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Timber Industry Presents Magazine

Genetic breakthrough?

A new DNA marker resource for genome-assisted breeding of pine trees.

A new perspective on pesticides

The revised FSC® Pesticide Policy means new rules for foresters to follow, but will it change the way they think?

The enemy of my enemy is my friend!

Using natural enemies of insect pests for their management is an old and generally well-known approach. But is this approach still valuable within the current and future plantation forestry landscape?

FORESTRY SOUTH AFRICA™

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CARRYING ON IN THE FACE OF ADVERSITY

THE FORESTRY SECTOR ONCE AGAIN SHOWCASING ITS DETERMINATION, ADAPTABILITY AND RESILIENCE

> “*...across the globe, Forestry is a challenging sector to be in. On a daily basis we need to deal with economic, social, biotic and abiotic challenges.*”

I BEGAN the introduction to the last issue of TIP-Mag, stating how, “*across the globe, Forestry is a challenging sector to be in. On a daily basis we need to deal with economic, social, biotic and abiotic challenges.*” At the time of writing this, I could not have imagined the challenges 2020 and the COVID-19 pandemic had in store for the Industry, the Sector, South Africa, and the World.

Yet, while the pandemic has brought a multitude of additional COVID-19-related health, financial, logistical, and social challenges, the Sector still needed to continue to adapt, evolve and grow in response to the existing abiotic challenges that clearly did not get the memo about going into isolation.

If anything, 2020 has placed even more emphasis on the Sector's ability to adapt, its need to be both proactive and progressive and applying a holistic approach to forest management, utilising new technology and embracing the implementation of innovation. As I previously mentioned, “*none of this would be possible without the Sector's strong knowledgebase, generated by world class research and supported by financial investment into Research and Development.*” This investment is still more than double the country average despite the increasingly challenging economic situation the Sector currently faces.

The Industry's strong research-base will be critical in moving the Sector forward as it negates the challenges currently faced and, if the Sector's role in the globally identified green economic response to the COVID-19 crisis is realised, it will also play a role in South Africa's economic recovery.

As FSA Director of Research and Protection, 2020 has made me even more proud of the world class research being conducted by the Sector, both in house and by our research partners, especially when we consider the 2020 restrictions that have had to be put in place.

As mentioned in the opening statement, the dissemination of the research being produced by the Sector is still one area that needs to be worked upon. This is critical in enabling its implantation and increase our return on investment. Back then, I suggested that this is where the TIP-Mag may constructively contribute to the Sector as a whole, “*providing a platform for researchers and students involved in forest research to publish work that might not fit the criteria for peer-reviewed scientific journals but is still of exceptional quality and valuable to the Industry*”. Reading through the pre-published version of this issue, after receiving so much positive feedback from the first, I feel TIP-Mag not only has provided this platform it has also made innovative and complex science available to a far wider audience. This is something that everyone involved in both publications should be immensely proud of.

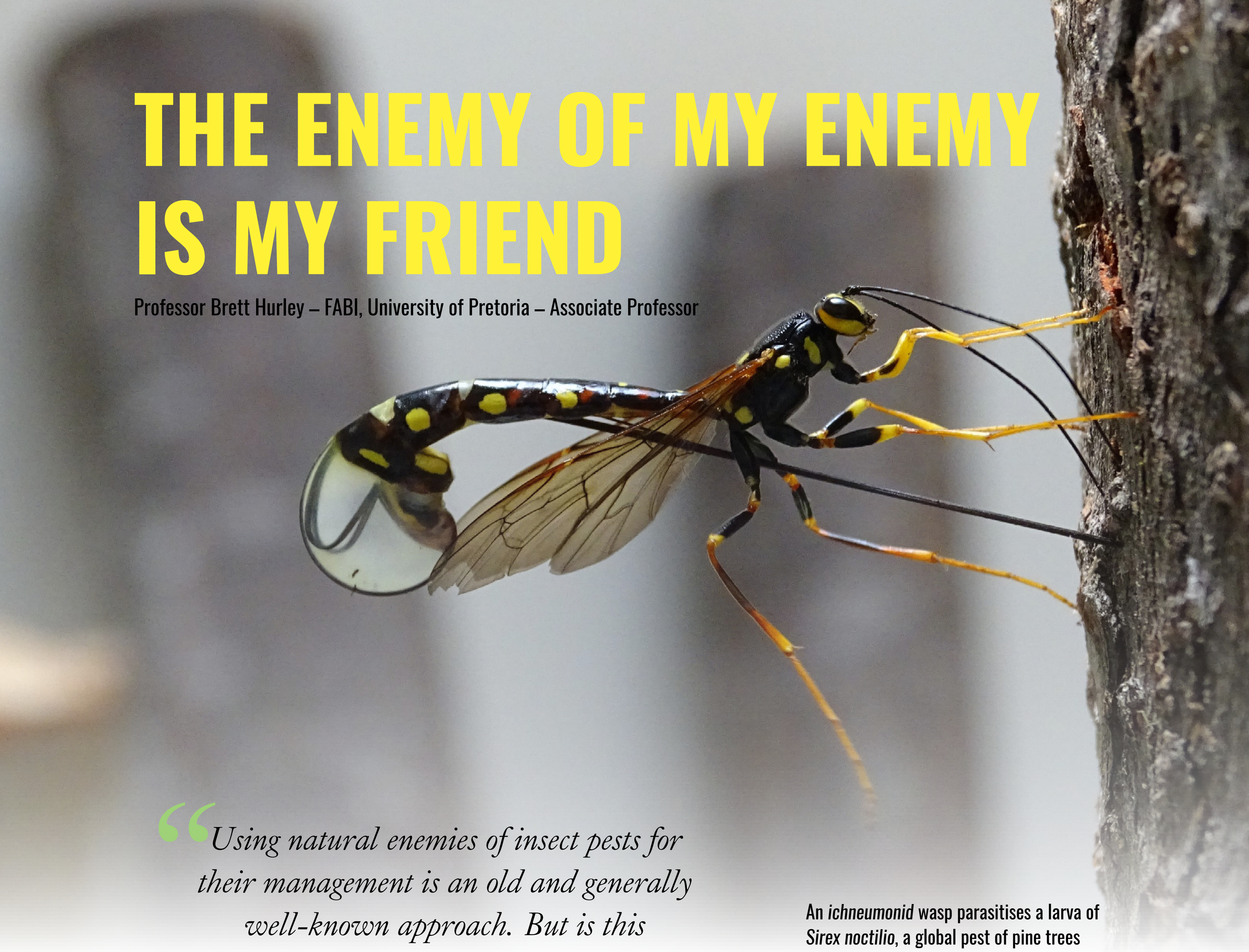
After reading through this issue, I am excited about the next two that are scheduled for publication in 2021 and urge everyone who is conducting research in the Sector to consider contributing. For those not actively involved in forestry research, I urge you to find a few minutes to thumb through this second issue of TIP-Mag and to get acquainted with some of the incredible work that will no doubt shape our Sector in the future.

May you enjoy reading and interrogating this second issue of TIP-Mag.

Dr Ronald Heath
FSA Director:
Research and Protection

THE ENEMY OF MY ENEMY IS MY FRIEND

Professor Brett Hurley – FABI, University of Pretoria – Associate Professor



An *ichneumonid* wasp parasitises a larva of *Sirex noctilio*, a global pest of pine trees

> *"Using natural enemies of insect pests for their management is an old and generally well-known approach. But is this approach still valuable within the current and future plantation forestry landscape?"*

The opinion shared in this article are the views of the author and not necessarily those of the University of Pretoria

THE PROVERB '*the enemy of my enemy is my friend*' is known from as early as the 4th century BC, where it is mentioned in the ancient Indian Sanskrit treatise, the Arthaśāstra. The doctrine underpinning this proverb has subsequently been referred to and followed in numerous major historic events, perhaps most notably the cooperation between the Allies (US and Britain) and the Soviet Union against the common Nazi enemy in World War II.

This proverb is also useful in explaining the pest management approach known as biological control. Simply put, this involves the use of a pest's natural enemies to reduce the population of that pest in the environment where it is not wanted. This approach is nearly as old as the proverb itself, being first reported around the 3rd century AD.

But is this approach still valuable as a pest management approach? Especially within today's plantation forestry environment, where new pests are being reported at an exponential rate and budgets to manage these pest incursions are tight.

THE VALUE: CURRENT AND POTENTIAL

Interestingly, biological control is particularly suited for use in plantation forests, due to the longer rotations in forestry, as compared to most agricultural crops, which offers

Prof Brett Hurley

> *"The action of these unsung, and largely unknown, heroes likely saves the forestry sector millions every year."*

more stability in the environment for the natural enemies to establish. Thus, establishment rates and successes of natural enemies are higher on pests of woody plants than on pests of herbaceous plants.

Most of the biological control used in South African plantation forests has been classical biological control (CBC) which involves the intentional introduction of biological control agents, usually non-native, with no further intervention. This approach is generally used as a tactic against non-native pests. A recent example is the release of a parasitic wasp (*Selitrichodes neseri*) of the Eucalypt gall wasp (*Leptocybe invasa*). Limited releases of hundreds of these parasitic wasps have resulted in their establishment (of likely millions or trillions) across eucalypt growing areas of the country – at very little initial cost.

Perhaps the best-known biological control programme in South African plantation forests is that of the Sirex woodwasp. Here, a form of augmentative biological control has been used, involving the mass production and annual inoculations of thousands of Sirex-infested trees with a parasitic nematode (*Deladenus siricidicola*). Contrary to a CBC programme, this augmentative approach involves continual expenses associated with the nematode production and in-field releases. That said, evaluations of the South African Sirex Control Programme showed that the reduction in losses associated with the use of biological control exceeded the costs by approximately R350 million over ten years.

Inoculations of a parasitic nematode to control populations of the *Sirex* woodwasp

Besides the economic value of biological control, this approach also offers an alternative to the use of insecticides when the use of the latter is not feasible due to environmental, regulatory or cost considerations. In some cases, biological control is the only effective management option available.

What is generally not considered is the massive value provided by natural biological control. This refers to the effect of indigenous biological control agents already present in the environment. For example, naturally occurring small tetraviruses that are thought to regulate populations of the pine emperor moth, causing muscular flaccidity and internal liquefication of the larvae. The action of these unsung, and largely unknown, heroes likely saves the forestry sector millions every year.

But perhaps of greater relevance is the potential to improve the role of these agents in regulating the pest populations. A conservation biological control approach involves modifying the environment or existing practices to protect and enhance the populations of existing natural enemies. This could be by providing a food source or shelter for the natural enemies. Such approaches are used very successfully for forestry pests in countries such as Colombia but remain largely unexplored in South Africa.

THE CHALLENGE… AND PROPOSED SOLUTIONS

Of course, if biological control was the 'silver bullet' for pest management, we would have no pest problems – and that is certainly not the case.

One of the benefits of biological control is also one of its challenges

– biological control agents, at least in a CBC approach, are host-specific. That's great news in terms of not having non-target effects, an attribute that often makes biocontrol preferable over insecticides. But it means that a biological control programme needs to be developed for each insect pest. This can be a costly and lengthy process; and, of course, not guaranteed to result in successful control of the pest.

A commonly acknowledged strategy within the biological control field to reduce this challenge is collaboration. The growing movement and establishment of many of the major insect pests is in a sense a saving grace, as it means there are many institutes and countries around the world with the same problem. Sharing of information and even the biological control agents themselves is commonplace between research programmes working on insect pests of plantation trees, thanks to initiatives such as BiCEP (Biological Control of Eucalypt Pests, bicep.net.au).

Changes in climate, new pest introductions and further introductions of new diversity of current pests complicate biological control approaches. Broader collections of biological control agents need to be considered and tested, to better match the agent with the pest population and climate. Collaborative networks can help to offset these challenges.

As with insecticides, the use of biological control needs to be regulated. Introduction of non-native species need to be tested for potential non-target effects on native or beneficial species, and new biological control products, such as entomopathogens, need to be registered. This can be a timely and very costly process.

In my experience, engaging with the government and other relevant bodies to provide input for the development of responsible and efficient regulatory and implementation policy is critical. Not many governments have the capacity to develop policy in response to the growing and changing needs

Release of minute parasitic wasps imported from Australia for the control of an invasive eucalypt pest

associated with pest management options. Here, internationally relevant guidelines can be helpful, such as the recent publication of "Guide to the classical biological control of insect pests in planted and natural forests" by the Food and Agricultural Organization (FAO) of the United Nations (http://www.fao.org/documents/card/en/c/CA3677EN/).

But perhaps the biggest challenge with biological control is this – sometimes it just doesn't work. The reality is that although biological control is often a key tool for the management of forestry pests, this is not always the case. And even for insects where biological control has been used successfully, the addition of other approaches could be very valuable. Thus, an integrated approach, considering other viable control options such as host resistance, silvicultural control and chemical control is a must.

LOOKING AHEAD

Despite its long history, I believe the potential of biological control as a pest management strategy remains largely untapped. There have been many success stories of biological control to manage forest insect pests, in South Africa and abroad. But for this approach to remain a viable tool in a rapidly changing environment, we will need to adapt. Increased collaborations, including with relevant government departments; improved monitoring and optimizing of biological programmes to address potential drops in efficacy from new pest strains or a changing climate; and better use of natural enemies that are already present.

We will certainly face an increasing number of pest 'enemies' in the future. Some of these could threaten the sustainability of the forestry sector. It will be important not to forget our friends.

ADDRESSING COMMON CHALLENGES ON SOUTH AFRICAN SOIL: AN INTERNATIONAL COLLABORATION EFFORT

Noxolo Ndlovu – Nelson Mandela University – PhD student

"and though a man might prevail against one who is alone, two will withstand him – a threefold cord is not quickly broken"

– Ecclesiastes 4v12

Although this verse was addressed to man, the principle holds for the global forestry sector – together, we are stronger!

GLOBALLY, forest plantations face common challenges: from uncertainties associated with climate change; to the growing negative impacts of pests and diseases; and pesticide use restrictions posed by certification bodies. The list is extensive.

International collaboration provides an opportunity to address common problems using a diverse array of divergent mindsets, differing experiences, backgrounds, and approaches. This inevitably results in richer ideas and fast tracks progress towards finding solutions, especially if resources beyond intellect are pooled.

NEW ZEALAND: LEADING THE WAY WHEN IT COMES TO ENVIRONMENTAL PESTICIDE FATE

In the past two decades, increased emphasis has been placed upon reducing reliance on pesticides. For certified plantations and those seeking certification, this has meant sometimes discontinuing the use of pesticides that provide the only viable (cost-effective and efficient) option for the management of an economically important pest or disease, because of their hazard classification by certification bodies. Needless to say, these prohibitions pose challenges to forest managers and the sustainability of the global forestry sector.

The hazard classification criteria of pesticides, used by certification bodies, is often founded on laboratory-based studies and/or field studies conducted in climates and/or use patterns which are not relevant to forest plantation conditions. As a result, pesticides that might pose little or no risk when used within the plantation landscape, in adherence to legislation and best practice, have been banned.

Following the restriction or prohibition of hexazinone and terbuthylazine, two of the most important herbicides for use within New Zealand (NZ) forest plantations, New Zealand forestry stakeholders took the initiative to implement a series of trials. These trials not only aimed to find alternative 'environmentally acceptable' vegetation management methods (Rolando, Gous & Watt 2011a) but also aimed to determine the actual environmental fate and risk posed by these two herbicides when used under NZ environmental conditions and industry-use patterns (Baillie, Neary, Gous & Rolando 2015; Baillie 2016; Garrett, Watt, Rolando & Pearce 2015; Garrett, Watt & Pearce 2016). When compared to vegetation management with terbuthylazine and hexazinone, alternative methods were associated with growth losses of up to 30% (Rolando, Todoroki & Watt 2015) and reduced internal rate of return ranging from 0.5 to 1.7% (Rolando, Watt & Zabkiewicz 2011b). In addition, and perhaps more importantly, environmental fate trial results showed that, under NZ forestry use patterns and conditions, hexazinone and terbuthylazine pose a low and short-term risk to aquatic and terrestrial environments. This led to the reinstatement of these two herbicides in New Zealand, almost eight years after the restriction (Rolando *et al.* 2015). This was a great relief to the industry especially since alternative methods proved less economically viable and using herbicides under derogation meant applying herbicides as a spot-spay (instead of the normal practice of broadcast). Spot spray applications posed challenges in that they were limited by labour availability and suitability of the area (Rolando *et al.* 2011b).

SOUTH AFRICA: FOLLOWING IN NEW ZEALAND'S FOOTSTEPS AND TAKING THE RESEARCH FORWARD

The South African forestry industry is already limited in terms of the

Noxolo Ndlovu

Conducting stream flow measurements

number of pesticides registered for use within the forestry landscape. This has been further reduced in certified plantations as a result of the restrictions and prohibition put in place by certification bodies to protect human health and reduce environmental impact. Yet, with no pesticide environmental fate studies related to South African silvicultural regimes and environmental conditions, it is difficult to know the impact of pesticides on aquatic and terrestrial environments. Consequently, without this information, it is impossible for national legislation and certification bodies to make informed decisions regarding the actual impact certain pesticides will have in South African forestry conditions.

To address this gap, a partly Forestry South Africa (FSA) funded study investigating the fate of key, operationally used pesticides was initiated as a collaboration between Nelson Mandela University (South Africa) and New Zealand forestry pesticide fate experts. With NCT Forestry, Nelson Mandela University and FP&M SETA also providing funding for this crucial work.

Water and sediment sampling

In 2019, PhD student Noxolo Ndlovu visited New Zealand to interact with numerous pesticide environmental fate experts, including Dr Brenda Ballie and Dr Carol Rolando. Noxolo drew knowledge from guided experience in trial implementation, data collection procedures, tools required, mistakes to avoid and tips for project success from an established team of experts who had designed, developed, implemented and improved many of the methodologies that would be employed during the South African study. Trials in South Africa began in January 2020, with data collection still ongoing. The first output of the project will be submitted for publication in December 2020. The project is due for completion in December 2021. Despite challenges relating to COVID-19 restrictions, the study looks set to yield several interesting results that have the potential to alter the way pesticide use is governed in South African certified plantations.

International collaboration, between scientists, working ultimately towards the same goals just in different continents, has been key to the success of this project. The guidance, support, patience, and expertise of supervisors working in separate times zones and yet equally invested in the success of the project is illustrative of the potential international collaborations can produce. The question now is, *where to next?*

REFERENCES

Baillie B. 2016. Herbicide concentrations in waterways following aerial application in a steepland planted forest in New Zealand. *New Zealand Journal of Forestry Science* 46:16.

Baillie BR, Neary DG, Gous S, Rolando CA. 2015. Aquatic fate of aerially applied hexazinone and terbuthylazine in a New Zealand planted forest. *Journal of Sustainable Watershed Science & Management* 2: 118–129.

Garrett LG, Watt MS, Pearce SH. 2016. Environmental fate of terbuthylazine and hexazinone in a planted forest steepland Recent Soil, New Zealand. *New Zealand Journal of Forestry Science* 46:17.

Garrett LG, Watt MS, Rolando CA, Pearce SH. 2015. Environmental fate of terbuthylazine and hexazinone in a New Zealand planted forest Pumice soil. *Forest Ecology and Management* 337: 67–76.

Rolando C, Todoroki C, Watt M. 2015. Minimising the environmental impact of forest weed management in New Zealand: final report on field trials. *Scion Publication No.* S0015. Rotorua: New Zealand Forest Research Institute Limited.

Rolando CA, Gous SF, Watt MS. 2011a. Preliminary screening of herbicide mixes for the control of five major weed species on certified Pinus radiata plantations in New Zealand. *New Zealand Journal of Forestry Science* 41: 165–175.

Rolando CA, Watt MS, Zabkiewicz JA. 2011b. The potential cost of environmental certification to vegetation management in plantation forests: a New Zealand case study. *Canadian Journal Forest Research* 41: 986–993.

A NEW PERSPECTIVE ON PESTICIDES

Manushka Moodley – FSC Global Development GmbH – Southern African Manager

"A revised FSC Pesticides Policy means new rules for foresters to follow. But more importantly, it should change the way we think."

Pesticides are a feature of many parts of our lives, and as time goes by, we are learning more about less obvious, chronic, cumulative and interacting effects of their use. Sadly, global average pesticide use per area of cropland is much higher now than it was in the 1990s. Agriculture may be the major player in pesticide usage, but how can we in the forestry sector contribute to turning the tide? How can we ensure that our forests remain full of life, and that they support livelihoods without invisible threats to the health of forest workers?

Surely very few forest managers use pesticides uncritically – even if our motivations were purely financial, pesticides might be cheaper than some other control options, but they still cost money. Maybe there are a few people out there who apply pesticides just because that's what they've always done, but on the most part, those applying chemical control agents are going through some sort of thought process beforehand, even if only to decide whether it is worth incurring the cost. With responsible forest managers already thinking far more broadly about environmental and social costs. The challenge is how we take that thinking to the next level, and make sure that all foresters are thinking along the same lines?

The revised FSC pesticides policy is intended to facilitate this, by changing the way we think about pesticides.

PRINCIPLES AND POLICIES – WHAT ARE THEY FOR?

The FSC Principles and Criteria already require forest managers to use integrated pest management and silviculture which avoids, or aims at eliminating, the use of chemical pesticides. They forbid the use of any chemical pesticides prohibited by FSC policy, and when pesticides are used, they require forest managers to prevent, mitigate, and/or repair damage to environmental values and human health. There is a clear role for the FSC pesticides policy here, but what else does it bring to the mix?

An important emphasis of the policy was, and remains, on the control of the use of Highly Hazardous Pesticides (HHPs). It is worth noting that this is not a concept unique to FSC – both the Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) refer to HHPs – but, through the pesticides policy, FSC aims to ensure that the concept is applied consistently and robustly in FSC-certified forests across the world. The aims of the policy go further, the stated short-term objectives are to:

- Promote best practices to minimise associated risks to human health and the environment when using chemical pesticides.
- Reduce the overall volume and number of chemical pesticides in use.
- Eliminate the use of the most hazardous chemical pesticides.

NOT ALL PESTICIDES ARE EQUAL...

In the previous iteration of FSC's policy and procedures, a HHP was a HHP was a HHP – there was no distinction between them, and all were subject to the same controls. The revised policy recognises three different categories of Highly Hazardous Pesticides i.e.: restricted, highly restricted, and prohibited.

Prohibited HHPs are the worst of the worst – chemical pesticides so dangerous that the risks associated with them cannot be realistically managed. Using international conventions regulating global pesticide use, they have been identified on the basis of their inherent properties: if they are acutely toxic and can induce cancer, if they contain dioxins, or if they contain heavy metals. FSC permits their use only in emergency situations or if mandated by a government order.

Restricted and highly restricted HHPs can be used in FSC-certified forests if the requirements of the Policy are met. Restricted HHPs fall into one of the hazard groups acute toxicity, chronic toxicity, or environmental toxicity – highly restricted HHPs fall into two or three of these groups.

Manushka Moodley

This categorisation recognises that there is considerable variation among Highly Hazardous Pesticides, and allows us to take a more nuanced approach to manage the risks they present – this should steer us towards the least hazardous options, and to the most effective management of risk.

NO MORE DEROGATIONS? THANK GOODNESS!

The revised policy does away with the previous system of derogations, whereby anyone who wanted to use a Highly Hazardous Pesticide had to apply to FSC International for temporary permission to deviate from FSC's rules – a derogation. It is fair to say that this system was pretty unpopular. There was a charge for the processing of derogation applications, and decisions were made by a central committee which realistically could not be aware of all the subtleties of every national situation. New policy requirements remove the need for charges and shift a lot of decision making to the national level.

A CONSISTENT APPROACH TO RISK

To replace the centralised derogation system, the revised policy introduced a framework of environmental and social risk assessment (ESRA), with elements operating at international, national and management unit levels.

Broad rules are set by FSC International, including what factors should be considered, and how. The South African Standard Development Group, with support from TIPWG, will adapt the finalised international generic indicators for HHPs and assist with the development and roll-out of the ESRAs.

The Policy requires the identification and assessment of key environmental and social risks associated with each restricted or highly restricted HHP used or likely to be used in the country. Thankfully, the bulk of this work has already been completed by TIPWG for South Africa. Once the approved international generic indicators are released by FSC, standard developers will undergo an assessment of each HHP utilised in South Africa and will engage with stakeholders in this process, which represents the key shift from international to national deliberation.

Individual certificate holders will eventually have to comply with these national indicators and thresholds, alongside the policy ESRA requirements. TIPWG has developed environmental and social risk assessments for South Africa to identify the lowest risk option to control a given pest, weed or disease, giving preference to non-chemical methods over chemical pesticides, non-HHPs over HHPs, and restricted HHPs over highly restricted HHPs. Before foresters apply any chemical pesticide, they must incorporate the results of the ESRA into operational plans, identifying site-specific risks and adapting generic mitigation and monitoring measures accordingly.

Risk assessments might sound bland, but this is powerful stuff, bringing new and consistent thinking to integrated pest management. Every one of us will have to think in terms of hazard levels, and in terms of a broad range of environmental and social risks. We'll even have to discuss those issues at a national level, making informed decisions about what Highly Hazardous Pesticides we think it is acceptable to use in our country. The revised policy might not bring about wholesale change overnight, but it will at least ensure that we are all thinking the right thoughts and moving in the right direction.

BUT WILL IT REALLY CHANGE THE WAY WE THINK IN SOUTH AFRICA?

In short, yes, I hope so. We are already seeing change within the sector with greater levels of risk-based thinking being integrated into Certificate holder's integrated pest management strategies and operational activities. This accompanied with the research into alternatives for restricted chemicals and other HHPs will hopefully yield long term positive results for the foresters, workers and the environment.

WE ALL NEED TO SEE PESTICIDES FROM A NEW PERSPECTIVE

If 2020 has taught us anything, it is that nothing can be taken for granted. A pandemic is just one symptom of the profound influence we have on the natural environment. Pesticides have revolutionised the way we interact with that environment, and while we have gained from their use, we also now face problems entirely of our own making, including threats to our own health. It is likely that some of the damage we have caused cannot now be undone. Are you ready to change your perspective on pesticides before things get worse?

CAN WILD POPULATIONS HELP OUR EUCALYPTS ADAPT TO CLIMATE CHANGE?

AN ASSESSMENT OF WILD *EUCALYPTUS GRANDIS* POPULATIONS IN SEARCH OF ADAPTIVE GENETIC VARIATION

Marja Mostert-O'Neill and Professor Zander Myburg – Forest Molecular Genetics (FMG) Programme, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria

Any opinions shared in this article are the views of the authors and not necessarily those of the University of Pretoria

FOR A CENTURY since the first import of *Eucalyptus grandis* seed in 1896 (then still incorrectly identified as *E. saligna*), the species and its hybrids have been the darlings of eucalypt forestry in South Africa. Throughout its cultivation history, foresters and breeders had to navigate several challenges and, in some cases, resorted to hybrid breeding to overcome them. For instance, in response to stem canker disease, the species was hybridized with disease-tolerant *E. urophylla*, and in order to cultivate *E. grandis* in cooler regions, *E. grandis* x *E. nitens* hybrids were developed. An even greater challenge faces us today in the form of rapid climate change. Weather conditions are forecast to become more extreme and the already erratic distribution of pests and diseases will become harder to predict. As such, varieties – be it improved families or hybrid clones – that have been favoured in the past may no longer perform as anticipated under future climate conditions. To navigate the expected change in abiotic and biotic threats, readily available genetic diversity is required.

The natural species range of *E. grandis*, along the east coast of Australia, spans several climate zones from subtropical provenances in the north to temperate areas in the south (Fig. 1). Only a fraction of this genetic diversity has been imported and advanced in South African breeding programmes. Despite its commercial importance, not only in South Africa but also globally in subtropical and temperate regions, little is known about the untapped, potentially adaptive, genetic diversity harboured in the natural species range. To this end, we set out to profile wild *E. grandis* populations in terms of genomic diversity and adaptation to geographic, climatic, and soil-related variables in a study published in *Molecular Ecology* (Mostert-O'Neill *et al.*, 2020). We imported and germinated 193 individual tree seed families from 33 provenances spanning the species distribution (Fig. 1). The seedlings were later established by Mondi Ltd in a Gene Conservation Park in the KwaZulu-Natal Midlands (Fig.3).

Marja Mostert-O'Neill

> *"These "hybrid provenances" may represent valuable variation for future genetic infusion into South African breeding programmes."*

We combined population genomics (genetic profiling of hundreds of individuals using approximately 20 000 molecular markers that cover the entire length of the 11 chromosomes of *E. grandis*) with environment association analysis to uncover genes that may underlie adaptive differences between provenances. As would be expected, the genes identified were enriched for abiotic and biotic stress response functions. We also identified

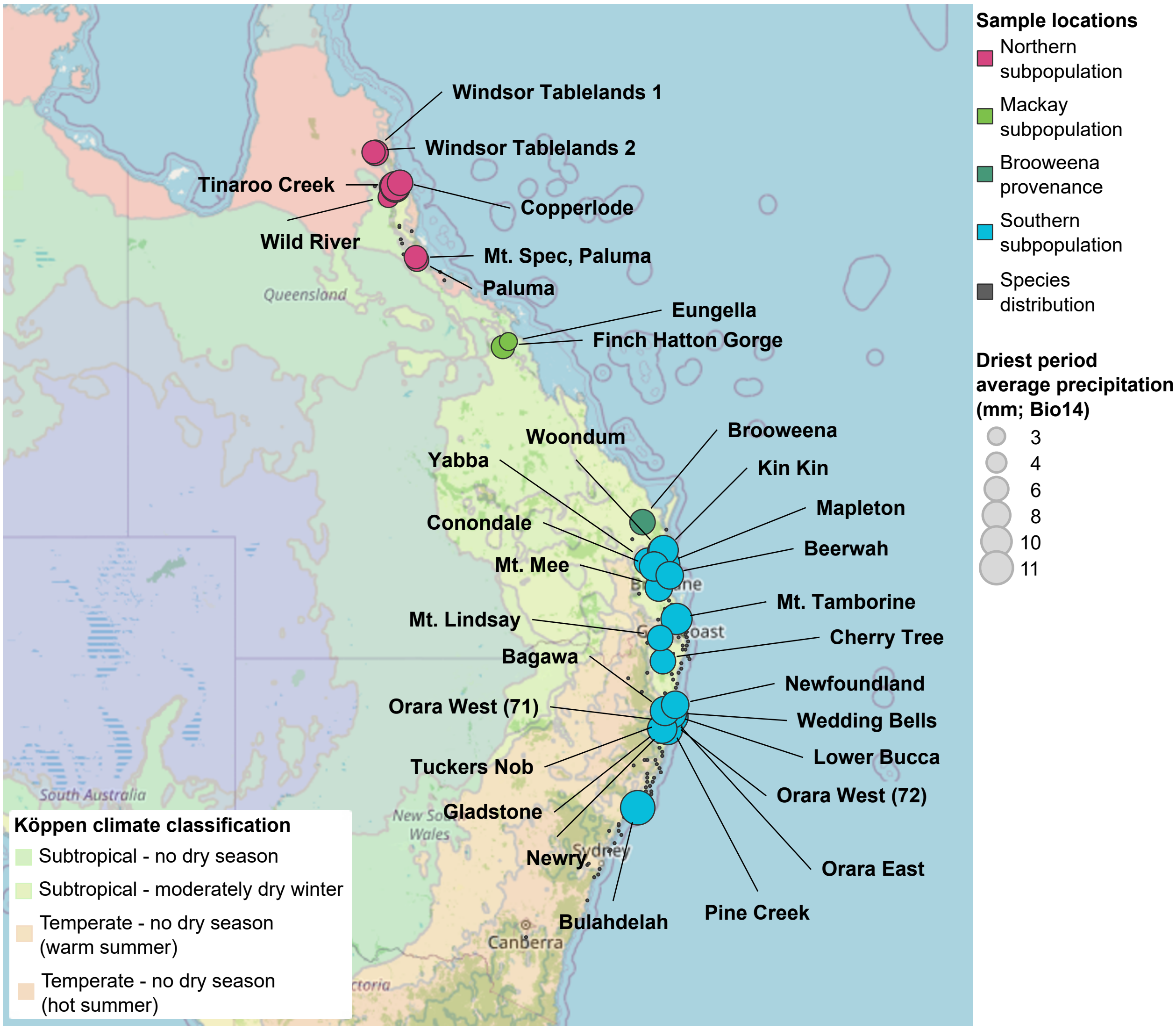


Fig.1. Sampling locations of wild *E. grandis* populations along the east coast of Australia. Three provenances (green circles), Eungella and Finch Hatton Gorge in the Mackay subpopulation, and Brooweena at the northern edge of the Southern subpopulation, had extensive introgression from one or more unknown, non-*E. grandis* sources. Circle circumferences indicate average precipitation during the driest period. Map shading indicates Köppen climate classification with the four main climate zones sampled given in the colour legend.

provenances that carry genetic footprints of natural introgression from other (non-*E. grandis*) species, thought to be in response to increased aridity (Fig. 1). These "hybrid provenances" may represent valuable variation for future genetic infusion into South African breeding programmes.

To investigate adaptive hybridization further, we modified a method to map the genetic ancestry (species origin) of blocks of DNA throughout the genome. This method, called ancestry mapping, allowed us to trace which segments of the genome originated from *E. grandis* and which originated from the hybrid partner/s in natural populations (Fig.2). This method can also be used in hybrid breeding programmes to trace the inheritance of genomic segments, harbouring genes with adaptive or economically important functions, originating from hybridizing partner species such as *E. urophylla*. Furthermore, it allows us to identify cryptic or unintended hybrids within pure species populations, even if the identity of the introgressing species is unknown.

This study revealed that wild *E. grandis* populations contain adaptive genetic variation that may assist us in navigating future climate conditions in South Africa. It also showed that hybridization remains an important means of introducing adaptive genetic variation, which may allow a species to prevail beyond its predicted niche. Lastly, advances in eucalypt genomics and genetically profiled reference populations enable us to trace the genetic origin of individual genome segments. In collaboration with Sappi Forests and

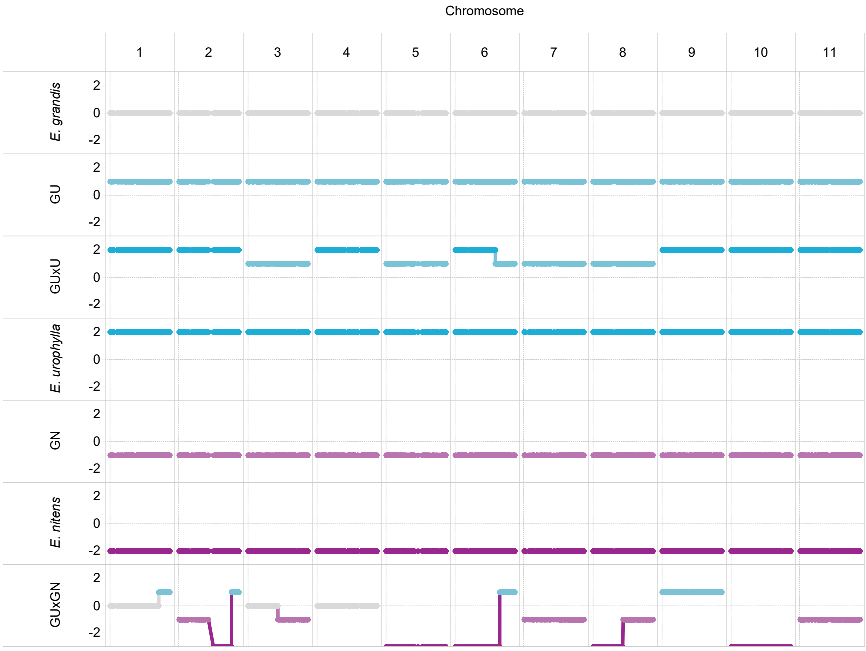


Fig.2. Ancestry maps of DNA segments for pure species and hybrid *Eucalyptus* individuals of known ancestry. Ancestry assignments along the 11 chromosomes are given for seven individuals; pure *E. grandis*, *E. grandis* x *E. urophylla* F1 hybrid (GU), *E. grandis* x *E. urophylla* backcrossed to *E. urophylla* hybrid (GUxU), pure *E. urophylla*, *E. grandis* x *E. nitens* F1 hybrid (GN), pure *E. nitens*, and a three way hybrid (GUxGN). Each colour dot represents a single DNA marker (7736 markers across 11 chromosomes). Grey shade indicates that both alleles originate from *E. grandis* (value of 0); while blue (value of 2) and purple (value of -2) shades indicate that both alleles have non-*E. grandis Latoangulatae* and *Maidenaria* ancestry, respectively. Intermediate values indicate mixed ancestry (one allele originates from *E. grandis* and the other allele originates from another species). A value of -2.5 indicates that one allele assigned to non-*E. grandis Latoangulatae* and the other allele to *Maidenaria* ancestry. The non-*E. grandis Latoangulatae* ancestral reference population used in this analysis included *E. urophylla* and *E. saligna* individuals. The *Maidenaria* ancestral reference population included *E. dunnii*, *E. nitens* and *E. viminalis*.

Mondi Ltd, 190 families used in this study are being established in two large common garden trials in Zululand and the KwaZulu-Natal Midlands, with ~3400 individuals each. In addition, other genotype-by-environment (GxE) interaction trials are being established in various locations around the world by Camcore using 108 of these seed families. These trials will be used for landscape genomics studies whereby genetic profiles, phenotypes and environmental variables are combined to model genotype-by-site compatibility.

ACKNOWLEDGEMENTS:

This study was made possible through financial support from the National Research Foundation (NRF) of South Africa - Bioinformatics and Functional Genomics Programme (BFG Grant UID 97911), the Technology and Human Resources for Industry Programme (THRIP Grant UID 96413), the Department of Science and Innovation and Technology Innovation Agency (DSI/TIA, Strategic Grant for the *Eucalyptus* Genomics Platform), the Forestry Sector Innovation Fund (FSIF *Eucalyptus* Genome Diversity Atlas grant) and by the Forest Molecular Genetics (FMG) Industry Consortium at the University of Pretoria. We would also like to thank Sappi Forests and Mondi Forests South Africa for support towards seed acquisition and establishment of the Gene Conservation Park, respectively. Lastly, we would like to acknowledge Camcore (North Carolina State University) and the Department of Agriculture and Fisheries (Queensland, Australia) for assistance with seed imports.

Fig.3. Mondi Ltd personnel establishing wild *E. grandis* germplasm in a Gene Conservation Park in the KwaZulu-Natal Midlands (photo credit: Mr Mmoledi Mphahlele)

GENOMIC SELECTION, A REVOLUTION IN TREE BREEDING

A COMPARISON OF TRADITIONAL AND GENOMIC BREEDING STRATEGIES IN EUCALYPTUS GRANDIS

Mmoledi Mphahlele – Mondi South Africa – Research and Development Department

Professor Zander Myburg – Forestry and Agriculture Biotechnology Institute (FABI) – Director of Forest Molecular Genetics (FMG) Programme

A lay-summary of research recently published.

Mphahlele, M.M., Isik, F., Mostert-O'neill, M. M., Raynolds, S. M., Hodge, G. R., and Myburg, A. A. (2020). Expected benefits of genomic selection for growth and wood quality traits in Eucalyptus grandis. Tree Genetics & Genome 16, 49. https://doi.org/10.1007/s11295-020-01443-1

AS CLIMATE change and global trade accelerate, associated biotic and abiotic challenges are increasingly affecting commercial forestry plantation areas in South Africa. In particular, the commercial deployment of *Eucalyptus grandis*, formerly a premier species for pulp and paper production, has dramatically declined due to pest and pathogens. Similar threats are imminent for many of South Africa's other commercial forestry species. This, together with the increasing demand for sustainable wood products, means faster and more agile breeding approaches are needed to mitigate risk factors while ensuring gains to sustain the growth in the wood-derived bioenergy, biomaterials (including pulp, paper and timber) and biochemical (biorefinery) industries. Traditional tree breeding approaches face many challenges, including long rotation cycles associated with late reproductive maturity, delayed expression of commercially important traits (rotation age volume, density, pulp yield and timber quality) and the cost of land for long-term field trials. There is a need to fast track tree breeding to accelerate gains in growth, wood quality and tolerance to biotic and abiotic stressors.

"A complete reconfiguration of the breeding steps of the traditional breeding approach is warranted."

Mmoledi Mphahlele

Genomic selection (GS) is a breeding approach that predicts the genetic merit of individuals, based on their genetic make-up as determined using DNA markers that detect genetic variation throughout all of the chromosomes of the tree (the genome). Genomic estimated breeding values can be obtained for trees while they are still seedlings, i.e. without having to wait many years to determine the tree's genetic merit based on field trial measurements. Genomic prediction of tree breeding values is based on statistical modelling of genomic (DNA marker) and phenotypic (trait) data of individuals in a training population. Adopting GS as an alternative or a complementary tree breeding strategy for growth and wood quality traits requires a practical demonstration of its benefits compared to traditional breeding approaches.

E. grandis is one of the "Big Nine" eucalypt species (together with *E. urophylla, E. tereticornis, E. camaldulensis, E. saligna, E. dunnii, E. pellita, E. nitens, E. globulus,* and their hybrids) planted around the globe. The availability of a reference genome sequence (Myburg *et al.* 2014) and development of a *Eucalyptus* single nucleotide polymorphism (SNP) marker chip (used to simultaneously genotype hundreds of individuals using 10,000s of DNA markers) have created new opportunities for genetic analysis of *Eucalyptus* breeding populations.

In a study recently published in *Tree Genetics and Genomes* (Mphahlele *et al.*, 2020), the authors interrogate the benefits of adopting a GS breeding approach for growth and wood quality traits (fibre length, fibre width, cellulose, SG ratio, density, diameter and height) in an *E. grandis* breeding programme. The training population consisted of 1,575 individuals, phenotyped for growth and wood quality traits, and genotyped for 15,000 genome-wide SNP markers. This resulted in prediction accuracies ranging from 0.47 (for diameter growth) to 0.67 (for wood fibre width), which are achieved at the seedling age and equivalent to what breeders would achieve at rotation age.

Using potted orchards and flower induction using chemical treatments (such as paclobutrazol), tree breeders can reasonably achieve a four-year GS breeding cycle compared to the eight-year traditional breeding cycle for *E. grandis*, thereby greatly improving gains per unit time. The relative efficiency which is the ratio of the four-year GS breeding cycle compared to the eight-year traditional breeding cycle ranged from 1.20 (20%, for wood density) to 1.62 (62%, for fibre length). The efficiency increases with even shorter GS breeding cycle times, pointing to the importance of early flower induction approaches. The benefit ratio in terms of accumulated genetic gains over an 18-year period including three GS cycles and two traditional breeding cycles ranged from 1.53 (for diameter) to 3.35 (for wood density). Together these results suggest that GS breeding approaches can significantly improve genetic gains per unit time in *E. grandis*.

Expanding the traditional and genomic breeding approaches to include commercial deployment opportunities, we show that over an 18-year period the traditional breeding approach should result in three clonal trials and clonal seed orchards (CSO) as demonstrated in Figure 1. Clonal seed orchards will allow the deployment of commercial seedlings, whereas the clonal trials will result in improved clonal varieties. Over the same period, the GS approach should result in four clonal trials and CSOs, as demonstrated in Figure 2. In the GS approach, the

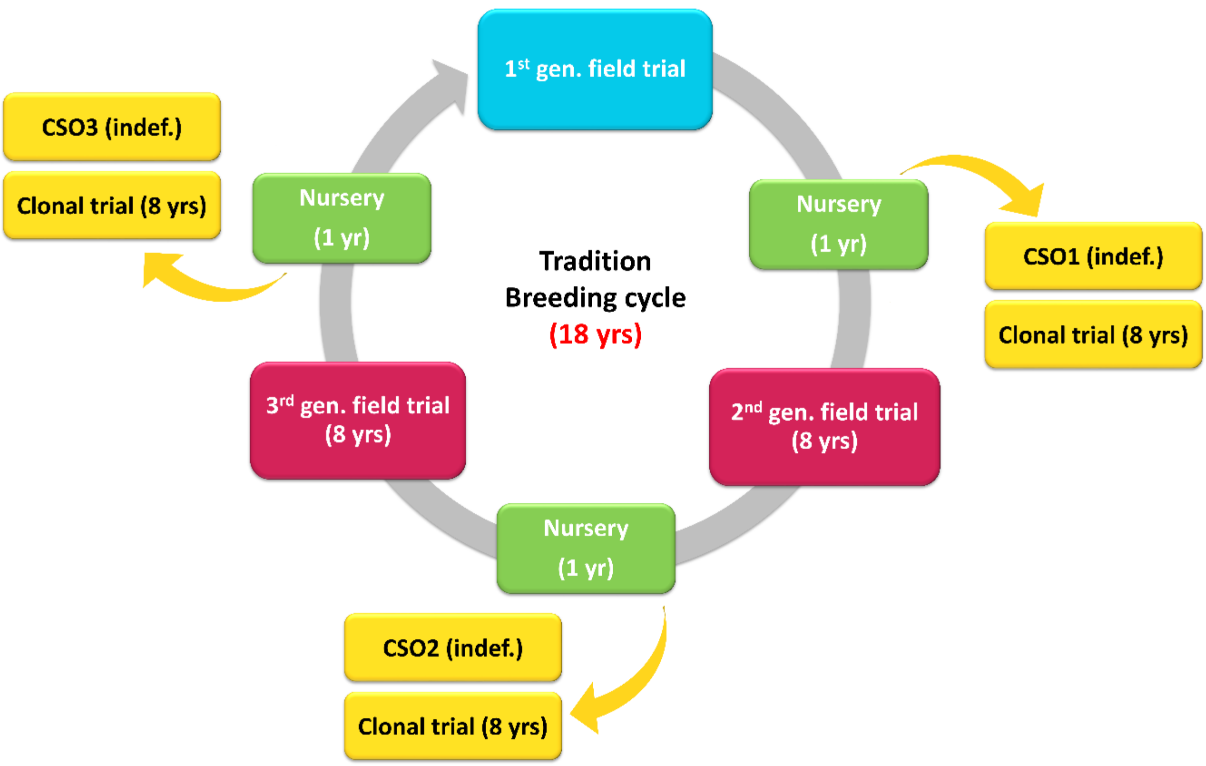


Figure 1. Illustration of the traditional breeding cycle of *E. grandis* and associated commercial deployment opportunities over 18 years. The cycle includes two eight-year full rotation open-pollinated progeny trials (red) with corresponding nursery activities to turn over the generations (green). Over 18 years, the tree breeder can establish three clonal seed orchards (CSO, yellow) for commercial seed deployment and three clonal trials to identify improved clonal varieties (yellow).

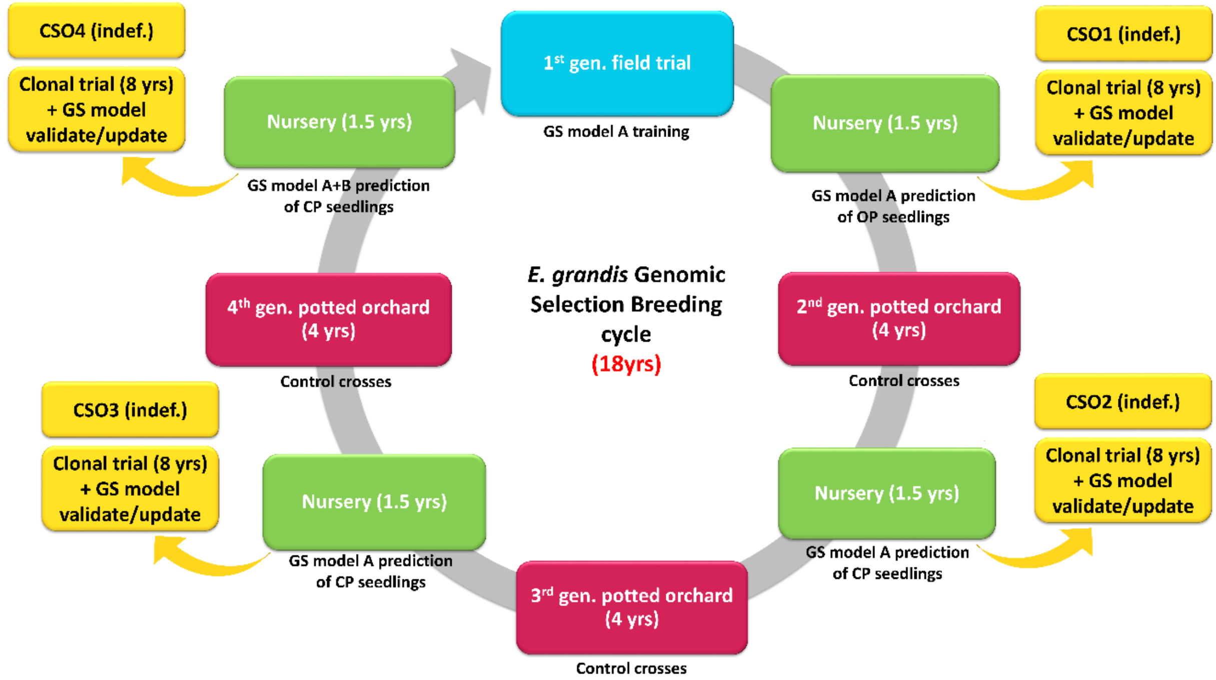


Figure 2. Plausible genomic selection breeding cycles for *E. grandis* over an 18 year period. The breeding approach starts with an open-pollinated training population (blue) to train the initial GS model (A). The cycle includes three four-year periods involving the establishment of potted orchards (red) for control pollination (CP) crosses. The nursery activities (green) to turn over the generation includes the genomic selection of CP seedlings using GS model A. The four established clonal seed orchards will provide commercial seed for deployment, whereas the four clonal trials (yellow) will serve to identify improved clonal varieties and to update the GS model (A+B) leading to further increases in accuracy and efficiency of selection.

clonal trials can also be used to validate the current GS model (A) and improve the model (A+B).

For tree breeders to realise the benefits of implementing genomic selection approaches of selection accuracy, selection efficiency, as well as improved genetic gains per unit time in *E. grandis*. A complete reconfiguration of the breeding steps of the traditional breeding approach is warranted.

It is important to note that this reconfiguration of breeding strategies is associated with additional operational and genotyping (DNA marker analysis) costs. Although these investments will lead to improved genetic gains, they may increase the cost per unit genetic gain in the short-term. Long-term, as genotyping costs continue to decline, the cost per unit genetic gain will also decline. Traditional and genomic breeding strategies can advance as two separate but complementary breeding activities with objectives such as maintaining genetic diversity and accelerating genetic gains, respectively. Looking forward, GS approaches will provide breeders with critical information to achieve breeding goals and produce elite clonal varieties and seed sources for commercial deployment.

A NEW DNA MARKER RESOURCE FOR GENOME-ASSISTED BREEDING OF PINE TREES

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SUMMARY

Pine tree breeders have for many years been searching for ways to speed up breeding cycles and make it cheaper and more efficient to improve specific traits. Besides selecting for desirable traits such as growth and wood quality, breeders have to deal with the fact that plantations have become more vulnerable to biotic and abiotic stresses linked to rapid climate change. Another problem is that commercially important traits such as wood quality are only fully expressed at maturity and can typically only be selected after 8-10 years in field trials. The significant time and cost associated with traditional tree improvement methods, and recent successful application of DNA-based (genomic) technologies in animal and crop plant breeding, have stimulated the international tree breeding community to explore the implementation of these genomic breeding approaches. Using tens of thousands of DNA markers spread out across all of the chromosomes of the plant, the genetic merit or breeding value of trees can be predicted when they are still seedlings. This can potentially shorten the breeding/selection cycle by a decade for pine trees. However, until recently, there has been a lack of resources to analyze large numbers of DNA markers for genomic breeding of pine trees, partly due to the enormous size of pine tree genomes (over 20 billion base pairs – seven times larger than human genomes).

Single nucleotide polymorphisms (SNPs) are the most common type of natural genetic variation which leads to biological variation among individual trees. These differences in DNA building blocks can ultimately influence a variety of traits including growth, wood properties and disease resistance. We used targeted genome and gene sequencing to identify a set of 50,000 informative SNP markers for the development of a commercial DNA marker chip for tropical pine trees (Pitro50K). Used as a genome-wide genotyping technology, the chip can uncover genetic differences for a wide range of applications in tree breeding. These include DNA fingerprinting for identification of individuals and clonal varieties, and parentage analysis for pedigree reconstruction, e.g. identifying the seed and pollen parents of superior individuals. The SNP chip can also be used for species and hybrid identification, genetic resource management and genome-assisted breeding.

In the future, the SNP chip can aid in fast-tracking the pine breeding cycle. Large numbers of informative SNPs can be incorporated into models for genomic prediction of breeding values at seedling stage. The use of inter-species hybrids to improve growth, wood properties, adaptability, or disease tolerance is imminent. An example in South Africa is the *P. patula* x *P. tecunumanii* hybrid, which has shown excellent growth and significantly better tolerance to pitch canker disease (caused by *Fusarium circinatum*) relative to the widely-planted pure species *P. patula*. The SNP chip will be invaluable for species and hybrid discrimination, and tracking of growth, wood property and disease resistance loci, thereby increasing the accuracy of pine hybrid breeding. The SNP chip and future genome sequencing efforts may also enable the analysis and prediction of genotype by environment (GxE) interactions, allowing us to potentially make climate-specific selections and breed for future environments. The development of suitable experimental populations for genomic mapping and modelling is a high priority that will be the focus of research in the next five to ten years.

INTRODUCTION

Pine tree species are economically important primarily because their wood is used for timber products, including furniture, window frames, paneling, floors and roofing. Tropical pine species play an important role in plantation forestry in South Africa. Eight tropical and subtropical pine species were included in this study due to their commercial and ecological importance: *P. oocarpa,*

P. patula, P. tecunumanii, P. greggii, P. caribaea, P. elliottii, P. maximinoi and *P. pseudostrobus*. These species can be deployed in a variety of commercial settings and can readily hybridize and produce viable offspring (Gwaze 1999; Hongwane et al. 2018). As a result, pine breeders can develop hybrid breeding populations for specific niche environments and for disease resistance.

Traditional pine tree improvement is a slow, laborious and expensive process. Therefore, the tree breeding community is exploring the possibility of implementing genome-assisted breeding technologies that can speed up breeding cycles. Next-generation DNA sequencing methods have revolutionized the field of genetics by facilitating the identification of natural genetic variation (DNA markers) and the development of high-throughput, low-cost marker genotyping assays. Single nucleotide polymorphisms (SNPs) have become the DNA marker of choice due to their abundance in plant and animal genomes, amenability to automation, low cost of genotyping and being co-dominant in nature. *P. taeda* is the best characterized pine reference genome, but the current assembly of the 22 Gb megagenome still consists of over 2.8 million sequence fragments ("contigs") with >80% repetitive content (Wegrzyn *et al.* 2014). Whole genome sequencing of many pine trees required for SNP discovery efforts therefore remain both costly and computationally intractable. Instead, methodologies based on reduced representation sequencing, for example transcriptome and targeted genome capture sequencing are being used for DNA marker discovery in pines (Telfer *et al.* 2019).

The lack of high-throughput, low-cost genome-wide genotyping resources has created a bottleneck in pine tree genetics research. To advance both commercial breeding programs and academic research, we have successfully designed the Pitro50K SNP array (DNA marker chip) with 49,674 markers carefully selected to optimize the number of polymorphic markers within each species and include sufficient unique markers for species and hybrid identification and ancestry reconstruction. This array provides at least 10,000 usable markers for each of the commercially grown tropical pine species and their F1 hybrids, and large numbers of these markers should be transferable to other closely related species in the Pinus section Trifoliae, subsections Australes and Ponderosae enabling wider genetic investigations.

MATERIALS AND METHODS

An automated bioinformatics pipeline for SNP discovery was established using (i) RNA sequencing (RNA-seq) data from five tropical pine tree species (*Pinus patula, P. tecunumanii, P. oocarpa, P. greggii* and *P. maximinoi*) and (ii) targeted capture sequencing (capture-seq) data across 85 provenances from the natural range in Mexico and Central America representing six species (the five species mentioned above plus *P. caribaea*) (Figure 1a). An aggregate of 200 million paired-end RNA-seq reads were aligned back to the five respective transcriptome (expressed gene) assemblies (Visser et al., 2018). A total of 5.3 million RNA-based SNPs were detected and identified 1.3 million that could be targeted for SNP genotyping. In parallel, 30 million paired-end capture-seq reads were aligned back to the *P. taeda* (loblolly pine) reference genome assembly. Out of the 3.3 million capture-seq SNPs identified, 1.02 million were detected in at least two of the provenances and 415K SNPs were shared among two or more species. From these two sets, a total of 563K could be targeted for genotyping. After in silico scoring for SNP assay design criteria, we selected a final set of 300K RNA-seq derived SNPs and 120K capture-seq derived SNPs for tiling on a 420K screening array (ThermoFisher Scientific, Figure 1b).

The screening array was used for genotyping of 576 individual trees from the 85 provenances and from commercial breeding material representing eight species, including *P. elliotti* and *P. pseudostrobus*. Samples that passed quality control filtering (98.3% of the samples) were subjected to genotype calling by Thermo Fisher's Axiom Analysis Suite software. Besides evaluating assay performance for each SNP probe set, the main aim with the screening array was to estimate the allele frequency and pattern of sharing of each marker across species. This information was crucial for selecting a set of 50K SNP markers that maximized the representation of each species on the final commercial Pitro50K array (Figure 1c). We prioritized SNPs that were informative (with allele frequencies greater than 5%) in three or more species. Since *P. greggii* and *P. maximinoi* were under-represented in this set, we also included markers unique to *P. greggii* and *P. maximinoi* (but still with allele frequencies greater than 5%). Finally, a set of 966 diagnostic SNPs (that vary greatly in allele frequency among species) were included to aid in future discrimination of species, sub-species and their F1 hybrids.

RESULTS AND DISCUSSION

Gene and genome targeted SNP discovery towards the development of a genome-wide, multi-species genotyping array for tropical pines were performed. In total, 49,674 SNP markers were selected for the 50K commercial array: roughly 20K polymorphic SNPs were selected for *P. patula, P. tecunumanii, P. oocarpa* and *P. caribaea*; 15K for *P. greggii* and *P. maximinoi*; 13K for *P. elliottii*; and 8K for *P. pseudostrobus*. These markers are shared in different combinations among the species. Together with the 966 species diagnostic SNPs, a set of 9700 SNPs are predicted to be ancestry informative markers that will be valuable for species and hybrid discrimination. In total, 25% of the SNPs were polymorphic in only one species and 75% were shared by two or more species.

Using the screening array results and the selected 50K SNPs, the following analyses were performed: (i) a principal component analysis (PCA) where the genotypes observed per sample were reduced to a few summary coordinates (Figure 2a), and (ii) an admixture proportion inference analysis, assuming 11 ancestral populations, to produce a visual summary that revealed the existence of population structure across all of the trees and within each group (Figure 2b). The PCA and structure plot resolve most species and subdivisions into unique genetic groups. For example, in the PCA (Figure 2a), *P. maximinoi* and *P. pseudostrobus* (in the Ponderosae subsection) behave as expected and cluster distantly from all of the the Australes species. The markers successfully partitioned P. *caribaea, P. elliottii*, and their F1 hybrids into distinct clusters, with the F1 hybrid exactly intermediate. *P. greggii* and *P. tecunumanii* resolve into their subdivision (North/South and High/Low elevation) categories well, but there is complete overlap between the two *P. patula* subspecies. The structure plot (Figure 2b) reveals that for *P. oocarpa*, geographically the most wide-spread of the species and most likely the ancestral species for the Australes subsection, we can discriminate between southern, central and northern provenances, as the (trees) in this plot are ordered according to latitude (South to North) of the sampled provenance.

One of the most important uses for the Pitro50K array will be genomic breeding in hybrid populations. Large numbers of informative SNPs can be incorporated in genomic prediction models, obtained from training populations with genotype and phenotype data available (Figure 3a). These models utilize accurate genomic relationships and modelled marker-trait associations to estimate the genetic merit of individual trees (Grattapaglia *et al.*, 2018). Once a predictive model is shown to provide adequate accuracy, it can be used to fast-track the breeding cycle via genomic selection, by predicting breeding values for seedlings with only genotypic information available (Figure 3b). Top ranked seedlings can then be used for top grafting, leading to early flower induction and subsequent inter-mating of the selected seedlings to create the next generation of improved genotypes for breeding. Finally, subsets of these seedlings can also be planted in field trials where their phenotypes can later be measured and used to update the predictive models.

CONCLUSION

The Pitro50K SNP array has utility for population genomics and molecular breeding in this group of pine species, that together with their hybrids, represent the majority of tropical and subtropical pine plantations globally. The DNA marker array provides a crucial resource for gene conservation; genetic resource management; rapid identification and deployment of resistant genotypes in response to pests and diseases; genotype by site matching; quality control of seed and clonal propagation; and genome-assisted breeding for growth, wood quality and resilience to pests and diseases and climate change.

Footnote: A full length article will be published with more detail.

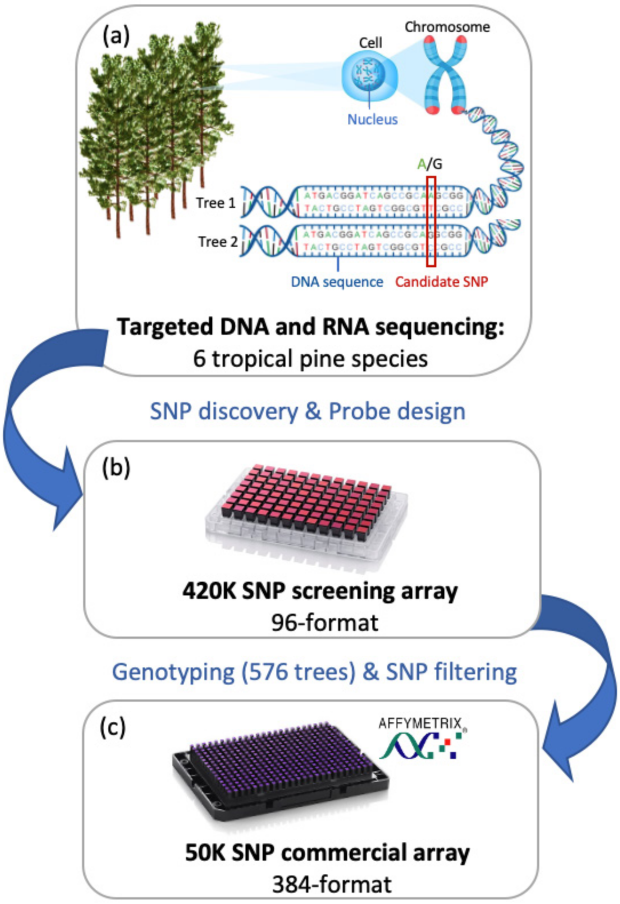

Figure 1. Sequencing data of >500 tropical pine trees from different species and provenances were used to identify single nucleotide polymorphisms (SNPs). A 420K format screening array was developed and used to identify the best set of 50K SNP markers for tiling on the Pitro50K commercial array (available from ThermoFisher Scientific) which will allow genotyping of batches of 384 trees.

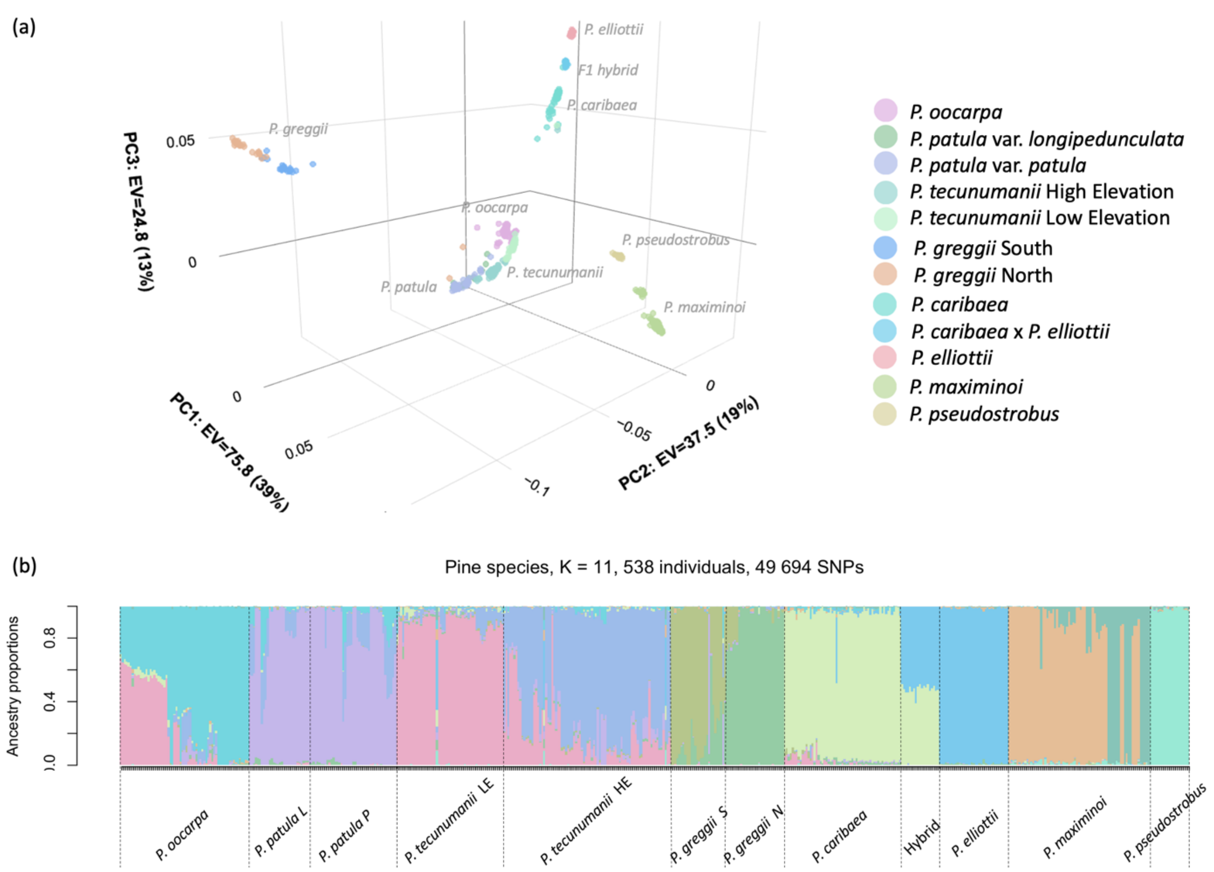

Figure 2. Principal component analysis (PCA) and structure plot of genome assignment (K=11 genetic structures) based on the data from the 50K SNPs produced for 538 trees. The PCA (a) shows that it will be possible to discriminate among the tropical pine species, sub-divisions and their F1 hybrids. The structure plot (b) shows that the species and subspecies (ordered by provenance from South to North) all represent unique genetic groupings, but shared ancestry is also evident (e.g. some provenances of *P. oocarpa* and *P. tecunumanii*) and may represent gene flow in areas of geographic overlap.

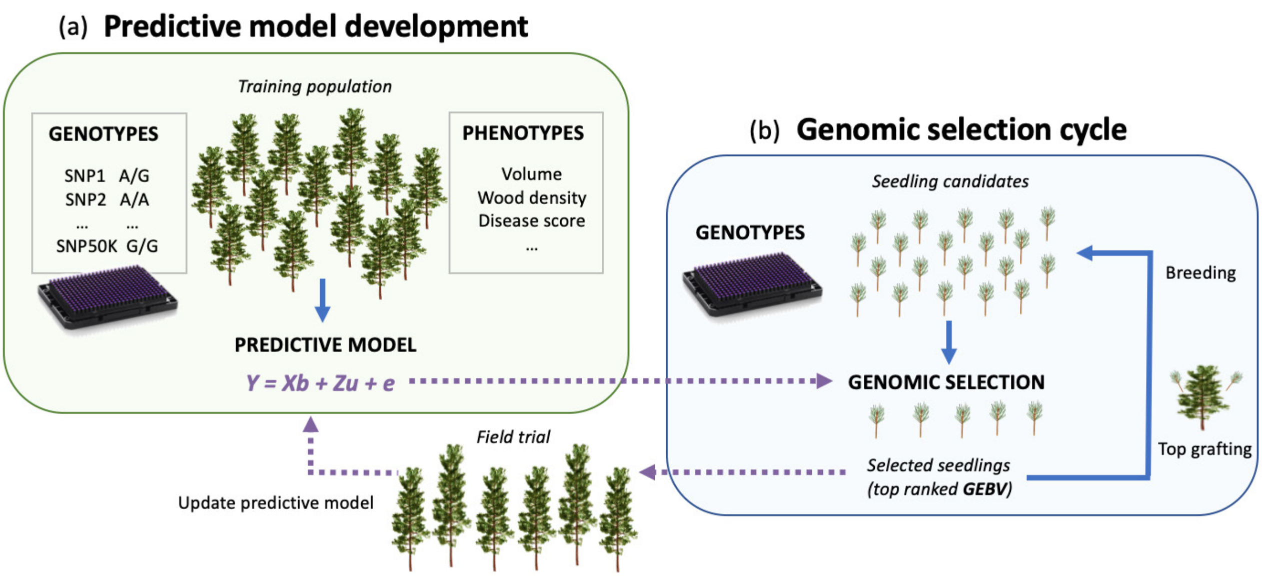

Figure 3. Genomic selection begins with the development of a predictive model for the traits of interest in a training population with genotype and phenotype data available. The model is then used to predict genome estimated breeding values (GEBVs) on seedlings, based on genotype data only, in genomic selection cycles; the model is progressively updated with each cycle.

GLOSSARY

- SNP: A single nucleotide polymorphism (SNP, pronounced “snip”) is a single position variant in a DNA sequence (which is formed from a chain of four nucleotide bases: A, C, G, and T) that can differ among individuals. For example most individuals may carry a C nucleotide at a specific position in the genome (DNA sequence), but some individuals may carry an A nucleotide at this position. Such a SNP will have two possible nucleotide variants (C or A) also referred to as “alleles”.
- DNA marker analysis or genotyping: Genotyping is the process of determining the unique marker allele combinations (AA, AC or CC in the example above) carried by an individual by examining the individual’s DNA sequence and comparing it to another individual’s sequence or a reference sequence. It reveals the combination of marker variants or alleles that an individual has inherited from its seed and pollen parents.
- F1 hybrids: Filial 1 (F1) is the first generation offspring resulting from a cross between populations, breeds, or cultivars between or within species. This flow of genetic material between populations can be referred to as hybridization.

REFERENCES

Grattapaglia D, Silva-Junior OB, Resende RT, Cappa EP, Müller BSF, Tan B, Isik F, Ratcliffe B, El-Kassaby YA. Quantitative genetics and genomics converge to accelerate forest tree breeding. Frontiers in Plant Science 2018; 9(1693).

Gwaze DP. Performance of some F1 interspecific pine hybrids in Zimbabwe. Forest Genetics 1999; 6(4): 283–289.

Hongwane P, Mitchell G, Kanzler A, Verryn S, Lopez J and Chirwa P. Alternative pine hybrids and species to *Pinus patula* and *P. radiata* in South Africa and Swaziland. Southern Forests. 2018; 80(4): 301-310.

Telfer E, Graham N, Macdonald L, Li Y, Klápště J, Resende M, Neves LG, Dungey H, Wilcox P. A High-Density Exome Capture Genotype-by-Sequencing Panel for Forestry Breeding in *Pinus Radiata*. PLOS ONE. 2019; 14(9): e0222640.

Visser EA, Wegrzyn JL, Myburg AA and Naidoo S. Defence transcriptome assembly and pathogenesis related gene family analysis in *Pinus tecunumanii* (Low Elevation). BMC Genomics. 2018; 19(1): 1–13.

Wegrzyn JL, Liechty JD, Stevens KA, Wu LS, Loopstra CA, Vasquez-Gross HA, Dougherty WM, et al. Unique features of the loblolly pine (*Pinus taeda L.*) megagenome revealed through sequence annotation. Genetics. 2014; 196(3):891–909.

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QR CODE WITH LINK TO YOUTUBE VIDEO

The video highlights how this tropical pine DNA marker resource will benefit both the industry and the scientific community: https://youtu.be/G-D5VXd0zSM

REVOLUTIONISING THE WAY LUMBER IS TRANSPORTED BY ROAD

PERFORMANCE-BASED STANDARD: MOVING FROM PILOT PROJECT TO PRACTICAL SOLUTION

Francois Oberholzer and Dr Katherine Louise Johnson
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"The conversion to PBS vehicles has the potential to benefit the Industry, South African road users and the environment."

SUMMARY

Performance-Based Standard (PBS) vehicles are likely to revolutionize the transport of timber, by allowing the Forestry Sector to move larger loads at a time and thus reducing the number of trips made, kilometres travelled, and fuel used. The PBS concept enables the use of bigger vehicles by regulating how the vehicle behaves on a road network, setting static and dynamic performance standards, rather than prescribing set vehicle mass and structural dimensions like the current South African legislation that governs the use of heavy vehicles on our roads.

The possible incorporation of the PBS approach into the South African national transport framework was initiated with a pilot project in 2007 by the Council for Scientific and Industrial Research (CSIR). The Forestry Sector was the first to join and currently has 99 PBS vehicles, travelling over 15 million kilometres and delivering over 2.4 million tons of timber annually. The next step towards incorporating the PBS system into our national legislation requires the PBS Steering Committee to show the net effects PBS vehicles have on the Transport Sector, including the forecast uptake of PBS vehicles within various subsectors including forestry.

To determine this, a survey of the eight largest forestry companies, responsible for moving 74% of the total timber volume in South Africa, was conducted. The survey predicted that 85% of timber will be moved annually by road in the future. Of this, 58% is predicted to be moved by PBS trucks, almost four times the amount expected to be transported by conventional trucks. Based on PBS configurations, the predicted volumes to be moved and the individual lead distances reported, a 58% adoption of PBS vehicles could result in a 19% saving in the number of trips from compartment to mill. This, in turn, would result in a 13.22 million km annual reduction, translating into a 16.7% saving on fuel and, in combination with the other reductions, could yield an approximated R215.9 million annual saving.

Beyond the economic benefits to the industry, all road users will benefit from the reduction in trips and therefore less forestry trucks on the road and the reported 45% reduction in accident rates of PBS vehicles. What's more - the predicted 16.7% saving in annual CO_2 emissions will help towards South Africa's pledge to cut CO_2 emissions, as part of the Draft Green Transport Strategy.

This study echoes that of the CSIR and shows that PBS offers an innovative solution yielding economic, social and environmental benefits.

INTRODUCTION

PBS differ significantly from the current South African legislation governing the use of heavy vehicles on the South African road network. Heavy vehicle use in South Africa is controlled by prescriptive regulations, where vehicle performance is governed by regulating vehicle mass and structural dimensions. These regulations can result in some inherently unsafe vehicle designs. The PBS principle does not stipulate dimension, but rather focuses on how the vehicle behaves on the road network. These behaviours are captured under static and dynamic performance standards which are then used to design PBS demonstration vehicles. They cover:

- **Startability –** the ability to commence forward motion on specified upgrade.
- **Gradeability A** – the ability to maintain forward motion on specified upgrade.
- **Gradeability B** – the ability to maintain a minimum speed on a specified upgrade.
- **Acceleration capability** – the ability to accelerate either from rest or to increase speed on a road with no grade.
- **Frontal swing** – the maximum width of the frontal swing swept path in a prescribed 90º low-speed turn.
- **Maximum of difference** – the maximum of the difference between the frontal swing-out distances between adjacent vehicle units in a prescribed 90º low-speed turn.
- **Difference of maxima –** the difference between the maximum frontal swing-out distances between adjacent vehicle units in a prescribed 90º low-speed turn.
- **Tail swing** – the maximum outward lateral displacement of the outer rearmost point on a vehicle unit during the initial and final stages of a prescribed 90º low-speed turn.
- **Low-speed sweep path** – the maximum width of the swept path in a prescribed 90º low-speed turn.

- **Steer-tyre friction demand** – the maximum friction level demanded of the steer tyres of the hauling unit in a prescribed 90º low-speed turn.
- **Tracking ability on a straight path** – the total swept width while travelling on a straight path, including the influence of variations due to cross fall, road surface unevenness and driver steering activity.
- **Static rollover threshold** – the steady-state level of lateral acceleration that a vehicle can sustain without rolling over during turning.
- **Rearward amplification** – the degree to which the trailing unit(s) amplify the lateral acceleration of the hauling unit.
- **Yaw damping coefficient** – the rate at which "sway" or yaw oscillations decay after a short duration steer input at the hauling unit.
- **High-speed transient off-tracking** – the lateral distance that the last-axle on the rearmost trailer tracks outside the path of the steer axle in a sudden evasive manoeuver.

This approach allows more flexibility for vehicle designers to utilize innovative solutions and the latest available technology to meet the required performance standards with improved safety outcomes and more effective use of the road infrastructure. PBS also improves transport productivity through increased payloads, thereby reducing costs associated with the transport of goods (Nordengen *et al.* 2018). Improvements in transport productivity lead to decreased fuel usage per ton moved and subsequent reductions in CO2 emissions

The Forestry Industry, therefore, sees PBS vehicles playing a key role in supporting and developing a road transport system that:

- Promotes safer roads
- Reduces road wear
- Is more efficient in terms of payload
- Having a role in the Industry's commitment to reducing carbon emissions along the value chain.

The PBS concept is currently operating as a pilot project under the Abnormal Load dispensation (DoT, 2020). This project was initiated in South Africa in 2007 by the CSIR. Since its inception, various industries have come on board, including coal, sugar, beverages, general freight and mining. The Forestry Industry was the first to join and currently has the most vehicles, 99 in total, involved in the project. These vehicles travel over 15 million kilometres, delivering over 2.4 million tons of timber, annually.

The CSIR compiled an evaluation report of the results of the pilot project and have submitted this document for review to the Abnormal Load Technical Committee for their review. Once this review process is completed, the Director-General of the Department of Transport will be approached to incorporate the PBS concept into the National Road Traffic Act.

Part of the move from a pilot project to a solution incorporated in the national transport framework is the need to show the net effect of PBS vehicles on the Transport Sector. The first step in this process is to forecast the uptake of PBS vehicles within the various subsectors, including Forestry, which is why this project was initiated.

MATERIALS AND METHODS

To quantify potential uptake of PBS vehicles with the South African Forestry Sector, Forestry South Africa (FSA) commissioned a survey of the eight largest forestry companies in the country. The survey was limited to these eight companies due to the large timber volume moved by these companies (74% of the total timber volume in South Africa), and because smaller members do not have the economy of scale to commission PBS fleets dedicated to them. It is also not financially viable for smaller members, due to the costs and logistical constraints associated with PBS vehicles which includes:

- Road Transport Management System (RTMS) certification – a requirement for PBS vehicles (DoT, 2020).
- Provincial and district road restrictions on PBS vehicles.
- Long-term contract obligations required by PBS transport operators, to depreciate their vehicles under the contract period.

A closed-answer questionnaire was sent out to the eight forestry companies on 25 June 2020, asking for the following information:

- The total annual volume of timber in tons they anticipated moving by road in the future.
- Volume they anticipate will be moved by PBS vs conventional trucks.
- Configuration of PBS trucks they anticipate using – different PBS vehicles offer different payload capacity. It is important to know the capacity to work out the rate and fuel use per ton.
- The lead distance for each configuration – the lead distance refers to the one-way distance between the plantation and the mill. It is needed to calculate the rate and fuel consumption.

All surveys were returned by September 2020.

Due to variations between participating companies concerning vehicle ownership models including leased vehicles; financed vehicles under buy-back guarantees;

and vehicles financed under different timescales, individual costing models for each company would be needed to accommodate this. A costing model collating all the business costs was applied and these figures were then divided by the expected productivity to give Rand/ton value or divided by the value for lead distance to give Rand/km value. The new Mercedes Benz Arocs series were used for the calculations, with the Arocs 3345 for conventional vehicles and the Arocs 3352 for PBS. These universal values could then be combined and compared.

RESULTS AND DISCUSSION

Analysis of the data projected that approximately 9.763 million tons (85%) of timber will be moved annually by road in the future – although it is worth noting that participants commented that they would prefer to transport higher volumes by rail, but predictions had to be based on current realities. Of this, 7.684 million tons (79%) is predicted to be moved by PBS trucks, almost four times the amount expected to be transported by conventional trucks (2.079 million tons). While this data cannot be extrapolated for the Industry as a whole, due to the constraints on smaller members already mentioned, the data from these eight companies can be assumed to be representative of the Sector's total PBS uptake. On this assumption, it is anticipated that 58% of South Africa's total timber moved by road will be done using PBS vehicles in the future.

Total tonnage moved by road by participants: 9 762 686

Total volume (tonnage) moved by participants: 11 500 000

Road as % of total = 84.9%

Total volume (tonnage) moved by the industry = 15 500 000

Total Road by industry (84.9%) = 13 158 402

Total PBS by participants: 7 683 824

PBS as % of road = 7 683 824 / 13 158 402 = 58%

Two PBS configurations were identified by all respondents:

- PBS configuration one – Gross combination Mass of 67.5 tons and average payload of 47 tons.
- PBS configuration two – Gross combination Mass of 75 tons and an average payload of 51 tons.

Using anticipated PBS configurations, volumes moved by PBS and the individual lead distances reported by the participants which ranged from 30km to 275kms, it can be estimated that a 58% adoption of PBS vehicles could result in:

1. **A 19.8% saving in the number of trips from compartment to mill (estimated 53 264 trips)**
2. **A 13.22 million km annual reduction**
3. **A 16.7% saving on fuel, totally 5.73 million litres**
4. **R215.9 million in annual savings.**
5. **A 15 130-ton reduction in annual CO2 emissions, resulting in a 16.7% saving.**

CONCLUSION

With an anticipated R215.9 million annual saving, the switch to PBS vehicles is likely to have a significant impact on a company's bottom line. With PBS offering an innovative solution to the constant battle forestry companies face to keep increases in logistic costs below inflation. The reduced cost of logistics could have an additional spinoff, with value-adding opportunities becoming viable.

While the Forestry Industry will be the main beneficiary of the financial savings made by moving to PBS vehicles, all road users will benefit from the reduction in trips and therefore forestry trucks on the road. With PBS vehicles shown to have a 45% reduction in crash rates when compared with conventional vehicles, there is also a safety aspect to the switch that will benefit South African road users. Finally, there is the potential to help towards South Africa's pledge to cut CO_2 emissions as part of the Draft Green Transport Strategy.

The conversion to PBS vehicles has the potential to benefit the Industry, South African road users and the environment. We now wait to see if the findings of this survey and others conducting across various sectors will aid in the incorporation of the PBS concept into current South African legislation by the National Department of Transport.

REFERENCES

DoT (Department of Transport), 2020. *Smart truck programme: Rules for the development and operation of smart trucks as part of the performance-based standards research*, National Department of Transport, Republic of South Africa, Pretoria.

Nordengen, P. A., de Saxe, C., Berman, R.J., and Steenkamp, A. 2018. 'Improving heavy vehicle safety and road transport efficiency: A performance-based standards approach in South Africa', in *Proceedings of the 7th Transport Research Arena TRA, 16-19 April 2018*. Vienna.

CSIR (Council for Scientific and Industrial Research), 2020. *Smart Trucks & Performance-Based Standards (PBS) for Road Freight Transport in South Africa: Pilot Project Evaluation Report 2007-2020*, National Department of Transport, Republic of South Africa, Pretoria.

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