SUNFLOWER DISEASES - RUST

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The objective of these studies was to try to elucidate any possible gene for gene relationship in the rust and resistance in the host. M. Jabbar Miah did most of the experimental work. He ran into some real troubles both in the germination of teliospores in doing genetic studies on the rust and in the reproducibility of the reactions of various races on specifid differentials. We worked with individual head lots of material all of which came originally from Eric Putt. We used three groups of lines as our differentials. Sometimes we had to increase seed stocks of some of them by selfing and the next generation did not always give us results that we expected.

Because we have to try to get reproducible results both in the uredospore stage and also to try to germinate teliospores, we investigated various environmental factors on the development of the rust working with temperature, light duration and light intensity amongst other things. This work was done by Liang and by Sood. At the present time we are interested with Sood in the infection process, especially in the initial stages of it.

Miah ran into considerable trouble because almost every time I gave him a lot of seed that I had worked with the results were not what we expected. We had been used to working with populations and with statistical results. That is we got most of the plants in a given lot behaving in a certain way. When we used another sample, presumably the same genotype or at least phenotype, we expected that our results would be comparable. They were not. Because of the difficulties it became necessary to treat not just every sample but every plant as an individual.

In order to be sure of our results we inoculated each plant with our four test races, what I have referred to as the Canadian races 1, 2, 3 and 4. They are race groups and I would not be surprised if any one of them could be split into two or 200 if you had sufficiently good differentials. We inoculated each plant with the four standard races, plus any race that we happened to be interested in testing. I would like to show you the results in the first slide. Here are the differentials that I have talked and written about. The universal suscept is S37-388 which Eric used as the female inbred. Cr 29 is presumably the equivalent of the R_2 resistant gene (factor 88), one of the earliest sources of resistance. Cr 69 or CM90RR has the R_1 gene (factor 22) and this "factor 41" is the situation when you have both resistant genes at the same time. Here are rust race groups 1, 2, 3, 4. On the universal suscept you get a good reaction. On R_2, 1 is not pathogenic, 3 is not pathogenic, 2 and 4 are. On factor 22 or R_1, 1 and 2 cannot attack, 3 and 4 can. On factor 41 where you have both resistant genes present as we understood them, or think we understand them sometimes, 1, 2 and 3 do not attack, 4 does.

What I have used frequently for the R_1 gene or the 22 factor was a cross 69 from Morden. When we increased it and tried to work with it again we found that we did not get the results that we expected. I am showing two things on the second slide. First, that if we are very careful we can put as many as 8 different inoculations on one leaf, 16 on a pair of leaves. We would not normally try to do this but it can be done. Working again with the four races 1, 4, 2 and 3 you can see that 4 gives you results. These are all resistant reactions except for 4 yet this is on a line which should have been susceptible to races 3 and 4. My second point is that we often run into difficulty. We very rarely get segregations for obvious susceptible outcrosses. It is almost as rare to recover uniform expected resistant reactions when we try and increase by selfing. The lines that we had as differentials often come up with unknown resistance.

The technique worked out by Miah is as follows: He uses a suspension of spores, or a leaf with rust pustules on it, or dry spores in a petri dish. He moistens tiny bits of absorbent cotton (plugs) in sterile distilled water, takes them with a pair of tweezers, just touches the moist cotton to the rust, puts the plugs in the appropriate place on the leaf, and goes through the whole series with 2 or 4 individual leaves. Then we incubate the plants in the usual way overnight or 24 hours. We have used plastic bags as incubators. We often use garbage cans. They are excellent incubators. After the initial incubation the plants are kept either in the greenhouse or under controlled environmental conditions.

The technique has some drawbacks. Sometimes you may not have enough moisture on the bit of cotton and it may dry out. If you keep the plants in incubators too long, or if there is too much condensation, or if the change of temperature to induce condensation is too abrupt, you can sometimes get a water film and a spore transfer so that you get contamination. These difficulties are quite easily overcome and technicians have learned to use this technique quite well.

We went on to determine the environmental factors that were most significant in getting germinable teliospores. We haven't answered that particular question yet. It is rather difficult to get exactly what we want. We sometimes get excellent results and sometimes with what we think are essentially the same conditions we get poor results.

Next we tried to determine the conditions under which we can get reproducible results with just uredospores. We investigated temperature, light duration, day length and light intensity. We have not yet studied light quality. We tend to work with cool white, very high output fluorecent lamps. We used pustule density as a criterion in temperature studies, counting the pustules per square centimetre of inoculated leaf surface. The variability was sufficiently great that we didn't get significant differences in the different temperatures we used. For various reasons of convenience and the germinability of the uredospores produced we found that a day temperature of about 25 degrees centigrade and a night temperature of about 18 were good standard conditions.

Then we went on to light duration or day length. Here, again, we got

quite variable results in pustule density with different tests using different races and varieties, and we were not satisfied that we really had a good answer. Using subsequent germinability of the spores produced as the criterion we decided that approximately 16 hours daylight was as good as anything. It was a convenient interval to use so when we are not studying day length specifically we use 16 hours as a standard condition.

The effects of light intensity on density of pustules on the leaf surface were rather variable. Various other criteria indicated an intensity anywhere between 800-foot candles to about 1600-foot candles to be satisfactory. We have settled on 1000 to 1200. So these are the routine conditions we tend to use. We have not tried all the possible variations but we have used a lot of permutations and combinations and these conditions are quite satisfactory. This work on environmental factors was done by Miss Liang whose M.Sc. thesis was submitted last year.

The current work is being done by Sood who is studying several things at the same time: The effects of environmental factors on initial infection (histological studies); effects on subsequent development of infection; the germinability of spores as a result of conditions during formation; spore germination under various environmental conditions. His final project is to produce viable germinable teliospores.

The thing that I find most interesting at the moment is the effect of light on the germination of rust uredospores produced under our standard conditions of 1600-foot candles in 16-hour days at day and night temperatures of 25° and 18°C respectively. We use races 1 and 3; two host lines, the universal suscept and line CM90RR, most plants of which behave essentially as the R_1 for us, that is the 22 factor. It is susceptible to races 3 and 4, and resistant to 1 and 2.

Some of the germination tests are done on water agar. We find that water agar as a medium for germination gives us fairly consistent results. Differences in light intensity don't have too much effect when you germinate spores on agar. There isn't too much difference whether you germinate spores on the universal suscept or on the resistant host when you are studying only germination and appresorium formation. Race 1 germinates on the resistant host as well as it does on the susceptible. So host substrate is not significant.

There is an almost incredibly large difference between races 1 and 3 in the ways they react to various stimuli. Race 1 seems to germinate better, to start with higher germination when produced under our standard conditions. The germination of race 1 under adverse conditions on leaf discs does not fall off nearly as rapidly as does the germinability of race 3 in a 16-hour period, at temperatures of 20°C + 0.5°C, in the various light conditions: complete darkness; 200-foot candles; 500-foot candles; and 800-foot candles. Germination is best in the dark, about 80 to 85 per cent. It drops off a little with 200-foot candles, perhaps by about 5% or 10%. Between 200-foot candles and 500-foot candles we get a very striking decrease in germination percentage. It may drop from about 75 down to 55 for race 1, to 25% for race 3. Between 500-foot candles and 800-foot candles the drop again is relatively slow. What we get is a curve that

comes down sharply and smooths out again. The interesting thing here is the difference between races 1 and 3. We are investigating this further.

The germination on the leaves is done on discs cut with a cork borer from the test leaves at about the same position in each case and then maintained on moist filter paper in petri dishes in our test environment.

Experiments were made by Liang in 1964-65 and by Sood in 1965-66 on the effect of preconditioning on spore germination. They exposed plants inoculated with race 1 or 3 to various day lengths and light intensities. The spores produced under the various conditions were germinated on water agar. Race 3 appeared to be affected more than race 1 by differences in day length, and less by light intensity.

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DOWNY MILDEW

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Our objective was to try to determine the biochemical mechanisms involved in the symptom expression of downy mildew (Plasmopara halstedii). It is quite obvious that amongst other things there is obviously a violent upset in the hormone balance of the affected plants. Quite often the symptoms are very much like those of the hormone-type herbicides 2,4-D and related chemicals. We did not achieve the particular objective. We did get some other results on the biology of the spores themselves and we worked out a very nice technique for getting standard results, inoculating sunflower seeds in order to be able to determine their resistance or susceptibility to downy mildew. Now I find that the people at Krasnodar have been using with better results a system that is essentially the same. It differs only in time of application or inoculum.

The slide shows a typical downy mildew plant with chlorotic areas on the upper leaf surface that, under humid conditions, would correspond with the profuse sporulation on the lower surface. Other symptoms include stunting of the plant, essentially the normal number of leaves but the internodes very short so that the plant is short, the leaves often approximately normal size, the heads sometimes normal size, pointed upward instead of bending over, and the seeds empty.

We tried to induce infection by sowing seeds in naturally infested soil which we got from the Canada Department of Agriculture Station at La Pocatiere, Quebec, where there is very, very high infection with downy mildew every year. It is quite a humid area. Downy mildew was a limiting factor in the area and made them go out of sunflowers as a forage crop. Under greenhouse conditions we got relatively poor results. We got infection but it wasn't consistent enough to evaluate mildew reaction of varieties. Quite often we found that there were 10 or 20 or 30% of the seedlings showing symptoms. We had expected much more infection so we checked our results by cutting sections of symptomless plants and found that many were infected with mycelium of Plasmopara. We placed seedlings which had grown for 2 weeks in this soil to a saturated atmosphere over-