Hosts of Synchytrium australe Speg.

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Synchytrium is the largest of the chytrid genera and includes at present more than 150 reported species, all of which are parasites of algae, mosses, ferns and flowering plants. Ever since the genus was established by De Bary and Woronin in 1863 most investigators have had a tendency to create new species for each host infected in nature. This has been particularly evident in the last decade during which more than 50 new species have been created primarily on this basis. This tendency is further reflected in the literature reports that of all known species, 114 have been recorded on only one host. As the author (1954 A) pointed out previously only eight species have been studied to determine their host range and host specificity, and of these only four have been investigated intensively. Since host, host range, and host specificity have been used as criteria for identification and classification it is obvious that intensive studies along these lines are essential for all species of *Synchytrium* which appear to be morphologically similar and occur on the same or different hosts. The author's (1954 D) host range study of *S. aecidioides* indicates how significant such investigations may be in relation to species which have been reported on several different hosts.

The present study concerns the host range of *Synchytrium australe* which Spegazzini (1881) and Cook (1945) reported as a parasite of *Modiola caroliniana (M. prostrata)* in Argentina and Louisiana, respectively. Cook described it as a new species, *S. modioliensis,* but the author (1954 C) believes that Cook's species is identical to *S. australe.* This species and its host were collected at Baton Rouge, La. by the author in March, 1953 and 1954, and transferred to the greenhouses at Purdue University where both have flourished remarkably well. During the spring and summer of these years, plants and seeds of various wild and cultivated malvaceous

*) This study has been supported by a grant from the National Science Foundation. — The author is very grateful to Drs. A. T. Guard. Purdue University, John J. Sperry and M. D. Whitehead, Texas A. & M. College, C. A. Brown, Luisiana State University, and Mr. B. T. Lingappa for identification of the host plants and collections of seeds.

species were collected and grown in the greenhouses for use in determining the host range and host specificity of *S. australe.*

In making these cross inoculations it was soon found that a healthy and viable inoculum of sporangia and zoospores had to be maintained constantly. This was accomplished by periodically inoculating cotyledons and the first leaves of seedlings of *Modiola caroliniana, Althaea rosea, Hibiscus esculentus* and *Malva rotundifolia* which are highly susceptible. This insured a constant and fresh supply of inoculum. The technique and procedure employed in testing the susceptibility of various plants were tested first on *M. caroliniana* to determine their effectiveness. If infection occurred on this host the methods were assumed to be effective. As a further check the inoculum was examined microscopically to insure the presence of healthy sporangia and their ability to produce viable zoospores. As far as was possible emerging leaves of mature plants as well as cotyledons and first leaves of seedlings were inoculated for each species tested. As soon as the leaves emerged they were wetted thoroughly, and a heavily infected piece of leaf with healthy sporangia was inserted between them. The leaves and the inoculum were swathed in a tuft of water-absorbent cotton, and the plants were then covered with a bell jar to maintain a moist environment. Care was taken to prevent the leaves and inoculum from becoming water-logged. After the first week examinations were made every two days over a period of several weeks to determine whether or not infection and gall development had occurred. In doubtful cases the inoculated leaves were removed and examined under the low power stereoscopic microscope. Also, free hand sections were made and studied as an adjunct to the macroscopic examinations.

The results of these inoculations are shown in table 1 which relates to inoculated emerging leaves of grown plants, and cotyledons and first leaves of seedlings.

It is to be noted in table 1 that the results of inoculating emerging leaves of grown plants are negative except for *Althaea rosea, Hibiscus esculentus, Malva rotundifolia, Modiola caroliniana, Gossypium herbaceum, G. arboreum, Impatiens sultana, Circaea latifolia* and *Oenothera biennis* var. *Winslow.* In the last five species named, however, only a few aborted galls were found. Infection of cotyledons and the first leaves of seedlings, on the other hand, was common and abundant. As shown in the second column of table 1 seedlings of 10 species in 6 genera of the Malvaceae besides *Modiola caroliniana* were infected. These results confirm the common observations of investigators in the field that infection by *Synchytrium* occurs usually at the seedling stage of the respective hosts.

Only three non-malvaceous species, *Circaea latifolia, Impatiens sultana* and *Oenothera biennis* var. *Winslow* were infected, but the few

galls present had aborted in the early stages of development. On *Oenothera biennis* var. *Winslow* the infections occurred primarily at the base of the trichomes, and the resulting galls were greatly elongate, conical or columnar in shape. Apparently, these non-malvaceous species are weakly susceptible to infection but resistant to the development of the parasite. In the family Malvaceae, *Modiola caroliniana, Hibiscus esculentus, Althaea rosea, A. officinalis,* and *Malva rotundifolia* were the most susceptible to infection and development of the parasite under the conditions of these experiments, but on some seedlings of *A. rosea* and *H. esculentus* as high as 20 and 8 per cent, respectively, of the galls and parasites aborted, as was noted previously by the author (1954 B). Accordingly, these two species appear to be highly susceptible to infection but more resistant to the development of the parasite. *Gossypium herbaceum, G. arboreum, Hibiscus syriacus* and *Thespesia populnea,* on the other hand, appear to be weakly susceptible to infection and also resistant to the development of the parasites. All tested species of *Sida* except *S. carpinifolia* were resistant both in the seedling and mature stages under the conditions of these experiments, but later *S. cordifolia* was found to be weakly susceptible under flooded conditions.

Particularly significant was the lack of infection of *Callirrhoe involucrata* because *S. australe* was reported and collected on this host by Jennings (1890) and Bartholomew (1902, 1927) in Texas and Kansas, respectively, according to herbarium specimens distributed by them. The author has examined their specimens, and these appear to be morphologically similar to *S. australe* on *Modiola caroliniana.* In light of the results obtained in the above inoculations it seems quite possible that biological races of *S. australe* exist in nature, and that the one on *Modiola* will not infect *C. involucrata.* On the other hand, they might prove to be distinct species. The author has searched for the species on the latter host in Texas where Jennings collected it but has not yet found it.

Whether or not susceptibility and resistance of these various hosts to infection is associated with differences in karyotype and chromosome number is not certain. As Skovsted (1935) has shown the basic number of chromosomes vary in *Abutelon, Althaea, Malva, Callirrhoe, Sida, Urena, Hibiscus, Thespesia* and *Gossypium,* and in only 15 of the malvaceous hosts used by the author are the chromosome numbers known.

The leaves of many of the malvaceous species used in these tests are covered with numerous trichomes and because of this it is difficult ordinarily to moisten or wet the surface of the leaves thoroughly. It was believed that this might have a direct bearing on the lack of infection of some of the hosts. Accordingly, the cotyledons and

leaves of seedlings and emerging leaves of grown plants were washed with a 0.5% solution of a wetting agent, "Tween 80" and then rinsed with distilled water to remove the detergent. Immediately afterwards they were inoculated in the manner described for the previous experiments. The results obtained were largely negative. The non-susceptible species remained resistant, and only in the case of *H. syriacus, H. trioneum, Gossypium herbaceum* and *H. arboreum* were the aborted galls slightly more numerous than in the previous tests.

Synchytrium australe is difficult to maintain on *Modiola caroliniana* and other highly susceptible hosts in the greenhouse during the hot months of July, August and September by the inoculation methods described above. *Modiola caroliniana* appears to become partially dormant during these months, and it was very difficult to infect the young leaves although the plants were kept in pots in a shallow cement trough or bench whose bottom was occasionally flooded with water. However, it was noted that young leaves on creeping stems which had grown into the flooded bench became heavily infected during the summer. On such leaves apparently *S. australe* maintained itself throughout the hot summer months. When the bench was flooded periodically zoospores, apparently, were discharged from mature sporangia and infected the leaves as they emerged. This observation led to another inoculation experiment involving periodically flooded or submerged seedlings.

Seeds of *Abutelon indicum* (5), *A. graveolens* (5), *Callirrhoe involucrata* (4), *Hibiscus syriacus* (12), *H. trioneum* (18), *Modiola caroliniana* (20), *Sida cordifolia* (43), *S. rhombifolia* (12) and *S. spinosa* (10) were germinated on filter paper in petri dishes. As the cotyledons emerged from the seed coats a heavily infected leaf of *M. caroliniana* was placed in each dish, and it as well as the seedlings were flooded with $^1/_4$' of non-chlorinated tap water. The dishes were allowed to stand in the laboratory 24 hours after which the roots of the seedlings were covered with soil. After two weeks the seedlings were examined carefully under the stereoscopic microscope for infection.

The results were negative except for 18 seedlings of *Modiola caroliniana* and 7 of *Sida cordifolia.* The cotyledons of *M. caroliniana* were heavily infected whereas those of *S. cordifolia* had only very few aborted and mature galls. In two cases only one gall was found on each seedling.

This experiment was repeated with approximately the same number of seedlings except that the dishes of seedlings were placed in the greenhouse bench next to heavily infected creeping stems of

*) The number in parentheses after each species indicates the number of seeds which germinated and were exposed to the inoculum.

Hosts	Emerging Leaves of Mature Plants			Cotyledons and First Leaves of Seedlings		
	No. Plants Inoculated	Results		No. Plants Inoculated	Results	
Malvaceae						
Abutilon theofrastii	12	0	12—	24	6	18—
A. graveolens				2	0	2—
A. indicum				2	0	2—
Althaea officinalis	15	0	15—	100	82	18—
A. rosea	20	16	4—	100	92	8—
Callirrhoe involucrata	8	0	8—	14	0	14—
Gossypium herbaceum	15	Few aborted galls		15	Numerous aborted galls	
G. arboreum	15	Few aborted galls		15	Few aborted galls	
Hibiscus esculentus	15	4	11—	60	40	20—
H. lasiocarpus				12	0	12—
H. militaris	12	0	12—	12	0	12 —
H. moschatus	8	0	8—	8	0	8—
H. palustris	8	0	8—	8	0	8—
H. sabdariffa				5	0	5—
H. syriacus	15	0	15—	30		
H. trioneum	15	0	15—	30	Few aborted galls	
H. aculeatus	10	0	10—		Few aborted galls	
Kosteletzkya virginica				12	0	12—
Malva rotundifolia	10	8	2—	30	30	0—
Sida ascuta				4	0	4—
S. carpinifolia				12	4	8—
S. cordifolia	12	0	12—	12	0	12—
S. hastata	30	0	30—	30	0	30—
S. indica	20	0	20—	20	0	20—
S. rhombifolia	20	0	20—	20	0	20—
S. spinosa	30	0	30—	30	0	30—
Thespesia populnea	4	0	4—	4	Few aborted galls	
Urena lobata				12	0	12—
Amaranthaceae						
Amaranthus retroflexus	4	0	4—			
Balsaminaceae						
Impatiens sultana	4	Few aborted galls				
Bignoniaceae						
Bignonia sp.	4	0	4—			
Chenopodiaceae						
Chenopodium album	5	0	5—			
Euphorbiaceae						
Ricinus communis	5	0	5—	5	0	5—
Geraniaceae						
Geranium carolinianum	12	0	12—	12	0	12—
G. maculatum	12	0	12—	12	0	12—
G. pusillum	12	0	12—	12	0	12—
Labiatae						
Monardia fistulosa	12	0	12—	12	0	12—
Leguminosae						
Amphicarpaea bracteata	12	0	12—	12	0	12—
Crotalaria stricta	12	0	12—	12	0	12—
Pueraria thunbergiana	12	0	12—	12	0	12—
Onagraceae						
Circaea latifolia	6	Few aborted galls				
Oenothera biennis var. Williamsburg	5	0	5—			

Hosts	Emerging Leaves of Mature Plants			Cotyledons and First Leaves of Seedlings		
	No. Plants Inoculated	Results		No. Plants Inoculated	Results	
O. biennis var. Winslow	5	Few aborted galls				
Rosaceae						
Potentilla recta	4	0	4—			
P. canadensis	4	0	4—			
Duchesnia indica				5	0	5—
Salicaceae						
Populus deltoides	3	0	3—			
Solanaceae						
Petunia axillaris	5	0	5—			
Umbelliferae						
Hydrocotyle canbyi	12	0	12—			
H. umbellata	12	0	12—			
Urticaceae						
Urtica dioica	12	0	12—			
U. chamaedryoides	12	0	12—			
Vitaceae						
Parthenocissus quinquefolia	4	0	4—			

Table 1 showing results of inoculating emerging leaves of mature plants and cotyledons and first leaves of seedlings with *Synchytrium australe.*

M. caroliniana. These and the entire bench were flooded to a depth of three inches four times during the course of three weeks. Again, the results were negative except for 8 out of 12 seedlings of *M. caroliniana* and 2 out of 9 seedlings of *S. cordifolia.*

In this connection it may be noted that *Dichondra repens, Duchesnea indica, Lythrum lanceolatum* and *Rubus trivealis* spread from their pots onto the bed of soil among the infected creeping stems of *Modiola caroliniana.* Also, seedlings of *Stellaria media, Cerastium vulgatum, Oxalis repens, Lepidium campestre* and *Linaria vulgaris* grew up in the bed, and these were flooded at the same time so that they had excellent opportunities for infection by zoospores. However, over the course of two years none of them became infected.

It is apparent from these inoculations that *S. australe* from Louisiana will infect a large number of malvaceous species besides *Modiola caroliniana* under greenhouse experimental conditions. However, with the possible exception of *Callirrhoe involucrata* noted above, it has not been reported on any of these additional hosts in nature. It is possible that if such hosts grew in close proximity to heavily infected *M. caroliniana* plants in nature the susceptible ones would become infected. However, of these hosts only *Callirrhoe involucrata* and *Malva rotundifolia* are prostrate and creeping species which grow close to the ground where moisture conditions are most favorable for infection.

Summary.

Synchytrium australe Speg., which occurs as a parasite on *Modiola caroliniana* in nature, was tested on 28 malvaceous and 24 non-malvaceous hosts in greenhouse inoculation experiments. Twelve of the malvaceous species, including *Abutelon theofrastii, Althaea officinalis, A. rosea, Gossypium herbaceum, G. arboreum, Hibiscus esculentus, H. syriacus, H. trioneum, Malva rotundifolia, Sida cordifolia, S. carpinifolia* and *Thespesia populnea,* were infected under these conditions. However, only aborted galls were formed on *Gossypium herbaceum, G. arboreum, Hibiscus syriacus, H. trioneum* and *Thespesia populnea.* Of the nonmalvaceous hosts tested only *Circaea latifolia, Impatiens sultana,* and *Oenothera biennis* var. *Winslow* were infected, but only aborted galls developed on these hosts.

Infections occurred more commonly on cotyledons and the first leaves of seedlings than on emerging leaves of mature plants. Except for *Althaea rosea, Gossypium herbaceum, G. arboreum, Hibisculus esculentus* and *Malva rotundifolia* no infections occurred on emerging leaves of mature plants. Although *Synchytrium australe* will infect a large number of malvaceous species under greenhouse conditions it has not been reported on any of these hosts except *Modiola caroliniana* and *Callirrhoe involucrata* in nature.

References.

Bartholomew, E., 1927. The fungus flora of Kansas. Spec. Res. Bull. Agric. Exp. Sta. Kansas State Agric. Coll. pp. 5—46.

Cook, M. T., 1945. Species of *Synchytrium* in Louisiana. I. Description of species found in the vicinity of Baton Rouge. Mycologia **37**: 284—294.

De Bary, A. and M. Woronin, 1863. Beitrag zur Kenntnis der Chytriden. Ber. Nat. Gesell. Freiburg **3**: 22—61.

Karling, J. S., 1954a. Host reaction, host-parasite relationship, hosts and taxonomic criteria in *Synchytrium.* Mycologia **46**: 293—313.

— 1954b. *Synchytrium australe* Spegazzini and *Synchytrium modioliensis* Cook. Ibid. 46: 529—533.

— 1954c. The cytology of host reaction to infection by *Synchytrium australe.* Amer. Jour. Bot. 41: 651—663.

— 1954d. Host range of *Synchytrium aecidioides.* Sydowia 8: 355—359.

Skovsted, A., 1935. Chromosome numbers in the Malvaceae. Jour. Genetics 31: 263—296.

Spegazzini, C., 1881. Fungi Argentini, pt. 4. Ann. Soc. Cient. Argentina 11: 1—38.