Proceedings of the Eighth International Symposium on Biological Control of Weeds
2-7 February 1992, Lincoln University, Canterbury, New Zealand.
Delfosse, E.S. and R.R. Scott (eds). DSIR/CSIRO, Melbourne, pp. 437-44 (1995).

Pathogens Attacking Groundsel Bush, *Baccharis halimifolia*, in Florida

R. Charudattan,[1] Uma Verma,[1] J.T. DeValerio,[1] and A.J. Tomley[2]

[1] Plant Pathology Department, University of Florida, Gainesville, FL 32611, USA
[2] Alan Fletcher Research Station, Queensland Department of Lands, Box 36, Sherwood, Queensland 4075, Australia

Groundsel baccharis (*Baccharis halimifolia* L.; Asteraceae) is a serious weed in Queensland and New South Wales, Australia, and in parts of the southwestern United States. It is native to the United States and is abundantly distributed throughout Florida. Through field surveys and pathogenicity screening in Florida, we have identified 3 pathogens: a *Phomopsis* sp., associated with a frequent twig die-back disease; *Puccinia evadens*, a common, macrocyclic, autoecious fungus that causes a foliar and stem rust; and an ascomycete, tentatively identified as *Dothidea puccinioides*, which causes the infrequently seen black pustule disease. In the field, all 3 diseases are associated with mortality of infected branches or the entire plants. Based on field observations, greenhouse determinations of pathogenicity and preliminary host range, and laboratory experimentation, the rust and the black pustule fungi are considered candidates deserving of further study. In inoculation trials, the *Phomopsis* sp. was weakly pathogenic and is therefore not considered a suitable candidate.

Introduction

Groundsel baccharis (= eastern baccharis, groundsel bush, groundsel tree, manglier, sea myrtle; *Baccharis halimifolia* L.; Asteraceae) is a woody perennial shrub or small tree that is native to the United States and is abundantly distributed throughout Florida. It is regarded as an invasive weed in pastures and rangelands of Texas and Louisiana in the United States (Boldt 1989).

It was first recognized as a potential weed in Australia and recorded as being naturalized in 1888 (Bailey 1900). It is a declared pest plant in Queensland and New South Wales, and landholders are legally obliged to control it. Infestations now occur along 1000 km of coastal plain and adjacent hinterland on the mid-eastern coastline of the continent (Tomley 1989). Although smaller infestations are found outside this zone, the plant appears to have reached the limits of its optimum ecological range.

The weed rapidly colonizes weakened native or improved pastures and any other disturbed areas. It also invades undisturbed native plant communities in coastal wetlands, often becoming the dominant substory, and as such it is a serious problem in National Parks. An assessment of its economic impact is complicated by changing patterns of land use, including recent urbanization of considerable areas of its present range. It remains a significant weed problem on land used for grazing purposes. Where these areas interface with zones of human population, conflicts arise over the use of herbicide (2,4-D [(2,4-dichlorophenoxy) acetic acid]), which is the main means of control. In spite of this, a recent survey showed that groundsel bush was still perceived by the majority of people as a weed that should be controlled (Csurhes and Chippendale 1990). This is probably due to its conspicuous appearance, particularly when in flower. Also, the pollen and seed have been blamed for the occurrence of respiratory allergies in humans, although this has not been substantiated by medical tests in Australia (Morrison 1984).

Attempts to find insect biological control agents have spanned 25 years, with 35 separate introductions being made. Of these, only five are permanently established in the field, and further control is needed. Here we report on the first attempts to seek and evaluate plant pathogens as biological control agents for *B. halimifolia*.

The Rust Fungus, *Puccinia evadens*

Identification of the Rust

Three rust species, *Puccinia baccharidis* Diet. and Holw., *P. evadens* Harkness, and *P. pistorica* Arthur have been recorded on *B. halimifolia* and *Baccharis* spp. in Florida herbaria. However, the validity of the Florida records of *P. pistorica* appears questionable to us, and it has been suggested that *P. baccharidis* and *P. evadens* are conspecific (Mordue 1979). Nonetheless, it is clear that *P. evadens* is the most common of the 53 species of rust fungi known on the genus *Baccharis*. The rust specimens we collected were identified as *P. evadens* (Fig. 1) and confirmed by Dr. Joe Hennen, Curator, Arthur Rust Herbarium, Purdue University. Our identification was based on symptomatology and on the morphology of various spore forms of rust samples collected from seven different locations in Florida and one in Georgia.

Life Cycle and Host Damage

Puccinia evadens is a macrocyclic, autoecious rust (Mordue 1979; Verma *et al.*, unpublished): it produces all 5 spore types, namely, basidiospore, pycniospore, aeciospore, urediniospore, and teliospore, and all spore stages are produced on the same host, concurrently or at different times in the year. The rust occurs throughout the year in Florida: generally, pycnia and aecia occur in the spring and uredinia and telia occur in late spring, summer, fall, and winter. The rust incidence is generally low during the summer months and high from December to February. During the latter months, uredinial and telial stages are predominant, but a few aecial galls also form then. Uredinia are succeeded by dark brown telia, about 15-18 d after the uredinia erupt. Commonly, the teliospores geminate *in situ* in telia and produce basidiospores. The latter are also readily produced from germinating teliospores on glass slides. Teliospores germinate either from one or both cells and produce one 4-celled promycelium per cell. Up to 4 basidiospores are produced on each promycelium. The cohabitation of the different spore stages in nature and in greenhouse studies, the frequent presence of pycnia as well as basidiospore-bearing teliospores on plants, and the ready germinability of teliospores and the formation of basidiospores suggest that there is potential for polycyclic infections in nature.

Although good morphological and taxonomic descriptions of *P. evadens* exist (Mordue 1979), details of host damage are lacking. The following narrative is provided to explain the potential impact of this rust as a biological control agent. Pycnia and aecia inflict more damage on the plants than do uredinia and telia. The pycnia occur on the leaves and tender stems, causing swellings on the infected tissue. Usually pycnia are surrounded by aecia; the latter occur on leaves and on tender as well as small woody stems, always causing fusiform swellings, thickening, and malformation of the infected tissue. The uredinia are hypophyllous, scattered, and occur as small, circular, bright yellow to orange colored isolated spots (0.5-1 mm) or coalesce to form bigger pustules, mainly on the abaxial leaf surfaces and less frequently on tender shoots. The uredinia are surrounded by chlorotic regions and not swollen tissue. The telia are also pustulate, scattered or confluent, and similar to uredinia in host damage.

Experimental Work

Preliminary inoculation trials were made using aeciospores, urediniospores, or teliospores as inoculum. Upon successful infection, the former 2 spore types produced uredinia and the latter gave rise to pycnia (presumably via basidiospore formation and subsequent infection by the basidiospores). Further attempts were made with freshly collected urediniospores suspended in deionized water. About 0.1 ml of a spore suspension (*ca.* 900 spores/cm^2 of leaf

surface or 10^5/ml of inoculum) was uniformly applied to the abaxial surface of leaves. The number of pustules per leaf and the percentage of leaf area covered with rust pustules were determined for 10 inoculated leaves from one or more plants. In separate studies, a humectant (0.5% Metamucil®, a *Psyllium* hydrophilic mucilloid; Proctor & Gamble, Cincinnati, OH) was added to the inoculum suspension, the humectant was allowed to swell, and the inoculum was applied with a brush to the leaf surfaces. Also, urediniospore suspensions were applied to the leaf surfaces without the humectant and the inoculated plants were incubated in an atmosphere containing 200 ppm nonyl alcohol, a spore-germination stimulant. In the 2 latter inoculations, plants were incubated at 30°C and 100% RH for 24 h in a dew chamber. Finally, dry spores or spore suspensions were also tested using an incubation regimen of 20°C and 100% RH for 48 h. After inoculation and incubation in the dew chamber, the plants were transferred to greenhouse benches.

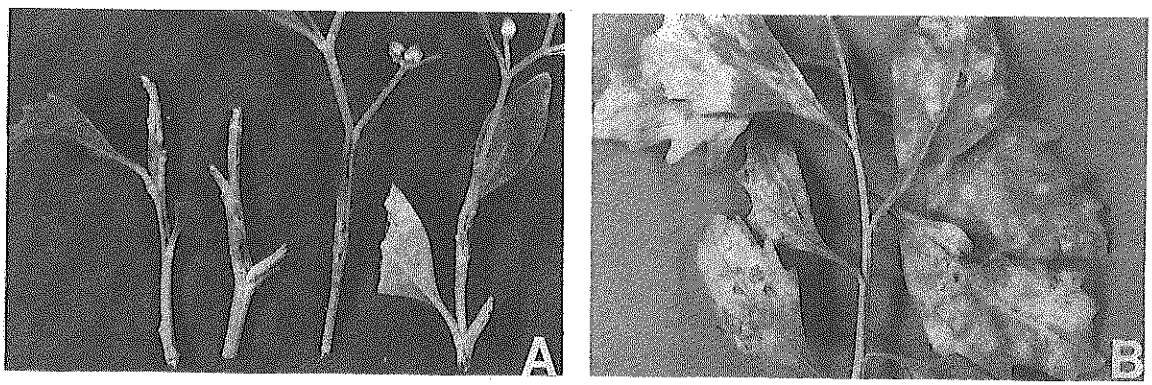

Figure 1. The rust fungus *Puccinia evadens*. A) Pycnial and aecial galls on *Baccharis halimifolia* twigs. B) Undersides of leaves covered with aecial and uredinial sori.

Small, pin-head-sized circular chlorotic spots developed on the adaxial leaf surface 11-12 d after inoculation. After 18-20 d, bright yellow uredinia were seen on the abaxial surface; these uredinia erupted after one month. In some uredinia, teliospores were formed 15-18 d after uredinial formation. The application of dry urediniospores on moist leaf surfaces as well as the use of urediniospore suspension were equally effective in inducing infection, resulting in an average of 7.4 and 7.6 rust pustules per leaf, respectively. Higher inoculum level (3×10^5 spores/ml) resulted in higher levels of leaf infection or average pustule numbers per leaf (10/leaf) as compared to lower inoculum level (4.8 – 7.6 pustules/leaf; 9.8×10^4 spores/ml). The use of 0.5% Metamucil® with urediniospores did not promote infection (20% incidence of leaf infection with the humectant compared to 19.2% without). In a spore-germination study, an average of 17.7% urediniospores in 0.5% the humectant suspension germinated after a 24-h incubation, compared to 19.3% in the control water suspension. In separate inoculations, aeciospores produced an average of 28.6 pustules per leaf (up to 50% incidence of leaf infection), whereas urediniospores produced an average of 12.6 pustules per leaf (up to 30% incidence). Inoculations with teliospores (2.5×10^4 spore/ml) gave rise to chlorotic spots with pycnia 15 d after inoculation; however, no aecia were formed later.

Preliminary Host Range Studies

The plants listed in Table 1 were tested against *P. evadens* for a preliminary assessment of host range. Twenty-two-day-old seedlings (except *Baccharis* spp. and *Olearia nernstii*) were used. Greenhouse-grown, pot-bound *B. halimifolia* plants (20-month-old in the first trial and 60-d-

old in the second) and 2- to 3-month-old plants of *Baccharis* spp. and *O. nernstii*, raised from rooted cuttings of plants from Texas (*Baccharis*) and Australia (*O. nernstii*) were included. Depending on plant size and species, 1-5 plants per pot and 4 replicate pots per host type were used, and the host-range trials were repeated thrice. Inoculated plants were incubated at 20°C and 100% RH for 48 h and returned to the greenhouse. Plants were monitored for 2 months and rated as indicated in Table 1. The average number of pustules per leaf was also determined from 5 infected leaves per plant.

Table 1. A preliminary assessment of the host-range of *Puccinia evadens*.

Plants inoculated	Infection type (0-4) [1]	Average number of pustules/leaf
Baccharis halimifolia (Florida biotype)	4	7.7
B. halimifolia (Australia biotype)	4	8.9
B. neglecta	4	4.9
B. pilularis	0	0
B. salicifolia	0	0
B. salicina	4	14.6
B. sarothroides	4	5.7
Dahlia pinnata sp. (*cv.* Rainbow Mix)	0	0
Callistephus chinensis (*cv.* Totempole)	0	0
C. chinensis (*cv.* Dwarf Border)	0	0
Helianthus annuus (*cv.* Mammoth)	0	0
H. annuus (*cv.* Giant Grey Stripe)	0	0
Olearia nernstii	0	0

[1] 0 = Immune, nearly immune, or hypersensitive; no uredinia; 1 = resistant; minute uredinia surrounded by necrosis; 2 = moderately resistant; small to medium-sized uredinia; 3 = moderately susceptible; medium-sized uredinia; no necrosis; some chlorosis; 4 = very susceptible; large uredinia, often coalescing; no necrosis; some chlorosis.

The results confirmed that both the Australian and Floridian biotypes of *B. halimifolia* were susceptible to the rust; no differences could be detected in the level of susceptibility of these 2 biotypes to the rust (Table 1). Also, *Baccharis neglecta, B. salicina*, and *B. sarothroides*, were susceptible. These, recognized as distinct species (Kartesz and Kartesz 1980, Nesom 1990), belong to 2 infrageneric sections, *Baccharis* (*B. halimifolia, B. neglecta*, and *B. salicina*) and *Sergilae* (*B. sarothroides*) (Nesom 1990). None of the other tested hosts in Asteraceae was susceptible.

Baccharis salicina and *B. sarothroides* have been previously recorded as hosts to this rust (Mordue 1979); our report herein of the susceptibility of *B. neglecta* appears to the first record for this host-rust combination. In addition to *B. halimifolia, B. salicina,* and *B. sarothroides,* the rust has been reported on *B. articulata, B. consanguinea, B. dracunculoides, B. floribunda, B. pilularis, B. platypoda, B. rosmarinifolia,* and *B. thesioides* (Mordue 1979). Of these hosts, we screened only *B. pilularis* but no infections resulted. Since this finding is inconsistent with the known occurrence of *P. evadens* on this host in California (Farr *et al.* 1989) and elsewhere (Mordue 1979), we will endeavor to confirm our results and explain the reason for this discrepancy.

The Black Pustule Fungus, *Dothidea puccinioides*

Causal Organism

The causal agent of the black pustule disease on *B. halimifolia* has been described as *Dothidea puccinioides* Fr. (= *Systremma baccharidis* [Cke.] Th. & Syd.) of the Order Dothideales, Class Loculoascomycetes, Subdivision Ascomycotina (Loeffler 1957). However, there is no definitive etiological study of the disease caused by *D. puccinioides.*

Because the taxonomy of the fungus is unclear, we are tentatively placing it in *D. puccinioides sensu* Loeffler (Loeffler 1957).

Symptoms

The disease is characterized by striking foliar black pustules consisting of leathery, shiny black, and raised (0.25-1 mm) stroma over the adaxial leaf surface (Fig. 2). Less noticeably, the pustules also occur on petioles of very young leaves. They are discrete or in groups of 2 to 5. The symptoms suggest that the infection is systemic although the disease may not always be expressed in 100% of the foliage. On the infected plant, the foliar spots appear soon after the leaves unfold from the bud. The infected leaves are attenuated, deformed, and slightly curled. Sometimes 90-100% of the leaf surface is covered with pustules, and young growing branches develop mild symptoms of witches' broom. The symptoms are persistent throughout the year; infected plants often became completely defoliated, although healthy regrowth may occur in some infected plants.

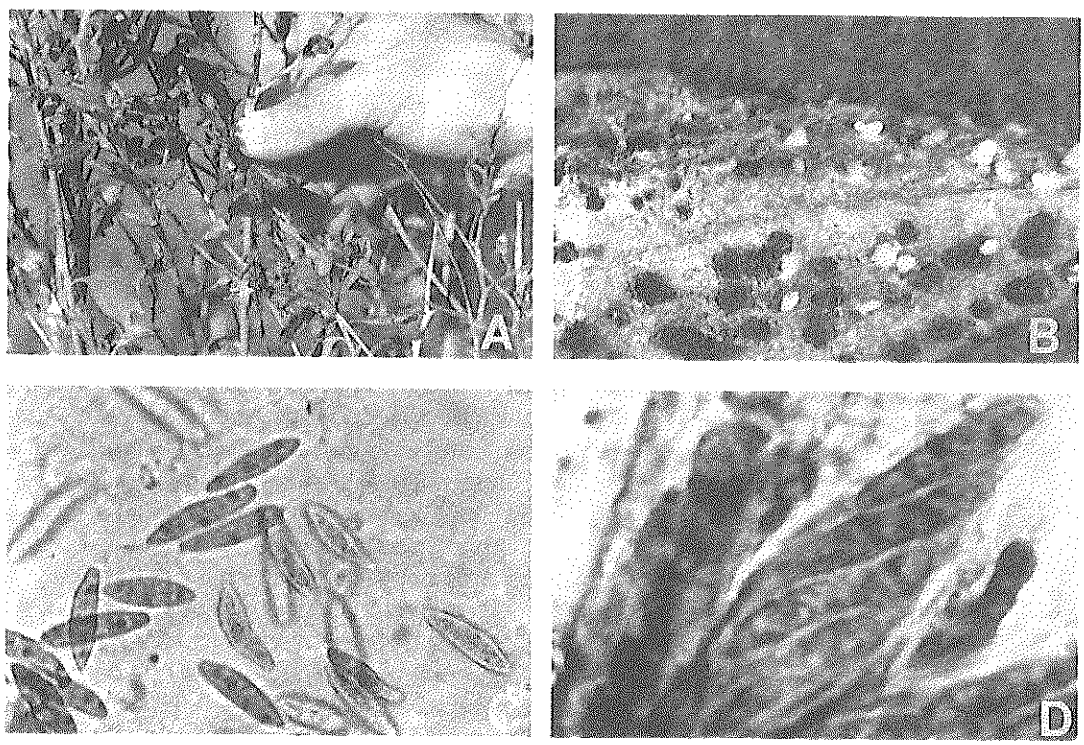

Figure 2. The black pustule disease caused by *Dothidea puccinioides*. A) Diseased shoots. B) Black, pustulate pycnidiostromata with white masses of dried conidia. C) Hyaline conidia. D) Asci with ascospores.

The effect of the black pustule disease on host mortality was studied in the field by tagging individual infected plants. Of the 25 plants (ranging in size from 1-5 m tall) kept under observation in 3 widely separated counties of Florida, 5 died within 3-19 months after we first recorded them as being infected with the disease. Nine of the remaining plants are in a state of decline with about 20-80% of their branches dead or dying. Therefore, it appears that the black pustule disease is lethal to *B. halimifolia*, suggesting that the pathogen *D. puccinioides* is an effective biological control agent.

Morphology and Taxonomy of the Fungus

Two types of spore are associated with the black pustule fungus: the asexual conidia (the anamorph) and the sexual ascospores (the teleomorph) (Fig. 2). The conidial stage occurs in field-collected and greenhouse-inoculated specimens as white incrustation on the black stromatic tissue that forms the black pustule. The incrustation consists of the conidia which are apparently exuded from the stroma and dry *in situ*. The conidia are smooth, hyaline, cigar-shaped with pointed or round ends, sometimes slightly curved, and 1-5 (usually 2) septate. Size: 17.60-39.60 x 4.4-8.8 (avg. 28.60 x 8.51 µm); mature spores are light brown. We have *tentatively* assigned the conidial stage to the genus *Dothistroma*.

The foliar pustules in some field-collected specimens yield both the asexual hyaline conidia and the sexual ascospores. The ascostromata, which are indistinguishable from the pycnidiostromata, are scattered, nearly round (0.5-1 mm dia), raised, black, erumpant, parenchymatous, and multilocular. Each stroma contains a number of ascigerous locules that are obovoid or elliptic (88-176 µm broad and 66-132 µm high; avg. 106 x 91 µm). The asci arise from a basal mass of hyaline parenchyma, are erect, cylindric, clavate, bitunicate, thick-walled, and broader near the middle. They are 4-8 spored, usually 6 spored, and aparaphysate. Size: 55-121 x 8.8-18.7 (avg. 79.30 x 12.65) µm. Ascospores are biseriate, often overlapping, hyaline when young, and turning pale brown when mature. They are 2-celled, smooth, ellipsoid, and straight, with obtuse ends and a slightly constricted middle. Size: 15.4-31.9 x 5.5-8.8 (avg. 24.64 x 7.11) µm and covered with a gelatinous or mucilaginous material. We have *tentatively* placed this ascospore stage under *Dothidea puccinioides*.

Inoculation Trials

To establish proof of pathogenicity and to evaluate the infectivity and virulence of the 2 spore forms, a number of inoculation trials were carried out as follows on greenhouse-grown *B. halimifolia* plants from Florida and Australia. Leaves and twigs of field-collected *B. halimifolia* with black pustules were crushed in water and a strained suspension containing fungal cells was sprayed on plants and injected into young stems. A suspension of hyaline, asexual spores (1.5×10^4 spores/ml) was applied with a brush on the adaxial and abaxial surfaces of leaves and on young tender stems. The inoculated plants were incubated in a dew chamber at 28°C and 100% RH for 48 h before moving them to a greenhouse at 25±5°C. Under these conditions, the black pustule symptoms appeared in 60-90 d after inoculation.

In addition, a suspension of fungal cells from freshly crushed, infected leaves + 0.5% Triton X-100; hyaline spores (5×10^5) scraped from fresh, black-pustule-infected leaves; and crushed, infected leaves with pycnidia were tried as inocula. These inocula were applied with a brush. Also, suspensions containing fungal mycelia and spores were sprayed as well as injected into the plants. The plants were incubated at 20°C for 48 h at 100% RH in a dew chamber and then kept in the greenhouse at 25±5°C.

Trials with conidia collected off the infected tissue were successful and produced black pustules in about one month after inoculation. Infected leaves from these plants produced black pustules which yielded the hyaline conidia comparable to those used as the inoculum. Fungal cultures obtained from inoculated diseased leaves were slow-growing and morphologically identical to the single-spore cultures derived from the hyaline spores.

Inoculum consisting of a suspension of the *Dothidea*-type ascospores (5.75×10^4 spores/ml) containing the hyaline spores (4.7×10^3/ml) as a contaminant + 0.5% Triton X-100 was applied to *B. halimifolia* leaves. In another trial, the *Dothidea*-type ascospore suspension (4×10^5/ml) containing 0.5% Triton X-100 was applied to the leaves and injected into young stems. The plants were incubated at 25°C or 48 h at 100% RH in a dew chamber and then kept in a greenhouse (25±5°C) for subsequent observations. So far none of the ascospore inoculations have yielded infections.

Host-Range Studies

The hyaline conidia, which were infective and produced the black pustule disease in

greenhouse trials, were used as inoculum for a preliminary determination of the host range against the plants listed in Table 2. The plants were grown as described under *P. evadens.* Twenty-five grams of infected leaves were crushed in 100 ml water and the suspension was strained. The filtrate containing fungal cells, pycnidia, and spores was centrifuged twice and the pellet was resuspended in 10 ml of deionized water and used as inoculum consisting of 1.94×10^5 (first trial) or 2.65×10^5 (second trial) spores/ml. Triton X-100 at 0.05% was used as a surfactant, and the inoculum was applied with a brush on both leaf surfaces of the different plants. Control plants received similar treatment except that only Triton X-100 (without spores) was applied. Inoculated plants were incubated at 25°C for 48 h at 100% RH in a dew chamber and then removed to a greenhouse.

Table 2. A preliminary assessment of the host range of *Dothidea puccinioides.*

Plants inoculated	Infection[1]
Baccharis halimifolia (Florida biotype)	+
B. halimifolia (Australia biotype)	+
B. neglecta	–
B. pilularis	+
B. salicifolia	–
B. salicina	–
B. sarothroides	+
Dahlia pinnata (*cv.* Rainbow Mix)	–
Callistephus chinensis (*cv.* Totempole)	–
C. chinensis (*cv.* Dwarf Border)	–
Helianthus annuus (*cv.* Mammoth)	–
H. annuus (*cv.* Giant Grey Stripe)	–
Olearia nernstii	–

[1] + = Development of black pustule disease in about 4-8 wk after spraying with conidial inoculum;
– = No disease developed.

Both the Australian and Floridian biotypes of *B. halimifolia* became infected and the symptoms appeared 6 and 4 wk after inoculations in the 2 trials. In addition, *B. pilularis* (section *Baccharis*) and *B. sarothroides* (section *Sergilae*) also developed black pustule symptoms 4 wk after inoculation. Both these species appear to be new host records for *D. puccinioides.* None of the other Asteraceae plants tested became infected.

In summary, the black pustule disease, described as being caused by *Dothidea puccinioides*, has an anamorph with hyaline pycnidioconidia (Loeffler 1957). In inoculation trials, spores of this anamorph were infective and caused the typical black pustule disease. The spores of the teleomorph were seen in some field-collected disease specimens. Pycnidial stroma and ascostroma were also found on the foliage, sometimes simultaneously on the same leaf. Cultures obtained from these two spore types were slow growing, and sporulation could not be induced in any of these cultures.

Phomopsis sp. Associated with Twig Die-back

A *Phomopsis* sp. has been consistently isolated from *B. halimifolia* plants with twig die-back, which is a very prevalent disease of this plant in Florida. Several pure cultures of this *Phomopsis* are in our collection; but to date none of our attempts to initiate the die-back disease under controlled conditions has succeeded. Efforts are continuing, but because of the lack of confirmation of pathogenicity and the apparent weak parasitism, this fungus is not considered a suitable biological control agent for groundsel bush.

Conclusions

The literature on the life cycle, taxonomy, and pathology of *P. evadens* is substantially complete. In addition, our host range results, although preliminary, suggest that this fungus is likely to be restricted to the genus *Baccharis.* Hence, we believe that after further comprehensive host range testing this fungus could be considered in the near future for introduction into Australian quarantine for the mandatory prerelease safety assessment. Data to support an application for importation of this fungus are being assembled.

More information must be gathered on the taxonomy of *D. puccinioides* and the etiology and epidemiology of the black pustule disease. Evidence on hand suggests that this pathogen is likely to be an effective biological control agent. At this time we do not have sufficient information to suggest whether this fungus might be host-specific to the genus *Baccharis*. Further studies on these 2 fungi are warranted and justified.

Acknowledgments

This research was supported by a grant to R. Charudattan, University of Florida, provided by the Queensland Department of Lands, Alan Fletcher Research Station, Sherwood. We thank Mr. Paul Boldt, USDA-ARS, Temple, Texas for providing several *Baccharis* spp. used in the host range studies. Florida Agricultural Experiment Station Journal Series No. N-00628.

References

Bailey, F.M. 1900. *The Queensland Flora 3.* A. Diddams and Company, New York, p. 813-4.

Boldt, P.E. 1989. *Baccharis (Asteraceae), A Review of Its Taxonomy, Phytochemistry, Ecology, Economic Status, Natural Enemies and the Potential for Its Biological Control in the United States.* MP 1674, Texas Agriculture Experiment Station, Texas A & M University, College Station, 32 p.

Csurhes, S.M. and J.F. Chippendale. 1990. *An Assessment of the Significance of Groundsel as a Woody Weed in Queensland.* Queensland Department of Lands Unpublished Report.

Farr, D.F., G.F. Bills, G.P. Chamuris, and A.V. Rossman. 1989. *Fungi on Plants and Plant Products in the United States.* APS Press, St. Paul, MN, 1252 p.

Kartesz, J.T. and R. Kartesz. 1980. *A Synchronized Checklist of the Vascular Flora of the United States, Canada, and Greenland. Volume 2, The Biota of North America.* The University North Carolina Press, Chapel Hill, 498 p.

Loeffler, W. 1957. Untersuchungen uber die Ascomyceten-Gattung *Dothidea* Fr. *Phytopathol. Z.* **30**:349-86.

Mordue, J.E.M. 1979. *Puccinia evadens. CMI Description of Pathogenic Fungi and Bacteria.* No. 620. Commonwealth. Mycological. Inst., Commonwealth. Agricultural. Bureaux, Surrey, England, 2 p.

Morrison, I.C. 1984. The changing face of pollen allergy in Queensland. *Medical Journal of Australia 1984 - Special Supplement, Sept. 1, Allergy in Australia; Symptoms Diagnosis and Treatment*, Baldo, B.A. and C.W. Wrigley (eds.).

Nesom, G.L. 1990. Infrageneric taxonomy of North and Central American *Baccharis* (Asteraceae; Asterae). *Phytologia* **69**:40-6.

Tomley, A.J. 1989. Biological Control of Groundsel bush. *Paper presented at the 5th Biennial Noxious Plants Conference*, Lismore, Australia, 73-5.