Growing confidence in forestry's future Research Programme

SCION FORESTS PRODUCTS INNOVATION

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Technical Note

Management options to increase radiata stress tolerance – progress to date

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Summary: The New Zealand planted forest estate will be under increasing levels of stress from climate and intensification over the next century. Plants tend to overreact to stress, and this response frequently does more damage to the plant than the stress itself. Soil bacteria the produce the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase can reduce this effect by attenuating the response of plants to stress, allowing plant to essentially ignore transient or low levels stresses that they would otherwise respond to in a damaging way. We have conducted a range of studies involving ACC deaminase activity, and have determined that variations in stress tolerance across different genotypes of radiata pine, and indeed various species of conifer, is likely associated with differences in their ability to recruit soil bacteria that produce this enzyme. Recently we have also determined that it is possible to manipulate soil to enhance the gene copy numbers for ACC deaminase, but the implications of this for stress tolerance in radiata pine are yet to be determined.

Introduction

The next century will see the New Zealand planted forest estate exposed to a shifted climate. Increased temperatures, increased incidence of drought, and increased incidence of extreme rainfall events are all predicted for New Zealand[1]. These changes will directly stress the planted forest estate, and the many of the processes that support the sustainability of forests[2]. In addition, climate change has the potential to drive an array of other ecological responses that may further enhance the stresses placed on our managed forests. These include:

- Increased insect pest activity
- Increased pathogen activity
- Increased range of pest and pathogen spread
- Emergence of new pathogen and pest species
- Increased vigour of weed species
- Emergence of new weed species

A further complicating factor is the drive to increase forest productivity[3]. Enhancing growth rates through the use of fertiliser products and the deployment of stock optimised for productivity may come at the cost of reduced allocation of resources to the *in planta* processes that maintain health, such as immune response. This has the potential to reduce the general robustness of the planted forest estate, increasing susceptibility to the new threats that are likely to emerge[4].

The response of trees to these stresses will reduce productivity if unchecked. A stressed plant produces ethylene at vastly elevated rates, triggering biochemical pathways the put the tree into a "survival mode", massively reducing the allocation of resources to growth. This process also results in the production of hydrogen cyanide (HCN), which can cause significant tissue damage. This ethylene response is triggered relatively easily, and therefore it is common that the response of plants to a minor or transitory stress does more damage to the plant than the stress itself. This process appears to be irrational, but as plants cannot move out of the path of an oncoming threat, this all-or-nothing response has proven to be an effective pathway to safeguard the survival of a given plant – but at a cost to growth.

Over the last decade Scion has conducted a series of studies exploring the potential to utilise soil microbes to provide some degree of influence over this stress response. Some soil microbes express the enzyme

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1-aminocyclopropane-1-carboxylate (ACC) deaminase, which breaks down the precursor of ethylene. This slows the stress response and allows the plant to maintain productivity when challenged by either a transitory or minor stress. In this technical note these past studies will be summarised, and the latest results from current trials will be presented.

ACC deaminase – mode of action

Ethylene is an important plant hormone is constantly synthesised from ACC at very low levels by the plant. ACC is very “leaky” and is readily realised into the soil around the roots, which prevents too much ethylene from being produced under normal conditions. However, when the plants are stressed, ACC production increases massively, and the exuded ACC builds up in the soil around the roots until no more can be released. This allows ACC to then accumulate in the plant, enabling a surge in ethylene and HCN production.

Soil bacteria that produce ACC deaminase can “eat” the ACC in soil, causing the plant to release more. This restricts the build-up of ACC in the plant, and extends the window of time before ethylene production increases. This additional time is critical, as it allows the plant to determine if the stress has abated, or stabilised at non-threatening levels.

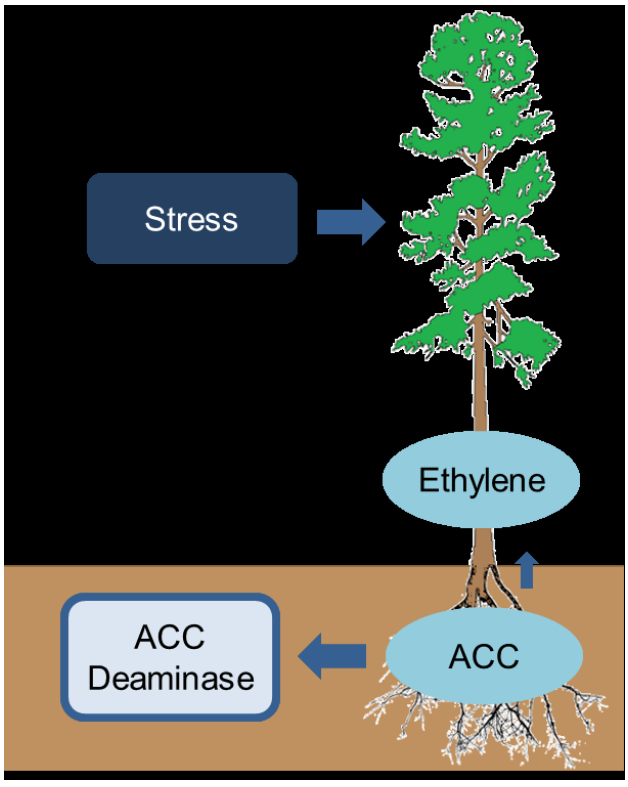

Figure 1 Stress causes the tree to produce ACC in greater quantities, which is released by the roots into the soil. ACC deaminase acts of continuously break this exuded ACC down, allowing more to be released, and reducing the pool of ACC in the plant that is available to convert into ethylene and HCN.

Manipulating ACC deaminase activity with stock selection

It is already known that different genotype of radiata pine can display varying levels of tolerance to stresses such as drought. While physiological adaptions exhibited by the genotypes are clearly a factor in tolerance to various kinds of stress, it was also considered possible that some genotypes were able to augment their innate stress tolerance by associating with soil bacteria that produce ACC deaminase. This was explored by examining the ACC deaminase activity from soils collected under a three clones at a drought prone site in Canterbury,

The results determined that the highest level of ACC deaminase was found under the clone that was performing best at the site (Clone 3), while the least activity was associated with the clone that was performing the worst. The clone selected as an average performer at the site was associated with an intermediate level of activity,

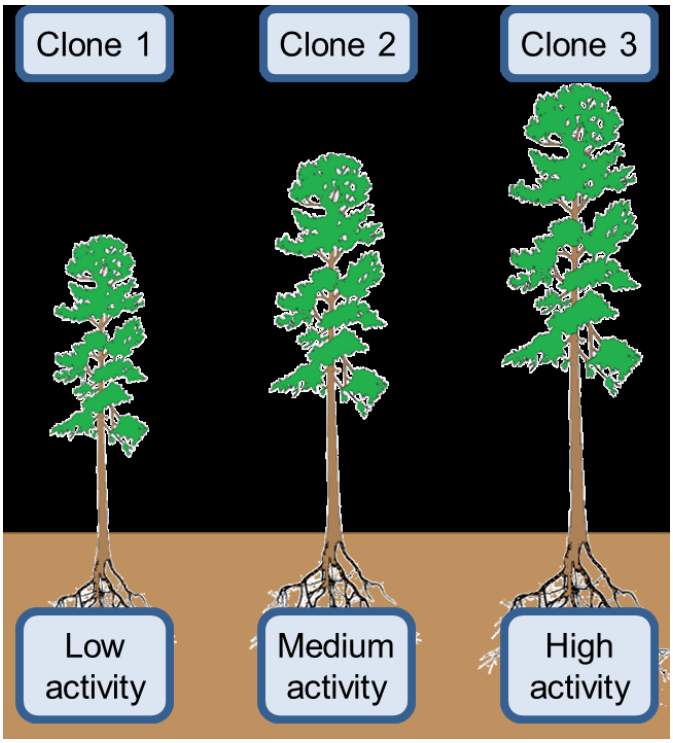

Figure 2 ACC deaminase activity was found to vary with clone, and aligned with performance at the site.

This finding suggests that some clones are more effective at “attracting” soil bacteria that produce ACC deaminase. The presence of these bacteria then likely contributed to the ability of that clone to adapt to the dry site.

This research was extended by assessing the ACC deaminase activity associated with a range of exotic forest species known to be able to colonise marginal land that would be considered stressful for many plant species. The pine species studied were Lodgepole pine, Mountain pine and Scots pine, while Douglas-fir was also assessed. Soil samples were

collected from underneath seven individuals of each species at a single location in the Waimakiriri Basin, central South Island. Analysis of the samples determined that ACC deaminase activity, determined by the ability of the soil bacteria to convert ACC into alpha ketobutyrate, was greatest in mountain pine, and least in Scots pine.

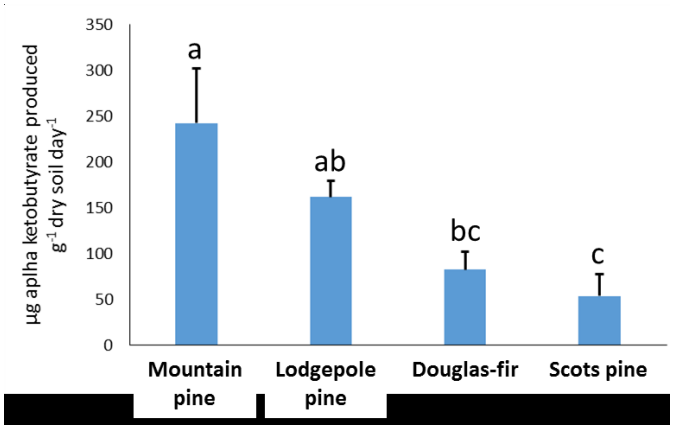

Figure 2 Variation in the ACC deaminase activity associated with different tree species. Letters indicate statistical differences.

Further analysis indicated that the ACC deaminase activity under the various species was very closely correlated with the local altitude limit of each species. As increasing altitude increases the stress placed upon the plants, this suggests that the ability of the trees to recruit ACC deaminase producing bacteria was deriving their ability to spread vertically into harsher terrain.

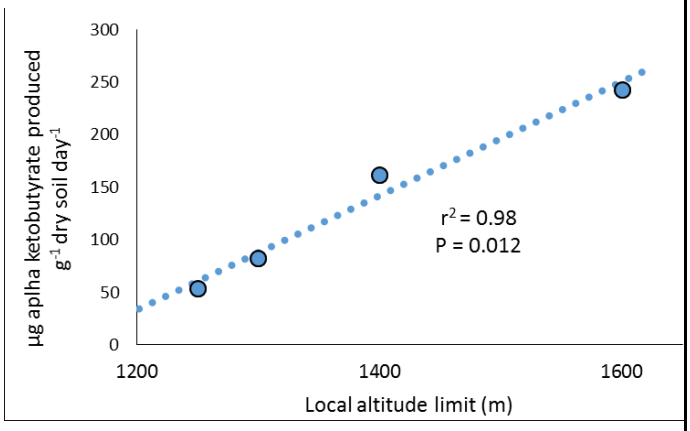

Figure 3 Strong correlation between ACC deaminase activity and the local altitude limit of the four species.

These outcomes indicate the clear sensitivity of ACC deaminase activity to stock selection.

Influence of site treatments on ACC deaminase activity

In order to extend the utility of the ACC deaminase process, it was necessary to determine if it could be enhanced through other methods that could be applied once the trees were already established. To address this knowledge gap, the activity of ACC deaminase in bulk soil was examined several years the imposition of weed control and nitrogen fertiliser treatments at a dryland radiata pine planation in Canterbury. Soils samples were collected form the bulk soil in previously established plots, then analysis for ACC deaminase activity as described above.

It was observed that the use of weed control had increased ACC deaminase activity by an average of 55% across the 16 plots used in the study, while the use of fertiliser increased ACC deaminase activity by 32%. To provide some information regarding the impact of this on productivity, plot-level regressions with growth data were performed. Overall it was determined that ACC deaminase activity was related to the growth metrics, but only in the plots that did not receive fertiliser[5].

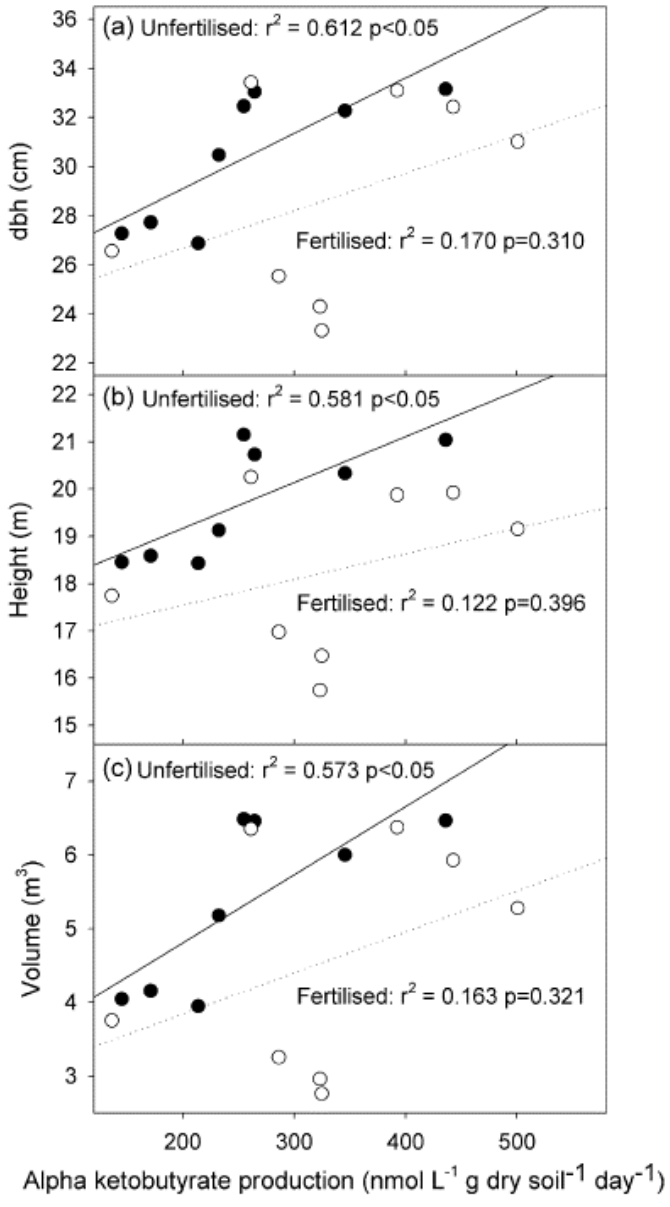

Figure 4 Degree of correlation between bulk soil ACC deaminase activity and radiata growth varies between unfertilised plots (closed circles and solid line) and fertilised plots (open circles and dotted line). (a) Correlations with mean dbh. (b) Correlations with mean height. (c) Correlations with mean tree volume.

Overall, this result indicated that fertiliser application was increasing the capability of the soil bacteria to produce ACC deaminase activity, but it also suggested that unless forced to (as in the analysis procedure) the bacteria were not using the enzyme. Therefore, the relationship with growth was only significant when fertiliser was not used. It is suggested that this is due to the greater availability of nitrogen in the fertilised plots, as ACC deaminase provides the bacteria with an additional nitrogen source. If nitrogen is already available, then expression of the enzyme would be less advantageous.

The response to weed control (conducted as glyphosate applications) was likely due to the differences in organic matter and chemical inputs into the soil due to the decreased variety and biomass of understory plants. Identifying the rationale for this response was outside the scope of the study, so this was not resolved.

In order to extend the research a new methodology was developed to quantify the copy number of the gene for ACC deaminase in the soil bacterial community. This provided a more robust benchmark to assess the impact of treatments across a wider range of conditions. This methodology was first used during the end of rotation assessments at the Woodhill and Tarawera Long Term Site Productivity (LTSP) trials.

These analyses found that fertiliser additions (which ceased at least a decade prior to this analysis) had significantly increased ACC deaminase gene copy number at Woodhill, but not at Tarawera. This variation may be due to the greater amount of fertiliser added to Woodhill, while Woodhill is also an inherently a more stressful site. It is also possible that ACC deaminase gene copy was elevated by fertiliser use at Tarawera around the time of application, but has subsequently decreased to initial levels.

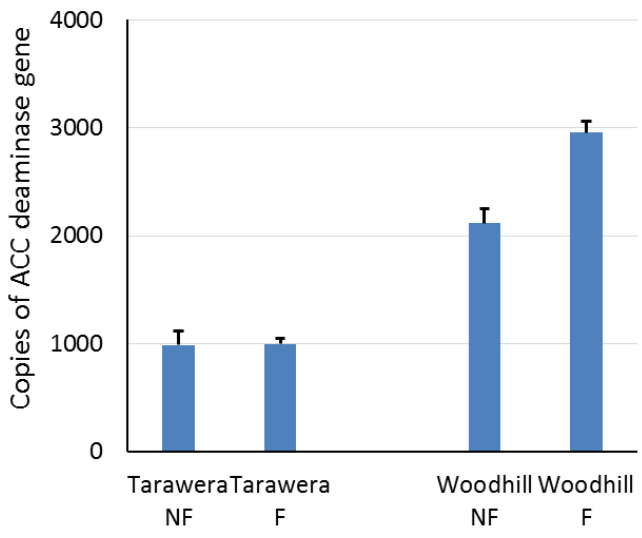

Figure 5 Effect of historic fertiliser use on ACC deaminase copy numbers.

Directed manipulation ACC deaminase gene copy numbers

Based on the response of ACC deaminase to radiata pine genotype and fertiliser use, a new trial was established to determine if these ad-hoc observations could be replicated in dedicated experiments. Four clones with known variations in stress tolerance were grown in the nursery then planted out into pots at age one, while an additional set of stock was potted and treated with nitrogenous fertiliser application. After one year the effect of genotype and the fertiliser treatment on ACC deaminase gene copy numbers in the soil was determined.

It was determined that the clones known to have relatively greater levels of stress tolerance (clones A and B) were associated with greatest ACC deaminase gene copy numbers. Clone D, which exhibited low levels of stress tolerance had the lowest ACC deaminase copy numbers. The addition of fertiliser to clone D (+ Fert) dramatically increased ACC deaminase gene copy numbers, approaching levels equivalent to the three other clones.

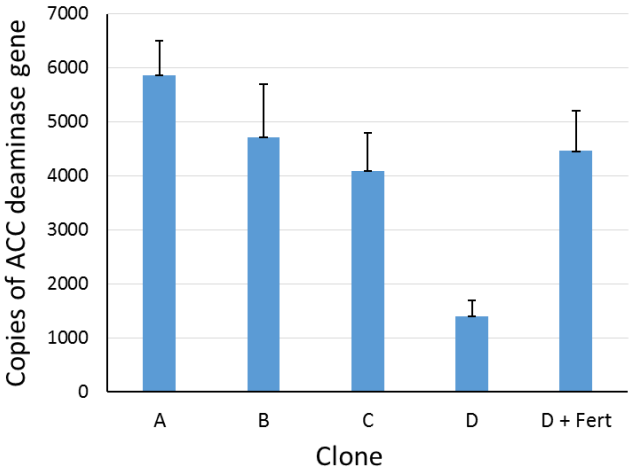

Figure 6 Effect of genotype and the fertiliser treatment on ACC deaminase gene copy numbers after one year.

Further research

The results of the directed manipulation trial indicate that ACC deaminase gene copy numbers are sensitive and can respond relatively quickly. This does not, however, prove that they have enhanced the tolerance of the plants to stress. This will be tested in the next few months, and the results reported as they become available.

Acknowledgements

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