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Current and Potential Impacts of Pitch Canker in Radiata Pine

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Pitch Canker: A South African Perspective

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ABSTRACT

Pitch canker, caused by *Fusarium subglutinans* f.sp. *pini* (FSP), is a serious disease of pines. Since the first report of pitch canker in North Carolina in 1945, the fungus has been reported from California, Haiti, Japan, Mexico and South Africa. There is now concern that FSP will spread to other countries where pines are native or where they have been established as exotics in plantations. In this paper, we summarise the knowledge gained from a wide range of studies undertaken on the pitch canker fungus in South Africa. FSP was first discovered infecting *Pinus patula* seedlings in a nursery where it caused serious losses. This has led to a series of intensive studies on FSP in South Africa. These have been facilitated by the fact that the FSP population is highly fertile. It was thus possible to develop useful mating tester strains that could be used in population genetic and taxonomic studies. We have thus been able to show that FSP represents a sexually outcrossing population and have proceeded to describe mating population H to represent this species. The FSP population in South Africa has been shown to be genetically diverse and this is either due to multiple introductions of genotypes of the fungus or active sexual recombination. Furthermore, various techniques to distinguish FSP from other mating populations in *Gibberella fujikuroi* have been developed. These are based on histone gene sequences and mating type genes.

Introduction

Pitch canker of pines, caused by *Fusarium subglutinans* f.sp. *pini* (FSP), was first discovered in the South Eastern United States in 1945 (Hepting and Roth 1946). The disease, however, remained fairly unimportant until it reached epidemic proportions in seed orchards and pine plantations during the 1970s (Dwinell and Phelps 1977; Dwinell et al. 1977). In 1986, pitch canker was discovered in California on *Pinus radiata* near Santa Cruz (McCain et al. 1987). The first report of the disease from outside the United States was from Japan where it was found on *P. luchuensis* in 1988 (Muramoto et al. 1988).

In 1991, a devastating root and root collar disease of *P. patula* was reported from one of the largest commercial pine seedling nurseries in South Africa (Viljoen et al. 1994a). The responsible fungus was identified as *Fusarium subglutinans*. This discovery was enigmatic for a number of reasons as follows:

- There was no prior record of *Fusarium subglutinans* causing root rot of pine seedlings in nurseries.
- The only form of *F. subglutinans* known to be associated with pine disease was FSP, the causal agent of pitch canker on pine seedlings and trees. This disease was not known in South Africa.
- There was no prior record of FSP on *P. patula*.

Presented by Michael Wingfield

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- In the South African nursery situation, *F. subglutinans* was causing dramatic mortality to *P. patula* seedlings but caused virtually no disease on *P. elliottii*, which is a well recognised host of FSP in the south eastern United States.

- *Fusarium subglutinans* is a well-known pathogen of maize and mangoes grown in close proximity to the pine nursery where the seedling disease was active.

The extent of the disease in the South African nursery and the unusual association of this disease with *F. subglutinans* led to an intensive study of isolates of the fungus associated with *Pinus* spp., which has now continued for almost a decade. Studies were initially focussed on identification of the causal agent of the pine seedling disease (Viljoen et al. 1994a,b; 1995; 1997). Subsequent studies have concentrated on (1) the population structure of the fungus in South Africa; (2) its probable origin; (3) techniques to enhance our ability to identify FSP rapidly and with confidence and; (4) strategies to ensure that disease tolerant planting stock is available to the South African forestry industry if field outbreaks of the disease should occur in the future.

In this paper, we summarise knowledge gained through a wide range of studies of FSP in South Africa. While we focus largely on the South Africa situation, we also consider the likely implications of the outbreak of FSP in the country.

The Fungus

One of the most complicated issues relating to the pitch canker fungus is that it cannot be identified with confidence based on morphological characteristics alone. Where isolates of *F. subglutinans* have been collected from cankers on mature trees, it has been assumed that they represent FSP. The only exception was isolates from *Gladiolus* which were previously also thought to represent the pitch canker fungus (Dwinell and Nelson 1978). The reported occurrence of the pitch canker fungus on *Gladiolus* led to the view that the fungus might have a host range beyond pine, and thus, pathogenicity tests would not provide a definitive basis for identification of the fungus. This problem led Viljoen et al. (1995) to reconsider isolates from *Gladiolus* that had been identified as *F. subglutinans*. The study showed that the fungus isolated from *Gladiolus* represents *F. proliferatum*, which is morphologically similar to *F. subglutinans*.

Complications with the identification of forms of *F. subglutinans* led Viljoen et al. (1997a) to undertake pathogenicity tests on pines using South African pine isolates and those from other crops. These, as well as detailed morphological studies and molecular comparisons (Viljoen 1994; 1997a), led to a definitive conclusion that the fungus associated with pine seedling mortality in South Africa was the pitch canker pathogen. The fact that *P. patula*, which is native to Mexico, was considerably more susceptible to this fungus, remained enigmatic.

One of the most interesting early discoveries relating to the discovery of FSP in South Africa, was the appearance in some cultures of small numbers of perithecia of *Gibberella fujikuroi*, which is the known teleomorph of FSP. This made it possible to select mating tester strains, and thus to cross isolates from various origins. Perithecia in culture had previously been found associated with the pitch canker fungus (Kuhlman et al. 1978), and the discovery of the teleomorph in South Africa was, therefore, not unique. However, what was of particular interest was the fact that Kuhlman (1982) had been able to obtain perithecia from crosses between isolates from pine and a mating tester strain from the B mating population. This, and the fact that the pine fungus had been found on hosts other than pine (e.g. *Gladiolus*) led to a general view that the pitch canker fungus belonged to the B mating population of *G. fujikoroi*. Correll et al. (1992) attempted to obtain crosses between B group mating testers of *F. subglutinans* and pitch canker isolates without success. Using selected single ascospore strains from South African FSP isolates, Viljoen et al. (1997a) also failed to repeat the crosses reported by Kuhlman (1982). Correll et al. (1992) suggested a *forma specialis* for *F. subglutinans* isolates pathogenic to pine species. Viljoen et al. (1997a) suggested that this *forma specialis* group represents a distinct biological species.

Coutinho et al. (1995) conducted studies with single ascospore isolates of FSP from South Africa to produce highly fertile, hermaphrodite tester strains. These tester strains and other isolates obtained from a pine nursery were used by Britz et al. (1998) to determine the female fertility and mating type distribution in the population of FSP. In further and related studies, Britz. et al. (unpublished) were able to obtain fertile crosses between one of the South African FSP mating tester strains and one of the B population testers. This result would have confirmed the findings of Kuhlman (1982) but would also have added considerable confusion to the evolving view that FSP represents a distinct mating population of FSP. An analysis of vegetative compatibility in the progeny from a cross between FSP and the B tester strain, showed the cross was a result of homothallism (Britz, et al. unpublished). Furthermore, fertile crosses between isolates from South Africa and Florida and California and Florida added further weight to the view that FSP represents a distinct inbreeding group within the *F. subglutinans* complex. This has recently been designated as FSP mating population H consistent with the standard system of nomenclature for this group of fungi (Britz et al. 1999).

Numerous authors with a commitment to studying pitch canker and using a wide range of techniques, have contributed to the view that FSP represents a distinct taxon. In a recent phylogenetic study of *Fusarium* spp., O'Donnell et al. (1998) supported this view, that has emerged from various laboratories over the past few years. Based on a single fertile cross between a California and a South African isolate of FSP, Nirenberg and O'Donnell (1998) chose to describe the fungus which they have called *F. circinatum* (teleomorph *Gibberella circinata*). Although this name must logically be adopted by researchers studying pitch canker, we believe that it is unfortunate that a more thorough study of a wide range of isolates and crosses was not undertaken prior to the description. A single cross could conceivably have originated from a homothallic cross as shown by Britz et al. (unpublished) with the B mating tests of *G. fujikuroi*. This would confuse the description and call into question the validity of the designated holotype. Additional crosses would also have ensured that teleomorph structures were not described based on very limited population data. Furthermore, such detailed studies would have ensured that researchers interested in pitch canker were provided with definitive morphological characteristics on which to base identification of cultures. At the present stage, we are unconvinced by the morphological characters that Nirenberg and O'Donnell (1998) have used to characterise *F. circinatum* and are presently studying a wider group of isolates in order to resolve this dilemma.

Pathogen identification Pitch canker is an important disease that appears to be spreading globally. It is, therefore, essential that tools are available for the reliable identification of the fungus. The fact that various host specific groups of *F. subglutinans* occur world-wide and that these are, in the view of many mycologists, morphologically identical to FSP, gives rise to a significant dilemma.

Characterisation of strains using pathogenicity tests and crosses with tester strains is possible, but this approach is time consuming and tedious. We have, therefore, focussed some of our attention on providing reliable tools to distinguish between host specific groups of *F. subglutinans*. Sequence data from the ITS region of the ribosomal DNA operon has not proved to be particularly reliable for this purpose (Britz 1997; O'Donnell et al. 1998). However, Steenkamp et al. (1999a) has effectively separated all of these groups based on H3 and H4 histone gene sequence. O'Donnell et al. (1998) have separated pine isolates from various other species based on b tubulin gene sequence. In our laboratory, we find the use of RFLP profiles derived from restriction enzyme polymorphisms of the H3 and H4 histone gene sequence provides a rapid, useful and reliable method to identify large numbers of isolates from pine (Steenkamp et al. 1999a).

The Disease

FSP is clearly well established in South Africa. However, pitch canker as it is known in North America is not present in South Africa. Various intriguing questions arise from this unusual situation. It is, for example, of importance to know whether pitch canker is likely to develop on established commercially planted pine trees in coming years. In addition, it would be useful to know how the pathogen entered South Africa, and where the local population might have originated. Such knowledge would be helpful in preventing future introduction of this fungus and other tree pathogens.

A study of the genotypic diversity of isolates of FSP from a nursery in South Africa (Viljoen et al. 1997b) showed that the pathogen was represented by a relatively high level of genetically diverse entities. A total of 27 different Vegetative Compatibility Groups (VCG's) were identified in a population of 74 isolates. The presence of sexual reproduction amongst isolates would also suggest that outbreeding was occurring and that the number of genetic entities of the pathogen might increase with time. However, the appearance of sexual reproduction in isolates soon after the first appearance of the disease is consistent with the high level of female fertility amongst South African isolates as shown by Britz et al. (1998), and it might be expected that the population would change towards being less fertile over time, due to a reduction in hermaphrodites in the FSP population.

In order to manage pitch canker, it is important to assess the fitness of the fungal population. For this purpose some indication of the ability of the fungus to outcross would be of interest. Traditionally, mating studies would be performed to determine the distribution of mating types in the population. Steenkamp et al. (1999b) investigated the possibility of developing a rapid technique for identifying the mating types in FSP. Thus, part of the MAT 1 and MAT 2 mating type genes were cloned and sequenced of FSP as well as other biological species of *Fusarium* section *Liseola*. Based on MAT gene sequence data, we are now able to distinguish between all sexually outcrossing groups and to rapidly screen isolates for mating type (Steenkamp et al. 1999b).

The high numbers of VCGs amongst South African FSP isolates is similar to the situation in Florida where the population is also diverse (Correll et al. 1992). It is, however, very different to that in California where only 8 VCGs are known to exist (Correll et al. 1992; Gordon et al. 1996). Data from California are fully consistent with the view that the pathogen has been introduced recently. The severe disease situation on *P. radiata* in California is also consistent with a recently introduced pathogen, infecting native plants with low levels of tolerance. The sudden, unprecedented and unexpectedly dramatic death of seedlings in a large commercial seedling nursery in South Africa suggests strongly that the pathogen was introduced into the nursery. However, the relatively diverse pathogen population might be considered inconsistent with this hypothesis. Our view is that a highly fertile population of the pathogen which included both mating types was probably introduced into the nursery on seed and that sexual reproduction has led to a diverse number of genetic entities of the fungus being present.

The origin of the South African as well as other populations of FSP remains unknown and is subject to considerable debate. The disease is well-established on native pines in Mexico (Guerra-Santos and Tovar 1991) and a current hypothesis, which we support, suggests that the fungus is native to that country and perhaps other parts of Central America. It could easily have entered California from Mexico, which is geographically adjacent to it. While South Africa is distant from Central America, the country has imported considerable quantities of seed from that part of the world. Storer et al. (1998) has recently shown that FSP is common on seeds, while the fungus has also recently been collected from cones of native populations of *P. patula* and *P. greggii* in Mexico (Wingfield, unpublished). Although seed importations are treated with care, the chance of a breach in seed management could easily have occurred. This is particularly so in the case of a pathogen such as FSP, which is internally seed-borne. The occurrence of the first documented outbreak of the pathogen in a nursery might also not be purely co-incidental.

Since the first outbreak of *Fusarium* root rot of pine seedlings in a single and very large pine nursery in the Mpumulanga Province of South Africa, there have been numerous and severe outbreaks in other nurseries in the area. These continue to appear and to cause very significant losses, particularly to *P. patula*. Certainly, FSP has now become the single most important pine nursery pathogen in South Africa.

Outbreaks of pitch canker have been expected in South Africa for some years. This is due to the fact that two of the most susceptible species, *P. patula* and *P. radiata*, are extensively planted in the country. There has, however, been only one suggested field outbreak and, although a *Fusarium* sp. was isolated from symptomatic tissue, this disease was not confirmed as being caused by FSP. It is difficult to predict whether FSP will become established under field conditions. In California, the disease appears to be strongly linked to the presence of cone and twig infesting bark beetles (Storer and Dallara 1992). These insects are not present in South Africa and this might explain the lack of disease on mature trees. However, one of the insects that has been associated with pitch canker in the south eastern United States is *Pissodes nemorensis* and this insect is a well established and serious pest of pines in South Africa. The presence of damage caused by *P. nemorensis* might, in the future, lead to field outbreaks of pitch canker in the country.

Future Prospects

Pitch canker has become one of the most important diseases of pines in the world. The devastation caused to *P. radiata* in California is of particular concern. Here it appears to behave in a manner typical of a virulent pathogen introduced into an area where host plants have a high degree of susceptibility. In this sense, it could be compared to pine pathogens such as white pine blister rust (Boyce 1961) and the pine wood nematode (Mamiya 1983; Wingfield 1987) that have caused substantial devastation in North America and Asia, respectively. The consequences of this disease in the longer term could be devastating.

Two of the most widely planted *Pinus* spp. in the world are *P. radiata* and *P. patula* (Harrington and Wingfield 1998). Significant plantation industries have been established with these species in many countries of the Southern Hemisphere. They also appear to be highly susceptible to FSP. South Africa is the only country in the Southern Hemisphere growing pine, where FSP has been encountered. Future developments in this country might, thus, provide some indication of how the pathogen would behave if it was introduced into countries such as Chile, New Zealand or Australia. As mentioned previously, the absence of shoot and cone feeding insects could restrict FSP to nurseries. At this stage, it is impossible to foresee the likely course of events in the future. However, very close monitoring, and an effort to ensure that disease tolerant planting stock is available, would seem to be logical actions for the immediate future.

Seed importations would seem to be a likely vehicle of introduction of FSP into new areas. One reaction to the risks associated with seed importation might be to impose a total moratorium on seed movement into countries where FSP is not already present. While this might prevent the introduction of the pathogen, it could also lead to a situation where the genetic diversity of pines remains highly uniform. Our view would be to support limited importations of seed that are treated as thoroughly as possible, and that are planted under strict quarantine situations. It should thus be possible to introduce new genetic material into countries without also introducing new pathogens. An alternative, but perhaps prohibitively expensive route would be to restrict importations of plants in sealed sterile containers and generated via tissue culture.

In the past, it was not possible to easily identify FSP and screening seed for the pathogen was virtually impossible. Accurate and relatively rapid techniques have now been established to allow reliable identification of FSP from seed. It should thus be possible to screen important seed lots for the pathogen and to eliminate it from those collections that might be infected. We would strongly support the introduction of such standards.

At the present stage, very little is known regarding the levels of disease tolerance to FSP amongst provenances, and families of important species such as *P. radiata* and *P. patula*. Inoculation studies are already underway in South Africa to screen key breeding stock for susceptibility to FSP. We believe that both field and greenhouse screening are being undertaken in California (T. Gordon, pers. com.) and possibly in other parts of the United States. Ultimately, it would be desirable to seek reliable genetic markers linked to FSP susceptibility. In this way, it will be possible to rapidly screen breeding stock and to eliminate susceptible material in a timely fashion.

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Question and Answer

Claudio Balocchi: Mike, do you have the disease close to the radiata pine plantations, where it could become infected?

Mike: Yes, not extensively. You know South Africa, so I sense this is a loaded question. The extensive plantings of *Pinus radiata* are in the Mediterranean areas, down in the Cape. There are lots of dead tops, lots of *Diplodia*, not pitch canker fungus. There are also of course a lot of ornamental plantings. I think it would have got there if it could have. So what makes it like it is here? My theory is insects, these little things that are bitting on the shoots, it has to be.

Sharon Clark: Do you have Christmas tree farms in South Africa?

Mike: No we don't.

Sharon: Because that might be one way it could move from a nursery situation out into the forest.

Mike: Because they shear the trees? What are you thinking?

Sharon: No because they grow them from seed in the nursery you may have some sharing of seedlings.

Mike: We've certainly spread this fungus into our plantation areas. There's a lot one could say about this. There's a lot of tension and controversy between planting contractors and nurseries. The fungus is well distributed.

Sarah Covert: I have first a point of information. You made the comment about the morphological identification of the species. I've not attempted that myself, but I saw Dave Geiser last week who's now at the Fusarium stock center at Penn State. He said that he gets those little curly que structures to form on all of the isolates he has from pine.

Mike: Those curly structures are there, but I've also seen them in the mango isolates. They are also in the pineapple isolates. Sarah I can't do it. They're there, but I don't think they're diagnostic.

Sarah: Other people have said they don't see them at all.

Mike: Well medium is important. A diagnostic has to be very good to be useful.

Sarah: The question was, on the isolates from Mexico, have you had a chance to check the female fertility of those isolates?

Mike: Tom can talk some about this, remind me Karen and Tom. You did the crosses. Only a couple of isolates produced perithecia. It's a very small sample. Tom will tell you . . . there's about 30 isolates.

Karen Wikler: There's only two VCGs.

Mike Carson: Without offending any of the plant pathologists here, including yourself, I wanted to ask how important, considering the situation in Australia, New Zealand and Chile, where we don't know what we have. How important are morphological, vegetative, or molecular based evidence of differences among the *Fusarium* species or whatever they are, compared with the pathogenicity among the strains? In terms of what to do about this problem? What I seem to be hearing is that we may have a *Fusarium,* which under the right environmental conditions, infect some of the pine species that we have. So it seems to me that pathogenicity is the only thing of concern to us, or the first thing lets say. Pathologists worry about what is genetic variation and what is a species when you're talking about fungi.

Mike: I don't think this is as serious an issue as you're making it out to be, at least in my opinion. There are lots of people here with similar opinions and more information than I have. *Fusarium subglutinans pini* or *F. circinatum* is a very very serious pathogen of pine. It's different from all other pine *Fusariums*. I don't think you should get away from that at all. This fungus is different to all the other forms of *Fusarium* even in the Section list. You can separate it out, it's a mating population, it's sexually isolated. It's a species, it's a good strong species. Nobody's arguing about that. And it's hugely virulent in comparison to any other *Fusarium* that you would isolate from a pine nursery, *F. sporotrichioides*, and others. Let's go to the other part of your question, and that is within *F. subglutinans pini* or *F. circinatum*, how much variation in virulence of many many isolates? I have not done that work, but Tom has done that work and perhaps others. I'm saying, if you want to screen your material perhaps you should do it in South Africa, because there's more VCGs; we have more genetic diversity than you have in California. And Tom will say there's actually very little variation in virulence amongst the set of isolates that he has tested. So it looks as if there's a relatively uniform level of virulence amongst a large set of isolates.

Sarah: In California?

Dave Dwinell: I think it is true in the Southeast also.

Mike: Sharon I think you said you've tested isolates from elsewhere also, under carefully controlled conditions.

Don Owen: I was curious about the control of the fungus in nurseries. If you cannot eradicate it in the greenhouse, would you ever be able to eradicate it? And how do you think the fungus is surviving?

Mike: I can answer some of it, some of it I just don't know. In the last couple of years we haven't had big outbreaks in any of the nurseries. And then last year we had a huge outbreak in a new nursery. Where did it come from? Where has it been sitting in the mean time? In the soil perhaps? In that first nursery we were eventually able to eradicate it to a point where the disease wasn't really serious. It was in the water, it did get into water sources. It got into the containers. Plastic containers were put into replace polystyrene containers because the polystyrene got . . . , the entire nursery, I mean this is hectares and hectares and hectares of containers. All the polystyrene was totally penetrated by the fungus. It was in the potting medium. So one had to go through every step of the process and get rid of it. But it's there, it's coming back into nurseries.

Dave Dwinell: Outbreaks of this disease really challenge our thinking, because it doesn't act like other diseases. For instance, we had two loblolly pine seed orchards in Katabra, North Carolina, which were side by side the same clones. One got shoot die back and the other had no disease at all. The only thing I've been able to come up with in my 32 years of working with this disease is: one, outbreaks can't be predicted, outbreaks don't happen in the same place twice, and outbreaks can't be explained.

Mike: I'm not perhaps quite as cynical as that.

Tom Gordon: I just wanted to continue along the lines of the potential role of the nursery, which you've alluded to a number of times, as a site for initial establishment. A couple of things worth mentioning there, like where does the fungus hang out. It does survive in the soil remarkably well. If you compare it to something like *F. oxysporum* which makes chlamydospores, it's not a good survivor. But we've been able to get it out of Christmas tree farm soil. We've inoculated soil in the greenhouse and we've been able to get it back out, even dry soil, a year later. The numbers are way down, but it's still there; so it can persist in soil. The other thing I wanted to mention is the situation in California really fits with the idea that the nursery is where it got started. Because if we look at the pattern of distribution of VCGs in California, and how that distribution has changed over time, it's very consistent with that. Of course this is only circumstantial evidence, but the pattern fits very well. The greatest VCG diversity that we've ever seen was in a Christmas tree farm. We have a particular VCG which initially we found only in a Christmas tree farm. We know that the owner of that farm shipped material from a Christmas tree farm in Los Angeles to Santa Barbara, where we then found only that VCG. And that VCG then moved from Santa Barbara to the entire south coast. So virtually that entire infestation is from that one VCG which we can trace to a Christmas tree farm. Incidentally, Dave Adams referred to that new infestation in Solano County at a Christmas tree farm, and it's this same VCG. So I think it really is consistent with your idea, and justifies the need for nurseries being a focal point for concern. That coupled with the fact that it can often be misdiagnosed when it's strictly a seedling pathogen. It's not necessarily obvious that it's the pitch canker pathogen.

Dave Wood: Is there anyone following these seedlings into the outplanting areas? Is there a surveillance program for looking at seedlings which die soon after planting?

Mike: Surveillance in sort of an *ad hoc* way. We have threatened court cases every now and then, we have a problem right at the moment. We have an outbreak in a nursery, and contractors are suggesting that they've been given poor material. I had one in my office last week or the week before last, and he is absolutely convinced. This last year he's getting huge mortality, and he's been blamed for this. Of course, we have a problem because we look after the companies and we can't get involved in these discussions. So we say go and talk to somebody else, we can't talk to you. But people watch. Certainly where there have been problems in the nursery, there are very substantial problems in the planting out situation.

Dave: So are you systematically tracking these.

Mike: No we haven't been systematic about it. You need a lot of people to do this. My responsibilities are to look after diseases in 1.6 million hectares of plantations, so with the team that I have, we haven't been doing this.

Scott Templeton: Did that nursery keep records of how much they spent to get rid of the disease?

Mike: We know more or less. I got enough money to build a new laboratory out of that particular situation. It's hard to compare in dollars because we deal with rands; a person who earns a dollar here makes about a rand there. You can't really compare in money, but the losses over four years were about 15 million rands. Very, very, very substantial! It certainly helped my research program somewhat.

Scott: That was their losses, but they also spent to get rid of it. Is that right?

Mike: For four years that particular nursery could not produce *Pinus patula* seedlings at all. That's a potential of about 15 million plants, a huge, huge problem. We have that going on at the moment now with another nursery.