final report

Project code: **SHP.015**

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Date published: **June 2008**

ISBN: **9781741917277**

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Responses to quorum sensing signals in pasture plants

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

This study investigated whether bacterial quorum sensing (QSS) signals could be used as potential treatments to enhance the performance of pasture plants. QSS are used by bacteria to coordinate behaviours important for host infection. Previous work found that QSS are perceived specifically by plants, and it was therefore hypothesised that plants could use QSS as cues for detecting and responding to the presence of specific soil bacteria. This study found that treatment of plants with specific QSS could enhance seed germination and plant growth. In addition, QSS altered plant microbe interactions, including nodulation of legumes, interactions with Pseudomonads and interaction with pathogenic fungi and oomycetes. The effects dependent on growth conditions, the type and concentration of QSS and their mode of application. The results suggests that certain QSS could be trialled for use as seed dressings or leaf sprays to enhance the growth of pasture plants.

Executive Summary

This project was initiated as a discovery project to investigate the potential of bacterial quorum sensing signals (QSS) as plant treatments to enhance pasture plant growth and health. It sought to establish a proof of concept for the effectiveness of QSS in improving plant growth and plant interactions with soil microbes.

QSS are chemical signals (acyl homoserine lactones) synthesised by most gram negative bacteria. Bacteria use these signals to regulate behaviours that require bacterial cooperation, for example when infecting a host. This has been demonstrated to be important for infection of plants with pathogenic bacteria (for example soft rot bacteria) as well as symbiotic bacteria, (for example rhizobia). Different bacterial species produce different chemical variations of QSS.

The background for the work was established by previous research showing that purified or synthetic QSS from bacteria can alter gene expression in plants when applied to roots (Mathesius et al., 2003). This suggested that plants can perceive these bacterial signals, and perhaps use them as cues to detect bacteria by their QSS 'signature' in the rhizosphere and to prepare for interactions with these bacteria, whether symbiotic or pathogenic. However, it had not previously been tested whether this ability to detect the signals and respond at a molecular level results in altered plant performance. The gene expression studies showed that genes important in plant defence, metabolism and hormone control were affected by QSS. This suggested that QSS could (i) alter plant defence responses and thus influence subsequent plant microbe interactions and (ii) influence plant hormone metabolism that could result in changes to plant growth. These hypotheses were tested in selected pasture plant species: *Lolium perenne* (perennial ryegrass), *Trifolium subterraneum* (subclover) and *Medicago truncatula* (barrel medic). Under sterile and non-sterile conditions, these plants or seeds were exposed to synthetic QSS that are known to be synthesised by soil/rhizosphere bacteria. Their subsequent germination, growth, nodulation (where appropriate) and interaction with pathogenic soil microbes was assessed.

The project established a proof of concept that certain QSS can affect plant growth, germination and plant interaction with soil microbes.

First, application of certain QSS accelerated seed germination under sterile conditions in all three plant species. The effect depended on the concentration and structure of the QSS. Under non-sterile conditions, a smaller effect on germination was shown. However, this effect was transient and plant growth was no longer accelerated after one to three weeks of growth.

Second, application of QSS as seed dressings or as a regular leaf spray altered plant growth. In some cases, leaf and root growth (weight) was significantly increased by the application of QSS. This effect again depended on the plant species, the structure of the QSS and the growth conditions and suggests specific recognition of QSS by plants.

Third, pre-treatment of the two legumes, subclover and medic, with QSS influenced their ability to form nodules in symbiosis with rhizobia. Whereas QSS did not enhance nodulation under sterile growth conditions, nodulation could be enhanced in semi-sterile soil experiments. This also resulted in improved plant growth. The QSS synthesised by the specific rhizobial symbionts were most effective at enhancing nodulation, whereas other QSS were ineffective.

Fourth, the effect of QSS on the interaction of plants with fungal or oomycete pathogens were tested. Pathogens selected for this study were those that have been shown to limit pasture plant growth and included *Rhizoctonia solani* AG 2.1, *Phytophthora medicaginis* and root knot nematodes (*Meloidogyne javanica*). The hypothesis behind this was that treatment with QSS could affect general defence responses in the plant that could then prepare the plant for a subsequent attack by pathogens. The results showed no significant effect of QSS treatment on the development of root galls by root knot nematodes in medic, and a small reduction of gal numbers and plant growth in subclover. Both species were similarly infected by this pathogen. *Rhizoctonia solani* appeared to colonise both subclover and medic, but subclover showed no inhibition of growth in response to Rhizoctonia, whereas medic was significantly affected. Certain QSS enhanced growth of Rhizoctonia infected subclover roots. Certain QSS had a small beneficial effect on Rhizoctonia-infected medic when used as a regular leaf spray but not when used as a seed dressing. *Phytophthora medicaginis* severely infected medic and killed plants within 1-2 weeks. This was accompanied by production of a specific flavonoid in the roots that was used to monitor infection. Certain long chain QSS inhibited the accumulation of that flavonoid and alleviated early symptoms, but did not prevent the eventual devastating effect of *P. medicaginis*.

This project was designed to focus on the basic research on plant perception of QSS. It was only four years ago that the first reports emerged that indicted that plants can perceive and respond to QSS. Therefore, this research is still in a stage of discovery, with limited applications unless further research is carried out. To benefit from this research, QSS would need to be tested under field conditions with the local soil and pasture plants. The research done in this project could guide those tests by providing information about active QSS that could be tested, how they could be applied to the plants and what outcomes might be expected. However, since this project found that plant responses to QSS depended strongly on the exact structure and concentration of the QSS, as well as on the growth condition and plant species studied, the final outcome of the QSS-plant interaction in the field is difficult to predict.

Farmers interested in testing QSS for potential to enhance their pasture plant growths could benefit from the results. Many QSS are commercially available and could be tested as seed dressing before pasture plants are sown into the soil. This might enhance seed germination and could potentially benefit plants by being able to outgrow pathogens present in the top soil layers. The QSS could also be further tested by seed companies or research and development organisations.

In addition, the observation that subclover was not affected by *Rhizoctonia solani* AG2.1 under the conditions used could be further investigated to determine the factors that could make subclover somewhat resistant. Since the application of certain QSS further enhanced growth of Rhizoctonia infected subclover, their potential in alleviating this important fungal pathogen disease could be further tested.

Contents

1 Background

1.1 Background

1.1.1 Quorum sensing in bacteria

In recent years the importance of the rhizosphere in plant growth and health has been recognised. Rhizoshere organisms can be beneficial or harmful to plants. They can contribute to nutrient cycling, nitrogen fixation, disease suppression, growth promotion and specific signalling with the host. Studies of signaling in the rhizosphere have started to identify the plant and bacterial signal molecules important for plant-microbe interactions (Hirsch et al., 2003). An important signalling system in the rhizosphere (as well as in other microbial habitats) is the communication among bacterial cells via quorum sensing signals (QSS). Quorum sensing is the population density-dependent regulation of gene expression that controls various behaviours in bacteria (Whitehead et al., 2001). QS enables single celled bacteria to communicate and coordinate gene expression, which can result in multi-cellular structures of bacteria, for example in biofilms and fruiting bodies. Single bacteria synthesise QSS which diffuse into the environment and are detected by receptors in other bacteria. Once a certain threshold ("quorum") of bacteria is present, QSS concentrations reach a level at which they trigger changes in gene expression. Many different behaviours are subject to QS regulation in bacteria, for example enzyme production, biofilm formation, and infection of eukaryotic hosts. Gene expression studies have suggested that up to 20% of bacterial genes could be regulated by QSS (e.g. Schuster et al., 2003).

Most gram negative bacteria use acyl homoserine lactone (AHL) quorum sensing signals. These conatin a homoserine lactone ring bound to a variable acyl side chain. Different bacterial species synthesise AHLs of different structures, although most bacteria appear to synthesise more than one AHL (Whitehead et al., 2001).

1.1.2 Detection of QSS by plants

If bacterial pathogens and symbionts of plants depend substantially on quorum sensing to colonize and infect their hosts, it is likely that plant hosts could have evolved strategies to detect these signals and take advantage of the information contained in QS signals. Several recent studies have found evidence that plants (and animals) can detect and respond to QSS from bacteria (Bauer and Mathesius, 2004). A proteomic analysis of *Medicago truncatula* found that >150 proteins in roots were altered by application of nanomolar amounts of purified QSS from *Sinorhizobium meliloti* and *Pseudomonas aeruginosa* (Mathesius et al., 2003). These changes included multiple defence related, as well as hormone-induced and metabolic proteins. Microarray studies have confirmed changes in gene expression in response to QSS in tomatoes (Schuhegger et al., 2006). This study also showed that the application of QSS at the roots had systemic effects in shoots that led to reduced infection of shoots by fungal pathogens. In addition, Joseph and Phillips (2003) found that watering roots of bean plants with homoserine lactone, a breakdown product of QSS that is present in the rhizosphere, increased stomatal conductance and transpiration.

Interestingly, plants have evolved strategies to interfere with QSS in bacteria by producing mimic compounds of QSS (Teplistki et al, 2000). These mimics have the potential to inhibit or prematurely stimulate the expression of QS regulated genes in bacteria, but they have not been identified so far.

These studies suggest that plant can detect QSS specifically (various structures and concentrations). This project investigated whether plants gain any advantage through exposure to these QSS in their ability to grow and their interaction with bacterial and fungal microbes in the soil.

2 Project Objectives

2.1 Project Objectives

The general project objectives are the following:

1. By (month 2)
   Evaluation and validation of sterilisation techniques and growth assays on different plant species to provide basis for the following tasks.
2. By (month 7)
   Assessment of plant growth responses to different quorum sensing signals under different growth conditions.
3. By (month 12)
   Assessment of plant microbe interactions of selected plant species and beneficial and harmful bacteria in the presence and absence of quorum sensing signals.
4. By (month 18)
   Assessment of plant colonisation by bacteria and fungi in the presence and absence of quorum sensing signals.
5. By (month 24)
   Test whether plant growth in response to plant growth promoting rhizobacteria can be improved by AHL treatment
6. By (month 30)
   Assess whether plant protection can from soil pathogens by plant growth promoting bacteria can be improved by treatment with AHLs.

3 Methodology

3.1 Methodology

The detailed methodology for the project can be found in the respective milestone reports.

3.1.1 Source of seeds, microbes and signal compounds.

Seeds of *Medicago truncatula* cv Jemalong A17 was received from SARDI, Adelaide. Seeds of *Lolium perenne* were received from Dr Yvonne Cheng, CSIRO Plant Industry, Canberra. Seeds of *Trifolium subterraneum* cv. Kerridale was purchased from Cleanseeds, Bungendore.

Cultures of rhizobia were maintained in the laboratory from ongoing cultures.
Cultures of *Rhizoctonia solani* AG2.1, *Meloidogyne javanica* and *Phytophthora medicaginis* were received from SARDI, Adelaide.

AHLs were either purchased from Sigma Aldrich or received from Professors Dietz Bauer (University of California, Davis) and Anatol Eberhard (Cornell University). AHLs were dissolved in ethyl acetate : acetic acid (1000:1) and diluted with sterile water to the final concentration.

3.1.2 Plant growth conditions

Seeds were sterilised in 6% hypochlorite for 5 min with shaking, washed 6 times with sterile water, and shaken in a Falcon tube for 6 h in shaker at 28 °C in 200mg/L of the antibiotic Augmentin. After six sterile water washes, seeds were placed on Fahreaus agar plates and germinated O/N at 28 °C, then plated on larger plates. Plants were incubated in a growth chamber at 25°C, 16 h day, 8 h night, 120 μE light intensity. When grown on agar plates, roots were protected from light with black cardboard

Sterilised soil experiments were done with a 1:1 mixture of washed river sand and vermiculite which was autoclaved before use.

Experiments in field soil were done with non-sterile Wallaroo field soil, received from Drs Richard Simpson and Yvonne Cheng, CSIRO Plant Industry, Canberra.

Rhizobium leguminosarum bv. *trifolii* and *Sinorhizobium meliloti* were maintained as a freezer cultures and freshly cultured in liquid Bergensen's modified medium at 28 °C in a shaking incubator until they reached an OD600 of 0.1. Pots were inoculated with 1 ml of this liquid culture; individual roots grown on plates were inoculated with 10 μL each of the culture.

Meloidogyne javanica was maintained in Medicago truncatula. When eggs emerged from galls, they were picked and placed into a drop of sterile water and incubated at 25 °C until juvenile worms hatched. These were re-inoculated onto fresh *M. truncatula* or *T. subterraneum* roots.

Rhizoctonia solani was grown on Potato Dextrose Agar plates by transferring a plug of an old culture onto a fresh plate. Cultures were maintained at room temperature. For inoculation of plants in pots, a 1x1 cm plug was cut from a freshly grown plate and placed 2 cm deep into the middle of a pot of soil. This was left to incubate for one week before planting seeds.

Phytophthora medicaginis was grown on V8 agar to sporulation stage (this was done in collaboration with Prof. Adrienne Hardham, Research School of Biological Sciences, who works on plant infection by Phytophthora species). Spores were diluted in sterile water to final concentrations of between 250 and 20 000 spores / ml. *M. truncatula* seeds were germinated as detailed above and four day-old seedlings grown on Fåhreus agar plates were inoculated with the *Phytophthora* spores by dipping roots into Eppendorf tubes containing the spores for 1 min. After that, seedlings were placed back onto the agar plates and incubated at the normal growth conditions. Seedlings were monitored daily for 1 week. All infected plants had died within 10 days.

3.1.3 Treatments with QSS

QSS were either applied to seeds by soaking freshly sterilised and washed seeds in a solution of AHLs or by spraying leaves repeatedly (usually 3 times per week) with a sterilised atomiser. In some experiments, AHLs were incorporated into agar plates by adding aliquots of AHLs to autoclaved media before pouring into agar plates. AHLs were used at different concentrations (between 10^{-6} and 10^{-10} M). Equivalent concentrations of solvent were used as a no AHL control.

3.1.4 Microscopy

Seedlings inoculated with *P. medicaginis* and *M. javanica* were monitored for accumulation of fluorescent flavonoids under an epifluorescence microscope (Leica DMLB, Wetzlar, Germany) using a UV excitation filter (excitation maximum at 365 nm, 425 nm longpass filter). Images were taken with a mounted CCD camera (RT Slider, Diagnostic Instruments).

3.1.5 High pressure liquid chromatography

To analyse flavonoid content flavonoids were extracted and separated by HPLC. Roots were excised, weighted and ground in liquid nitrogen. 1 ml of 80% methanol was added per 100 mg fresh weight to each sample and extracted overnight (~16h) at room temperature (20 degrees) in the dark on a rotating wheel. Tubes were centrifuged at 14,000 rpm on a bench centrifuge at room temperature for 30 min. The supernatant was taken into a new tube and evaporated in a speedy-vac. The pellets were redissolved in 45% methanol at 1ml/g fresh weight. Flavonoids were separated on a Shimadzu LC-10 VP series HPLC, equipped with a diode array UV/VIS detector and a fluorescence detector, using an Alltec Altima C18 5u reverse phase column (250 x 4 mm, Alltech). We loaded 50 μL of each sample. Solvents were A MilliQ water + 0.1% acetic acid and B acetonitrile + 0.1% acetic acid. Separation was over 70 min, as follows: 0-5 min 100 % A, 5-70 min 0-80% B. Absorbance was recorded between 190 nm and 700 nm, and fluorescence emission was detected at 450 nm (excitation at 365 nm).

3.1.6 Real time PCR

Root samples were ground in liquid nitrogen to a fine consistency and RNA extracted using a standard Trizol extraction protocol. Extracted RNA was treated with DNase to remove any contaminating DNA, and then processed with an RNA clean-up kit to remove degraded DNA and other possible contaminants. 500 ng of the clean RNA from each biological replicate was used to synthesize cDNA. The resulting cDNA was diluted 1:10 for use in quantitative real time-PCR.
Primers were made using the Primer 3 program, using the TC sequences for the genes of interest. Commercial control primers (for Secret Agent: an O-GlcNAc transferase) were used for amplification of a reference gene. All primers were optimised before use to determine efficiency. Using SYBR Green and a standard amplification protocol, each cDNA sample underwent 65 rounds of amplification followed by melting point determination. The relative amounts of cDNA for each gene in each sample could then be determined (normalized to the reference gene).

4 Results and Discussion

4.1 Results and Discussion

4.1.1 Effects of QSS on seed germination

Treatment of seeds with various QSS led to increased seed germination rates of plants grown under sterile conditions on agar plates. This depended on the concentration and structure of the QSS. For example, treatment of *Lolium perenne* with 3-oxo-C16:1-HSL (an AHL synthesised by Sinorhizobium meliloti, a symbiont of medics) significantly enhanced seed germination speed. Treatment of medics with C8-HSL significantly increased seed germination rate at concentrations between 10^{-10} and 10^{-6}

M. In (non-sterile) soil experiments, seed germination of subclover was initially enhanced, leading to a higher germination rate measurable two weeks after sowing.
These experiments suggested that QSS affect the early development of the seed, that this response is specific for certain AHLs and concentration dependent. The enhancement and acceleration of seed germination might be useful in field situations where plants need to outgrow pathogens located in the top layers of the soil.

4.1.2 Effects of QSS on plant growth

Plant growth was assessed in sterile situations (growth on sterile agar plates and in sterilised soil) as well as in non-sterile field soil. Root and shoot weight, leaf numbers and hypocotyl length were increased after different treatment with QSS, but again this depended on the exact structure of the QSS and the species that was treated. Short term growth enhancement was seen inn plants whose seeds had been treated with AHLs before sowing. Longer term effects were seen in plants repeatedly sprayed with QSS on the leaf surface. The treatment of leaves clearly showed positive effects in roots, this could either indicate a systemic effect, as reported in other studies (e.g. Schuhegger et al., 2006; Joseph and Phillips, 2003), or it could be an indirect effect caused by increased shoot growth.

4.1.3 Effects of QSS on plant-symbiont interactions

We assessed the effect of AHLs on nodulation in subclover and medic. When seedlings were grown under sterile conditions on agar plates, AHLs did not have any, or any positive, effects on nodulation. Plants grown in sterilised soil showed a significant increase in nodule numbers after leaf treatment with certain QSS. The active signals were in particular those that were synthesised by the rhizobial symbionts of the respective legumes. This suggests that the plant might recognise the specific AHLs of its symbiont and use the information to enhance the interaction with the symbiont, for example by down-regulation of defence responses, increased expression of Nod factor receptors or other reasons. Since the enhanced nodulation in soil was accompanied by increased plant growth, it is also possible that the increased nodule numbers were an indirect result of increased carbon availability in the plant. However, the overall effect of QSS treatment was a positive effect on growth of symbiont - inoculated plants.

4.1.4 Effects of QSS on plant-pathogen interactions

A first experiment examined the growth responses of plants grown in field soil containing native pathogens (Walloaroo field soil). Treatment of subclover with C8-HSL in this soil initially stimulated seed germination and increased the numbers of leaves within two weeks of sowing. After four weeks, plant growth was slightly enhanced in the QSS treated plants, but this was not statistically significant. Experiments with medic showed a decrease in plant growth after treatment with AHLs. This suggests that whereas treatment of plants under sterile conditions with AHLs can increase plant growth, this is not necessarily the case under conditions where pathogens are present in the soil.

The second experiment assessed infection of subclover and medics with root knot nematodes. Treatment of medics with C8-HSL did not change the number of galls formed on the root system. In subclover, C8-HSL decreased the number of galls significantly, and this was accompanied by decreased root and shoot weight. Whether the reduction in gall formation is an indirect effect of reduced plant growth is unknown although it is unlikely because C8 HSL treatment of uninfected plants caused enhanced plant growth in previous experiments. Gall formation can sometimes be

associated with increased plant growth, possibly through alteration of auxin metabolism by the nematodes, so it is possible that this was the case in control plants containing more galls.

A third experiment analysed the effect of QSS on infection of medic with *Phytophthora medicaginis*. This pathogen infected medic roots very severely and killed all treated plants within approximately ten days. Initially the minimum number of spores that caused infection was estimated (approximately 750 spores/plant). When inoculated with these numbers of spores, all plants showed symptoms of fluorescence compound accumulation (visible with fluorescence microscopy) within 48 h. This yellow fluorescent compound was identified as the flavonoid 7,4'-dihydroxyflavone (DHF). It accumulated in epidermal and cortical cells at sites of hyphal growth and was used to quantitatively assess the amount of damage caused to plants by the pathogen using HPLC. Growth of infected plants on agar containing various QSS showed differences in the amount of DHF accumulated, with the lowest accumulation in plants treated with long chain QSS. This suggests that certain AHLs might be able to alter plant defence responses that may alleviate pathogen infection. The mechanism will have to be further investigated.

A forth experiment investigated the effect of QSS on infection of subclover and medic by *Rhizoctonia solani* AG2.1. While medic growth was significantly reduced by Rhizoctonia infection, subclover was not, even though both species were colonised by the fungi on the root surface. Gene expression analyses showed that two defence genes, PR10 and prg2, were induced by Rhizoctonia infection in medic.

Treatment of soil grown subclover plants with C8-HSL increased growth in Rhizoctonia infected plants compared to non-AHL treated infected, and uninfected plants.

We also observed that hyphal growth of Rhizoctonia on plates was severely inhibited by the presence of *Sinorhizobium meliloti* and *Pseudomonas aeruginosa* wild type strains (QSS deficient *P. aeruginosa* strains were less inhibitory). Therefore we tested whether the presence of these bacteria had any effect on plants grown in the presence of Rhizoctonia. In subclover grown in soil, *P. aeruginosa* wild type had a positive effect on uninfected plants, but in Rhizoctonia infected plants this positive influence of *P. aeruginosa* was not significant. In medic grown in the presence of *S. meliloti*, the bacteria significantly increased plant growth of Rhizoctonia infected plants irrespective of QSS treatment. This was only the case in an experiment where QSS were applied to seeds at the beginning of the experiment. When seedlings were repeatedly sprayed with QSS, QSS also had a positive effect, and *S. meliloti* inoculation had no additional beneficial effect over QSS treatment.

Quantification of gene expression of selected defence-related genes did not show any clear correlation between QSS treatment of plants and gene expression that could explain any of the positive or negative effects of QSS treatment on growth of *R. solani* infected plants.

Overall, the experiments with pathogens suggested that in specific cases, QSS application could alleviate some of the damage caused by pathogens. However, each pathogen showed different requirements for AHLs and varied between plant species. Therefore, to develop any effective treatment, further experiments will need to be done to optimise AHL structure, concentration and application for certain plant species. AHLs can also break down in the soil, and this is highly dependent on soil pH (stable under acidic but labile under alkaline conditions). This should be taken into account when trying to apply these results to field studies.

5 Success in Achieving Objectives

5.1 Success in Achieving Objectives

5.1.1 Evaluation and validation of sterilisation techniques and growth assays on different plant species to provide basis for the following tasks

Different seed sterilisation techniques were trialled to be able to carry out experiments on sterile plants. A combination of hypochlorite and antibiotic treatment was effective in removing almost all bacteria and fungi from growing seedlings. These techniques were subsequently used to ensure that no endophytic bacteria that produce AHLs interfere with QSS application. This milestone was relevant only for the first experiments, to establish a proof of concept that it is the QSS themselves that produce beneficial effects. In later experiments, non-sterile plants were used to demonstrate QSS effects in realistic field situations.

5.1.2 Assessment of plant growth responses to different quorum sensing signals under different growth conditions

Growth responses of plants to QSS were tested in agar plates, in sterile soil and in non-sterile field soil. The results showed variable effects in the different growth conditions. In general, positive effects were found in sterilised soil that was later inoculated with rhizobia. Additional application of QSS as a leaf spray enhanced plant growth under these conditions.

5.1.3 Assessment of plant microbe interactions of selected plant species and beneficial and harmful bacteria in the presence and absence of quorum sensing signals

Interaction of subclover and medic with their respective rhizobia, as well as with *Pseudomonas aeruginosa* was investigated.
Nodulation experiments with plants grown on sterile agar plates did not show any enhancement after AHL application.
Experiments with *Pseudomonas aeruginosa* and three different *P. aeruginosa* AHL-defective mutants had mainly negative effects on root growth in *T. repens* and *L. perenne*, with a slight positive effect on shoot growth in *L. perenne*. In *Medicago truncatula*, wild type *P. aeruginosa* significantly stimulated root and shoot growth. The ability of *P. aeruginosa* to synthesise AHLs affected its ability to change root and shoot growth.

5.1.4 Assessment of plant colonisation by bacteria and fungi in the presence and absence of quorum sensing signals

Colonisation of roots and nodules with rhizobia was not affected by AHL treatment.
Colonisation of roots with *Phytophthora medicaginis* was reduced after treatment with long chain AHLs.
Colonisation of roots with root knot nematodes was not affected by AHL treatments.

5.1.5 Test whether plant growth in response to plant growth promoting rhizobacteria can be improved by AHL treatment

Experiments with rhizobia showed that in soil grown plants, nodulation of subclover and medic could be increased by treatment of plants with AHLs, in particular with the AHLs synthesised by their respective symbionts.

5.1.6 Assess whether plant protection can from soil pathogens by plant growth promoting bacteria can be improved by treatment with AHLs

The ability of QSS to alleviate symptoms of *Rhizoctonia solani*, *Meloidogyne javanica* and *Phytophthora medicaginis* was evaluated in medic and/or subclover. No significant effect on root knot nematode infection could be found in medic, whereas in subclover, numbers of root gals were reduced by AHLs. Long chain AHLs alleviated the early symptoms of infection of *M. truncatula* with *P. medicaginis* but did not prevent eventual infection. *R. solani* had a negative effect on *M. truncatula* but not on *T. subterraneum*. An AHL had further positive effects on infected *T. subterraneum* and minor positive effects on *M. truncatula* when applied repeatedly as a leaf spray. Inoculation of *R. solani* infected plants with rhizobia significantly enhanced plant growth in one experiment with medic. Inoculation of *R. solani* infected plants with *Pseudomonas aeruginosa* did not significantly enhance plant growth.

6 Impact on Meat and Livestock Industry – now & in five years time

6.1 Impact on Meat and Livestock Industry – now & in five years time

While this project was not designed to deliver immediate applications, it has established a proof of concept that QSS could be used as potential plant treatments to improve pasture plant growth. If further research is undertaken within the next five years, certain QSS could be developed for specific applications as germination enhancers or for the protection of plants from rhizosphere pathogens. For example, QSS could be used as seed dressing before sowing seed or as leaf sprays in established pastures.

7 Conclusions and Recommendations

7.1 Conclusions and Recommendations

7.1.1 Potential for QSS to enhance seedling growth

QSS were shown to accelerate seed germination under certain conditions. Further research is necessary to determine whether this effect is relevant in the field and in different plant species. If this holds true for relevant plant species under soil conditions, the application of QSS as seed dressings could be beneficial in the field. In particular, this study showed that QSS from symbiotic bacteria, for example rhizobia, were the most beneficial for plant growth enhancement.

7.1.2 Potential for QSS to alleviate damage by rhizosphere pathogens

Rhizosphere pathogens, especially fungi and oomycetes, are major constrains of pasture plant performance. Therefore, any improvement in plant defence or resistance to these pathogens could contribute to better pasture plant health. It would be worth further investigating the use of specific QSS to protect plants from pathogens present in native pasture soils. Because this project showed that specific QSS are effective only in interaction of plants with specific microbes, it would be beneficial to undertake such research in combination with the newly developed identification tests for soil microorganisms by researchers at SARDI, so that QSS application could be tailored for pastures with specific pathogens.

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