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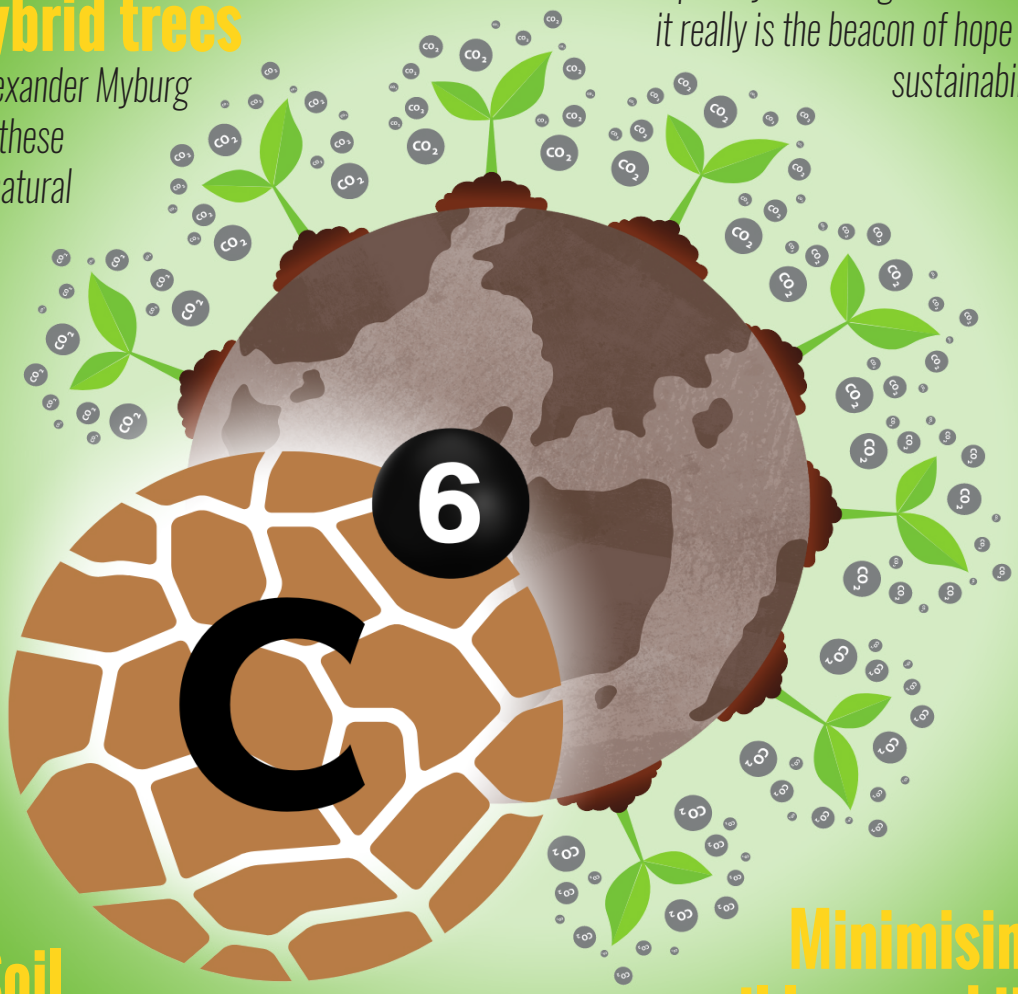
Timber Industry Presents Magazine

Discovery of genomic rearrangements in eucalyptus hybrid trees

Anneri Lotter and Professor Alexander Myburg consider the potential of using these rearragements as a source of natural variation for tree improvement

Biomimicry

Jacqui Meyer investigates whether it really is the beacon of hope for sustainability



Near-Infrared Spectroscopy

Professor Mark Laing & Dr Sascha Beck-Pay evaluate the opportunities, limitations and future applications

The Long-term Soil Monitoring Network

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Kate van Zyl unveils micro-catchments

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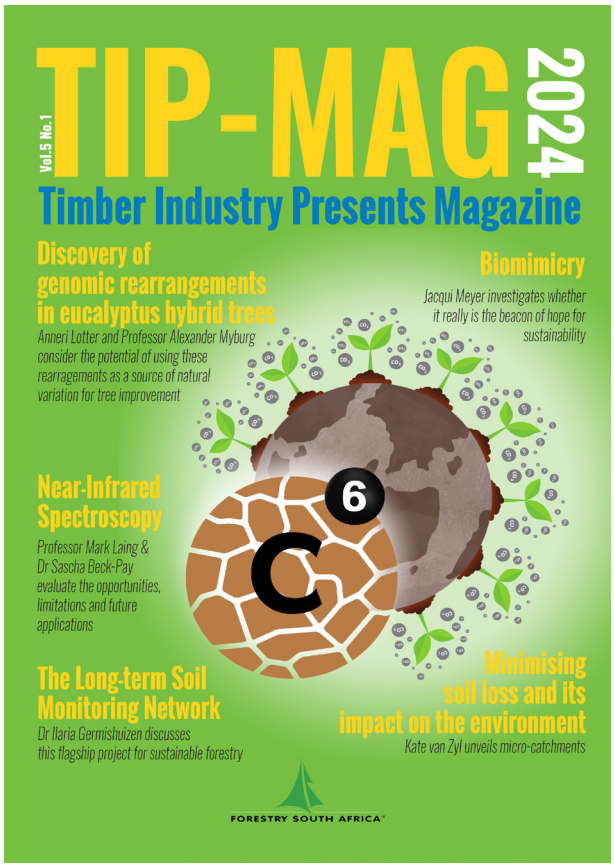

FROM THE EDITOR

OPINION

"For commercial forestry to remain viable, in South Africa and around the world, there is an urgent need to recognise knowledge gaps and vulnerabilities, and to adapt and alter management practices and strategies to match the increasing economic, environmental and social challenges – like climate change – we will face."

CHANGING THE WORLD WITH A SINGLE THOUGHT

THE IMPORTANCE OF SHARING OPINIONS

> *"If you want to learn something, read about it. If you want to understand something, write about it. But if you want to master something, teach it."*
> *- John C Maxwell*

JOHN C. MAXWELL's quote can be applied to most things but fits few better than it does the world of research – especially academic-based research – where we actively practice all three. Indeed, good research is built on these three steps, we first read about a subject extensively, to identify needs and knowledge gaps. Then, once the data has been collected and analysed it is written up to make sense of the findings and understand their implications. Finally, we teach the findings of our research to those charged with practically implementing it. Put simply, researchers learn, they understand and then they master.

While at first glance, TIP-MAG appears to be a conduit facilitating the second step in this process – allowing researchers to write about their findings and therefore providing an opportunity to enhance their understanding. I would argue that TIP-MAG should be viewed as facilitating the final step – providing a platform for researchers to teach a receptive, yet non-scientific, audience about their research and in the process further mastering their own subject. TIP-MAG requires authors to process their findings in a way that ensures their accessibility to the lay-reader rather than a scientific peer, in doing so, authors must adapt the way they communicate the science that goes beyond simply writing about it and moves into the teaching arena. I believe this is what makes TIP-MAG both unique and valuable, as it provides a platform for interested lay-people to access the science and enables the contributors to master it.

This is the first issue that only contains opinion pieces, something Jacqui and Katy were initially concerned about, despite the diverse subject matter making each opinion piece distinct and captivating. It is also important not to undervalue the importance of opinion pieces. They are the cumulation of countless hours of scientific reading and writing, that is now being expressed as an opinion piece, or, as I interpret it, 'a teaching' from some of the brightest minds in our Sector. So, while an issue exclusively comprising of opinion pieces should be the exception rather than the rule, I find it fitting that in a magazine that focuses on informing – in other words teaching – a much wider non-scientific community about the science that is moving the sector forwards, we have issues that really promote opinions and ultimately the teachings of those at the forefront of forestry science.

It might be too much to say TIP-MAG, and this issue in particular, is a lesson in mastery, but it certainly would not be over exaggerating the point to say those who contribute to this issue are masters in their respective disciplines and key role players in our Sector's continued success.

Dr Ronald Heath
FSA Director:
Research and Protection

THE LONG-TERM SOIL MONITORING NETWORK

A FLAGSHIP PROJECT FOR A SUSTAINABLE FORESTRY SECTOR

By Dr Ilaria Germishuizen, Director of Research ICFR

"Due to lack of available science-based data, reporting is currently based on global guidelines and conversion factors, compromising the accuracy of the reporting."

Dr Ilaria Germishuizen

PLANTATION forests are particularly vulnerable to climatic risks due to the long rotations and breeding cycles. Projected changes in climate, in particular, the trend towards more frequent extreme climatic events, suggest that even within the current rotational period climate change could affect forest productivity and operations. These climatic changes and their impact on plantation forests are likely to result in increased pest and disease risks, increased risk of fires and other abiotic threats. The adoption of adaptation strategies in forest management is therefore critical for sustained productivity in response to the changing environment. Through well managed plantation forests, the Sector can contribute to mitigating carbon emissions and play an intrinsic role in the development of a low-carbon and climate resilient nation.

One of the instruments adopted by the South African government to stimulate a low carbon economy and combat climate change is the Carbon Tax, which became effective in June 2019. Strictly linked to the Carbon Tax is the legislation around carbon accounting and non-carbon greenhouse gases (GHG) emissions, the reporting of which is a requirement for all timber growers with 100ha or more of planted land. Due to lack of available science-based data, reporting is currently based on global guidelines and conversion factors, compromising the accuracy of the reporting.

IMPROVING ACCURACY BY CREATING A NETWORK

In 2018, Sappi and Mondi proactively initiated a new project, the long-term soil monitoring plots network (LTSMP), based at the ICFR. The aim of which, was to produce a database and reporting system for monitoring and detecting changes in site and soil properties that determine plantation

Figure 1: Soils samples are taken just outside the four corners of the plot, at three depths, and analysed at the ICFR analytical laboratory facility.

growth over multiple rotations. This flagship project generates data needed for the development of Sector level standards for GHG reporting, by providing more accurate estimates of emissions and sinks within the plantation forestry environment. Multiple benefits can be derived from the development of a standardised, Sector level reporting system for GHG emissions and carbon accounting at a regional (Tier 2) level. These go beyond ensuring the fair application of the Carbon Tax on all participants of the Sector and include the promotion management practices that alleviate emissions and accelerating the shift towards a low carbon economy.

The soil monitoring network is based on a simple design that requires minimum input. Ten by ten tree plots are established in compartments close to felling date, when the site is the least disturbed. Soils and litter samples are taken from the four corners just outside the plot and analysed at the ICFR laboratory. Trees are then measured (Dbh; Ht) when the plot is established and annually thereafter, with soil and litter samples taken again when the re-established compartment reaches rotation age. These samples provide a snapshot of the soil and litter nutrient profile at every rotation. From 2018 to 2022, the project established just over 50 plots in eucalypt compartments, representative of the most prevalent climate zones and lithologies within the Sappi and Mondi landholdings. Eucalypt stands were chosen over pine and wattle due to resource constraints, as they represent the "worse case" scenario, having a shorter rotation and thus a faster rate of nutrient uptake and removal.

The development of a database for Sector level reporting, however, requires data from pine and wattle stands. This was made possible in 2023, through funding from the Forest Sector Innovation Fund (FSIF) accessed by Forestry South Africa (FSA) to support a suite of projects under the GHG Emissions and Carbon Accounting research umbrella, based at the ICFR, Stellenbosch University (SU), and the University of KwaZulu-Natal (UKZN). One of the projects to receive this funding was the LTSMP, which has used it to quickly expand the network of plots by attracting new companies to join the project on a co-funding basis. Here the companies benefit by receiving one FSIF funded plot on their plantations for every two company funded plots. In the six months since the FSIF funding was granted, NCT, TWK, UCL, PG

> *"The positive impact of the FSIF funding cannot be overstated, with projections indicating that the monitoring network will achieve the target of 100 pine, wattle and eucalyptus plots in total, covering the most representative climate zones and lithologies found within these stands by the end of the FSIF funding cycle."*

Figure 2: Litter samples are taken using a 30 cm x 30 cm grid where soil samples are also taken and analysed to quantify levels of N, P, K, and other macro and micro nutrients.

Figure 3: Soil bulk density samples are taken to determine important soil physical properties at each site.

"Beyond understanding the potential mitigation role of plantation forests on climate change, this information will plug current information gaps.... ... improving the accuracy of carbon stock reporting."

Bison, and MTO have committed to the project, with other companies showing interest in joining.

The positive impact of the FSIF funding cannot be overstated, with projections indicating that the monitoring network will achieve the target of 100 pine, wattle and *eucalyptus* plots in total, covering the most representative climate zones and lithologies found within these stands by the end of the FSIF funding cycle.

MOVING THROUGH THE TIERS

The next step is to improve GHG reporting in South Africa, this will be achieved by moving to a Tier 2 system, which ensures fairer application of the Carbon Tax on all participants. To move from the current Tier 1 reporting to a more accurate and country specific Tier 2 system, several knowledge gaps need to be filled.

The expansion of the network of LTSM plots to cover the edaphic and climatic diversity of the forestry landscape, varying silviculture management, crop cycle and planted genus would generate data for an objective evaluation of the changes over time of carbon stocks in the soil and litter layer at regional level. This would provide the Forestry Sector with the knowledge required for a standardised and accurate reporting at Tier 2 Level of its soil and litter carbon fluxes over time. Should the LTSMP network demonstrate that over time, under certain site management practices, soil and litter carbon remains stable or on positive balance, it would provide the basis for exemption for this component from the annual carbon fluxes reporting requirements (IPCC Decision 2/CMP.7). The findings would also support the development of guidelines for climate smart, low carbon plantation forest management, a prerequisite for compliance with forest certification bodies such as the Forest Stewardship Council (FSC) and the Programme for Endorsement of Forest Certification (PEFC) and is in line with the goals of the United Nations Development Programme for Sustainable Development and the South African National Land Care Program.

TAKE HOME MESSAGE

Thanks to FSIF funding the LTSMP network has grown exponentially and will in time provide an objective benchmark of the impact of plantation forests and their management on soil nutrient pools and productivity over multiple rotations. Beyond understanding the potential mitigation role of plantation forests on climate change, this information will plug current information gaps that could see GHG emissions reporting move from a Tier 1 process – based on international standards – to a Tier 2 process – based on regional knowledge – improving the accuracy of carbon stock reporting.

Figure 4: Long term soil monitoring site at Sutton, Southern KZN.

BIOMIMICRY: A BEACON OF HOPE FOR SUSTAINABLE PLANTATIONS AND BIODIVERSITY CONSERVATION

By Jacqui Meyer, Forestry Consultant

AS A parent the urgency of the biodiversity crisis strikes a personal chord with me. The prospect of a world where my son, now ten, might face scarcity of healthy, fresh foods and clean water by the time he reaches 37 is both alarming and deeply unsettling. It compels me to reflect on our responsibility as custodians of the Earth and our obligation to ensure a sustainable future for generations to come.

In South Africa, where vast expanses of plantation forestry coexist with diverse ecosystems, the forestry sector is taking steps to integrate conservation and sustainability into its practices. Thousands of hectares conserved as natural areas, including indigenous forests, grasslands, and wetlands, are carefully managed to preserve biological diversity. However, the global challenge of biodiversity loss persists, exacerbated by climate change, land-use changes, and unsustainable resource extraction.

The UN Convention on Biological Diversity (CBD) meeting in Kunming, China, serves as a stark reminder of our collective failure to meet biodiversity targets set three decades ago. The need for transformative change is clear, as emphasized by experts who advocate a shift away from traditional economic metrics toward a balanced approach that values both human development and nature.

Enter biomimicry, a groundbreaking concept that offers a beacon of hope in our quest for sustainability. Inspired by nature's brilliance, biomimicry is a science and design approach that holds the key to creating resilient plantation ecosystems with high levels of diversity. The late Claire Janisch, a personal friend and pioneering figure in biomimicry, emphasized the importance of learning from nature and emulating its ingenious solutions.

> *"The prospect of a world where my son, now ten, might face scarcity of healthy, fresh foods and clean water by the time he reaches 37 is both alarming and deeply unsettling."*

Biomimicry challenges us to reconsider our role on this planet and recognize that nature has already provided solutions to many of the challenges we face. By embracing biomimicry, we can tap into the wealth of knowledge accumulated over millions of years of evolution. The practice involves three essential elements: Emulate, Ethos, and (Re) Connect. It encourages us to learn from nature's forms, processes, and ecosystems, adopt a philosophy that supports and sustains life, and reconnect with our place in the interconnected web of life.

The potential applications of biomimicry are vast and inspiring. Examples like the Eastgate Mall in Zimbabwe, designed to mimic termite mound cooling mechanisms, and Japan's bullet trains, inspired by the

Jacqui Meyer

Kingfisher bird, showcase the practicality and efficiency of nature-inspired design.

As we grapple with the need to increase food production by 70 percent and wood supply by three- to fourfold by 2050, biomimicry emerges as a powerful ally. While planted forests are often criticized as "green deserts,", they have the capacity to be multifunctional landscapes that support biodiversity, mitigate climate change, and meet human needs sustainably.

The New Generation Plantations initiative, led by the World Wide Fund for Nature (WWF), exemplifies a collaborative approach to sustainable forestry. By integrating conservation and restoration of native forests into business models, this initiative demonstrates the potential for economic benefits while preserving nature.

The adoption of biomimicry in forestry practices can contribute to net-zero emissions objectives, aligning with global goals for climate change mitigation. For example, inspired by the need for innovative reforestation methods, Bruno Rutman Pagnoncelli's vision led to the creation of Nucleário, a groundbreaking reforestation solution. Mimicking natural forest succession, Nucleário won the 2018 Ray of Hope Prize® for its leaf litter-like design, preventing soil leaching and enhancing seedling survival without extensive maintenance. By reducing the main bottleneck in forest restoration—seedling maintenance—Nucleário enables more efficient tree planting, aligning with global environmental goals like the Bonn Challenge and the Paris Agreement. This biomimicry-driven breakthrough showcases tangible progress in biodiversity conservation and climate change mitigation.

By combining biomimicry with innovative governance models, forest plantations can become integral components of sustainable, multiple-use landscapes that benefit local communities and the environment.

"While planted forests are often criticized as "green deserts", they have the capacity to be multifunctional landscapes that support biodiversity, mitigate climate change, and meet human needs sustainably."

In conclusion, biomimicry offers a transformative path forward, urging us to view ourselves as part of biodiversity rather than external actors. By learning from nature and applying its ingenious strategies, we can create sustainable plantations and ecosystems where all forms of life not only survive but flourish. As Chief Seattle, a Native American leader, wisely stated, "We don't inherit the earth, we borrow it from our children." It is our duty to ensure that when we return what we borrowed, it is in a better state than when we received it.

MINIMIZING SOIL LOSS AND ITS IMPACT ON THE ENVIRONMENT

(MICRO-CATCHMENTS UNVEILED: DECODING SOIL LOSS IN FOREST MANAGEMENT)

An interview with Kate van Zyl

WHY IS THIS RESEARCH IMPORTANT?

As a Sector, we pride ourselves in the provision of ecosystem services, such as the crystal-clear streams that leave our headwaters. We also understand the importance of the soil substrate and its crucial role in ensuring future plantation productivity. As much of the forest landscape is located in upper catchments, we are some of the first curators of water, and thus have a responsibility to ensure not only our own aquatic habitats and wetlands are thriving, but also those downstream have the best possible starting point through the provision of high-quality water coming out of forestry land. While no forester wants to be associated with

Karkloof plantation with stunning waterfall you can hike up from Bushwillows caravan park.

Katy van Zyl

"As a Sector, we pride ourselves in the provision of ecosystem services, such as the crystal-clear streams that leave our headwaters."

Sediment clogs streamflow measurement weir in Jonkershoek plantation after the 2021 fire.

potentially controversial environmental problems – that's not what we signed up for – despite our best-efforts soil erosion, washaways, sedimentation of streams or discolouration does happen, and it is our responsibility to both respond quickly when it does happen and manage our landholdings proactively to guard against this in the future.

HOW DID THE PROJECT COME ABOUT?

Forestry South Africa's Environmental Management Committe have been looking for funding for this project for a few years. In 2022 they succeeded in securing funding through the Department of Science and Innovation's (DSI) Forest Strategic Innovation Fund (FSIF), for this project and several others. While the project is coordinated by the Department of Forest and Wood Science at Stellenbosch University, FSA's Environmental Management Committee will provide active guidance and monitoring.

WHAT WILL IT INVOLVE ?

The first part of this new project aims to try to find out why we still see – in some cases – quite serious examples of erosion and sedimentation happening in plantation forestry. This is a bit unexpected because the topic is well researched, erosion agitators and mechanisms are generally well

"...it is a question of whether practitioners do not have the requisite knowledge on the topic, whether information has been made available in an appropriate form, or whether there are other reasons for intentionally or unintentionally down-prioritising soil conservation."

understood, and there are numerous regulations and guidelines that have been developed over the years with the aim of curbing this problem. Also, over 80% of South Africa's forestry landscape is Forest Stewardship Council® (FSC®) and/or Programme for Endorsement of Forest Certification (PEFC) certified, both have their own principles and criteria in relation to this that certificate holders need to adhere to.

HOW WILL YOU GO ABOUT THIS?

To start with, a gap analysis will be conducted to try to better understand the reasons that the knowledge / best operating practices are not having the desired effect in the field. Broadly speaking, it is a question of whether practitioners do not have the requisite knowledge on the topic, whether information has been made available in an appropriate form, or whether there are other reasons for intentionally or unintentionally down-prioritising soil conservation. In the latter case, the study will attempt to categorise those reasons into areas that potentially could be dealt with through various interventions. This information will be collected through qualitative means using an online questionnaire.

At the same time, the existing regulations (obligatory) and guidelines will be evaluated and recommendations made on how these can be implemented to a greater degree at an operational level.

WILL IT MOSTLY BE A DESKTOP STUDY THEN?

No not at all. While it is important to establish the framework that we are operating in, which needs to be done at a desktop level, we also need to get out into the field to observe operational practices to better understand the potential challenges and barriers. Erosion typically occurs on sites between harvesting and when the new seedlings have properly taken root. Although there are other operational causes such as firebreaks and infrastructure like roads, especially when they involve stream crossings. The study will also look at surface (sheet) erosion and at gulley erosion, which is more difficult to bring under control. The study will also involve collecting research on the impact of soil erosion and the effects of suspended material or sedimentation on the aquatic habitats.

> *"This is an incredibly forward-facing project... ... will enable the identification of gaps in current environmental guidelines, and their implementation, ultimately improving the sustainability of forestry."*

WHAT KIND OF EQUIPMENT, INSTRUMENTS OR TOOLS WOULD ONE NEED?

Erosion needs to be measured both at the source (loss) and at the deposit- either in the riparian vegetation or in the stream itself. In the stream, it ends up as sediment although the finer clays remain in suspension and that is what you typically see when a stream has a brown or orange look to it.

To evaluate loss, we will use frequently sampled ultra-high resolution (5 mm) terrain models. This will enable loss to be calculated at a high spatial resolution and enable cross-season comparisons to be made. One of the external consultants, Bennie van der Waal, is an expert in modelling and will facilitate us to develop compartment level models for predicting soil loss.

Sediment traps, at different stages of the sediments journey, will be used to evaluate sediment deposition. Geotextiles traps can be used in streams as they retain sediment while allowing water to pass, enabling the comparison of sediment loads between the micro-catchments feeding the stream. Private consultant, Gerhard Diedericks, will assist with turbidity measurements, sediment trapping and in assessing the effect of sediment load on invertebrates and other aquatic fauna.

ANY CLOSING THOUGHTS ?

This is an incredibly forward-facing project, harnessing technological advances to better understand the intricacies of soil mobility and loss. This will enable the identification of gaps in current environmental guidelines, and their implementation, ultimately improving the sustainability of forestry practices.

For commercial forestry to remain viable, in South Africa and around the world, there is an urgent need to recognise knowledge gaps and vulnerabilities, and to adapt and alter management practices and strategies to match the increasing economic, environmental and social challenges – like climate change – we will face. Research, like this, will play an integral role in how forestry is proactively managed moving forwards, to ensure the longevity, health and wellbeing of both our Industry and the natural resources – soil and water – upon which we all depend.

DISCOVERY OF GENOME REARRANGEMENTS IN EUCALYPT HYBRIDS – A SOURCE OF NATURAL VARIATION FOR TREE IMPROVEMENT?

Anneri Lötter and Prof Alexander A. Myburg

Forest Molecular Genetics (FMG) Programme, Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria

MAKING wide crosses to create hybrids within or between species has been a useful approach in the quest to develop new high-yielding varieties in plant breeding. Different kinds of hybrids can be produced depending on how related the parents are and how inbred, or outbred they are. The fitness and performance of such hybrids are thought to be determined by different genetic mechanisms depending on the nature of the crosses. Although wide crosses can lead to genetic incompatibilities, it is often possible to identify superior hybrid individuals that combine favourable characteristics of both parents and that can outperform the parents, a phenomenon known as heterosis. As an example, most high-yielding maize varieties today are hybrids of specific pairs of parental lines that were bred to produce superior hybrid varieties.

Hybrid superiority in outbred organisms such as forest trees likely involves a more complex set of genetic mechanisms, but ultimately also combine the best genetics of the two parents to produce superior hybrid varieties. Fast-growing interspecific hybrids of *Eucalyptus* tree species have become important for plantation forestry in subtropical and temperate regions. These varieties are a feedstock for pulp, paper and other renewable bio-based products in South Africa. Hybrids of *Eucalyptus grandis* and *E. urophylla* make up the majority of pulpwood plantations in subtropical regions by combining the fast growth and good form of *E. grandis* with the fungal disease resistance imparted by *E. urophylla*. The success of the *E. grandis x E. urophylla* (GU or UG) hybrids in subtropical plantation forestry has made them a target for genetic studies to better understand hybrid superiority and to design molecular breeding strategies.

> *"...it is often possible to identify superior hybrid individuals that combine favourable characteristics of both parents and that can outperform the parents."*

To date, studies on genome diversity in crop plants and forest trees have mainly focused on single nucleotide changes, or polymorphisms

Zander Myburg

Anneri Lötter

(SNPs), the most abundant form of genetic diversity in plant genomes. More recently, new long-read DNA sequencing technologies (typically producing reads of >10,000 bp) have allowed researchers to detect larger scale rearrangements, or structural variants (SVs), in plant genomes. Researchers can now begin to assess how such SVs in parental genomes may affect gene expression and function, which will provide greater insight into the genetic basis of hybrid performance and superiority. In crop plants such as tomato, genome SVs have been shown to affect multi-billion dollar production traits (Alonge *et al.*, 2020) placing new emphasis on the need to understand genome sequence and structure available for plant improvement.

The application of long-read sequencing to study SVs in highly diverse and outbred forest trees, like *Eucalyptus* species and hybrids, promises to produce groundbreaking insights into the evolution, diversity and genome biology of these trees. In our recent paper, Lotter *et al.* (2023, *GigaScience*), we used Nanopore long-read DNA sequencing technology (Oxford Nanopore Technologies, Oxford, UK) and a trio-binning method to study SVs present within an F1 hybrid and its *Eucalyptus urophylla* and *E. grandis* parents (**Figure 1**). Trio-binning is a unique approach to genome assembly whereby the maternal and paternal genomes within an F1 hybrid can be identified (or phased) and separately assembled as individual genomes. By using short-read DNA sequencing (reads of 100-150 bp) of the paternal genomes, the parental origins of the long-reads from the F1 hybrid can be traced and the corresponding parental genomes within the hybrid assembled independently to reveal sequence and structural differences between the parental genomes (**Figure 1**).

We successfully assembled the maternal (*E. urophylla*) and paternal (*E. grandis*) genomes inherited by an F1 hybrid individual. These assemblies (total length of 545 Mb and 567 Mb, respectively) represent the 11 chromosome pairs of *Eucalyptus* trees.

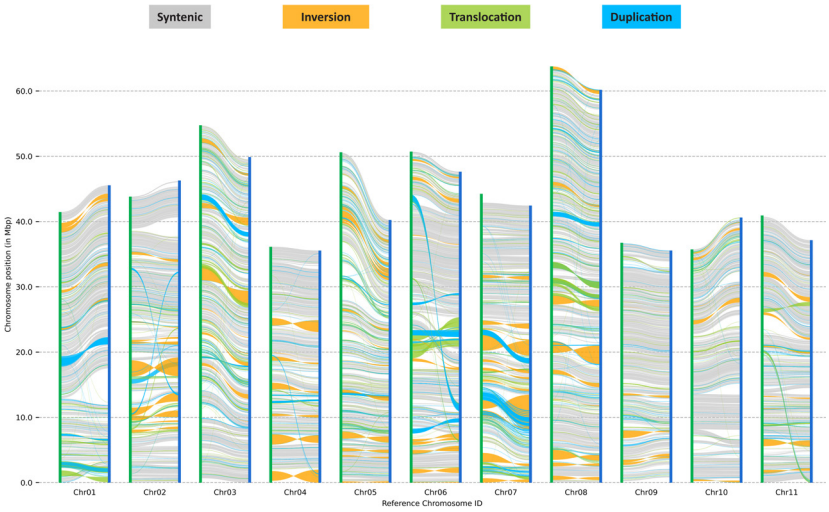

Figure 1: Trio-based assembly and comparison of the *E. urophylla* and *E. grandis* haplogenomes in an F1 hybrid. Long-read sequencing data of the F1 hybrid is separated into parental read bins based on unique markers in the parental short-read sequencing data. The binned long reads were assembled into pseudo-chromosomes. The assembled genomes were compared to identify regions that may contain differences in DNA sequence, but no change in structure (i.e. syntenic DNA) and regions with structural variants such as those with changed orientation (inversions), sequences that have increased or decreased copy numbers (duplications) and sequences that have moved to another location (translocations). Figure adapted from Koren *et al.* (2018) and *Eucalyptus* trees from https://rooweb.com.au/.

> *"The discovery of 10,000s of novel SVs and detailed insight into genome rearrangement within and between Eucalyptus species will provide a foundation for a better understanding of the genetic basis of hybrid vigour in these trees."*

Alignment of the two assemblies revealed 48,729 SVs including inversions, duplications and translocations (**Figure 2**) none of which have been described before. Despite the extensive number of structural variations encompassing 35% of the genes in the genome, the overall structure of the two species' genomes is well enough preserved to make the species interfertile and able to produce viable F1 hybrid progeny. How these SVs (and 8.4 million single nucleotide differences that we also discovered) affect gene expression and F1 hybrid performance is still not understood and will be the focus of future work.

The discovery of 10,000s of novel SVs and detailed insight into genome rearrangement within and between *Eucalyptus* species will provide a foundation for a better understanding of the genetic basis of hybrid vigour in these trees. Our study provides basic knowledge, but also paves a path to the design of targeted breeding strategies for superior hybrids in *Eucalyptus.* Studies in our laboratory are already under way to sequence the haplogenomes of additional species

and F1 hybrids in order to understand which SVs are shared and which are unique to different crosses.

CONCLUSIONS

The use of a trio-binning approach in combination with long-read DNA sequencing has resulted in high-quality haplo-genome assemblies for two key eucalypt species. Comparing these genomes has revealed substantial sequence and structural differences between them. Approximately 35% of genes are contained in rearranged genomic regions. Knowing how these parental genes are mixed and matched in F1 hybrids could help us in the future to create new, stronger, and more resilient forest tree varieties. The next step is to expand this study to include more individuals allowing us to construct a more comprehensive catalogue of genomic variation in the parental species (a pan-genome view). We also want to better understand how SVs influence gene regulation and ultimately affect complex trait variation to develop novel breeding strategies and molecular technologies for *Eucalyptus* plantation forestry and the Forestry Bioeconomy in South Africa.

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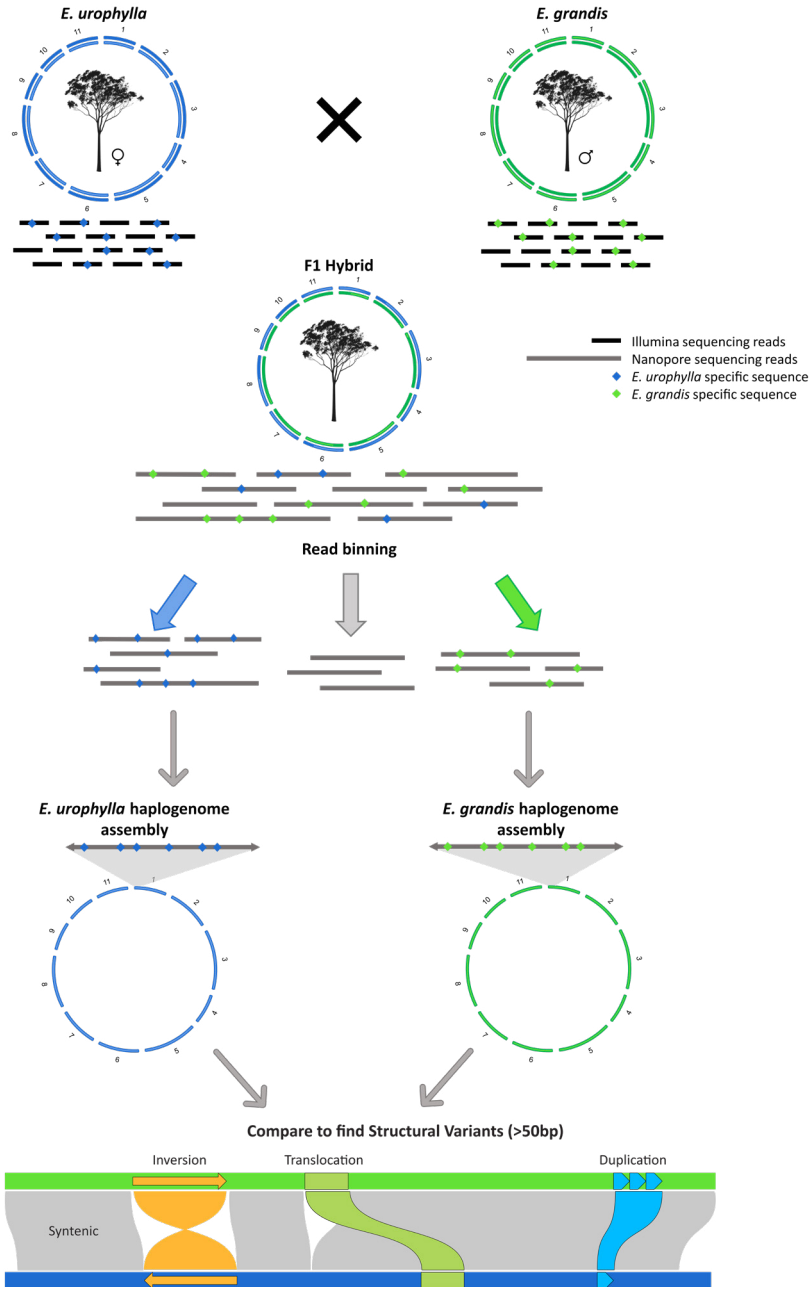

Figure 2: Genome-wide comparison of *E. grandis* (green) and *E. urophylla* (blue) haplogenomes. The eleven chromosome pairs are shown on the x-axis and the position of the structural variants are shown on the y-axis (in millions of base-pairs, Mbp). Structural variant types are colour-coded as shown in the legend. Differences between the *E. grandis* and *E. urophylla* haplogenomes can be due to fixed genetic differences between the two species (i.e. that will differ among all members of the two species), but some may be due to intraspecific variation present in the parents of the particular F1 hybrid individual (and will only differ among some members of the parental species).

GLOSSARY BOX

Outbred: Organisms like forest trees that freely breed (e.g.via open – pollination) with other members of the same species inherit and combine genetic variation from their seed and pollen parents and therefore accumulate large amounts of genetic variation. Their progeny vary widely due to the genetic differences in their genomes. This has great advantages for adapting to environmental change and for resilience to pests and diseases.

Inbred: Organisms that are restricted to close-mating (often imposed by breeders via self – pollination), inherit the same genetic variation from the two parents and therefore accumulate less genetic variation. Their progeny are less variable and can even be genetically uniform, which can be useful for developing new true-breeding varieties

Genome: The complete set of genetic information encoded by the DNA of the organism packaged into 11 chromosome pairs in the case of *Eucalyptus*. Interspecific hybrids carry a full set of 11 chromosomes from each of the parental species (as shown in **Figure 2**).

NEAR-INFRARED SPECTROSCOPY (NIRS)

OPPORTUNITIES, LIMITATIONS AND FUTURE APPLICATIONS

Prof Mark Laing and Dr Sascha Beck-Pay

NEAR-INFRARED SPECTROSCOPY (NIRS) is a light-based analytic technology that is used to characterize organic compounds. The technology is mature, but its implementation has been slow and expensive and requires highly skilled researchers with advanced knowledge of chemometrics. Only very large companies could afford to use the technology. Over the past five years, significant transformations have unfolded in the landscape. A pivotal breakthrough has materialized through the creation of cost-effective, compact, and portable Near-Infrared (NIR) devices, facilitating remote analysis. Concurrently, strides in computer technology, machine learning, and artificial intelligence have revolutionized the landscape of chemometrics, making the technology more accessible and potent for the broader populace. Anticipated future advancements are poised to expand its applications across various domains, extending its reach for online or at-line quality monitoring.

This spectroscopy technique, which uses the reflectance or absorbance of light waves at the wavelength range of 700–2500 nm, offers a rapid, non-destructive, cheap, is less labor-intensive, and can address some of the challenges faced by the seedling production industry (e.g. varietal purity screening, seed germination assessment, detection of pathogens, foliar nutrition analysis, screening for drought and frost tolerance, and the prediction of hybrid root development during clonal propagation of commercial clones) with on-site, real-time analysis (García-Martínez *et al.*, 2012; Van Maarschalkerweerd and Husted, 2015).

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>
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WHY NIRS?

Traditional standard laboratory methods developed to analyze plant materials can be accurate, but they are slow and expensive, making the cost of analyzing large numbers of samples inaccessible to most users. If the complex analytic equipment is not managed properly, or if the technician running the analyses is poorly trained, then the results will not be accurate. In contrast, NIRS technology allows

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for low-cost, fast, accurate, and non-destructive quality assessments to be performed, without the need for complex sample preparation, or the deployment of expensive, highly trained technicians. Furthermore, the NIRS results are very consistent once a good calibration model is created. The use of the NIRS technology is safe as there are no moving parts in the equipment.

However, NIRS is an indirect form of measurement that requires a database of samples to be scanned and analyzed by conventional analytic tools initially. After this step, the NIR spectra and the reference results need to be correlated using advanced statistical tools to create a calibration model. Once the calibration model is created, tested, and shown to be accurate, then NIRS can take place using the calibration model to estimate the expected results. The accuracy of the estimated values is usually in the region of 90-99% reliability, using current systems.

HOW DOES NIRS ANALYSIS WORK?

NIRS is based on the absorbance of light waves at molecular vibrational frequencies occurring for the O-H, N-H, and C-H groups and for the C-C, C-O, C-N, and N-O groups in organic materials. Overtone and combination vibrations of the first group dominate the NIR region (700–2500 nm), while those of the second group absorb in the mid-infrared region (MIR) (2500–25,000 nm, which is used in soil science and mineralogy) (Cortésa *et al.*, 2019). Near-infrared spectroscopy (NIRS) has been used to measure quality parameters in agricultural materials such as foods, agricultural products, and forest products. **Figure 1** shows the NIR spectra of apple, chocolate, kiwifruit, spinach, and wood.

The key concept is that all organic compounds absorb near-infrared (NIR) light, but each molecule has a unique "fingerprint" of absorbance. What is needed is to identify that "fingerprint" as a pattern of NIR absorbance reliably. This is the process of creating the calibration model. Once this has been done, then it is captured and can be used repeatedly by users. And now that the NIRS devices are portable, and linked to cellphones by Bluetooth, the users can be anywhere with cellphone connectivity.

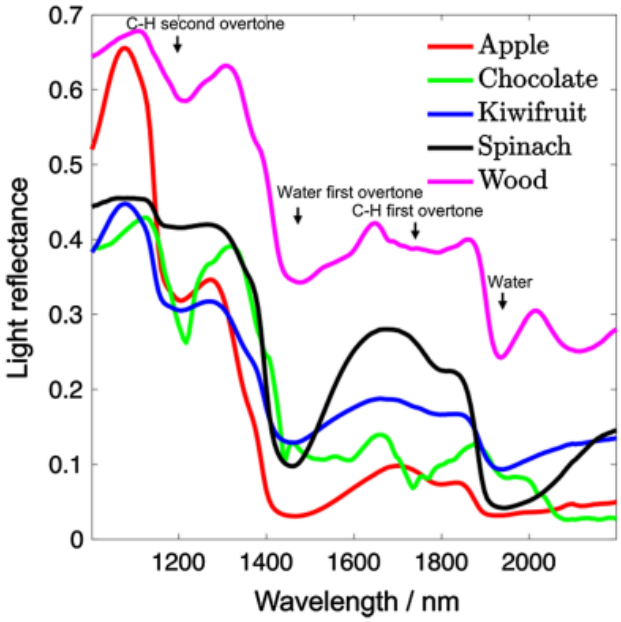

Figure 1: NIR spectra of different agricultural products

"NIRS technology allows for low-cost, fast, accurate, and non-destructive quality assessments to be performed, without the need for complex sample preparation, or the deployment of expensive, highly trained technicians."

DEVELOPING NIRS CALIBRATION MODELS IN PRACTICE

NIRS calibration models require 60-300 samples to create a reliable model. These samples need to be scanned and analyzed using the standard method of analysis. For example, if one wants to measure seed germination in cabbage seedlots using NIRS, then one needs to start

Figure 2: The laboratory/bench top device, such as the Bruker device

with 100-300 different seedlots of variable ages and germination potential. These seedlots then need to be scanned with the selected NIR device, and tested for germination, using standard International Seed Testing Association (ISTA) protocols for measuring seed germination of Brassica seed. Once this is completed, a spreadsheet is created with the sample numbers, the germination results, and the NIR scans of each seedlot. This spreadsheet will then be sent to Frequai, our analytic partner, who uses Machine Learning (artificial intelligence tools) to create the calibration model. Subsequently, they will integrate the cabbage germination calibration into their database of calibration models stored in the cloud on the internet. This comprehensive database will be easily accessible to Seedling Growers Association of South Africa (SGASA) users through a dedicated mobile app on their smartphones. The end-user (e.g. nurseryman, farmer, industry rep.) can then scan any cabbage seedlot using their portable NIR device, and they will get back a result in seconds.

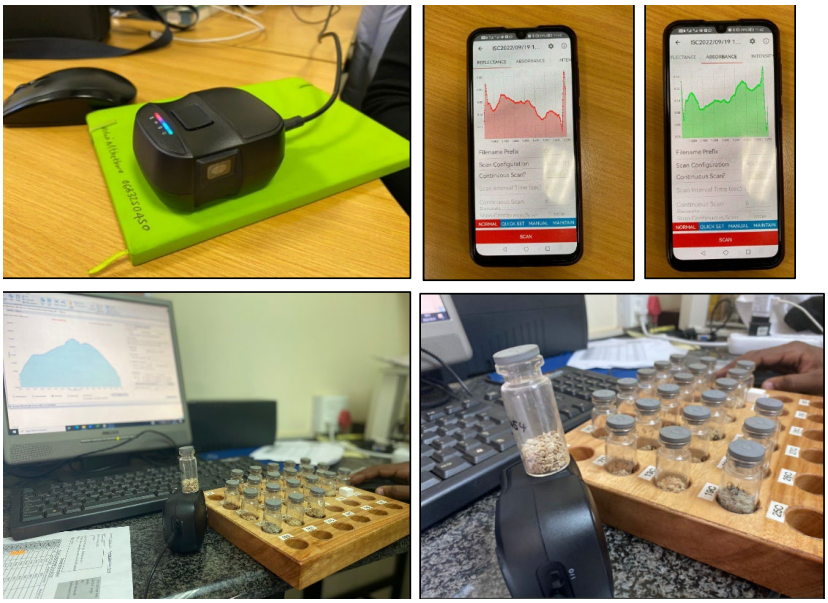

Figure 3: (A) Portable InnoSpectra NIR-S-G1 device. (B) Scans are captured using a smartphone, or (C) laptop. (D) Scanning of tomato seeds.

BENCHTOP VERSUS PORTABLE DEVICES FOR NIRS ANALYSIS

A varied selection of NIRS devices are available: laboratory/benchtop devices, sorting and grading units, and portable devices.

- The laboratory/benchtop devices, such as the Bruker device housed at the ICFR (**Figure 2**), retail at around R2.7 million, whereas the low-cost, portable NIRS devices such as the InnoSpectra NIR-S-G1 retail at around R30-40 000. The desktop devices scan a wider NIR spectrum than the portable units and can generate more accurate calibration models.
- However, portable devices can generate results that are close in accuracy, and portable devices can be used to capture scans of samples in the field. They are controlled either through a laptop or a smartphone, so they are easy to use in the field and can scan at least 1000 scans a day. One practical example is that portable NIR devices are being used in France to evaluate grapes for their maturity, so they get picked at exactly the right stage of ripeness (**Figure 3**).

"NIRS stands out as an immensely potent tool that, for decades, faced limited adoption due to cost constraints and intricacies in its application. However, these barriers are swiftly diminishing, ushering in a new era where NIRS is poised to become a standard feature in various human endeavors."

Sorting and grading devices are available that can be used to scan products on a conveyor belt, and then the samples can be sorted according to their NIR scans. The NIR "camera" is a fixed NIR device sitting over the conveyor belt, scanning the samples as they go past. Most pharmaceutical pills are scanned for their ingredients using this process. It can be used to scan sugar cane delivered at mills for its sucrose content, wood chips for the lignin: cellulose ratio, or to sort rancid macadamia nuts from good nuts.

HOW ARE SAMPLES ANALYZED – PRACTICAL EXAMPLE, BENCHTOP VS PORTABLE?

1. Laboratory/benchtop device:

- Once a robust calibration model is crafted, it will reside on the computer linked to the desktop device. The administration and oversight of this model will be handled by a laboratory service provider equipped with a benchtop device in their laboratory.
- A grower will send in samples. These will be scanned by the service provider and compared against the calibration model, and then the results will be sent back to the end-user.

2. Portable device (innospectra nirs applications are currently under development by a company called frequai with whom we are collaborating):

- Models that have been developed are being made accessible to the end-user through a Cloud-based interface.
- A user will scan their sample with their portable NIR device, using an app on their smartphone or laptop.
- Through the app, they will select what analysis they desire, upload their scan, and the results are delivered within minutes either through the app or via email.

LIMITATIONS

- We have discovered that Huawei cellphones (made in China) are not compatible with the software on the InnoSpectra NIR devices (made in Taiwan).
- We are working with Frequai to make the user interface easy for users to access and use.
- Bluetooth connections can be challenging, occasionally posing difficulties when pairing a cellphone with an InnoSpectra NIR device. Currently, we are actively troubleshooting this issue in collaboration with the

manufacturer to ensure a seamless and reliable connection.

- The quality of the calibration model depends on the number of training samples scanned in to create the calibration model, plus the quality of the original reference chemistry results. This is a moving target and the model can always be improved with more data.
- The scanning process must follow a standard protocol, so the NIR scans are captured in the same way each time.

EXAMPLES OF CURRENT RESEARCH PROJECTS RELEVANT TO NURSERY APPLICATION OF NIRS

1. Seed Moisture, Seed Germination and Seed Vigour Estimates:
The quality of seed significantly influences seedling production, particularly in terms of uniform germination and the development of robust young seedlings. Aging or excessively moist seeds tend to exhibit poor germination, resulting in the production of weak seedlings that may need to be discarded as substandard. Consequently, the seed and seedling industry places great importance on obtaining precise measurements of seed moisture, seed quality, and seed vigour for each seedlot across various crops. For a nurseryman, the germination rate of a seedlot needs to be determined before sowing to determine optimal sowing rates and to ensure that seedling trays are full. This is critical for efficient and cost-effective seedling production. Various protocols have been described by the International Seed Testing Association (ISTA), which are used by seed companies for quality control at production and packaging centres. At the point of planting at nurseries, seed germination rate is typically measured by subjecting batches of seed sub-samples to normal germination conditions, and then counting the numbers of sprouted seedlings. However, this is a laborious process that delays planting and seedling production by at least a week. The alternative is to use NIRS to screen seed, using it to estimate these germination parameters. Such a rapid, non-destructive, high-throughput screening method for seed germination would reduce labor costs and testing time, to estimate the optimal sowing rate determination at nurseries on the day of planting.

SGASA is coordinating a project to develop calibration models for tomato seed moisture, germination percentage and viable seedling percentage. This project is progressing successfully and will be completed early in 2024.

Within the forestry sector in South Africa, two student projects at UKZN/ICFR are currently addressing the use of NIR within nurseries. The first project has already shown that NIR on a benchtop system can successfully predict seed germination and accurately estimate the rate of germination for a variety of forestry and Brassica species. This will be taken further to include NIR scans using the InnoSpectra portable NIR device. Projects are set to be completed in 2024.

2. Nutrient Analysis:
Using NIR to scan leaves allows for direct measurement of the crop's nutrient status, which bypasses soil or foliar nutrient analysis by conventional chemical analysis (slow and expensive), which will allow growers to use this information for fertilizer management. Moreover, NIR enables the detection of hidden deficiencies and prevents the application of excessive levels of fertilizers (Van Maarschalkerweerd and Husted, 2015). This would allow growers to be proactive in their nutrient management by maintaining the synchrony between nutrient demand from the crops and the supply from the soil or growing medium, consequently reaching optimum yields and increasing profits, while reducing unnecessary input costs and the environmental footprint of the agricultural enterprise. Key findings from a review by Prananto *et al.* (2020) were that: (1) macronutrients (N, P, K, S, Ca, Mg) and micronutrients (Fe, Zn, Mn, Cu) can be predicted accurately using NIRS; (2) macronutrients are better predicted than micronutrients; (3) NIRS can detect macronutrients such as N, P, and S directly because they are major constituents of NIRS-sensitive organic compounds, whereas

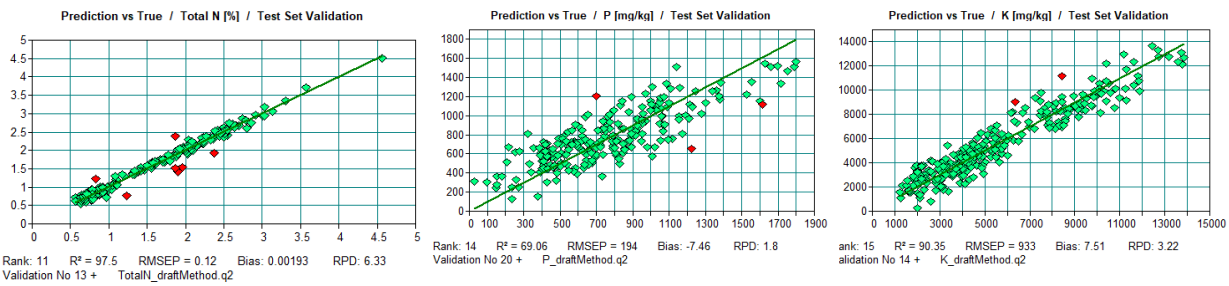

Figure 4: NPK is the most used nutrient supplement with other micro/macronutrients, the NIR spectroscopy can successfully predict the nutrient level on the foliar material.

- A1=Acacia SP644
- A2=Acacia FW54
- E1=G x NPP 2017
- E2=G x U 400
- E3=GxU488
- E5=GL222

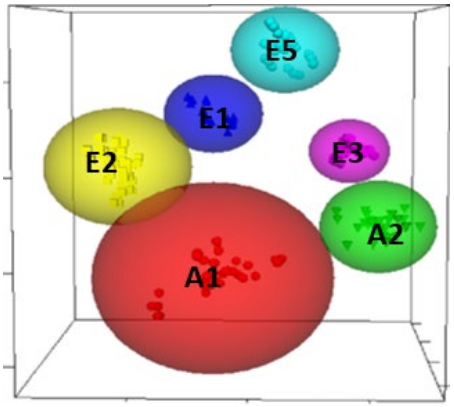

Figure 5: NIR cluster model showing unique identification of clonal samples and differentiation between varieties.

micronutrients and macronutrients exist mostly in their inorganic forms such as Ca, Mg, and K, but are detected by NIRS through their association with organic compounds, and their indirect correlations with these organic compounds. Overall, the review suggests that there is great potential for the field application of NIRS to be used for in situ nutrient analysis of plant leaf tissue (Prananto *et al.* 2020).

Within the forestry sector in South Africa, a student project at ICFR/ UKZN tested forestry seedlings, using NIRS for the early detection of foliar nutrient deficiencies or excesses, and preliminary results have shown that NIRS can successfully predict the nutrient levels of NPK and other minerals (**Figure 4**). This is being written up as a MSc thesis.

3. Varietal Identification

Forestry varieties: A benchtop clonal varietal purity prediction model with a 98% accuracy has been successfully developed for two Acacia and four Eucalyptus species. Currently, the InnoSpectra portable device is being tested to see if it can discriminate between clones and varieties as accurately as the benchtop NIR (MSc Project (UKZN, ICFR) (**Figure 5**).

Avocado varieties: A similar project is being run on avocados and it has shown that Hass and Fuerte can be identified accurately using NIRS. This is being expanded to all popular avocado cultivars.

Macadamia varieties – there is an opportunity to use NIRS to ensure true-to-type grafting material of the correct scion is being used in the macadamia industry.

4. Varietal Rooting Potential

In an ICFR/UKZN project Eucalyptus and wattle varieties are being evaluated for their ability to root as cuttings. These are being scanned with NIR. The researchers will then determine whether they can generate strong calibration models for rooting ability in the varieties. Given that rooting is a physiological response that is based on plant hormones. The likelihood of the NIRS model being robust is quite high.

5. Disease Detection:

The South African Avocado Association (SAAGA is running a project with UKZN to detect Avocado Sunblotch Viroid disease in avocado trees using NIRS. The initial model using a laboratory NIR device is strong and can differentiate between diseased and healthy leaf samples. A calibration model using the InnoSpectra portable device is now under development.

Other potential disease detection projects could use NIRS to detect hard-to-detect diseases such as Turnip mosaic virus in Brassicas, downy mildew in several crops, bacterial diseases in Brassicas and tomato, anthracnose fungi in beans and tomato seed, viruses in peppers and chilis, and citrus greening in citrus crops.

SUMMARY

NIRS stands out as an immensely potent tool that, for decades, faced limited adoption due to cost constraints and intricacies in its application. However, these barriers are swiftly diminishing, ushering in a new era where NIRS is poised to become a standard feature in various human endeavors. In agriculture, numerous possibilities arise for leveraging NIRS to consistently assess various facets of agricultural practices. The present challenge lies in envisioning the potential applications and subsequently developing precise calibration models for each specific use case.

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