities proceed at the phylloplane. Generally, the spores germinate and grow in response to two stimuli: a physical contact stimulus that directs the growth of the main hyphae, and the chemical stimulus resulting from a diffusible substance, which arrests hyphal growth and triggers appressorium formation (Ingram, Sargent, and Tommerup 1975). The appressorium is the flattened, thickened tip of a hyphal branch by which some fungi attach to their host. Formation of the appressorium marks the end point of the second phase of the infection process, growth on the surface.

The initiation of the third phase of infection is host penetration. Penetration of the host by a fungus can occur in three ways: (a) directly through the intact surface (generally by cellulase and/or pectinase production), (b) through natural openings such as stomates, and (c) through wounds. Submersed aquatic plants have few natural openings; therefore, direct penetration and wounds are the principal methods of invasion. The production of disease symptoms in the host is the final phase of infection and is mediated by toxin or lytic enzyme release from an outgrowth of the appressorium called the infection peg (Flentje 1959).

Specificity may be confirmed at any phase of the infection process and is dependent upon a number of factors, such as the presence or absence of lectins, enzyme cofactors, morphological and chemical inhibitors, or even environmental factors. The rust fungus *Puccinia coronata*, for example, will only form appressoria on gelatin in the presence of zinc ions (Sharp and Smith 1952). The plant often releases substances onto the surface of its tissues that influence spore germination (Brown 1922; Walker, Link, and Angell 1929). High phosphorus, calcium, and potassium are believed to reduce the infection capability of several fungi (Gaumann 1950).

Environmental factors may positively or negatively influence plant susceptibility to infection. For example, high or low temperature, or high or low light intensity, may increase or decrease plant susceptibility to several fungal pathogens (Gaumann 1950). The *Rhizobium*-legume association mediated by specific plant lectins (glycoprotein) demonstrates a potential means of specific attachment of microbes to aquatic plants (Dazzo and Hubbell 1975).

The lectin hypothesis was advanced some years ago (Bohlool and Schmidt 1974). Lectins are proteins or glycoproteins that bind to cell surfaces via specific oligosaccharide components. The best known are the phytohemagglutinins, which agglutinate red blood cells (Matsumoto and Osawa 1969). The hypothesis is that lectins provide a site on the surface of the plant that interacts with a specific, distinctive oligosaccharide on the surface of the microorganism, as described in the *Rhizobium*-legume association.

Strominger and Ghuysen (1967) suggested that, since certain oligosaccharides that react with plant lectins are constituents of both bacterial and fungal cell walls, lectins may play a role as determinants of specificity.

Lectins are a poorly defined group of molecules. They contain 0 to 22 percent carbohydrates and make up as much as 10 percent of total protein in legume seeds. There has been much speculation as to the physiological role lectins play within a plant. The list of functions of plant lectins includes the following: (a) act as plant antibodies to neutralize bacteria and fungi, (b) serve to protect plants against fungal attack by inhibiting fungal enzymes, (c) play a role in transport and storage of sugars in plants, (d) are responsible for the organization of glycopeptides possessing enzymatic activity in multi-enzyme systems, (e) play a role in differentiation and development of cells, and (f) act as *Rhizobium*-legume specificins (Sharon and Lis 1972; Liener 1976).

Three classes of lectins have been observed. In Type I lectins, all subunits are identical. Type II lectins are those with two different pairs of promoters (two different sized subunits). Type III lectins are composed of four subunits with four oligosaccharide binding sites (Broughton 1978).

Most rhizobial-lectin experiments have used seed lectins because of the relative ease of extraction of lectins in legume seeds. Root lectins are poorly characterized as to their similarity with seed lectins of the same plant, their location on the root system, and their occurrence at infection sites that eventually become nodulated. Pueppke et al. (1978) determined that the root lectin of clover was no longer detectable in roots of 2- to 3-week-old plants.

Bauer (1977) suggests that resistance in plants is a dominant trait, while the virulence of pathogens is inherited as a recessive trait. In this model the potential pathogen becomes virulent by escaping recognition by the plant. Lectins have been proposed for the recognition molecules found in plants (Bauer 1977; Callow 1984). The association of plant lectins with infectivity of a pathogen was first demonstrated by Sequeira and Graham (1977). The study involved *Pseudomonas solanacearum*, a pathogen of potato and tobacco. Potato lectin was shown to bind to all 34 avirulent and none of the 55 virulent strains of *P. solanacearum*. The binding site for the lectin was the lipopolysaccharide (LPS) component. Lack of agglutination was attributable to the extrapolymer polysaccharides (EPS) of the virulent strains.

The researchers demonstrated that the potato lectin would bind with the glycosylated receptor LPS of both the virulent and avirulent strains as well as the EPS of the virulent organisms. This same scenario has been demonstrated in several other species of *Pseudomonas* and *Erwinia* with virulent and avirulent organisms on apple, red beans, and tobacco (Anderson and Jasalavich 1979).

Pistole (1981) stated that studies using isolated lectins support the role of lectins in host defense. Plant proteins resembling lectins have been found to inhibit the activity of polygalacturonases from fungi pathogenic for these plants (Albersheim and Anderson 1971; Anderson and Albersheim 1972).

Purpose

The purpose of this investigation was to gain a basic understanding of the initiation of the infection process between fungal plant pathogens and submersed aquatic plants. The specific attachment mechanism of plant lectins could offer a potential method of screening for host-specific pathogens. Lectins have also been implicated in the host defense system as a recognition factor.

The objectives of this investigation were (a) to demonstrate pathogenicity of fungal plant pathogens currently being maintained in cryoculture, which have been isolated previously from hydrilla and Eurasian watermilfoil, (b) to search for lectins and/or agglutinins in the roots, foliage, and tubers/seeds of hydrilla and Eurasian watermilfoil tissues, and (c) to determine the role lectins may play in the host-pathogen association mechanism using known pathogens of each target plant species.

Pathogenicity was evaluated to determine the specificity of association of a fungus with a target plant. Fungal attachment was determined in order to relate pathogenicity to the presence or absence of lectin. Lectin activity was studied to gain insight into the function of lectins as it relates to the specific association of aquatic plants with fungal pathogens and to provide evidence toward the validation or rejection of the theory that lectins are mediators of plant defense mechanisms.

2 Materials and Methods

Experimental Plant Material

Hydrilla and Eurasian watermilfoil were collected from field sites in Florida and Tennessee, respectively, and maintained in greenhouse cultures. Plants were rinsed with tap water, and 10.0-cm apical portions were excised. The apical portions were planted in flats containing autoclaved pond sediment, 30 apical segments per flat. Flats were placed in fiberglass tanks 1.5 by 0.75 by 1 m deep containing 200 gal (760 l) of Smart and Barko solution (Smart and Barko 1985) made with reverse osmosis water. Chillers were used to maintain the water temperature at 25 °C, and atmospheric CO₂ was bubbled into the tanks to maintain an adequate supply of inorganic carbon.

Culture Media

Potato dextrose agar (PDA) was used for the propagation of fungal cryo-isolates evaluated in the rapid screening bioassay. Dehydrated Difco PDA concentrate was used according to recommended procedures. Chloramphenicol was added at a concentration of 250 mg/ml to inhibit bacterial contamination.

Richard's V8 agar (V8A) was used for plating fungal pathogens from which 2.5-cm agar plugs were taken to inoculate flasks of V8 broth.

Richard's V8 broth (V8B) was used for the propagation of fungal inocula used in the pathogen bioassay, attachment assay, and fungal growth assay.

Double-strength B5 medium was prepared according to Gamborg et al. (1968) and used in the fungal growth assay.

Fungal Cultures

Fusarium roseum 'Culmorum' was provided by Dr. R. Charudattan, University of Florida, Gainesville, FL. This organism was originally isolated

from diseased *Stratiotes aloides* in the Netherlands (Charudattan and McKinney 1978). *Macrophomina phaseolina* (Tassi) Goid. was originally isolated from hydrilla collected from a lake in southern Texas as part of a biocontrol study for the U.S. Army Engineer Waterways Experiment Station (Joye 1990).

Acremonium curvulum and *Colletotrichum gloeosporioides* were isolated from watermilfoil collected from Lake Wingra, Wisconsin (Andrews, Hecht, and Bashirian 1982; Sorsa, Nordheim, and Andrews 1988), and provided by Dr. Craig Smith. *Mycocleptodiscus terrestris* was collected from a lake in western Massachusetts (Gunner 1983) and provided by Ecoscience Laboratories, Inc., of Amherst, MA.

All other fungal isolates used in this study were obtained as existing cryocultures from earlier field surveys of hydrilla and Eurasian watermilfoil in Florida, Louisiana, Texas, and Vermont.

Rapid Screening Bioassay

The bioassay used the 5.0-cm apical segment of leafy, healthy hydrilla and watermilfoil tissues obtained from greenhouse cultures. These apical segments were placed one each into 40 ml of sterile Smart and Barko medium in glass petri dishes. Four agar plugs were cut from a 7-day-old PDA culture of the test fungus using a sterile No. 3 cork borer, then placed face down on the plant material (two plugs on the stems and two plugs on the leaves). Inoculated plant tissues were prepared in triplicate and incubated for 14 days at room temperature on a 10:14 light/dark cycle. Agar plugs were checked daily to ensure that the test isolate remained in physical contact with the plant.

Plant samples were qualitatively evaluated at the end of the 14-day incubation period for visible signs of necrosis, chlorosis, stunted new growth, and tissue death. Any fungal isolates exhibiting measurable pathogenesis were verified by Koch's postulates.

Lectin Isolation, Purification, and Characterization

Plant preparation

The leaves, stems, and roots of hydrilla and watermilfoil from greenhouse cultures were harvested and washed twice with tap water, and twice with distilled water to remove algae and other superficial associates. Hydrilla tubers were harvested from the sediment in which hydrilla was cultured, and then cleaned in the same way. Eurasian watermilfoil seedpods were harvested from mature topped out plants grown in ponds at the Corps of Engineers Lewisville Pond Facility, Lewisville, TX, and processed in the same manner as hydrilla tubers.

Plant material was gently pressed between absorbent paper towels to remove excess surface moisture. After blot drying, leaf, stem, and root tissues were cut into 1- to 2-cm pieces, weighed, and placed in a Waring blender containing an equal volume of 0.05 M phosphate buffered saline (PBS) (pH 7.2, containing 0.75 mM phenylmethylsulfonyl fluoride to inhibit protease activity) and 5 percent weight/volume of polyvinylpyrrolidone to bind phenolic compounds that are known to decrease protein content after cell lysis. PBS was adjusted to pH 7.20.

Hydrilla tubers and watermilfoil seedpods were placed in the blender whole. The plant tissues were homogenized at high speed for 2 min. The resulting pulpy mixture was further macerated using a mortar and pestle against sterile masonry sand and sonicated for 3 min at a setting of 60 with an Artek Sonic Dismembrator model 150. The resulting slurry was centrifuged at 10,000 rpm for 30 min at 5 °C. The supernatant was filtered through four layers of cheesecloth, and then through Whatman Glass Microfibre Filters GF/F to remove remaining particulate matter in preparation for ammonium sulfate precipitation.

Ammonium sulfate precipitation

The supernatant was subjected to a 25-percent ammonium sulfate precipitation to remove the larger protein fraction. The ammonium sulfate was slowly added to the supernatant, which was maintained at 0 to 5 °C in an ice bath with constant stirring for 1 hr. The mixture was then centrifuged at 15,000 rpm for 20 min at 5 °C. The supernatant, which contained the smaller protein fractions including the lectins, was decanted for a second ammonium sulfate precipitation at 80 percent.

The pellets from both ammonium sulfate precipitations were resuspended in 50 ml of 0.05 M PBS (pH 7.20). The pellets from both precipitations were assayed for total protein; the protein pellet from the 25-percent precipitation was stored at -80 °C. The pellet from the 80-percent precipitation was processed further for lectin characterization.

Desalting

Ammonium sulfate and other low molecular weight compounds were removed from the resuspended 80-percent pellet by passing the sample through a column of polyacrylamide 6,000-dalton desalting gel (P-6DG), a product of Bio-Rad Laboratories (Rockville Centre, NY).

Protein quantification

A Bio-Rad Protein Assay Kit-II (Bio-Rad Laboratories, Rockville Centre, NY) was used to assay for protein activity before and after each step of

purification. The assay is based on the absorbance maximum (λ max) for an acidic solution of Coomassie Brilliant Blue G-250, which shifts from 465 to 595 nm when bound to protein (Fazekas de St. Groth et al. 1963; Reisner, Nemes, and Boucholtz 1975; Sedmak and Grossberg 1977).

Affinity chromatography studies

The desalted mixed protein fraction containing potential lectin was applied to seven sugar agarose affinity gels (Sigma Chemical Company, St. Louis, MO) at a rate of 3 ml/hr (Allen and Johnson 1977). The agarose gels used in affinity chromatography studies were β -D-mannose, α -D-lactose, β -D-glucose, β -D-galactose, α -L-fucose, N-acetyl-D-galactosamine, and N-acetyl-D-glucosamine. These seven sugars were selected for initial affinity chromatography studies because they are the most frequently reported free sugar haptens in lectin literature (Goldstein, Hollerman, and Merrick 1965; Archibald and Coapes 1971; Hammarstrom and Kabat 1971; Bauer et al. 1974; Hall and Rowlands 1974; Horisberger 1976; Barkai-Golan, Mirelman, and Sharon 1978; Pistole 1981).

Polyacrylamide gel electrophoresis (PAGE)

Proteins were separated by gel column electrophoresis. Molecular weight was determined using native (nondenaturing) polyacrylamide and sodium dodecyl sulfate (SDS) linear gradient gels at 0.5-percent increments ranging from 4.5 to 10 percent. The molecular weight standards, in kilodaltons, for nondenaturing PAGE were α -lactalbumin (14.2 kda), carbonic anhydrase (29 kda), chicken egg albumin (45 kda), bovine serum albumin (monomer-66 kda, dimer-132 kda), and urease (trimer-272 kda, hexamer-545 kda) (Davis 1964). SDS PAGE standards were lysozyme (14.3 kda), α -lactoglobulin (18.4 kda), trypsinogen (24.0 kda), pepsin (34.7 kda), egg albumin (45.0 kda), and bovine plasma albumin (66.0 kda) (Weber and Osborne 1969). The molecular weight standards were obtained from marker kits that are products of Sigma Chemical Company, St. Louis, MO.

Host Penetration Specificity

Light microscopy

A pathogen of hydrilla (*M. phaseolina*) and a pathogen of Eurasian water-milfoil (*M. terrestris*) were allowed to infect leaf and stem fragments of the respective plant for 1 hr, 1 day, 3 days, and 7 days. Infected plant fragments were fixed in fluoroacetic acid for 18 hr at room temperature and then washed in distilled water for 15 min. The tissues were then placed in a saturated aqueous solution of chloral hydrate, warmed to the boiling point, and allowed to cool to room temperature. Tissues were stained with a mixture of

0.025 percent aniline blue and 0.025 percent trypan blue dissolved in lactophenol for 16 hr at room temperature and then rinsed in distilled water for 15 min.

The plant tissues were returned to saturated chloral hydrate for 1 to 2 hr to remove excess stain and improve transparency. The tissues were then mounted on glass slides in lactophenol for examination.

Pathogen bioassay

Healthy apical hydrilla and watermilfoil fragments, approximately 10.0 cm in length, were placed in test tubes containing 50 ml of sterile Smart and Barko medium (Smart and Barko 1985). Two known fungal pathogens of hydrilla (*F. roseum* and *M. phaseolina*) and two known fungal pathogens of watermilfoil (*C. gloeosporioides* and *M. terrestris*) were grown in petri plates containing PDA for 7 days.

Six plugs were cut with a No. 2 cork borer from PDA cultures of the fungal pathogens and used to inoculate 150 ml of sterile V8B. The inoculated flasks were incubated on a rotary shaker at 25 °C for 4 days on a 12:12 light/dark cycle. The liquid cultures were macerated with a Sorval blender for 3 min at top speed while the container was submerged in an ice bath.

Ten replicates of each plant species were inoculated with 3 ml of the macerated hyphal slurry. Plant tissues were examined for disease symptoms at 3, 5, and 7 days after inoculation, and the causative agent was reisolated from infected tissue.

Damage to the plant was qualitatively assessed using a damage index of 0 to 5, where the values are defined as follows:

- 0 = No visible necrosis or other tissue damage except brown, naturally senescent tissues near the oldest nodes.
- 1 = Necrosis/damage to less than one-third of the leaves or stem, with any new growth being normal in appearance.
- 2 = Necrosis/browning is evident over one-third to two-thirds of the plant, with new growth appearing normal.
- 3 = More than two-thirds of the plant is necrotic or browning and the new growth is stunted and/or chlorotic.
- 4 = Necrosis of entire plant or nearly so, and there is no new growth.
- 5 = Plant death characterized by complete necrosis and mushy and/or brittle plant parts.

Lectin Activity Assays

Erythrocyte agglutination assay

Purified lectin collected from the affinity chromatography columns was tested for its ability to agglutinate a 2-percent suspension of washed red blood cells in 0.85-percent sodium chloride at a minimum lectin concentration of 45 $\mu\text{g/ml}$. Equal volumes (50 μl) of purified plant lectin and types A, B, and O human erythrocytes were combined on sterile depression glass slides and incubated for 1 hr at room temperature (Pereira and Kabat 1974). The slides were then examined by phase-contrast microscopy for evidence of cell clumping with a Leitz Diaplan light microscope.

Fungal attachment to intact plants

Dazzo and Hubbell (1975) demonstrated that Phase I attachment occurs in a few hours when attachment is specific, as in the case of *Rhizobium* and legumes. Joye and Paul (1992) have determined that *M. phaseolina* requires between 8 and 16 hr to attach to hydrilla. Therefore, 16 hr was allowed for the specific attachment of known pathogens to the target species.

Plant leaf and stem segments, 1.0 cm long, were incubated in a 10-percent solution of hydrogen peroxide for 1 min and rinsed three times in sterile water to remove naturally occurring epiphytes. The segments were then rinsed twice in sterile buffered tween (SBT) and allowed to remain in SBT for 30 min to allow dissipation of residual hydrogen peroxide. Six plant fragments were placed in 20 ml of sterile Smart and Barko solution and dipped into a 3-ml mycelial suspension of each of the four fungal pathogens for 0 and 16 hr. The fungal pathogens were grown according to the procedure described earlier in the pathogen bioassay. Controls were treated with 3 ml sterile V8B and chloramphenicol.

Each plant fragment was thoroughly agitated and rinsed three times in sterile water to remove unattached cells and then sonicated for 3 min with an Artek Sonic Dismembrator model 150 in 7.5 ml of sterile water at a setting of 48 to remove the tightly bound microbes.

Fungal attachment numbers were established by plating the water from the sonications on PDA containing chloramphenicol. An attachment index was used to evaluate the results. The index represents the number of colony forming units (CFU) per milliliter of water containing fungal propagules detached from the plant fragments.

Fungal agglutination assay

Hyphal suspensions (2 ml) harvested from 7-day-old V8B cultures of the pathogens were mixed with purified lectin (50 μ l) to test for agglutination. The hyphal suspensions were homogenized to a fine slurry using a Sorval Omni-Mixer. The isolates and lectin were combined on a sterile glass depression slide and incubated for 24 hr at room temperature. The slides were examined for evidence of agglutination with a Leitz Diaplan standard light microscope equipped with phase-contrast optics at 100X and 400X magnification.

Fungal growth assay

Lectin from seeds of watermilfoil was dialyzed against B5 growth medium (Leatherman and Pueppke 1985), filter sterilized, and protein concentration determined. Lectin concentration was adjusted to 7.5 μ g/ml, the concentration present in cotyledons of watermilfoil leaves and stems. Controls contained B5 growth medium only.

Equal volumes of molten 3 percent water agar and double-strength B5 medium were aseptically placed into 30-mm-diam petri plates maintained at 60 °C on a slide-warming tray. One-third of the plates contained hydrilla lectin, one-third contained watermilfoil lectin, and one-third contained no lectin. The final volume of medium in each plate was 1.5 ml. Each plate was inoculated with a 5-mm-diam plug taken from the advancing edge of a colony of each of the four fungal pathogens. The plates were sealed and incubated in darkness at 25 °C.

Colony diameters were measured at regular intervals of 12, 24, and 36 hr. Radial growth was determined by dividing the sum of two perpendicular colony diameters minus the diameter of the original plug by four. Three replicates were measured for each treatment.

Statistical Methods

Analysis of variance of data was performed using the F-test for determination of overall variance. The t-test was used to determine whether significant differences existed between two treatments for the attachment assay. A pairwise comparison of treatments based on Fisher's Least Significant Difference was used to determine significant differences in the pathogen bioassay and the rapid screening bioassay. Analysis was performed by computer application of PC-SAS statistical software package. The level of significance was determined within a 95-percent confidence interval ($p < 0.05$).

3 Results

Rapid Screening Bioassay

The rapid screening bioassay evaluated a total of 344 fungal isolates: 154 hydrilla isolates and 190 watermilfoil isolates. This assay resulted in the identification of 16 new potential pathogens on hydrilla (Table 1) and 15 new potential pathogens on Eurasian watermilfoil (Table 2).

Each new pathogen identified has been assigned a virulence grouping of 1 to 3. Placement within groups can be interpreted as follows:

- 1 = Pathogen's mean damage index was 4 or greater.
- 2 = Mean damage index of at least 2 but less than 4.
- 3 = Mean damage index is greater than the least significant difference (1.2431 for hydrilla and 1.6153 for watermilfoil) but less than 2 (weakly virulent pathogen).

Isolate Name	Virulence Group
FHy26	1
4F3	1
12F1	1
FHy17	1
10F11	1
8F7	2
9F3	2
9F8	2
8F8	2
FT2	2
FQ4	2
FQ9	2
FR7	2
FHy19	2
FT3	3
FQ6	3

Isolate names correspond to an internal system of nomenclature; fungi have not been classified taxonomically.

Lectin Isolation, Purification, and Characterization

Protein content of plant tissues

The total protein content in shoots and stems of hydrilla averaged 56.15 mg per 100 g wet weight of tissue (Table 3).

Root tissues contained approximately one-fourth the protein of the leaves and stems, 14.1 mg protein per 100 g wet weight. Tuber tissues contained nearly three times the protein content of leaves and stems, averaging 179.8 mg per 100 g wet weight.

The total protein content in leaves and stems of watermilfoil was approximately 46.2 mg per 100 g wet weight of tissue (Table 3). Root tissue contained extremely low levels of protein at 0.75 mg per 100 g wet weight. Watermilfoil seed contained 21.6 mg of protein per 100 g wet weight, half that of leaves and stems.

Table 2
New Eurasian Watermilfoil Pathogens

Isolate Name	Virulence Group
FH14	1
FE1	1
FH12	1
FMy3	1
FM1	2
LMy2	2
FMy25	2
LMy9	2
LMy1	2
FH9	2
FI14	2
FF8	2
FMy5	3
FMy37	3
LMy10	3

Affinity chromatography

Protein assays of fractions collected from the carbohydrate affinity columns revealed the presence of a fucose-specific protein isolated from leaves and stems, and tubers of hydrilla (Table 3). Extremely low levels of fucose-specific protein were detected from hydrilla root tissues but could not be obtained in sufficient quantities to produce detectable banding with disc gel electrophoresis.

Table 3
Protein Content of Hydrilla and Watermilfoil Tissues

Tissues	Total Protein/100 g, mg	Lectin/100 g, mg	Lectin Protein, %
Hydrilla			
Stems and leaves	56.15	2.33	4.15
Roots	14.1	0	0
Tubers	179.8	2.15	0.86
Watermilfoil			
Stems and leaves	46.2	0.755	1.64
Roots	0.75	0	0
Seeds	21.60	0.3	1.41

Fucose lectin comprises approximately 4.15 percent of the total protein in hydrilla leaves and stems and 0.86 percent of the total protein in tubers (Table 3). However, leaves/stems and tubers contain approximately the same concentration of lectin.

Protein assays of watermilfoil leaves/stems and seeds collected from the affinity columns verified the presence of fucose-specific proteins as well. There was no definitive indication of any carbohydrate-specific protein isolated from watermilfoil root tissues.

Fucose lectin content in watermilfoil leaves and stems is approximately 1.64 percent of total protein (Table 3). Root tissue lectin could not be detected. Watermilfoil seed lectin was approximately 1.41 percent of the total protein. Leaf/stem lectin was approximately twice that of seeds by total wet weight.

Lectin characterization

Native polyacrylamide gel electrophoresis of fucose-specific hydrilla lectin from stems/leaves and tubers consistently revealed two bands (Figure 1) with molecular weights corresponding to 210,000 and 145,000 daltons, respectively (Figure 2). SDS PAGE revealed two protein subunits with molecular weights of 50,000 and 67,000 daltons (Figures 3 and 4).

Native PAGE of fucose-specific watermilfoil lectin displayed two polypeptides, with molecular weights of 200,000 and 100,000 daltons, respectively (Figures 5 and 6). SDS PAGE revealed one band at 48,000 daltons (Figures 7 and 8).

Host Penetration Specificity

Light microscopy

To establish an adequate time period for the attachment assay, it was necessary to know the time required for initiation of infection. Joye and Paul (in press) determined, by transmission electron microscopy, that *M. phaseolina* will attach to hydrilla and begin the infection process within 16 hr.

For watermilfoil, initiation of infection was observed by light microscopy. Attachment was observed 24 hr after inoculation of watermilfoil with *M. terrestris*. Within 5 days the fungus had formed an appressorium and penetrated the plant tissues (Figure 9).

Pathogen bioassay

Pathogen bioassays were conducted to verify the virulence of infection of the four fungal pathogens being evaluated and their relative specificities for the target species. Three days after inoculation, only hydrilla inoculated with *M. phaseolina* displayed significantly more damage than the control (Table 4). In 7 days, all four pathogen treatments were significantly different from the control (Figure 10). *Macrophomina phaseolina*-treated plants displayed significantly more damage than those inoculated with the other fungi.

Pathogen	Time After Inoculation	
	3 Days	7 Days
<i>M. phaseolina</i>	0.7 A ²	3.0 A
<i>M. terrestris</i>	0.2 B	1.4 B
<i>F. roseum</i>	0.0 B	1.3 B
<i>C. gleosporioides</i>	0.0 B	1.3 B
Control	0.0 B	0.0 C

¹ Numbers represent mean damage index for each treatment.
² Means followed by the same letter are not significantly different at the 95-percent confidence level (alpha = 0.05). N = 10.

Three days after inoculation, *M. phaseolina* and *M. terrestris*-treated water-milfoil sprigs displayed significantly more damage than the controls as well as the other two pathogen treatments (Table 5). On the seventh day, plants inoculated with *M. phaseolina*, *M. terrestris*, and *C. gleosporioides* were significantly different from those inoculated with *F. roseum* and from the controls (Figure 11). Plants treated with *M. phaseolina* again exhibited the greatest damage. Damage from *F. roseum* was not significantly different from the controls.

Lectin Activity Assays

Erythrocyte agglutination

A common characteristic of most lectins is their ability to agglutinate members of the ABO blood group. Purified hydrilla glycoprotein and purified watermilfoil glycoprotein were tested for their ability to agglutinate the ABO human blood group at concentrations one-half the physiological lectin concentration (equal to the physiological concentration) and twice the physiological concentration in each plant species. Hydrilla lectin failed to agglutinate

Table 5 Pathogen Bioassay on Watermilfoil Sprigs¹		
Pathogen	Time After Inoculation	
	3 Days	7 Days
<i>M. phaseolina</i>	0.9 A ²	2.6 A
<i>M. terrestris</i>	0.6 A	1.7 B
<i>C. gloeosporioides</i>	0.2 B	1.5 B
<i>F. roseum</i>	0.0 B	0.9 C
Control	0.0 B	0.1 C

¹ Numbers represent mean damage index for each treatment.
² Means followed by the same number are not significantly different at the 95-percent confidence level (alpha = 0.05). N = 10.

Types A and B blood at either of the concentrations tested, but Type O blood was agglutinated at every concentration tested (Table 6, Figure 12). The same was true for the watermilfoil lectin (Table 6, Figure 13).

Table 6 Lectin Agglutination of Human ABO Blood Groups¹			
Plant	Type A	Type B	Type O
	0.5 1 2	0.5 1 2	0.5 1 2
Hydrilla ²	- - -	- - -	+ + +
Eurasian ³	- - -	- - -	+ + +

¹ Equal volumes of 2 percent red blood cells and plant lectin at 0.5, 1, and 2 times that contained in 1 g of leaves and stem tissues were mixed on a depression slide and incubated at room temperature for 1 hr.
² Hydrilla leaves and stems contain 23.3 µg/g wet weight of tissue.
³ Watermilfoil leaves and stems contain 7.55 µg/g wet weight of tissue.

Fungal attachment to intact plants

The attachment assay was conducted to determine specificity and frequency of attachment for the four pathogens. All four plant pathogens tested on hydrilla tissues exhibited significant attachment between 0 and 16 hr of incubation (Table 7, Figure 14). The watermilfoil pathogen *C. gloeosporioides*, attached at the highest density, with an average of 3,924.5 points per 1-cm fragment of hydrilla tissue (261.6 CFU/ml). The other three pathogens attached at levels comparable to each other: 112.75 points of attachment by

Table 7 Attachment of Pathogens on Hydrilla and Watermilfoil After 16 hr Incubation			
Pathogen	Diff ¹	t	P > t
Hydrilla			
<i>F. roseum</i>	8.9 B ³	4.2079	0.0084
<i>M. phaseolina</i>	3.5 B	5.6936	0.0021
<i>C. gloeosporioides</i>	261.6 A	14.5821	0.0001
<i>M. Terrestris</i>	7.5 B	3.6761	0.0115
Watermilfoil			
<i>F. roseum</i>	142.2 A	3.7476	0.0131
<i>M. phaseolina</i>	26.7 C	7.9471	0.0005
<i>C. gloeosporioides</i>	89.7 B	7.1129	0.0001
<i>M. terrestris</i>	10.7 C	3.3607	0.0194
¹ Diff = U16 - U0 = attachment density index at 16 hr minus attachment density index at 0 hr. Attachment density index equals CFU/ml of water in which plant fragments were sonicated to dislodge attached pathogen propagules. The mean total number points of attachment per plant fragment can be determined by multiplying total volume (7.5 ml) by the attachment index. ² Test of H0 : U0 = U16 using Satterthwaite approximation for unequal variance. ³ Means followed by the same letter are not significantly different at p < 0.05 level of significance.			

M. terrestris, 132.75 points of attachment by *F. roseum*, and 52.5 points of attachment by *M. phaseolina*.

All four pathogens tested on watermilfoil tissues exhibited significant attachment (Table 7). The hydrilla pathogen *F. roseum* attached with the highest density, averaging 2,132.25 points of attachment per plant fragment. *Collectotrichum gloeosporioides* averaged 1,346.0 points of attachment per plant fragment. *Macrophomina phaseolina* and *M. terrestris* averaged 401.0 and 159.75 points of attachment, respectively.

The attachment rate of *F. roseum* was significantly greater than that of the other three, and that of *C. gloeosporioides* was significantly greater than that of *M. phaseolina* and *M. terrestris* (Figure 15).

Fungal agglutination assay

None of the pathogens were agglutinated by hydrilla lectin at physiological concentration (Table 8). Three of the four fungal pathogens were agglutinated

Table 8
Lectin Agglutination of Fungal Pathogens¹

Pathogen	Lectin ²	
	Hydrilla ³	Watermilfoil ⁴
<i>F. roseum</i>	-	+
<i>M. phaseolina</i>	-	-
<i>C. gloeosporioides</i>	-	+
<i>M. Terrestris</i>	-	+

¹ Equal volumes of a 1-percent mycelial suspension of the four pathogens and plant lectins at concentrations contained in 1 g of leaf and stem tissues were mixed in a depression slide and incubated at room temperature for 1 hr.

² Plus sign indicates agglutination; minus sign indicates no agglutination.

³ Hydrilla leaves and stems contain 23.3 $\mu\text{g/g}$ wet weight of tissue.

⁴ Watermilfoil leaves and stems contain 7.55 $\mu\text{g/g}$ wet weight of tissue.

by watermilfoil lectin at physiological levels in stems and leaves (Table 8). The only fungus not agglutinated by watermilfoil lectin was *M. phaseolina*.

Fungal growth assay

Physiological concentrations of active watermilfoil lectin failed to significantly alter the radial growth rates of the four fungal pathogens (Table 9). Radial growth rates were linear regardless of the treatment.

Since hydrilla lectin failed to agglutinate any of the fungi, growth assays were not conducted.

Table 9
Watermilfoil Lectin Fungal Growth Assay¹

Pathogen	12 hr	24 hr	36 hr
<i>F. roseum</i>	-0.37	-0.25	+0.50
<i>M. phaseolina</i>	0.00	+0.18	+0.56
<i>C. gloeosporioides</i>	-0.12	+0.19	+0.31
<i>M. terrestris</i>	0.00	-0.12	-0.06

¹ Numbers represent mean radial growth differences (mm) between fungal colony grown in presence of lectin minus fungal colony grown in absence of lectin (+ = growth in treated plates was greater than the control, - = growth in treated plates was less than the control). Lectin-treated plates contained 7.5 $\mu\text{g/ml}$ Eurasian lectin in B5 medium. There was no significant difference for any treatment.

4 Discussion

Based on polyacrylamide gel electrophoresis, the hydrilla L-fucose lectin appears to be made up of a complex of two proteins. The first is a tetramer of approximately 210,000 daltons (da) with monomeric subunits of 50,000 da; the second is a dimer of 145,000 da with subunits of 67,000 da.

The watermilfoil L-fucose lectin is similar in molecular weight and polymeric structure to the tetrameric protein of hydrilla. It was isolated as a tetramer of approximately 200,000 da and a dimer of 100,000 da with a monomeric subunit of 48,000 da.

The anti-H agglutinins of Type O blood are agglutinated by the phytohemagglutinins of *Ulex europaeus* and *Lotus tetragonolobus* (Allen and Johnson 1977). In fact, every lectin reported to date that has demonstrated an affinity for L-fucose has likewise agglutinated the anti-H agglutinins of Type O blood. The L-fucose-specific lectins of hydrilla and watermilfoil are Type O blood group-specific as well (Table 10).

Lectin	Source	Molecular Weight, kda	Blood Type	Yield mg/100g
<i>Lotus tetragonolobus</i>	Asparagus pea ¹	35	O	394
<i>Ulex europaeus</i>	Furze ¹	40-46	O	4.5
<i>Hydrilla verticillata</i>	Hydrilla ²	50 and 67	O	2.3
	Hydrilla ³	50 and 67	O	2.1
<i>Myriophyllum spicatum</i>	Watermilfoil ¹	48	O	0.3
	Watermilfoil ²	48	O	0.8

¹ Seed lectin.
² Leaf and stem lectin.
³ Tuber lectin.

Lectin content in hydrilla leaves/stems and tubers, 2.33 mg and 2.15 mg/100 g wet weight of tissue, respectively, is comparable to that of seeds of *U. europaeus* (Table 10). Watermilfoil seeds contain much lower levels of lectin than other L-fucose lectin plants. Normally, lectin content in seeds is much higher than in other tissues. However, watermilfoil seed lectin content is approximately half that of leaves and stems. This may be a result of the method of seed harvest. A blender was used to macerate the seedpods to free the minute seeds. The resulting pulp was washed through several sieves to separate the seeds from the pulp. While it is likely that some seeds were destroyed during the blending, it is also true that the recorded weight for watermilfoil seeds consisted of seedpod, seed coat, and seed, whereas only the seeds devoid of coat and pod were actually processed for affinity chromatography. Seed lectin content should be verified in further studies.

Although all four of the fungi tested were pathogenic on hydrilla, one cannot assume that the same would occur in the field. By design, the pathogen assay is advantageous to the fungus. The plants are under stress in that they are not rooted in sediment and no aeration is provided to maintain good water quality and provide carbon dioxide for photosynthesis. These conditions allow the fungus every opportunity to infect the plant, whether it is a true pathogen or an opportunist (Smith et al. 1989). This assay demonstrates that all are biotrophs or opportunists on hydrilla, but makes no distinction. It is highly unlikely that these conditions would be met in the field to allow the opportunists to impact hydrilla except under the most extreme climatic conditions.

This experimental design provides a rapid preliminary screening method to evaluate the ability of isolates to infect the target plant. It is appropriate for purposes of this investigation to make comparisons among disease symptom production, attachment, and lectin presence and activity.

All but *C. gloeosporioides*, which attaches nonspecifically to most surfaces, attached at low levels on hydrilla tissues. None of the fungi were reactive with hydrilla lectin at physiological concentrations (Table 11). The role of hydrilla lectin in the infection process is consistent with the findings of Sequiera and Graham (1977) and Pistole (1981). Further studies should be conducted using both virulent and avirulent strains of potential fungal pathogens to clarify the interaction between plant lectins and fungal isolates.

Eurasian lectin was implicated in the specific attachment of *F. roseum* to watermilfoil tissues. Three of the four, including *F. roseum*, were agglutinated by watermilfoil lectin (Table 12). However, *F. roseum* was the only fungus to attach at high levels. Even *C. gloeosporioides* attached at significantly lower levels.

Even though *C. gloeosporioides* and *M. terrestris* were reactive with watermilfoil lectin in vivo, specific attachment did not occur. This would imply that the lectin does not reside at the cell surface, that the glycoconjugate

Table 11 Summary Table of Pathogen Association with Hydrilla Tissues			
Fungus	Pathogenicity ¹	Attachment ²	Lectin Affinity ³
<i>F. roseum</i>	Moderate	Low	-
<i>M. phaseolina</i>	High	Low	-
<i>C. gloeosporioides</i>	Moderate	High	-
<i>M. terrestris</i>	Moderate	Low	-

¹ Pathogenicity rate based on mean pathogen damage index after 7 days (Table 4).
² Attachment rate based on mean difference attachment index after 16 hr incubation (Table 7).
³ Lectin affinity was determined by agglutination or nonagglutination (-) of fungal hyphal tissues in the presence of plant lectin.

Table 12 Summary Table of Pathogen Association with Watermilfoil Tissues			
Fungus	Pathogenicity ¹	Attachment ²	Lectin Affinity ³
<i>F. roseum</i>	None	High	+
<i>M. phaseolina</i>	High	Low	-
<i>C. gloeosporioides</i>	Moderate	Moderate	+
<i>M. terrestris</i>	Moderate	Low	+

¹ Pathogenicity rate based on mean pathogen damage index after 7 days (Table 5).
² Attachment rate based on mean difference attachment index after 16 hr incubation (Table 7).
³ Lectin affinity was determined by agglutination (+) or nonagglutination (-) of fungal hyphal tissues in the presence of plant lectin at concentrations equal to leaves and stems.

(L-fucose) is not readily accessible for attachment to the lectin on the surface of the plant, or that the process of isolating conditions the lectin to allow attachment to occur. Further research is necessary to locate the lectin in watermilfoil and hydrilla tissues.

The results support the theory that watermilfoil lectin is involved in plant recognition for purposes of plant defense. When a high level of specific attachment is verified and lectin affinity is demonstrated, as is the case with *F. roseum* on watermilfoil, pathogenicity does not occur. On the other hand, when attachment occurs and lectin affinity is negative, pathogenicity can occur, as is the case with *M. phaseolina*.

Colletotrichum gloeosporioides was moderately pathogenic on watermilfoil and attached at a moderate level when compared to the attachment of *F. roseum* on watermilfoil tissues. However, the moderate level of attachment of *C. gloeosporioides* on watermilfoil tissues may be due to the fungus' ability to

attach to virtually all surfaces (Smith et al. 1989). Since neither is pathogenic on the plant to which it attaches at high densities, specific attachment may be a mechanism of disease resistance for hydrilla and watermilfoil.

Since hydrilla and watermilfoil are vastly different plants physiologically (monocotyledon and dicotyledon, respectively), the presence of L-fucose lectin in both hydrilla and watermilfoil tissues may be indicative of a common primary purpose in aquatic plants. This investigation provides evidence that microbial recognition is a function of both plant lectins.

That lectins do function to recognize specific microorganisms in the environment is now incontrovertible (Pistole 1981). In some cases this may be a prelude to specific physiological interactions, as in the case of legume plants and the *Rhizobium* symbionts. In other cases this recognition may play a key role in host defense. This investigation supports the host defense theory.

5 Conclusions

The existence of lectins in the aquatic plants *Hydrilla verticillata* and *Myriophyllum spicatum* has been demonstrated. The validity of the thesis that plant lectin plays a role in aquatic plant/pathogen association through attachment and/or recognition has been confirmed for Eurasian watermilfoil. The results are inconclusive for hydrilla.

Though the role of lectin-microbe recognition in aquatic plants requires further investigation, evidence is provided in support of the theory that recognition in the case of watermilfoil is a host-defense mechanism.

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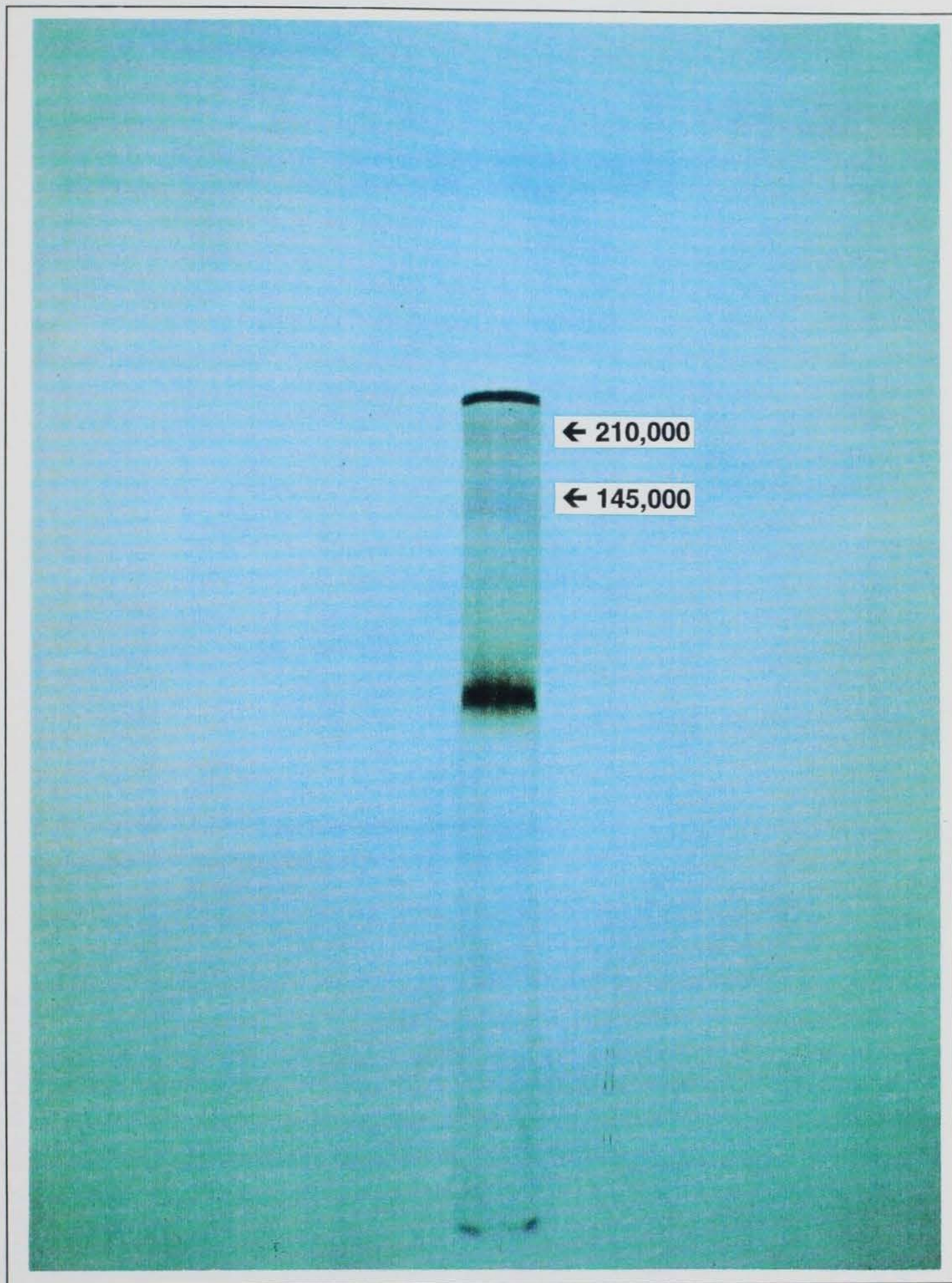


Figure 1. Polyacrylamide gel identifying a hydrilla L-fucose-specific lectin. Two protein bands were identified in the upper portion of the gel

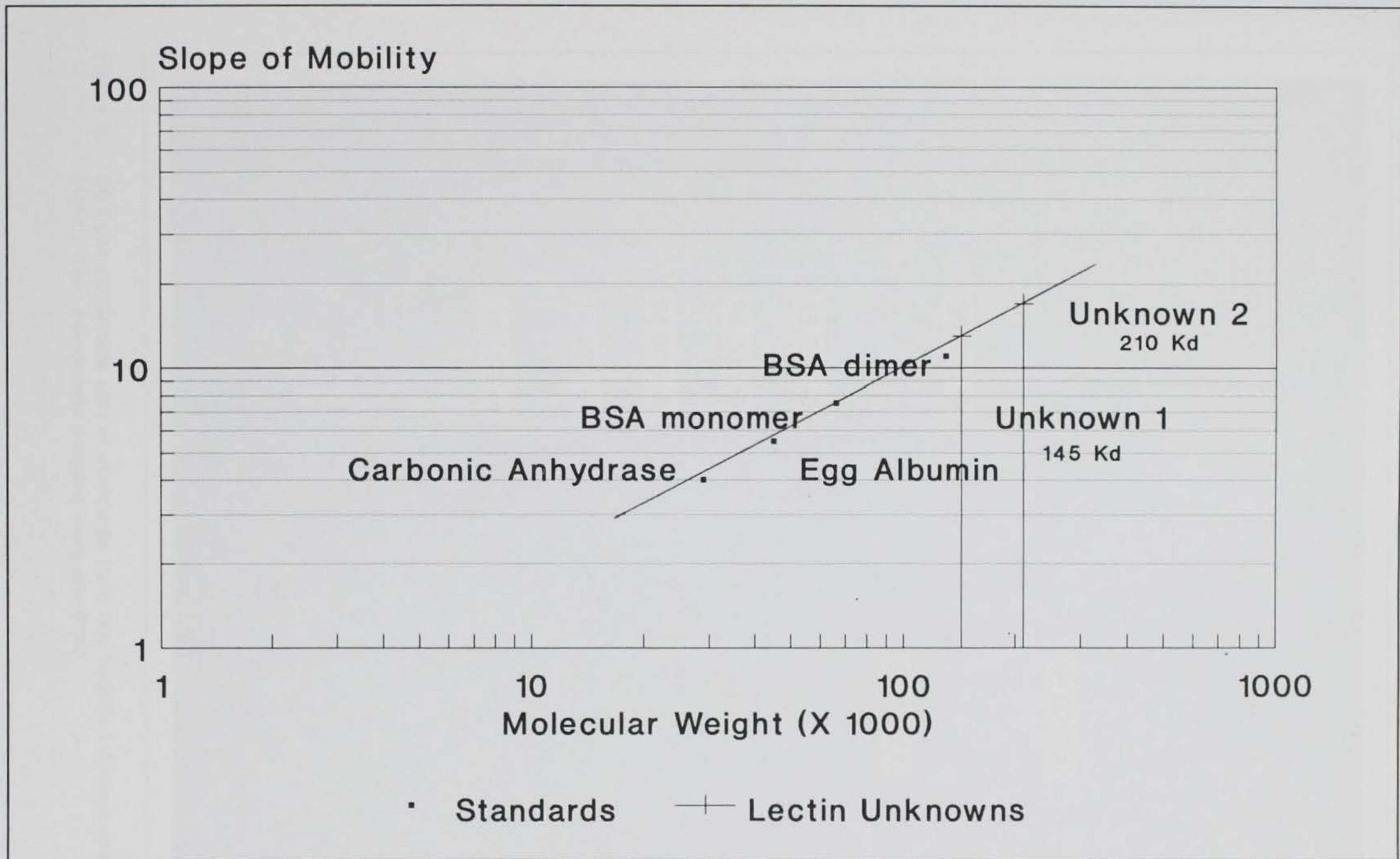


Figure 2. Graph of hydrilla lectin depicting molecular weights of standards and hydrilla L-fucose lectin. Two polymeric forms of the lectin were isolated



Figure 3. SDS polyacrylamide gels of standards (left) and hydrilla L-fucose-specific lectin (right). Two monomeric proteins were identified

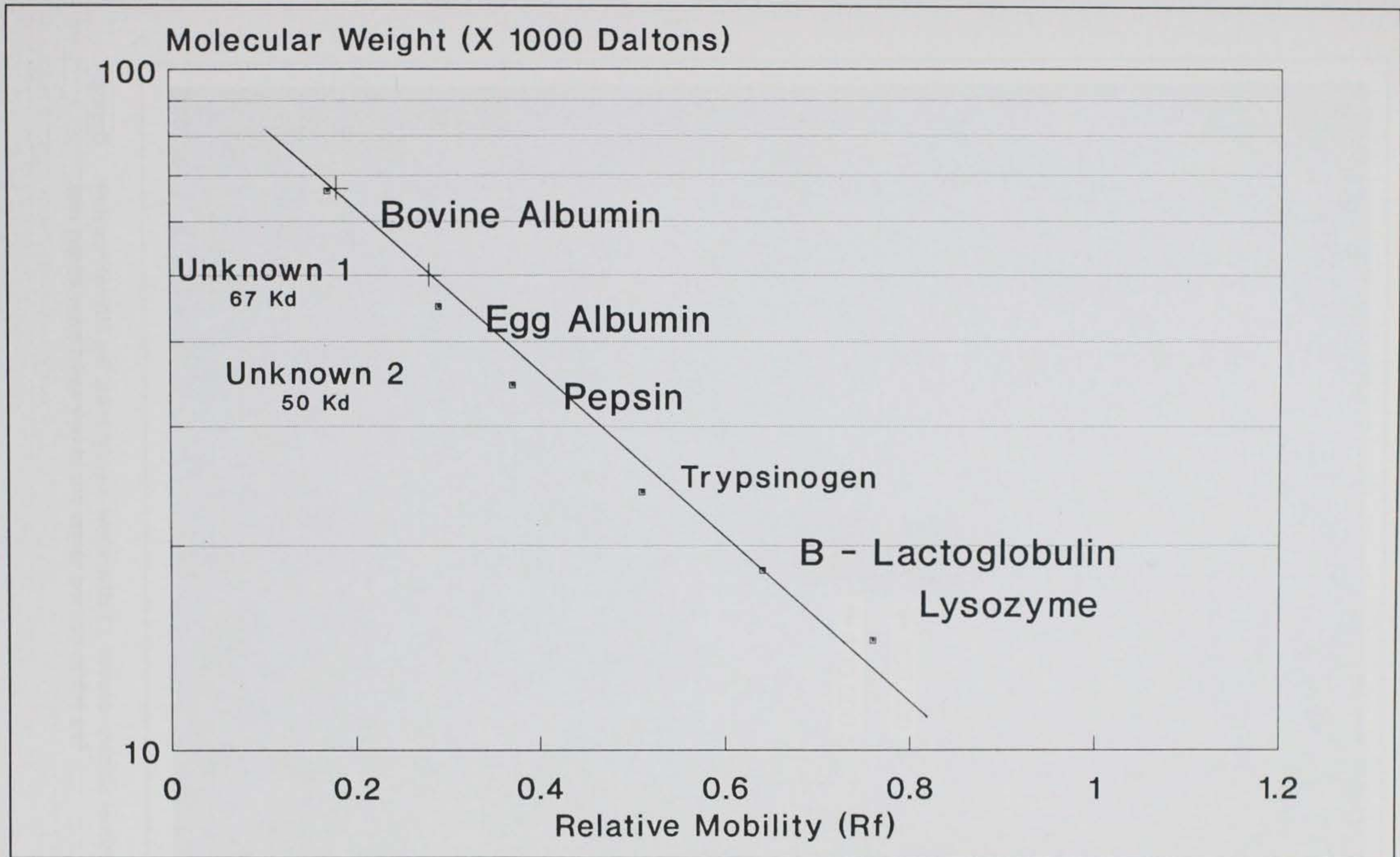


Figure 4. Graph of hydrilla lectin identified by SDS PAGE. Two monomeric subunits were isolated



Figure 5. Polyacrylamide gel identifying a watermilfoil L-fucose-specific lectin. Two protein bands were observed in the upper portion of the gel

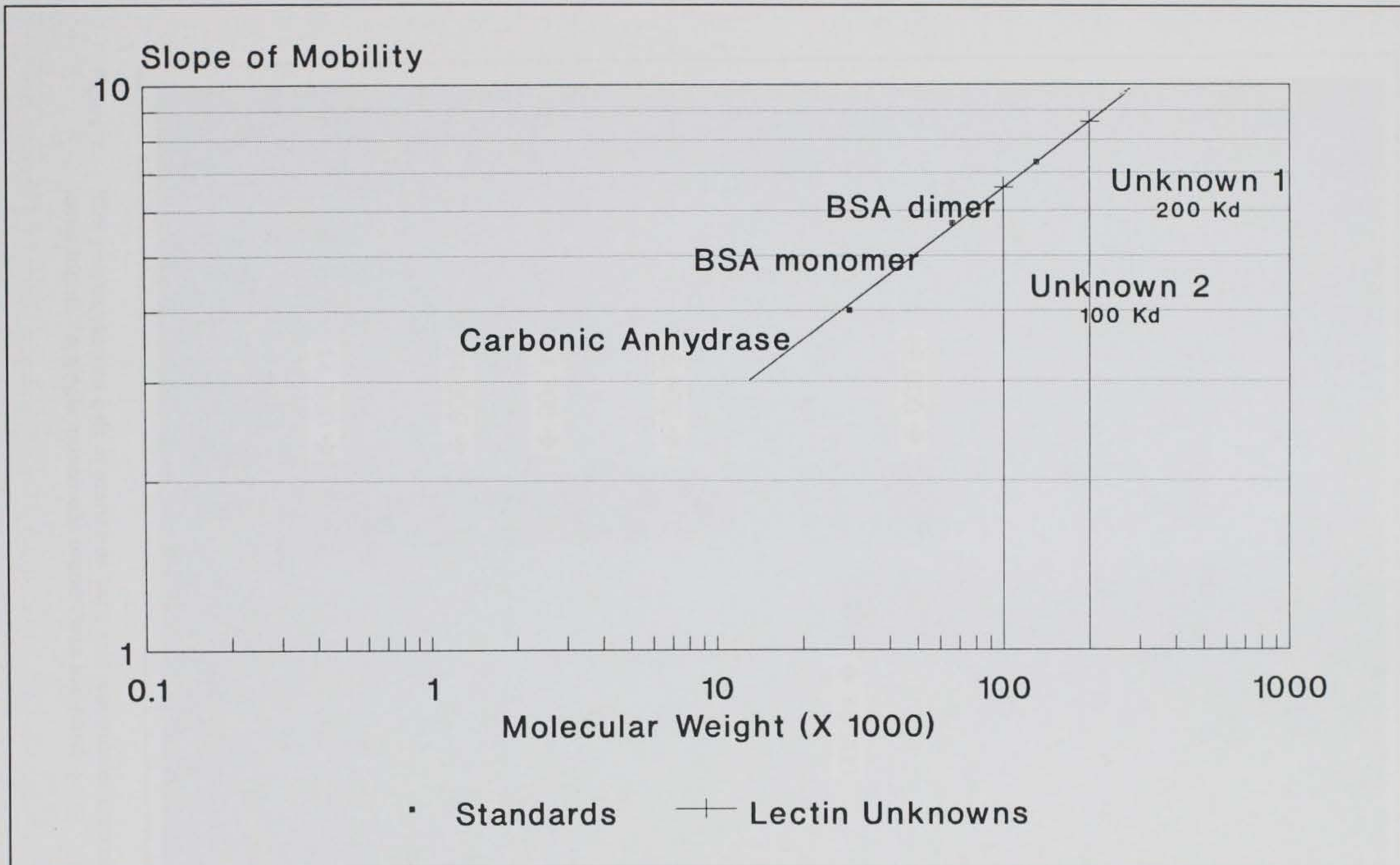


Figure 6. Graph depicting molecular weights of watermilfoil L-fucose-specific lectin. Two polymeric bands were isolated weighing approximately 200,000 and 100,000 daltons

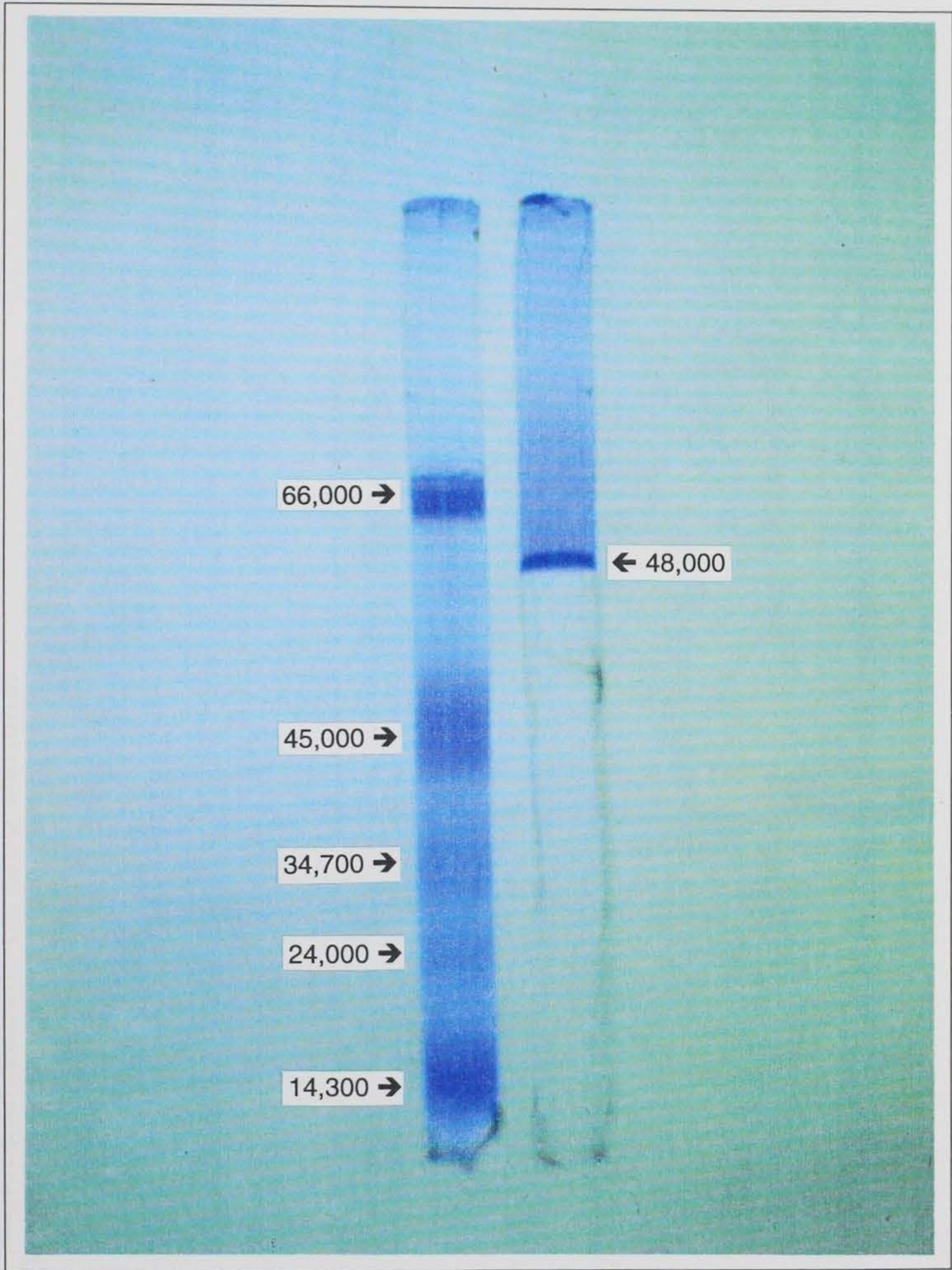


Figure 7. SDS polyacrylamide gels of standards (left) and watermilfoil L-fucose-specific lectin (right). A single monomeric protein was identified

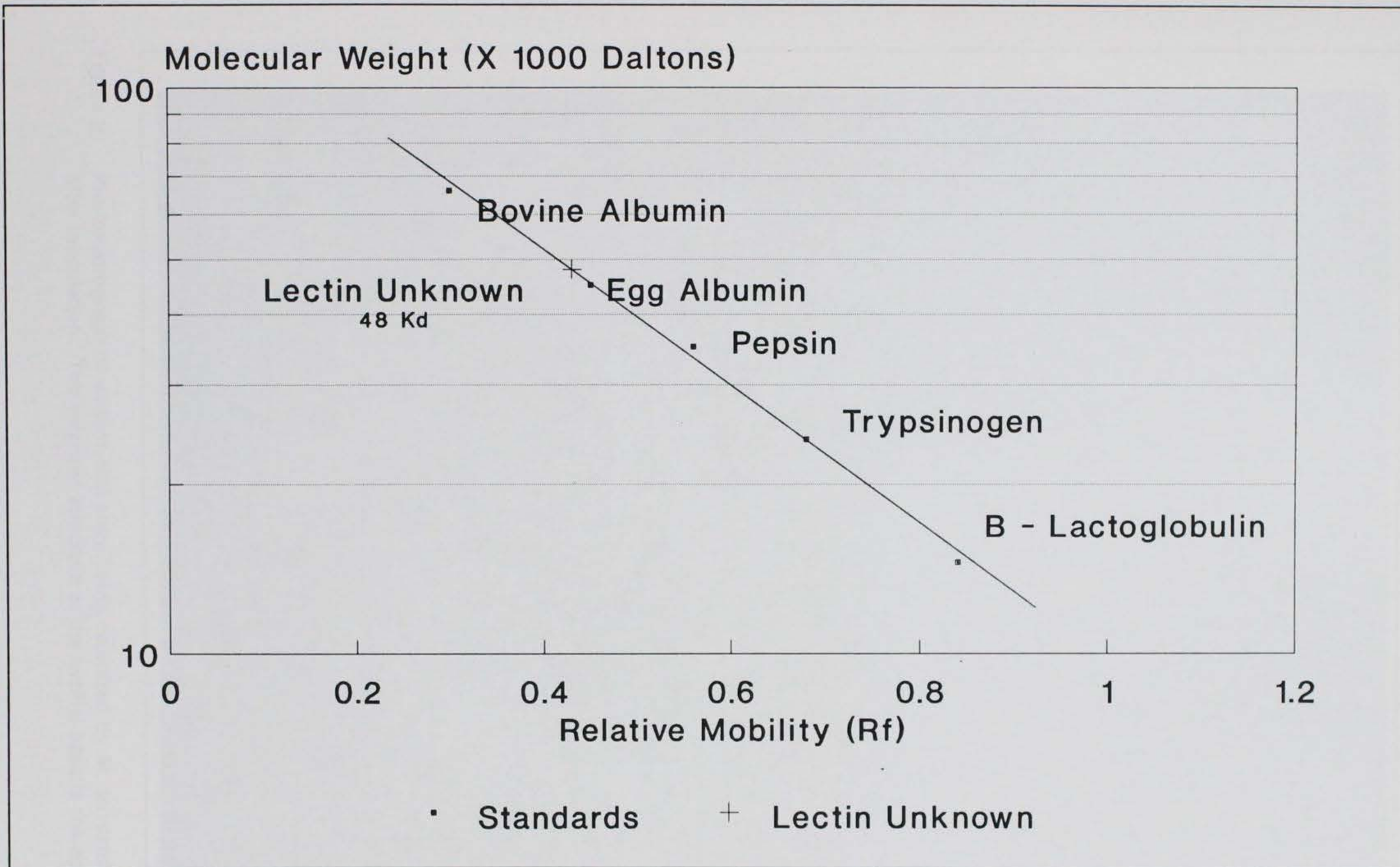


Figure 8. Graph depicting the molecular weight of the watermilfoil L-fucose-specific lectin

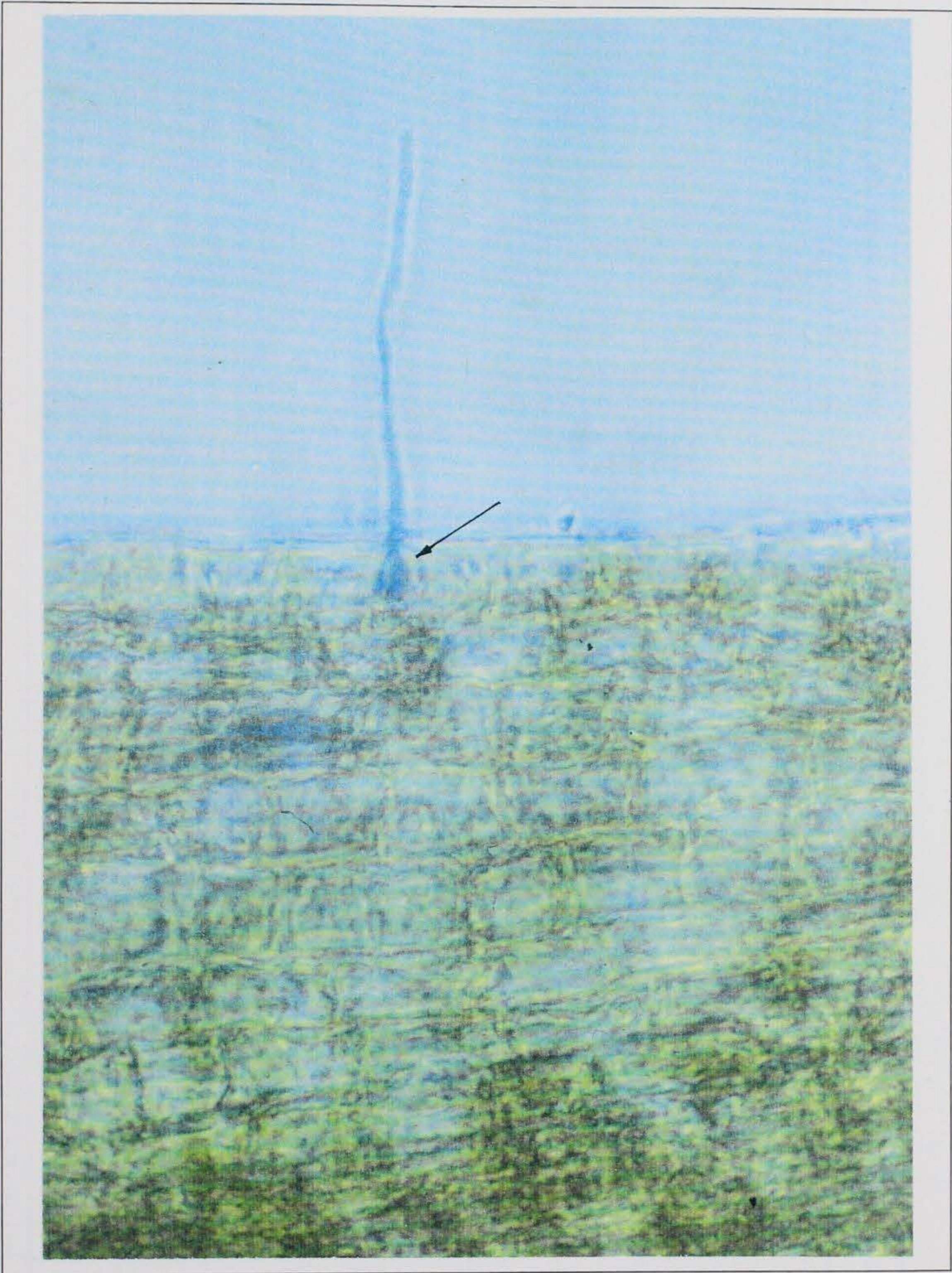


Figure 9. Photomicrograph of watermilfoil stem being colonized by *M. terrestris* 5 days after inoculation. The enlarged structure at the hyphal base is the appressorium

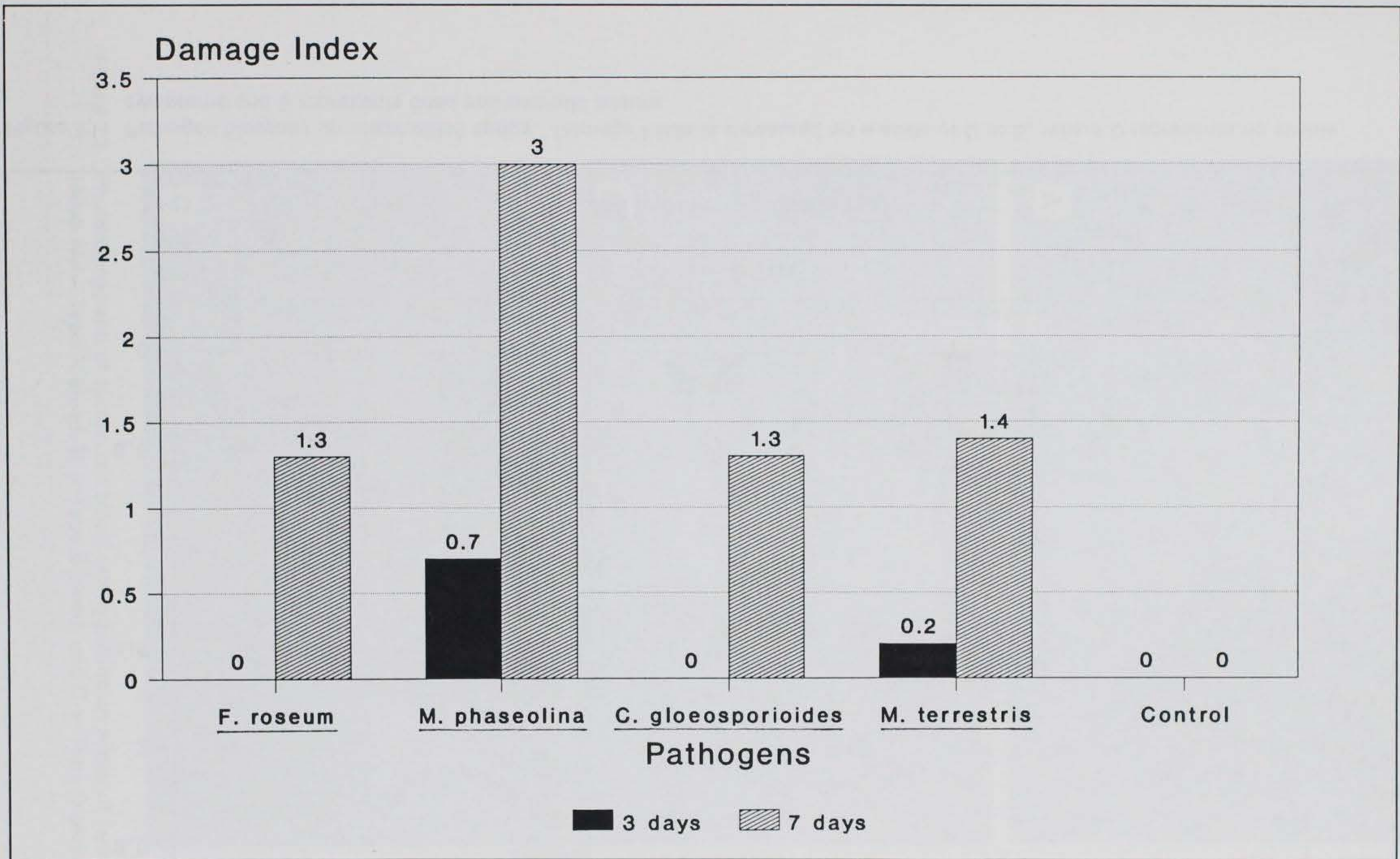


Figure 10. Pathogen bioassay on hydrilla sprigs. Damage index is measured on a scale of 0 to 5, where 0 represents no visible symptoms and 5 represents dead and necrotic tissues

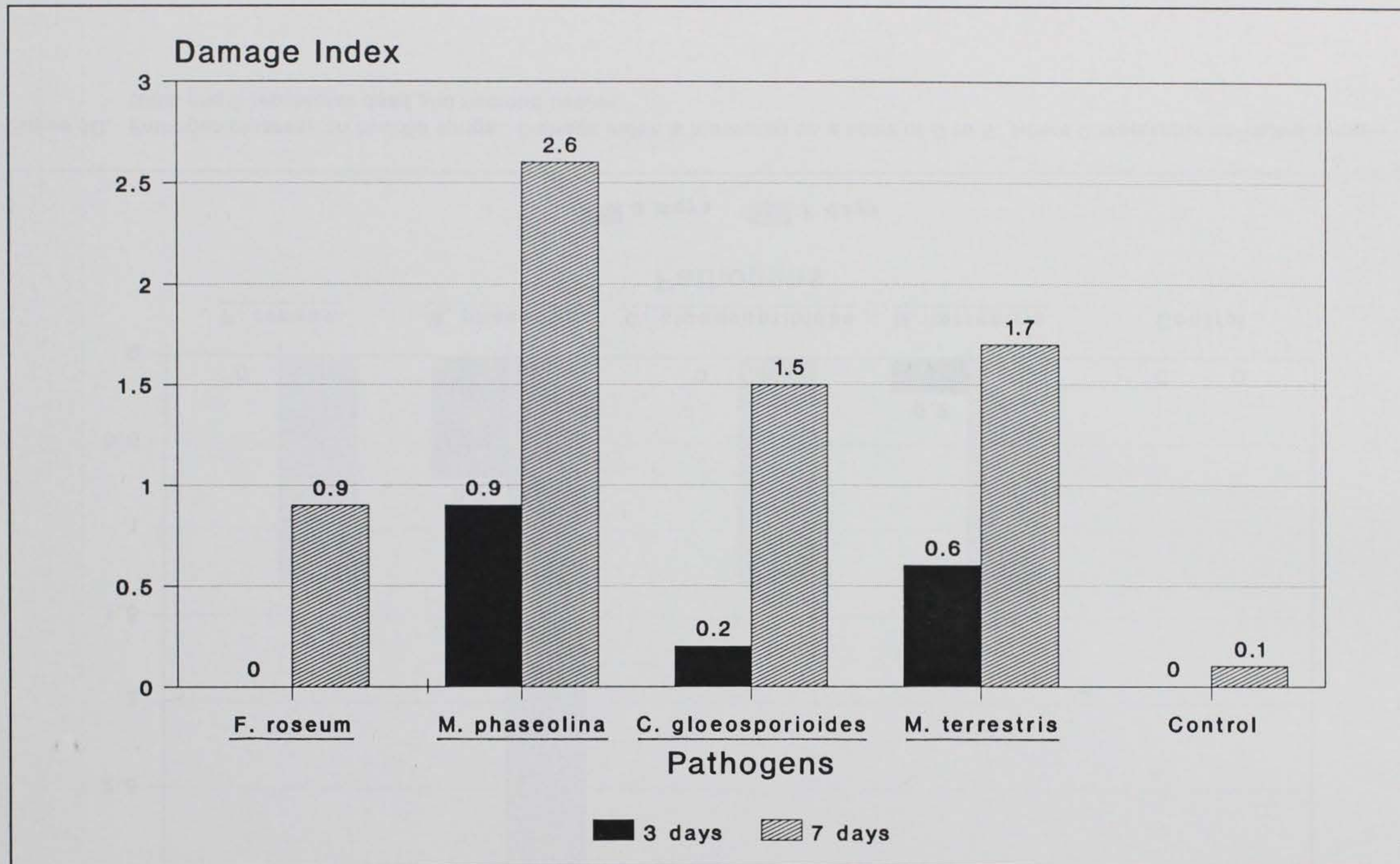


Figure 11. Pathogen bioassay on watermilfoil sprigs. Damage index is measured on a scale of 0 to 5, where 0 represents no visible symptoms and 5 represents dead and necrotic tissues

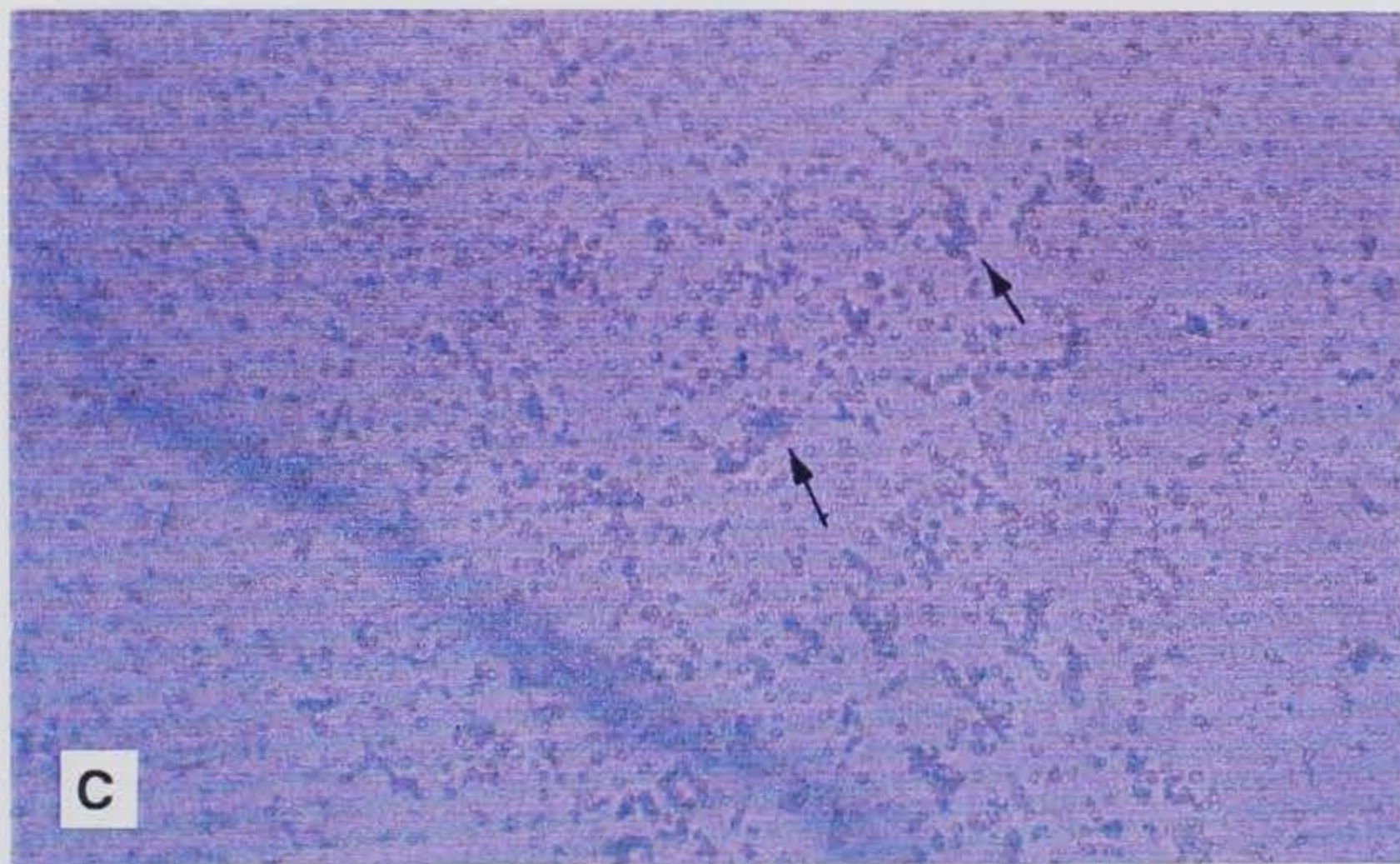
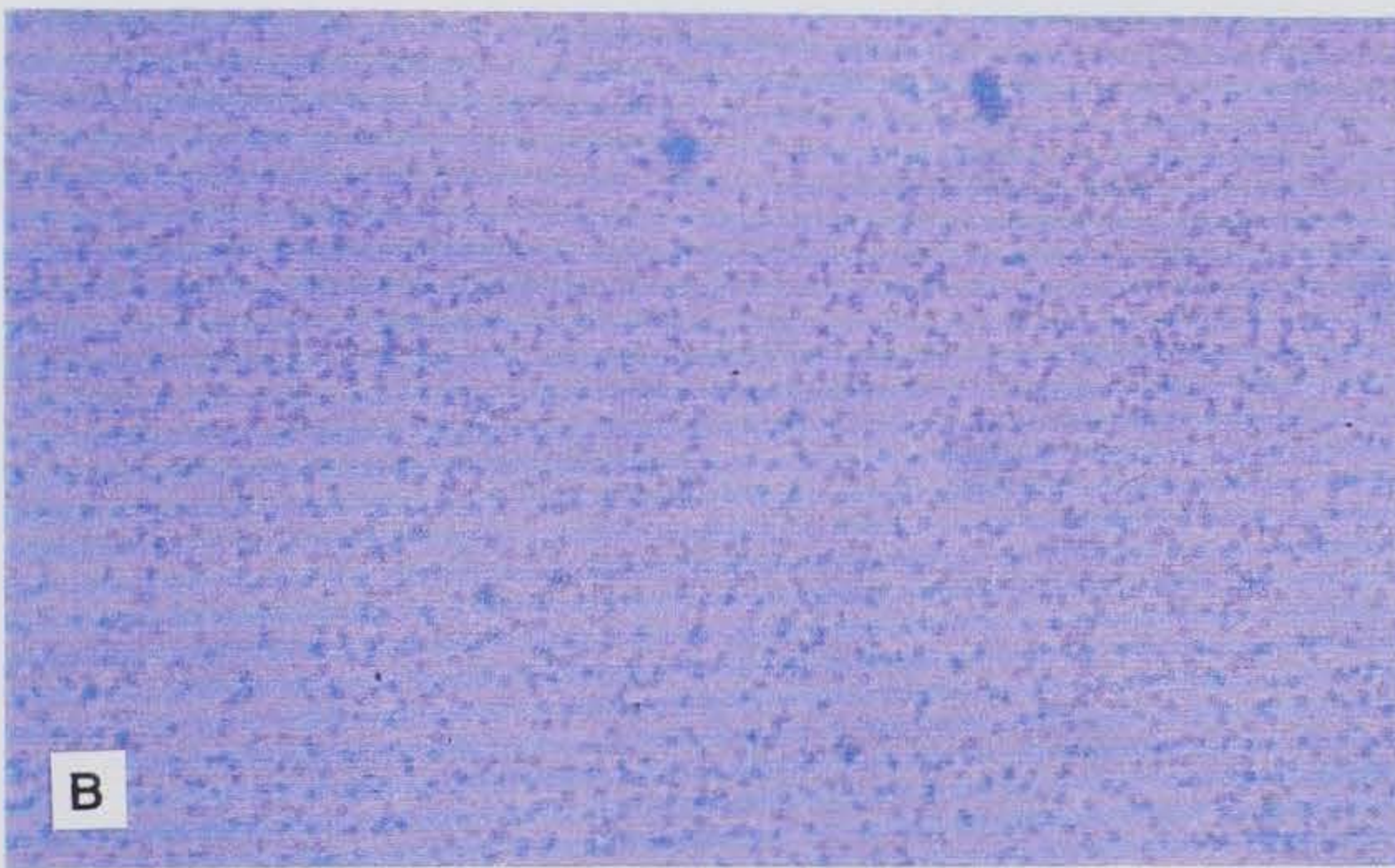
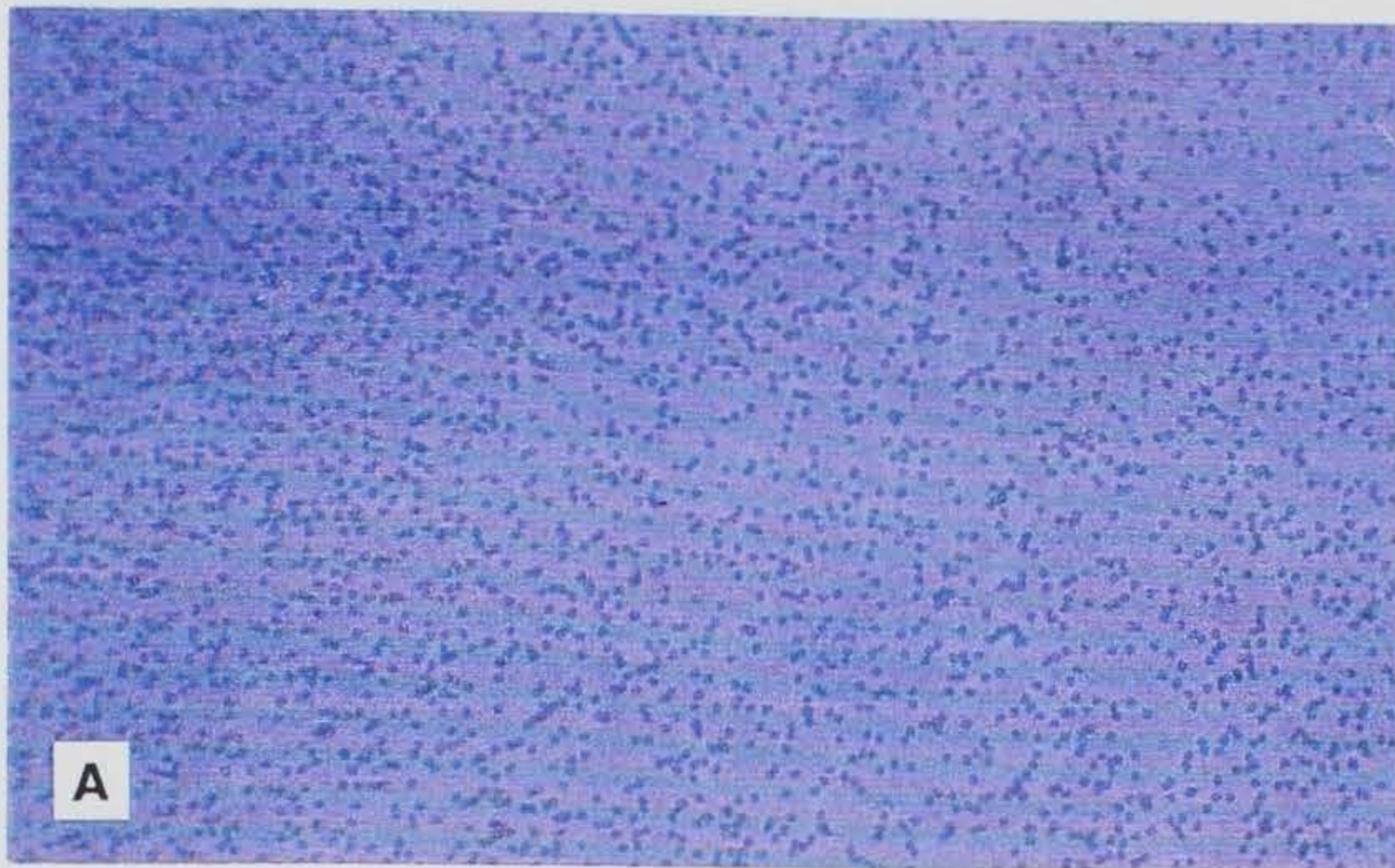


Figure 12. Photomicrograph of human red blood cell agglutination assay of hydrilla lectin (40X) (A = Type A blood, B = Type B blood, and C = Type O blood)

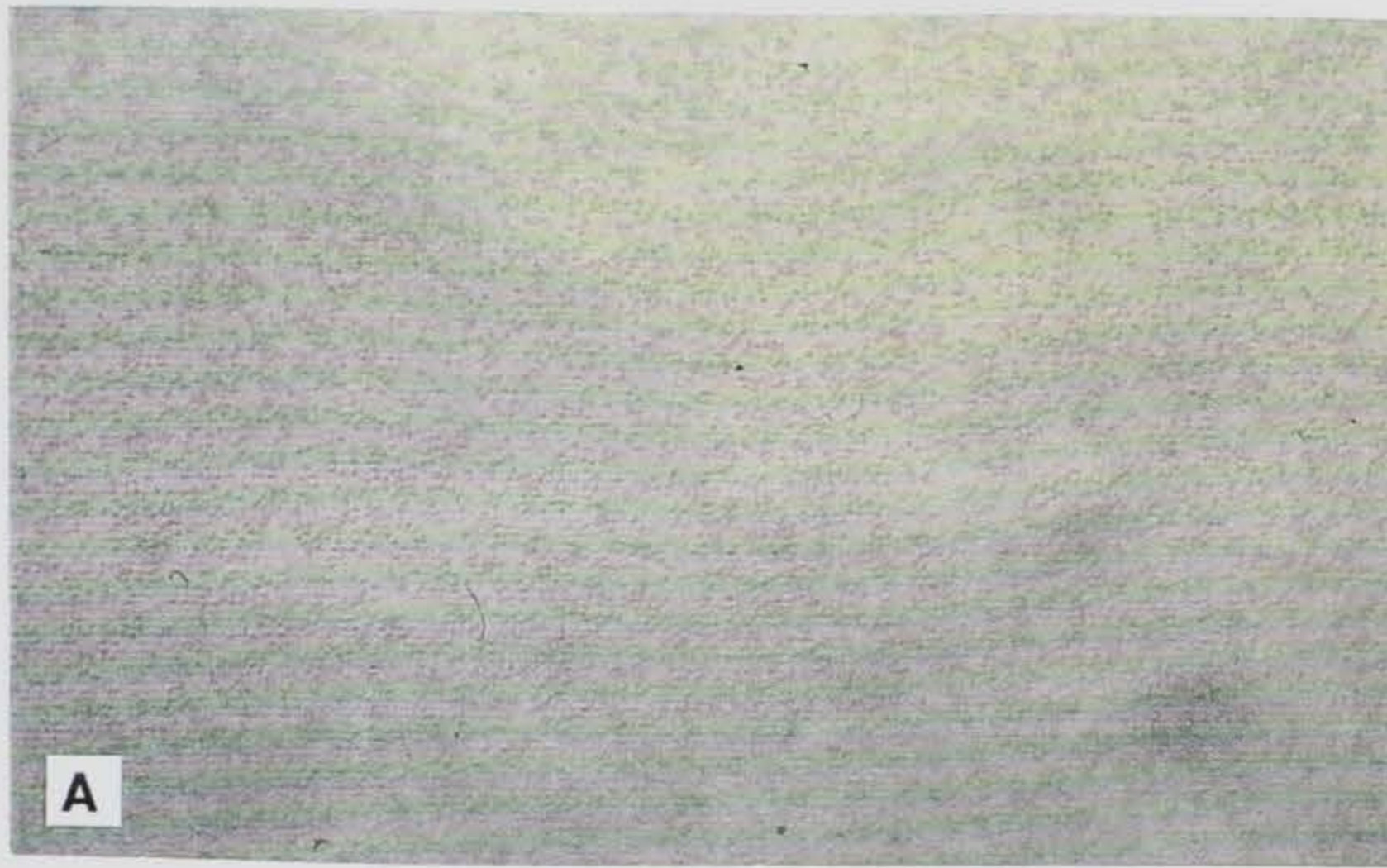


Figure 13. Photomicrograph of human red blood cell agglutination assay of watermilfoil lectin (40X) (A = Type A blood, B = Type B blood, and C = Type O blood)

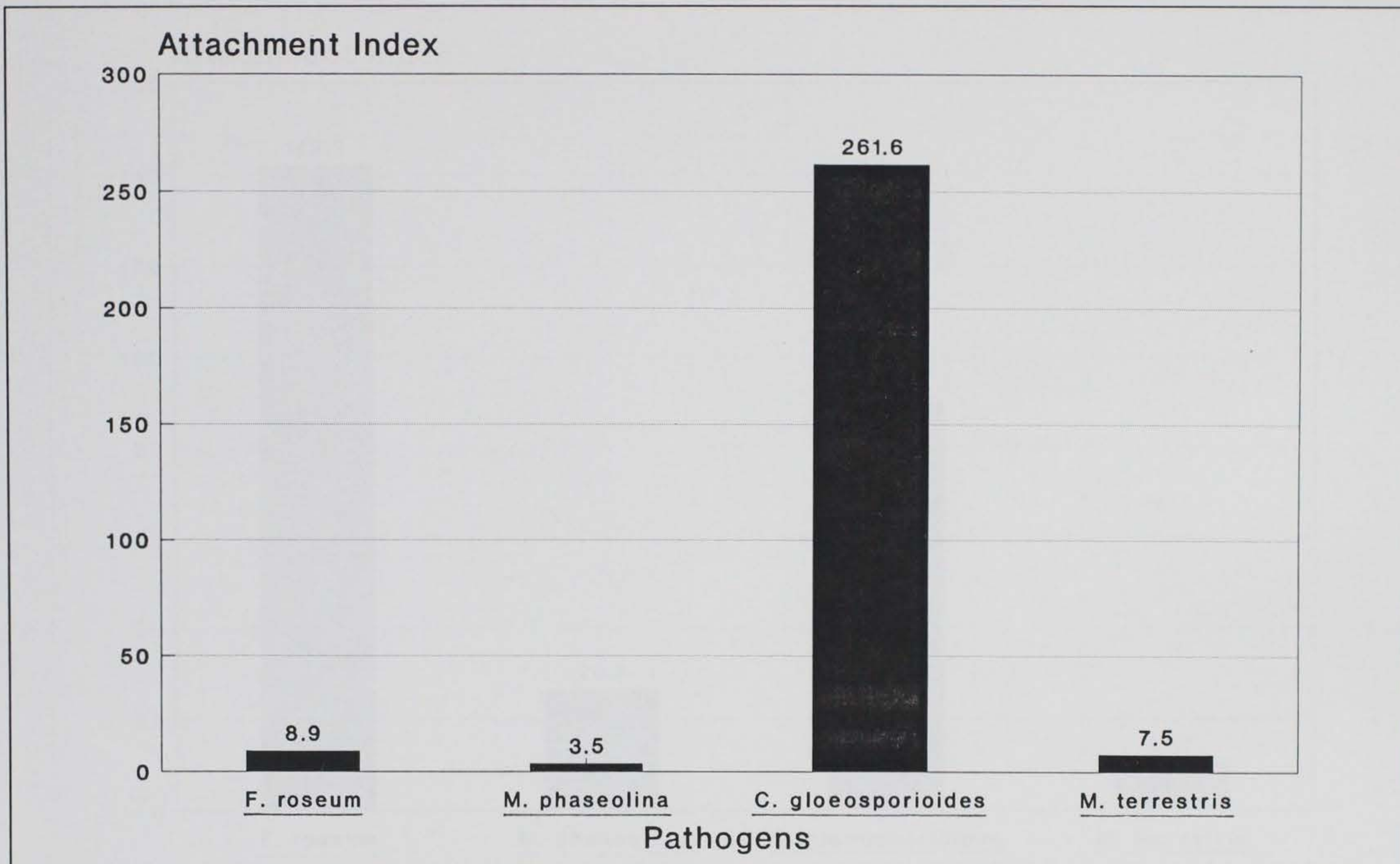


Figure 14. Fungal attachment assay on hydrilla sprigs. Attachment index is the number of fungal CFU/ml detached from a 1-cm fragment in 7.5 ml of sterile water after 16 hr

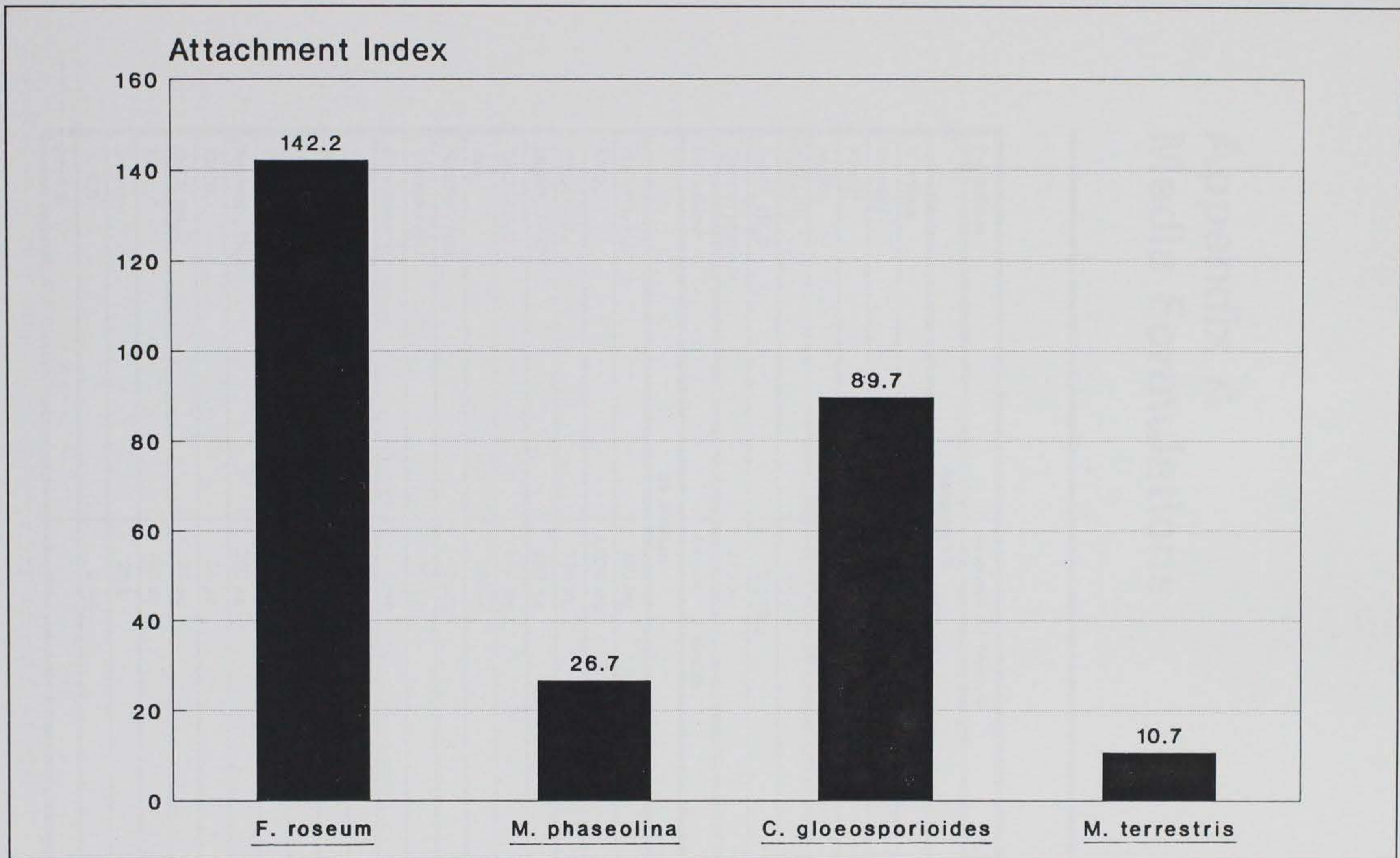


Figure 15. Fungal attachment assay on watermilfoil sprigs. Attachment index is the number of fungal CFU/ml detached from a 1-cm watermilfoil fragment in 7.5 ml of sterile water after 16 hr

Appendix A

Media Formulations

Ingredients	Amount/1.5 l Medium
Richard's V8	
V-8 juice	200 ml
Sucrose	45 g
KNO ₃	15 g
MgSO ₄	7.5 g
CaCO ₃	4.5 g
FeSO ₄ 7H ₂ O	0.015 g
Chloramphenicol	375 mg
Ingredients	Amount/1 l Medium
B5 Medium	
NaH ₂ PO ₄ H ₂ O	150 mg
KNO ₃	3,000 mg
(NH ₄) ₂ SO ₄	134 mg
MgSO ₄ 7H ₂ O	500 mg
CaCl ₂ 2H ₂ O	150 mg
Iron	28 mg
Nicotinic acid	1 mg
Thiamine HCl	10 mg
Pyridoxine HCl	1 mg
m-Inositol	100 mg
MnSO ₄ H ₂ O	10 mg
H ₃ BO ₃	3 mg
ZnSO ₄ 7H ₂ O	2 mg
Na ₂ MoO ₄ 2H ₂ O	250 µg
CuSO ₄	25 µg
CoCl ₂ 6H ₂ O	25 µg
KI	750 µg
Sucrose	20 g
2, 4-D	2 mg
pH:5.5	

REPORT DOCUMENTATION PAGE

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13. ABSTRACT (Maximum 200 words) The submersed aquatic plants <i>Myriophyllum spicatum</i> L. (Eurasian watermilfoil) and <i>Hydrilla verticillata</i> (L. fil.) Royle (hydrilla) are a nuisance in waterways of the United States. Biological control with plant pathogens is a proven method for the management of aquatic plants. This study describes the identification, isolation, and characterization of lectins of Eurasian watermilfoil and hydrilla as a potential mechanism for specific attachment of fungal plant pathogens to the target plants. Lectins specific for α -L-fucose were isolated from both Eurasian watermilfoil and hydrilla. The watermilfoil lectin is a Type I lectin having a monomeric subunit molecular weight of 48 kilodaltons (kda) and exists in both dimeric and tetrameric form. The hydrilla lectin is a Type II lectin composed of two monomeric subunits having molecular weights of 67 and 50 kda, which persist as a dimer and a tetramer, respectively. Two fungal pathogens of Eurasian watermilfoil and two fungal pathogens of hydrilla were evaluated for their ability to attach and infect the target species and for their capacity to be agglutinated by physiological concentrations of purified lectin from each plant species. Findings of this study support the theory that microbial recognition by plant lectins is a mediator of host defense. This study also reports 16 new potential pathogens against hydrilla and 15 against Eurasian watermilfoil.				
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