

- Unique Floral Kingdom. Fernwood Press, Cape Town.
25. Donaldson J.S. and Scott G. (1994). Aspects of human dependence on plant diversity in the Cape mediterranean-type ecosystem. *S. Afr. J. Sci.* 90, 338–342.
  26. Richardson D.M., Macdonald I.A.W., Holmes P.M. and Cowling R.M. (1992). Plant and animal invasions. In *The Ecology of Fynbos: Nutrients, Fire and Diversity*, ed. R.M. Cowling, pp. 271–308. Oxford University Press, Cape Town.
  27. Cowling R.M. and Hilton-Taylor C. (1994). Patterns of plant diversity and endemism in southern Africa: an overview. In *Botanical Diversity in Southern Africa*, ed. B.J. Huntley, pp. 31–52. National Botanical Institute, Pretoria.
  28. Scott D.F., Le Maitre D.C. and van Wilgen B.W. (1991). Report on the problems relating to the fire site on Devil's Peak and proposals toward their solution. Report C72, CSIR Division of Forest Science and Technology, Pretoria.
  29. Scott D.F. and van Wyk D.B. (1990). The effects of wildfire on soil wettability and hydrological behaviour of an afforested catchment. *J. Hydrol.* 121, 239–256.
  30. African National Congress (1994). *The Reconstruction and Development Programme*. Umanyano Publications, Johannesburg.
  31. Repetto R., Magrath W., Wells M., Beer C. and Rossini F. (1989). *Wasting assets: Natural resources in the national income accounts*. World Resources Institute, Washington, DC.
  32. Lutz E. (1993). *Toward improved accounting for the environment*. A UNSTAT-World Bank Symposium. The World Bank, Washington, DC.
  33. Higgins S.L., Turpie J.K., Costanza R., Cowling R.M., Le Maitre D.C., Marais C.M. and Midgley G.F. (in press). An ecological economic simulation model of mountain fynbos ecosystems: dynamics, valuation and management. *Ecologic. Econ.*
  34. Dethlefsen V., Jackson T. and Taylor P. (1993). The precautionary principle — towards anticipatory environmental management. In *Clean Production Strategies: Developing Preventive Environmental Management in the Industrial Economy*, ed. T. Jackson, pp. 41–62. Lewis Publishers, Boca Raton.

## Pitch canker of pines — a contemporary review

A. Viljoen<sup>1</sup>, M.J. Wingfield<sup>1</sup>, W.F.O. Marasas<sup>2</sup> and T.A. Coutinho<sup>1</sup>

<sup>1</sup>Department of Microbiology, University of the Free State, P.O. Box 339, Bloemfontein, 9300 South Africa and <sup>2</sup>Medical Research Council, Programme on Mycotoxins and Experimental Carcinogenesis, P.O. Box 19070, Tygerberg, 7505 South Africa.

*Fusarium subglutinans* f. sp. *pini* (F. s. *pini*) has, for many years, been recognised as the causal agent of pitch canker of pines in the southern United States. Recent occurrences of the disease in new geographical areas has led to a revival of pitch canker research. F. s. *pini* can be separated from other non-pine isolates of *F. subglutinans* in its ability to attack pines, as well as on the basis of its genetic and molecular structure. Sub-specific populations that are essentially biological species may, therefore, exist within *F. subglutinans*. Pitch canker has been reported from four new geographical areas, including California in the United States, Mexico, Japan and South Africa, during the course of the last eight years. Incidence and spread of pitch canker in Mexico suggests that the country might be regarded as a possible centre of origin of F. s. *pini*. The population structure of F. s. *pini* in Florida, where the disease is well established, is genetically diverse and represents a large number of vegetative compatibility groups (VCGs). In California and South Africa, where the fungus is believed to have been introduced recently, a small and a large number of VCGs have been reported in the former and in the latter, respectively. The populations in California and South Africa suggest that the pathogen has most likely been introduced from different sources. It is expected that pitch canker will spread to new areas and become more severe in future.

Pitch canker is a serious disease of pines (*Pinus* L.) caused by *Fusarium subglutinans* (Wollenw. & Reinking) Nelson, Tousoun & Marasas. The disease was first observed on Virginia pines (*P. virginiana* Mill.) in North Carolina (United States) in 1945,<sup>1</sup> and by 1974 had reached epidemic proportions in seed orchards and plantations in the southern United States.<sup>2,3</sup> In 1985, it was suggested that pitch canker is endemic to the southern United States.<sup>4</sup>

Pitch canker has been viewed as a complex, rather than a discrete canker disease. This is because *F. subglutinans* from pines infects a variety of vegetative and reproductive plant structures at different stages of host maturity, and produces a diversity of disease symptoms.<sup>4</sup> Damage includes tree mortality, growth sup-

pression, stem deformation, seed and cone loss, and loss of seedlings. The pitch canker fungus infects various pine species, enters wounds in trees made by insects, and exploits mechanical injuries in seed orchards, weather-related injuries and those caused by fusiform rust galls.<sup>4</sup> The fungus spreads rapidly, is wind-borne, seed-borne and vectored by insects. The impact of the disease can be reduced through proper management and selection of trees for resistance.<sup>5</sup>

Two reviews on pitch canker have been published in the past.<sup>4,6</sup> These specifically deal with pitch canker in the southern United States. The aim of this review is to provide an update on recent developments relating to the taxonomy, geographic distribution and genetic structure of the pitch canker fungus.

### The fungus

*Fusarium subglutinans* is a diverse and cosmopolitan species defined by simple morphological characteristics such as microconidial ontogeny and conidiogenesis.<sup>7</sup> The fungus has a wide host range including maize, mango, pineapple, pine and sorghum.<sup>8,9</sup> Inoculation data for *F. subglutinans* has shown that isolates from pine are virulent to pine seedlings.<sup>10–12</sup> Isolates from non-pine hosts (sorghum, maize, sugarcane, rice, pineapple, dracaena, mango, reeds and grass), in contrast, are avirulent to pine seedlings in greenhouse pathogenicity tests.<sup>12–14</sup> Isolates of *F. subglutinans* are regarded, therefore, as the pitch canker pathotype, only if their virulence to pines has been shown. An exception here is in isolates from gladiolus corms which have been reported to be weak to moderately pathogenic to slash (*P. elliotii* Engelm. var. *elliotii*) and loblolly (*P. taeda* L.) pines.<sup>14</sup>

Strains of fungi, having the physiological ability to parasitise specific hosts, but which are morphologically indistinguishable from saprophytic strains of the same species, are termed special forms or *formae speciales*. *Formae speciales* have been proposed predominantly for strains of *F. oxysporum* Schlecht. emend. Snyd. & Hans., but also for strains of *F. solani* (Mart.) Appel & Wollenw., *F. lateritium* Nees and several other *Fusarium* spp.<sup>8</sup> In greenhouse pathogenicity tests, isolates of *F. subglutinans* from pine appear to represent a distinct sub-population within *F. subglutinans*. Accordingly, Correll *et al.*<sup>13</sup> proposed that the pitch canker pathogen be recognised as a separate *forma*



*specialis*, *Fusarium subglutinans* f. sp. *pini* (*P. s. pini*). Isolates from gladiolus corms pathogenic to pine seedlings would have complicated such a designation, but have since been assigned to *F. proliferatum* (Matsushima) Nirenberg.<sup>15</sup> Isolates of *F. subglutinans* from pine have also been shown to be significantly less virulent to gladiolus corms than isolates of *F. proliferatum* from gladiolus.<sup>15</sup>

Isolates of *F. s. pini* have a similar DNA genome,<sup>12,16</sup> implying that they have a common ancestry. Restriction fragment length polymorphisms (RFLPs) were absent among pine isolates as they use four different restriction enzymes.<sup>16</sup> Non-pine isolates had mtDNA restriction fragment patterns that differed from pine isolates, but that were co-incident with host origin. Random amplified polymorphic DNA (RAPD) data have supported the view that isolates of *F. s. pini* are homogeneous, and unrelated to non-pine isolates.<sup>12</sup> Vegetative compatibility groups (VCGs), representing isolates from different pine hosts and geographical areas, were also similar in their DNA banding patterns.<sup>13</sup>

*Fusarium subglutinans* is heterothallic<sup>8</sup> and, therefore, fertile crossings can occur between compatible strains belonging to different mating types. Two different mating populations within the section *Liseola* have been assigned to *F. subglutinans* and are known as the B- and E-mating populations.<sup>17</sup> The teleomorph *Gibberella subglutinans* (Edwards) Nelson, Toussoun & Marasas has, however, never been recorded on pines under natural conditions. Kuhlman *et al.*<sup>18</sup> successfully initiated sexual crosses between five *F. s. pini* isolates and testers belonging to the B-mating population. A subsequent attempt to produce fertile perithecia with known sexually compatible tester strains within the section *Liseola*, as well as reported testers of the pitch canker fungus, have failed.<sup>13</sup> Viljoen *et al.*<sup>12</sup> were also unable to demonstrate sexual compatibility between pine isolates and compatible testers belonging to the B- and E-mating populations, but reported sexually fertile crosses between isolates belonging to the South African and United States populations of *F. s. pini*.

### Geographical distribution

Pitch canker has, for many years, been known in the southern United States and Haiti.<sup>6,19</sup> Since 1987, the disease has been reported from four new geographical areas. The occurrence of the disease in new areas may have a serious impact on native or exotic pine species. A relatively mild occurrence of pitch canker, however, may indicate that the fungus has evolved with its hosts and may have remained undetected for many years.

Pitch canker was discovered in Santa Cruz county in California in the summer of 1986.<sup>20</sup> The disease was associated with landscape plantings in a limited geographic area,<sup>21</sup> but quickly spread to trees along a major freeway corridor and into nearby residential areas and state parks.<sup>22</sup> The chronology of disease spread, its distribution and VCG structure suggests that it has been introduced into California recently.<sup>13</sup>

Pitch canker symptoms in California are restricted to die-back of branch tips, entire branches and even tree tops.<sup>20</sup> These symptoms can be seen throughout the year, suggesting that infection occurs over an extended period of time. Pitch canker has been associated primarily with Monterey pine (*P. radiata* D. Don.), but has now been found naturally infecting nine additional pine species, six of them native to California.<sup>23</sup> The disease has also been identified in several tree nurseries and Christmas tree farms.<sup>13</sup> Trees with pitch canker have been found in ten counties with the largest infestation in Santa Cruz and Alameda counties.<sup>23</sup> Insects such as the engraver beetles *Ips mexicanus* Hopkins and *I. paraconfusus* Lanier are able to vector and transmit *F.*

*s. pini* to mature pines and seedlings.<sup>22,24</sup> A number of other insects are also strongly implicated in the transmission of the fungus.<sup>23</sup>

In Mexico, pitch canker was first reported on *P. douglasiana* Martinez, a species indigenous to San Andres Milpillas in Nay.<sup>25</sup> *F. s. pini* has now been associated with many pine species in Mexico where the disease is severe in some areas.<sup>26</sup> The pine species most affected are *P. maximinoi* H.E. Moore, *P. pringlei* Shaw and *P. pseudostrobus* Lindl. Insects are implicated in the transmission of the disease.<sup>26</sup>

Pitch canker in Japan was first observed on larch pine (*P. luchuensis* Mayr.) on Amamiooshima Island in 1988.<sup>27</sup> The disease has since been reported from Kagoshima Prefecture, Okinawa Ishigaki, Iriomote, Kume, Miyako Islands and Okinawa Prefecture.<sup>28,29</sup> Symptoms include both die-back of shoots and branches, and cankers on the main stems.<sup>11</sup> No primary source of infection, dispersal season or vector of *F. s. pini* is known in Japan. One possible means of spread is on infected pine seedlings. Since pine seedlings are not produced in Miyako Island, the disease and pathogen might have been introduced to the island from Okinawa Island on infected seedlings.<sup>30</sup> In Kagoshima Prefecture, *F. s. pini* has been isolated in many areas from the air, bark and needles of non-diseased trees, and proved to be pathogenic to larch and Japanese black (*P. thunbergii* Parl.) pines.<sup>31</sup> The disease is, therefore, expected to become more severe in Japan in the future.

In South Africa, the pitch canker fungus has been responsible for a devastating root disease of containerised *P. patula* Schlecht. & Cham. seedlings in a forest nursery in Mpumalanga.<sup>32</sup> There have been no reports of outbreaks in plantations as yet, but the major species planted in the country (*P. patula*, *P. elliottii* and *P. radiata*) are all susceptible to the fungus.<sup>33</sup> The origin of *F. s. pini* in South Africa has not yet been established, but it is believed that the fungus was introduced into the country.<sup>34</sup> A major concern at present is the potential spread of the fungus to plantations. *Pissodes nemorensis* Germar., an insect with a long history of association with *F. s. pini* in the United States, is present and infests pines in South Africa.<sup>35</sup> This increases the potential for the spread and development of pitch canker in South Africa.

Pitch canker incidence and spread in California, South Africa and Japan favours the hypothesis that *F. s. pini* has been newly introduced into these areas. The fungus, however, appears to be well established in Mexico, where pitch canker is reported to be widespread. In 1953, it was suggested that *F. s. pini* was introduced in the southern United States from Haiti, where pitch canker was abundant on Western Indian pine (*P. occidentalis* Swartz).<sup>36</sup> Mexico might possibly be regarded as another centre, other than Haiti, of origin of pitch canker.

### Genetic structure of the pathogen

Vegetative compatibility groups (VCGs) are useful markers to understand diversity and genetic drift of fungal populations. Fungi reproducing asexually usually have a limited number of VCGs.<sup>37</sup> Given the fact that source populations are usually much more diverse than introduced populations, any appreciable frequency of related isolates in areas where pitch canker is well established may indicate the origin of an introduced population. Sexual reproduction would, however, lead to increased genotypic diversity that would make detection of clonal lineages difficult. In Florida, where pitch canker has been known for at least 15 years, a high VCG diversity of *F. s. pini* has been found.<sup>16</sup> This reflects a well-established fungal population representing



both mating types with regular sexual recombination and segregation of the loci that control vegetative compatibility.

VCG diversity in the Californian population of *F. s. pini* has been shown to be limited.<sup>16</sup> From a total of 209 isolates examined, only five distinct VCGs were identified. One VCG predominated, representing almost 70% of the sampled population.<sup>16</sup> Indications are that the founder population was recently introduced into California and thus consists of a small number of VCGs. Different sexual mating types apparently do not occur, or conditions for sexual reproduction are unfavourable. The low number of VCGs reflects a single introduction of a few individuals, most likely from neighbouring Mexico.

In South Africa, it is believed that *F. s. pini* was introduced recently.<sup>12</sup> The large number of VCGs representing both mating types in South Africa<sup>34</sup> is either from more than one migration event, or from the single migration of many individuals of *F. s. pini*. The presence of *F. s. pini*, initially in only one nursery, favours the hypothesis that the fungus was introduced during a single migration event, probably on infected seed.<sup>32</sup>

The VCG diversity in the South African population is more similar to the Florida population than to the Californian. At least four VCGs dominate the South African population.<sup>34</sup> Sexual reproduction in the fungus has been achieved under laboratory conditions.<sup>12</sup> Under favourable environmental conditions, newly introduced isolates of *F. s. pini* representing different VCGs of opposite mating types could have led to a large number of VCGs through sexual reproduction in a relatively short period in South Africa.

## Conclusions

Recent developments in pitch canker research lead us to conclude that pitch canker of pines is becoming an important disease of pines internationally. The disease is spreading rapidly to new geographical areas, and now threatens pines worldwide. We, expect, therefore, that reports of pitch canker from new pine-growing countries will continue, and that the disease will become more significant in future.

Molecular and genetic techniques are now available to study *F. s. pini*, its origin and worldwide distribution. It is possible to characterise the worldwide population, and to predict and minimise future outbreaks of the disease. The population structure of *F. s. pini* in Haiti and Mexico could explain many unanswered questions regarding the origin and evolution of the pitch canker fungus, and deserves further study.

The association of *F. s. pini* with insects appears to be important in the epidemiology of the fungus. This aspect of pitch canker is also likely to be important in the future.

- Hepting G.H. and Roth E.R. (1946). Pitch canker, a new disease of some southern pines. *J. For.* **44**, 742-744.
- Dwinell L.D. and Phelps W.R. (1977). Pitch canker of slash pine in Florida. *J. For.* **75**, 488-489.
- Dwinell L.D., Ryan P.L. and Kuhlman E.G. (1977). Pitch canker of loblolly pine in seed orchards. *Proc. 14th. South. For. Tree Improv. Conf.*, pp. 130-137. Gainesville, Florida.
- Dwinell L.D., Barrows-Broadbent J. and Kuhlman E.G. (1985). Pitch canker: A disease complex. *Plant Dis.* **69**, 270-276.
- Blakeslee G.M., Dwinell L.D. and Anderson R.L. (1980). Pitch canker of southern pines: Identification and management considerations. *USDA For. Rep. SA-FR 11*.
- Dwinell L.D., Kuhlman E.G. and Blakeslee G.M. (1981). Pitch canker of southern pines. In *Fusarium: Diseases, Biology, and Taxonomy*, eds P.E. Nelson, T.A. Toussoun and R.J. Cook, pp. 188-194. The Pennsylvania State University Press, Pennsylvania.
- Nelson P.E., Toussoun T.A. and Marasas W.F.O. (1983). *Fusarium species: An illustrated manual for identification*. The Pennsylvania State University Press, Pennsylvania.
- Booth C. (1971). *The genus Fusarium*. Commonwealth Mycological Institute, Kew, Surrey.
- Nirenberg H. (1976). Untersuchungen über die morphologische und biologische Differenzierung in der *Fusarium*-Sektion *Liseola*. *Mitt. Biol. Bundesanst. Land-Forstwirtschaft. Berlin-Dahlem* **169**, 1-117.
- Dwinell L.D. (1988). Comparative pathology of *Fusarium subglutinans* isolated from Monterey pine in California and southern pines. (Abstr.). *Phytopathology* **78**, 1607.
- Muramoto M. and Dwinell L.D. (1990). Pitch canker of *Pinus luchuensis* in Japan. *Plant Dis.* **74**, 530.
- Viljoen A., Marasas W.F.O., Wingfield M.J. and Viljoen C.D. (1997). Characterization of *Fusarium subglutinans* f. sp. *pini* causing root disease of *Pinus patula* seedlings in South Africa. *Myc. Res.* **101**, 437-445.
- Correll J.C., Gordon T.R., McCain A.H., Fox J.W., Koehler C.S., Wood D.L. and Schultz M.E. (1991). Pitch canker disease in California: Pathogenicity, distribution and canker development on Monterey pine (*Pinus radiata*). *Plant Dis.* **75**, 676-682.
- Dwinell L.D. and Nelson, P.E. (1978). Susceptibility of slash and loblolly pines to strains of *F. moniliforme* and its variety *subglutinans*. (Abstr.). *Phytopathol. News* **12**, 207.
- Viljoen A., Wingfield M.J., Marasas W.F.O. and Coutinho T.A. (1995). Characterization of *Fusarium* isolates from gladiolus corms pathogenic to pines. *Plant Dis.* **79**, 1240-1244.
- Correll J.C., Gordon T.R. and McCain A.H. (1992). Genetic diversity in California and Florida populations of the pitch canker fungus, *Fusarium subglutinans* f. sp. *pini*. *Phytopathology* **82**, 415-420.
- Leslie J.F. (1991). Fungal vegetative compatibility. *Ann. Rev. Phytopathol.* **31**, 127-151.
- Kuhlman E.G., Dwinell L.D., Nelson P.E. and Booth C. (1978). Characterization of the *Fusarium* causing pitch canker of southern pines. *Mycologia* **70**, 1131-1143.
- Hepting G.H. and Roth E.R. (1953). Host relations and spread of the pine pitch canker disease. (Abstr.). *Phytopathology* **53**, 475.
- McCain A.H., Koehler C.S. and Tjosvold S.A. (1987). Pitch canker threatens California pines. *Calif. Agric.* **41**, 22-23.
- McCain A.H., Correll J.C. and Gordon T.R. (1988). Pitch canker in California. (Abstr.). *Phytopathology* **78**, 1539.
- Fox J.W., Wood D.L. and Koehler C.S. (1990). Distribution and abundance of engraver beetles (Scolytidae: *Ips* species) on Monterey pines infected with pitch canker. *Can. Entomol.* **122**, 1157-1166.
- Storer A.J. and Dallara P.L. (1992). Pitch canker disease in California. *Calif. Dep. For. Fire Protect. Tree notes* **15**.
- Fox J.W., Wood D.L., Koehler C.S. and O'Keefe S.T. (1991). Engraver beetles (Scolytidae: *Ips* species) as vectors of the pitch canker fungus, *Fusarium subglutinans*. *Can. Entomol.* **123**, 1355-1367.
- Rodrigues R.G. (1989). Pitch canker on *Pinus douglasiana* pines indigenous to San Andres Milpillas, Municipal of Huajicori, Nay. For. Parasit. Symp. V. Summary: 28. City of Juarez, Chihuahua, October 4-6.
- Santos J.J.G. and Tovar D.B. (1991). Algunos aspectos sobre el cancro resinoso de los pinos. (Abstr.). VI Simposio Nacional Sobre Parasitología Forestal, October 1991. Unidad de Congresos del Colegio de Postgraduados Montecillos, Edo, Mexico.
- Muramoto M., Minamihashi H., Mitsuishi K. and Kobayashi T. (1988). Occurrence of resiniferous damage on *Pinus luchuensis* Mayr. — symptoms and analysis of the damage. *Proc. 99th Ann. Meet. Jpn. For. Soc.*, pp. 513-514.
- Kobayashi T. and Kawabe Y. (1991). Research on tree diseases on Miyako Island, Kyushu. *Jpn. For. Pests* **40**, 219-224.
- Kobayashi T. and Muramoto M. (1989). Pitch canker of *Pinus luchuensis*, a new disease in Japanese forests. *For. Pests* **38**, 169-173.
- Kobayashi T. and Kawabe Y. (1992). Tree diseases and their causal fungi in Miyako Island. *Jpn. J. trop. Agric.* **36**, 195-206.
- Muramoto M., Tashiro T. and Minamihashi H. (1993). Distribution of *Fusarium moniliforme* var. *subglutinans* in Kagoshima Prefecture and its pathogenicity to pines. *J. Jpn. For. Soc.* **75**, 1-9.
- Viljoen A., Wingfield M.J. and Marasas W.F.O. (1994). First report of *Fusarium subglutinans* f. sp. *pini* in South Africa. *Plant Dis.* **78**, 309-312.
- Viljoen A., Wingfield M.J., Kemp G.H.J. and Marasas W.F.O. (1995). Susceptibility of pines in South Africa to the pitch canker fungus, *Fusarium subglutinans* f. sp. *pini*. *Plant Pathol.* **44**, 877-882.
- Viljoen A., Gordon T.A., Wingfield M.J., and Marasas W.F.O. (1997). Genetic diversity in a South African population of the pitch canker fungus, *Fusarium subglutinans* f. sp. *pini*. *Plant Pathol.* **46**, 590-593.
- Borthwick R.B. and van Rensburg N.J. (1993). Insect pests in South African forest plantations. In *Forestry Handbook*, ed. H.A. van der Sijde, pp. 249-266. Southern Afr. Inst. For., Pretoria.
- Berry C.R. and Hepting G.H. (1959). Pitch canker of southern pines. *USDA For. Serv. For. Pest Leaflet* **35**.
- Leslie J.F. (1993). Mating populations in *Gibberella fujikuroi* (*Fusarium* Section *Liseola*). *Phytopathology* **81**, 1058-1060.