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

Table 3 Effect of substrate on effectiveness of inoculum of Sclerotium batáticola on sunflowers.

Substrate	Percentage plants killed
Sand : cornmeal 1:1 (wt.)	100
Sand : cornmeal 10:1 (wt.)	71
Soil : cornmeal 4:1 (wt.)	20
Vermiculite : cornmeal 3:1 (vol.)	100
Vermiculite : V-8 juice 3:1 (vol.)	15
Vermiculite : PD decoction 3:1 (vol.)	9
Controls	0

We praise the names of Pawlowski and Hahn in our laboratory because they came out with a new technique, which they used and described for the study of possible toxin production by Sclerotinia sclerotiorum. It consists of splitting the bases or the apices of sunflower seedlings. The slide shows an un-inoculated seedling. Then we inoculate one side using various methods. We use the technique of taking inoculum produced on vermiculite, or soil or sand or whatever we are studying and then just wrapping it in moist cotton around the stem and enclosing it in polyethylene film. We also use Young's toothpick method where we grow the inoculum directly on boiled wooden toothpicks and then insert a toothpick into the stem. The next slide shows a closeup of rot occurring on one side, the inoculated side - nothing on the un-inoculated side. You will see later on the disease progresses. Not all the leaves are affected but you can follow the pattern of phyllotaxis. Apparently symptoms appear on the leaves supplied by the vessels from the inoculated area. If you catch these affected, wilted leaves early, before they show necrotic breakdown, and put them in water, they will recover. If you wait too many days before you try this they will recover in part but not at the edge. Once they reach the final stage, of course, they don't recover in water. But if you merely sever the water connection by cutting partly through the stems you get wilting but never this necrotic effect. At least we do not induce necrosis by cutting the stems.

The next slide shows the other technique we use, splitting the stems. This happened to be the other method of inoculation as well, the toothpick method, but the rot does not progress very far. You will see that we got wilting on the inoculated portion of the stem, but nothing on the other at an early stage. The next slide shows the same plant at a later stage. You can see that the inoculated half stem was killed, and necrotic spots in the leaf lamina of the un-inoculated stem. There was usually no wilting of leaves on the un-inoculated half of the stem, but whatever induced leaf necrosis travelled down to the junction of the two stem halves, then up the un-inoculated portion. Yet when we made isolations from these plants afterwards the pathogen itself had moved only about one centimeter in all from the point of inoculation. We considered this to be fairly definite proof that there is some toxic material which moved from the point of inoculation. It does not move with the transpiration stream because it obviously goes down

and then comes up again, although we suspect that most of the movement is through the xylem.

Now we are trying to get at what is happening here. We have no final answers, but we have learned a few things. We are studying various inocula. We inoculate with the pathogen together with the medium on which it is grown and we get very striking results. When we use the medium in which it is grown as the inoculum without the pathogen, we get little or no effect in most cases. There is some effect that we can attribute to possible enzyme activity. We have had some indication of the production of possible toxic material, other than enzymes, but we cannot be nearly as sure of that as we are of the fact that the pathogen, independent of any metabolic byproducts which are left in the medium is capable of inducing all the observed symptoms. It doesn't have to have its food base present in order to induce disease. We don't know yet the nature of the material that appears to be translocated from the inoculated to the control stem half. We hope to know in another year or so, if Chan continues to be lucky.

DISCUSSION

Kinman: It has been proven rather conclusively that this organism does act directly as a pathogen and doesn't require some predisposing factor, then. Remember we had quite a discussion of this and I think Luciano and I were both of the opinion that there must be some predisposing factors since in most cases this is not a very good pathogen.

Sackston: I agree with you 100%, Murray. What I have shown here is that under conditions suitable for this kind of experiment you can demonstrate that this is a first-class pathogen. In the field it can be present, the soil can be thoroughly infested and unless conditions are right, or the predisposing factors are there the plants grow as well as though the pathogen had never been heard of. That is why I am interested in environmental factors involved in pathogenesis of this organism because I am quite convinced that in the field it is not a factor unless everything is right for it. But when conditions are right for it it is really a good pathogen.

Kinman: Now what I am getting at is that you were showing, pretty conclusively, that no other organism need be involved.

Sackston: Oh, I'll agree to that, I know that Sclerotium can attack and kill sunflowers without the intervention of other pathogens.

Kinman: We were supposing, and that is about all it was, that some other organism probably did the initial damage which allowed this thing to get started. This doesn't rule it out.

Sackston: We do not rule it out.

Kinman: But you have shown that it doesn't require another organism.

Sackston: It doesn't, but others may be present. One of the things that

I found - I got interested in this thing first the year I worked in Uruguay - that quite often when I isolated this pathogen and I was convinced that it was the effective pathogen, I also got from the same cultures, very characteristically Fusarium oxysporum but when I inoculated plants I got drastic results with the Sclerotium bataticola and very, very negligible results from the Fusarium under experimental conditions with that particular isolate. I'm convinced that environmental factors are critical in the activity of this organism as a major pathogen causing economic loss but it is certainly able to attack without any other pathogen present with the conditions just being relatively favorable for it.

Orellana, R. G.: I wonder if this pathogen would infect the sunflower without injury?

Sackston: Oh, yes. The toothpick method worked very well but it didn't spread nearly as fast as when we just put the pathogen with no medium, just the mycelium itself directly on the stem and provided the moisture.

Orellana: On the intact stem?

Sackston: Yes, intact, not wounded. Incidentally we have gone further. Our current work now is involving excised or detached leaves and putting the inoculum on very, very gently so that we hope not more than a few leaf hairs are broken, and we get beautiful invasion.

Orellana: Tissue maceration too?

Sackston: It involves blackening and tissue maceration, yes.

Orellana: Enzyme?

Sackston: Yes, perhaps. We haven't isolated the enzyme. We certainly know that this thing produces pectinases. What else it produces we don't know.

* * * * * *