SUNFLOWER DISEASE RESEARCH AT MACDONALD COLLEGE

By

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Rust

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Inheritance of pathogenicity.

The work done by Miah for his Ph.D. showed that inheritance of resistance to rust in sunflowers, and of pathogenicity in the rust, were comples, and difficult to study (Miah and Sackston 1970a, 1970b). This work is being continued by Mr. Hennessy, who finds the problem as involved as Miah indicated.

Hennessy has worked out a method for studying the genetics of sunflower rust on detached leaves, and of inducing germination of teliospores with reasonable ease (Hennessy and Sackston 1970). Like Miah, he has found that avirulence tends to be dominant on the differential variety CM90RR - but recessive in some cases - and inheritance is 'maternal' on the differential "Cross 29".

Hennessy has found a yellow mutant in a collection of race 2. Its pathogenicity was the same as that of the original collection. The yellow color appeared to be recessive to brown in crosses with the parent race. Populations in F^2 are too limited to permit many definite conclusions. It is already apparent, however, that segregation occurs within individual aecial cups, and that the unit of inheritance is the aeciospore. The number of cups per cluster has been too small to permit calculating reliable segregation ratios. Single pustule uredial cultures of the F_2 hybrids continued to segregate for four uredial generations. Germination and infection success with single spores has been low, so few single-spore cultures have been established. Segregation for spore color has, however, been observed in single spore cultures.

Texas Collections.

Rust and seed samples were collected from 114 locations in Texas and central states in 1969, from four wild annuals and one perennial *Helianthus spp.* Data for geographic distribution of race types determined on the Canadian differentials are not yet complete, but there seems to be most variability in pathogenicity in collections from the Red River area of northeast Texas. As might be expected, there appear to be new reaction patterns, indicating the existence of "races" not previously identified. The four wild annual *Helianthus spp.* are segregating for reaction to the four standard Canadian rust races and to the collections from Texas.

Histology.

Dr. Sood found that the early stages of infection: spore germination; appressorium formation; and penetration by race 1; were identical on a susceptible and a resistant variety. Haustoria were numerous in the susceptible variety, few in the resistant one. Mycelial growth was faster and more extensive in the susceptible variety, and host cells appeared uninjured until urediospore formation started. Growth was restricted in the resistant variety, and host cell collapse became apparent 4 days after inoculation.

Daylength.

Our earlier work indicated that a daylength of 16 hours was satisfactory for rust development. Sood investigated the effect of providing this daylength for inoculated plants in various combinations such as 16 hours light, 8 hours dark (16 L 8 D); 8 L 4 D; 4 L 2 D; and also the reverse, 8 L 16 D. Other conditions were the standard 24°C during the light and 18°C during the dark period. Germinability of spores produced under these conditions was determined immediately after "harvesting", and after storage for two months at -16°C. Difference due to light regimes were statistically significant, but were small. Differences between races 1 and 3 were small and not consistent.

Light Intensity.

Light intensity about 1000 to 1200 foot candles from V.H.O. cool white fluorescent tubes has proved satisfactory for our routine studies. Sood investigated the effect of producing rust at intensities from 200 to 4000 foot candles. The effect on germinability of fresh spores was small, although some of the differences were statistically significant. Germinability was tested again after storage at -16°C for 1 and 2 months. Germination percentage decreased fairly rapidly in storage in these experiments, particularly for race 3 stored for 2 months. The effect of light intensity on germinability of stored spores was highly significant, but not consistently linear. Germination percentages were highest, however, for spores produced under 200 to 1300 foot candles, and lowest for those produced under 3000 to 4000 foot candles, particularly for race 3 (Table 1).

Temperature.

We found previously that rust developed well on sunflowers grown at about 25°C day temperature and 18°C at night. Sood investigated the effect of day temperatures of 16°, 21°, and 27°C, keeping night temperatures at 18°C in all cases, on the germinability of fresh and stored (2 months at -16°C) urediospores.

The effect of temperature during production on germinability of fresh spores was negligible. The effects on stored spores were large and very highly significant (Table 2). As with most other factors, the effect was much more pronounced on race 3 than on race 1.

Longevity.

Sunflower rust differs from other *Puccinia* spp. in its ability to survive protracted storage in a freezer at -15 to -20°C, and even in a

TABLE 1. Percentage germination of fresh and stored urediospores of Puccinia helianthi races 1 and 3 produced at various light intensities.[1/]

Light intensity (Foot candles)	Fresh		Stored [2/]			
			1 month		2 months	
	Race		Race		Race	
	1	3	1	3	1	3
200	86.3	89.7	79.0	82.7	73.3	69.2
700	84.0	84.1	77.9	78.2	72.3	64.9
1000	86.3	85.5	79.2	76.8	73.4	67.0
1300	83.2	83.5	78.2	74.7	70.0	62.8
1600	83.1	82.6	77.8	72.9	67.6	63.4
2000	85.6	89.5	75.0	74.5	69.2	65.0
3000	86.5	85.1	74.0	71.3	69.3	59.7
4000	88.5	85.1	73.8	67.9	66.0	52.0
L.S.D. 5%	2.6	2.6	1.9	1.9	2.4	2.4
1%	3.5	3.5	2.3	2.3	3.2	3.24

1/ Average of two experiments.

2/ Stored at -16°C.

TABLE 2. Percentage germination of fresh and stored urediospores of Puccinia helianthi races 1 and 3 produced at various day temperatures.[1/]

Day temperature °C	Fresh		Stored[2/]	
	Race		Race	
	1	3	1	3
16	83	85	77	57
21	84	85	82	78
27	82	83	70	46
L.S.D. 5%	8.4		6.1	
1%	11.3		9.3	

1/ Average of 3 replications; 300 to 400 spores counted per replication for each treatment.

2/ Stored 2 months at -16°C.

refrigerator at about 4°C (Sackston 1960). Sood produced the four Canadian races under standard conditions, then determined their longevity at various storage temperatures. Races 3 and 1 still germinated 2 to 5% after 3 months storage at 23°C, 0.5 to 4% after 3-1/2 years at 4°C, and 43 to 64% after 3-1/2 years at -16°C. Races 2 and 4 retained even higher germinability. The longevity of spores produced under standard conditions in these experiements was higher at room and refreigerator temperatures than that of the field collections previously studied; it was somewhat lower at freezer temperature. Spores produced under standard conditions and stored 2 months at freezer temperature varied in germination percentage in various experiments. We have therefore not succeeded in determining longevity in storage. We have shown, however, that spores produced under controlled conditions can survive as long in storage as spores collected in the field.

Charcoal Rot

Yu-Ho Chan and W. E. Sackston

Dr. Chan has continued the studies on mechanisms of pathogenesis in Sclerotium bataticola which were first reported at the Sunflower Conference in 1966. We now know definitely that this pathogen produces a necrosis-inducing toxin which can be translocated in the host plant (Chan and Sackston 1969). We have not isolated and characterized the toxin, but I hope we will be able to do that before long. Our current work indicates that the toxin is not specific, and its production may not be closely correlated with pathogenicity of the fungus on sunflowers. It does not affect all the plant species we have tested. We still have a lot of work to do with it.

Chan's studies on the enzymes produced by the fungus, and their role in pathogenesis have been going well. S. bataticola produces a range of pectolytic enzymes in culture and in host plant tissues. Pectin methylesterase (PME), endopolygalacturonase (endo-PG), exopolygalacturonase (exo-PG), and polygalacturonase trans-eliminase (PGTE), were all found in culture and in extracts of diseased host plants. Pectin trans-eliminase (PTE) was not detected in culture, and its presence in diseased plants was doubtful. Only PME was found in extracts of healthy plants as well as in diseased plants. The cellulolytic enzymes cellulase and cellobiase were also found in fulture filtrates, and in extracts of diseased, but not of healthy plants. The evidence is therefore strong that these enzymes play a role in disease development (Chan and Sackston 1970a).

Three isolates of S. bataticola virulent on sunflowers, one moderately avirulent, and two avirulent were studied intensively. The avirulent isolates could be re-isolated from inoculated plants even though they did not induce symptoms. Dialyzed culture filtrates of all six isolates showed exo-PG and endo-PG activity; the activity was highest in filtrates from virulent cultures. Activity of both exo-PG and endo-PG was detected in extracts from plants inoculated with the four isolates which induced symptoms, but not from plants inoculated with avirulent isolates, or uninoculated control plants (Chan and Sackston 1970b).

Downy Mildew

M. A. Viswanathan and W. E. Sackston

Dr. Viswanathan reported to the Sunflower Conference in 1968 on his work with the growth substances indole acetic acid (IAA), and gibberellic acid (GA_3). He has since extracted seedlings inoculated with Plasmopara holstedii, and uninoculated control seedlings, and determined the content of gibberellin-like substances by paper chromoatgraphy. Various part of the chromatograms were eluted and bioassayed on dwarf corn and dwarf peas. The gibberellin content, converted to GA_3 equivalents, was found to be slightly higher in diseased than healthy plants when the corn bioassay was used, and slightly lower with the pea bioassay.

Root exudates of diseased and healthy plants were extracted and chromatographed to determine if cytokinins were present. Activity in various fractions was converted to a kinetin equivalent. Extracts from diseased plants had much higher kinetin-like activity than healthy plants.

Kinetin was supplied to germinating sunflower seeds. The resulting plants were dwarfed. When kinetin was added to inoculated seedlings, the dwarfing effect was greater than that induced by either inoculation or kinetin alone. It is therefore possible that cytokinins may play some role in the stunting of sunflowers induced by early infection with the downy mildew pathogen.

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