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.

T1966PAT02

DOWNY MILDEW

W. E. Sackston and P. G. Goossen

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-

night. The slide shows the upper and lower surfaces of the true leaves and cotyledons of a small and of a large seedling respectively. They had been kept in a moist chamber for approximately 24 hours. It is often enough to maintain the saturated atmosphere overnight. Good results were obtained at temperatures from 16° to 20°C.

Using naturally infested soil obviously wasn't good enough when not all the seedlings gave this sort of result. We did not really know whether we had resistance or susceptibility. We had to get a better technique.

We could induce very heavy sporulation with this moist chamber exposure. We had the spores so we worked with them. We tried various ways to inoculate plants. We made spore suspensions of the zoosporangia and put the suspension on seeds, on the surface of pasteurized soil, then covered them with 2 cms. of soil. We grew the inoculated seeds at about 20°C for 7 days, to 3 weeks. Then exposing the seedlings to the humid environment gave very high percentages of infection indeed.

Table 1 shows the kind of infections we got. These are the reactions of USSR sunflowers in one test using this technique. These results do not necessarily correspond with the results that are obtained at Krasnodar, there I understand Peredovik gives essentially 100% infection and their technique of seedling, not seed inoculation, gives them very close to 100% infection of all susceptible material. The values for the Canadian varieties were not from this experiment, they were taken from another experiment under comparable conditions for the sake of comparison.

While we had this material we were interested in what we could do with these spores. It isn't always convenient to maintain a supply of inoculum on plants in the greenhouse or growth cabinets, so we tried longterm storage. We made spore suspensions and kept them at room temperature, in a refrigerator, and in a freezer. At room temperature they still gave us infection after three days but if kept longer the bacterial growth in them was so high that they were completely polluted and we discarded them. We got decreasing infection from the spores kept in the refrigerator about a week. We could freeze the spore suspensions solid in the freezer and then use them afterwards but this technique had some disadvantages. We had to thaw the suspension before we could use it. We had to thaw it relatively slowly, and this took time. We modified this technique, by storing spores on the leaves. We just snip them while they are still moist, put them in petri dishes in the freezer and find that the spores from them still give a very good infection after about three months. It has dropped off quite a bit after 12 weeks but we still get quite good germination and infection and we think that this particular technique has some promise for those people who have to work with Plasmopara.

I will be surprised if the Soviet delegation did not have further evidence to amplify or correct some of the things I have said. I have learned from them on the trip that they have had better, even higher percentages of infection with the technique they worked out during the same period that we were working.

Table 1 Reactions of Canadian "check" sunflower varieties and of USSR sunflower varieties and hybrid lines to inoculation with Plasmopara halstedii

W. E. Sackston, Macdonald College, P. Q.
February 1964

Host		Plants infected per cent
Canadian varieties		
CM 5		66
S37-388RR		7
USSR varieties		
Peredovik		64
Smena		80
VNIIMK	6540	96
	8863	80
	8931	92
USSR hybrids		
VNIIMK	20697	56
	25104	78
	25144	74
	25210	69

DISCUSSION

Bergen: You mention the symptom of downy mildew is often similar to that of chemicals such as 2,4-D. Now we have a very widespread condition in sunflower here this year that we assume, or some of us assume to be 2,4-D or MCP chemical. Now, have you seen any of our sunflowers, have you observed this condition, or am I not making the correct assumption?

Sackston: Without seeing them I would say that your assumption is most likely correct. My statement taken as it was given was most likely misleading. What I meant was that along with the other symptoms that make us sure we have Plasmopara infection we often get a leaf distortion, veinal effects that people would recognize as being something similar to the kind of distortion or damage that you get from the hormone chemicals. You would not likely say that it was hormone when actually it was Plasmopara, just that the kind of effect was reminiscent of the effect that you get with hormone disturbance. My statement was not sufficiently well qualified.

Panchenko (translation): Are the symptoms shown in the first picture in your opinion induced by primary infection from the soil presumably from oospores or could it result from secondary infection from zoosporangia which fell on the plant after it had germinated?

Sackston: I have talked with the USSR delegates and I know they have results indicating that they can get essentially this sort of symptom with secondary infection after the plant has germinated. I am answering now not on the basis of what you do in the USSR, but of what I have found. These symptoms to me have always meant primary infection from the soil. Now one of the things that I did not mention, incidentally, one of the other things that we worked on was how long zoosporangia would live in the soil because we did a whole series of plantings to see how long soil which we had inoculated would stay infective. In the earlier Russian work, they said if you get the infected seedlings out within two weeks of emergence and appearance of symptoms you would not have any build up of inoculum because oospores would not have formed. We found that we did get a certain build up of infection but we tried tests where we put zoosporangia suspensions, as we used them to inoculate seedlings, on pasteurized soil, on unpasteurized soil and on sterile soil, and didn't put any seed in for various intervals. Well those zoosporangia in unpasteurized normal soil were mostly non-infective, after about a week. We got practically no infection. In pasteurized soil and sterilized soil they were still infective after 10 or 12 days, by two weeks they were gone so anytime we got infection in soil which had no sunflowers in it for more than two or three weeks, we assumed that we had oosporangial inoculum to start with. We were able to demonstrate the formation - the presence of oosporangia in seedlings within two weeks of sowing and inoculation. So we assumed that most of our inoculum here is in fact oosporangia and certainly our infection when it is significant in the field is from the soil, not secondary.

Panchenko: Yes, the same. In two weeks seedlings we have seen oospores.

Sackston: Well our results check in this particular case.

Dueck: I have more of a comment than a question. I don't know whether you are aware of it but Leith, Romig and Rowell of Minnesota who developed a liquid nitrogen method for the storage of ground rust spores and this method requires very little space and they use a cyclone-type of collector for the spores, and store the spores in small ampules although it requires a liquid nitrogen facility and storage is for an indefinite period of time as far as they know. This might be different for this organism but I think it would be worth looking into.

Sackston: It would be worth looking into and we hope that eventually we would be able to try it with rust from sunflowers and we are quite confident it would work. We don't have the facilities, therefore we haven't used it.

Kinman: We have obtained some extremely large cattle semen ampules and stored uredospores in quite large volume in liquid nitrogen in this

manner, it is really too soon to tell how successful it is going to be. So far it has been alright.

Sackston: One of the reasons we have not gone to liquid nitrogen apart from the fact that we do not have a liquid nitrogen storage unit, is the fact that ordinary freezer storage at minus 20 degrees centigrade has given us excellent results with sunflower rust. It is still perfectly germinable after five years so because we had the freezer and we did not have the liquid nitrogen we continued to use the freezer.

Kinman: And vice versa for us.

Hoes: Dr. Sackston, would you say that a reaction of variety of downy mildew would be similar when being affected through the roots? Would it then be similar to local infection on the foliage.

Sackston: We find it very difficult to find what you could distinguish as local infection. We tried various ways to get infection on fully expanded leaves and on plants that were well emerged, not just the young, very tiny seedlings. We got infection and we did demonstrate in some cases that the mycelium grew down through the hypocotyl and the roots where we knew there had been no soil infection. We got systemic infection starting from local infection on the leaves but we had so much trouble trying to get local infection regularly, what we would call secondary infection, that we did not investigate that any further. I am quite sure you would get essentially the same results, it is just that the method in our hands did not work as well for us as inoculating the seeds.

Orellana, R. G.: I would like to go back to the inoculum for a minute. I was wondering under what conditions you can induce the ejection of the zoospores out of the zoosporangium, and if zoosporangia can induce or cause infection?

Sackston: Yes. We studied the effects of temperature on germination of zoosporangia. First we had to find suitable media for such germination. When I had the pleasure of spending a month at the Institute at Krasnodar I went off to an experiment station not far from there where the USSR authority on plasmopara, Novatelnova, was working. She took me to the field at 6 a.m. to collect freshly formed "conidia" or zoosporangia and dew and rainwater in which to study germination and various factors affecting germination. We do not like getting up at 6 a.m. at Macdonald College so we tried various ways of germinating these spores in water, etc. and we found that the germinability in tap water and distilled water was very, very low. The spores just burst, they lysed, so we figured this might be an osmotic phenomenon. We tried various agents to raise the osmotic level. Saline wasn't any good. Then the very next one we tried was sugar which worked like a charm. We found that approximately 1/10th of 1% sugar wasn't good enough, ½% wasn't too bad, 1% or 2% sugar solutions gave us excellent germination. When we got up to 4% sugar we often had good germination and often had poor germination, but we found that one to two per cent was quite good. Coming back to temperature: the zoospores formed from zoosporangia at low temperatures, but more rapidly at 12 to 16, 18 and even at 20 degrees. We also found that there was quite a range of time over which they would germinate then encyst, have their walls thicken, and then produce the germ tube. The earliest

members of that population would be starting to germinate from the encysted zoospore producing the germ tube when there were still motile zoospores in the suspension. Then for the last part of your question: We did occasionally, I think it was just a few cases per thousand get direct germination by germ tubes from zoosporangia at temperatures over 20, 22 to 24. It was not a frequent phenomenon and we did not investigate it sufficiently to say that it was like or not like Phytophthora infestans, or had different threshhold values. All we did was observe direct germ tube production by zoosporangia at the higher temperatures that we used infrequently.

Orellana: What about infection in the tissues?

Sackston: I can't answer that. We were hoping to study the penetration of the spores but this was one of the things that did not materialize.

Orellana: How does the infection start in the field from zoospores?

Sackston: We assume they are from oospores in the soil which germinate to produce oospores and we have no data of our own to question the early work back in the 1920's of Nishimura and others.

T1966PAT03

CHARCOAL ROT

W. E. Sackston, S. B. Mathur and Y.-H. Chan

This is the work of two students, one a post-doctoral who worked for a couple of years on Sclerotium bataticola on sunflowers and other hosts with isolates from a wide range of hosts. He determined various environmental factors involved in infection. Now another man has just completed his masters working with the same pathogen and is continuing for the Ph.D. studying the mechanism of pathogenesis.

The slide shows what happens to sunflower seedlings about two weeks old when we inoculate them with an appropriate isolate of Sclerotium bataticola. Isolate N of the pathogen, 14 days old, kills the seedlings. You can see that the isolates differ only at the lowest temperature, 25°C. Under these conditions M was the most virulent, although more recently it does not always kill most plants. All three gave very good results at 30° and 35°C.

Table 2 shows effect of inoculum age using a sand cornmeal medium in which the fungus had grown for 4 days, 7 or 14 days respectively applied around the base of the young seedling.

Kinman: And watered in?

Sackston: Normal watering, they were not kept dry, but it wasn't watered in deliberately.

The inoculum was put in place and covered over with sterilized soil