DEVELOPMENT OF A NEW RISK ASSESSMENT PROCEDURE FOR PINEWOOD NEMATODE IN EUROPE

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ABSTRACT

Research, partly funded under the EU PHRAME (Plant Health Risk And Monitoring Evaluation) program has provided new information on the biology and ecology of pinewood nematode (PWN), *Bursaphelenchus xylophilus*, in Portugal. Studies have been carried out by eight partner research teams in six countries (UK—coordinator, Austria, France, Germany, Portugal, Spain). Data gathered have improved our understanding of the interaction of PWN, its vectors and host trees in both a European and a global context. Specifically, *Monochamus galloprovincialis*, has taken on the role of vector in Portugal and results have indicated that it has a single generation a year with a well-defined flight period. Collection of strains of *B. xylophilus* and their mass culture has enabled biological and molecular studies to be carried out, suggesting that there are two strains of the nematode in Portugal. Detailed studies of early infestation of seedling trees in both Germany and Portugal have provided excellent information on rapid movement of nematodes in the tree, followed by onset of wilt symptoms. Data from all partners are being incorporated in a new risk model based on existing process-based models of tree growth developed in the UK. These are driven by the differences between actual and potential transpiration in host trees and the interactions with site, temperature, moisture regimes and presence of nematodes in the tree. Predictions from the new models are being tested using parameters from the known infested area of Portugal. This new approach should allow refined assessment of the likelihood of wilt expression both in Europe and internationally.

Background to the PHRAME program

Pinewood nematode (PWN), *Bursaphelenchus xylophilus (*Nematoda: Aphelchoididae*)*, is native to North America where it exists primarily in a saprophytic mode linked closely to the oviposition activities of vectors in the genus *Monochamus* (Coleoptera: Cerambycidae). Nematodes will also enter the tree through maturation feeding wounds made by adult *Monochamus*. Under certain conditions, thought to be mainly driven by high summer temperatures and availability of susceptible tree species, trees can exhibit rapid wilting symptoms followed by early mortality. This is rare in North America, but has been highly prevalent when the nematode has established in new regions, notably in Japan, China, Korea and, from 1999, in Portugal. In each new situation the local species of *Monochamus* has taken on the role of vector and in most cases have proved highly effective in transmission of nematodes from tree to tree, usually at local scales.

Although there have been studies of how *B. xylophilus* compromises the vascular system of host trees resulting in loss of water flow in the xylem, cavitation and wilt expression, it has not been possible to determine precisely how the nematode results in such rapid effects, even on large mature trees.

The EU research program PHRAME (Plant Health Risk And Monitoring Evaluation) has addressed the whole spectrum of interactions between PWN, its vectors and tree susceptibility in the context of the recent establishment of the nematode in Portugal. The partnership involved is indicated in the following table:

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More detailed information on the Work Packages within the program can be found on the consortium website: *http://www.forestresearch.gov.uk/fr/INFD-63KGEF.* The key findings from research carried out since the program started in February 2003 are summarized here. Contact with the respective partners should be made if further information is required on particular topics.

Vector identification and biology

Within Portugal, Edmundo Sousa and colleagues, have studied PWN infested trees and have examined a wide range of bark and wood-boring beetles associated with those trees. Although many beetle species had colonized the trees, the only insect known to be an effective vector of PWN was *Monochamus galloprovincialis*, which is found in several European countries. Emergence commences in mid-May and is usually complete by late August; flight periods extend this by up to 1 month. Within the known PWN-infested area, up to 75 percent of *M. galloprovincialis* captured in traps were found to carry nematodes. However, surveys by Maria Arias and colleagues in Spain did not detect any *B. xylophilus* in the *M. galloprovincialis*. Feeding activity by *Monochamus galloprovincialis pistor* was studied by Christian Tomiczek and co-workers in Austria. Feeding was greatest in the first 2 weeks after emergence and the adults showed preferences for cut branches compared with whole seedling trees. This rapid early feeding phase is also coincident with the peak of nematode transmission by the beetles (Edmundo Sousa).

Surveys have provided collections of a range of *Bursaphelenchus* species and these have been categorized and many are now in culture collections maintained by Manuel Mota in Portugal. This valuable resource is providing material for molecular analysis of the variation in different populations of *B. xylophilus* which is being carried out by Wolfgang Burgermeister in Germany and Philippe Castagnon-Sereno in France. RAPD and satellite DNA techniques are being used in a complementary fashion to determine the phylogeny of the strains of nematodes present in Portugal. This will provide information to help determine the likely origin and pathway of entry of nematodes to Europe.

Work by Sam Evans and Makihiko Ikegami in the UK is concentrating on novel methods for assessing the likelihood of wilt expression in trees where the nematode has been introduced by maturation feeding. The basis for this approach

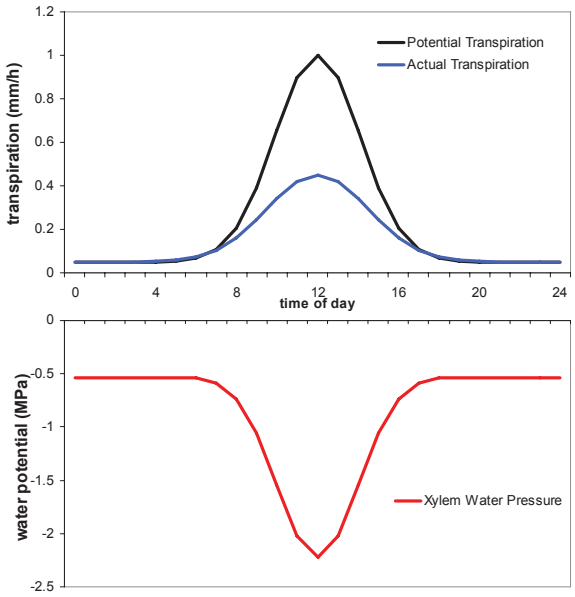

Figure 1: The difference between potential transpiration and actual transpiration expressed as xylem pressure (MPa).

is to employ process-based models of tree growth that have already been developed by staff at our institute. The key variables are those related to potential and actual transpiration by the host tree. This is illustrated in Figure 1, where the difference between potential and actual transpiration is expressed as xylem pressure in increasingly negative MPa (megapascals).

As water demand rises in a situation where water supply is constant the xylem pressure increases (greater negative value) and there is a risk of cavitation in xylem vessels. In most situations trees will reduce the deficit by reducing transpiration through stomatal closure during peak demand when the plant is photosynthesising in daylight. Recovery, even from quite severe reductions in xylem pressure and potential cavitation usually takes place during the hours of darkness. However, when PWN is present some of the xylem vessels are irreversibly blocked and this compromises the ability of the tree to recover from episodes of high-negative xylem pressure. The process based models of tree growth have been adapted to simulate the presence of PWN under a range of eco-climatic conditions. Current simulations have included (i) normal temperatures and water availability;

(ii) higher than average temperatures but normal water availability; and (iii) reduced water availability but normal temperatures all with and without the presence of PWN. Early indications from simulations runs of the model are that high temperature conditions, with normal water availability tend to result in onset of irreversible cavitation and onset of wilt expression. Interestingly, high temperatures, but higher than normal water availability, did not result in irreversible wilt, even though there was a sharp decline in xylem pressure. Similarly, low water availability (drought conditions) but normal temperatures did not always result in irreversible wilt; we speculate that under low water availability this is due to effective shutting down of transpiration and under high water availability water uptake can compensate for all levels of transpiration.

We are now exploring a wider range of parameters to test the models and also including the exploitation of carbon by the tree, with a particular view to simulating how the tree apportions and subsequently employs carbon for either growth or storage/defence. Early results suggest that apart from immediate effects of water availability, there may be carry-over effects on carbon usage that could compromise early growth and bud burst in the spring following nematode infestation in the previous summer or autumn.

Future Work

Although the new process-based models offer a novel procedure for assessing risks based on input of eco-climatic parameters, they still need to be verified in a field situation. This work is likely to be carried out during 2006 when field inoculation with PWN will be carried out on 10-year-old trees in Portugal and detailed measurements of a range of physiological parameters relevant to the input criteria for the models will be carried out. These data will be used to improve the models and to extend their use to refine the Pest Risk Analysis for PWN in Europe and globally.

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