HOST SPECIFICITY IN THE RED ALGAL PARASITES
BOSTRYCHIOCOLAX AUSTRALIS AND *DAWSONIOCOLAX BOSTRYCHIAE* (CHOREOCOLACACEAE, RHODOPHYTA)[1]

Giuseppe C. Zuccarello[2]

Department of Plant Biology, University of California, Berkeley, California 94720

and

John A. West

School of Botany, University of Melbourne, Parkville, Victoria 3052, Australia

ABSTRACT

The determinants of host specificity, which are poorly understood in red algal parasites, were studied in the red algal parasites Bostrychiocolax australis *Zuccarello et West and* Dawsoniocolax bostrychiae *(Joly et Yamaguishi-Tomita) Joly et Yamaguishi-Tomita. Culture studies were performed to determine host range, sites of host resistance, and genetics of transmission of resistance. Both species parasitize* Bostrychia radicans *(Montagne) Montagne, whereas* Bostrychiocolax australis *also parasitizes* Bostrychia moritziana *(Sonder ex Kützing) J. Agardh and* Stictosiphonia kelanensis *(Grunow ex Post) R. J. King et Puttock. Isolates of* B. radicans *resistant to both parasites were found worldwide, often within the same population as susceptible isolates. On resistant* Bostrychia *species and isolates, specificity was manifested at three stages: 1) host penetration, in which the spore germ peg failed to penetrate the host cuticle/wall; 2) parasite–host cell fusion, in which the fusion cell died and the parasite died; and 3) growth, in which parasites grew but soon died; parasites rarely reproduced and infections did not continue in culture. Resistance to parasite infection was usually transmitted as a dominant trait and did not segregate as a single locus during meiosis. In certain crosses, transmission of resistance was non-mendelian.*

Key index words: Bostrychia radicans; Bostrychiocolax australis; Dawsoniocolax bostrychiae; *genetics; host specificity; parasite–host interactions; Rhodophyta*

Red algal parasites are host-specific biotrophic algae, growing on only one or a few host species (Goff 1982). Few published studies examine host range and factors that may influence this host specificity.

In culture, red algal parasites infect algae other than their native host with varying degrees of success. The adelphoparasite (i.e. parasite is closely related to its host) *Janczewskia morimotoi* Tokida infected two *Laurencia* species other than its natural host (Nonomura and West 1981), but on *L. subopposita* (J. Agardh) Setchell, parasite growth usually stopped before reproductive maturity. The adel-

phoparasite *Asterocolax gardneri* (Setchell) Feldmann et Feldmann, which is found on several Delesseriacean hosts, is divided into "physiological races" able to infect other natural hosts with varying degrees of success (Goff 1982).

As with all parasite–host interactions, host specificity (i.e. the ability of the parasite to successfully grow and reproduce on a host) rests in the ability of the parasite to overcome host defenses at each stage of the interaction. These stages were outlined by Goff (1982) and include spore attachment, spore germination, host wall penetration, and resistance to any harmful products formed by the host. In addition, the parasite must gain access to a source of nutrition. A detailed study of red algal parasite development on both natural and closely related hosts may reveal which events are important in the determination of host specificity.

The basis of parasite–host specificity in fungal pathogens and plant hosts has been the subject of much research. Heath (1981, 1991) described a model in which two types of specificity operate. The first is basic to all organisms, called basic (nonhost) resistance. This is a response to any foreign invader and is not parasite-specific. All host genotypes respond to all parasite genotypes at this level. This defense can involve preformed chemical factors (Schönbeck 1976) or physical features of the nonhost, and it occurs before specific resistance (Heath 1992). The next level of resistance is parasite genotype and host genotype specific, involving postulated gene-for-gene interactions, and is expressed after basic resistance. These types of interactions have been demonstrated or suggested in many other biotrophic parasitisms, involving a wide variety of host organisms (summarized in Vanderplank 1982:84).

A common feature of red algal parasite–host associations is the formation of host–parasite fusion cells by secondary pit connection formation (Goff and Coleman 1984, 1985). These authors have proposed that this process was necessary for the establishment of a host–parasite nutritional relationship. Feldmann and Feldmann (1958) suggested that compatibility between cytoplasmically linked hosts and parasites could account for the occurrence of parasites on only certain hosts. Degrees of cytoplasmic

compatibility during the fusion of different strains of an organism have been well documented in fungi (Glass and Kuldau 1992) and have been observed in algae (Koslowsky and Waaland 1984).

The red algal alloparasite (i.e. parasite is not closely related to its host) *Bostrychiocolax australis* Zuccarello et West was first reported by West and Calumpong (1988a) from Queensland, Australia, as *Dawsoniocolax bostrychiae* (Joly et Yamaguishi-Tomita) Joly et Yamaguishi-Tomita growing on *Bostrychia radicans* (Montagne) Montagne. *Dawsoniocolax bostrychiae* was originally described from a Brazil specimen (Joly and Yamaguishi-Tomita 1967, 1969) growing on *B. radicans*, *B. calliptera* (Montagne) Montagne, and *B. montagnei* Harvey (as *B. scorpioides* var. *montagnei*). Differences in early development in culture served as the basis for the creation of the new genus *Bostrychiocolax* for the Australian parasite (Zuccarello and West 1994). Early development of *Bostrychiocolax australis* and *Dawsoniocolax bostrychiae* can be separated into three stages: 1) spore adhesion and host cuticle/wall penetration, 2) parasite–host cell fusion, and 3) growth after the host–parasite connection has formed (Fig. 1).

The purpose of this study was to determine the host range of these two parasites of the red algal genus *Bostrychia* Montagne both among populations of a species and between species of *Bostrychia* and the closely related genus *Stictosiphonia* J. D. Hooker et Harvey. Detailed observations of parasite infection on various hosts was employed to assess the progress of parasite infection on susceptible and resistant hosts. Genetic analysis of resistance was then investigated using genetic crosses between cultured isolates.

FIG. 1. Schematic of early developmental stages in A) *Bostrychiocolax australis* and B) *Dawsoniocolax bostrychiae*. Development is divided into three stages: Stage 1 = spore penetration; Stage 2 = parasite–host cell fusion; Stage 3 = growth from infected host cell and feeding phase. Stippling indicates parasite cytoplasm. Black circles = parasite nuclei. Clear circles = host nuclei. Arrow indicates first host–parasite cell fusion (for more details see Zuccarello and West 1994).

MATERIALS AND METHODS

The genera *Bostrychia* and *Stictosiphonia* (Rhodomelaceae, Ceramiales) include mostly pantropical mangrove-dwelling algae. A recent monograph of the genera (King and Puttock 1989) recognized 11 species of *Bostrychia* and 6 species of *Stictosiphonia*. For the present study, algal cultures of 10 species of *Bostrychia* and 3 species of *Stictosiphonia* were collected and maintained as outlined in West and Calumpong (1988b). *Murrayella periclados* (C. Agardh) Schmitz was used to test infection on a distantly related mangrove alga. Culture numbers and exact collection locations for all isolates are available upon request.

Bostrychiocolax australis growing on *Bostrychia radicans* (isolate 2837) was collected from an intertidal rock (4 June 1987) from the northern end of Florence Bay, Magnetic Island, Queensland, Australia. The parasite culture was derived from a single tetrasporophytic plant. It was maintained on its host at 25° C, 12:12 h LD, and 20–30 μmol·m⁻²·s⁻¹ photon flux density.

Inoculations with B. australis. Reproductively mature tetrasporophytic and cystocarpic parasites were used to inoculate uninfected hosts. Uninfected host plants were placed on Nitex screen (150 μm mesh) floating on the culture medium. Parasites that were releasing spores were placed on uninfected hosts for 2–4 days and then removed. Hosts with attached spores were maintained in culture for observation. Infections were scored only when many (>50) spores were seen on or near test plants. All test p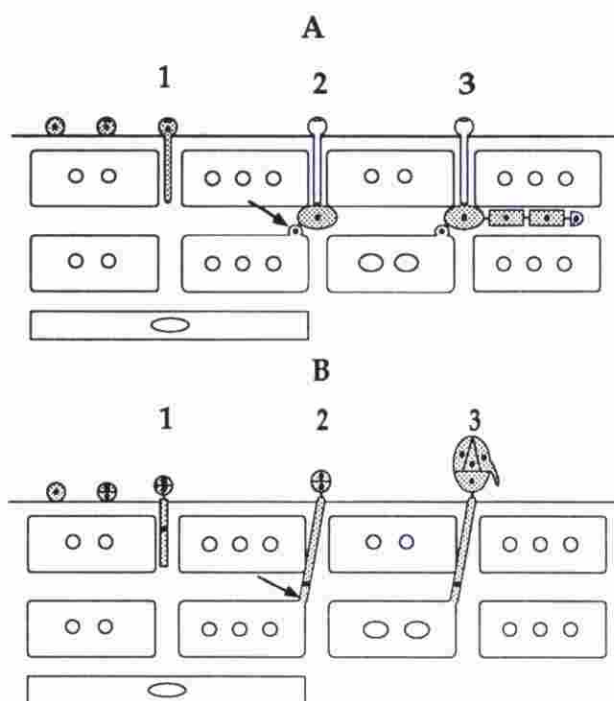lants were observed weekly, for up to 2 months, from the time of removal of the inoculating parasite. Seventy-five percent of all attempted infections were repeated two or more times. Interactions were consistent with a particular isolate in all repeat infections and among spores in any one infection. When both tetrasporophytes and gametophytes of a host isolate were available, the tetrasporophyte and at least one gametophyte (male or female) were tested.

Dawsoniocolax bostrychiae, growing on *Bostrychia radicans*, was collected on 11 April 1990 from Rio Sitio Grande, Ilha do Cardoso, São Paulo, Brazil, from *Rhizophora mangle* L. prop roots. All parasite cultures were derived from one tetrasporophyte. This isolate was maintained in culture not on its original host specimen but on a *B. radicans* isolate (3058) from Rio Sitio Grande, Ilha do Cardoso. Parasite–host cultures were maintained on a rotary shaker (70 rpm) at 20°–25° C, 12:12 h LD, 20–30 μmol·m⁻²·s⁻¹ photon flux density.

Inoculation with D. bostrychiae. Spore release was unpredictable in this parasite. Test hosts were placed in a 250-mL culture dish with *B. radicans* (3058) with large parasites and placed on a rotary shaker (70 rpm) until a sufficient number of parasite spores (>50) had been released (3 days to 3 weeks) and were seen on the test plants. The test plant was examined and placed in a culture dish containing fresh culture media. Test plants were observed weekly for up to 2 months. Due to the sporadic reproduction and spore release of *D. bostrychiae*, many infections were not repeated, but all interactions were consistent between repeat infections and among spores in any one infection.

Host genetic crosses. To test for transmittance of host resistance, crosses were performed between resistant and susceptible isolates of *B. radicans* from Brazil and Peru, which were interfertile and variable in parasite susceptibility. Crosses were performed by placing male and female plants, about 1 cm in length, in 100-mL

culture dishes containing 30‰ quarter-strength Provasoli en-richment seawater medium at 25° C, 12:12 h LD, and 30–40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photon flux density on a rotary shaker (60–75 rpm) for approximately 2 weeks until cystocarps started to form. Male plants were removed from the female plant, and the female was incubated until maturity and release of carpospores. Carpospores were individually isolated and placed in culture dishes. Hybrid tetrasporophytes were tested for parasite susceptibility when about 2 cm in length, as previously outlined. Tetraspores from these hybrids were also isolated, and the gametophytes were tested for parasite susceptibility.

Cytology. All tissue was fixed in a modified Karnovsky seawater fixative (Sol A: 4 g paraformaldehyde, 50 mL distilled water, 30 mL 0.2 M cacodylate buffer, pH 6.8; Sol B: 10 mL 50% glutar-aldehyde, 10 mL 0.2 M cacodylate buffer, pH 6.8; Sol C: 100 mL sterile seawater) for 2 h at room temperature or overnight at 4° C. Tissue was then washed in cacodylate buffer solution (pH 6.8, osmolality 500 mOsm·g⁻¹). Tissue for fluorescence micros-copy was processed as in González and Goff (1989) and observed with a Zeiss Axiophot epifluorescence microscope. Fluorescent photos were taken on Fujichrome 400 film.

Tissue was softened in saturated chloral hydrate and stained with 0.25% acidified aniline blue in 60% Karo® syrup. The host cuticle was stained with Fast green (general protein stain) (McMully et al. 1980).

Fluorescein isothiocyanate-lectin staining. Tissue was fixed in 2.5% paraformaldehyde and 0.25% glutaraldehyde in phosphate buffer solution at pH 7.45 for 30 min. Tissue was washed in 30‰ sea-water, three times for 20 min each, and placed in 50 $\mu g \cdot mL^{-1}$ fluorescein isothiocyanate (FITC)-labeled lectin (wheat germ ag-glutinin [WGA]; concavalin [Con A]; *Bauhinia purpurea* aggluti-nin [BPA]; peanut agglutinin [PNA]; *Griffonia simplicifolia* agglu-tinin II [GSII]) (E & Y Labs, San Mateo, CA) for 30 min. Tissue was washed in lectin buffer, prepared as per E & Y Labs, three times for 15 min each. Positive controls for lectin staining were prepared by adding 0.2 M of hapten (N-acetyl-D-glucosamine [for WGA and GSII]; mannose [for Con A]; N-acetyl-D-galactosamine [for BPA]; lactose [for PNA]) to lectin for 30 min prior to addition of tissue.

RESULTS

Bostrychiocolax australis

Host range. The host range of this parasite on spe-cies and isolates of *Bostrychia*, *Stictosiphonia*, and *Mur-rayella* is summarized in Table 1. Of 13 potential host species, 105 isolates were tested. Susceptible host isolates were observed in *B. radicans*, *B. morit-ziana* (Sonder ex Kützing) J. Agardh, and *Stictosi-phonia kelanensis* (Grunow ex Post) R. J. King et Put-tock. Susceptible hosts were defined as hosts in which the parasite was able to grow, reproduce, and con-tinue infections in culture. *Bostrychiocolax australis* was able to successfully infect 21 of the 57 isolates of *Bostrychia radicans* tested. Susceptible *B. radicans* isolates were found over a wide geographic range (Australia, Philippines, northeastern United States, Brazil, Peru, Micronesia, and South Africa). Within most geographic areas, susceptible and resistant iso-lates occurred together. In one population on Ilha do Cardoso, São Paulo, Brazil, three isolates were susceptible and four isolates were resistant to infec-tion. On the other hand, all 19 isolates from ap-proximately 5000 km of coastline in Pacific Mexico (states of Baja California Sur, Sinaloa, Jalisco, and

Nayarit) were resistant. Parasite development on re-sistant hosts was arrested at three stages: before host wall penetration, at the first parasite–host cell fusion stage, or during the growth of the parasite pustule.

Host penetration. Parasite spores did not penetrate the host cuticle in any isolates of *Murrayella pericla-dos*, *Stictosiphonia hookeri* (Harvey) J. D. Hooker et Harvey, *Bostrychia montagnei* Harvey, *B. tenella* (La-mouroux) J. Agardh, *B. tenuissima* R. J. King et Put-tock, *B. calliptera* (Montagne) Montagne, and *B. pin-nata* J. Tanaka et M. Chihara (Table 1). Parasite spores also did not penetrate one isolate each of *S. kelanensis* and *S. tangatensis* (Post) R. J. King et Put-tock. Spores that attached to the host cuticle could not be removed by agitation on a rotary shaker. Upon germination, however, the elongating germ tube pushed the spore body away from the host surface (Fig. 2). Occasionally a spore appressorium was formed on the surface of the host (Fig. 3), but no penetration of the host was observed (Fig. 4).

The site of spore attachment was random in all hosts except *Bostrychia moritziana*. In *B. moritziana*, spores or parasites were never observed on mono-siphonous lateral branches, although they were found on monosiphonous branches in other species (*B. bispora* West et Zuccarello, *B. simpliciuscula* Har-vey ex J. Agardh and *B. tenella*). Lectins were used to test the polysaccharide moieties on the host sur-face (Table 2). All hosts (*Bostrychia radicans*, *B. morit-ziana*, *B. pinnata*, *B. simpliciuscula*, and *B. bispora*) stained with lectin Con A (Fig. 5). Monosiphonous lateral branches of *B. moritziana* showed only weak staining with Con A and none with WGA (Fig. 6), whereas polysiphonous branches of this species stained strongly with both lectins. Addition of hap-tens to lectins significantly reduced or eliminated lectin binding.

The cuticle of *B. radicans* is proteinaceous (stains with Fast green), whereas the underlying cell wall does not stain (Fig. 7). In all these algae, the cuticle was very tough and remained intact during cytolog-ical preparations.

Parasite–host fusion. In *Bostrychia radicans*, *B. morit-ziana*, *B. bispora*, *B. simpliciuscula*, and all but one isolate of *Stictosiphonia kelanensis* and *S. tangatensis*, parasite spores germinated on the host surface. A small percentage was able to penetrate the host cu-ticle and outer cell wall. The germ tube penetrated the host cuticle, and then the tip expanded within the host tissue. Although all parasite spores germi-nated, only a small percentage (approximately 10–20%) penetrated the host. Parasite–host cell fusions were formed by most spores (>80%) that penetrated the host's outer wall. On *B. bispora* and *B. simpli-ciuscula*, parasite spores that penetrated the host cu-ticle were associated with dead host cells within 3 days. These infected host cells first lost their red pigmentation, turned green, and became slightly plasmolysed (Fig. 8). In *B. bispora* and *B. simplicius-cula*, parasite development always stopped at this
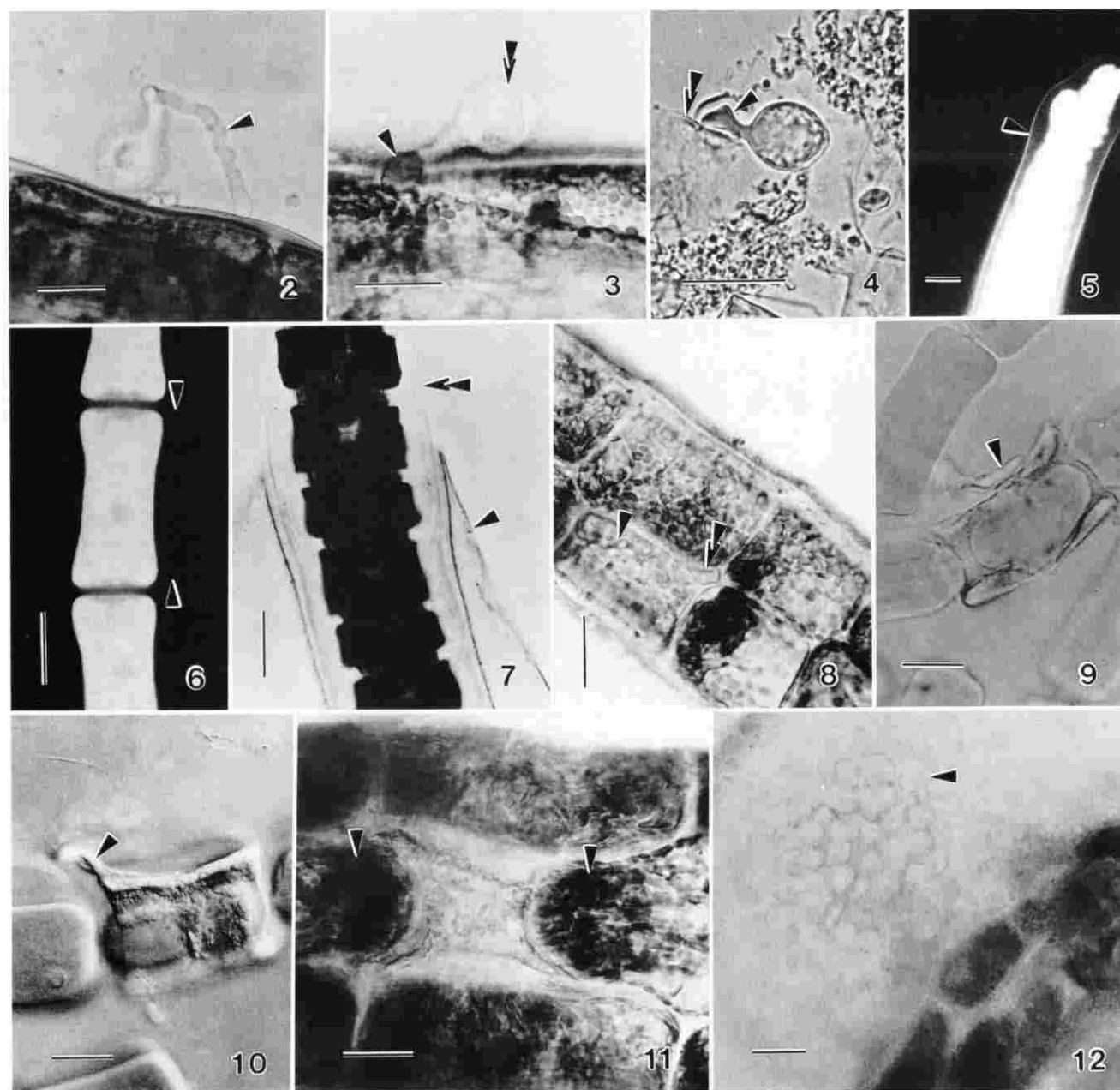
TABLE 1. *Host range of* Bostrychiocolax australis *on species of Bostrychia, Stictisiphonia, and Murrayella. S = susceptible (infects host and complete life history normally); R^a = resistant (spore germ tube does not penetrate outer host cuticle; no development beyond spore germination); R^b = resistant (limited parasite development; spore penetrates host outer cuticle but host–parasite fusion cell aborts and parasite development ceases); R^c = resistant (occasional parasites become mature and release spores, but infection cycle does not continue in culture).*

Host species	Location	No. of isolates	Infectivity
Bostrychia radicans	Queensland, Australia	2	S
B. radicans	Queensland, Australia	1	R^c
B. radicans	Northern Territories, Australia	2	S
B. radicans	Northern Territories, Australia	2	R^c
B. radicans	São Paulo, Brazil	6	S
B. radicans	São Paulo, Brazil	6	R^c
B. radicans	Edo Sucre, Venezuela	1	R^c
B. radicans	Northeastern United States	3	S
B. radicans	Northeastern United States	3	R^c
B. radicans	Philippines	1	S
B. radicans	Guam	2	R^c
B. radicans	Micronesia	1	S
B. radicans	Baja California Sur, Mexico	15	R^c
B. radicans	Mainland Pacific Mexico	4	R^c
B. radicans	Tumbes, Peru	1	S
B. radicans	Tumbes, Peru	2	R^c
B. radicans	Natal, South Africa	5	S
B. moritziana	Victoria, Australia	2	S
B. moritziana	Victoria, Australia	2	R^c
B. moritziana	Venezuela	2	R^c
B. moritziana	South Africa	3	R^c
B. moritziana	Micronesia	1	R^c
B. bispora	Darwin, Australia	2	R^b
B. simpliciuscula	Singapore	2	R^b
B. simpliciuscula	Queensland, Australia	1	R^b
B. pinnata	Queensland, Australia	2	R^a
B. pinnata	Singapore	1	R^a
B. pinnata	Tumbes, Peru	1	R^a
B. calliptera	São Paulo, Brazil	3	R^a
B. tenuissima	Australia	6	R^a
B. tenella	Queensland	1	R^a
B. tenella	Philippines	2	R^a
B. tenella	Belize	1	R^a
B. montagnei	Florida, United States	1	R^a
Stictosiphonia hookeri	Cape Province, South Africa	2	R^a
S. kelanensis	Darwin, Australia	2	R^c
S. kelanensis	Darwin, Australia	1	R^a
S. kelanensis	Singapore	2	R^c
S. kelanensis	Micronesia	1	S
S. tangatensis	Natal, South Africa	1	R^a
S. tangatensis	South Africa	2	R^c
Murayella periclados	Queensland, Australia	1	R^a
M. periclados	Singapore	1	R^a

stage (stage 2 in Fig. 1) and was always associated with a dead host cell. Occasionally, host cell death occurred before host–parasite cell fusion (Fig. 9), but in most cases host cell death was associated with fusion of parasite and host cells (Fig. 10). Later, these host cells plasmolysed, and uninfected host cells on either side of them produced repair cells that grew through these plasmolysed host cells (Fig. 11) and fused to form a new cell within the wall of the dead cell.

Development on resistant hosts. In certain isolates of *B. radicans*, *B. moritziana*, *S. kelanensis*, and *S. tangatensis* (Table 1), parasite spores penetrated the host cuticle and fused with underlying host cells. Occasionally the host cell at this stage died and parasite development stopped, but in most cases parasite development continued. Parasite development was initially similar to that on susceptible isolates, but it eventually stopped and the parasite died. Cessation of development involved progressive vacuolization (Fig. 12) and subsequent plasmolysis (Fig. 13) of parasite cells. Death of the parasite occurred at any stage of development, although on the majority of resistant hosts small parasite pustules (50–200 μm) were visible (Fig. 14). Parasites formed reproductive structures (tetrasporangia, spermatangia, and pro-

FIGS. 2–12. *Bostrychiocolax australis* on resistant hosts. Figures 2–4 and 8–11, scale bars = 20 μm; Figures 5–7 and 12, scale bars = 25 μm. FIG. 2. *B. australis* spore on the surface of *Bostrychia tenuissima*. Arrowhead indicates the germ tube. Live preparation. FIG. 3. *B. australis* spore (double arrowhead) on *Murrayella periclados*. Arrowhead indicates appresorium. Live preparation. FIG. 4. *B. australis* on *Bostrychia tenuissima*. Arrowhead indicating appresorium still attached to host cuticle (double arrowhead). Squash preparation. FIG. 5. *B. radicans* staining with FITC-labeled WGA. Cuticle-stained (arrowhead), host cell autofluorescence also visible. Fluorescence microscopy. FIG. 6. Monosiphonous axis of *B. moritziana* stained with FITC-labeled WGA. No staining of cuticle (indicated between arrowheads). Fluorescence microscopy. FIG. 7. Protein staining (Fast green) of *B. radicans* cuticle. Stained cuticle (arrowhead) peeled back revealing unstained cell wall (double arrowhead). FIG. 8. Slightly plasmolysed infected host cell of *Bostrychia simpliciuscula* (arrowhead). Parasite cell visible (double arrowhead). Live preparation. FIG. 9. Germ tube of parasite spore (arrowhead) apparently not fused to dead host cell of *Bostrychia bispora*. Squash preparation. FIG. 10. Fusion between parasite germ tube (out of focal plane) and host cell. Parasite–host fusion visible (arrowhead). Dead host cell wall visible. Squash preparation. FIG. 11. Host repair cells (arrowheads) growing through dead host cell that is associated with a parasite spore (out of focal plane). Live preparation. FIG. 12. Dead vacuolate parasite pustule on resistant *B. radicans*. Vacuolate cells indicated (arrowhead). Live preparation.

carps) in less than 25% of the plants that formed visible pustules, but most of these plants died before reproduction could take place. Parasite death was always associated with the death of underlying infected host cells (Fig. 15). Tetrasporic parasites occasionally released a small number (<30) of tetraspores that produced very few gametophytic pustules. Infections with these tetraspores on resistant strains

TABLE 2. *FITC-labeled lectin binding to host cuticles of Bostrychia species.* + = *strong binding of lectin;* ± = *weak binding of lectin;* − = *no lectin binding;* ** = *not tested.*

Host species	Lectin type				
	Con A	WGA	BPA	PNA	GSII
B. radicans	+	+	±	±	±
B. moritziana					
Polysiphonous laterals	+	+	+	±	−
Monosiphonous laterals	±	−	−	−	−
B. pinnata	+	**	**	−	−
B. simpliciuscula	+	**	−	−	+
B. bispora					
Polysiphonous laterals	+	**	**	**	**
Monosiphonous laterals	+	**	**	**	**

TABLE 3. *Susceptibility of B. radicans F_1 sporophytes to B. australis and D. bostrychiae infection. S = susceptible; R = resistant; F_1 tetrasporophyte = susceptibility of F_1 tetrasporophytes; No. crosses tested = number of separate crosses of different pairs of gametophytes tested.*

Parental strains	F_1 tetrasporophyte	No. crosses tested
B. australis		
Female (S) × male (S)	S	3
Female (R) × male (R)	R	3
Female (S) × male (R)	R	4
Female (R) × male (S)	R	8
D. bostrychiae		
Female (S) × male (S)	S	5
Female (R) × male (R)	R	1
Female (S) × male (R)	R	6

produced a low percentage of mature gametophytes. Mature males often released spermatia, which attached and fused to trichogynes. Females produced a normal number, approximately 50, of trichogynes. Of the hundreds of fertilized procarps observed, only a small number (approximately 10%) produced cystocarps. Developing cystocarps usually aborted before spore release (Fig. 16), but occasionally a few carpospores were released. Mature tetrasporophytes were never observed to develop from these released carpospores. On many resistant hosts, particularly *B. moritziana* and *S. kelanensis*, host cells would become greatly hypertrophied (Fig. 17), and later these infected host cells would die.

Transmission of parasite resistance. Tetrasporophytes originally isolated from the field, and gametophytes derived from the tetrasporophytes, showed the same susceptibility or resistance to *Bostrychiocolax australis*. F_1 tetrasporophytes of a cross between any two susceptible gametophytes of *B. radicans* were susceptible to infection by *B. australis* (Table 3). F_1 sporophytes derived from two resistant parents were also resistant. These hybrid sporophytes showed greater resistance to *B. australis* than either of the parents (i.e. parasite pustules were smaller when death occurred).

Crosses between resistant and susceptible parents demonstrated that resistance was transmitted as a dominant trait to F_1 sporophytes (Table 3). Transmission of resistance to gametophytes derived from hybrid tetrasporophytes (one resistant and one susceptible parent) revealed segregation of resistance (Table 4). Segregation deviated significantly from a one-to-one ratio in two of the three crosses tested (chi-square test).
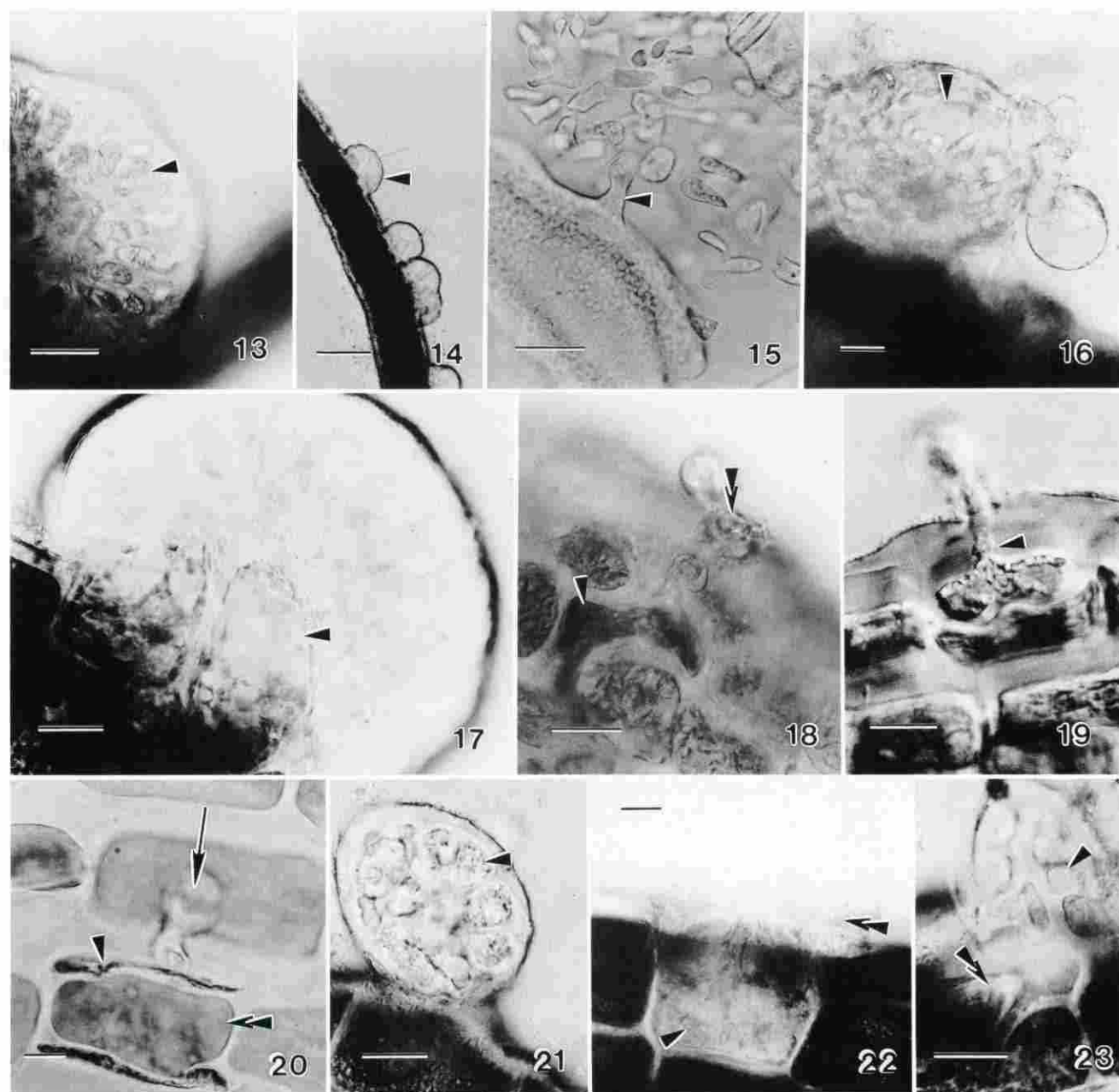
Dawsoniocolax bostrychiae

Host range. Forty-nine isolates of 11 species of *Bostrychia, Stictosiphonia,* and *Murrayella* were inoculated with *D. bostrychiae* spores (Table 5). Susceptible hosts were only observed in *B. radicans*. Many of the inoculations could not be repeated due to the variability of spore release by *D. bostrychiae*. Parasite development was arrested at three different stages.

Host penetration. Spore penetration of the host cuticle was not observed on *Murrayella periclados, B. montagnei,* and certain isolates of *B. calliptera, B. tenella,* and *B. pinnata* (Table 5). Spore germination was low on *M. periclados, B. montagnei,* and the *B. tenella* isolates from Brazil and the Philippines. On *B. calliptera* from Colombia, most spores progressed through the first stages of germination including division and extension of a germ tube, although no host penetration was observed.

Parasite–host fusion. In all other species and isolates, parasite spores germinated on the host surface and were able to penetrate the host cuticle. The spore divided on the host surface, and the cell nearer the host produced a germ tube that penetrated the host cuticle and cell wall and fused directly with an underlying cell. In certain hosts (Table 5), host cell wall penetration was associated with the death of an underlying host cell (Fig. 18). Squash preparation clearly revealed that host cell death was associated with the fusion of the parasite germ tube (Fig. 19).

TABLE 4. *Susceptibility of F_1 tetrasporophytes and ratio of susceptibility in F_1 gametophytes of Bostrychia radicans to B. australis and D. bostrychiae infection. S = susceptible; R = resistant; nd = not determined; S:R = segregation of susceptibility (S) and resistance (R) in F_1 gametophytes derived from the F_1 sporophytes;* * = *significant deviations from one-to-one ratio, P < 0.05.*

Parental strains (female × male)	F_1 tetra-sporophyte (susceptibility)	F_1 gameto-phytes S:R
B. australis		
Brazil 2649 (S) × Brazil 3058 (S)	S	nd
Brazil 2649 (S) × Brazil 3017 (R)	R	1:5
Brazil 3017 (R) × Brazil 2649 (S)	R	1:13*
Brazil 3058 (S) × Brazil 3017 (R)	R	0:5*
Brazil 3162d (R) × Brazil 3107 (R)	R	nd
D. bostrychiae		
Brazil 3058 (S) × Peru 3043 (R)	R	0:5*
Brazil 3040 (S) × Brazil 3058 (S)	R	0:2
Brazil 3058 (S) × Brazil 3017 (R)	S	2:0
Brazil 3017 (R) × Brazil 3058 (S)	R	nd
Brazil 3039 (S) × Brazil 3017 (R)	S	nd
Brazil 3040 (S) × Brazil 3017 (R)	R	nd
Brazil 3159G (S) × Brazil 3017 (R)	R	nd

FIGS. 13–23. *Bostrychiocolax australis* and *Dawsoniocolax bostrychiae* on resistant hosts. Scale bars = 20 μm. FIG. 13. Abortive plasmolysed tetrasporophytic pustule of *B. australis* on resistant *Bostrychia radicans*. Arrowhead indicates a tetrasporangium. Live preparation. FIG. 14. *Bostrychiocolax australis* pustules on *Stictosiphonia kelanensis*. Arrowhead indicates a female pustule. Live preparation. FIG. 15. Female *B. australis* pustule attached to dead host cell wall of resistant *Bostrychia radicans*. Arrowhead indicates host–parasite cell fusion (parasite pustule had plasmolysed cells before fixation). Squash preparation. FIG. 16. Abortive cytocarp (arrowhead) of *Bostrychiocolax australis* on resistant *B. radicans*. Live preparation. FIG. 17. Hypertrophied host cells (arrowhead) and associated *B. australis* pustule. Live preparation. FIG. 18. *Dawsoniocolax bostrychiae* spore associated with plasmolysed cortical cell of *Bostrychia tenella* (arrowhead). Double arrowhead indicates second parasite cell. Live preparation. FIG. 19. *D. bostrychiae* spore fused to plasmolysed host cell. Parasite–host fusion indicated (arrowhead). Squash preparation. FIG. 20. *D. bostrychiae* spore (arrow) associated with dead host cell wall (arrowhead). Repair cell clearly visible (double arrowhead). Squash preparation. FIG. 21. *D. bostrychiae* pustule on resistant host. Parasite cells with many small vacuoles (arrowhead) and slightly plasmolysed. Live preparation. FIG. 22. *D. bostrychiae* pustule (double arrowhead) on resistant host. Infected host cell (arrowhead) less pigmented than surrounding host cells. Live preparation. FIG. 23. Plasmolysed *D. bostrychiae* pustule (arrowhead) on resistant host, associated with dead and repair (double arrowheads) host cells. Live preparation.

Cells on either side of the dead host cell cut off small "repair" cells that grew through the dead host cell and fused to replace that cell (Fig. 20). Parasite development always ceased with the death of the host cell (two-celled stage); occasionally, another parasite cell division occurred before the parasite died (three-celled stage). On other resistant hosts, host cell and parasite death sometimes occurred at this stage, but parasite development usually continued.

Development on resistant hosts. In certain resistant

TABLE 5. *Host range of* Dawsoniocolax bostrychiae *on species and isolates of* Bostrychia, Stictosiphonia, *and* Murrayella. *S = susceptible (parasite completes life cycle in culture and establishes new infections); R^a = resistant (parasite spores do not penetrate host cuticle); R^b = resistant (parasite development arrested at two- to three-celled stage); R^c = resistant (parasites develop, but most abort before becoming reproductively mature). Occasionally becoming reproductive in culture, but reinfections are not established.*
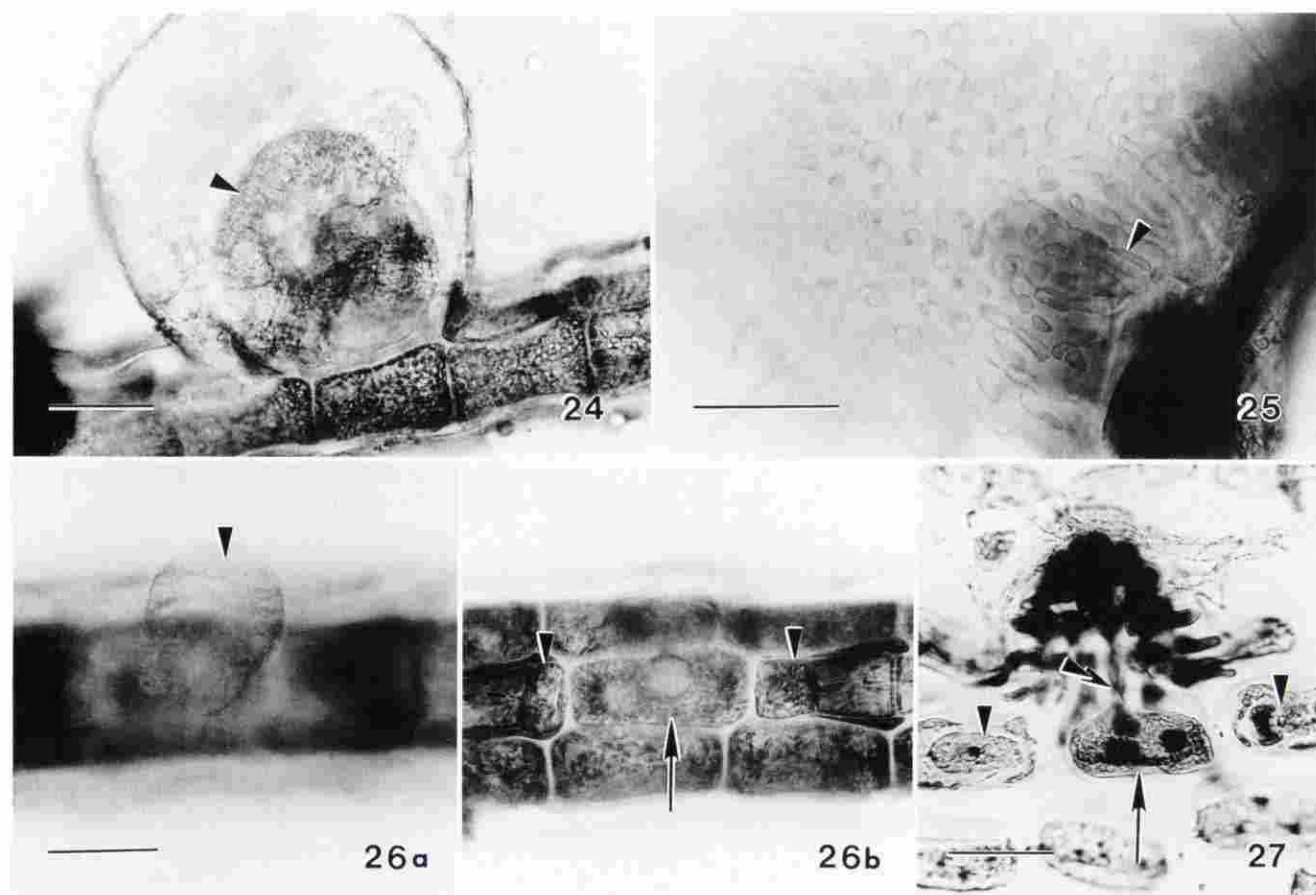
Host species	Location	No. of isolates tested	Infectivity
Bostrychia radicans	São Paulo, Brazil	6	S
B. radicans	São Paulo, Brazil	6	R^c
B. radicans	São Paulo, Brazil	1	R^b
B. radicans	Australia	2	R^c
B. radicans	New Jersey, United States	1	R^c
B. radicans	Florida, United States	1	S
B. radicans	Baja California Sur, Mexico	4	R^c
B. radicans	Baja California Sur	1	R^b
B. radicans	Edo Sucre, Venezuela	1	R^c
B. radicans	Edo Sucre	1	R^b
B. radicans	Tumbes, Peru	1	R^c
B. radicans	Tumbes, Peru	1	R^b
B. radicans	Natal, South Africa	1	S
B. radicans	Guam	1	R^c
B. moritziana	Victoria, Australia	2	R^c
B. moritziana	Edo Sucre, Venezuela	2	R^c
B. mortiziana	Cali, Colombia	1	R^b
B. calliptera	São Paulo, Brazil	1	R^c
B. calliptera	São Paulo, Brazil	2	R^b
B. calliptera	Cali	1	R^a
B. montagnei	São Paulo, Brazil	2	R^a
B. tenella	Belize	1	R^b
B. tenella	Philippines	1	R^a
B. tenella	Bahia, Brazil	1	R^a
B. simpliciuscula	Singapore	1	R^b
B. bispora	Northern Territory, Australia	1	R^c
B. tenuissima	South Australia	1	R^b
B. pinnata	Singapore	1	R^b
B. pinnata	Tumbes, Peru	1	R^a
Stictosiphonia kelanensis	Singapore	1	R^c
S. kelanensis	Northern Territories, Australia	1	R^b
Murrayella periclados	Singapore	1	R^a

isolates of *B. radicans, B. moritziana, B. calliptera, B. bispora,* and *S. kelanensis* (Table 5), parasite spores penetrated the host cuticle and fused with underlying host cells. Parasite death was not immediate and development continued. Parasite reproductive structures formed rarely; none were observed releasing spores. On these hosts, incipient parasite death was first marked by the appearance of vacuoles within the parasite cells (Fig. 21), which eventually plasmolysed. Parasite death was often associated with changes in the appearance of underlying infected host cells, including changes in pigmentation of infected host cells from red to green, loss of pigments (Fig. 22), increase in vacuoles, and eventual death of underlying host cells (Fig. 23). In some infections, host cell hypertrophy was observed prior to cell death (Fig. 24). Later, the parasite cells would plasmolyse (Fig. 25). In many of the parasite infections on resistant *B. radicans,* host cells on either side of the infected host cell died (Fig. 26), while the infected host cell remained viable. The cells on either side of the dead host cell produced repair cells that grew

through the space of the dead host cell but did not appear to fuse with the infected host cell (Fig. 27). Eventually, the infected host cell and its attached parasite died.

Transmission of parasite resistance. Crosses between two susceptible parents produced susceptible F_1 progeny (Table 3); crosses between two resistant parents were resistant. Six crosses between susceptible females and resistant males were also resistant. Transmission of resistance to gametophytes derived from hybrid tetrasporophytes were significantly different from a one-to-one segregation (Table 4).

Inheritance of resistance to *D. bostrychiae* did not always follow the pattern of *Bostrychiocolax australis* resistance (Table 4). In one cross between two susceptible hosts from São Paulo, Brazil (3040 × 3058), the tetrasporophyte was resistant. In a cross between a resistant female (3017) and a susceptible male (3058), the F_1 sporophyte was resistant, but in the reciprocal cross (3058 × 3017) the sporophyte remained susceptible. In another cross with the same male (3017), the F_1 sporophyte was also susceptible.

FIGS. 24–27. *Dawsoniocolax bostrychiae* on resistant hosts. Scale bars = 50 μm. FIG. 24. Hypertrophied host cell (arrowhead) of *Bostrychia moritziana* associated with parasite pustule. Live preparation. FIG. 25. Plasmolysed cell of *D. bostrychiae* on resistant *B. radicans*. Arrowhead indicates downward-growing rhizoids characteristic of *D. bostrychiae*. Live preparation. FIG. 26. *D. bostrychiae* on resistant *B. radicans*. Focal series: a) parasite pustule (arrowhead). b) Infected host cell (arrow) still viable. Adjacent repair cells (arrowheads) visible growing through dead host cell walls. Live preparation. FIG. 27. *D. bostrychiae* on resistant *B. radicans*. Parasite–host fusion (double arrowhead) connecting parasite to infected host cell (arrow). Note enlarged host nuclei in infected host cell, typical of these infections. Repair cells (arrowheads) have grown through dead host walls of infected cells. Squash preparation.

DISCUSSION

Host Range

The parasite *Bostrychiocolax australis* was able to grow and reproduce in culture on *Bostrychia radicans*, *B. moritziana*, and *Stictosiphonia kelanensis*. Algal host susceptibility to parasite infection may be the result of a similarity between the hosts at the biochemical level (Feldmann and Feldmann 1958) and may reflect host phylogeny. Parasitic interactions have been used to determine taxonomic relationships between host species in vascular plants (Savile 1971, McCain and Hennen 1982). A taxonomic revision of *Bostrychia* by King and Puttock (1989) resurrected the genus *Stictosiphonia* to include organisms with three or more tier cells. Earlier work by Post (1936) placed the three host species mentioned previously into a subgroup within the genus *Bostrychia* based on the presence of specialized attachment organs (cladohaptera). The susceptibility of these species to parasite infection may reflect a common ancestry, which would support Post's classification.

Within all three susceptible species, resistant isolates were observed. Resistance of these isolates usually occurs late in the infection process, at or before reproduction of the parasite. An exception was an *S. kelanensis* isolate from Australia in which parasite spores do not penetrate the cuticle. This isolate is distinct morphologically and in growth habit in culture from other *S. kelanensis* isolates (pers. observ.), which may indicate that it was misidentified.

Susceptible and resistant isolates of the same species were found in geographically distant populations and within a population (*B. radicans* at Ilha de Cardoso, Brazil). It is believed that host resistance is superimposed on basic compatibility between host species and their parasites (Heath 1981). Compatible interactions involve a series of complex "accommodations" of the parasite to the host that are less likely to have evolved multiple times than resistance, which can involve a simple change in the host to render it resistant (Heath 1981). It is possible then that *B. australis* has a basic compatibility with *B.*

radicans and that resistance has evolved multiple times in various populations.

The parasite *D. bostrychiae* was able to grow in culture only on *B. radicans*. Joly and Yamaguishi-Tomita (1967) first described *D. bostrychiae* on two other species of *Bostrychia* (*B. calliptera* and *B. montagnei*). Specimens have never again been reported from these species (Guimarães 1993), and multiple collections of the parasite in Brazil and a survey of the Universidad do São Paulo herbarium by one of us (J.A.W.) did not find parasites on these species, so it is possible that the original report was based on a misidentification. In culture, the parasite was not able to grow on these two *Bostrychia* species.

Resistance Mechanisms

Although differences exist in early spore development between the parasites *Bostrychiocolax australis* and *Dawsoniocolax bostrychiae*, similarities in the resistance patterns on various hosts clearly indicate areas where parasite–host recognition and host specificity is manifested. Host specificity was determined at three points: at the host surface, during cytoplasmic fusion between host and parasite, and during parasite growth.

Nonhost-specific or basic resistance as defined by Heath (1981, 1991) is a generalized resistance that all hosts have to all nonspecific parasites and can be thought of as a generalized mechanism of resistance to any symbiont. For example, cuticular sloughing in *Prionitis lanceolata* (Harv.) Harvey removes epiphytes that are not able to penetrate its cuticle (González and Goff 1989). Spore adhesion is a prerequisite to infection on a host (Nicholson and Epstein 1991). The inability of *B. australis* and *D. bostrychiae* spores to penetrate the cuticle and outer wall of the Rhodomelacean genus *Murrayella periclados*, distantly related to *Bostrychia*, suggests that this is a nonhost type of resistance reaction.

The initial attachment and penetration stages of parasitic algae are poorly understood, and both mechanical and chemical means of spore penetration have been proposed (Goff 1982). The inability of *B. australis* to penetrate the host cuticle of some *Bostrychia* species (*B. pinnata*, *B. calliptera*, *B. tenella*, *B. tenuissima*) and *Stictosiphonia hookeri* may be due to its inability to attach firmly and/or its inability to produce the right set of cell wall degrading enzymes. The host cuticles contain carbohydrates (lectin staining) and proteins (Fast green staining) as do other algal cuticles (Hanic and Craigie 1969). Lectin–carbohydrate interactions (Sharon and Lis 1989) and glycoproteins (Epstein et al. 1985) have been proposed as important in spore adhesion and infection for microorganisms and fungi. *Bostrychiocolax australis* spores are unable to attach to the monosiphonous lateral branches of *Bostrychia moritziana*, which have different lectin-binding properties than the polysiphonous branches, possibly due to specific cuticular carbohydrates that may be important in spore attachment. *Dawsoniocolax bostrychiae* spores show variability in their ability to penetrate host cuticles between isolates of one species. Some *Bostrychia calliptera*, *B. tenella*, and *B. pinnata* isolates were resistant to spore penetration whereas others were penetrable. This variability may reflect differences in the composition of the cuticle or cell wall of these different isolates.

Resistance to parasite infection was also seen during parasite–host cell fusion, where the product of this fusion (heterokaryotic cell) quickly dies. This host cell reaction is analogous to the hypersensitive response (HR) seen in some incompatible fungal–plant interactions (Aist and Bushnell 1991). In certain fungi (e.g. powdery mildews), HR occurs when the haustorium of a parasite penetrates the lumen of the host cell and comes into contact with the host plasma membrane. Although not the only site where host specificity is determined (Clifford et al. 1985), contact between the fungal pathogen cell and the plant plasma membrane is believed to be important in the determination of host specificity in many interactions (Brian 1976).

Feldmann and Feldmann (1958) hypothesized that incompatibility between cytoplasmically connected red algal parasites and hosts could be the basis of host specificity. Red algae normally undergo developmental cell fusions (Goff and Coleman 1986, Goff et al. 1992) and form cell fusions during wound repair (L'Hardy-Halos 1969, Waaland and Cleland 1974, Kim et al. 1988, West et al. 1992). Fusions between certain isolates of *Griffithsia pacifica* Kylin lead to cytoplasmic incompatibility reactions (Koslowsky and Waaland 1984), which involved fusion and lysing of plastids. It is likely that red algae have means of recognizing vegetative cytoplasms similar to somatic compatibility in fungi (Glass and Kuldau 1992). The ability of a parasite cytoplasm to reside in a host cytoplasm suggests that a mechanism exists by which the host recognition response can be circumvented.

The ability of a parasite to grow successfully on its host also rests in its ability to draw nutrients from that host. Any limitations of this nutrient conduit could lead to less vigorous parasite growth and/or death. It has been proposed that physiological changes in infected host cells are important in the transfer of nutrients to the parasite (Goff and Coleman 1985, Zuccarello and West 1994). The inability of the parasite to maintain a compatible interaction was followed at a cytological level. In many resistant isolates of *B. radicans*, host cell cytoplasms fused to parasite cells lost their normal pigment composition, turning from red to green, and became vacuolate, whereas on susceptible hosts infected cells retained their pigmentation, even in nutrient-limited conditions (Zuccarello and West 1994). This was accompanied by changes in the parasite cytoplasm. The dense cytoplasm became extremely vacuolate, and subsequently the parasite cells plasmolysed. The

parasite was apparently unable to induce the proper physiological changes in the host cell that lead to nutrient availability.

In certain infections of *D. bostrychiae* on resistant *B. radicans*, host cells surrounding the infected host cell died, while the infected cell remained viable. Interestingly, only host cells with pit connections to the infected host cell responded in this way. Death of pit-connected but uninfected host cells, and not of other adjacent host cells, indicates that there is some means of communication between cells via the pit connections. The transfer of substances through pit connections has been hypothesized (Wetherbee 1979, Broadwater and Scott 1982), but there is no direct evidence of such transfer.

To determine whether or not the formation of hybrids would affect susceptibility to the parasites (by causing cytoplasmic instability), crosses between two susceptible isolates were made. These F_1 tetrasporophytes remained susceptible to infection. In all crosses of *Bostrychia radicans*, resistance to *Bostrychiocolax australis* was transmitted as a dominant trait in F_1 tetrasporophytes. This mode of transmission is commonly observed in crosses between resistant and susceptible isolates of plant hosts to fungal pathogens (Crute 1981, Vanderplank 1982), although nondominant transmission is also observed (Newton and Crute 1989). Because resistance transmission deviates significantly from a one-to-one ratio in F_1 gametophytes, it may be inferred that resistance genes are not at a single locus. In the host, more than one locus must be involved in resistance to parasite infection; each may act at a different stage of the infection. Crosses of two resistant hosts give rise to progeny that are more resistant than either parent. Resistance genes may be additive in their action.

In the response to infection by *Dawsoniocolax bostrychiae*, most crosses showed dominant transmission of resistance, and, in one cross, the segregation of resistance into F_1 gametophytes deviated significantly from a one-to-one ratio. On the other hand, a cross between two susceptible strains (3040 × 3058) produced resistant F_1 sporophytes. When susceptible isolate 3058 was crossed with resistant isolate 3017, the F_1 sporophyte was susceptible if 3058 was female and 3017 was male but resistant if 3058 was male and 3017 was female. A second cross involving a male 3017 also produced susceptible offspring. For these two isolates, resistance was not transmitted paternally from isolate 3017. The picture was further complicated by two additional crosses in which a paternal 3017 crossed with susceptible females (3040 and 3159G) did confer resistance to the F_1 sporophytes. The possibility exists that a compatibility element ("gene") may be found in an organellar genome and that transmission of this genome is different between different strains. The organellar genome of the male 3017 may have been transmit-

ted in crosses with female 3040 and 3159G but not in others (3058 and 3039).

Flor (1942) was the first to present a model for the genetic interaction between hosts and parasites. The gene-for-gene hypothesis stated that a resistance gene in the host is only effective when it encounters a corresponding pathogenic (avirulence) allele in the parasite. The gene-for-gene hypothesis has been assumed or proposed in many biotrophic interactions (Vanderplank 1982). It assumes that a basic compatibility between a host and a parasite has evolved upon which the interaction between resistant and avirulent genes is superimposed (Heath 1981). These studies involve genetic analysis of both host and parasites. Resistance observed in certain host isolates in this study may be due to genes expressed in the parasite that were recognized by the host. A gene-for-gene interaction can only be confirmed by genetic studies of multiple isolates of the parasite, with varying host ranges, and a corresponding genetic study of the host.

In summary, our results reveal that *Bostrychiocolax australis* had a broader host range than that observed in the field, and this may reflect an underlying phylogenetic relationship between susceptible hosts. Our isolate of *Dawsoniocolax bostrychiae* has a host range that includes only *Bostrychia radicans* and not species from which it has been reported. Within susceptible species, resistant and susceptible host isolates were found worldwide and within the same populations. Resistance was manifested at three main stages in both parasites: during host penetration, during parasite–host cell fusion, and during parasite growth. Parasite resistance was transmitted as a dominant genetic phenotype in most crosses and does not segregate as a single locus. Resistance was also transmitted in a non-mendelian fashion in certain crosses, which may be due to organellar DNA.

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