January 2023

Exploiting Genetic Diversity in Potato (*Solanum tuberosum*) to Overcome Soil Constraints to Tuber Yields

This thesis is being submitted for the degree of PhD in Agricultural Science

Lancaster Environment Centre, Lancaster University

Patrick Skilleter

BSc (Hons) Biology, Biosciences and Chemistry, Sheffield Hallam University, UK

MSc Crop Improvement, School of Biosciences, University of Nottingham, UK

Author’s Declaration

I declare that the contents of this thesis are the product of my own work, except where cited or credit otherwise given. No part of this thesis has been submitted in the same form for a higher degree elsewhere or published as research.

I also declare that the word count of this Thesis totals 29,368 words, including data, figure legends, and text incorporated into diagrams, tables, and figures. This value does not include words outside of the Thesis, such as references, appendices, and the abstract.

Patrick Skilleter

Lancaster University

January 2023.

Acknowledgements

This thesis would not have been produced with the quality both of data and production without the help of a great many people. I would firstly like to thank my Academic Supervisor Professor Ian Dodd for his constant advice and support, and for encouraging me to go beyond what I would have otherwise done by myself. I would also like to thank my Industrial Supervisor Doctor David Nelson for giving me insight into the industrial side of potato growing, his practical help with field trials, and his advice on both writing up and planning my experiments. I am grateful to the BBSRC and Waitrose for funding this project, and to Roz Wareing, Carly Stevens, and Amanda Weston for providing support throughout my project.

I would also like to thank those who have helped me expand my knowledge and skills throughout my PhD, allowing me to learn a vast array of new practical skills without which this thesis would not be here today. Specifically, I'd like to thank Arnauld Thiry, Jaime Puértolas, Muhammad Yahya Khan, Sarah Donaldson, Rachel Baxter, Katharina Huntenburg, Robert Kempster, Cristina McBride-Serrano, Eric Mensah, Vasileos Giannakopoulos, Vassil Karloukovski, Shane Rothwell, John Quinton, and Ryan Edge. I'd also like to thank the support staff of the LEC for ensuring my work has gone smoothly from the background, including Sam Jones, Maureen Harrison, Annette Ryan, and many more.

Finally, I would like to thank my parents, Lois and John Skilleter, my brother, Robert Skilleter, and my friends Anastasia Sokolidi, Ashley Swanson, Christopher Marsh, Conner Fenton, Kane Griffiths, and Peter Crosby for providing constant moral support and belief that I could see this through to the end.

Abstract

Exploiting Existing Genetic Diversity in Potato to Overcome Soil Constraints to Tuber Yields – Patrick Skilleter

Potato fields are highly susceptible to soil compaction, with two-thirds of UK fields severely affected. Compacted soils impede root growth and decrease crop yields. This thesis's goal was to identify ways to improve potato yields in compacted soil.

Genotypic diversity in compaction tolerance was determined by growing six potato cultivars in loose and compacted soil in containers. Compacted soil delayed emergence, reduced leaf area and root length, and increased root diameter differently between cultivars. When cultivars varying in compaction tolerance were reciprocally grafted, rootstock affected leaf area and root growth more than the scion, indicating the root system's importance.

Root production of, and sensitivity to, ethylene were measured in cultivars that showed high (Charlotte), intermediate (Maris Piper), and low (Pentland Dell) sensitivity to soil compaction. Soil compaction and cultivar did not affect root ethylene evolution. Exogenous ethylene increased root diameter of Maris Piper and Charlotte similarly, but Pentland Dell was unresponsive. Applying ACC deaminase-containing rhizobacteria lowered root ethylene evolution of Maris Piper roots and increased root growth in compacted soil to comparable levels as roots in uncompacted soil. Thus, variation in ethylene production and ethylene sensitivity mediate root growth in compacted soil.

Two cultivars with higher (Inca Bella) or lower (Maris Piper) sensitivity to soil compaction were grown in field trials comparing compacted and uncompacted soil. Irrespective of initial soil resistance, soil resistance consistently increased to cultivar-dependent maxima during the growing season. Compacted soil decreased Inca Bella yields, but not Maris Piper yields. Inca Bella better maintained shoot growth whilst Maris Piper root growth was unaffected by compaction. Thus, maintaining root growth is more important for maintaining yields in compacted soil produced by pressure on the soil surface.

This thesis is the first to identify compaction- and cultivar-dependent changes in soil resistance in potato fields, and to utilise ACC deaminase-containing rhizobacteria to enhance potato tolerance to compaction.

Contents

Commonly Used Abbreviations

Ψ	Water potential
5C-2	*Variovorax paradoxus* 5C-2
ABA	Abscisic acid
ACC	1-aminocyclopropane-1-carboxylic acid
ANOVA	Analysis of variance
KPa	Kilopascals
MPa	Megapascals
TR	Transpiration rate
WHC	Water holding capacity
WT	Wild type

Chapter 1 General Introduction

1.1 Introduction

Potato plants (*Solanum tuberosum*) have been a mainstay in the agricultural industry of the British Isles since the 16th century. The United Kingdom was the twelfth largest potato producing country in the world in 2020, yielding over 5.5 million tons of the crop, with China and European nations such as Germany, Poland and Russia being the largest producers (FAO, 2022). Potato crops harvest indices typically vary between 0.7 and 0.8 (Mazurczyk *et al.,* 2009), with most cereal crops having a maximum of 0.6 (Unkovich *et al*., 2010), indicating more biomass is being allocated to edible parts of the plant. In addition, potato yields are typically far higher than other crops, with maximum expected cereal yields in the UK reaching 10 t/ha, and potato yields 40 to 50 t/ha (FAO, 2022), although the high water content of tubers means dry matter yields are typically 8 t/ha to 12 t/ha. Due to the importance of this crop, it is vital that high yields are maintained.

As the human population brushes past 8 billion people and continues to rise, demand for food also increases. In order to meet food production targets, farming is likely to become more intensive (Haverkort *et al*., 2015), with increasing reliance on farm machinery to manage crops throughout the growing season. As farming machinery continues to get larger and heavier (Johansen *et al*., 2015), cropland becomes increasingly susceptible to soil compaction. Potato fields globally are frequently afflicted with soil compaction (Figure 1-1) that restricts root growth and decreases yield by more than 30% (Stalham *et al*., 2007; Johansen *et al*., 2015; Ghosh and Daigh, 2020), with two-thirds of UK potato fields containing regions that restrict over 90% of root growth. This is most notably an issue in developed nations, such as those in the EU, due to increased reliance on heavy agricultural machinery (Johansen *et al*., 2015). It is therefore necessary to understand how and why this compaction occurs, apply methods to minimise its presence, and to adapt or overcome the constraints compaction causes.

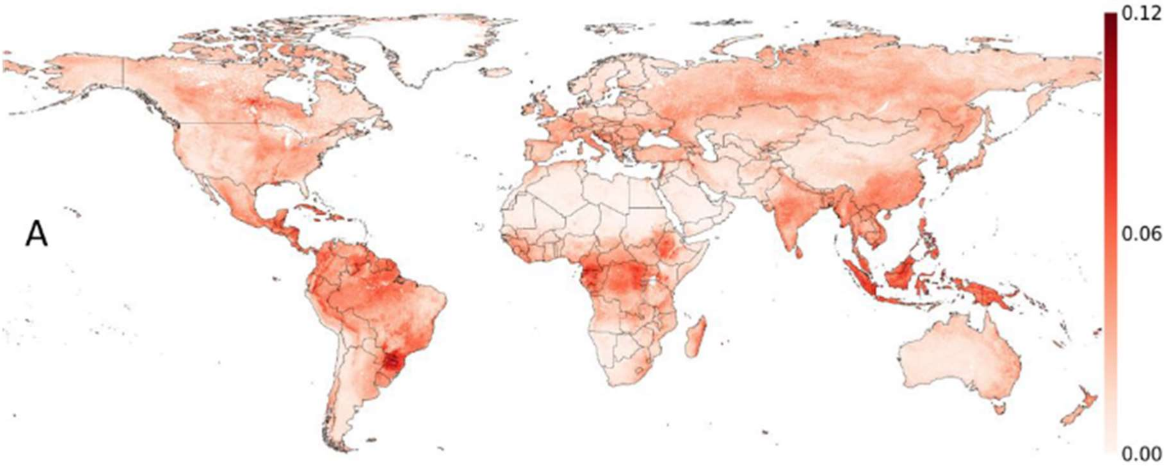


Figure 1-1: Global productivity losses (% loss/ha/yr) caused by soil compaction and erosion from high-throughput agriculture. Taken from Sonderegger and Pfister, 2021.

1.2 The Causes and Mitigation of Soil Compaction

Soil compaction is the process by which soil particles are forced closer together. Compaction is typically measured either by soil resistance (resistance of the soil to deforming and breaking) or bulk density (dry soil mass per unit volume). Soil resistance is a term used to define how much force is required to penetrate through the soil (Ajayi *et al*., 2009), and is a measure of the effect soil compaction has upon plants growing in it (Passioura, 2002). Bulk density can also be used to determine soil strength by measuring the mass of solid particles within a specific volume. As compaction decreases the volume of air space between soil particles (Ghosh and Daigh, 2020), bulk density naturally increases as soil becomes more compact. Whilst increases in bulk density and soil resistance tend to correlate, they are not comparable measurements. Bulk density is primarily used to compare levels of compaction within a single soil, as differences in soil texture affect bulk density. Soil resistance is comparable between soil types as it measures force required to penetrate, but unlike bulk density, resistance is affected by environmental factors, such as soil water content.

Soil compaction can originate from many sources. The most common cause in agricultural land is the use of heavy farming equipment on fields. The pressure of massive machinery being driven over soil causes soil to compact, especially on wet soil (Johansen *et al*., 2015). This problem has become more serious in recent years, with farm machinery becoming larger and heavier (Duiker, 2004). A second cause is slumping. Tilling soil causes it to become very loose, but structurally unstable (Batey, 2009). When tilled soil is subjected to a rapid wetting event, the sudden change in conditions can cause the structure to collapse, with soil particles collapsing into each other, compacting the soil (Hao *et al*., 2011). This is most likely in sandy soils, as these contain larger, heavier particles that are more prone to collapse, and can hold more water. Finally, the specialisation of farming machinery has caused its own issues as different machinery has different track widths (Johansen *et al*., 2015). Instead of avoiding compaction within seed beds by following the same tracks, seed beds must be narrowed, reducing growing space and yield, or wider vehicles must be driven across seed beds. This causes compaction within the seed beds, directly inhibiting crop growth. Although methods to alleviate compaction are necessary, an ideal solution does not necessarily exist.

Compaction in the topsoil can be reduced or removed entirely with the use of tilling or subsoiling. This involves breaking up the soil structure, which removes compaction. However, this is merely a temporary measure, as compaction can easily recur, especially if other steps are not taken to avoid recompacting the soil (Batey, 2009). In addition, tilling soil, especially when wet, can create regions of high soil resistance (known as plough pans) just below the tillage depth, as the soil is forced downwards by the machinery (Kubo *et al*., 2008). These pans have higher resistance than the surrounding soil (Figure 1-2) and create a major barrier to root growth. To conclude, whilst subsoiling can remove compaction, it is typically a temporary measure, and can lead to subsoil compaction, that is very difficult to remove (Johansen *et al*., 2015).

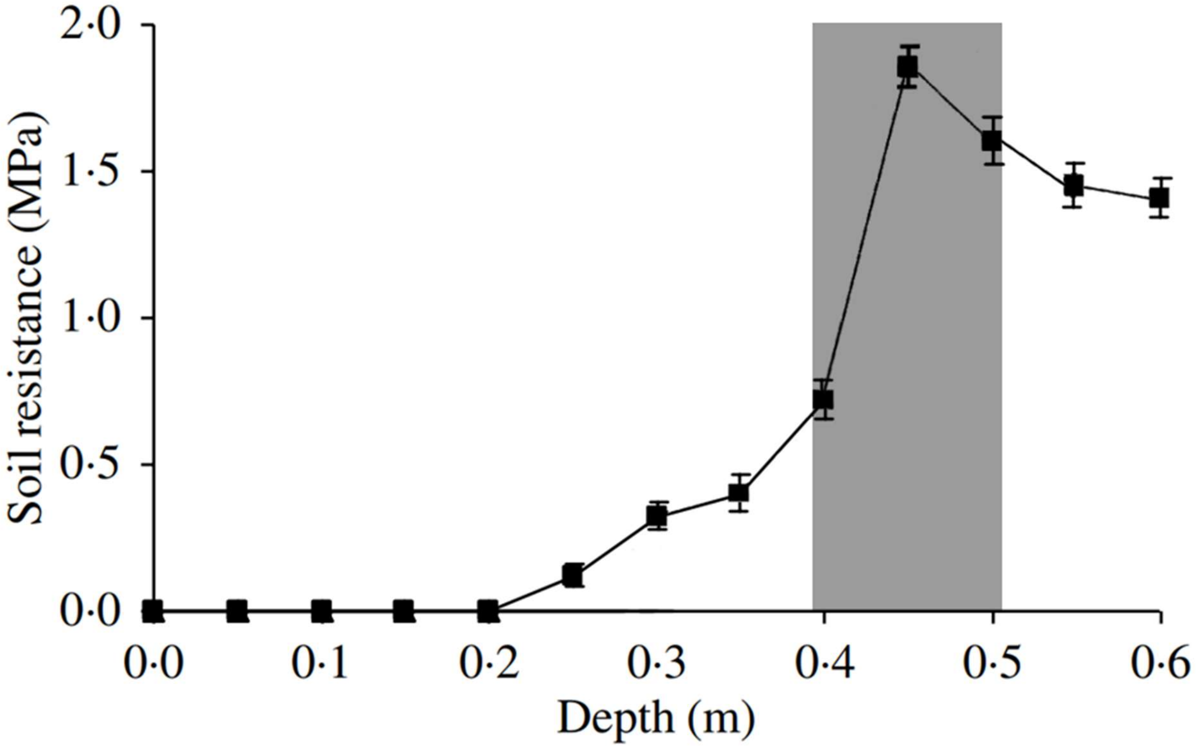


Figure 1-2: A soil resistance graph for a potato field affected by a plough pan caused by destoning (shaded region). Depth is relative to the top of the de-stoned bed. Error bars indicate standard error with 95% confidence limits. Modified from Stalham *et al*., (2007).

A second option is applying additional organic carbon to the soil by methods such as spreading manure. Increased soil organic carbon decreases bulk density and soil resistance (Sakin, 2012), and has a minor effect at resisting compaction by improving the soil's ability to rebound from pressure (Pereira *et al*., 2007). However, the effects are very variable and limited, and thus the benefits are questionable. Furthermore, such methods are costly, with the organic carbon being most easily obtained from sources such as manure. The cost of such large amounts of manure and the equipment to spread it is not insignificant and must be factored into any decision. In addition, the use of machinery to apply organic carbon is likely to further increase the risk of compaction if not done when the soil is dry, further limiting effectiveness. If this method provides only limited benefits, is unlikely to be profitable under many circumstances.

While mechanical subsoiling applied to alleviate soil compaction can actually cause soil compaction, biological subsoiling, otherwise known as biological drilling, is a potentially viable option. This approach utilises cover crops that produce a high density of bio pores in compacted soil, particularly tap-rooted species (Chen and

Weil, 2010), such as *Raphanus sativus* (forage radish) and *Lupinus* spp. (lupins) (Ghosh and Daigh, 2020). The bio pores remain after plant death, leaving channels for other roots to access. Whilst there is evidence of this being a practical method of alleviating compaction (Besson *et al.*, 2013), issues still arise. For example, cover crops used for this purpose need to have a similar root diameter to the grown crop or contact between root and soil within the pore is limited, reducing uptake of water and nutrients (Ghosh and Daigh, 2020). Finally, the planting and harvesting of potatoes requires excavation of the topsoil, which disrupts the soil structure. This would also remove the bio pores that are present in the excavated soil, reducing effectiveness.

A final option is to manage the soil using conservation agriculture. Conservation agriculture utilises minimum or no tillage methods of farming and mitigates the risk of compaction by ensuring that the farmland is constantly covered by crop plants or their residues, with subsequent crops directly drilled into the residue of the previous crop. These factors promote soil stability and aeration whilst reducing the potential of compact soil forming in the long term (Kassam *et al.*, 2009). Constant soil coverage also increases soil organic matter content over time (Djaman *et al.*, 2022). However, the lack of tillage leads to increased soil resistance and bulk density (Naab *et al.,* 2017; Djaman *et al.*, 2022). Whilst conservation agriculture is a viable option, yield responses are inconsistent, with some studies reporting a 20% increase in marketable yield, whilst others report a 20% yield loss compared to conventional tillage in potato (Djaman *et al.*, 2022). However, alternative tillage strategies, such as the use of raised beds (Essah and Honeycutt, 2004) or performing shallow tillage in the regions where tubers are planted (Pierce and Burpee, 1995) have also improved yield over conventional tillage, implying that other alternatives, such as limiting subsoiling to regions that are compacted at times when the soil is dry may be better than reduced or zero-tillage in potato cropland.

Overall, there is no simple way to entirely remove and prevent compacted soil, and most solutions are costly and temporary. It is therefore likely to be more cost effective to adapt to soil compaction rather than investing money in constant repairs through methods such as deep tillage. Since tillage damages soil structure, such methods should not be undertaken frequently (Bhogal *et al.*, 2011), and are only a temporary measure, as re-compaction occurs within a few years (Botta *et al.*, 2006). Whilst soil compaction should be managed by minimising the use of heavy machinery on wet soil and aiming to use the same wheel tracks as much as possible (Knight *et al*., 2012), farmers should adapt to the realities of its existence by producing or utilising crops capable of tolerating soil compaction. Understanding the effects of compaction on both soil and plant are key steps to do this.

1.3 The Effects of Soil Compaction on Soil Properties

Soil compaction forces soil particles closer together, removing soil pore space (Figure 1-3) and reducing soil porosity (ratio of air space to solid particles) (Pandey *et al*., 2021). This affects other soil properties, changing how the soil is affected by the environment.

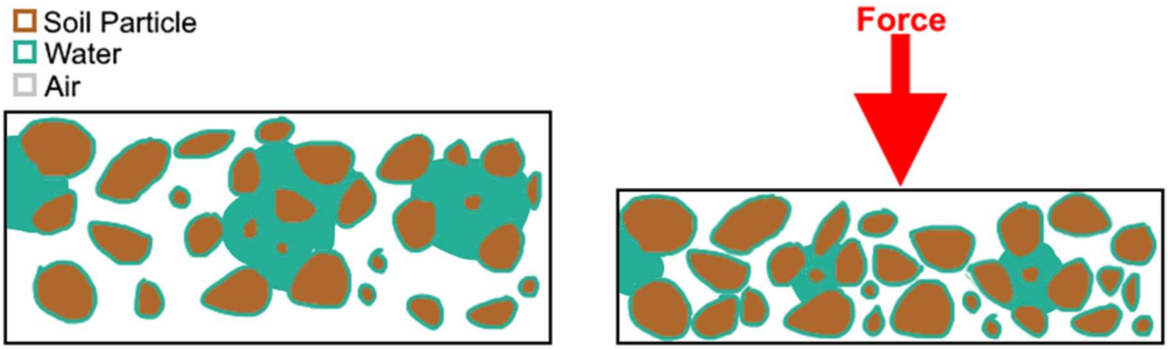


Figure 1-3: Soil structure in an uncompacted (left) and compacted (right) soil. The increase in particle density, and the reduction in pore number and size is clearly visible in the compacted soil compared to the uncompacted soil.

Decreased porosity of compacted soil affects its interactions with water. For example, compacted soils have a much slower water infiltration rate. Pores in compacted soil are smaller and less numerous than those in uncompacted soil, and as a result are much less connected. Water therefore moves through the soil far more slowly (Ghosh and Daigh, 2020), reducing infiltration. Where cropland is irrigated by hose reel, or if the field is subjected heavy rainfall, this can flood the soil (Batey, 2009). In addition, the water holding capacity (WHC) of soil is significantly reduced when compacted (Figure 1-4). Water is typically held in the gaps between soil particles, and the loss of these spaces restricts the ability of soil to hold water. This loss is detrimental to the crop, as it lowers the maximum available water in the soil. This reduces WHC, causing leaf water deficit if irrigation is infrequent, as the soil is more quickly depleted of water, causing reduced shoot growth (Xue *et al*., 2017). Reduced WHC also reduces nutrient availability in the soil by forcing nutrients out of solution into a solid state, preventing uptake by roots (Xue *et al*., 2017).

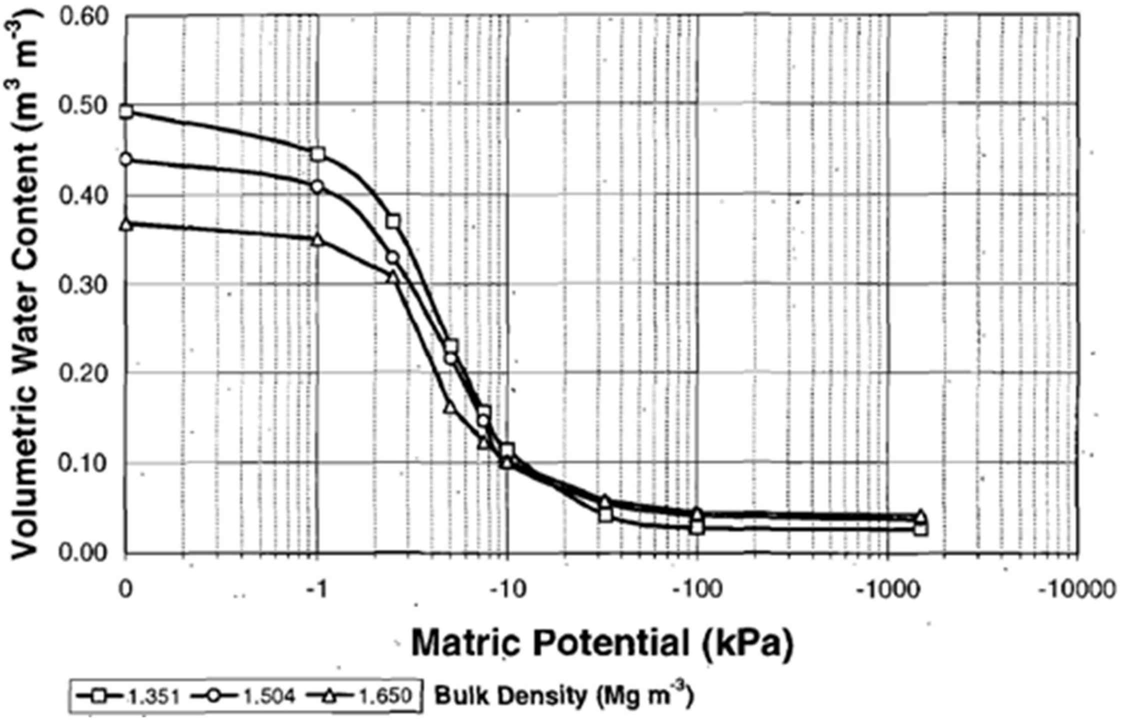


Figure 1-4: The relationship between matric potential and soil water content for different bulk densities of soil (key at the lower-left of the graph). Bulk density units are equivalent to g/cm^3. Taken from Smith *et al*., (2001).

Compacting soil also restricts aeration. The reduced pore space limits the amount of air that can be held in the soil, and its ability to diffuse through the soil profile (Pandey *et al*., 2021). This limits the available oxygen within the soil, causing hypoxic conditions. Under hypoxic conditions, roots are forced to utilise inefficient anaerobic metabolic pathways, which also produce harmful by-products, such as ethanol (Magneschi and Perata, 2009). In species that are intolerant of anaerobic conditions, this can result in cell death (Mancuso and Marras, 2006). Even in species tolerant of such conditions, root growth is almost completely inhibited, making the soil impossible to colonise (Biemelt *et al*., 1998). Furthermore, decreased aeration also promotes anaerobic activity of microbes within the soil, including denitrification (Ruser *et al*., 2006). In well-drained soil, aerobic activity is favoured due to increased efficiency of ADP to ATP conversion per molecule of glucose. However, under anaerobic conditions, aerobic processes cannot occur. Denitrifying bacteria can utilise nitrates (NO_3^-) to respire by converting them to nitrogen and nitrous oxides (Rana *et al*., 2019). This leads to decreased soil nitrogen content and thus an increase in fertiliser demand.

The changes to soil physical properties that compaction causes make it difficult for crops to grow within them. Combined with the reduced water holding capacity, the susceptibility of compacted soils to poor aeration makes it challenging to ensure adequate water is provided for crops to avoid drought stress, whilst avoiding flooding the plant roots. Furthermore, plants growing in compacted soil exhibit changes in morphology and physiology, even when otherwise well managed.

1.4 Morphological and Physiological Effects of Compaction

Compacted soil causes a variety of issues for plant roots, particularly regarding root growth. As roots grow through soil, they encounter resistance due to the forces required to move soil particles from their path. The more resistant the soil, the more force this requires. Therefore, roots must exert more turgor pressure to pass through the soil, which is generally achieved through an influx of water into the root cells by reducing Ψ_{root} by increasing ion concentration (Jin *et al*., 2013). The rate of root elongation rate in response to increased soil resistance has been correlated with rate of reduced elongation rate in response to reduced Ψ_{root} in *Pisum sativum* (pea), *Zea mays* (maize), *Arachis hypogaea* (peanut) and *Gossypium arboreum* (cotton) (Bengough *et al*., 2011), although the relative change in Ψ_{root} depended on the study. This implies that roots that can maintain greater elongation rates whilst increasing turgor pressure by reducing Ψ_{root} are able to better penetrate compacted soil. Soil compaction also increases root diameter (Rich and Watt, 2013), the root's resistance to bending against a high level of resistance (Materechera *et al*., 1992), and reduces the risk of buckling (Colombi *et al*., 2017b). A soil that has a higher resistance than the force exerted by the root can overcome results in root growth being inhibited.

Whilst changes to root architecture in compact soil such as increased diameter and decreased root growth rates are well known and accepted, not all changes are consistent. An example of this is root branching density. root branching of maize was promoted when soil resistance increased from 3.5 MPa to 5.7 MPa (Konôpka *et al*., 2009) and pea when soil resistance increased from 0.037 MPa to 0.506 MPa (Tsegaye and Mullins, 1994). These differences in response may depend on developmental stages. When growing *Triticum aestivum* (wheat) under compact conditions, Rich and Watt (2013) found that root growth through compact, pore-free soil increased branching only during early root development. There is also evidence that, when soil resistance increased from 0.3 MPa to 1.0 MPa, lateral root growth in *Hordeum vulgare* (barley, cv. Prisma) increases as a compensatory measure, but this change was not observed in wheat (cv. Alexandria) grown in the same conditions (Bingham and Bengough 2003). This evidence suggests that increased root branching in compact soil only occurs during the early stages of root growth in some species such as wheat but is a constant response from others such as barley. Both rice and maize crops increased branching during the first few weeks of development in compact soil compared to uncompact soil (Ijima and Kono 1991). However, in maize, root branching rates dropped significantly after four weeks within the compact soil whilst still increasing in uncompact soil. Rice root branching in compact soil was still significantly higher than in loose soil at the end of the experiment, but a similar pattern to maize was emerging. Overall, this suggests that compact soil promotes early root branching potentially to avoid compaction by seeking regions of lower resistance. However, root branching is limited after initial development, likely since insufficient root elongation growth does not warrant further branching.

Soil compaction also influences shoot growth and physiology. Compacted soil decreased stomatal conductance of barley without any change in leaf water potential, implying the change is due to chemical signals from roots in compacted soil rather than a leaf water deficit (Mulholland *et al*., 1996a). Shoot growth is inhibited when pressure is applied to the roots of maize seedlings in the absence of other stresses (Sarquis *et al*., 1991), meaning that the signal is not caused by stress, but by impedance to root growth. Wheat seedlings grown in soil with a resistance of 5.5 MPa (megapascals) had 33% less leaf area than those grown in soil with a resistance of 1.5 MPa (Masle and Passioura, 1987). This reduction was visible almost immediately upon the first leaf growing, despite seeds germinating in a layer of loose soil above the compacted layer, meaning that root growth was not yet limited by compaction. This implies that plants respond to the presence of compacted soil, rather than impedance of root growth caused by compaction. When *Solanum lycopersicum* (tomato) plants were grown in split-pots with compacted (1.5 g/cm^3) and uncompacted (1.1 g/cm^3) soil, leaf area decreased whilst Ψ_{leaf} was not affected, even though total root growth was not limited due to the availability of uncompacted soil (Hussain *et al*., 1999). Thus, compacted soil limits shoot growth, and hence decreases photosynthesis and biomass production, likely due to a root-sourced signal being produced when roots encounter compacted soil.

The primary factor determining penetrative ability remains uncertain. Whilst there is evidence to suggest that the increase in root diameter correlates with improved penetration (Materechera *et al*., 1992), and is not causative. Rice (*Oryza sativa*) mutants that do not exhibit increased root diameter in compacted soil better penetrate that soil than wild type cultivars (Pandey *et al*., 2021), supporting this theory. Instead, recent research has attributed penetrative ability to other factors, such as reduced cortical thickness (outer layer of the root) (Chimungu *et al*., 2015), the angle of roots relative to compaction interface, with angles closer to vertical improving penetration (Vanhees *et al*., 2022), and root sensitivity to the hormone ethylene (Pandey *et al*., 2021).

1.5 Hormonal Responses to Soil Compaction

Ethylene is a gaseous hormone that generally inhibits root growth by causing root cells to expand laterally instead of vertically, thereby reducing root growth rates and increasing root diameter (Okamoto *et al*., 2008). As roots respond similarly to ethylene as they do to compacted soil, ethylene has been proposed to play a major role in the root's response to compaction. Ethylene is a stress hormone, and roots encountering compacted soil may increase its production (Moss *et al*., 1988; Sarquis *et al*., 1991). When ethylene production is inhibited in maize plants by applying chemical inhibitors, the roots do not exhibit ethylene related compaction responses (decreased elongation rates and increased root diameter) (Sarquis *et al*., 1991). Furthermore, the *Arabidopsis* mutant *eto1-1* (an ethylene overproducing mutant) responded identically to wild type plants (increased root diameter and decreased cell length) when exposed to compact soil, but ethylene insensitive mutant *aux1-7* did not exhibit ethylene related responses (Okamoto *et al*., 2008). However, it is debatable whether ethylene production and responses genuinely improve penetrative ability and compaction tolerance. Whilst ethylene evolution increased over five-fold in response to soil compaction in both whole maize plants (Sarquis *et al*., 1991), and tomato leaves (Hussain *et al*., 1999), it may be associated with compaction-related stresses such as hypoxia (Liu *et al*., 2022) or drought (Naing *et* *al*., 2022). Instead, ethylene is trapped around the roots due to limited diffusion in compacted soil (Pandey *et al*., 2021), inhibiting root elongation and increasing root diameter by increasing biosynthesis of auxin and promoting root tip swelling via increased abscisic acid (ABA) production (Huang *et al*., 2022). Roots are hypothesised to grow directly through compacted soil with ease by penetrating soil via pre-existing pores, with ethylene insensitive mutants of rice better penetrating compacted soil without any increase in root diameter compared to wild type plants (Pandey *et al*., 2021). In addition, improved penetrative ability in different maize cultivars have been associated with relative ethylene insensitivity (Vanhees *et al*., 2022). This would indicate that compaction-induced ethylene production may not be a desirable trait for compaction tolerant cultivars.

Alternatively, it may be possible to overcome compacted soil using cultivars that exhibit reduced ethylene sensitivity. Ethylene insensitive roots have been found to better penetrate compact soil (Pandey e*t al*., 2021), which may translate into improved tolerance to compaction. However, they may be less able to exploit the soil. Root hairs are vital structures for maximising the uptake of water and nutrients from the soil, and for stabilising the root within the soil (Bengough *et al*., 2016), and ethylene promotes root hair formation and elongation (Zhu *et al*., 2006). As low yields in compacted soil are associated with reduced nutrient and water uptake (Ghosh and Daigh, 2020), the increased penetrative ability of ethylene insensitive cultivars may be associated with insufficient root hair density to fully exploit available nutrients and water in the soil profile.

Inhibiting root ethylene production may improve plant growth in compacted soil. Canopy growth of transgenic, low ethylene tomato genotype ACO1$_{AS}$ was unaffected by soil compaction in split plots containing compacted (1.5 g/cm^3) and uncompacted soil (1.1 g/cm^3) treatments, whilst canopy growth was reduced for the wild type cultivar. Applying silver ions (an inhibitor of ethylene perception) to the roots of the wild type restored root and shoot growth of plants grown in split pots (Hussain *et al*., 1999). However, applying heavy metals is not viable in cropland due to risk of environmental contamination (Ratte, 1999), and non-metallic inhibitors are costly, therefore the economic benefit to growers is limited.

The use of ACC deaminase-containing rhizobacteria is a potential alternative. These bacteria cleave the ethylene precursor ACC into α-ketobutyrate and ammonia, which are then metabolised by the bacteria for energy (Chandwani and Amaresan, 2022). When applied to potato plants, these bacteria enhance tolerance to drought stress, salt stress, heat stress, and anoxic stress (Chandwani and Amaresan, 2022), and improved tuber yields under drought stress (Belimov *et al*., 2015). However, their effectiveness at improving tolerance to soil compaction has not been investigated.

1.6 The Susceptibility of Potato to Soil Compaction

The first report on the effect of soil compaction on potato yield, in which penetration resistance typically doubled compared to plots without traffic, correlated increased soil resistance from traffic with a mean yield loss of 20% in trafficked plots (Struchtemeyer *et al*., 1963). Removing soil compaction via subsoiling typically improves crop yield by between 20% and 50% in species including maize, wheat, barley, *Brassica napus* (oilseed rape) and *Glycine max* (soybean) (Bogunovic *et al*., 2018). However, it is not clear why subsoiling in potato has seen little to no effect on yield, which Stalham *et al*., (2005) attributed to deficiencies in experimental design, such as not confirming the presence of compaction before using subsoiling treatments, or compacting during planting, although re-compaction from soil slumping is also possible (Batey, 2009). Whether or not these hypotheses are correct, it remains uncertain whether potato yields benefit from growing in loosened soil, and why their yield responds differently to other crops.

Compared to other crop plants, the root system of potato plants is poorly equipped to deal with soil compaction and strong soils. Potato root systems tend to grow very shallow, with two-thirds of root growth occurring in the top 20 cm of soil, compared to only 45% in maize, 53% in wheat, and 52% in sugar beet (Yamaguchi and Tanaka, 1993). Shallower root systems increase soil resistance, as water uptake from the topsoil is increased, reducing soil water content and therefore increasing soil resistance. This increased resistance reduces root growth rates, preventing root growth into deeper soils where water is more available (Colombi *et al*., 2018), leading to a greater proportion of root growth in the topsoil, and further soil drying. Whilst irrigation can mitigate this issue, potato plants are very sensitive to water stress. Potato leaf stomata will rapidly close even when only a moderate loss of leaf water potential (from -0.6MPa to -0.8MPa) is detected (Figure 1-5) (Vos and Oyarzun, 1987). This differs from other crop plants such as sunflower, tomato and wheat (Figure 1-5), where stomatal closure is more moderate when leaf water potential falls (Tardieu *et al.*, 1996). Furthermore, high soil water content prevents common scab from forming on tubers, improving yield (White *et al.*, 2005). These factors mean that potato cropland must be constantly kept wet, making the soil consistently susceptible to compaction throughout the growing season.

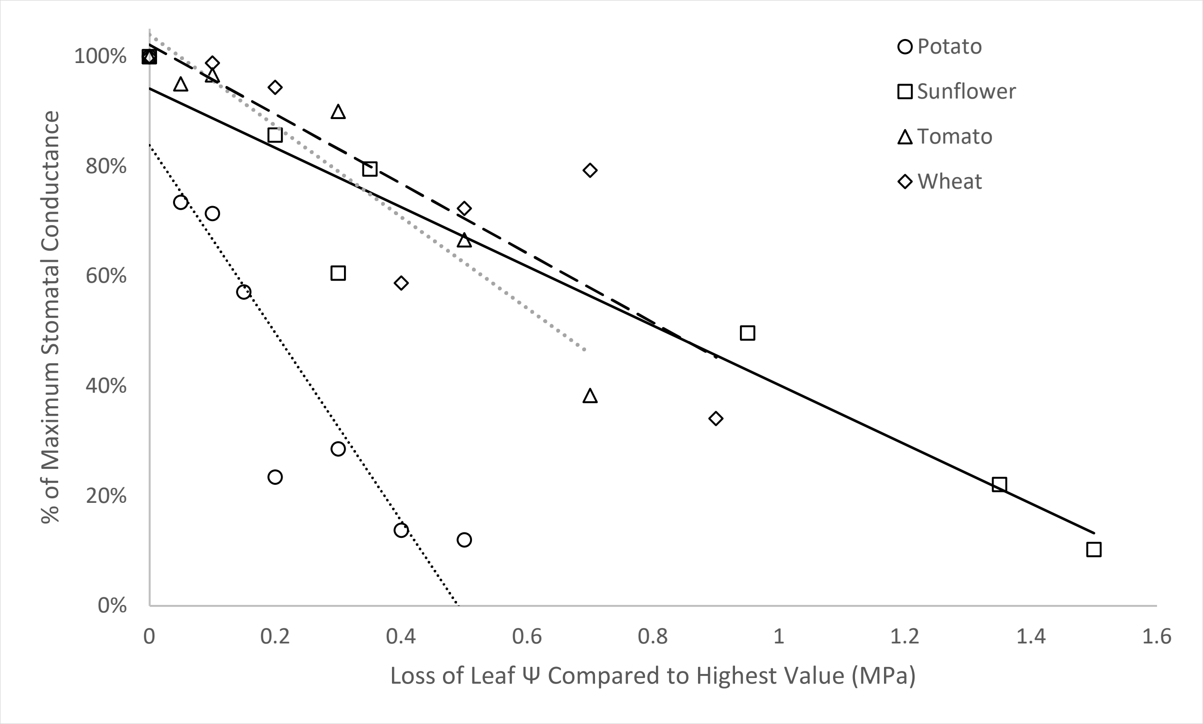


Figure 1-5: The relationship between leaf water potential and stomatal conductance in potato (circles), sunflower (squares), tomato (triangles) and wheat (diamonds). These data were collected from a variety of different experiments, causing the variation in maximum values. Linear regressions for potato (thin line), sunflower (thick line), tomato (dotted line) and wheat (dashed line) are also included.
Modified from Garcia *et al.*, (2007), Liang *et al.*, (2002), Liu *et al.*, (2005), Quarrie and Jones, (1979), Tang and Boyer, (2006), Tardieu *et al.*, (1996), Vos and Oyarzun, (1987).

The increase in soil resistance caused by compaction impedes the passage of roots through the soil, decreasing root growth rates. The penetrative ability of roots through compacted soil can be measured as the penetrometer resistance value at which root growth rates are half that of roots in uncompacted soil, with higher values indicating a greater degree of penetrative ability. There is strong evidence of interspecific variation in compacted soil, with penetrative ability values including 2.5 MPa in peanut (Atwell, 1993), 1.4 MPa in pea, 3 MPa in *Eucalyptus kochii* (oil mallee) (Azam *et al*., 2014), and 1.8 MPa in potato (Stalham *et al*., 2007). Most plant species exhibit exponential decay responses to increased soil resistance, with some root growth occurring at resistances exceeding 6 MPa. However, potato exhibits a linear decay, with complete root growth inhibition by 4 MPa (Stalham *et al*., 2007). Since most potato croplands have regions with soil resistance values exceeding 3 MPa (Stalham *et al*., 2007), this clearly limits root growth. Effects on tuber yield vary, but compacted soil typically decreases yield by between 25% and 50% (Stalham *et al*., 2007; Huntenburg *et al*., 2021).

Existing potato cultivars show substantial phenotypic variation in root growth. After removing the upper 30 cm of soil and determining root length and diameter of each variety, root diameter varied by 13% increase and the cultivar with the greatest root density was 1.7-fold larger than the cultivar with the lowest root density in a study of three purebred varieties, and ten varieties made by crossing potatoes with wild relatives (Iwama and Nishibe 1989). Furthermore, the difference in total root system length varied three-fold between the most and least vigorous of 28 potato cultivars (Wishart *et al*., 2013). The high level of variation in root traits within potato increases the probability that genotypic variation in compaction tolerance exists within existing cultivars.

1.7 Genotypic Variation in Response to Compaction

There is strong evidence of interspecific and intraspecific variation in plants' ability to penetrate compacted soil. Materechera *et al*., (1992) measured the proportion of roots successfully penetrating a layer of compacted soil of those that met it varied heavily between species, with the worst penetrating species (*Lolium rigidum*) having only 30% penetration, whilst the best penetrating species (*Carthamus tinctorius*) had 61% of roots penetrating the compacted layer. Similar variation was observed between cultivars for species including rice (Clark *et al*., 2002) and *Phaseolus vulgaris* (common bean) (Rivera *et al*., 2019). Species exhibiting a greater relative increase in mean root diameter had a greater proportion of roots capable of penetrating compacted soil (Materechera *et al*., 1992; Clark *et al*., 2002). Whilst these studies demonstrate genotypic variation in responses to compaction, the extent to which potato cultivars exhibit this variation remains unknown. Whilst reduced tuber yield in compacted soil is well reported (Stalham *et al*., 2005; Ghosh and Daigh, 2020; Huntenburg *et al*, 2021), comparisons between genotypes are rarely performed, and tend to produce limited differences in yield. Stalham *et al*., (2007) found that Maris Piper and Estima yield was decreased by 40% and 45% respectively in compacted soil (Stalham *et al*., 2007), whilst Miller and Martin (1987) had no significant differences to yield between plants grown in compacted soil, and those in subsoiled soil.

Long distance hormone signalling between roots and shoots allows an appropriate plant response to changes in resource availability (Thomas and Frank, 2019). Whilst plants growing in compacted soil exhibit reduced canopy growth and root growth (Huntenburg *et al*., 2021), whether root growth regulates shoot growth, or vice versa, is not clear. One method of resolving this uncertainty is to perform reciprocal grafts of two genetically different plants. By combining the influence of the shoot from one cultivar with the influence of the root of another, comparisons can be made to self-grafts to identify the source of differences. If the process in question is controlled by the scion, then chimeras would exhibit a similar phenotype to the self-grafted plants with the same scion. By comparing two varieties of potato and their chimeras (Figure 1-6) under drought stress, Jefferies (1993) found that root biomass distribution was primarily determined by the scion in ideal conditions, although this effect was less notable when the plants were droughted. Whether the scion determines root growth in compacted soil remains uncertain, especially since root growth is inhibited under these circumstances. Thus, it is unknown whether the shoot will continue to control root growth, or if root growth limitation would decrease canopy growth.

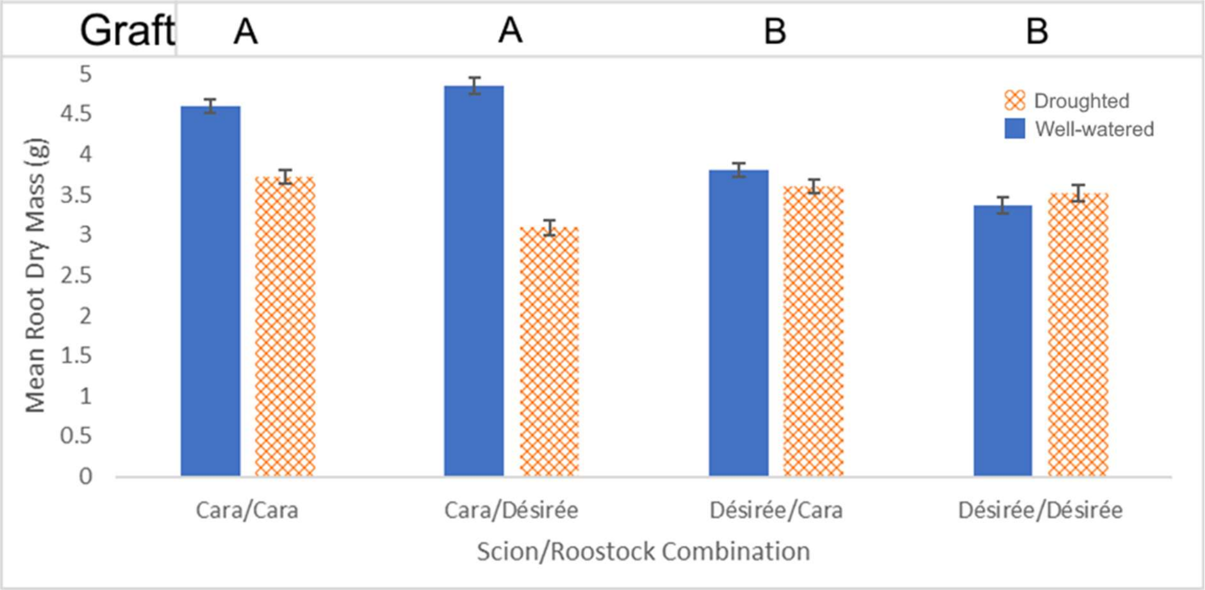


Figure 1-6: Root dry mass of different potato grafts 110 days after emergence. Plants were either well-watered (blue) or droughted (hatched orange). Error bars indicate least square differences with a p-value of 0.05. Letters of mean discrimination above plots indicate differences in root dry mass between grafting combinations. Produced using data from Jefferies (1993).

1.8 Thesis Structure

This thesis aims to determine whether genotypic variation in tolerance to compacted soils exists between potato cultivars, and whether this genotypic variation can be used to overcome yield losses due to soil compaction. Potato plants are particularly susceptible to soil compaction, and being able to provide low-cost, effective options to recover yield is important in ensuring sustainable yield of potato tubers in the future.

Chapter 2 determined the extent of genotypic variation in compaction tolerance. Six cultivars of potato (Andeana, Charlotte, Estima, Inca Bella, Maris Piper, Pentland Dell) were grown in compacted (3 MPa) and loose (0.3 MPa) soil, and key morphological and physiological traits (emergence rates, leaf area, root length, root diameter, water potential and transpiration rate) were measured to determine relative changes in compacted soil. Three cultivars (Andeana, Inca Bella, Maris Piper) were repeatedly measured over time in compacted and loose soil to identify any changes in the timing at which responses to compaction arose, as this has not previously been measured. Finally, two cultivars (Inca Bella, Maris Piper, chosen based on significantly different responses in earlier experiments) were reciprocally grafted to determine the extent to which root and shoot were responsible for determining tolerance to compaction soil by measuring the key morphological and physiological phenes previously listed, which has not previously been tested.

As Chapter 2 identified genotypic variation in morphological responses that was primarily root-sourced, Chapter 3 focussed on understanding the role of ethylene in tolerance to compaction in potato. The best performing (Pentland Dell), intermediate (Maris Piper) and worst performing (Charlotte) cultivars in compacted soil were selected. Root ethylene evolution under compaction was measured, and ethylene sensitivity determined to discriminate whether ethylene production, sensitivity, or both were responsible for morphological changes to potato roots in compacted soil. Finally, the ACC deaminase-containing bacterial species *Variovorax paradoxus* 5C-2 (5C-2) (Belimov et al. 2015) was applied to Maris Piper roots to determine whether inhibiting ethylene production would improve penetrative ability.

Chapter 4 aimed to determine whether genetic variation in compaction effects identified in pot experiments (Chapter 2) were comparable and consistent in field trials, using the cultivars Inca Bella and Maris Piper grown in compacted and uncompacted soil. The first trial was unirrigated and had a very light compaction treatment applied, whilst the second trial was irrigated and applied moderate compaction. Soil resistance and soil water content were measured to identify physical changes to the soil, whilst leaf area, root density and diameter, and tuber yield were measured to determine effects of soil compaction on the potato plants.

Chapter 2 Differences in physiological responses to soil compaction between potato cultivars

2.1 Introduction

Since alleviating soil compaction mechanically (e.g., through subsoiling) is financially expensive, there is considerable interest in identifying crops that are better adapted to grow in compact soil. This has led to numerous studies attempting to quantify the ability of different species and crop cultivars to tolerate and overcome the constraints caused by compacted soil. Within numerous crop species, different cultivars show up to eight-fold differences in the proportion of roots able to penetrate compacted soil layers in rice (Babu *et al*., 2001; Clark *et al*., 2002), wheat (Colombi and Walter, 2017), and common bean (Rivera *et al*., 2019), but similar studies have not been performed in potato. Thus, it is not clear whether genotypic variation in this crop can help overcome constraints to soil compaction.

In compacted soil, plant roots tend to grow more slowly, with cell expansion being promoted laterally, increasing root diameter (Bengough *et al*., 2006). Increased root diameter increases root turgor pressure (Atwell, 1993), increasing the force the root can apply to the soil, which increases its ability to penetrate compacted soil (Clark *et al*., 2003). Decreased root system size reduces water and nutrient uptake, and thus the leaf area the plant can support (Grzesiak *et al*., 2013). Plants growing in compacted soil are prone to dehydration, and their leaf water potential (Ψ_{leaf}) and stomatal conductance can be lower than in plants grown in loose soils (Tardieu *et al*., 1992; Grzesiak *et al*., 2013). However, this is not a consistent response, with many studies finding little or no change in Ψ_{leaf} or stomatal conductance between compaction treatments (Andrade *et al*., 1993; Huntenburg *et al*., 2021; Hussain *et al*., 2000). Limited root and shoot growth in response to compaction tends to decrease yield by between 20% and 60%, depending on severity, location in the soil profile, and crop species (Wolfe *et al*., 1995; Lipiec *et al*., 2003; Hatley *et al*., 2005; Chen and Weil, 2011; Correa *et al*., 2019). The loss of yield is typically attributed to reductions in water uptake, carbon capture, and nutrient uptake (Stalham *et al*., 2007). Thus, changes in root length and diameter, as well as leaf area, are key in identifying the ability of a plant to tolerate compacted soil. To conclude, soil compaction inhibits root extension growth, increases root diameter, and decreases leaf area, thereby decreasing yields.

Whilst the effect of compacted soil on plant morphology and physiology is well studied, the effects on the rate of change of physiological traits within the roots is less well understood. Most studies investigating soil compaction measure its effects at a single point in time, but it is important to understand how and when compaction limits growth. The effect of soil compaction on root growth rates has been estimated previously, but these have been estimates based on the effect of soil resistance on the rate of increase of maximum rooting depth, as opposed to actual root growth rates (Stalham *et al*., 2007). X-ray tomography has been frequently used to measure the effects of compaction on root growth more accurately, but only for ten days after emergence (Tracy *et al*., 2012) or once only rather than changes over time (Pandey *et al*., 2021). Soil resistance varies greatly throughout the soil profile even when the soil is not compacted due to changes in soil composition and soil water content, and pressure from the weight of the soil above (Kubo *et al.*, 2006). Furthermore, whilst roots take less than an hour to respond to increased compaction (Bengough and MacKenzie, 1994), it takes several days for them to respond to decreased compaction (Bengough and Young, 1993). By understanding how growth rates change over time in compacted and uncompacted soil, the effects of compacted layers in the field can be more clearly understood. Furthermore, comparing growth rates between cultivars may help understand the causes of differences in root system size. Changes in root diameter over time are also of particular interest. Whilst it is commonly accepted that increased soil resistance increases mean root diameter (Materechera *et al*., 1992; Correa *et al*., 2019), and that mean root diameter generally decreases over time as larger primary roots produce smaller secondary roots (Wu *et al*., 2016; Pages *et al*., 2020), how this relationship is affected by increased soil resistance is not clear. Correlating changes in root diameter and root growth rates may help determine the role that increased root diameter plays in determining the root growth rate in compacted soil.

To better understand how roots and shoots co-ordinate these changes in crop development, it can be valuable to have genetically distinct root and shoot genotypes. Plant roots and shoots communicate primarily by sending hormones through the xylem and phloem of the stem (Notaguchi and Okamoto, 2015) to cause a response in target locations, and it is often difficult to separate a locally mediated response to one influenced by distant organs. Grafting is a surgical technique that can separate the genetic influence of the shoot from that of the root of the plant by combining different above ground (scion) and below ground (rootstock) genotypes. Past studies with grafted potato varieties are few in number but provide some insights. Although tuber size was scion-dependent, tuber number and total yield did not differ between graft combinations (Odgerel and Banfalvi, 2021). In addition, Jefferies (1993) found that potato root matter distribution was heavily dependent on the scion rather than the rootstock. These studies indicate that many below-ground traits are dependent on the above-ground organs. Grafting has not been used to investigate the regulation of responses to soil compaction, and thus it remains uncertain to what extent the shoot determines the ability of a plant to tolerate compacted soils.

Overall, this chapter aims to identify any physiological changes in response to compaction in potato plants, whether these changes vary between cultivars, and whether they are primarily regulated by the roots or the shoots. Since other species show variation in root and shoot responses to soil compaction, it was hypothesised that potato cultivars would also exhibit decreased root length and leaf area, with increased mean root diameter. Secondly, since shoot growth and root growth of potato are correlated in ideal conditions (Jefferies, 1993), it was hypothesised that the cultivar that maintained the greatest proportion of root length was expected to also maintain more leaf area. Finally, since variation in potato canopy growth determined root length in grafting studies (Figure 1-6; Jefferies, 1993), it was hypothesised that the scion would determine differences in root length and leaf area in compacted soils. However, since root diameter changes in response to encountering impedance in the soil, it was hypothesised that root diameter was rootstock mediated.

2.2 Methodology

Plant and Soil Conditions

Potato tubers from six cultivars were obtained for these experiments. Inca Bella and Andeana were obtained from Branston Ltd. (Branston, UK) whilst Maris Piper, Estima, Pentland Dell, and Charlotte tubers were obtained from TLC Potatoes (Durris, UK). All tubers were between 15 mm and 25 mm in length.

Pots used in the experiment were custom made from Polyvinyl chloride pipe (Keyline, Northampton, UK) The pots had an interior diameter of 6.4 cm and a height of 26 cm, and were designed to have a diameter equal to the size of the press used to apply compaction treatments to the soil, ensuring a consistent application of pressure to the soil to produce an constant resistance throughout the post both laterally and vertically. Stainless steel wire mesh (0.7 mm aperture) from Mesh Direct (Stoke-on-Trent, UK) was melted into the pipe at one end to produce the base. Soil used in this experiment was silty loam (Heavingham, Norfolk UK). Soil particle size analysis was performed using an LS 13 320 particle size analyser (Beckman Coulter, High Wycombe, UK) with texture and nutrient status described in Table 2-1.

Table 2-1: The physical and chemical properties of Norfolk Topsoil. All data obtained by NRM (Cawood, Bracknell, UK) except for particle size data.

Soil Property	*Value*
% Clay	8.69
% Silt	79.81
% Sand	11.49
pH	7.1
Available P (mg/l)	30.3
Available K (mg/l)	321
Available Mg (mg/l)	51.8
Nitrate Nitrogen (mg/kg)	78.9
Ammonium Nitrogen (mg/mg)	1.76

Soil was prepared for compaction by being air dried, sieved using a 10 mm sieve to remove large particles such as rocks, and then re-wetted to the desired soil water content depending on requirements. Soil was compacted by the model PK3000 arbour press from Jack Sealy Ltd. (Bury St. Edmunds, UK) with a fitted metal disc that matched the interior diameter of the pots to ensure even pressure was exerted to the soil. Applied force was controlled using a torque wrench. Soil was compacted into the pots in 2 cm layers to ensure a consistent resistance throughout the pot. Compacted soil was applied until the soil level was 4 cm below the pot's top, at which point the potato tuber was placed on top and loose soil applied to cover the tuber. A loose soil treatment was produced by setting the torque wrench force to 10 Nm (compressive stress of 140 kPa (kilopascals)) and using air dried soil, whilst high compaction was produced by setting force to 40 Nm (compressive stress of 460 kPa) to soil with a 10% soil water content. Soil resistance was measured using a Van Walt hand penetrometer (Haslemere, UK). Resistance of loose soil was 0.3 ± 0.06 MPa (n = 5), whilst resistance in the compacted soil was 3.0 ± 0.16 MPa (n = 5).

Experimental Design

In all experiments, the potatoes were grown in a controlled environment room with a sixteen-hour day. Light during the day was provided by metal halide lamps (Osram Powerstar HQI-T, Munich, Germany) with a PPFD at pot height of 450 µmol/m^2/s. The daytime temperature was maintained at between 24^{o}C and 25^{o}C with a night temperature of 15^{o}C to 16^{o}C. Soil water content was maintained between 20% and 30% by watering at 10am on Monday, Wednesday, and Friday. Plants were placed directly under growth lights in columns 5 pots deep to ensure all pots received similar concentrations of radiation. All plants were distributed randomly within this area to avoid bias.

Experiment 2.1: Phenological responses to soil compaction in six potato cultivars

Tubers of six different potato cultivars (Andeana, Charlotte, Estima, Inca Bella, Pentland Dell, and Maris Piper) were planted in either loose or compacted soil, with five plants used per treatment. Plants were grown for four weeks post-emergence before harvesting. Four weeks was chosen as the experimental period, as this was this was the longest growth period in which no potato plants became pot bound in preliminary experiments.

Plant Measurements

The time for potato plants to emerged was measured daily, with a plant having been determined to emerge once its stem was visible above the layer of soil. Before harvesting, transpiration rates (TR) were measured by weighing the plant in its pot for thirty minutes, with any changes in weight assumed to have come from water transpired by the plant. Ψ_{root} was measured by placing the plant pot with cut shoot in a Scholander Pressure Chamber (Soil Moisture Equipment Corp., Santa Barbara, USA). The leaf area of each plant was measured using a LI-COR's LI-3100C leaf area meter (Cambridge, UK). The shoot of the plant was dried and weighed to determine dry mass. Next, roots were separated from the tuber and stolons and then washed. The roots were scanned using an Epson Expression 11000XL (Suwa, Japan) and analysed using WinRHIZO software (Reagent Instruments, Canada) to determine root length, volume and mean diameter. To help clarify whether root morphology was changing, root diameter was differentiated into ten equal size classes from 0 to 1 mm.

Experiment 2.2: Temporal responses to soil compaction in three potato cultivars

Tubers of three potato cultivars (Andeana, Inca Bella and Maris Piper) were planted in loose or compacted soil. Three plants of each treatment were harvested weekly for four weeks. Leaf area, shoot dry mass and root volume, length and mean diameter were measured as per experiment 2.1.

Experiment 2.3: Grafting Experiments

Inca Bella and Maris Piper tubers were grown for seven days post-emergence in loose or compacted soil. Each plant shoot was cut below the first leaf, and cleft grafting was performed to produce both self- and reciprocal-grafts. Plants were then covered with polythene bags for seven days to allow the graft union to heal. Bags were removed and plants that had failed to graft were removed from the experiment. Plants were grown for a further fourteen days. Root analysis and leaf area measurements were then undertaken as per experiment 2.1. Biomass distribution between shoot and root was measured by calculating the ratio between root length and leaf area for each plant.

Statistical Analysis

Statistical analysis was carried out using version 0.16 of JASP (University of Amsterdam, The Netherlands). For all statistical analyses, a p-value of less than 0.05 was deemed significant. To identify variables that responded differently between cultivars in Experiment 2.1, two-way analysis of variances (ANOVAs) were performed with cultivar and soil resistance as independent variables. Time to emerge, TR, Ψ_{root}, leaf area, shoot dry mass, root length and root diameter were the dependent variables. To identify causes of variation in Ψ_{root}, a linear regression with Ψ_{root} as the dependent variable, leaf area and root length as independent variables, and variety as a factor was performed. To identify whether different phenological traits were changing in the different cultivars, Tukey's tests were performed using soil resistance as the independent variable using emergence time, TR, leaf area, shoot dry mass, root length, and root diameter as dependent variables, when compaction was determined to have a significant effect by the ANOVA tests. To determine how root diameter was changing, linear regressions were performed with each diameter class as a dependent variable, and variety and resistance as independent variables. Experiment 2.2 used three-way ANOVAs to determine which traits responded differently to soil compaction over time and between cultivars. Leaf area, shoot dry weight, root length and root diameter were used as dependent variables, with cultivar, soil resistance and time since emergence were independent variables. Tukey's tests were performed to determine significance between cultivars where the ANOVAs were significant. To understand the effects of the scion and rootstock in the response to compaction in experiment 2.3, three-way ANOVAs were performed. Scion, rootstock and compaction were used as independent variables, with leaf area, root length and root diameter as dependent variables. To identify the sources of variation, Kruskal Wallis tests were performed for each variable where scion or rootstock had a significant effect. Linear regressions using leaf area, scion, and rootstock as independent variables with root length as a dependent variable were used to determine the effects of scion and rootstock on biomass distribution.

2.3 Results

Compaction affects morphology of potato plants

Whilst compacted soil did not affect plant water relations (transpiration and Ψ_{root}), it significantly delayed emergence, decreased root length and leaf area and increased root diameter (Table 2-2). While there was cultivar variation in all variables measured except transpiration rate, only root length response to soil compaction significantly differed between cultivars (Table 2-2).

Table 2-2: Two-way ANOVA table showing significances of results for Experiment 2.1. NS indicates not significant.

Variable	*Emergence*	*TR*	Ψ_{root}	*Leaf Area*	*Root Length*	*Root Diameter*
Compaction	0.002	NS	NS	<0.001	<0.001	<0.001
Cultivar	<0.001	NS	0.04	<0.001	<0.001	<0.001
Cultivar x Compaction	NS	NS	NS	NS	0.005	NS

Emergence time differed significantly ($p < 0.001$) between cultivars and was typically slowed by increased soil resistance ($p < 0.01$). Emergence rates of four cultivars, such as Inca Bella and Maris Piper were unaffected by compaction. Charlotte and Pentland Dell took 10 days and 15 days longer to emerge in compacted soil respectively, (Figure 2-1). Whilst the compaction treatment tended to cause different changes in emergence time between cultivars, this interaction was not significant (p = 0.07; Figure 2-1).

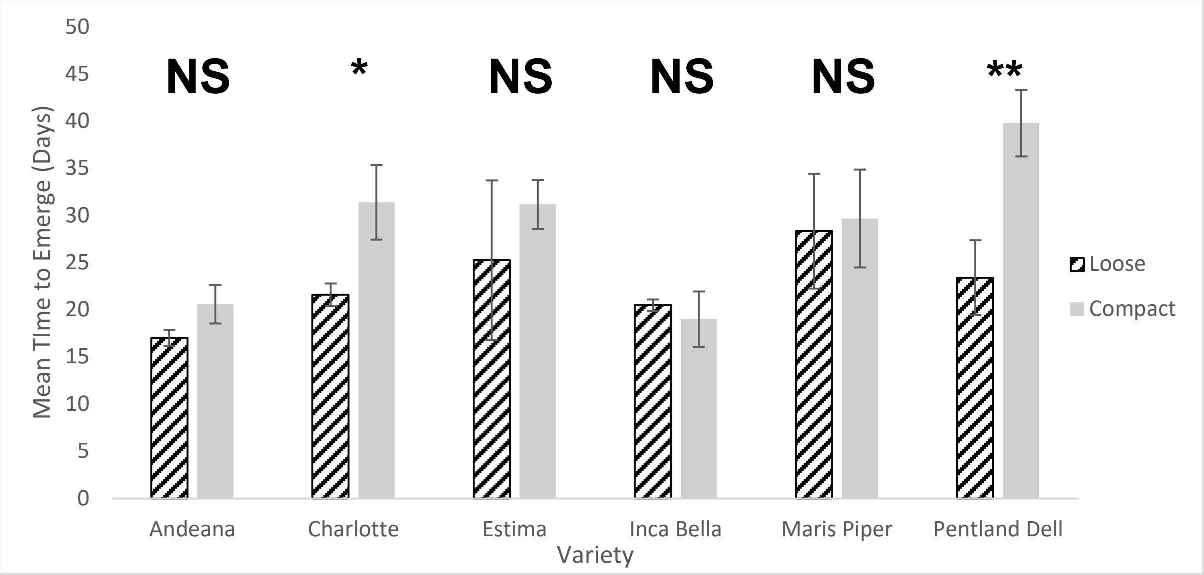


Figure 2-1: The effect of soil resistance and potato cultivar on plant emergence time. Each bar represents the mean of five plants. Bars with diagonal lines represent tubers grown in loose soil (0.3 MPa) whilst solid bars are tubers grown in compacted soil (3.0 MPa). Significance between compaction treatments from Tukey's tests is indicated using stars. NS not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Error bars show standard error with 95% confidence limits.

Neither cultivar ($p = 0.13$) nor soil compaction ($p = 0.47$) significantly affected transpiration rates (data not shown), and cultivars did not vary in their response to changes in soil resistance ($p = 0.87$). Ψ_{root} did not change due to soil compaction ($p = 0.74$). The highest Ψ_{root} was observed in Charlotte (-0.30 ± 0.023 MPa, n = 5) and the lowest in Estima (-0.41 ± 0.028 MPa , n = 5).

Leaf area was very different between cultivars ($p < 0.001$), with a four-fold increase in leaf area from the smallest (Charlotte) to the largest (Inca Bella). Soil compaction significantly ($p < 0.001$) decreased leaf area by a mean of 27% (Figure 2-2), with a consistent response across cultivars ($p = 0.11$ for cultivar x compaction interaction). Shoot dry mass followed the same trend as leaf area, with similar level of significances (data not shown).

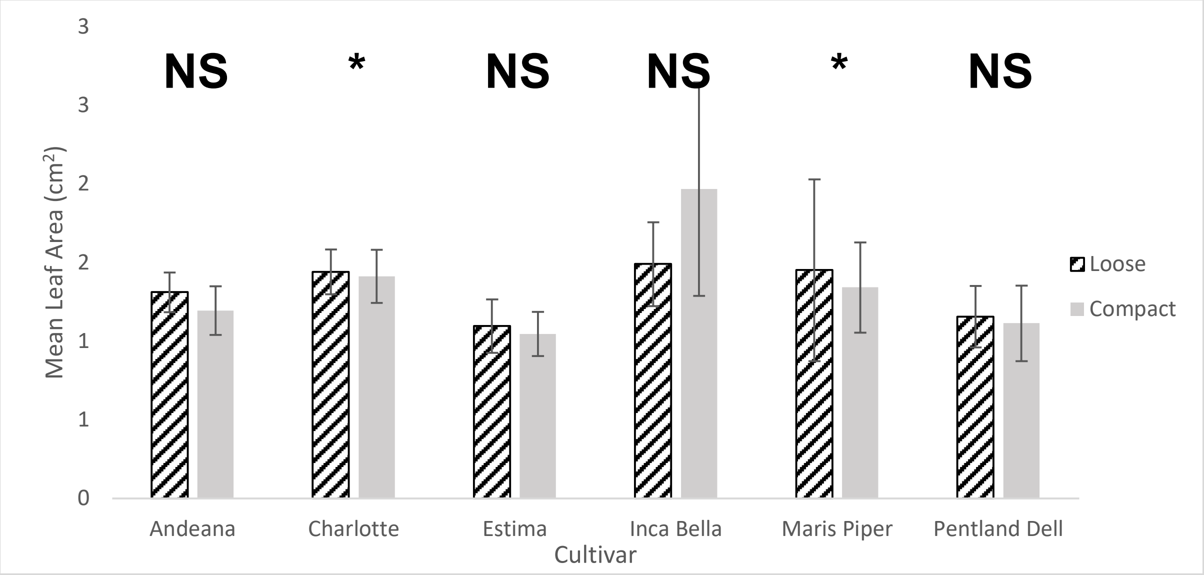


Figure 2-2: The effect of soil resistance and potato cultivar on leaf area. Each bar represents the mean of five plants. Bars with diagonal lines represent tubers grown in loose soil (0.3 MPa) whilst solid bars are tubers grown in a compacted soil (3.0 MPa). Significance between compaction treatments from Tukey's tests is indicated using stars. NS not significant, * p < 0.05, ** p < 0.01, *** p < 0.001. Error bars show standard error with 95% confidence limits.

Root length varied substantially between cultivars (p < 0.001), with the smallest mean root length in loose soil (Pentland Dell) being 40% the length of Andeana's. Root length decreased in response to soil compaction in all cultivars (p < 0.001). Cultivars varied in their response to soil compaction (p < 0.01 for cultivar x compaction interaction), with compaction decreasing root length of Pentland Dell by 25% and Charlotte by 70% (Figure 2-3).

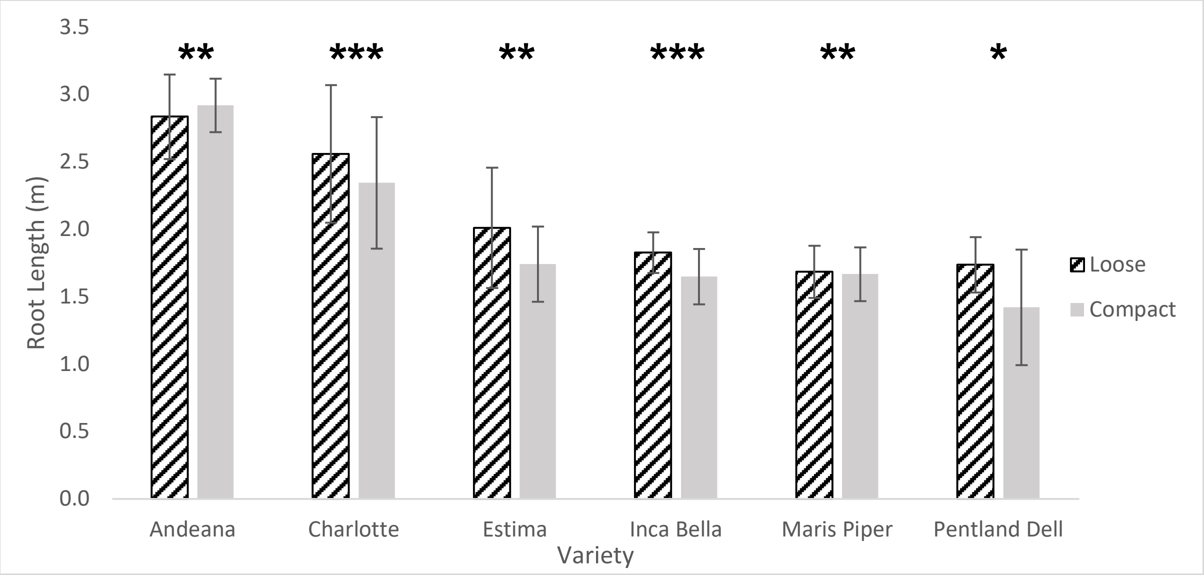


Figure 2-3: The effect of soil resistance and potato cultivar on root length. Each bar represents the mean of five plants. Bars with diagonal lines represent tubers grown in loose soil (0.3 MPa) whilst solid bars are tubers grown in a compacted soil (3.0 MPa). Significance between compaction treatments from Tukey's tests is indicated using stars. NS not significant, * p < 0.05, ** p < 0.01, *** p < 0.001. Error bars show standard error with 95% confidence limits.

Biomass distribution significantly differed between cultivars (p < 0.001) and compaction treatments (p < 0.001), shown in Figure 2-4. The ratio between leaf area and root length differed for each treatment (p < 0.001). Wheras Estima showed no change in root length as leaf area increased, root length of Charlotte plants increased exponentially as leaf area increased.

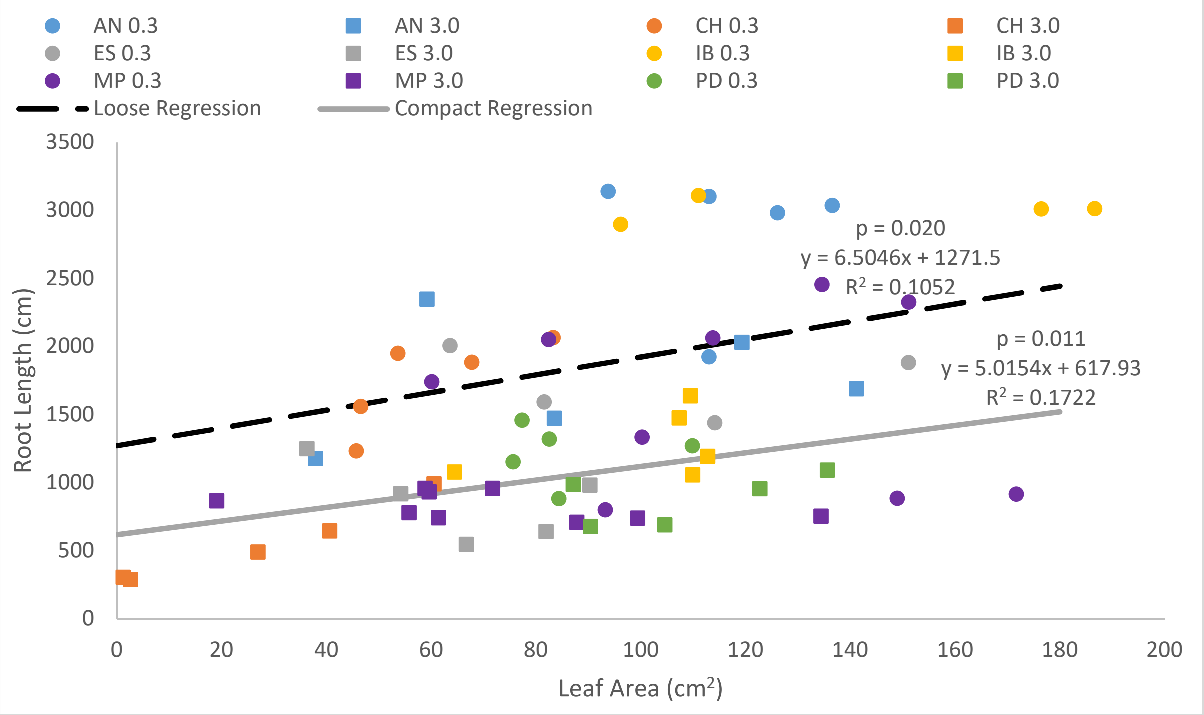


Figure 2-4: Biomass distribution categorised by the relationship between potato plant leaf area and root length. Each data point represents a single plant. Lines show linear regression of plants grown in loose (black, dashed line) and compacted (grey, solid line) soil, along with their significance, equation, and R^2 values. Regressions for individual cultivars not included for clarity.

Root diameter significantly differed between cultivars ($p < 0.001$), with a 60% difference between the thinnest roots (Maris Piper) and the thickest (Andeana). Diameter increased significantly in response to increased soil resistance ($p < 0.001$). Most cultivars had a similar increase in root diameter in response to increased soil resistance, typically between 15% and 25% ($p = 0.12$). However, some individual cultivars responded much more than others. Root diameter of Andeana, Estima Inca Bella and Pentland Dell did not change (Figure 2-5), whereas root diameter of Estima and Maris Piper increased by 20% and 40% respectively.

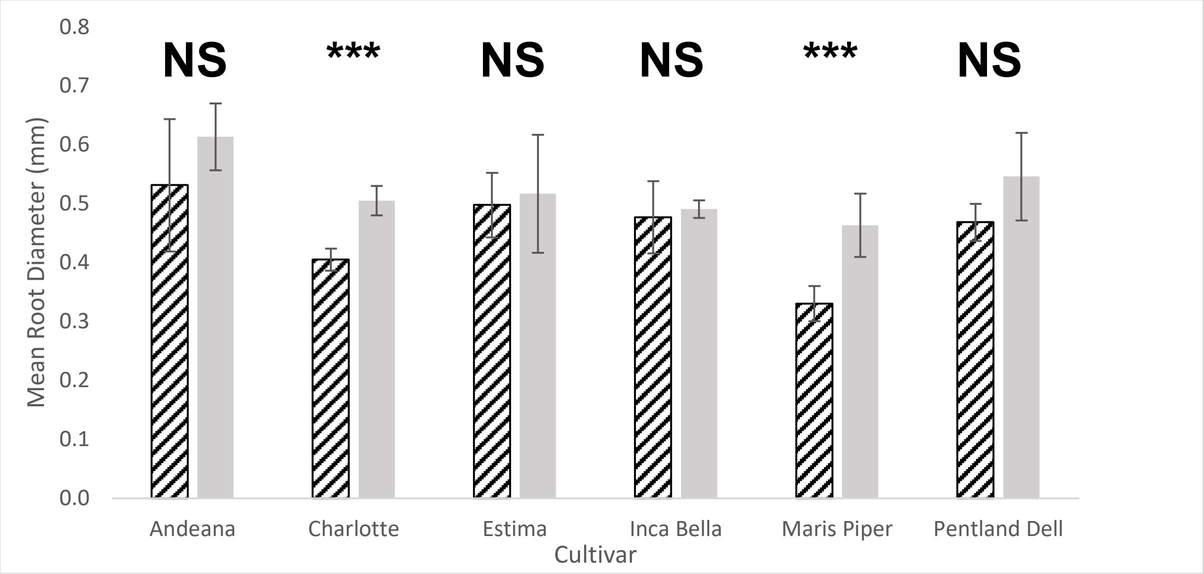


Figure 2-5: The effect of soil resistance and potato cultivar on root diameter. Each bar represents five tubers. Bars with diagonal lines represent tubers grown in loose soil (0.3 MPa) whilst solid bars are tubers grown in a compacted soil (3.0 MPa). Significance between compaction treatments from Tukey's tests is indicated using stars. NS not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Error bars show standard error with 95% confidence limits.

Root diameter distribution in compacted soil significantly changed in all cultivars except Estima ($p < 0.001$). Fine roots (0.2 mm) decreased in most cultivars whereas thicker roots (0.3 mm to 0.9 mm) showed a small increase (Figure 2-6).

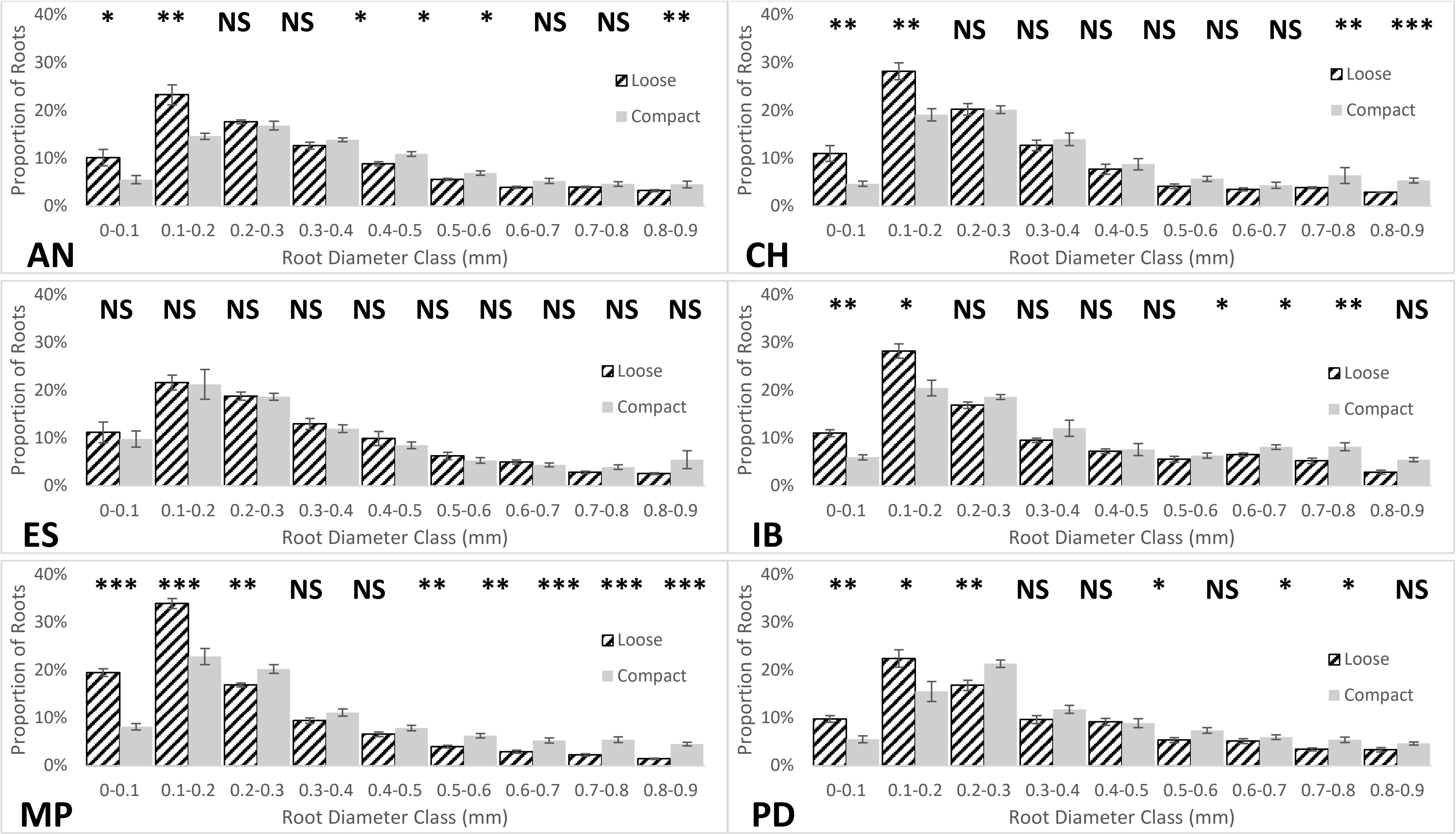
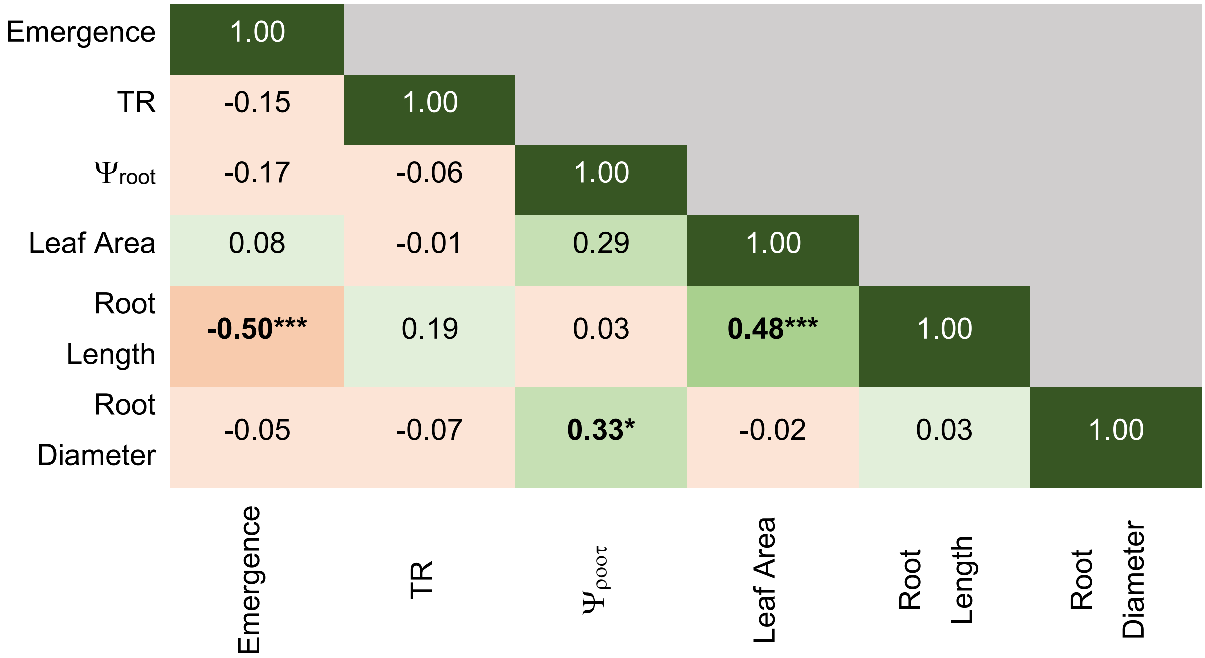


Figure 2-6: Changes in root diameter between compaction treatments. Bars with diagonal lines represent potato plants grown in loose soil, whilst solid bars represent those grown in compacted soil. Cultivar is indicated using letters at the lower left of each graph. Andeana (AN), Charlotte (CH), Estima (ES), Inca Bella (IB), Maris Piper (MP), and Pentland Dell (PD). Error bars indicate standard error with 95% confidence limits. Significance between compaction treatments from Tukey’s tests is indicated using stars. NS not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Error bars show standard error with 95% confidence limits.

Root length was greater when emergence time was shorter ($p < 0.001$; Table 2-3). Root water potential had a weak, positive correlation with root diameter ($p = 0.04$). Plants with greater leaf area also produced a larger root length ($p < 0.001$).



	Emergence	TR	Ψ_{root}	Leaf Area	Root Length	Root Diameter
Emergence	1.00					
TR	-0.15	1.00				
Ψ_{root}	-0.17	-0.06	1.00			
Leaf Area	0.08	-0.01	0.29	1.00		
Root Length	**-0.50*****	0.19	0.03	**0.48*****	1.00	
Root Diameter	-0.05	-0.07	**0.33***	-0.02	0.03	1.00

Table 2-3: Pearson coefficients for key morphological and physiological variables from Experiment 2.1. Significant correlations are indicated by bold text with asterisks indicating significance. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Potato cultivars exhibit different growth rate responses to compaction

Plant morphology changed drastically over the four weeks, with cultivar and soil compaction having significant effects (Table 2-4). While leaf area of all cultivars responded similarly to soil compaction (no significant cultivar x compaction interaction) as in Experiment 2.1, root responses varied between cultivars. Root length and diameter responses to compaction were consistent with Experiment 2.1, although mean root diameters were consistently 10% lower.

Table 2-4: Three-way ANOVA table for experiment 2.2. NS indicates no significance.

Variable	*Leaf Area*	*Root Length*	*Root Diameter*
Cultivar	<0.001	<0.001	<0.001
Compaction	<0.001	0.007	0.007

Time	<0.001	0.013	0.026
Cultivar x Time	NS	NS	0.003
Compaction x Time	NS	NS	NS
Cultivar x Compaction	NS	0.019	<0.001
Cultivar x Compaction x Time	NS	NS	0.010

Higher soil resistance significantly ($p < 0.01$) decreased root length, which exhibited a small but significant increase of 10% over four weeks ($p = 0.01$) when root length was measured weekly. Cultivars varied in their response to compaction ($p = 0.02$), with Inca Bella exhibiting a consistent 33% loss of root length, Andeana a constant 50% reduction, whilst Maris Piper only differed between compaction treatments in weeks 3 and 4, when higher compaction decreased mean root length by 25%, as shown in Figure 2-7.

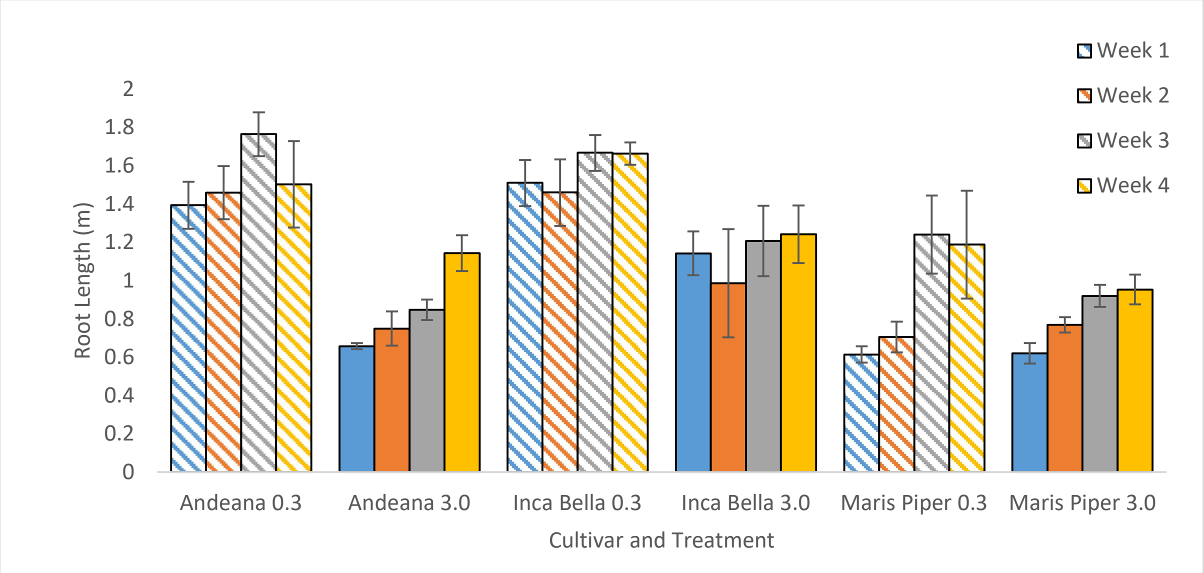


Figure 2-7: The effect of age and cultivar on root length on potato plants grown in loose (diagonal lines) and compact (solid bars) soil. Each bar represents three plants. Error bars show standard error with 95% confidence limits.

Soil compaction significantly ($p < 0.01$) affected root diameter, which notably differed ($p < 0.001$) between cultivars. Changes in root diameter differed between cultivars and compaction treatments over the four weeks ($p = 0.01$). Root diameter of Inca Bella did not change ($p = 0.32$) in compacted soil but increased by 20% over time in loose soil (p = 0.04) (Table 2-5). Mean root diameter of Maris Piper decreased by 18% in compacted soil (p = 0.01), and tended to decrease in loose soil (p = 0.07).

Root diameter of Andeana significantly (p < 0.01) decreased by 30% in compacted soil but did not change (p = 0.30) in loose soil. Root diameters in compacted soil were consistently 10% smaller compared to those obtained in Experiment 2.1, with mean diameter of 0.5 mm for Maris Piper, 0.57 mm for Inca Bella and 0.66 mm for Andeana from Experiment 2.2 compared to 0.46 mm, 0.49 mm, and 0.61 mm from Experiment 2.1, respectively, likely due differences in tuber age affecting vigour.

Table 2-5: The effects of cultivar and soil resistance on the changes in root diameter and root length for each treatment. Values are obtained by determining the mean change in value per treatment across all weeks. Asterisks represent significance, and significant changes are bolded for clarity. * p < 0.05, ** p < 0.01. ± values indicate standard error for each variable.

Treatment	*Mean Change in Root Diameter per Week (mm)*	*Mean Change in Root Length per Week (cm)*
Andeana 0.3 MPa	+0.020 ±0.007	+27 ±26
Andeana 3.0 MPa	**-0.057**** ±0.005	**+122*** ±35
Inca Bella 0.3 MPa	**+0.027*** ±0.026	+38 ±26
Inca Bella 3.0 MPa	+0.012 ±0.007	+25 ±14
Maris Piper 0.3 MPa	**-0.043*** ±0.014	**+144*** ±74
Maris Piper 3.0 MPa	-0.025 ±0.024	**+83**** ±47

Genotypic variation in potato morphology in compacted soil are root-sourced

Morphological comparisons were made between grafts of the potato cultivars Inca Bella and Maris Paper grown in either compacted or uncompacted soil for a total of 28 days after emergence, 21 of which were after grafting.

Overall, scion genotype did not significantly affect any of the measured variables, while rootstock genotype affected both root length and diameter, and leaf (Table 2-6). Both scion and rootstock influenced plant morphology in response to soil compaction. Plants with Inca Bella rootstocks exhibited a decreased leaf area in response to soil compaction, whilst plants with Maris Piper scions were not significantly affected.

Table 2-6: Three-way ANOVA table for this experiment. NS indicates a non-significant p-value. Non-significant p-values between 0.05 and 0.1 were included for clarity.

Variable	*Leaf Area*	*Root Length*	*Root Diameter*
Scion	NS	NS	NS
Rootstock	NS	<0.001	0.008
Compaction	0.024	<0.001	<0.001
Scion x Rootstock	NS	NS	NS
Scion x Compaction	0.048	NS	NS
Rootstock x Compaction	0.040	<0.001	<0.01
Scion x Rootstock x Compaction	0.056	<0.01	NS

The root length of Inca Bella rootstocks was reduced more by soil compaction than Maris Piper rootstocks (Table 2-7), and plants with Maris Piper scions retained more root length in compacted soil than plants with Inca Bella scions. Maris Piper rootstocks exhibited no changes in root length or diameter in compacted soil, unlike other Inca Bella rootstocks.

Table 2-7: Summary of the effects different graft sections have on key morphological variables when compacted. Variables either increased (↑), decreased (↓), or were unaffected (↔) by compaction. More arrows indicate a stronger effect.

Variable	*Inca Bella Scion*	*Inca Bella Rootstock*	*Maris Piper Scion*	*Maris Piper Rootstock*
Leaf Area	↓↓	↓↓	↓	↓
Root Length	↓↓	↓↓↓	↓	↓
Root Diameter	↑	↑↑	↑	↑↑

Soil compaction did not influence ($p = 0.06$) the relationship between leaf area and root length. Biomass distribution differed between rootstocks ($p < 0.001$), with root length of Maris Piper rootstocks not changing as leaf area increased ($p = 0.88$), whilst root length of Inca Bella rootstocks increased as leaf area increased ($p = 0.02$). For a given leaf area, Inca Bella rootstocks produced significantly ($p < 0.001$) more root length (Figure 2-8). Biomass distribution was not altered by the scion (p =

0.78), or the interaction of scion and rootstock (p = 0.86).

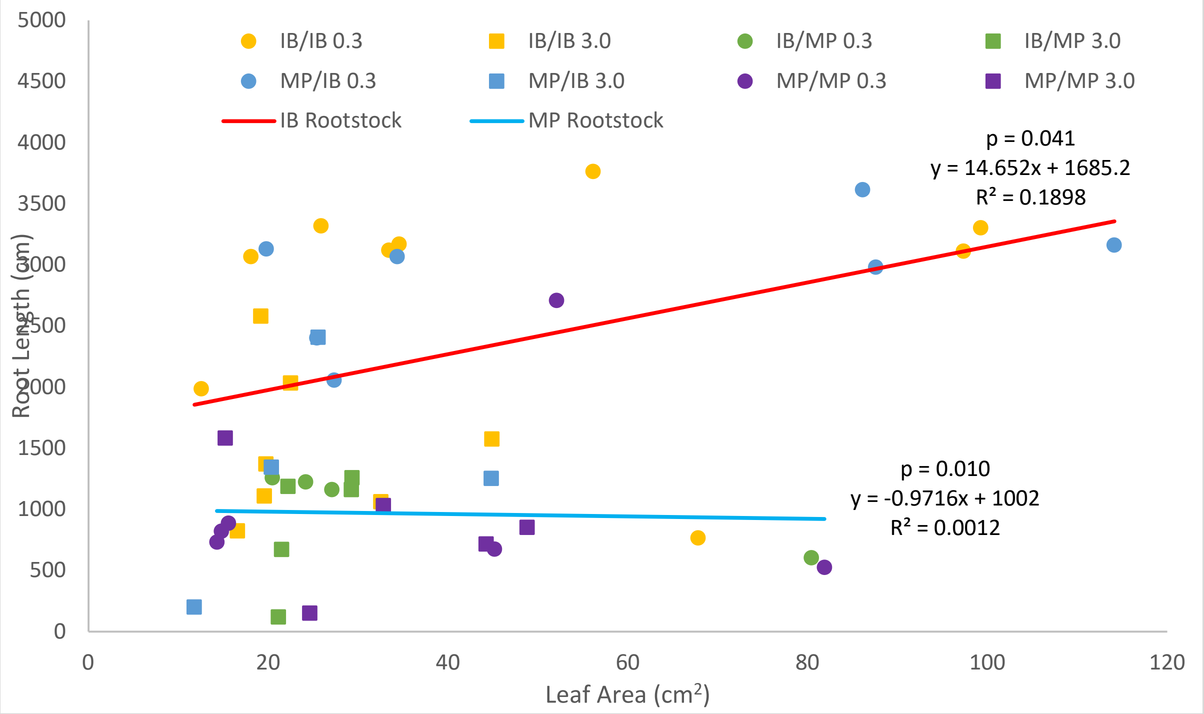


Figure 2-8: Biomass distribution categorised by the relationship between potato leaf area and root length. Each data point represents a single plant. Plants in legend are represented as scion/rootstock. Lines show linear regression of Inca Bella rootstocks (red line) and Maris Piper rootstocks (blue line), along with their significance, equation, and R^2 values. Scion linear regressions not included due to a lack of significance.

Compacted soil decreased total leaf area of Inca Bella scions by 50%, and Maris Piper scions by 20% to 30% (Figure 2-9). Leaf area decreased differently in response to compaction between different rootstocks ($p = 0.04$) and scions ($p < 0.05$), but not between all treatments ($p = 0.06$). Leaf area in compacted soil was 63% and 54% lower for Maris Piper and Inca Bella scions respectively. Plants with Maris Piper rootstocks lost 23% of their leaf area in compacted soil, whilst those with Inca Bella rootstocks lost 52%.

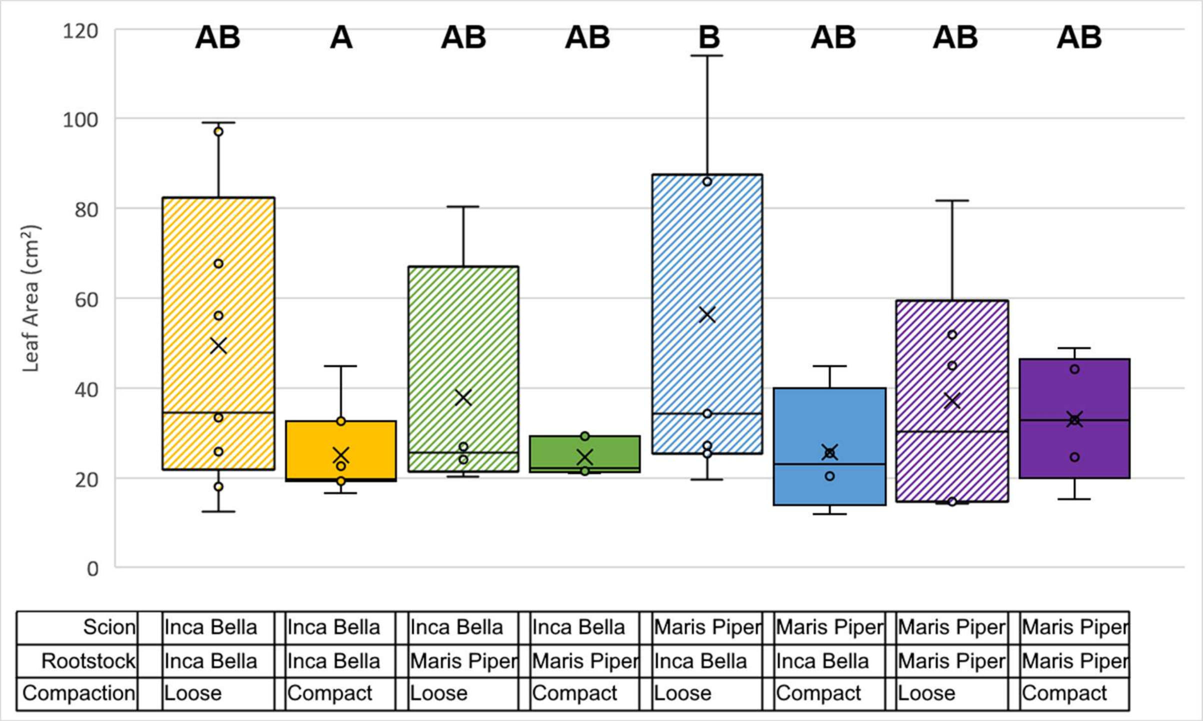


Figure 2-9: Box and whisker plot showing the distribution of total leaf area. Due to natural variation in survival rates, each plot represents between four and eight replicates. Letters above plots indicate significance with a p-value less than 0.05 based on a Kruskal-Wallis test.

Root length differed greatly in response to compaction ($p < 0.001$), with each graft combination producing a significantly different response ($p < 0.01$). Compact soil decreased root length of Inca Bella rootstocks by 60%, and Maris Piper rootstocks by 20%. However, Inca Bella rootstocks tended to produce a greater root length, even in compacted soil, with Maris Piper rootstocks having much smaller root systems even in loose soil (Figure 2-10).

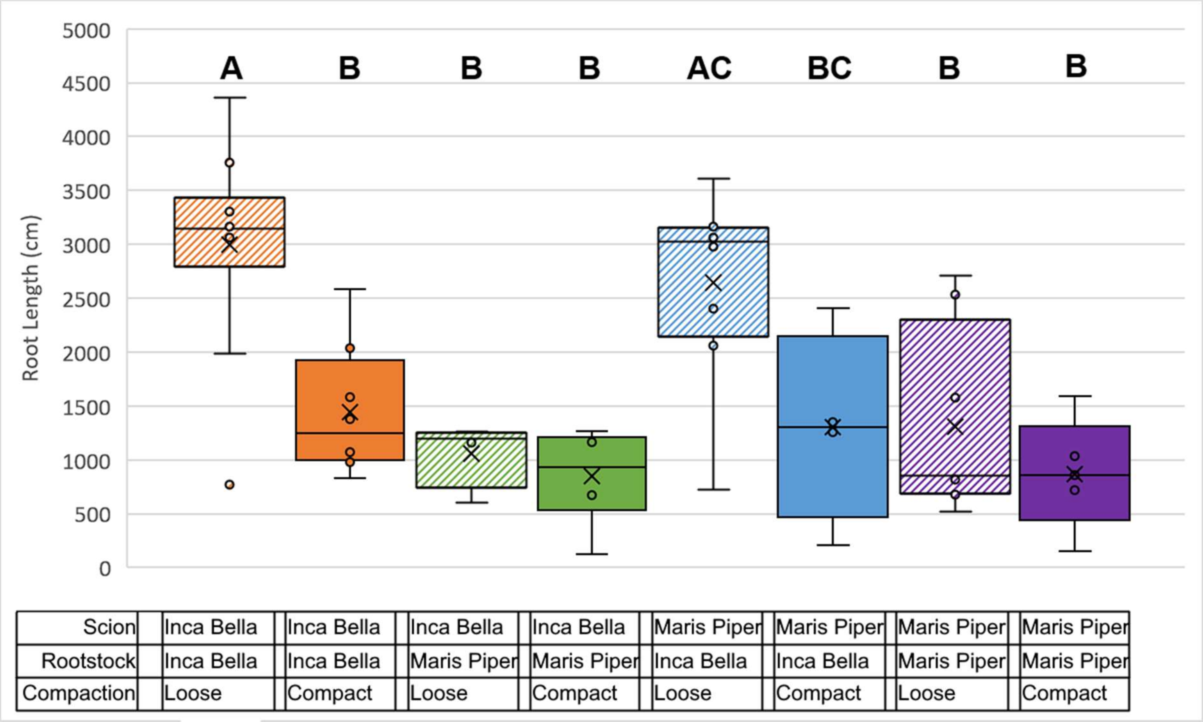


Figure 2-10: Box and whisker plot showing the distribution of root lengths observed across all treatments. Due to natural variation in survival rates, each plot represents between four and eight replicates. Letters above plots indicate significance with a p-value less than 0.05 based on a Kruskal-Wallis test.

Compact soil significantly ($p < 0.001$) increased root diameter (Figure 2-11). Rootstock determined changes in root diameter, with diameter of Inca Bella and Maris Piper rootstocks increased by 40% and 50% respectively.

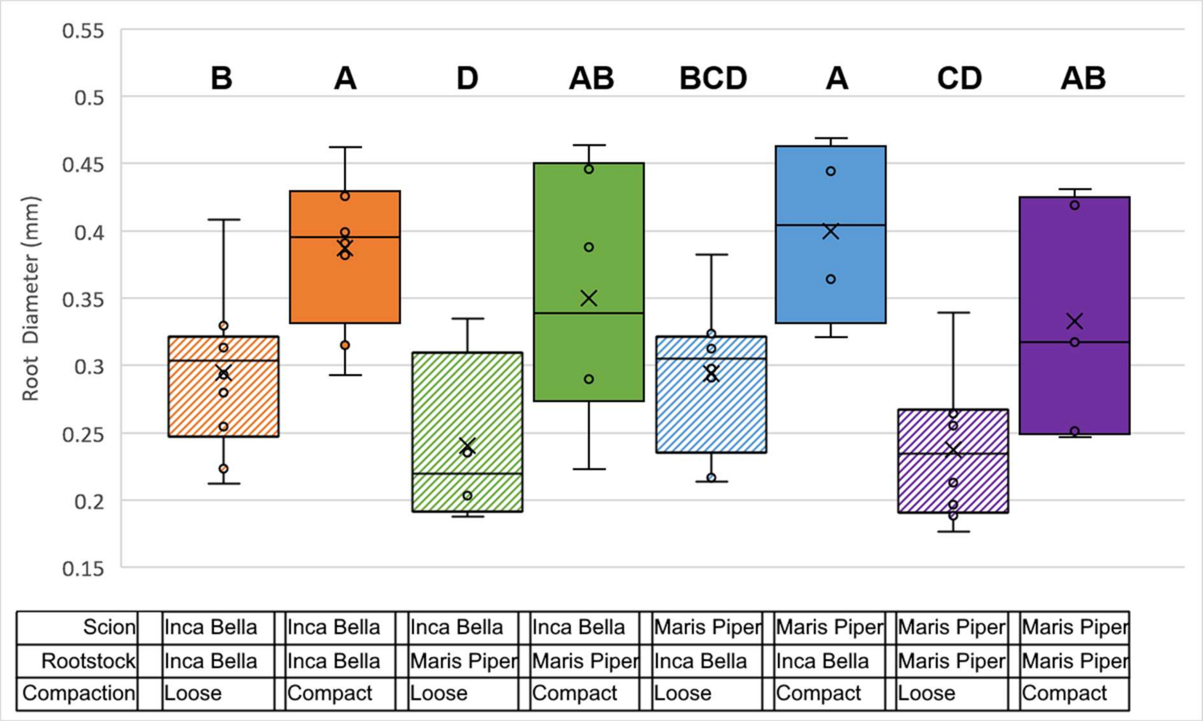


Figure 2-11: Box and whisker plot showing the distribution of root diameters observed across all treatments. Due to natural variation in survival rates, each plot represents between four and eight replicates. Letters above plots indicate significance with a p-value less than 0.05 based on a Kruskal-Wallis test.

2.4 Discussion

In this chapter, experiments were conducted to determine whether genotypic variation between cultivars of potato caused different morphological responses to soil compaction, and the relative importance of root and shoot in determining these responses. Genotypic variation in morphology, especially root length (Figure 2-3) and root diameter (Figure 2-4; Figure 2-6) was observed between cultivars. Changes in morphology were found to be primarily determined by the rootstock (Table 2-6; Table 2-7), suggesting that the genotypic variation in compaction responses were sourced from the root.

Potato Cultivars Exhibit Different Phenotypic Responses to Compaction

In experiment 2.1, the mean time it took for potato plants to emerge from compacted and uncompacted soil was compared for each cultivar, along with the number of plants that failed to emerge. Potato plants emerging from compacted soil did not exhibit any increase in mortality or emergence failures compared to plants emerging from loose soil. However, shoot emergence from tubers was delayed by up to 15 days in Pentland Dell, which exhibited the greatest delay in emergence when grown in compacted soil (Figure 2-1). Most studies find that increased soil resistance delays emergence and greatly increases seedling mortality (Kubota and Williams, 1967; Masaka and Khumbula, 2007), with up to 70% seeds failing to germinate in extreme cases (Nawaz *et al*., 2013). The high rate of mortality is typically attributed to soil compaction causing loss of pore space that inhibits soil aeration (Atkinson *et al*., 2019) which in turn limits root growth and reduces soil water holding capacity (Smith *et al*., 2001). However, tubers in these experiments were planted in a thin layer of loose soil above the compacted soil, thus these stresses were absent when the tubers sprouted. While this likely explains why all tubers planted emerged, it does not account for the observed delay. It is possible that the compacted layer causes ethylene (released by the tuber) to become trapped between the tuber and the soil, thereby extending dormancy. By restricting diffusion of ethylene, soil compaction causes it to accumulate next to the roots (Pandey *et al*., 2021). Tubers treated with the competitive inhibitor of ethylene 2,5-norbornadiene have been found to emerge more rapidly than those left untreated (Suttle, 1998), and long-term ethylene exposure (20 ppb to 20 ppm over a period of 35 days) inhibited sprout growth on tubers (Rylski *et al*., 1974). It is therefore likely that the compacted treatment extends tuber dormancy by trapping ethylene between the tuber and the compacted soil. Since emergence of cultivars such as Maris Piper and Inca Bella was not delayed by compacted soil, they may be less sensitive to ethylene overaccumulation than cultivars like Charlotte and Pentland Dell. This is supported by the negative correlation between emergence time and root length (Table 2-3), which indicates that tubers with delayed emergence also had lower root growth rates. As ethylene reduces root growth rates (Okamoto *et al*., 2008), this supports the hypothesis that these delayed tubers are sensitive to ethylene. Tubers that emerge rapidly in compacted soil may therefore be more tolerant of soil compaction.

While compacted soils reduced leaf area of the potato cultivars Estima (Stalham *et al*., 2007) and Maris Piper (Huntenburg *et al*., 2021), (Stalham *et al*., 2007) in field trials, and the majority of studied cultivars by 15-30% across all three pot experiments (Figure 2-2; Figure 2-9), a high degree of variation resulted in a lack of statistical significance. This was unexpected, possibly caused by the level of soil compaction experienced and/or other stresses such as water deficits. Dry summers, with much lower soil water potentials in compacted treatments, significantly decreased canopy coverage and foliar biomass of potato, but these effects were not present in years with wet summers (Van Loon *et al*., 1985). Nevertheless, decreased leaf area of cereals such as wheat (Masle and Passioura 1987) and barley (Mulholland et al. 1996a) in response to soil compaction could not be attributed to leaf water deficit, suggesting a direct response to the presence of compacted soil. Soil resistances greater than 3.0 MPa (applied in the pot experiments) may further limit leaf expansion, with soil compaction decreasing wheat leaf elongation rate exponentially above 4 MPa (Masle and Passioura 1987). Since such high soil resistances tend to prevent any root growth of potato (Stalham *et al*., 2007), the lower soil resistance applied (3.0 MPa) may explain the lack of significant leaf growth inhibition. Furthermore, ensuring the plants were well-watered irrespective of soil resistance may have minimised the effects on leaf area.

Genetic variation in root length inhibition in response to soil compaction was clearly observed (Figure 2-3), and other plant species such as rice (Clark *et al*., 2002) and lupin (*L. angustifolius*) (Chen *et al*., 2014) showed similar variation, lending credence to the hypothesis that these differences in penetrative ability are consistently driven by genotypic variation. Differences in root growth rates between cultivars (Figure 2-7) also support the existence of genotypic variation in root growth responses to compacted soil. Inca Bella did not show any significant increases in root length over time (Table 2-5), whilst Andeana roots grew rapidly in compacted soil, with little change in loose soil, and Maris Piper increased significantly over time in both treatments. The large initial root system size is likely due to the presence of the loose soil in which the tubers were planted. This meant the upper 2 cm of soil had no measurable resistance, allowing rapid initial growth before the roots encountered the more compact soil below. Whilst there is a lot of variation, even 0.3 MPa of soil resistance can significantly reduce root growth rates (Taylor and Brar, 1991; Stalham *et al*., 2007), which meant the loose soil treatment will have reduced root growth compared to soil that was not compacted at all, considering both treatments in these experiments was subjected to compacting force. Furthermore, since root growth occurs pre-emergence, it is likely that the measured root growth rates were considerably less than earlier in the experiments. Overall, changes in root length and growth rates indicate cultivar variation in responses to compaction.

Root Diameter Does Not Correlate with Root Length

Mean root diameter tended to increase in all cultivars in response to soil compaction (Figure 2-5), although the increase was only significant in two cultivars (Maris Piper and Charlotte). However, all cultivars except Estima had significantly lower proportions of smaller (<0.2 mm) diameter roots and higher proportions of larger (>0.8 mm) roots in compacted soil compared to loose soil. This indicates a loss of fine roots, with other roots thickening in response to compaction. In addition, the negative correlation between Ψ_{root} and root diameter (Table 2-3) implies that osmotic potential is decreasing, causing water to swell the roots to improve turgor pressure. This corroborates other studies that have observed increased Ψ_{root} in roots exposed to compaction, with the increased turgor pressure potentially improving penetration (Bengough *et al.*, 2011). Increased root diameter is strongly correlated with increased soil resistance (Atwell, 1993; Bengough *et al.*, 2006; Potocka and Szymanowska-Pulka, 2018). However, the lack of correlation between root length and either Ψ_{root} or root diameter (Table 3-3) implies that this increased root diameter may not improve penetrative ability. More recent research indicates that increased root diameter is merely a side effect of reduced aeration in compacted soil causing ethylene to over accumulate (Pandey *et al.*, 2021), and that increased ethylene sensitivity reduces penetrative ability in compacted soil (Vanhees *et al.*, 2021). The hypothesis that increased root diameter improves penetrative ability is disproved by the lack of correlation between root length and root diameter in Experiment 2.1 (Table 2-3). Furthermore, root diameter is likely to be genetically determined, as significant differences in root diameter between cultivars were observed (Table 2-2). However, it cannot be confirmed from these experiments if ethylene causes the observed variation.

Rootstock and Scion influence Compaction Responses

The root system supplies the nutritional and water requirements of the shoot, with drought stress promoting vertical root growth and nutrient deficiency promoting root branching (Scheible *et al.*, 1997; Delay *et al.*, 2013; Kudoyarova *et al.*, 2015).

However, Experiment 2.3 with grafted plants determined that when compacted soil impedes root growth, the roots affect shoot growth (Figure 2-9), with the rootstock significantly affecting leaf area (Table 2-6). Typically, scion growth is less affected by rootstocks than rootstock growth is by scions (Chen *et al.*, 2003), whilst in this experiment, the rootstock primarily determined plant growth. This is likely due to compacted soil limiting root growth (Stalham *et al.*, 2007), which was not overcome by signals from the scion. Even when plants roots are impeded by pressure but not stressed, shoot growth is inhibited (Sarquis *et al.*, 1991), possibly by a root-sourced ABA signal sent in response to impedance (Mulholland *et al.*, 1996a; Grzesiak *et al.*, 2016). Nevertheless, there is evidence to suggest that increased foliar ABA improves leaf growth in compacted soil, as the ABA-deficient barley mutant Az34 produced a much lower leaf area than wild type in compacted soil, and a strong correlation between xylem ABA concentration and leaf area in compacted soil (Mulholland *et al.*, 1996b).

Since root length was different between all grafting combinations, penetrative ability may not entirely depend on root traits. Whilst the effect of the scion in determining responses to compacted soil is not significant, plants with Inca Bella scions were more vigorous than plants with Maris Piper scions, whilst plants with Maris Piper rootstocks retained more root length in compacted soil (Figure 2-10). The lack of response to compaction by Maris Piper self-grafts may be because these plants had the lowest measured root length of all the treatments. Root growth in compacted soil tends to exploit pre-existing soil pores, with low total root length suggesting that pores were sufficient to maintain similar root growth to plants in loose soil. The scion-based differences are due to the increased rooting requirements of the larger Inca Bella canopy than Maris Piper (Figure 2-2), (Figure 2-9), which would necessitate an increase in root system size, promoting root vigour to maintain water relations (Tandonnet *et al.*, 2010; Harrison *et al.*, 2016). The lack of change in root length between Maris Piper self-grafts may be due to the reduced root system size. Maris Piper root length in compacted soil is comparable between experiments (Figure 2-3; Figure 2-7; Figure 2-10), so the comparable root growth in uncompacted soil may be due to stress or wounding caused by grafting rather than genotypic variation.

Root length data were mostly comparable between Experiments 2.3 and 2.1. Inca Bella rootstocks were vigorous in loose soil and exhibited a 60% reduction in root length in compacted soil. Plants with Maris Piper rootstocks and Inca Bella scions produced root lengths comparable to Maris Piper plants, with a 25% loss of root length in compacted soil, but Maris Piper self-grafts were unaffected by compaction. Root diameter was very different in Experiment 2.3 compared to 2.1. All plants had smaller mean root diameters when grafted, with around a 20% decrease in Maris Piper rootstocks compared to ungrafted Maris Piper plants, and a 35% decrease in Inca Bella rootstocks. Furthermore, Inca Bella rootstocks exhibited a 30% increase in mean root diameter that was not observed in other experiments, whilst Maris Piper had a mean 35% increase, which was comparable to Experiment 2.1 (Figure 2-5). The reduced root diameter of grafted plants may be caused by drought stress. Grafting causes the xylem to be open to the elements, with uncontrolled water loss from the stem. This can lead to rapid water loss from the roots until the graft has fully healed, potentially causing drought stress. Potato root diameter decreases when droughted in order to maximise water uptake by increasing hydraulic conductance (Quandahor *et al*., 2021). Whilst steps were taken to reduce water loss as much as possible, it is still possible that water loss was sufficient after the stem was cut to increase the production of fine roots.

Contrary to expectations, biomass distribution was solely affected by rootstock. Inca Bella rootstocks caused a clear, positive correlation between root length and leaf area, whilst Maris Piper rootstocks showed no significant relationship between the two variables. Previous studies have found that root to shoot ratio is primarily determined by the scion, with the rootstock only having a minor effect (Jefferies, 1993). Different graft combinations may have their own ideal environmental conditions, such as humidity and temperature (Mir and Kumar, 2011), and exhibit reduced shoot growth and healing time when recovering in less ideal conditions (Petropoulos *et al*., 2012). Leaf area may have recovered more slowly than root traits due to Maris Piper rootstocks being grown in conditions that were less suitable, whilst Inca Bella rootstocks were better adapted.

2.5 Conclusions

Although compacted soil had limited effects on leaf area, it decreased root system size of all tested cultivars, and root diameter of most cultivars, with substantial genetic variation in response to soil compaction in root traits. It is likely that leaf area was maintained by avoiding stresses such as drought that are commonly caused by compact soil, and potentially due to the resistance of the soil being lower than required to cause a significant change in leaf area. However, root thickening may result from encountering impedance rather than indicating penetrative ability, as many of the cultivars investigated in this experiment did not exhibit an increase in root diameter in compacted soil (Figure 2-5) but varied in their ability to maintain root growth (Figure 2-3). Most responses to compaction are determined by the roots, with control of leaf area, root length and root diameter having larger differences between rootstocks than scions. Differences in these responses between grafted and ungrafted plants may result from wounding stresses caused by grafting, and the stress caused by compacted soil impeding root growth. Since ethylene overaccumulation is at least partially responsible for inhibiting root growth (Pandey et al. 2021), measuring ethylene production or sensitivity required to test the role of ethylene in genotypic variation in the ability of roots to grow through compacted soil. To better understand the genotypic variation observed, ethylene production and sensitivity were investigated in Chapter 3.

Chapter 3 Ethylene sensitivity determines compaction tolerance in potato

3.1 Introduction

Ethylene has a variety of roles within the root tip, including the control of root elongation (Stepanova and Alonso, 2009). Increases in root ethylene evolution are associated with decreased root length and increased root diameter by promoting lateral elongation of cells in root tips (Osborne, 1976; Potocka and Szymanowska-Pułka, 2018). These root responses are also observed when roots encounter compacted soil, implying that ethylene causes these responses (Clark *et al*., 2003). Furthermore, chemical inhibition of ethylene production in maize plants prevents typical root responses to compaction, with root growth through compacted soil increasing as a result (Sarquis *et al*., 1991). Finally, the *Arabidopsis* mutant *eto1-1* (an ethylene overproducing mutant) had comparable root ethylene evolution to wild type plants grown on agar with simulated impedance. Both *eto1-1* and wild type plants exhibited identical responses to the simulated impedance (increased root diameter and decreased cell length), but root morphology of the ethylene insensitive mutant *aux1-7* did not change (Okamoto *et al*., 2008). Based on these studies, it seems likely that root ethylene production increases in response to impedance from compacted soils, with roots requiring a functioning ethylene sensing pathway to adapt to the presence of compacted soil impeding root growth.

Traditionally, it has been accepted that increased ethylene production improves penetrative ability. Ethylene evolution increased by over 300% in corn seedlings subjected to 100 kPa of impedance (Sarquis *et al*., 1991). Increased ethylene evolution caused increased root diameter (Potocka and Szymanowska-Pułka, 2018), which was correlated with more roots successfully penetrating compacted soil across multiple crop species, including faba bean, pea, wheat, and barley (Materechera *et al*., 1992). Likewise, rice cultivars which exhibited a greater degree of root thickening in response to impedance had roots more capable of penetrating a resistant wax layer (Clark *et al*., 2002). These data indicate a strong correlation between ethylene production, increased root diameter, and increased penetrative ability.

Whilst it is generally accepted that ethylene is involved in the roots' response to soil compaction, the mechanics remain uncertain. Studies comparing wild type and ethylene-insensitive mutants of *Arabidopsis* have found that key genes promoting ethylene production such as the ACC oxidase gene Ethylene Forming Enzyme and Ethylene Response Factor genes ERF1a and ERF4 were significantly upregulated in impeded compared to unimpeded roots (Jacobsen *et al*., 2020). However, there is also evidence that ethylene accumulation in the soil, not its production *in planta*, changes root morphology. Reductions in pore space and aeration within compacted soil greatly reduce the diffusion rate of ethylene produced by roots, potentially causing an overaccumulation of ethylene and increased activity of the ethylene reporter gene OsEIL1 (Pandey *et al*., 2021). In turn, this upregulates auxin and ABA biosynthesis which are transported to the root elongation zone, leading to an increase in root cell expansion perpendicular to the direction of root growth which causes root diameter to increase and inhibits root elongation (Huang *et al*., 2022). The precise ambient concentration of ethylene required to get this response differs between species, but 0.25 ppm in *Rumex palustris* and *Rumex thyrsiflorus* (Marsh Dock) (Visser *et al*., 1997), and 0.7 ppm in *Vicia faba* (broad beans) (Kays *et al*., 1974) significantly decreased root elongation rates. Root diameter of ethylene insensitive rice mutants (Pandey *et al*., 2021) and maize cultivars (Vanhees *et al*., 2021) did not change in response to impedance, with root system size maintained within compacted soil. Based on these data, plants with reduced sensitivity to ethylene are likely to be more successful at adapting to compacted soil. Understanding whether increased ethylene production or accumulation is responsible for the compaction response is necessary to determine whether compaction tolerance can be produced by inhibiting ethylene production, or attenuating ethylene sensitivity.

Irrespective of whether ethylene production or sensitivity determines root penetrative ability, mitigating the effects of ethylene may improve root growth in compacted soil. One potential method is to reduce ethylene production by applying ACC deaminase-containing bacteria to roots. These bacteria form a symbiotic relationship with plants, taking up excess ACC from plant roots as a nutrient source by using ACC deaminase to cleave ACC into α-ketobutyrate and ammonia (Belimov *et al*., 2015). ACC is the precursor to ethylene in plants, and the bacterial enzyme ACC deaminase cleaves this precursor. This inhibits ethylene production by 50% to 75% (Glick, 2005), improving root growth. Applying the ACC deaminase-containing bacterium *Variovorax paradoxus* 5C-2 enhanced growth of potatoes in drying soil, with potato root biomass being 30% larger and tuber yield increased by 10% in both controlled environment experiments and field trials (Belimov *et al*., 2015). Although potato fields are susceptible to, and frequently heavily afflicted with, soil compaction (Stalham *et al*., 2007), using these bacteria to inhibit the effects of ethylene in compacted soil has not been attempted.

This chapter has two key aims. Whilst previous studies correlated penetrative ability with ethylene production (Sarquis *et al*., 1991; Okamoto *et al*., 2008), ethylene insensitive mutants grew better in compacted soil than their respective wild types (Pandey *et al*., 2021). Using potato cultivars that differed in their ability to grow in compacted soil based on data from Experiment 2.1, root ethylene production and sensitivity to ethylene were measured in attempting to discriminate their relative importance in mediating physiological responses to compaction. The second aim is to evaluate whether applying ACC deaminase-containing bacteria to potato plants growing in compacted soil can recover root growth. Irrespective of whether the production of, or sensitivity to, ethylene determines penetrative ability, inhibiting root ethylene production should lower ethylene accumulation rates, allowing greater root growth.

3.2 Methods

Plant and Soil Conditions

Potato tubers of the cultivars Pentland Dell, Maris Piper, and Charlotte were used, based on data from Chapter 2. Pentland Dell exhibited the least morphological changes in response to compaction, Charlotte had the greatest loss of root length in compacted soil, whilst soil compaction increased Maris Piper root diameter the most. All tubers were obtained from TLC Potatoes (Durris, UK) and were between 15 mm and 25 mm in length. The soil used for these experiments was Norfolk Topsoil from Baileys of Norfolk (Hevingham, UK), as described in Chapter 2. Plants were grown and maintained as described in Chapter 2.

Experiment 3.1: Cultivar variation in ethylene production rate

Three potato cultivars (Charlotte, Maris Piper and Pentland Dell) were grown in soil with either high or low compaction, with nine replicates of each treatment. In order to reduce experiment time, and to avoid root systems becoming too large to fit into vials for ethylene evolution analysis, plants were grown for 16 days post-emergence before harvesting.

When the plants were harvested, leaf area of each plant was measured using a leaf area meter (LI-COR's LI-3100C, Nebraska, USA). The roots were separated from the tuber, and quickly washed to remove soil. Excess moisture was removed from the roots with a paper towel, and the roots were then weighed to obtain root fresh weight. The roots were incubated in 50 ml glass vials for 60 minutes (± 10 minutes) to produce ethylene, with the volume of ethylene in the vials measured using an Ethylene Analyser (ETD-300, Sensorsense, Nijmegen, The Netherlands). The roots were then dried in a drying oven and weighed to determine dry mass. Root ethylene production was calculated by dividing the ethylene volume by either the dry or fresh mass and the incubation time.

In order to account for increased ethylene production due to wounding (Wang *et al*., 2002), all roots were carefully excavated from the soil to limit damage, with the only wounding site being excision from the tuber. Preliminary experiments examined ethylene evolution at twenty-minute intervals and determined that increased ethylene evolution due to wounding was comparable across all cultivars used in the experiment within 120 minutes of being excised. As roots from all treatments were treated identically, including incubation time, ethylene evolution from wounding likely increased similarly in all cultivar/treatment combinations. Extraction of roots was done by washing soil from within the pots using a tap. Compacted soil was harder to erode, but all root samples used in this experiment were placed in vials within 8 minutes from the start of root extraction, with the only root damage being separation from the tuber. One-way ANOVAs confirmed incubation time had no effect on ethylene evolution in experiments 3.1 ($p = 0.95$), 3.2 ($p = 0.52$) or 3.3 ($p = 0.80$).

Experiment 3.2: Cultivar variation in sensitivity to ethylene

The three cultivars from experiment 3.1 (Charlotte, Maris Piper and Pentland Dell) were planted in pots containing vermiculite. Eighteen plants were planted per cultivar, for a total of 54 plants. Upon emergence, the roots of each plant were scanned using an Epson Expression 12000XL (Suwa, Japan) before being analysed using WinRHIZO software (Regent Instruments, Canada) to determine root length, diameter and volume before being replanted in vermiculite. Plants received water until the vermiculite was saturated every Monday, Wednesday, and Friday until emergence, as well as immediately before being placed in the airtight containers. The vermiculite retained at least 85% of its water content between waterings, ensuring plants were not water stressed. The plants were then transferred to custom-made airtight containers (dimensions 325 x 235 x 145 mm) with a self-sealing injection port (Figure 3-1). Each container held 6 pots, and 9 containers were produced to contain the 54 plants used in the experiment. The plants from each cultivar were divided equally between three ambient ethylene concentration treatments. These were produced by injecting nitrogen gas containing 20 ppm of ethylene into the containers to produce concentrations of 0 ppm, 0.5 ppm, and 1.0 ppm into the airtight containers. The plants were then grown for three days in the containers before being harvested.

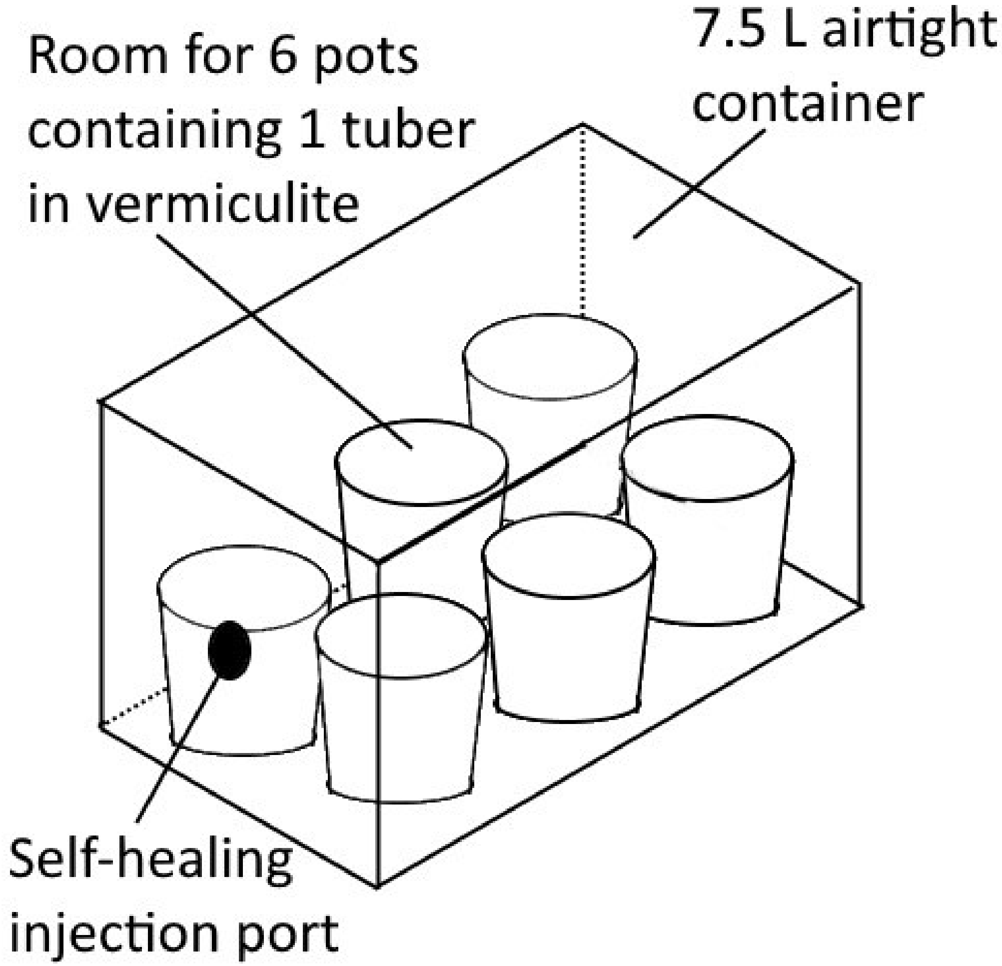


Figure 3-1: Airtight container used for ethylene sensitivity assay.

The root systems of harvested plants were washed, and then scanned using an Epson Expression 12000XL (Suwa, Japan) before being analysed using WinRHIZO software (Regent Instruments, Canada) to determine root length, diameter and volume. The change in these variables was calculated by dividing the end value by the start value.

Experiment 3.3: Impacts of ACC deaminase-containing bacteria on root growth

The ACC deaminase-containing bacterium *Variovorax paradoxus* 5C-2 was obtained from ARRIAM (St. Petersburg, Russia) and cultured in nutrient broth no. 1 (product code 70122, Sigma-Aldrich, Saint Louis, USA). Tubers of the cultivar Maris Piper were sterilised by washing with soap and planted into compacted or loose soil. Each pot was then watered with 30 mL of sterile water, with either 3 mL of sterile water added, or 3 mL of 5C-2 bacteria to a concentration of 10^8 cells mL^{-1} to produce a total of four treatments (loose and compacted, with and without bacteria). Each treatment had 12 replicates and were grown for 2 weeks in the same conditions as described in Chapter 2 until harvested.

Three replicates per treatment were used to determine colonisation of 5C-2. The roots of these plants were gently shaken to remove excess soil. The root and rhizosheath were ground using a mortar and pestle before being mixed into 100 mL of sterile distilled water. The supernatant (100 µL) was placed in 900 µL of sterile distilled water to perform serial dilutions to calculate the concentration of 5C-2 present in and around the root systems. These were then streaked onto Pseudomonas F Agar with 10 ml/l glycerol, 20 mg/l rifampicin, 30 mg/l kanamycin sulphate, and 40 mg/l nystatin added to ensure only 5C-2 successfully colonised. This method confirmed that 5C-2 treatments did not significantly differ in mean CFU concentration, with a mean ± SE value of $56 \pm 21 \times 10^4$ CFUs per gram (n=6). No CFUs were produced in treatments where 5C-2 was not added. The remaining 9 plants per treatment underwent root ethylene evolution analysis using the methodology outlined in Experiment 3.1. Root traits were then analysed, as described in Experiment 3.2.

Statistical Analysis

For all statistical analyses, a p-value of less than 0.05 was considered significant. To identify any variation in ethylene production between treatments in Experiment 3.1, two -way ANOVAs were performed using compaction treatment and cultivar as independent variables, with ethylene production rates per unit of either fresh or dry mass per hour as the dependent variable (Table 3-1). Significant differences between compacted and loose treatments were determined for each cultivar using T-tests. A linear regression was performed with fresh weight as the independent variable and ethylene evolution per unit of fresh weight as the dependent variable to determine whether root system size affected ethylene production rate. To determine if ethylene sensitivity varied between cultivars in Experiment 3.2, two-way ANOVAs were performed using ambient ethylene concentration and cultivar as independent variables. Change in root length, mean root diameter, and ethylene production rates were used as dependent variables (Table 3-2). Finally, to understand the effect that 5C-2 bacteria had on potato plants, two-way ANOVAs were performed using bacterial treatment and soil compaction as independent variables, and root length, mean root diameter, root ethylene production rates and leaf area (Table 3-4). Two-way ANOVAs were also performed for root diameter separated into 0.1 mm root classes to identify morphological changes to root morphology, and 5C-2 count to determine whether bacterial colonisation was affected by compaction.

3.3 Results

Soil compaction does not affect root ethylene evolution

Compacted soil affected root fresh weight and leaf area of each cultivar differently (Table 3-1). Ethylene evolution was not affected by compaction and tended to remain constant across all cultivars.

Table 3-1: Two-way ANOVA of cultivar and compaction for Experiment 3.1. Significant ($p < 0.05$) values are bolded for clarity.

Source of Variation	*Root Fresh Weight*	*Leaf Area*	*Ethylene Evolution*
Cultivar	**<0.001**	**<0.001**	0.07
Compaction	**<0.001**	**<0.001**	0.24
Cultivar x Compaction	**0.03**	**<0.01**	0.13

Compacted soil decreased root fresh weight of all cultivars (Table 3-1; Figure 3-2), but differently (p = 0.04) in each cultivar. Compacted soil decreased root fresh weight of Charlotte, Maris Piper, and Pentland Dell by 55%, 20% and 40% respectively compared to plants grown in loose soil.

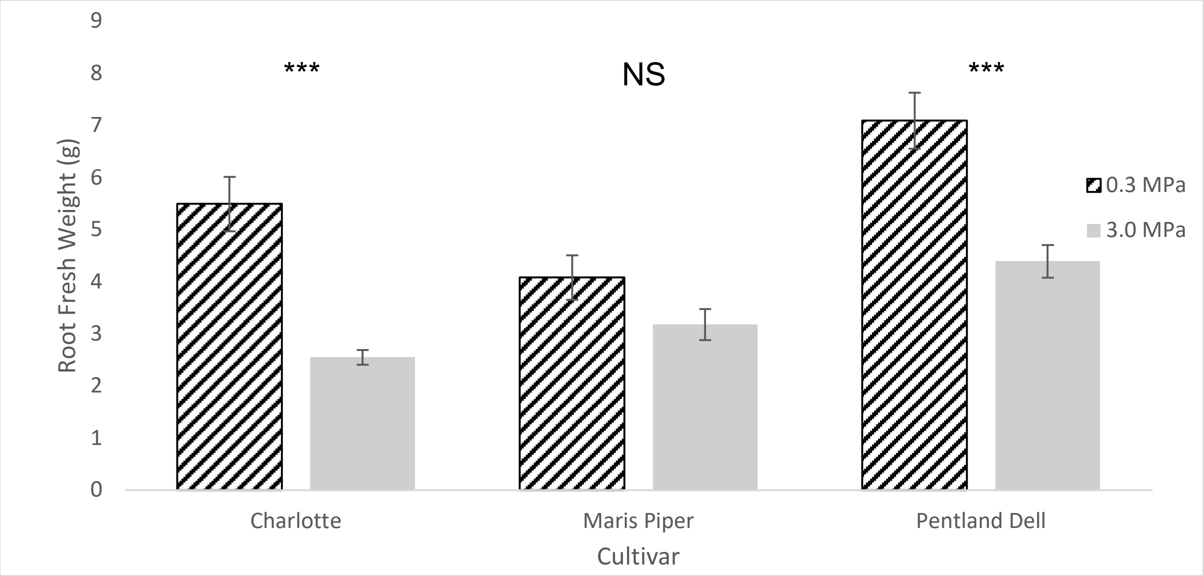


Figure 3-2: Changes in root fresh weight for three potato cultivars in loose (diagonal lines) and compacted (grey) soil. Error bars show standard error with 95% confidence limits. Each bar represents the mean value of nine replicates. Asterisks indicate significance between treatments for each cultivar. NS = no significance. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Compacted soil decreased leaf area of all cultivars (Figure 3-3), with different amounts of loss for each cultivar ($p < 0.01$). Compacted soil decreased leaf area of Charlotte, Maris Piper and Pentland Dell by 65%, 40% and 60% respectively compared to plants grown in loose soil.

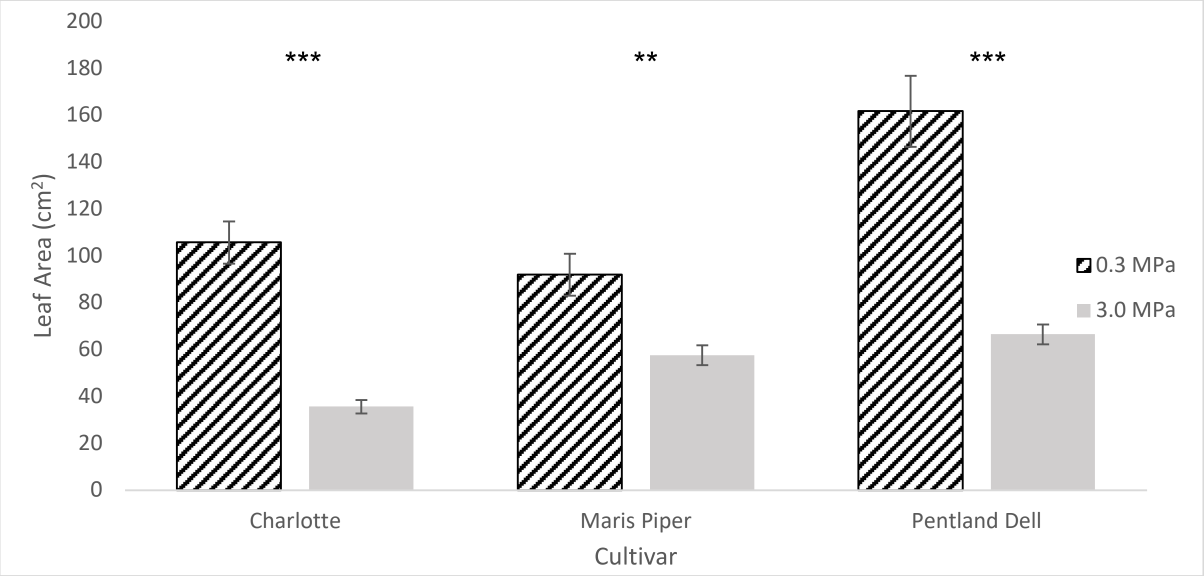


Figure 3-3: Changes in leaf area for three potato cultivars in loose (diagonal lines) and compacted (grey) soil. Error bars show standard error with 95% confidence limits. Each bar represents the mean value of nine replicates. Asterisks indicate significance between treatments for each cultivar. NS = no significance. * = p < 0.05, ** = p < 0.01, *** = p < 0.001.

Ethylene evolution was measured in nanolitres of ethylene produced per gram of fresh root weight per hour (nl/g fwt/hour). Ethylene evolution tended to be similar between cultivars (p = 0.07) and did not differ in response to compaction (Figure 3-4).

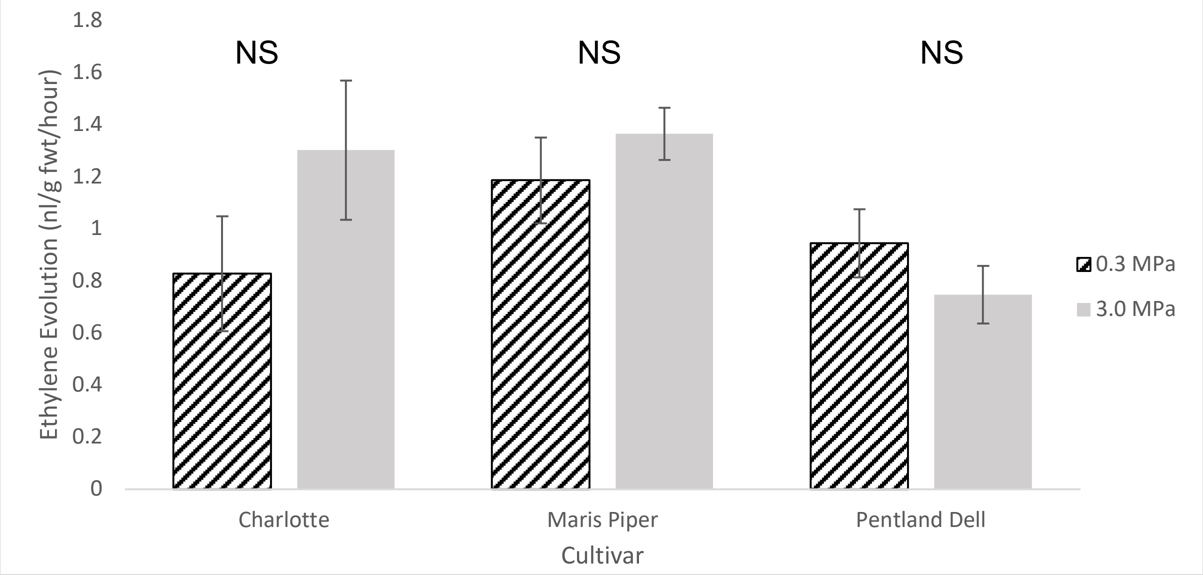


Figure 3-4: Changes in ethylene evolution per unit fresh weight for three potato cultivars in loose (diagonal lines) and compacted (grey) soil. Error bars show standard error with 95% confidence limits. Each bar represents the mean value of nine replicates. Asterisks indicate significance between treatments for each cultivar. NS = no significance. * = p < 0.05, ** = p < 0.01, *** = p < 0.001.

Genotypic variation in sensitivity to ambient ethylene

Root fresh weight, root diameter, and ethylene evolution exhibited a cultivar-dependent response when exposed to different ethylene concentrations (Table 3-2). Root length was consistent between cultivars and ethylene concentrations.

Table 3-2: Two-way ANOVA of cultivar and ambient ethylene concentration for Experiment 3.2. Significant (p < 0.05) values are bolded for clarity.

Source of Variation	*Root Fresh Weight*	*Root Length*	*Root Diameter*	*Ethylene Evolution*
Cultivar	**<0.01**	0.75	**<0.001**	**0.02**
[Ethylene]	0.17	0.68	0.37	0.52
Cultivar x [Ethylene]	**<0.001**	0.86	**<0.001**	**0.04**

Root fresh weight differed between cultivars. Fresh weight was 60% lower in Pentland Dell and 30% lower in Maris Piper than Charlotte (Figure 3-5). Cultivars responded differently to increasing ethylene concentration ($p < 0.01$). Root fresh weight decreased with increased ambient ethylene concentration in Charlotte, with no change in Maris Piper and Pentland Dell (Figure 3-5). Charlotte lost 40% of its root mass when exposed to 1 ppm ethylene, whilst Pentland Dell lost 60%, although this change was not significant due to high variation.

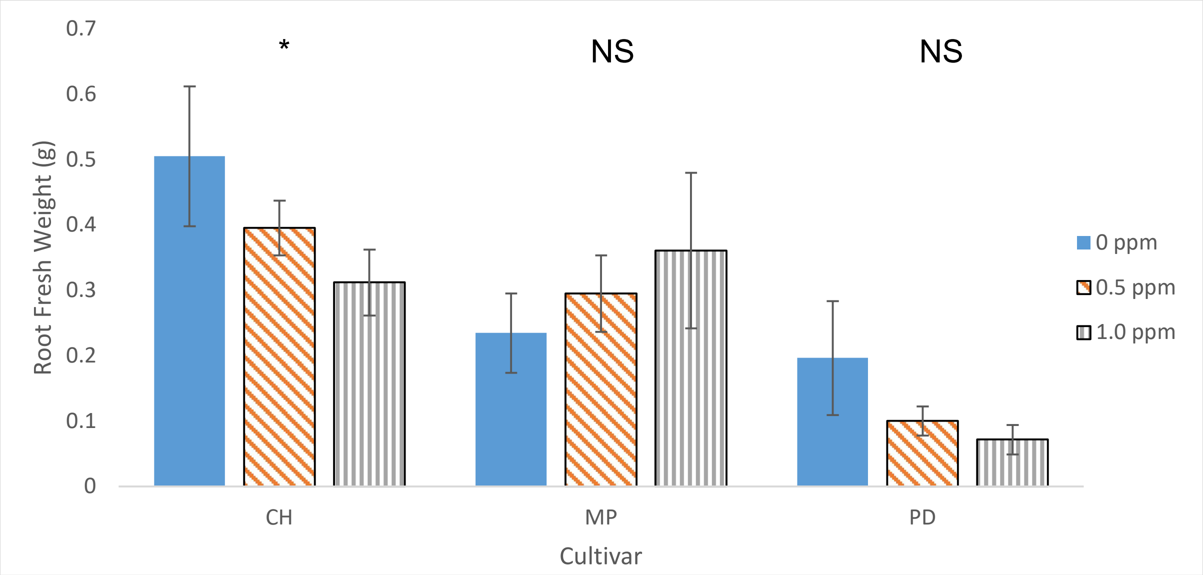


Figure 3-5: Changes in root fresh weight for three potato cultivars grown in airtight containers with ambient ethylene concentration of 0 ppm (solid blue), 0.5 ppm (diagonal orange lines), or 1 ppm (vertical grey lines). Error bars show standard error with 95% confidence limits. Each bar represents the mean of six replicates. Asterisks indicate significance between all treatments per cultivar. NS = no significance. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

All treatments and cultivars exhibited similar increases in root length during their growth in the airtight containers ($p = 0.75$). No significant differences were observed between any treatment for any cultivar (Table 3-2). The relationship between root length per root at the start and end of incubation differed between cultivars ($p < 0.001$). Maris Piper roots typically increased 1.6-fold during incubation, whilst Charlotte roots grew 1.4-fold (Figure 3-6). Charlotte roots increased just 1.05-fold. Ambient ethylene concentration had no effect on the change in root length size ($p = 0.65$). However, changes to root length per root differed between cultivars ($p < 0.001$). Pentland Dell exhibited no changes with increased ethylene concentration. Both Charlotte and Maris Piper roots grew a mean 1.7-fold in 1.0 ppm ambient ethylene compared to 1.35-fold in the absence of ethylene.

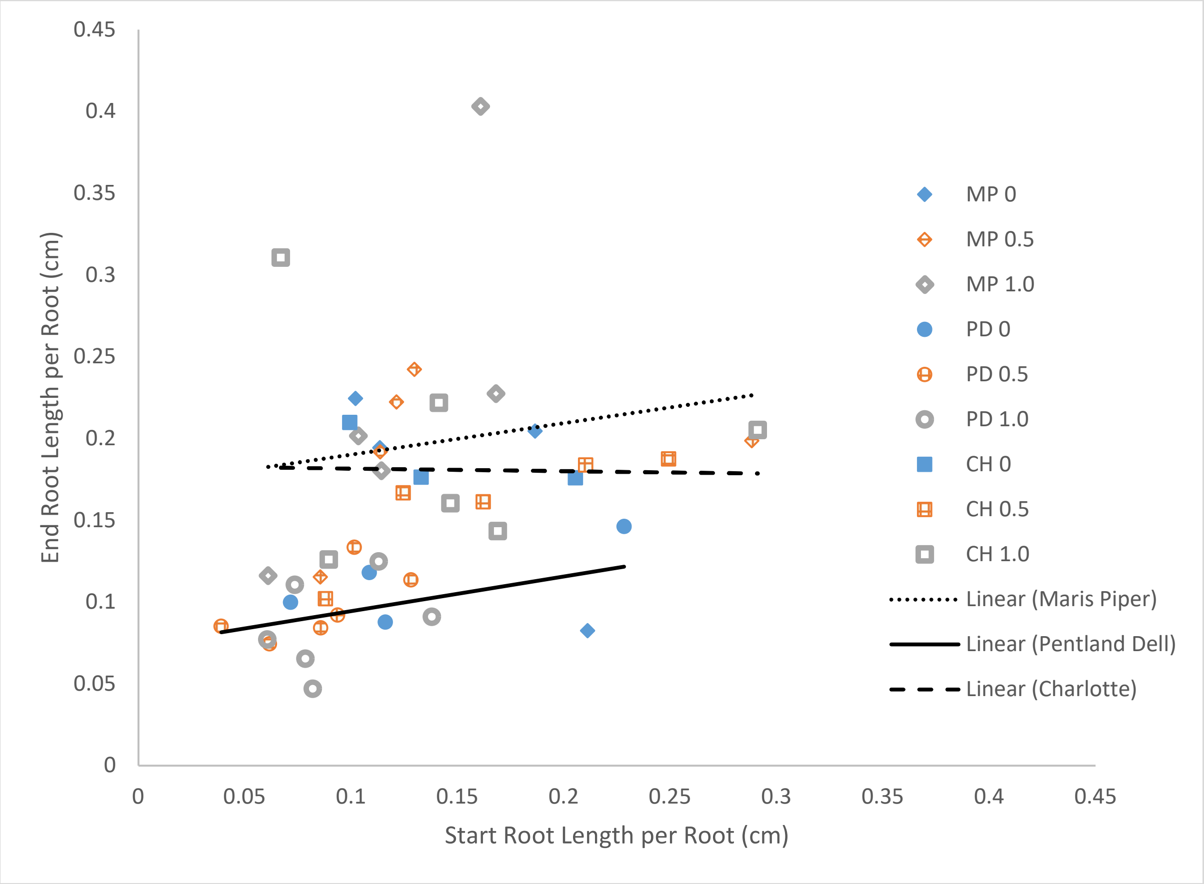


Figure 3-6: The relationship between root length per root at the start and end of incubation for Maris Piper (diamonds), Pentland Dell (circles), and Charlotte (squares) in ambient ethylene concentrations of 0 ppm (solid blue), 0.5 ppm (hatched orange), or 1.0 ppm (hollow grey). Linear regression lines are provided for Maris Piper (dotted line), Pentland Dell (solid line) and Charlotte (dashed line).

Changes to mean diameter differed between cultivars. Charlotte exhibited a mean 20% increase in root diameter during the incubation period, whilst Pentland Dell root diameter dropped 15%, although this was no significamt. Cultivars exhibited different changes in root diameter in response to changes in ambient ethylene ($p < 0.001$).

Charlotte and Maris Piper exhibited increased root diameter, whilst Pentland Dell root diameter tended to decrease (p = 0.08; Figure 3-7).

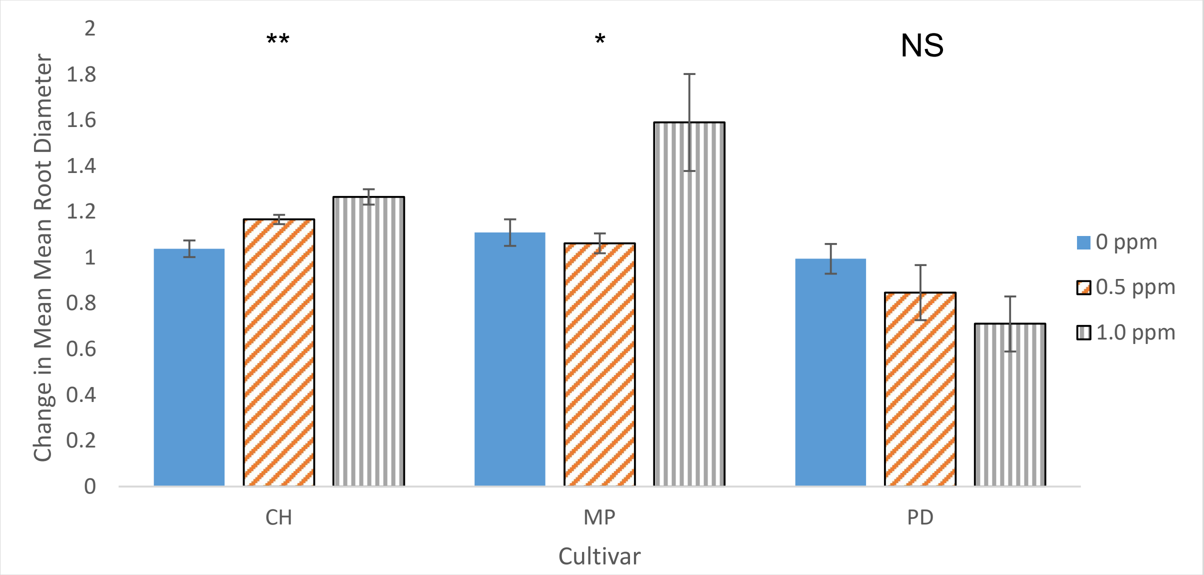


Figure 3-7: Changes in mean root diameter for three potato cultivars grown in airtight containers with ambient ethylene concentration of 0 ppm (solid blue), 0.5 ppm (diagonal orange lines), or 1 ppm (vertical grey lines). Each bar represents the mean of six replicates. Error bars show standard error with 95% confidence limits. Asterisks indicate significance between all treatments per cultivar. NS = no significance. * = p < 0.05, ** = p < 0.01, *** = p < 0.001.

Mean ethylene evolution differed between cultivars, with Charlotte having 30% lower evolution than Pentland Dell, whilst Maris Piper was 45% lower. Cultivars differed in their changes to ethylene evolution in response to ambient ethylene concentration (p = 0.04). Ethylene evolution was increased two-fold in Maris Piper plants grown in 1 ppm ambient ethylene (Figure 3-8), whilst no changes were observed for other cultivars. In general, ethylene evolution was not affected by increased ambient ethylene concentration (Table 3-2), with the only exception being Maris Piper plants in the 1 ppm ambient ethylene treatment.

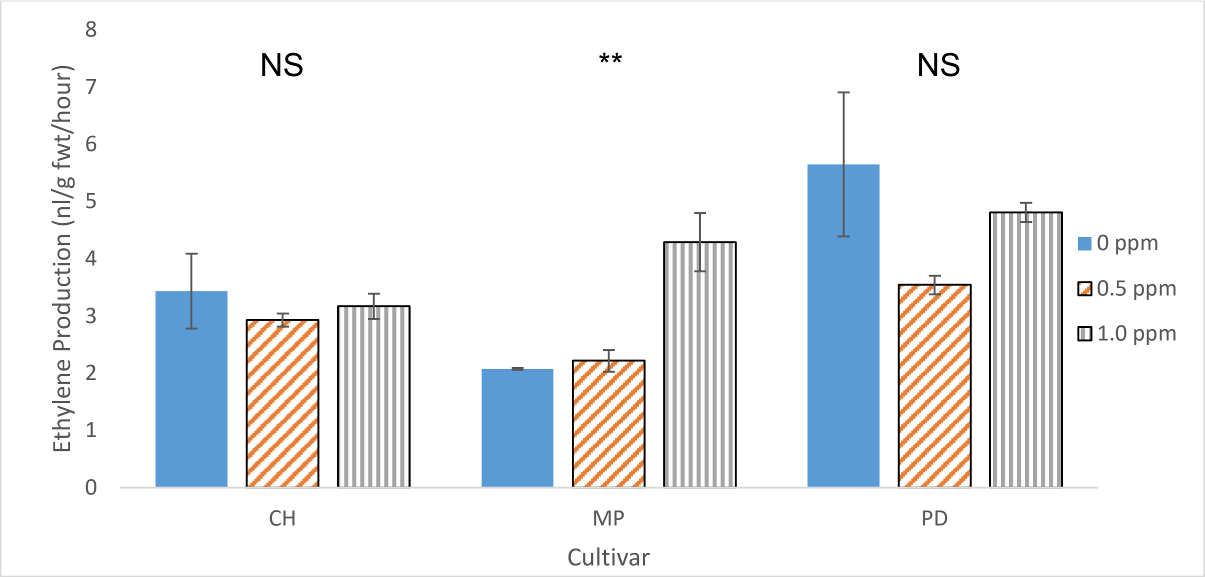


Figure 3-8: Changes in ethylene evolution for three potato cultivars grown in airtight containers with ambient ethylene concentration of 0 ppm (solid blue), 0.5 ppm (diagonal orange lines), or 1 ppm (vertical grey lines). Each bar represents the mean value of six replicates. Error bars show standard error with 95% confidence limits. Asterisks indicate significance between all treatments per cultivar. NS = no significance. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Greatest morphological responses to ambient ethylene are summarized in Table 3-3. Fresh weight and root length were expected to decrease when ambient ethylene increased, and root diameter was expected to increase. Pentland Dell exhibited the largest change in fresh weight, but was least sensitive with regards to root length and diameter, although none of these changes were significant due to high variation.

Table 3-3: Cultivars Inca Bella (IB), Maris Piper (MP), and Pentland Dell (PD) ranked by the ethylene-mediated responses for key morphological traits, with % changes between the 0 ppm and 1.0 ppm treatments included for each variable. Changes were significant ($p < 0.05$) where values are bolded.

	Fresh Weight	*Root Length*	*Root Diameter*
Most Sensitive	-64% (PD)	-3% (MP)	**+40% (MP)**
Median Sensitivity	**-39% (CH)**	+3% (CH)	**+22% (CH)**
Least Sensitive	+53% (MP)	+24% (PD)	-29% (PD)

ACC deaminase-containing bacteria recover root growth in compacted soil

5C-2 had no significant effect on morphological traits, but significantly reduced ethylene evolution (Table 3-4). In compacted soil, however, 5C-2 applications had a significant effect, reducing limiting the effect of compacted soil on the morphology of potato plants.

Table 3-4: Two-way ANOVA of rhizobacteria (5C-2) and compaction for Experiment 3.3. Significant ($p < 0.05$) values are bolded for clarity.

Source of Variation	*Fresh Weight*	*Root Length*	*Root Diameter*	*Leaf Area*	*Ethylene Evolution*
5C-2	0.38	0.66	0.56	0.25	**0.02**
Compaction	0.12	**0.03**	**<0.01**	**<0.01**	0.56
Compaction x 5C-2	0.21	**0.04**	**0.02**	**<0.01**	0.61

Evapotranspiration rates were 20% lower in compacted soil compared to loose soil ($p < 0.01$). Plants with 5C-2 applied exhibited a 40% higher transpiration rate than those without ($p = 0.04$). Evapotranspiration rates were unaffected by root mass for plants grown in compacted soil without 5C-2 ($p = 0.46$). Evapotranspiration rates were a mean 15% higher in plants with 5C-2 added in compacted soil than those without 5C-2 ($p = 0.01$). All treatments had different relationships between root fresh weight and evapotranspiration rates ($p < 0.001$; Figure 3-9).

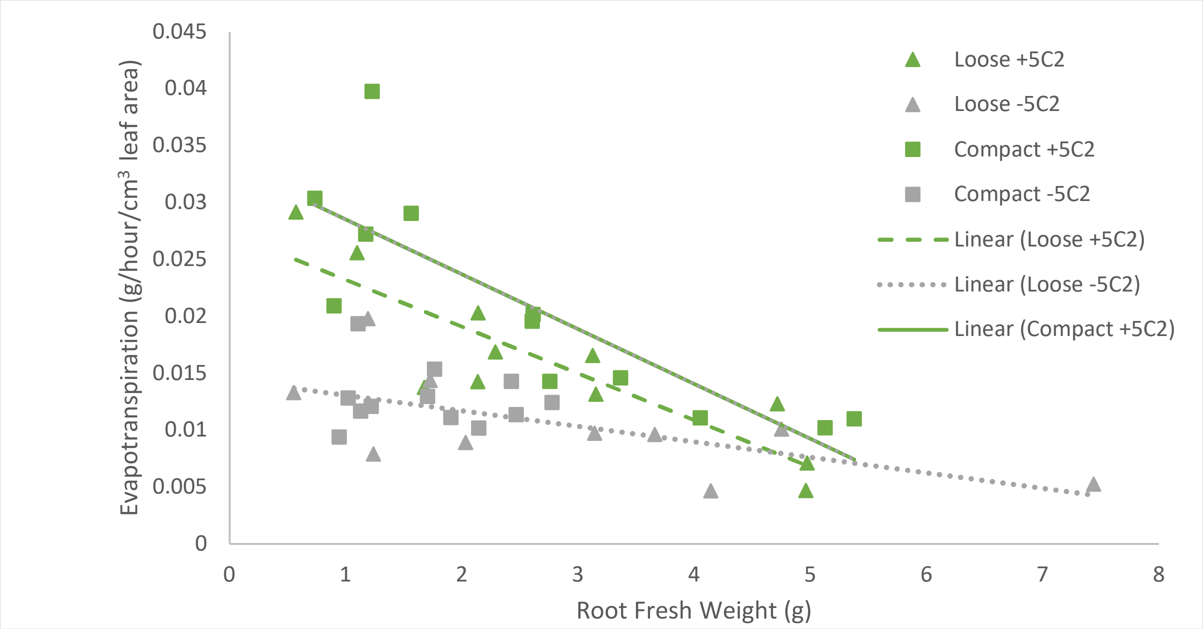


Figure 3-9: Effect of root fresh weight, soil compaction, and 5C-2 application on evapotranspiration rates measured over the last 48 hours before harvest. Each point represents a single measurement. The shape of points represents plants grown in loose soil (triangles) or compacted soil (squares). Colour represents bacteria applications. Plants with 5C-2 applied (green) or without (grey). Linear regression lines are included for treatments with a significant ($p < 0.05$) relationship. These were 5C-2 applied in loose soil (green dashed line, $R^2 = 0.69$), 5C-2 applied in compact soil (green solid line, $R^2 = 0.76$), and those without 5C-2 grown in loose soil (grey dotted line $R^2 = 0.40$).

Mean root length was 65% smaller in compacted soil than in loose soil. 5C-2 did not affect root length in loose soil but increased it by 35% in compacted soil (Figure 3-10).

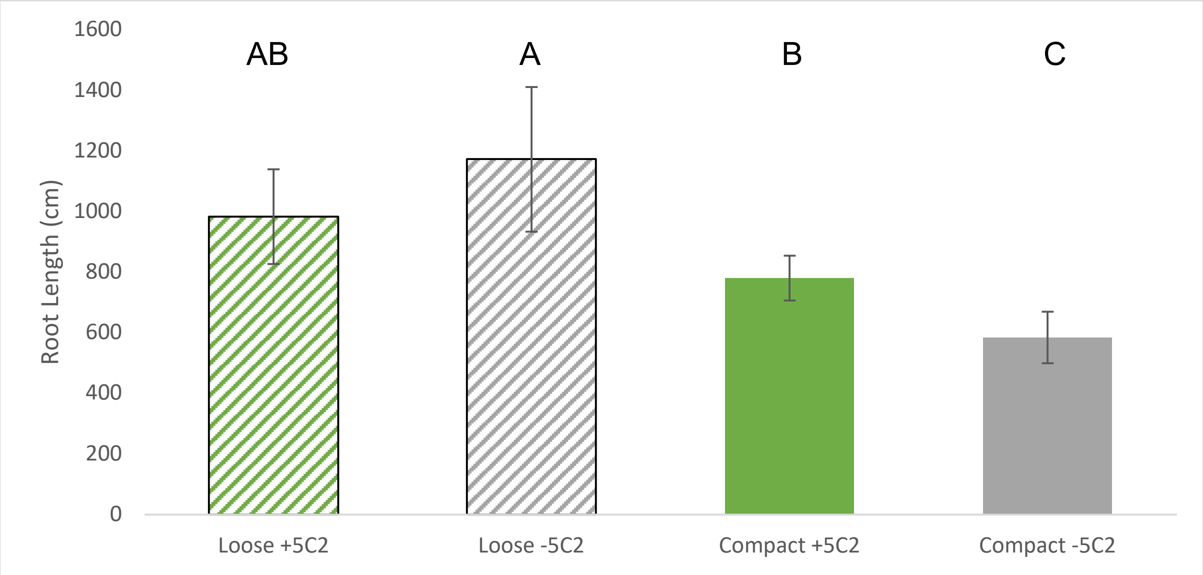


Figure 3-10: Changes in root length between potato plants grown in either compact (solid bars) or loose (diagonal lines) soil in the presence (green) or absence (grey) of 5C-2. Error bars show standard error with 95% confidence limits. Each bar represents the mean of nine replicates. Letters of mean discrimination indicate significant differences between treatments with a p-value of 0.05.

Root fresh weight did not differ between treatments overall (p = 0.21) but was 40% lower in the compacted treatment without 5C-2 application (Figure 3-11).

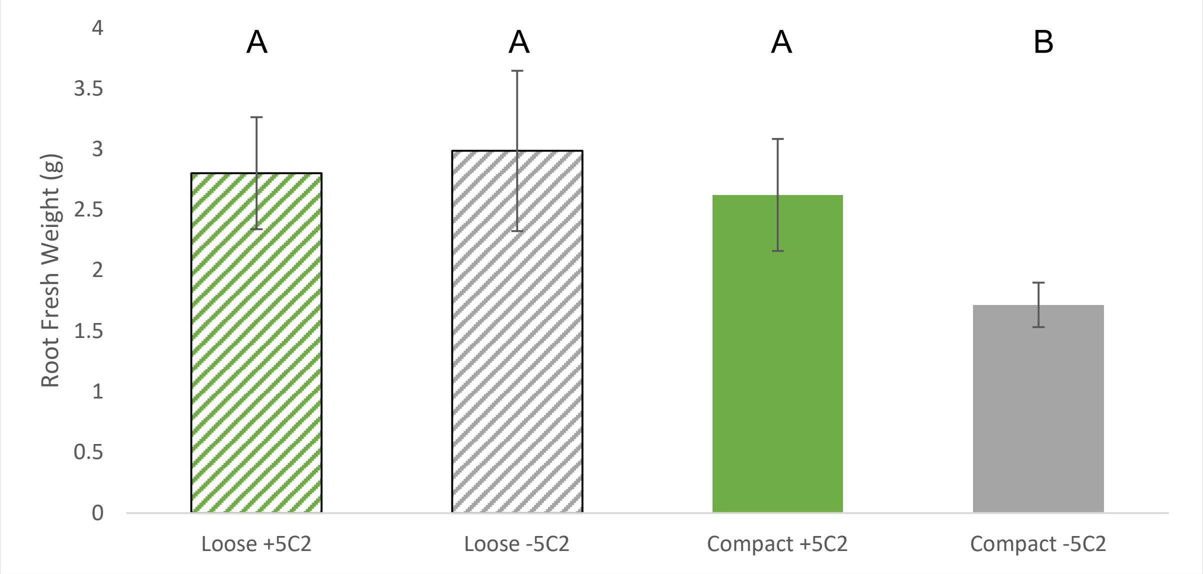


Figure 3-11: Changes in root fresh weight between Maris Piper plants grown in either compact (solid bars) or loose (diagonal lines) soil in the presence (green) or absence (grey) of 5C-2. Error bars show standard error with 95% confidence limits. Each bar represents the mean of nine replicates. Letters of mean discrimination indicate significant differences between treatments with a p-value of 0.05.

Root diameter was 20% higher in compacted soil than loose soil (Figure 3-12). Rhizobacteria did not affect root diameter (p = 0.35). However, presence of 5C-2 increased the proportion of fine (< 0.1 mm) roots in compacted soil (p = 0.03) and decreased the proportion of thicker (> 0.6 mm) roots (p < 0.01) (Figure 3-13)

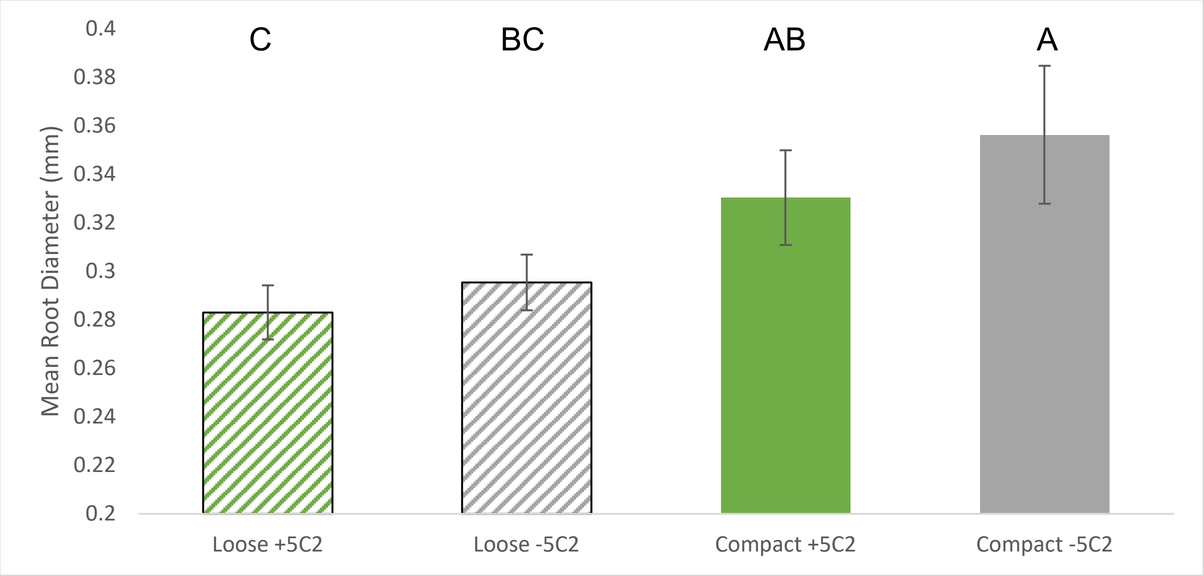


Figure 3-12: Changes in mean root diameter between Maris Piper plants grown in either compact (solid bars) or loose (diagonal lines) soil in the presence (green) or absence (grey) of 5C-2. Error bars show standard error with 95% confidence limits. Each bar represents nine replicates. Letters of mean discrimination indicate significant differences between treatments with a p-value of 0.05.

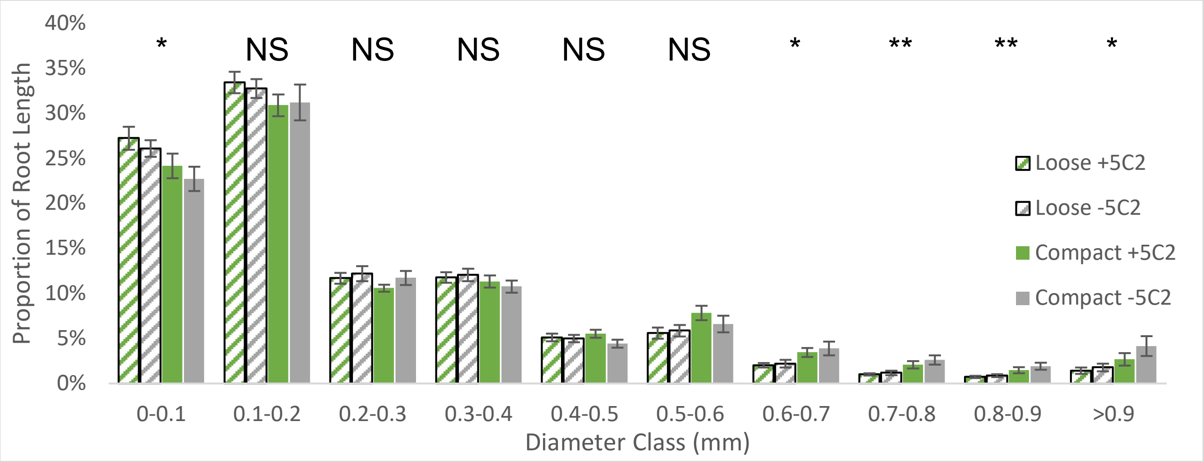


Figure 3-13: Changes in root diameter class proportions between Maris Piper plants grown in either compact (solid bars) or loose (diagonal lines) soil in the presence (green) or absence (grey) of 5C-2. Error bars show standard error with 95% confidence limits. Each bar represents the mean of nine replicates. Asterisks indicate significance between all treatments. NS = no significance. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Plants grown in loose soil had 55% larger leaf area than those grown in compacted soil (Figure 3-14), with the presence of rhizobacteria having no effect (p = 0.25). Leaf area of plants inoculated with 5C-2 was decreased by a greater amount by compaction than plants without 5C-2 (p < 0.01).

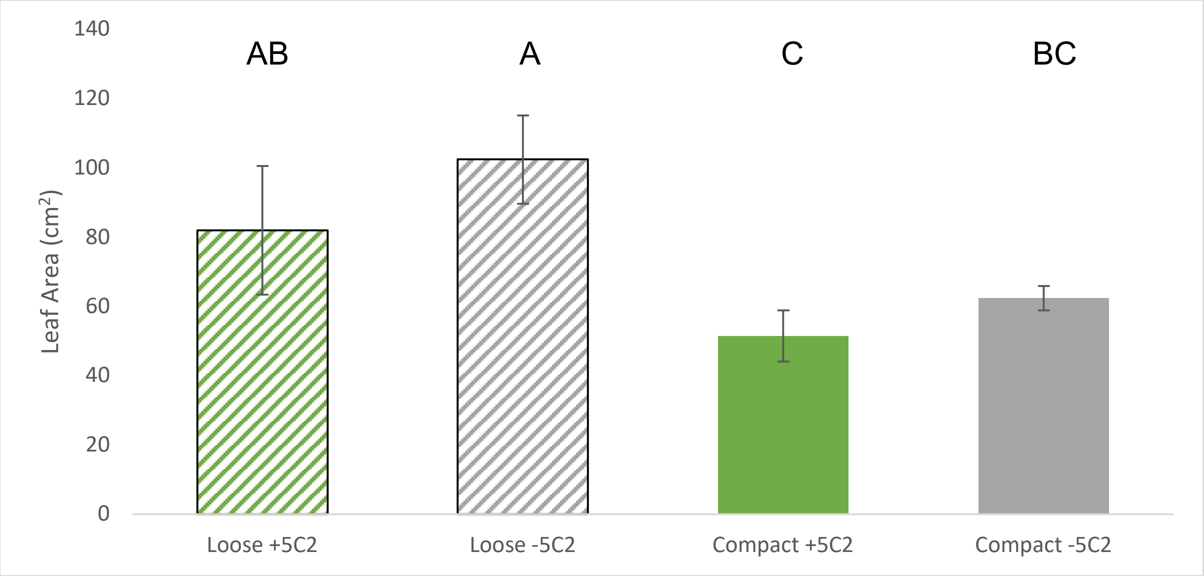


Figure 3-14: Changes in leaf area between Maris Piper plants grown in either compact (solid bars) or loose (diagonal lines) soil in the presence (green) or absence (grey) of 5C-2. Error bars show standard error with 95% confidence limits. Each bar represents the mean of nine replicates. Letters of mean discrimination indicate significant differences between treatments with a p-value of 0.05.

Root ethylene evolution did not differ between compaction treatments (p = 0.56), but was decreased by 45% by 5C-2 in both treatments (Figure 3-15).

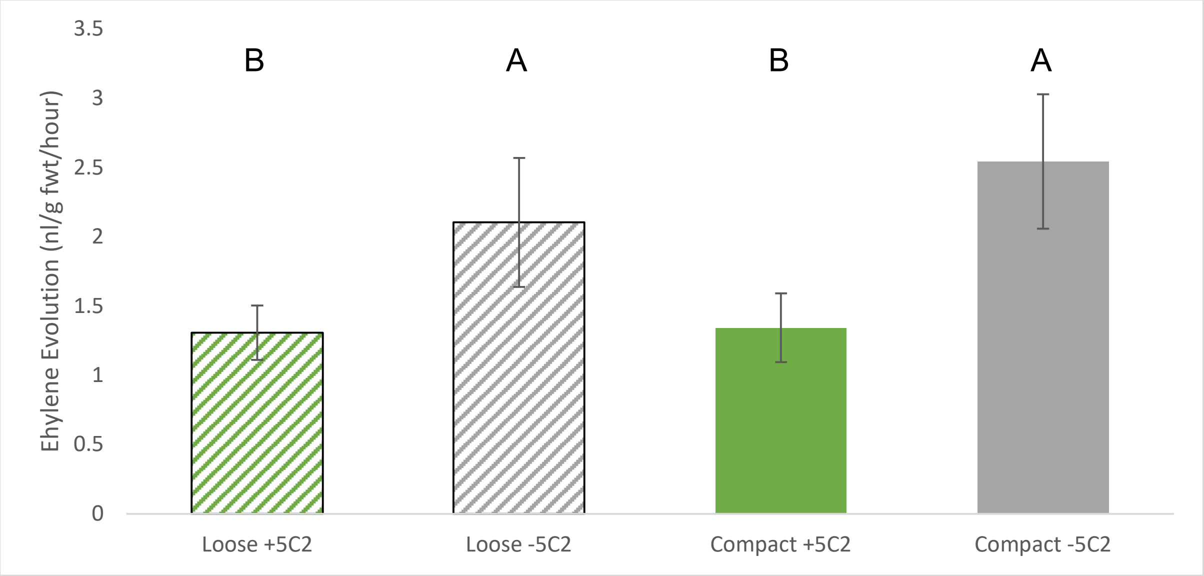


Figure 3-15: Changes in ethylene evolution between Maris Piper plants grown in either compact (solid bars) or loose (diagonal lines) soil in the presence (green) or absence (grey) of 5C-2. Error bars show standard error with 95% confidence limits. Each bar represents the mean of nine replicates. Letters of mean discrimination indicate significant differences between treatments with a p-value of 0.05.

3.4 Discussion

In this chapter, experiments were conducted to determine whether genotypic differences in ethylene production or sensitivity is responsible genotypic variation between cultivars in response to soil compaction. It was determined that root ethylene evolution was unaffected by soil compaction (Figure 3-4), but differences in root sensitivity to ambient ethylene affected root diameter and ethylene evolution (Figure 3-5; Figure 3-8). Applying ACC deaminase-containing bacteria reduced root ethylene evolution (Figure 3-15), which, when applied to potato plants grown in compacted soil, recovered root fresh weight and root diameter to levels comparable to plants grown without ACC deaminase-containing bacteria in loose soil (Figure 3-11; Figure 3-12). This suggests that genotypic variation in response to soil compaction may be due to differences in sensitivity to ethylene.

Soil Compaction does not affect Ethylene Evolution in Potato

Across all experiments, ethylene evolution did not exhibit significant changes in response to compacted soil (Table 3-1; Table 3-4), nor in response to simulated ethylene accumulation (Table 3-2). Unlike Experiment 2.1, leaf area of Pentland Dell was significantly reduced by compacted soil in Experiment 3.1. It is likely this is due to shorter growth time before harvest, as potato plants grown in compacted soil typically exhibit delays in canopy growth but can reach the same maximum growth (Huntenburg *et al*., 2021). Due to the limited growth caused by the small pot size, it is probable that this maximum was reached within four weeks in Experiment 2.1, but growth was still limited after 16 days in Experiment 3.1.

Although compacted soil decreased both leaf area (Figure 3-3) and root mass (Figure 3-2) of all cultivars in Experiment 3.1, root ethylene evolution was not affected by soil compaction (Figure 3-4; Figure 3-15). Root ethylene evolution has been found to increase for broad bean plants when encountering impedance and dropping upon the impedance being removed (Kays *et al*., 1974), and the use of small glass spheres to represent compacted soil caused a 2-fold increase in root ethylene evolution in maize (Moss *et al*., 1988). However, more recent research implies that ethylene increases in compacted soil are due to poor aeration rather than a direct response to impedance. Compacted soil has reduced pore density (Ghosh and Daigh, 2020), which reduces aeration and water holding capacity. In well-watered compacted soil, this may lead to anoxic environments around roots, as pores fill with water and air cannot diffuse effectively, causing carbon dioxide from respiring roots to accumulate, and oxygen levels to drop (Liu *et al*., 2022). Roots produce ethylene in response to oxygen deprivation and protects the roots from damage caused by oxygen deprivation by reducing oxygen demand, for example by limiting root growth and formation of reactive oxygen species that would cause damage to the roots (Liu *et al*., 2022). Soil compaction decreased ethylene evolution in *Eucalyptus todtiana* seedling roots (Benigno *et al*., 2012), whilst it would be expected to increase if impedance from compacted soil determined root growth in compacted soil, rather than the production of ethylene. This implies that increased ethylene evolution is not a direct result of impedance, but other stresses caused by impedance, including anoxia and drought. The presence of anoxia due to overwatering, or drought from low water availability (Jain *et al*., 2006) can explain increased the ethylene production in compacted soils in certain studies. For example, Moss *et al*., simulated compaction using dry glass beads for 24 hours, which is likely to have caused the root to dry. Simulating soil compaction by applying lateral pressure to the roots (Sarquis *et al*., 1991) likely stimulated wound-induced ethylene production (Wang *et al.*, 2002). Overall, it appears that ethylene production does not increase in potato plants in response to mechanical impedance.

Genotypic Variation in Ethylene Sensitivity Causes Differences in Compaction Responses

The genotypes differed in their response to increased ambient ethylene. Charlotte and Maris Piper exhibited similar root length responses to increasing ambient ethylene (Figure 3-6). However, root fresh weight of Maris Piper was unaffected by ambient ethylene, whereas Charlotte exhibited a significant decrease in root fresh weight with increased ambient ethylene. Pentland Dell also exhibited a decrease, although this was not significant (Figure 3-5; Table 3-2), it was only significant for Charlotte (Figure 3-5; Table 3-2). The large variation and limited significant differences in changes to root fresh weight (Figure 3-5) may be due to variation in initial root mass, which was small (<0.1 g), and could not be distinguished from tuber mass. Root length did not change in response to increased ethylene concentrations. However, this may be due to differences in root sensitivity to ethylene between cultivars. Root elongation sensitivity to compaction varies between species, with 0.5 ppm ethylene significantly decreasing root elongation in marsh dock (Visser *et* al., 1997) and maize (Moss *et al.*, 1988; Okamoto *et al.*, 2008), whilst 1 ppm ethylene did not change root length of broad bean (Kays *et al.*, 1974), or Phaseolus vulgaris (common bean) (Borch *et al.*, 1999). Overall, root fresh weight did exhibit cultivar-dependent changes in response to increased ambient ethylene, implying the existence of genotypic variation. However, as root length was unaffected by ambient ethylene, it is uncertain to what extent this affects penetrative ability in compacted soil.

Increased ethylene concentration increased root diameter of two cultivars (Figure 3-7), with Charlotte root diameter linearly increasing with ethylene concentration whilst Maris Piper root diameter increased only in the 1.0 ppm treatment. Despite root diameter typically increasing in response to increased ethylene concentration (Ghosh and Daigh, 2020), unexpectedly root diameter of Pentland Dell decreased (Figure 3-7) perhaps due to the initiation of new thinner roots. Ethylene insensitive plants maintain root growth in compacted soil, with no change in root diameter (Vanhees *et al.*, 2021), which is similar to the response of Pentland Dell. Pentland

Dell having low ethylene sensitivity may cause the reduced root diameter by promoting root growth. At low (<0.1 ppm) concentrations, ethylene increases root growth rates in Vicia faba (broad bean) (Kays *et al.*, 1974), which would cause an increase in young roots with smaller diameters. This is supported by root length data, where mean root length of Pentland Dell did not change (Figure 3-6) whilst other cultivars exhibited a significant increase. Whilst this response was not expected with the ethylene concentrations used, the decrease in sensitivity may cause Pentland Dell to not react to ambient concentrations of 1 ppm of ethylene. As increased ambient ethylene concentration did not change any root traits in Pentland Dell (Table 3-3) suggests it has a much lower level of ethylene sensitivity than other potato cultivars, which may make it an ideal cultivar to maximise yield in fields suffering from compaction.

ACC Deaminase-Containing Bacteria Improve Compaction Tolerance

Soil inoculation with 5C-2 increased potato root length (Figure 3-10; Figure 3-11) and decreased ethylene production (Figure 3-15) in compacted soil. Plants with smaller root systems had a higher evapotranspiration rate, especially those inoculated with 5C-2 (Figure 3-9). Increased evapotranspiration rates in plants of similar size is indicative of increased photosynthetic activity, as it is a sign of increased stomatal opening, which is required to ensure carbon dioxide can enter the leaf and undergo photosynthesis. An increase in evapotranspiration rate therefore suggests that a plant is less stressed, and more tolerant of compacted soil, which typically reduces transpiration rates due to stresses on the plant (Hussain *et al.*, 1999). The inhibition of root ethylene evolution suggests rhizobacterial ACC deaminase activity and is likely responsible for enhancing root growth and compaction tolerance, since compacted soil causes ethylene to over accumulate around roots due to reduced diffusion rates, leading to growth inhibition (Pandey *et al.*, 2021). By reducing ethylene production, the concentration of ethylene accumulating around the root should decrease, as a greater proportion of produced ethylene is able to diffuse away This is supported by changes to root diameter. Whilst the application of ACC deaminase-containing bacteria does not affect mean root diameter (Jiang *et al.*, 2012; Figure 3-12), uninoculated plants had a significantly reduced proportion of fine (< 0.1 mm) roots, and a greater proportion of thicker (> 0.6 mm) roots than those with 5C-2 applied (Figure 3-13). However, these changes were not observed in pea plants inoculated with 5C-2, where significant differences in root diameter were only observed for roots with a diameter between 3.5 mm and 4 mm (Jiang *et al*., 2012). However, these data did not include data from compacted soil, where differences in mean root diameter of plants inoculated with 5C-2 was twice as large as those grown in loose soil, indicating that 5C-2 had a greater effect on root diameter in compacted soil. These changes in root diameter did not affect the mean diameter, but the thickening of roots is a characteristic response to the overaccumulation of ethylene (Potocka and Szymanowska-Pułka, 2018), which is caused by compacted soil and therefore would cause the greatest change in root diameter if ethylene production was reduced. This supports the hypothesis that the inhibition of ethylene production lowers ethylene accumulation, and changes to root morphology. Therefore, applying 5C-2 enhances tolerance to soil compaction, as it does with many other abiotic stresses.

Application of 5C-2 is a possible method of improving potato yield in compacted soil. ACC deaminase-containing bacteria have been found to improve crop yield in many abiotic stress conditions in many species (Chandwani and Amaresan, 2022), including drought-stressed potato (Belimov *et al*., 2015). However, the effects on yield in compacted soil have not been confirmed in any species, although mean yield increases of 10% have been observed in dry soils, where soil resistance is higher (Belimov *et al*., 2015), although the extent to which this can be attributed to increased soil resistance is uncertain. Although 5C-2 application increasing root growth in compacted soil (Figure 3-10) implies improved yield, potato yield in compacted soil is more typically associated with improvements to shoot growth. Shoot biomass and leaf area index are strong predictors of potato yield, as the amount of canopy determines the potential carbon capture of the plant, and hence the biomass of tubers (Luo *et al*., 2020; Huntenburg *et al*., 2021). Whilst 5C-2 did not affect leaf area in this experiment (Figure 3-14), it did improve transpiration rates (Figure 3-9), indicating increased stomatal opening and possibly higher rate of photosynthetic activity (Luo *et al*., 2020). This may allow greater biomass production and thus a higher potential yield under stress conditions. Furthermore, plants inoculated with 5C-2 exhibited a mean 10% yield increase in controlled environments and under drought stress in field trials, but with no benefit in well-watered trials (Belimov *et al*., 2015). Whilst *Variovorax paradoxus* 5C-2 improved potato yield in water stressed environments (Belimov *et al*., 2015), and improved root growth when plants were grown in compacted soil in this experiment (Figure 3-10), it remains uncertain whether these relationships can directly benefit tuber yield in compacted soil.

3.5 Conclusions

Soil compaction itself does not cause an increase in root ethylene production in potato plants. However, root sensitivity to ethylene determines tolerance to soil compaction, with cultivars such as Pentland Dell which did not exhibit any significant responses to increased ambient ethylene concentrations and improved ability to maintain root length in compacted soil. However, root fresh weight decreased more in Pentland Dell plants than Maris Piper plants. This may have potentially been caused by Maris Piper increasing root diameter, retaining a greater proportion of total root biomass. As ethylene sensitivity was cultivar-dependent, it is likely that compaction tolerance can be bred into future cultivars. In addition, applying bacteria containing ACC deaminase recovered root growth in compacted soil by reducing root ethylene production. Inoculation of field-grown plants with these bacteria is a reasonable proposition (Belimov *et al*., 2015), assuming the benefits to yield make it economically viable. However, there is no certainty that either of these methods (plant breeding, rhizobacterial inoculation) will enhance yield in the field.

Chapter 4 The Effect of Soil Compaction on Yield of Potato Cultivars

4.1 Introduction

Soil compaction is a major issue affecting potato crops. Potato fields have to be kept well-irrigated throughout the growing season to avoid infections from diseases such as common scab (AHDB, 2018). Irrigation tends to form a layer of wet soil above a layer of dryer soil (Reyes-Cabrera *et al*., 2016), and the interface of these layers is especially sensitive to soil compaction (Batey, 2009). It has been estimated that up to two-thirds of UK potato fields suffer from compaction sufficient to inhibit potato root growth (Stalham *et al*., 2007), making compaction a major concern in potato cropland.

Soil resistance tends to increase between planting and harvest in potato fields (Boone *et al*., 1978), (Huntenburg *et al*., 2021). The precise cause of this is uncertain, but likely causes include changes in soil water content, growth of below-ground biomass, and soil slumping. Dry soil tends to have a higher soil resistance than wet soil (Gao *et al*., 2012), and plants tend to increase water uptake throughout the growing season, drying the soil out (Whalley *et al*., 2006), leading to increased soil resistance. This is a particular issue in areas with compacted soil. Due to the increased resistance, root growth is promoted at the surface, increasing water uptake, which leads to further increases to soil resistance (Colombi *et al*., 2017a). This is most likely to be visible in rainfed crops, where soil water content is not maintained to a specific level. Increases in soil resistance reduce soil water holding capacity (Torbert and Wood, 1992), which reduces the amount of available water in the soil, potentially causing further drying and greater increases to the soil resistance. Compaction may occur due to increased plant biomass in the soil. Tilled soils are often unstructured, resulting in a low pore density (White *et al*., 2007). In these conditions, roots are forced to create pores, which compacts the soil around them (Lucas *et al*., 2019). Whilst this compaction is detectable with roots as small as 0.25 mm (Lucas *et al*., 2019), tree roots with a diameter of over 3.5 cm can cause soil compaction over 4 cm from the root surface (Clemente *et al*., 2005). Whilst potato plants do not produce roots of this size, tubers can easily exceed it, and therefore may be able to notably increase soil resistance. Slumping is the process by which unstructured or loose soil is compacted by a single rainfall or irrigation event. The soil particles are forced closer together, reducing soil height and increasing bulk density, and therefore soil resistance (Hao *et al*., 2011). Unlike the other possibilities, the effect of soil slumping is determined by soil texture instead of biotic factors such as root growth.

Soil compaction creates many challenges to optimal plant growth. Compaction reduces the size and density of soil pores, in turn reducing the soil's water holding capacity, which also limits nutrient availability (Bengough *et al*., 2006), as well as reducing aeration within the soil, particularly at depth (Hakansson *et al*., 1988). Furthermore, the reduction in pore size and density directly limits root growth. Roots favour growth through pores and other gaps in the soil, rather than expanding through the soil structure (Rich and Watt, 2013). Soil compaction significantly inhibits root elongation (Stalham *et al*., 2007; Ghosh and Daigh, 2020), as well as tuber growth. Yield losses of up to 50% have been reported in trials comparing yield in compacted and uncompacted soils (Stalham *et al*., 2005), with some evidence that increased compaction also increased the proportion of malformed tubers, further reducing marketable yield (Timm and Flocker, 1966; McDole, 1975). It is therefore imperative to understand how to maintain yield in agricultural environments affected by soil compaction.

It is possible that genotypic variation in responses to compaction could be key to improving yield in affected fields by utilising cultivars that are better able to maintain growth in compacted soil. Root traits such as root length, weight and number correlate between pot experiments and field trials when pot size does not restrict growth (Wishart *et* al., 2013). Cultivar-dependent differences in root growth correlated well between pot and field experiments in response to drought stress (Puértolas *et al*., 2014), but similar experiments have not been performed regarding soil compaction. Potato yield correlates with shoot traits such as mid-season leaf area index (Luo *et al*., 2020) and mid-season biomass (Huntenburg *et al*., 2021). By comparing growth of potato cultivars with different amounts of tolerance to compaction in controlled environment experiments (identified in chapter 2) to those grown in the field, it can be determined whether compaction tolerance improves yield, or simply improves vegetative growth.

This chapter had three aims. The first aim was to determine why soil resistance increases in potato fields, and whether its effects can be mitigated through the selection of appropriate, compaction tolerant cultivars. It was hypothesised that the two most likely causes of increased soil resistance were, firstly, decreased soil water content in compacted soil, due to increased water uptake at the soil surface due to restricted vertical root growth (Colombi *et al.*, 2017a). Compacted soil has been previously observed to have increased soil water content at depth, even under irrigation (Huntenburg *et al.*, 2021). In addition, increased root density is also likely to cause increased soil resistance, both by directly compacting the soil (Lucas *et al.*, 2019) and increasing the rate of water uptake.

The second aim was to determine whether responses to soil compaction such as changes to canopy growth and root density were consistent between the field and controlled environment experiments, and whether they could be used to predict yield. It was hypothesised that the cultivar that produced the largest maximum leaf area in compacted soil would maintain more of its marketable yield in compacted soil compared to uncompacted soil, as shoot growth has been found to be a strong predictor of yield in compacted soil (Huntenburg *et* al., 2021).

The final aim was to identify genetic diversity between potato cultivars in their ability to maintain shoot and root growth in compacted soil. Genotypic variation in tuber yield has been previously reported (Stalham *et al.*, 2007), but genotypic differences in vegetative growth are less clear. Based on data from Chapter 2, Inca Bella would be able to retain more of its leaf area whilst Maris Piper would maintain a greater proportion of its root system size when exposed to high levels of soil compaction.

4.2 Methodology

Tubers Used

Two potato cultivars were chosen for these field trials: the tetraploid *tuberosum* cultivar Maris Piper, and the diploid *phureja* cultivar Inca Bella. All tubers were sourced by Branston Ltd. (Branston). The cultivars had exhibited differences in response to compaction, particularly in leaf area (Figure 2-2), root length (Figure 2-3), and root diameter (Figure 2-5) when analysed in controlled environment experiments, making them likely candidates to exhibit significantly different responses in the field.

Trial 1: Growth and yield responses of Inca Bella and Maris Piper to minor soil compaction applied at planting.

Trial Site Description

This field trial was performed on a field with silty soil (5.42% sand, 88.73% silt and 5.84% clay) with a pH of 7.65 and organic matter content of 11.5%. The previous crop was winter wheat. The field is found at a latitude of 53°20‘33” N and longitude of 0°40‘36” W near Branston Booths, Lincolnshire. The trial was performed between 6th May 2021 when the tubers were planted, and 22nd September 2021, when the trial was harvested. The trial area was cultivated to 25 cm, with bed forming and stone separation all being performed within 3 days from stubble. The trial area was left unirrigated for the duration of the season, being solely rainfed. Incident radiation, air temperature and rainfall were measured using an Automatic Weather Station (Sentek, Stepney, Australia). During the trial period, the trial area was sprayed with herbicide and blight spray. Desiccation of the crop was performed 3 weeks prior to harvest.

The trial area was divided using a randomised block pattern with three replicates per treatment. Each plot comprised a single ridge, with two rows of tubers planted 40 cm apart per ridge. Each ridge was 0.9 m in width. Each plot was ten tubers (3.6 m) long for a total of 20 tubers per plot. Plots were separated with a guard plot with a minimum size of 1.5 m of the cultivar Taurus.

Before planting, soil resistance was measured using a Van Walt (Hazelmere) Hand Penetrometer. Resistance measurements were taken at 5 cm intervals until soil resistance became too high for the penetrometer to penetrate (5 MPa) resistance once per trial plot. Tubers were then planted by hand at a depth of 5 to 10 cm. The compacted treatment was produced by applying a 0.61 kN/m^2 force by walking across the entire plot area twice, and soil resistance measured again. A final measure of soil resistance was made 102 days post-emergence (September 8th). Finally, Delta-T PR2/4 Profile Probes (Cambridge) were inserted into each plot along the centre line 60 cm from the edge of the plot by auguring using a Delta-T PR2 pilot auger (Cambridge) to create a 25.4 mm diameter hole, into which the PR2/4 profile probes were placed. This enabled soil moisture to be measured at depths of 10 cm, 20 cm, 30 cm and 40 cm without affected compaction of the surrounding soil. Soil moisture content was not measured at this time.

Growth Analysis

Once the plants had emerged (~ May 27th), visits to the field were made fortnightly. Moisture measurements were taken from the Profile Probes on each visit. Canopy coverage was recorded by taking a picture of a randomly selected 0.9 m x 0.9 m section of the plot. The percentage of green coverage was determined using Image J by the National Institutes of Health (Bethesda, USA). One plant from each plot was harvested each week by excising the plant stems at soil level. Leaf area was measured using a LI-COR (Cambridge) LI-3100C Area Meter. Shoot dry mass was measured by placing the shoots in a drying oven until a constant mass was reached. Root cores were taken adjacent to the excised plant stems. Cores had a mean diameter of 1.25 cm and were taken at 15 cm increments to a depth of 45 cm, where the soil was too resistant to core. Each core had a soil volume of 73.6 cm^3. Root length, volume and diameter were measuring using Regent Instrument's (Quebec, Canada) software WinRHIZO. Root density was then calculated using the formula:

$$Root\ density\ (cm^3/m^3) = \frac{Root\ Volume\ (cm^3)}{Soil\ Core\ Volume\ (cm^3)}.$$

Harvest

Tubers were harvested 116 days post-emergence (September 22nd). Prior to harvesting, soil resistance was measured following the same method as used when planting. All tubers were manually harvested by plot and weighed to calculate gross yield. Unmarketable tubers were removed, and marketable tubers underwent weight and size analysis, with size being based on the longest straight line that could be made on the tuber. Marketable yield was calculated by removing all tubers that would be unsuitable for sale, due to either being malformed, green, or by suffering infection by bacteria, viruses, or pests.

Trial 2: Growth and yield responses of Inca Bella and Maris Piper to heavy soil compaction applied at planting.

Trial Site Description

Trial 2 took place on a silt-loam soil (<0.01% sand, 81.81% silt and 18.19% clay), with a pH of 8.11. The previous crop was winter barley. The field is located at a latitude of 53°18’19” N, and a longitude of 0°27’67” W near Bucknall, Lincolnshire. The trial was performed between 27th April 2022 when the tubers were planted, and September 20th, 2022, when the tubers were harvested. The trial area was ploughed to a depth of 30 cm, with bed-tillage and stone separation after the previous crop was stubbled. The plots were irrigated with drip tape placed 5 cm below the level of the topsoil designed to apply 3.5 mm/day irrigation. Rainfall and temperature data was logged by an iMETOS 3.3 weather station (Pessl Instruments, Weiz, Austria). During the trial period, the trial area was sprayed with herbicide and blight spray. Desiccation of the crop was performed 3 weeks prior to harvest.

The trial area was a split plot with 4 treatments and 3 replicates per treatment for a total of 12 plots. Each plot comprised a single ridge, with two rows of tubers planted 40 cm apart per ridge. Each ridge was 0.9 m in width. Each plot was nine tubers (3.2 m) long for a total of 18 tubers per plot. Plots were separated with a guard plot with a minimum size of 1.5 m of the cultivar Taurus. As it was not possible to alternate compact and loose treatments using the compaction method, each treatment’s replicates were on the same ridge.

Before planting, soil resistance was measured as per trial 1. Delta-T PR2/6 Profile Probes (Cambridge) were then inserted into each plot along the centre line 60 cm from the edge of the plot, by auguring using a Delta-T PR2 pilot auger (Cambridge) to create a 25.4 mm diameter hole, into which the PR2/4 profile probes were placed. This enabled soil moisture to be measured at depths of 10 cm, 20 cm, 30 cm, 40 cm, 60 cm and 100 cm without affected compaction of the surrounding soil. Soil moisture content was not measured at this time. Tubers were then planted by hand. The compacted treatment was produced by applying a 4.25 kN/m^2 force to the topsoil by driving an Audi A4 Allroad vehicle across the whole plot area three times.

Growth Analysis

Once the plants had emerged (~ June 1st), growth analysis was undertaken as per trial 1.

Harvest

Tubers were harvested 111 days post-emergence (September 20th). All tubers were manually harvested by plot and weighed to calculate gross yield. Unmarketable tubers were removed, and marketable tubers underwent weight and size analysis, with size being based on the longest straight line that could be made on the tuber. Marketable yield was calculated as per trial 1.

Statistical Analysis

Each field trial utilised 2-way ANOVA with compaction and cultivar as independent variables, and leaf area, root density, initial and final soil resistance, soil water content, and yield as dependent variables. These tests were also performed for soil water content and root density data from the last visit before harvest for each trial, as well as leaf area data from each measurement date and yield. With the same variables, 3-way ANOVA was utilised but also including trial as an independent variable to detect any variation between trials. Least square differences were calculated for soil resistance at each measured depth for both trials for each set of measurement. The relationship between soil resistance and moisture for the two trials was determined using linear regression with soil moisture as a dependent variable, and soil resistance and trial as independent variables. For each trial, variation in soil water content was determined using linear regression with soil resistance, variety, and soil depth as covariates. Pearson correlation coefficients were calculated for each treatment from both trials with regards to initial and end soil resistance, soil moisture, leaf area, root density and yield. Linear regressions were used to determine the significance of these correlations. Differences in yield between treatments were determined using Tukey’s HSD tests.

4.3 Results

Changes in Soil Resistance Over Time

Initial soil resistance in trial 1 was three-fold lower than trial 2 (Figure 4-1). After compaction was applied, soil resistance in the compacted plots increased to double that of uncompacted plots ($p < 0.001$). There was no variation in resistance between cultivars at this stage (p = 0.67), (Figure 4-1). Soil resistance became too high to measure below 45 cm depth in most plots, irrespective of compaction treatment.

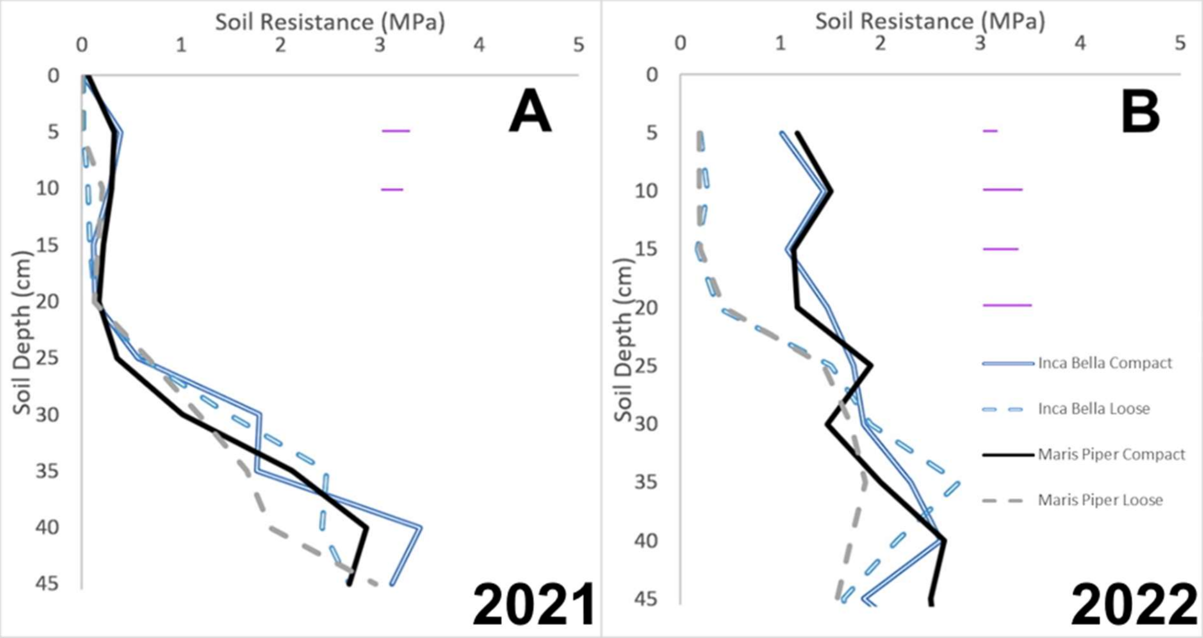


Figure 4-1: Soil resistance immediately after compaction was applied in trial 1 (A) and trial 2 (B). Inca Bella plots are coloured blue and have hollow lines, and Maris Piper coloured black. Compacted plots have solid lines, whilst uncompacted plots have broken lines. Each point represents the mean of three resistance measurements. LSD (5%) is given for each depth (purple horizontal lines) where treatment x resistance interaction had a p-value of less than 0.05.

Compared to uncompacted plots, soil resistance of the compacted plots at the end of trial 1 increased 2.5-fold in Inca Bella and 3-fold in Maris Piper (Figure 4-2). Across the entire soil profile in both trials, there were very clear differences in soil resistance within the upper 20 cm of the soil (p < 0.001). Cultivar-dependent differences in soil resistance first appeared 35 days post-emergence in trial 2 and differed between all treatments 49 days post-emergence (Table 4-1). Despite the 7-fold increase in initial compacting pressure, soil resistance in the upper 15 cm was similar (p = 0.43) at the end of trial 1 and 2 (Figure 4-2).

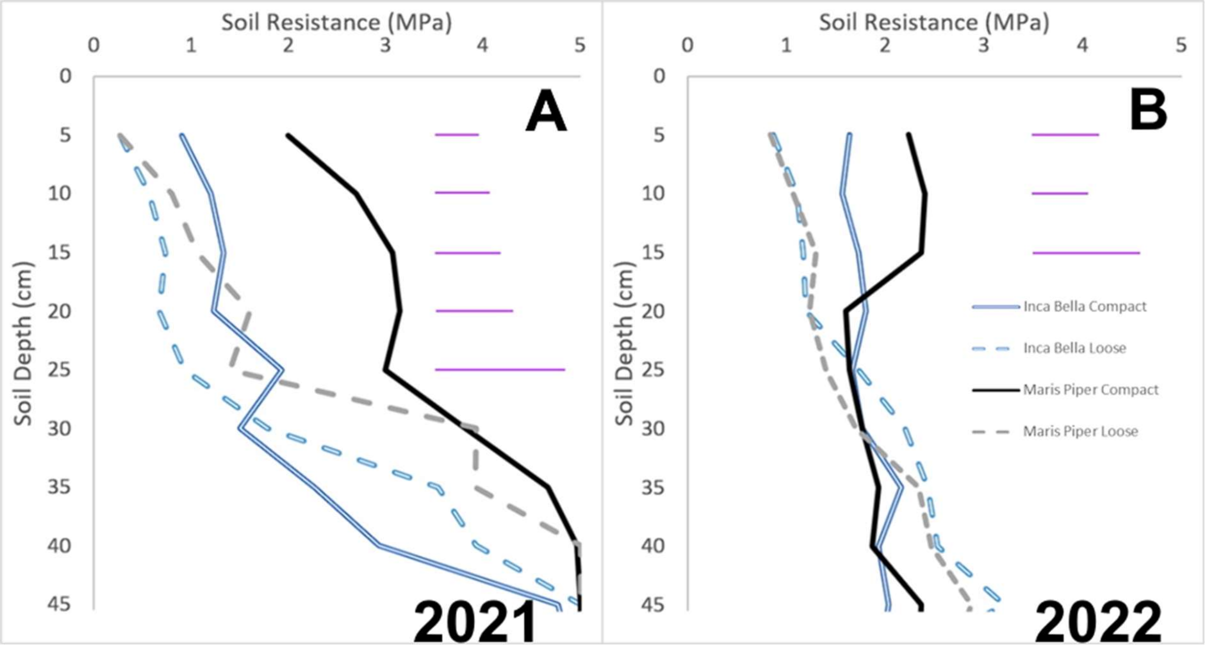


Figure 4-2: Comparison of soil resistances at the end of trial 1 (A) and trial 2 (B) for both Inca Bella (blue, hollow lines) and Maris Piper (black/grey). Compacted and uncompacted plots are indicated with unbroken and dashed lines respectively. Each point represents the mean of three resistance measurements. LSD (5%) is given for each depth (purple horizontal lines) where treatment x resistance interaction had a p-value of less than 0.05.

Table 4-1: Three-way ANOVA of cultivar, compaction, and measurement depth on ANOVA p-values of soil resistance measurements from the upper 20 cm of the soil from each data collection date in trials 1 and 2. Significant ($p < 0.05$) p-values are written in bold text for clarity.

Days After Emergence		*Compaction (Co)*		*Cultivar (Cv)*		*Depth (D)*		*Co x Cv*		*Co x D*		*Cv x D*		*Co x Cv x D*	
Trial 1	*Trial 2*	*Trial 1*	*Trial 2*	*Trial 1*	*Trial 2*	*Trial 1*	*Trial 2*	*Trial 1*	*Trial 2*	*Trial 1*	*Trial 2*	*Trial 1*	*Trial 2*	*Trial 1*	*Trial 2*
-21	*-23*	**<0.001**	**<0.001**	0.166	0.98	0.25	0.05	0.58	0.98	0.11	0.29	0.27	0.80	0.66	0.53
	7		**<0.001**		0.65		0.13		0.65		0.17		0.06		0.18
	21		**<0.001**		0.97		0.07		**<0.001**		0.32		0.89		0.83
	35		**<0.001**		**<0.001**		**0.02**		0.25		0.87		0.20		0.23
	49		**<0.001**		**<0.001**		**<0.01**		**<0.001**		0.63		0.89		0.45
	63		**<0.001**		**0.04**		0.06		0.07		0.63		0.74		0.59
	72		**<0.001**		0.51		**0.001**		0.08		0.97		0.94		0.69
102	*91*	**<0.001**	**<0.001**	**<0.001**	**0.04**	**0.03**	0.36	0.13	**0.02**	0.95	0.23	0.45	**0.02**	0.97	0.31

Soil Water Content

Soil water content did not differ between cultivars in either trial 1 or trial 2 ($p = 0.82$; $p = 0.75$). Soil water content was 55% lower in trial 1 than trial 2 across all treatments. Soil water content of uncompacted plots averaged 50% less ($p < 0.001$) than compacted plots in trial 1, and 30% in trial 2 (Table 4-2).

Table 4-2: Three-way ANOVA of cultivar, compaction, and measurement depth on ANOVA p-values of soil water content from each data collection date in trials 1 and 2. Significant ($p < 0.05$) p-values are written in bold text for clarity.

Days After Emergence		*Compaction (Co)*		*Cultivar (Cv)*		*Depth (D)*		*Co x Cv*		*Co x D*		*Cv x D*		*Co x Cv x D*	
Trial 1	*Trial 2*	*Trial 1*	*Trial 2*	*Trial 1*	*Trial 2*	*Trial 1*	*Trial 2*	*Trial 1*	*Trial 2*	*Trial 1*	*Trial 2*	*Trial 1*	*Trial 2*	*Trial 1*	*Trial 2*
7	*7*	0.83	**0.03**	0.34	0.79	**<0.001**	**<0.001**	0.63	0.40	0.65	0.21	0.43	0.99	0.66	0.71
22	*21*	0.89	0.07	0.29	0.68	**<0.001**	**<0.001**	0.86	0.22	0.14	0.38	0.21	0.80	0.74	0.85
36	*35*	0.65	**0.005**	0.16	0.56	**<0.001**	**<0.001**	0.37	**0.04**	0.25	0.34	**<0.01**	0.54	0.56	0.52
50	*49*	0.48	**<0.001**	**0.05**	0.94	**<0.001**	**<0.001**	0.54	0.26	0.08	0.34	**<0.01**	0.68	0.48	0.27
	63		**0.005**		0.52		**<0.001**		0.20		0.35		0.90		0.32
74	*72*	**<0.001**	**0.003**	0.74	0.71	**<0.001**	**<0.001**	0.98	0.06	**<0.001**	0.38	0.69	0.96	0.47	0.60
102	*91*	**<0.001**	**0.033**	0.76	0.93	**<0.001**	**<0.001**	0.95	0.35	**<0.001**	0.64	0.59	0.99	0.40	0.72

Leaf Area

Leaf area tended to be higher in Maris Piper than Inca Bella (p = 0.06) across both trials (Figure 4-3). Averaged across all treatments and measurement occasions, leaf area was 50% greater in trial 1 than trial 2. The leaf area of Inca Bella was substantially less than Maris Piper when measured up to 49 days after emergence in trial 1, and on all sampling dates except 49 days after emergence in trial 1 (Figure 4-3). Near the time of maximum canopy development in trial 1, Maris Piper plants grown in uncompacted plots had 15% larger leaf area than those in compacted soil, while soil compaction had no effect on Inca Bella leaf area (Figure 4-3). At a similar phenological stage in trial 2, Maris Piper plants in compacted plots had 15% larger leaf area than those in uncompacted plots, but Inca Bella plants grown in compacted soil had 25% less leaf area than those in uncompacted soil. Overall, the leaf area of Maris Piper tended to be more responsive to soil compaction than Inca Bella.

Trial	***	***	***	***	-	***	-
Cv	***	***	***	NS	**	***	**
Co	NS	NS	***	***	NS	*	NS
T x Cv	***	***	NS	NS	-	NS	-
T x Co	NS	NS	**	NS	-	NS	-
Cv x Co	NS	NS	***	***	*	NS	NS
T x Cv x Co	NS	NS	***	***	-	**	-

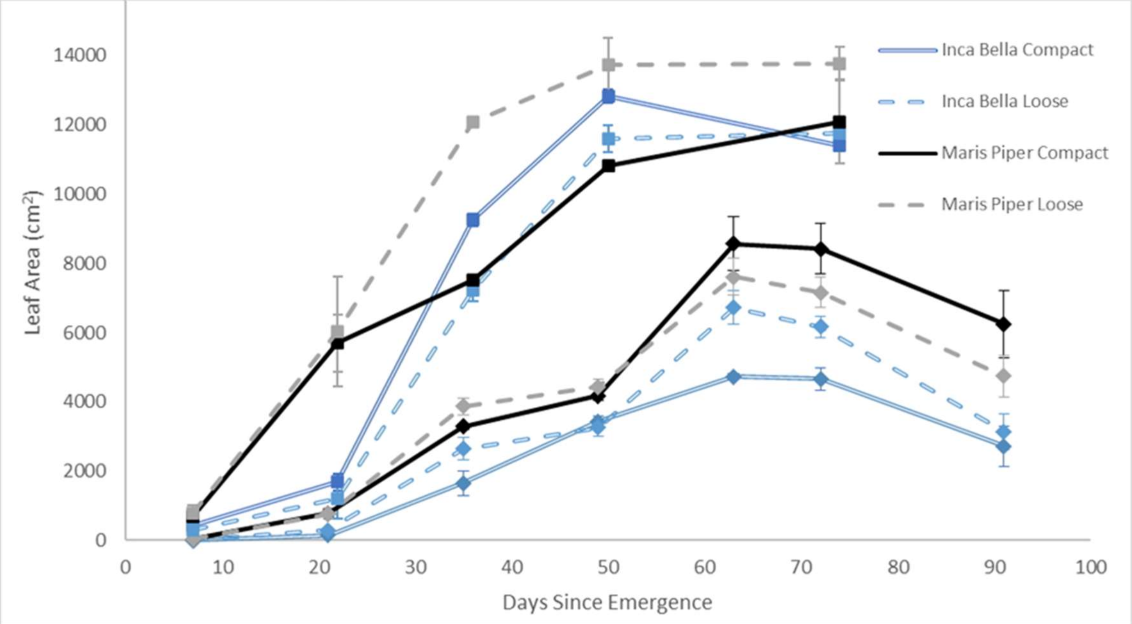
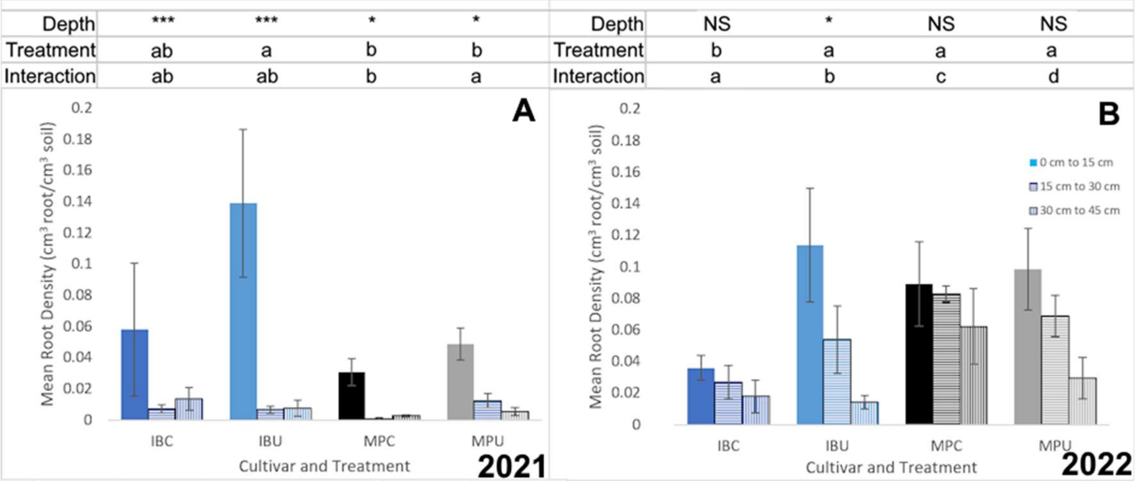


Figure 4-3: Changes in mean leaf area of plants over time for each treatment in trial 1 (squares) and trial 2 (diamonds). Inca Bella is represented in blue with hollow lines, with Maris Piper in black/grey. Compacted treatments have unbroken lines whilst uncompacted treatments have dashed lines. Each point represents the mean of 3 plants. Error bars show standard error with 95% confidence limits. Low error values are not visible on some points. Asterisks denote significances from three-way ANOVA on leaf area. - = not used, NS = no significance, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Root Density and Diameter

Root density did not differ ($p = 0.77$) between cultivars. At harvest, Inca Bella root density did not significantly differ between trials, but Maris Piper root density in trial 2 was 7-fold higher in the compacted treatment and 3-fold higher in the uncompacted treatment than in trial 1 (Figure 4-4). Inca Bella root density was similar in the upper 45 cm of soil between both trials. Compared to the topmost (0 to 15 cm) core, deeper (15 to 30 cm and 30 to 45 cm) cores had 60% less root density across both trials (Figure 4-4), with a 90% reduction in trial 1 and a 45% reduction in trial 2. Root density with depth was unaffected by soil compaction (Figure 4-4). Maris Piper maintained a similar root density in both compacted soil and uncompacted soil in both trials, whilst Inca Bella root density decreased in compacted soil (Figure 4-4). Overall, root density of Inca Bella was more sensitive to compaction than Maris Piper.



Depth	***	***	*	*
Treatment	ab	a	b	b
Interaction	ab	ab	b	a

Depth	NS	*	NS	NS
Treatment	b	a	a	a
Interaction	a	b	c	d

Figure 4-4: Mean root density from soil cores between on the last field visit before harvest: 102 days after emergence in trial 1 (A) and 89 days after emergence in trial 2 (B). 0 cm to 15 cm core is represented by solid bars, 15 cm to 30 cm by horizontally lined bars, and 30 cm to 45 cm by vertically lined bars. Each bar represents the mean of six replicates. Error bars show standard error with 95% confidence limits. Significance of mean root density in the upper 45 cm between treatments is represented using letters of mean discrimination with a p-value of less than 0.05. Asterisks denote significance of coring depth on root density. NS = no significance, * = $p < 0.05$, ** = $p < 0.01$, *** = p <

Total root density was typically similar between cultivars and compaction treatments, with significant differences in root density observed only at the start and end of trials (Figure 4-5). Root density typically reached its maximum between 40 and 60 days after emergence in both cultivars and tended to decrease ($p = 0.08$) later in the season.

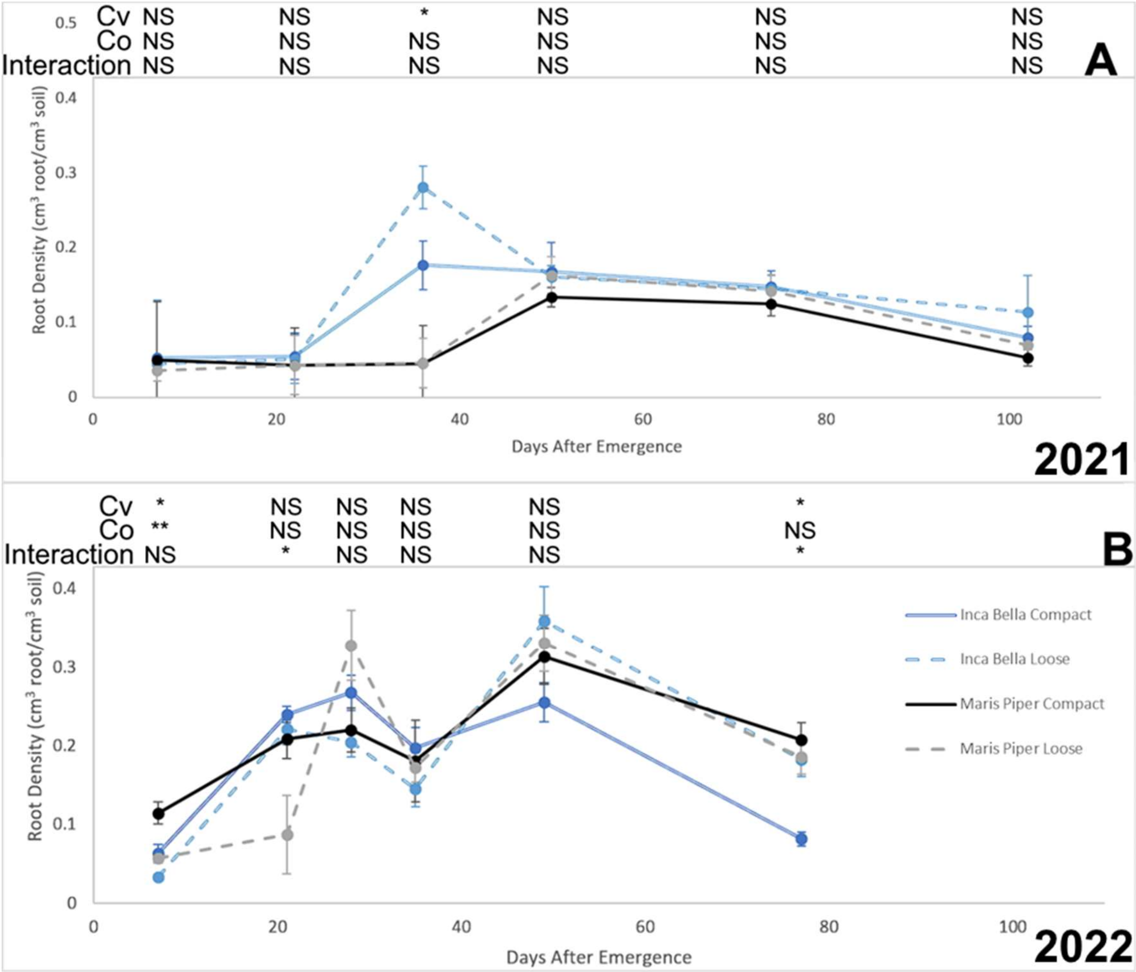


Figure 4-5: Root density from in the upper 45 cm of the soil in trial 1 (A) and trial 2 (B). Inca Bella (blue, hollow lines) and Maris Piper (black/grey) plants grown in either compacted (solid line) or uncompacted (dashed line) soil. Error bars show standard error with 95% confidence limits. 2-way ANOVA p-values are represented using asterisks. NS = no significance, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Mean root diameter did not differ between trials ($p = 0.77$) and was overall unaffected by compaction ($p = 0.21$). However, this is because the mean root diameter of each cultivar changed differently in response to soil compaction ($p < 0.001$), with compacted soil decreasing root diameter of Inca Bella by 20% in trial 1 but having no effect in trial 2 (Figure 4-6). Compacted soil significantly ($p < 0.01$) increased mean root diameter of Maris Piper by 60% and 35% in trials 1 and 2 respectively. Overall, root diameter responded differently to compaction between cultivars, but Maris Piper was more responsive.

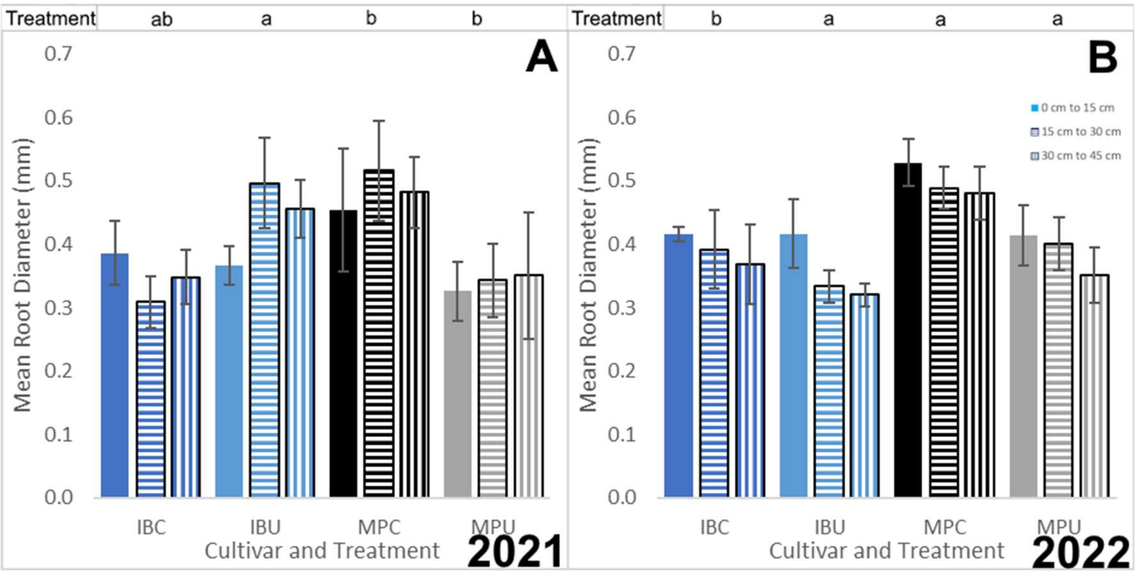


Figure 4-6: Mean root diameter from soil cores between on the last field visit before harvest: 102 days after emergence in trial 1 (A) and 89 days after emergence in trial 2 (B). 0 cm to 15 cm core is represented by solid bars, 15 cm to 30 cm by horizontal hatched bars, and 30 cm to 45 cm by vertical hatched bars. Each bar represents the mean of six replicates. Error bars show standard error with 95% confidence limits. Letters of mean discrimination indicate significant differences between treatments obtained using Tukey Tests with a p-value of 0.05.

Tuber Yield

Yield of Maris Piper plots was significantly ($p < 0.001$) higher than Inca Bella, by 2-fold and 1.2-fold in trials 1 and 2 respectively. Soil compaction decreased overall tuber yield ($p = 0.01$), primarily due to Inca Bella yields declining by 18% ($p < 0.01$), whilst Maris Piper yields were unaffected (Figure 4-7). In trial 2, Inca Bella had a 50% larger yield than trial 1 (figure 4-7).

All treatments for both trials had a mean 71% marketable yield, except Inca Bella in trial 1, where the marketable yield was 49%. Marketable yield was 25% lower in compacted soil in trial 2, and 15% less in Inca Bella than Maris Piper. Maris Piper yield did not differ between trials. Across both trials, Inca Bella yield was decreased by 20% in compacted soil, whilst Maris Piper remained unaffected. Inca Bella suffered from a significantly larger proportion of malformed tubers than Maris Piper (Table 4-3). Thus, Inca Bella yield was more affected by compaction than Maris Piper.

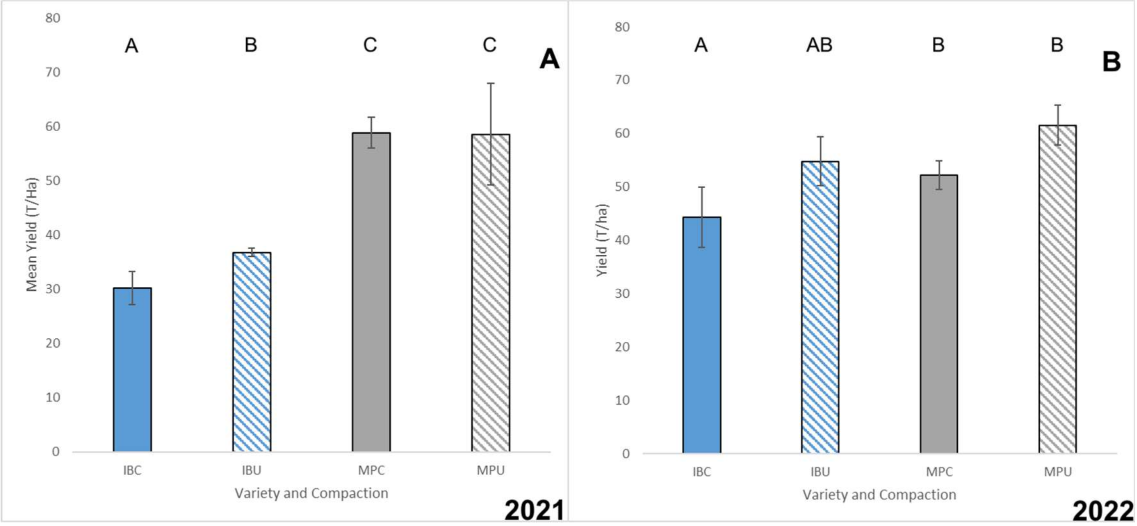


Figure 4-7: Mean gross yield from each treatment in trials 1 (A) and 2 (B). Each bar represents the mean of three plots. Error bars show standard error with 95% confidence limits. Letters of mean discrimination indicate differences between treatments within a graph with a p-value of less than 0.05.

Maris Piper tubers were shorter and heavier than Inca Bella tubers across both trials ($p < 0.001$ for both). Tubers in trial 1 were smaller and shorter than those in trial 2 ($p < 0.001$) for both cultivars (Figure 4-8; Figure 4-9). Overall, tubers tended to be smaller and lighter in trial 1 compared to trial 2.

Table 4-3: Two-way ANOVA table comparing proportions of unmarketable yield based on why the tubers were considered unmarketable. Significant ($p < 0.05$) values are bolded for clarity.

Factor	*Cultivar*		*Compaction*		*Cu x Co*	
	Trial 1	*Trial 2*	*Trial 1*	*Trial 2*	*Trial 1*	*Trial 2*
Malformed	**0.02**	0.06	0.15	0.12	0.15	0.42
Green	0.52	**0.001**	0.45	**0.001**	0.33	0.13
Infected	0.38	0.73	0.48	**0.006**	0.35	0.34
Pest Damage	**0.04**	0.30	0.07	0.58	0.41	0.12
Total	0.88	**0.001**	0.65	**0.02**	0.89	0.16

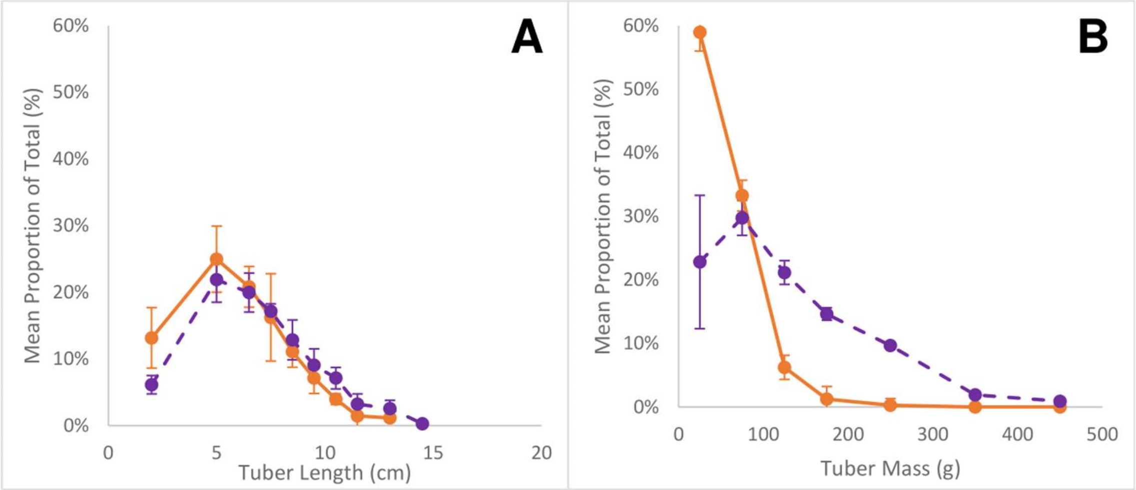


Figure 4-8: Differences in marketable tuber length (A) and mass (B) for Inca Bella in trial 1 (orange, solid line) and trial 2 (purple, dashed line). Error bars show standard error with 95% confidence limits.

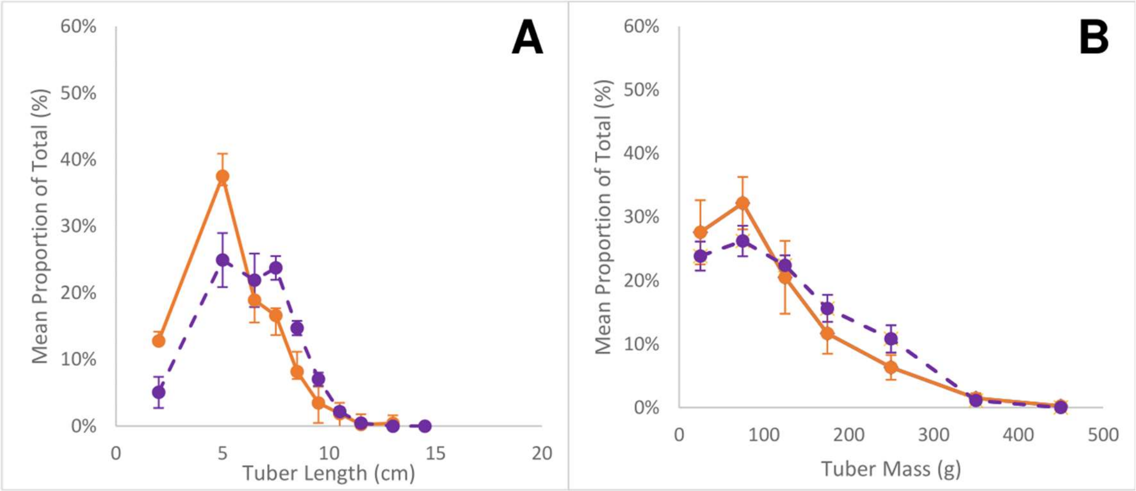


Figure 4-9: Differences in marketable tuber length (A) and mass (B) for Maris Piper trial 1 (orange, solid line) and trial 2 (purple, dashed line). Error bars show standard error with 95% confidence limits.

Of the measured variables in these field trials, most had very weak correlations. Increased end soil water content correlated with reduced maximum leaf area and increased initial soil resistance (Table 4-4). Whilst no variable had a significant correlation with tuber yield, leaf area was the best predictor.

Table 4-4: Pearson correlation coefficients for variables across all plots from both trials. Significant correlations in bold text with asterisks indicating level of significance. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

	Initial Resistance-	End Resistance-	Soil Water Content-	Leaf Area-	Root Density-	Yield-
Initial Resistance	1.00					
End Resistance	0.33	1.00				
Soil Water Content	**0.80****	0.50	1.00			
Leaf Area	-0.58	-0.02	**-0.82***	1.00		
Root Density	0.34	-0.30	0.43	-0.54	1.00	
Yield	-0.31	-0.08	-0.40	0.56	0.21	1.00

4.4 Discussion

In this chapter, field trials were conducted with the aim of determining whether selecting potato cultivars with better tolerance to compacted soil could be used to improve yields in fields afflicted with surface soil compaction, and why. Despite having different initial compaction treatments and initial soil resistances, the soil resistance compacted treatments in both trials increased to comparable, cultivar-dependent levels by the time of harvest. (Figure 4-1; Figure 4-2). The resistance increases did not correlate with changes in soil water content (Table 4-2) or root density (Figure 4-5). It is hypothesized that tuber growth was related to the changes in soil resistance, as Inca Bella plants yielded significantly less than Maris Piper plants in compacted plots (Figure 4-7) and had significantly lower soil resistance at harvest (Figure 4-2). Overall, Maris Piper plants exhibited higher tolerance to compacted soil and maintained more yield than Inca Bella plants when grown in compacted plots (Figure 4-7), which suggests that selecting compaction-tolerant cultivars can be a method of improving yield in fields susceptible to surface soil compaction.

Soil Resistance Increases Over Time

In both trials, soil resistance increased dramatically in compacted soil across the growing season. The final resistance was determined by both planted cultivar and the initial compaction, with loose soil treatments having lower end soil resistance, and plots that contained Maris Piper plants having higher soil resistance than Inca Bella plots, but only under compaction (Figure 4-2). The final soil resistance was comparable between treatments in trial 1 and 2, despite trial 2 having significantly higher initial compaction (Figure 4-1). This implies that the soil resistance at the end of the growing season was determined by the planted cultivar in compacted soil, with no effect in loose soil.

One potential cause of increased compaction is soil slumping. In low density soil, the soil particles are loosely bound together, and easily compacted by heavy rainfall (Cerda *et al*., 2021). Destoning potato fields is a common pre-cultivation practice that can decrease bulk density by up to 0.3 g/cm^3 (Stalham *et al*., 2012), leaving them vulnerable to slumping. Periods of heavy rainfall (>10 mm/hour) occurred nine times over the growing season in trial 1, with the heaviest resulting in 30 mm of rainfall in two hours. Increased root density improves the stability of soil (Gyssels *et al*., 2005), reducing the risk of slumping. However, no differences in root density were observed between cultivars in compacted soil in trial 1 (Figure 4-4) and Inca Bella had reduced root density in trial 2. This makes slumping unlikely to be responsible for observed changes to soil resistance, as the change in soil resistance would have been similar between cultivars in trial 1 due to comparable root densities, with increase resistance in Inca Bella plots in trial 2.

Soil drying correlates strongly with increased soil resistance (Batey, 2009). Compacted soils have reduced water holding capacity (Smith *et* al., 2001), and lose more water due to evaporation than uncompacted soils with identical soil moisture (An *et al*., 2018), making it likely that soil water content would be lower in compacted soil when bare. However, soil moisture was consistently higher in compacted plots compared to uncompacted plots in trial 2 and was similar or higher in trial 1. Since the compacted treatment tended to decrease root density (Figure 4-4) and leaf area (Figure 4-3), this likely decreased water uptake rates (Whalley *et al*., 2020). Since both treatments received the same irrigation treatment, this would cause soil moisture to increase in the compacted treatment. As a result, increased soil water content correlates with increased soil resistance in these trials with no cultivar variation. This means the cultivar-dependent increases in soil resistance cannot be explained by changes in soil water content.

Another possible factor is compaction by the plants themselves. Normally, roots do not compact soil, but when macropore density is low, the growth of roots through the soil can cause compaction as the roots force soil to move (Lucas *et al*., 2019). However, Maris Piper plots had a root density comparable to Inca Bella in trial 1, and a similar density in trial 1 (Figure 4-4). Furthermore, since the maximum root densities observed account for less than 0.3% of soil volume, this is unlikely to result in the large increases in soil resistance observed. Finally, root density reached its maximum in the middle of the growing season and dropped towards the end (Figure 4-5). As soil resistance continued increasing during this period, it is therefore unlikely that root density is responsible for increasing soil resistance.

Growing potato tubers may promote soil compaction. As the size of the tuber increases, soil is forced away from it, increasing the surrounding soil’s density. As cultivar-dependent differences in soil resistance between compaction treatments began to arise around 49 days after emergence (Table 4-1), approximately the time tuber bulking initiates (Pavlista, 1995), differences in tuber yield (figure 4-7) may explain these differences. Since Maris Piper had double the yield of Inca Bella plots in trial 1, this would result in roughly double the volume of tubers in the soil. Soil resistance in compacted Maris Piper plots was double that of Inca Bella plots in trial 1 (Figure 4-2), where Maris Piper tuber yield was double that of Inca Bella (Figure 4-7), proving a correlation between tuber yield and soil resistance. Furthermore, as soil below the tuber growth region (> 20 cm) had comparable soil resistances for both cultivars (Figure 4-2), the cause of the soil resistance increase originated within this upper 20 cm, which is where new tubers are formed. Overall, these factors may explain the increased resistance in both compacted and uncompacted soils observed in trial 1, and why the increase was greater in the compacted treatment, but not the lack of differences in trial 1.

Yield Responds to Soil Compaction

Although tuber length or mass did not differ between the compaction treatments in either trial, both cultivars produced heavier (Figure 4-8), larger (Figure 4-9) tubers in trial 2 than trial 1. Although this response was correlated with increases in soil resistance, it is likely to be drought related. Compacted soil does not alter tuber size distribution (Huntenburg *et al*., 2021), whereas droughted potato plants tend to produce smaller tubers (Aliche *et al*., 2018; Huntenburg *et al*., 2021). Overall, it seems likely that changes in tuber size and length distribution are primarily due to drought stress from the use of unirrigated fields in trial 1, rather than the presence of compacted soils.

Whilst compacted soil decreased yield (Figure 4-7), the response was more limited than expected. Inca Bella plants grown in compacted soil had an 18% smaller yield in trial 1 but there were no significant differences in yield between the compaction treatments for Maris Piper (Figure 4-7). Nevertheless, soil compaction can reduce Maris Piper yield, with losses of between 15% and 30% being typical (Ghosh and Daigh, 2020; Huntenburg *et al.,* 2021). However, there have been many trials where yield has not been affected by subsoiling potato fields, even those that are otherwise compacted. Less than half of subsoiling trials significantly improved yield, and those that do tend to be limited to under 5 t/ha improvements (Stalham *et al*., 2005). As previously stated, tuber bulking is likely causing soil compaction in the latter part of the growing season. Each cultivar in these trials reached a different maximum resistance, irrespective of initial compaction amount. The lack of change in uncompacted plots implies that a compact layer is required for this to occur, but as heavy machinery is being increasingly relied upon to apply fertilizer, weed and pest control, and irrigation to potato fields (Johansen *et al*., 2015), which are typically kept constantly moist, slight levels of compaction often inadvertently occur, and become more prominent later in the season. This compaction may prevent tuber growth increasing soil height, causing bulk density to increase instead. Increased Inca

Bella yield in compacted plots in trial 2 compared to trial 1 (Figure 4-7) was correlated with increased soil moisture (Figure 4-5) reducing soil resistance, allowing increased tuber growth before increases to soil resistance inhibits further bulking. The relative insensitivity of Maris Piper yields to soil compaction may be due to genotypic differences in tuber growth at higher soil resistances, which were insufficient to inhibit Maris Piper tuber growth but constrained Inca Bella tuber growth. It is therefore possible that genotypic differences in tuber tolerance to compaction primarily determines yield in compaction-susceptible soil.

Responses to Soil Compaction Differ Between Cultivars

In both trials, increased soil compaction did not change Maris Piper root density, but decreased Inca Bella root density (Figure 4-4). This indicates that root sensitivity to soil compaction is consistent between controlled and uncontrolled environments and can therefore be predicted from controlled environment experiments (Figure 2-3). Whilst these results are similar in these trials, it may not hold true for all field-based environments, but instead relies on roots being restricted to the topsoil. In both trials, soil resistance below 45 cm consistently exceeded 4 MPa (Figure 4-2), which is the maximum limit for potato root growth (Stalham *et al*., 2007). Roots typically utilize pre-existing pores when growing at depth (Hodgkinson *et al*., 2017), and as compaction reduces pore size and density (Bengough *et al*., 2006), root growth is further limited to the looser topsoil. As a result of these factors, root growth in controlled environment experiments may not be comparable to plants grown in fields where compacted soil does not limit vertical root growth. This has been observed for stresses such as drought (Puértolas *et al*., 2014), where roots tend to grow vertically to extract water from deeper soil (Pierret *et al*., 2016). It has also not been observed in penetrative ability between rice cultivars. Clark *et al*., (2002) found that many of the cultivars that best penetrated a resistance wax layer in controlled environment experiments performed poorly at penetrating compacted soil in the field. However, as this only compared a waxy layer screen rather than a like-for-like comparison using soil, its representation of field conditions was limited. Overall, these results show that root responses to compaction in the field can be predicted in controlled environment experiments.

Compacted soil caused cultivar-specific differences in mean root diameter to arise. In controlled environment experiments, Maris Piper plants had a 40% larger mean root diameter when grown in compacted soil, whilst Inca Bella plants exhibited no changes (Figure 2-6). Across both trials, compacted soil decreased Inca Bella mean root diameter by 20%, but increased Maris Piper root diameter by 45% (Figure 4-4). These different strategies of adapting to compacted soil may depend on the roots' ability to tolerate It. Whilst Maris Piper maintained root density in compacted soil (Figure 4-4), the root density of Inca Bella decreased. In compacted soil, pore density and size decreases (White *et al*., 2007). Roots tend to follow the path of least resistance, which in compacted soil directs them into soil pores (Atkinson *et al*., 2019). As compaction decreases soil pore size, root diameter decreases to compensate, allowing continued root growth. This may occur in Inca Bella plants, thereby decreasing root diameter. However, roots can also adapt to compaction by increasing in diameter. Increased root diameter is a common response to impedance and is believed to increase turgor pressure of the root, improving its ability to penetrate the soil (Bengough *et al*., 2006). The observed increase in Maris Piper mean root diameter is therefore likely due to the roots thickening upon encountering impedance. These differences in root responses indicate genotypic variation in methods to grow roots through compacted soil, with Inca Bella roots better adapting to the lower initial soil resistance in trial 1, whilst Maris Piper roots grew better than Inca Bella in the soil with greater initial resistance in trial 2.

In both trials, Maris Piper produced a larger canopy than Inca Bella. The enlarged canopy correlated well with final tuber yield (Table 4-4), explaining more of the variation in yield than other factors. Shoot growth has been correlated with potato yield, with mid-season shoot biomass predicting yield in compacted soil ($R^2 = 0.71$; Huntenburg *et al*., 2021), as well as leaf area index under ideal conditions ($R^2 = 0.77$; Luo *et al*., 2020). This indicates that canopy growth can be used to predict tuber yield.

However, leaf area in controlled environment studies from controlled environment studies (Figure 2-2) produced different results to these field trials. Inca Bella tended to produce a larger leaf area than Maris Piper three weeks post-emergence. Inca Bella may be better adapted to ideal conditions but is more susceptible to environmental stresses. Plants grown in pots quickly become pot bound, resulting in restricted growth rates and reduced plant sizes compared to those grown in outdoor environments. Furthermore, as controlled environments remove many of the stresses present outdoors, such as temperature variation and pest and disease damage (Poorter *et al*., 2016), plants in controlled environments will be healthier than those grown outdoors, resulting in a greater proportion of sequestered carbon being allocated to growth instead of repair, increasing initial growth rates (Poorter *et al*., 2016). Whilst there was no evidence of pest or disease damage affecting the cultivars differently, temperature stress is likely to have had some effect, particularly in trial 2, where temperatures approached 40°C during the growing season. *Phureja* group cultivars such as Inca Bella are typically more heat-sensitive than *Tuberosum* group cultivars (Hetherington *et al*., 1983), and as evidence suggests that Inca Bella roots are more compaction-sensitive (Figure 4-4), it is possible that these stresses combined to limit canopy growth. Overall, the data strongly suggests Maris Piper is more tolerant of stresses caused by soil compaction.

4.5 Conclusions

Tuber growth in potato fields increased soil resistance, which increased to a cultivar-dependent maximum resistance. These cultivar-dependent differences did not occur in uncompacted plots, implying that some surface compaction is required for this to occur. This surface compaction can easily occur by using farm machinery on the field and causes large increases in resistance across the growing season. This may explain why subsoiling has limited effects on tuber yield in compacted fields (Ghosh and Daigh, 2020), as resistance would nevertheless increase during the season and limit final yield. It is possible that increased irrigation during tuber bulking may recover lost yield by reducing soil resistance, allowing increased tuber growth before resistance reaches inhibitory levels. However, it is unknown whether this would be an economical solution.

Secondly, whilst cultivar variation in root density was maintained between controlled environment studies and field trials, leaf area was less consistent, possibly due to additional stresses in the field, such as heat stress. Despite these inconsistencies, leaf area remained the best predictor of tuber yield of the measured variables, as in other research. Thus, early vegetative vigour in the field may best indicate compaction-tolerant cultivars.

Finally, genotypic diversity was less than hypothesised, with root density being comparable between both cultivars across trial 1 and most of trial 2. However, there were clear genotypic differences in canopy growth, as well as tuber growth. Both gross and marketable yield exhibited clear genotypic differences in response to soil compaction. Compacted soil did not decrease gross yield of Maris Piper, but decreased Inca Bella gross yield by 20% across both trials. Marketable yield was similar for all treatments across both trials, except for a significantly lower value for compacted Inca Bella tubers in trial 1. Overall, whilst genotypic variation in response to soil compaction occurred, there was limited variation in morphological traits, including leaf area and root density, and limited change in Maris Piper yield compared to other studies where yields typically drop by over 20%. This implies that the plants in these trials were less stressed, perhaps due to high soil water content.

Chapter 5 General Discussion

5.1 Responses to Compaction are Consistent Between Controlled and Field Environments

One of the most important factors of any experiment is that its results can be consistently reproduced to reach the same conclusions. This may be challenging when comparing responses of plants grown in controlled environments with those grown in field-based environments. Comparisons between these have often seen inconsistencies when measuring responses to specific stresses and conditions, including root penetrative ability in rice (Clark *et al*., 2002), and physiological responses to drought stress in potato leaves (Puértolas *et al*., 2014). This is typically attributed to differences in soil conditions, with plants becoming pot bound (Puértolas *et al*., 2014), or the methods used to attempt to replicate soil conditions were not comparable to field environments, such as using waxy layers to represent compacted soil (Clark *et al*., 2002). However, this thesis demonstrated consistent, albeit varied, responses to soil compaction between field trials and controlled environments for each genotype (Figure 5-1). Higher variation in field trials is likely due to environmental differences across the two trial years, although the slightly larger sample size may also be partly responsible. However, both cultivars (Maris Piper and Inca Bella) exhibiting comparable decreases in root length (Figure 5-1A) and increased root diameter was comparable for Maris Piper (Figure 5-1B) in compacted soil treatments in both field trials and controlled environments. The consistency of the root responses to compaction were irrespective of differences in soil particle size distribution between the three soils used in experiments (controlled environment, trial 1, and trial 2), which implies that whilst soil type determines the susceptibility of a soil to compaction (Vorhees *et al*., 1987), it has little effect on the responses of plant roots to compaction. Overall, the proportion of root length retained in compacted soil tends to be greater when the relative increase in root diameter is also greater, as previously observed in compacted soil (Materechera *et al*., 1992; Clark *et al*., 2002). However, more recent research has found that increases to root diameter are incidental to increased root penetration (Vanhees *et al*., 2022), with ethylene sensitivity and root angle on meeting compaction being more important for determining penetrative ability.

Early leaf area development (within 4 weeks) appeared to be unaffected by compaction in field trials. Soil compaction typically reduces canopy growth rate in potato (Stalham *et al*., 2007; Huntenburg *et al*., 2021), although maximum canopy growth is comparable to plants in uncompacted soil. However, there was little difference in mean leaf area during the growing season of the field trials between treatments. As mid-season canopy growth explains a high percentage of the variation in tuber yield across treatments imposing a factorial combination of compaction and water stresses (Huntenburg *et al*., 2021), this would indicate no change in yield for Inca Bella plants in compacted soil, as leaf area was comparable between compacted and uncompacted plots (Figure 4-3). However, Maris Piper canopy growth tended to be comparable in both treatments, as was yield. Whilst the correlation was not significant, the correlation between leaf area four weeks post-emergence and final tuber yield explained 22% of variation indicating leaf area at this stage is a poor predictor of final yield. However, it explained a much larger amount of variation for Inca Bella plants (explaining 55% of variation) than Maris Piper plants (explaining 15% of variation). This is most likely due to different responses to compacted soil. Inca Bella yield was reduced by compacted soil, unlike Maris Piper, whilst the canopy growth of both cultivars was affected. In conclusion, some responses to soil compaction, such as changes to root length and diameter, are typically conserved between controlled environment experiments and field trials. The responses of a cultivar to soil compaction in the field can therefore be estimated based on controlled environment data.

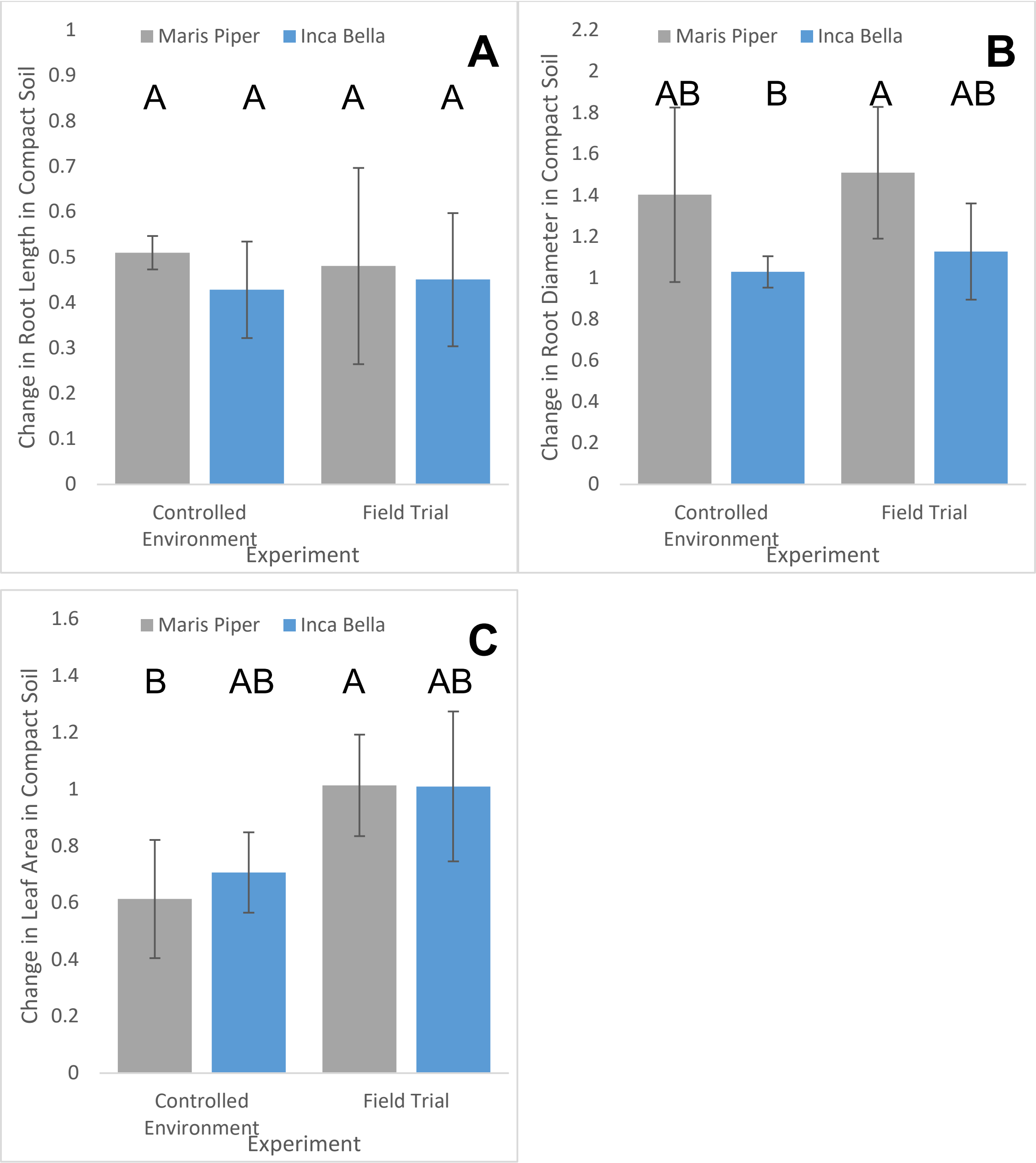


Figure 5-1: Changes in relative root length (A), root diameter (B) and leaf area (C) between compacted treatments for Maris Piper (grey bars) and Inca Bella (blue bars) four weeks post-emergence in controlled environments and field trials. Controlled Environment data obtained from experiment 2.1, and field trial data from Trial 1 and Trial 2. Error bars show standard error with 95% confidence limits. Field Trial bars represent the mean of six values, whilst bars for Experiment 2.1 represent five values. Letters of mean discrimination represent significance where $p < 0.05$.

5.2 Ethylene Sensitivity Correlates with Compaction Tolerance

To determine whether root penetrative ability is controlled by roots or shoots, grafting experiments were performed as part of this thesis (Experiment 2.3).

Previous studies with grafted potato determined that the scion controls root vigour (Iwama, 2008), including under drought stress (Jefferies, 1993), although the use of grafting to investigate growth responses in compacted soil has not previously been studied. Similar shoot vigour between cultivars made it difficult to determine the role of rootstock and scion on shoot growth regulation in compacted soil (Figure 2-9). However, the rootstock had no effect on leaf area of drought-stressed potato (Jefferies, 1993). Nevertheless, in this thesis, rootstock primarily controlled plant growth in compacted soil, with leaf area, root length and root diameter all exhibited different responses to compaction according to the rootstock, whilst only leaf area exhibited a scion-dependent response, and root length exhibited differing responses depending on both scion and rootstock (Table 2-6). However, the rootstock was especially important in regulating root morphology. Plants with Inca Bella rootstocks produced root systems three times larger than Maris Piper rootstocks, but with 70% less root length in compacted soil, whilst Maris Piper roots were not significantly affected (Figure 2-10). This relationship was also observed in field trials (Figure 4-5), indicating a consistent response. Furthermore, root diameter was also primarily determined by the rootstock, with only a small but significant effect from the scion. Whilst grafting studies solely involving potato have not previously determined control of root diameter, grafts of potato rootstock with tomato scions result in diameter being primarily influenced by the potato rootstock, with only a small effect from the scion (Theivasigamani *et al.*, 2021). This supports the hypothesis that that whilst scion has a small role in determining morphological root traits such as length and diameter (Figure 5-2) in compacted soil, root responses are being primarily determined by the roots themselves. Rootstock-determined changes to root length has been observed in tomato as a response to hypoxia (Agnello, 2018), and is likely an ethylene-mediated response, as ethylene causes increased root diameter and decreased root growth (Pandey *et al.*, 2021).

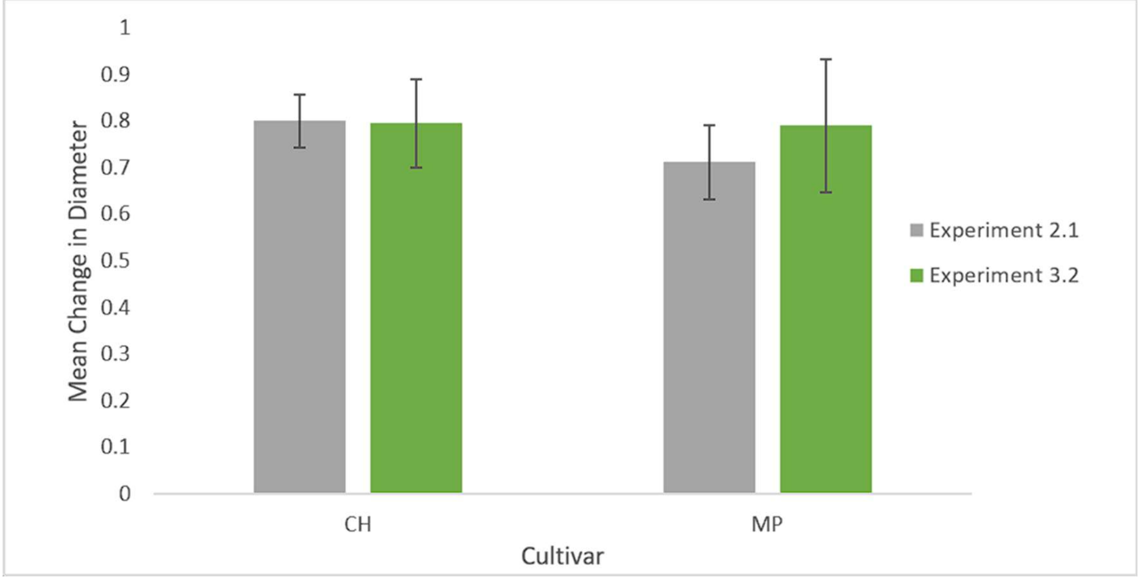


Figure 5-2: Relative changes in mean root diameter for Charlotte (CH) and Maris Piper (MP) between compacted and loose treatments in Experiment 2.1 (grey bars), and the 0 ppm 1.0 ppm ambient ethylene concentration treatments from Experiment 3.2 (green bars). Error bars show standard error with 95% confidence limits. No significance is observed between the values.

Whilst previous experiments have typically attributed increased ethylene production to differences in penetrative ability (Sarquis *et al*., 1991), this thesis is the first study to compare ethylene evolution in compacted and uncompacted soil between cultivars of potato. Ethylene evolution did not vary between cultivars or compaction treatments (Figure 3-15), indicating that, when the only stress present is impedance caused by compacted soil, root ethylene evolution is not affected. However, genotypic responses in ethylene sensitivity correlated with differences in penetrative ability. In experiment 3.2, Pentland Dell roots did not significantly respond to exogenous applications of ethylene, unlike Maris Piper and Charlotte. In this assay, Pentland Dell appeared to initiate new roots, as total root length increased (Table 3-3) whilst the mean root length remained unchanged (Figure 3-6). Charlotte and Maris Piper elongated existing roots (Figure 3-10). As increased ethylene production (*eto1-1* mutant) and response (*ctr1-1* mutant) inhibits branching and production of new roots in *Arabidopsis* (Negi *et al*., 2008), this implies that Pentland Dell is not as sensitive to ethylene as the other cultivars. This data is supported by Experiment 2.1. Pentland Dell exhibited minimal morphological changes in response to compaction, particularly within the roots, with both root diameter and leaf area being unaffected (Figure 2-3; Figure 2-5). Both Charlotte and Maris Piper exhibited increased root diameter and decreased root length and leaf area in compacted soil in this experiment, further supporting the hypothesis that ethylene sensitivity and not production is more important in regulating morphological responses to compaction.

Whilst increased ethylene sensitivity has previously been associated with decreased penetrative ability, Pandey *et al*., 2021 used ethylene insensitive mutants rather than commercially viable cultivars. This thesis is the first to investigate the genotypic variation of ethylene sensitivity in potato and the effects it has on responses to soil compaction. Whilst measuring ethylene sensitivity in Inca Bella would have allowed comparisons with compaction effects on root growth and yield, tuber supply issues prevented these experiments. Although Maris Piper exhibited the greatest increase in root diameter in response to ambient ethylene, compacted soil did not reduce its yield in the experiments reported in this thesis. This was unexpected as compacted soil often decreases Maris Piper yield (Stalham *et al*., 2007; Huntenburg *et al*., 2021), possibly due to reduced canopy growth, which was not observed in Chapter 4. Whereas Stalham *et al*., (2007) does not report canopy growth in experiments measuring yield, Huntenburg *et al*., (2021) observed significant delays in canopy growth within compacted soil. This implies that whilst sensitivity to ethylene restricts root growth, it may not necessarily limit yield, which is primarily associated with canopy growth (Huntenburg *et al*., 2021), although roots influence canopy growth in compacted soil (Table 2-6). This may be due to limited biomass production, caused by reduced water availability due to reduced root system size in compacted soil (Figure 2-3). However, compaction in field trials in Chapter 4 was not as high as resistances observed in many potato fields in the UK (Stalham *et al*., 2007), and the crop was kept well-watered (Table 4-1), which means that yield losses may have been observed in more hostile conditions. Soil water deficits could lead to root-derived stress signals such as ABA reducing canopy growth (Dodd, 2005), and thus yield.

The increased evapotranspiration rates observed in compacted soil by plants inoculated with 5C-2 (Figure 3-9) indicates that compacted soil is reducing stomatal opening (Hussain et al., 1999) and likely the rate of biomass production. The application of 5C-2 potentially decreases root to shoot signalling of ABA (Jiang *et al*., 2012). Ethylene promotes root ABA production (Huang *et al*., 2022), which may then be used as a root-sourced stress signal sent to the leaves, resulting in stomatal closure (Dodd, 2005). If 5C-2 does reduce the concentration of ABA sent to the shoot, this may explain why evapotranspiration rates were higher. Whilst there is evidence both of increased root to shoot ABA transport under compaction (Mulholland *et al*., 1995; Hussain *et al*., 1999), it remains uncertain if 5C-2 is limiting the root to shoot ABA signal, as ABA concentrations in the phloem were increased in inoculated plants (Jiang *et al*., 2012), which may imply that the signal was re-routed instead of reduced. To conclude, whilst ethylene sensitivity is primarily responsible for root responses to compaction, its effect on shoot responses and tuber yield remains uncertain.

5.3 Comparing Methods of Adapting to Soil Compaction

This thesis investigated two potential approaches to improving tuber yield in compacted soil. The first was to apply the ACC deaminase-containing rhizobacterium *Variovorax paradoxus* 5C-2 to the soil (Experiment 3.3). The second was to take advantage of existing genotypic variation to select cultivars with greater tolerance to soil compaction (Chapter 4). Whilst the benefits of PGPR in compacted soil had not previously been investigated, applying 5C-2 to drought-stressed potato plants significantly increased tuber yield by 10% (Belimov *et al*., 2015). Due to time constraints, field trials comparing the yield benefits of 5C-2 application were unable to be performed, but benefits can be inferred from controlled environment experiments, as responses to compaction were consistent between field trials and controlled environment experiments in this thesis.

Studies investigating genotypic variation in potato tuber yield changes in compacted soil are rare, and have reported little variation in yield loss (Stalham *et al*., 2007), whilst those that investigate changes to yield in other plants such as cereals find yield dramatically improves when compacted soil is tilled (Unkovich *et al*., 2023), indicating that this lack of response related either the potato crop, or how it is managed. Although potato yield responses to compaction are themselves very varied and inconsistent, this is primarily due to environmental effects, such as how compaction is applied, recompaction of control plots, and climate (Stalham *et al*., 2005). Many studies investigating yield responses simply comparing cultivated and uncultivated plots without confirming the presence of compaction (Stalham *et al*., 2005). Secondly, the use of subsoiling to remove compaction may have been done when the soil was wet, producing plough pans (Johansen *et al*., 2015). Subsoil compaction reduces yield (Huntenburg *et al*., 2021), which would account for reduced yields when this was performed. As detailed in Chapter 4, unstructured soils caused by subsoiling are susceptible to slumping (White *et al*., 2007), which recompacts the soil in the event of heavy rainfall or over-irrigation. Also, using heavy machinery after subsoiling can cause the soil to compact (Johansen *et al*., 2015). Without frequent resistance measurements, it cannot be certain if soil has become compacted after subsoiling, therefore preventing valid comparisons between treatments. Soil resistance naturally fluctuates throughout the growing season as soil water content changes (Huntenburg *et al*., 2021), which means soil water content must also be observed to determine if the soil is compacting or drying. Whilst steps were taken to ensure that these problems did not arise in field trials performed as part of this thesis, yield losses in compacted soil were still not observed for Maris Piper. This may indicate that applied compaction was insufficient to cause yield losses in Maris Piper, or that in otherwise ideal conditions, soil compaction does not limit yield significantly.

As Maris Piper (the most commonly cultivar grown in UK) was used within all Chapters in this thesis, and was sensitive to ethylene (Experiment 3.2), it was chosen to determine whether applying 5C-2 could mitigate root length loss under soil compaction. Applying 5C-2 significantly improved root growth in Maris Piper in compacted soil, reaching root lengths comparable to those obtained in uncompacted soil (Figure 3-10), and increased evapotranspiration rates (Figure 3-9). However, the benefits of these changes to yield are not certain. Previous research on predicting yield in potato finds that leaf area and green area are strong predictors of yield (Luo *et al*., 2020; Huntenburg *et al*., 2021), and that in the field, compaction does not affect stomatal closure

(Huntenburg *et al.*, 2021), which primarily determines transpiration rate. However, application of 5C-2 did not improve leaf area in compacted soil (Figure 3-14), so it is possible that yield improvements may not be forthcoming, with increased biomass allocation to the roots (Figure 3-11) rather than to improve yield.

On the other hand, selecting appropriate genotypes has shown potential to improve yield in field trials. Inca Bella plants yielded 20% less in compacted soil, whilst Maris Piper yields remained unaffected (Figure 4-7). This provides strong evidence of genotypic variation in yield responses that can be further investigated and exploited to improve yield in compacted soil, or soils susceptible to compaction. Overall, whilst genotypic variation to yield has been confirmed and can be exploited, the extent to which application of 5C-2 could benefit yield remains uncertain but is potentially helpful. It is likely that both methods can be applied simultaneously by selecting compaction tolerant cultivars, and then further improve plant growth via the application of 5C-2.

5.4 Closing Remarks

Overall, this thesis has confirmed intraspecific variation in tolerance to soil compaction and provided insight into the role of ethylene and ethylene sensitivity in determining tolerance in potato. Cultivars with reduced ethylene sensitivity were better able to penetrate compacted soil (Figure 5-2) and maintain greater leaf area than cultivars with a greater degree of ethylene sensitivity. Whilst work remains to determine whether differences in ethylene sensitivity are related to improved tolerance to compaction in the field, comparable morphological responses indicate that this is likely to be the case.

In addition, methods that take advantage of this knowledge to improve yield have been proposed, with genotypic variation in compaction tolerance altering yield responses in compacted soil, both in total and marketable yield. Overall, field trials showed that plants of the cultivar Maris Piper were better able to maintain root growth and tuber yield in compacted soil than those of the cultivar Inca Bella. Applying ACC deaminase-containing bacterium 5C-2 enhanced root growth and transpiration by decreasing root ethylene evolution in controlled environment trials, although the benefits to yield were not confirmed as the benefits to photosynthesis and tuber growth in the field remain unknown. To further support the conclusions reached in this thesis, field trials comparing the three cultivars grown in Chapter 3 would help in understanding the effect ethylene sensitivity has on yield. These field trials should use compacted and uncompacted soil in the presence or absence of rhizobacterial inoculation to understand the benefits of PGPR on yield.

References

AGNELLO, M., (2018). Role of vegetable grafting in the control of abiotic stresses and effects on yield and quality. Dissertation. Università Degli Studi di Catania.

AHDB, (2018). Early-season water management and common scab control in potato crops. Accessed 12/1/2022. https://ahdb.org.uk/knowledge-library/early-season-water-management-and-common-scab-control-in-potato-crops

AJAYI, A., SOUZA DIAS JR., M., CURI, N., *et al*., (2009). Comparison of estimation methods of soil strength in five soils. *Revista Brasileira de Ciência do Solo*. **33** (3), 487-495.

ALICHE, E., OORTWIJN, M., THEEUWEN, T., *et al*., (2018). Drought response in field grown potatoes and the interactions between canopy growth and yield. *Agricultural Water Management*. **206**, 20-30.

AN, N., TANG, C., XU, S., GONG, X., SHI, B., INYANG, H., (2018). Effects of soil characteristics on moisture evaporation. *Engineering Geology*. **239**, 126-135.

ANDRADE, A., WOLFE, D., FERERERES, E., (1993). Leaf expansion, photosynthesis, and water relations of sunflower plants grown on compacted soil. *Plant and Soil*. **149** (2), 175-184.

ATKINSON, J., HAWKESFORD, M., WHALLEY, W., ZHOU, H., MOONEY, S., (2019). Soil strength influences wheat root interactions with soil macropores. *Plant, Cell and Environment*. **43** (1), 235-245.

ATWELL, B., (1993). Response of roots to mechanical impedance. *Environmental and Experimental Botany*. **33** (1), 27-40.

AZAM, G., GRANT, C., MURRAY, R., NUBERG, I., MISRA, R., (2014). Comparison of the penetration of primary and lateral roots of pea and different tree seedlings growing in hard soils. *Soil Research*. **52** (1), 87-96.

BABU, R., SHASHIDHAR, H., LILLEY, J., *et al*., (2001). Variation in root penetration ability, osmotic adjustment and dehydration tolerance among accessions of rice adapted to rainfed lowland and upland ecosystems. *Plant Breeding*. **120**, 233-238.

BATEY, T., (2009). Soil compaction and soil management – a review. *Soil Use and Management*. **25**, 335-345.

BELIMOV, A., DODD, I., SAFRONOVA, V., *et al*., (2015). Rhizobacteria that produce auxins and contain 1-amino-cyclopropane-1-carboxylic acid deaminase decrease amino acid concentrations in the rhizosphere and improve growth and yield of well-watered and water-limited potato (*Solanum tuberosum*). *Annals of Applied Biology*. **167**, 11-25.

BENGOUGH, A., BRANSBY, M., HANS, J., MCKENNA, S., ROBERTS, T., VALENTINE, T., (2006). Root responses to soil physical conditions; growth dynamics from field to cell. *Journal of Experimental Botany*. **57** (2), 437-447.

BENGOUGH, A., LOADES, K., MCKENZIE, B., (2016). Root hairs aid soil penetration by anchoring the root surface to pore walls. *Journal of Experimental Botany*. **67** (4), 1071-1078.

BENGOUGH, A., MACKENZIE, C., (1994). Simultaneous measurement of root force and elongation for seedling pea roots. *Journal of Experimental Botany*. **45** (1), 95-102.

BENGOUGH, A., MCKENZIE, B., HALLETT, P., VALENTINE, T., (2011). Root elongation, water stress, and mechanical impedance: a review of limiting stresses and beneficial root tip traits. *Journal of Experimental Botany*. **62** (1), 59-68.

BENGOUGH, A., YOUNG, I., (1993). Root elongation of seedling peas through layered soil of different penetration resistances. *Plant and Soil*. **149**, 129-139.

BENIGNO, S., CAWTHRAY, G., DIXON, K., STEVENS, J., (2012). Soil physical strength rather than excess ethylene reduces root elongation of *Eucalyptus* seedlings in mechanically impeded sandy soils. *Plant Growth Regulation*. **68**, 261-270.

BESSON, A., SÉGER, M, GIOT, G., COUSIN, I., (2013). Identifying the characteristic scales of soil structural recovery after compaction from three in-field methods of monitoring. *Geoderm*. **204-205**, 130-139.

BHOGAL, A., BENTLY, C., PRICE, P., CHAMBERS, B., (2011). Characterisation of soil structural degradation under grassland and development of measures to ameliorate its impact on biodiversity and other soil functions. *DEFRA*. **BD5001**.

BIEMELT, S., KEETMAN, U., ALBRECHT, G., (1998). Re-Aeration following Hypoxia or Anoxia Leads to Activation of the Antioxidative Defense System in Roots of Wheat Seedlings. *Plant Physiology*. **116**, 651-658.

BINGHAM, I., BENGOUGH, A., (2003). Morphological plasticity of wheat and barley roots in response to spatial variation in soil strength. *Plant and Soil*. **250** (2), 273-282.

BOGUNOVIC, I., PEREIRA, P., KISIC, I., SAJKO, K., SRAKA, M., (2018). Tillage management impacts on soil compaction, erosion and crop yield in Stagnosols (Croatia). *CATENA*. **160**, 376-384.

BOONE, F., BOUMA, J., DE SMET, L., (1978). A case study on the effect of soil compaction on potato growth in a loamy sand soil. 1. Physical measurements and rooting patterns. *Netherlands Journal of Agricultural Science*. **26**, 405-420.

BORCH, K., BOUMA, T., LYNCH, J., BROWN, K., (1999). Ethylene: a regulator of root architectural responses to soil phosphorus availability. *Plant, Cell and Environment*. **22**, 425-431.

BOTTA, G., JORAJURIA, D., BALBUENA, R., *et al*., (2006). Deep tillage and traffic effects on subsoil compaction and sunflower (*Helianthus annus* L.) yields. *Soil and Tillage Research*. **91** (1-2), 164-172.

CERDA, A., DALIAKOPOULOS, I., TERROL, E., *et al*., (2021). Long-term monitoring of soil bulk density and erosion rates in two *Prunus Persica* (L) plantations under flood irrigation and glyphosate herbicide treatment in La Ribera district, Spain. *Journal of Environmental Management*. **282**, 111965.

CHANDWANI, S., AMARESAN, N., (2022). Role of ACC deaminase producing bacteria for abiotic stress management and sustainable agriculture production. *Environmental Science and Pollution Research*. **29**, 22843-22859.

CHEN, G., (2011). Root growth and yield of maize as affected by soil compaction and cover crops. *Soil and Tillage Research*. **117**, 17-27.

CHEN, G., FU, X., LIPS, S., SAGI, M., (2003). Control of plant growth resides in the shoot, and not in the root, in reciprocal grafts of flacca and wild-type tomato (*Lysopersicon esculentum*), in the presence and absence of salinity stress. *Plant and Soil*. **256**, 205-215.

CHEN, G., WEIL, R., (2010). Penetration of cover crop roots through compacted soils. *Plant and Soil*. **331**, 31-43.

CHEN, Y., PALTA, J., CLEMENTS, J., BUIRCHELL, B., SIDDIQUE, K., RENGEL, Z., (2014). Root architecture alteration of narrow-leafed lupin and wheat in response to soil compaction. *Field Crops Research*. **165**, 61-70.

CHIMUNGU, J., LOADES, K., LYNCH, J., (2015). Root anatomical phenes predict root penetration ability and biomechanical properties in maize (*Zea Mays)*. *Journal of Experimental Botany.* **66** (11), 3151-3162.

CLARK, L., COPE, R., WHALLEY, W., BARRACLOUGH, P., WADE, L., (2002). Root penetration of strong soil in rainfed lowland rice: comparison of laboratory screens with field performance. *Field Crops Research*. **76**, 189-198.

CLARK, L., WHALLEY, W., BARRACLOUGH, P., (2003). How do roots penetrate strong soil? *Plant and Soil*. **255**, 93-104.

CLEMENTE, E., SCHAEFER, C., NOVAIS, R., VIANA, J., BARROS, N., (2005). Soil resistance to penetration under the dynamic and predictive perspective of restriction to crop yield. 19th World Congress of Soil Science, Soil Solutions for a Changing World. *Australian Journal of Soil Research*. 43 (**2**), 139-146.

COLOMBI, T., BRAUN, S., KELLER, T., WALTER, A., (2017a). Artificial macropores attract crop roots and enhance plant productivity on compacted soils. *Science of the Total Environment*. **574**, 1283-1293.

COLOMBI, T., KIRCHGESSNER, N., WALTER, A., KELLER, T., (2017b). Root Tip Shape Governs Root Elongation Rate under Increased Soil Strength. *Plant Physiology*. **174** (4), 2289-2301.

COLOMBI, T., TORRES, L., WALTER, A., KELLER, T., (2018). Feedback between soil penetration resistance, root architecture and water uptake limit water accessibility and crop growth – A vicious circle. *Science of the Total Environment*. **626**, 1026-1035.

COLOMBI, T., WALTER, A., (2017). Genetic Diversity under Soil Compaction in Wheat: Root Number as a Promising Trait for Early Plant Vigor. *Frontiers in Plant Science*. **8**, 420.

CORREA, J., POSTMA, J., WATT, M., WOJCIECHOWSKI, T., (2019). Soil compaction and the architectural plasticity of root systems. *Journal of Experimental Botany*. **70** (21), 6019-6034.

DELAY, C., IMIN, N., DJORDJEVIC, M., (2013). *CEP* genes regulate root and shoot development in response to environmental cues and are specific to seed plants. *Journal of Experimental Botany*. **64** (17), 5383-5394.

DJAMAN, K., KOUDAHE, K., KOUBODANA, H., SAIBOU, A., ESSAH, S., (2022). Tillage Practices in Potato (*Solanum tuberosum* L.) Production: A Review. *American Journal of Potato Research*. **99**, 1-12.

DODD, I., (2005). Root-to-shoot signalling: Assessing the roles of ‘up’ in the up and down world of long-distance signalling *in planta*. *Plant and Soil*. **274**, 251-270.

DODD, I., THEOBALD, J., RICHER, S., DAVIES, W., (2009). Partial phenotypic reversion of ABA-deficient flacca tomato (*Solanum lycopersicum*) scions by a wild-type rootstock: normalizing shoot ethylene relations promotes leaf area but does not diminish whole plant transpiration rate. *Journal of Experimental Botany*. **60** (14), 4029-4039.

DUIKER, S., (2004). Effects of Soil Compaction. *Penn State College of Agriculture*.

ESSAH, S., HONEYCUTT, C., (2004). Tillage and seed-sprouting strategies to improve potato yield and quality in short season climates. *American Journal of Potato Research*. **81,** 177-186.

FAOSTAT (2022). Potato Crops (Production). Accessed 12/12/2018. http://www.fao.org/faostat/en

GAO, W., WATTS, C., REN, T., WHALLEY, W., (2012). The effects of compaction and soil drying on penetrometer resistance. *Soil and Tillage Research*. **125**, 14-22.

GARCIA, A., MARCELIS, L., GARCIA-SANCHEZ, F., NICHOLAS, N., MARTINEZ, V., (2007). Moderate water stress affects tomato leaf water relations in dependence on the nitrogen supply. *Biologia Plantarum*. **51** (4), 707-712.

GHOSH, U., DAIGH, A., (2020). Soil compaction problems and subsoiling effects on potato crops: A review. *Crop, Forage and Turfgrass Management*. **6** (1), e20030.

GLICK, B., (2005). Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. *FEMS Microbiology Letters.* **251** (1), 1-7.

GONZÁLEZ-GUZMÁN, M., GÓMEZ-CADENAS, A., ARBONA, V., (2021). Abscisic Acid as an Emerging Modulator of the Responses of Plants to Low Oxygen Conditions. *Frontiers in Plant Science*. **12**, 661789.

GRZESIAK, M., JANOWIAK, F., SZCZYREK, P., et al., (2016). Impact of soil compaction stress combined with drought or waterlogging on physiological and biochemical markers in two maize hybrids. *Acta Physiologiae Plantarum*. **38**, 109.

GRZESIAK, S., GRZESIAK, M., HURA, T., MARCINSKA, I., RZEPKA, A., (2013). Changes in root system structure, leaf water potential and gas exchange of maize and triticale seedlings affected by soil compaction. *Environmental and Experimental Botany*. **88**, 2-10.

GYSSELS, G., POESEN, J., BOCHET, E., LI, Y., (2005). Impact of plant roots on the resistance of soils to erosion by water: a review. *Progress in Physical Geography: Earth and Environment*. **29** (2), 189-217.

HAKANSSON, I., VORHEES, W., RILEY, H., (1988). Vehicle and Wheel Factors Influencing Soil Compaction and Crop Response in Different Traffic Regimes. *Soil and Tillage Research*. **11**, 239-282.

HAO, H., HARTMANN, C., APICHART, J., *et al*., (2011). Slumping Dynamics in Tilled Sandy Soils Under Natural Rainfall and Experimental Flooding. *Soil and Tillage Research*. **114**, 9-17.

HARRISON, N., PEREZ, N., PENNINGTON, B., CASCANT, E., (2016). Root system architecture in reciprocal grafts of apple rootstock-scion combinations. *Acta Horticulturae*. **1130**, 409-414.

HATLEY, D., WILTSHIRE, J., BASFORD, B., ROYALE, S., BUCKLEY, D., JOHNSON, P., (2005). Soil compaction and potato crops. *British Potato Council*. Ref R260.

HAVERKORT, A., STRUIK, P., (2015). Yield levels of potato crops: Recent achievements and future prospects. *Field Crops Research*. **182**, 76-85.

HUANG, G., KILIC, A., KARADY, M., *et al*., (2022). Ethylene inhibits rice root elongation in compacted soil via ABA- and auxin-mediated mechanisms. *PNAS*. **119** (30), e2201072119.

HUANG, G., KILIC, A., KARADY, M., *et al*., (2022). Ethylene inhibits rice root elongation in compacted soil via ABA- and auxin-mediated mechanisms. *PNAS*. **119** (30), e2201072119.

HUNTENBURG, K., DODD, I. C., STALHAM, M., (2021). Agronomic and physiological responses of potato subjected to soil compaction and/or drying. *Annals of Applied Biology*. **178** (2), 328-340.

HUSSAIN, A., BLACK, C., TAYLOR, I., ROBERTS, J., (1999). Soil Compaction. A Role for Ethylene in Regulating Leaf Expansion and Shoot Growth in Tomato? *Plant Physiology*. **121** (4), 1227-1237.

HUSSAIN, A., BLACK, C., TAYLOR, I., ROBERTS, J., (2000). Does an antagonistic relationship between ABA and ethylene mediate shoot growth when tomato (*Lycopersicon esculentum* Mill.) plants encounter compacted soil? *Plant, Cell and Environment*. **23**, 1217-1226.

IIJIMA, M., KONO, Y., (1991). Interspecific Differences of the Root System Structures of Four Cereal Species as Affected by Soil Compaction. *Japanese Journal of Crop Science*. **60** (1), 130-145.

IWAMA, K., NISHIBE, S., (1989). Comparison of Root Characters among Cultivated Potatoes (*Solanum tuberosum*) and Their Wild Relatives. *Japanese Journal of Crop Science*. **58** (1), 126-132.

JACOBSEN, A., XU, J., TOPPING, J., LINDSEY, K., (2020). Root growth responses to mechanical impedance are regulated by a network of ROS, ethylene and auxin signalling in Arabidopsis. *Preprint, unreleased*.

JAIN, M., NANDWAL, A., KUNDU, B., *et al*., (2006). Water relations, activities of antioxidants, ethylene evolution and membrane integrity of pigeonpea roots as affected by soil moisture. *Biologia Plantarum*. **50**, 303-306.

JEFFERIES, R., (1993). Cultivar responses to water stress in potato: effects of shoot and roots. *New Phytologist*. **123**, 491-498.

JIANG, F., CHEN, L., BELIMOV, A., *et al*., (2012). Multiple impacts of the plant growth-promoting rhizobacterium *Variovorax paradoxus* 5C-2 on nutrient and ABA relations of *Pisum sativum*. *Journal of Experimental Botany*. **63** (18), 6421-6430.

JIN, K., SHEN, J., ASHTON, R., DODD, I., PARRY, M., WHALLEY, W., (2013). How do roots elongate in a structured soil? *Journal of Experimental Botany*. **64** (15), 4761-4777.

JOHANSEN, T., THOMSEN, M., LØES, A., RILEY, H., (2015). Root development in potato and carrot crops – influences of soil compaction. *Acta Agriculturae Scandinavica, Section B – Soil and Plant Science*. **65** (2), 182-192.

KASSAM, A., FRIEDRICH, T., SHAXSON, F., PRETTY, J., (2009). The spread of Conservation Agriculture: justification, sustainability and uptake. *International Journal of Agricultural Sustainability*. **7** (4), 292-320.

KAYS, S., NICKLOW, C., SIMONS, D., (1974). Ethylene in relation to the response of roots to physical impedance. *Plant and Soil*. **40**, 565-571.

KNIGHT, S., KNIGHTLEY, S., BINGHAM, I., *et al*., (2012). Desk study to evaluate contributory causes of the current ‘yield plateau’ in wheat and oilseed rape. *AHDB*. Project Report No. 502.

KONÔPKA, B., PAGÈS, L., DOUSSAN, C., (2009). Soil compaction modifies morphological characteristics of seminal maize roots. *Plant, Soil and Environment*. **55** (1), 1-10.

KUBO, K., IWAMA, K., YANAGISAWA, A., *et al*., (2006). Genotypic Variation of the Ability of Root to Penetrate Hard Soil Layers among Japanese Wheat Cultivars. *Plant Production Science*. **9** (1), 47-55.

KUBO, K., UCHINO, H., JITSUYAMA, Y., IWAMA, K., (2008). Relationship between Deep Root Distribution and Root Penetration Capacity Estimated by Pot Experiments with a Paraffin and Vaseline Layer for Landraces and Recent Cultivars of Wheat. *Plant Production Science*. **11** (4), 487-497.

KUBOTA, T., WILLIAMS, R., (1967). The effects of changes in soil compaction and porosity on germination, establishment and yield of barley and globe beet. *Journal of Agricultural Science*. **68** (2), 227-233.

KUDOYAROVA, G., DODD, I., VESELOV, D., ROTHWELL, S., VESELOV, S., (2015). Common and specific responses to availability of mineral nutrients and water. *Journal of Experimental Botany*. **66** (8), 2133-2144.

LIANG, Z., ZHANG, F., SHAO, M., ZHANG, J., (2002). The relations of stomatal conductance, water consumption, growth rate to leaf water potential during soil drying and rewatering cycle of wheat (*Triticum aestivum*). *Botanical Bulletin*. **43**, 187-192.

LIPIEC, J., MEDVEDEV, V., BIRKAS, M., *et al*., (2003). Effect of soil compaction on root growth and crop yield in Central and Eastern Europe. *International Agrophysics*. **17**, 61-69.

LIU, F., JENSEN, C., SHAHANZARI, A., ANDERSEN, M., JACOBSEN, S., (2005). ABA regulated stomatal control and photosynthetic water use efficiency of potato (*Solanum tuberosum* L.) during progressive soil drying. *Plant Science*. **168** (3), 831-836.

LIU, Z., HARTMAN, S., VAN VEEN, H., *et al*., (2022). Ethylene augments root hypoxia tolerance via growth cessation and reactive oxygen species amelioration. *Plant Physiology*. **190** (2), 1365-1383.

LUCAS, M., SCHLUTER, S., VOGEL, H., VETTERLEIN, D., (2019). Roots compact the surrounding soil depending on the structures they encounter. *Nature Scientific Reports.* **9**: 16236.

LUO, S., HE, Y., LI, Q., JIAO, W., ZHU, Y., ZHAO, X., (2020). Nondestructive estimation of potato yield using relative variables derived from multi-period LAI and hyperspectral data based on weighted growth stage. *Plant Methods*. **16**, 150.

MAGNESCHI, L., PERATA, P., (2009). Rice germination and seedling growth in the absence of oxygen. *Annals of Botany*. **103** (2), 181-196.

MANCUSO, S., MARRAS, A., (2006). Adaptative Response of *Vitis* Root to Anoxia. *Plant Cell Physiology*. **47** (3), 401-409.

MASAKA, J., KHUMBULA, N., (2007). The Effect of Soil Compaction Levels on Germination and Biometric Characteristics of Coffee (*Coffee arabica*) Seedlings in the Nursery. *International Journal of Agricultural Research*. **2** (7), 581-589.

MASLE, J., PASSIOURA, J., (1987). The Effect of Soil Strength on the Growth of Young Wheat Plants. *Australian Journal of Plant Physiology*. **14**, 643-656.

MATERECHERA, S., ALSTON, A., KIRBY, J., DEXTER, A., (1992). Influence of root diameter on the penetration of seminal roots into a compacted subsoil. *Plant and Soil*. **144**, 297-303.

MAZURCZYK, W., WIERZBICKA, A., TRAWCZYNSKI, C., (2009). Harvest index of potato crop grown under different nitrogen and water supply. *Acta Scientiarum Polonorum series Agricultura*. **8** (4), 15-21.

MCDOLE, R., (1975). Influence of cultural practices and soil compaction on yield and quality of potatoes. *American Potato Journal*. **52**, 285-286.

MILLER, D., MARTIN, M., (1987). The effect of irrigation regime and subsoiling on yield and quality of three potato cultivars. *American Potato Journal*. **64**, 17-25.

MIR, M., KUMAR, A., (2011). Effect of different methods, time and environmental conditions on grafting in walnut. *International Journal of Farm Sciences*. **1** (2), 17-22.

MOSS, G., HALL, K., JACKSON, M., (1988). Ethylene and the responses of roots of maize (*Zea mays* L.) to physical impedance. *New Phytologist*. **109** (3), 303-311.

MULHOLLAND, B., BLACK, C., TAYLOR, I., ROBERTS, J., LENTON, J., (1996a). Effect of soil compaction on barley (*Hordeum vulgare* L.) growth. I. Possible role for ABA as a root-sourced chemical signal. *Journal of Experimental Botany*. **47** (297), 539-549.

MULHOLLAND, B., TAYLOR, I., BLACK, C., ROBERTS, J., (1996b). Effect of soil compaction on barley (*Hordeum vulgare* L.) growth: II Are increased xylem sap ABA concentrations involved in maintaining leaf expansion in compacted soils? *Journal of Experimental Botany*. **47** (4), 551-556.

NAAB, J., MAHAMA, G., YAHAYA, I., PRASAD, P., (2017). Conservation Agriculture Improves Soil Quality, Crop Yield, and Incomes of Smallholder Farmers in North Western Ghana. *Frontiers in Plant Science*. **8**, 996.

NAING, A., CAMPOL, J., KANG, H., XU, J., CHUNG, M., KIM, C., (2022). Role of Ethylene Biosynthesis Genes in the Regulation of Salt Stress and Drought Stress Tolerance in Petunia. *Frontiers in Plant Science*. **13**, 844449.

NAWAZ, M., BOURRIE, G., TROLARD, F., (2013). Soil compaction impact and modelling: a review. *Agronomy and Sustainable Development*. **33**, 291-309.

NEGI, S., IVANCHENKO, M., MUDAY (2008). Ethylene regulates lateral root formation and auxin transport in Arabidopsis thaliana. *The Plant Journal*. **55** (2), 175-187.

NOTAGUCHI, M., OKAMOTO, S., (2015). Dynamics of long-distance signalling via plant vascular tissues. *Frontiers in Plant Science*. **6**, 161.

ODGEREL, J., BANFALVI, Z., (2021). Metabolite analysis of tubers and leaves of two potato cultivars and their grafts. *PLoS One*. **16** (5), e0250858.

OKAMOTO, T., TSURUMI, S., SHIBASAKI, K., OBANA, Y., TAKAJI, H., OONO, Y., RAHMAN, A., (2008). Genetic Dissection of Hormonal Responses in the Roots of Arabidopsis Grown under Continuous Mechanical Impedance. *Plant Physiology*. **146**, 1651-1662.

OSBORNE, D., SARGENT, J., (1976). The positional differentiation of ethylene-responsive cells in rachis abscission zones in leaves of Sambucus nigra and their growth and ultrastructural changes at senescence and separation. *Planta*. **130** (2), 203-210.

PAGES, L., BERNERT, M., PAGES, G., (2020). Modelling time variations of root diameter and elongation rate as related to assimilate supply and demand. *Journal of Experimental Botany*. **71** (12), 3524-3534.

PANDEY, B., HUANG, G., BHOSALE, *et al*., (2021). Plant roots sense soil compaction through restricted ethylene diffusion. *Science*. **371**, 276-280.

PASSIOURA, J., (2002). Soil conditions and plant growth*. Plant, Cell & Environment*. **25***,* 311–318.

PAVLISTA, A., (1995). Potato Production Stages: Scheduling Key Practices. *Historical Materials from University of Nebraska-Lincoln Extension*. 1584.

PEREIRA, J., DEFOSSEZ, P., RICHARD, G., (2007). Soil susceptibility to compaction by wheeling as a function of some properties of a silty soil as affected by the tillage system. *European Journal of Soil Science*. **58**, 34-44.

PETROPOULOS, S., KHAH, E., PASSAM, H., (2012). Evaluation of rootstocks for watermelon grafting with reference to plant development, yield and fruit quality. *International Journal of Plant Production*. **6** (4), 481-492.

PIERCE, F., BURPEE, C., (1995). Zone tillage effects on soil properties and yield and quality of potatoes (*Solanum tuberosum* L.). *Soil and Tillage Research*. **35** (3), 135-146.

PIERRET, A., MAEGHT, J., CLÉMENT, C., MONTOROI, J., HARTMANN, C., GONKHAMDEE, S., (2016). Understanding deep roots and their functions in ecosystems: an advocacy for more unconventional research. *Annals of Botany*. **118**, 621-635.

POORTER, H., FIORANI, F., PIERUSCHKA, R., *et al*., (2016). Pampered inside, pestered outside? Differences and similarities between plants growing in controlled conditions and in the field. *New Phytologist*. **212**, 838-855.

POTOCKA, I., SZYMANOWSKA-PULKA, J., KARCZEWSKI, J., NAKIELSKI, J., (2011). Effects of mechanical stress on *Zea* root apex. I. Mechanical stress leads to the switch from closed to open meristem organization. *Journal of Experimental Botany*. **62** (13), 4583-4593.

POTOCKA, I., SZYMANOWSKA-PUTKA, J., (2018). Morphological responses of plant roots to mechanical stress. *Annals of Botany*. **122** (5), 711-723.

PUÉRTOLAS, J., BALLESTER, C., ELPHINSTONE, D., DODD, I. C., (2014). Two potato (*Solanum tuberosum*) varieties differ in drought tolerance due to differences in root growth at depth. *Functional Plant Biology*. **41** (11), 1107-1118.

QUANDAHOR, P., GOU, Y., LIN, C., COULTER, J., LIU, C., (2021). Comparison of root tolerance to drought and aphid (Myzus persicae Sulzer) resistance among different potato (*Solanum tuberosum* L.) cultivars. *Scientific Reports*. **11**, 628.

QUARRIE, S., JONES, H., (1979). Genotypic Variation in Leaf Water Potential, Stomatal Conductance and Abscisic Acid Concentration in Spring Wheat Subjected to Artificial Drought Stress. *Annals of Botany*. **44**, 323-332.

RANA, A., PANDEY, R., RAMAKRISHNAN, B., (2019). Chapter 3 – Enzymology of the nitrogen cycle and bioremediation of toxic nitrogenous compounds. Smart Bioremediation Technologies. *Academic Press*

RATTE, H., (1999). Bioaccumulation and toxicity of silver compounds: A review. *Environmental Toxicology and Chemistry*. **18** (1), 89-108.

REYES-CABRERA, J., ZOTARELLI, L., DUKES, M., ROWLAND, D., SARGENT, S., (2016). Soil moisture distribution under drip irrigation and seepage for potato production. *Agricultural Water Management*. **169**, 183-192.

RICH, S., WATT, M., (2013). Soil conditions and cereal root system architecture: review and considerations for linking Darwin and Weaver. *Journal of Experimental Botany*. **64**, (5), 1193-1208.

RIVERA, M., POLANIA, J., RICAURTE, J., BORRERO, G., BEEBE, S., RAO, I., (2019). Soil Compaction Induced Changes in Morpho-physiological

Characteristics of Common Bean. *Journal of Soil Science and Plant Nutrition*. **19**, 217-227.

RUSER, R., FLESSA, H., RUSSOW, R., SCHMIDT, G., BUEGGER, F., MUNCH, J., (2006). Emission of N_2O, N_2 and CO_2 from Soil Fertilised with Nitrate: Effect of Compaction, Soil Moisture and Rewetting. *Soil Biology and Biochemistry*. **38**, 263-274.

RYLSKI, I., RAPPAPORT, L., PRATT, H., (1974). Dual Effects of Ethylene on Potato Dormancy and Sprout Growth. *Plant Physiology*. **53**, 658-662.

SAKIN, E., (2012). Organic carbon organic matter and bulk density relationships in arid-semi arid soils in Southeast Anatolia region. *African Journal of Biotechnology*. **11** (6), 1373-1377.

SARQUIS, J. JORDAN, W., MORGAN, P., (1991). Ethylene Evolution from Maize (Zea mays L.) Seedling Roots and Shoots in Response to Mechanical Impedance. *Plant Physiology*. **96**, 1171-1177.

SCHEIBLE, W., LAUERER, M., SCHULZE, E., CABOCHE, M., STITT, M., (1997). Accumulation of nitrate in the shoot acts as a signal to regulate shoot-root allocation in tobacco. *The Plant Journal*. **11** (4), 671-691.

SMITH, C., JOHNSTON, M., LORENTZ, S., (2001). The effect of soil compaction on the water retention characteristics of soils in forest plantations. *South African Journal of Plant and Soil.* **18** (3), 87-97.

SONDEREGGER, T., PFISTER, S., (2021). Global Assessment of Agricultural Productivity Losses from Soil Compaction and Water Erosion. *Environmental Science and Technology*. **55** (18), 12162-12171.

STALHAM, M., ALLEN, E., HERRY, F., (2005). Effects of Soil Compaction on Potato Growth and its Removal by Cultivation. *British Potato Council*. Ref R261.

STALHAM, M., ALLEN, E., ROSENFELD, A., HERRY, F., (2007). Effects of soil compaction in potato (*Solanum tuberosum*) crops. *Journal of Agricultural Science*. **145**, 295-312.

STALHAM, M., ALLISON, M., FIRMAN, D., (2012). Improving cultivation practices in potatoes to increase the window of workability and soil structural stability. *British Potato Council*. Ref R452.

STEPANOVA, A., ALONSO, J., (2009). Ethylene signaling and response: where different regulatory modules meet. *Current Opinion in Plant Biology*. **12**, 548-555.

STRUCHTEMEYER, R., EPSTEIN, E., GRANT, W., (1963). Some effects of irrigation and soil compaction on potatoes. *American Potato Journal*. **40**, 266-270.

SUTTLE, J., (1998). Involvement of Ethylene in Potato Microtuber Dormancy. *Plant Physiology*. **118**, 843-848.

TANDONNET, J., COOKSON, S., VIVIN, P., OLLAT, N., (2010). Scion genotype controls biomass allocation and root development in grafted grapevine. *Australian Journal of Grape and Wine Research*. **16** (2), 290-300.

TARDIEU, F., LAFARGE, T., SIMONNEAU, T., (1996). Stomatal control by fed or endogenous xylem ABA in sunflower: interpretation of correlations between leaf water potential and stomatal conductance in anisohydric species. *Plant, Cell and Environment*. **19**, 75-84.

TARDIEU, F., ZHANG, J., KATERJI, N., BETHENOD, O., PALMER, S., DAVIES, W., (1992). Xylem ABA controls the stomatal conductance of field-grown maize subjected to soil compaction or soil drying. *Plant, Cell and Environment*. **15**, 193-197.

TAYLOR, H., BRAR, G., (1991). Effect of soil compaction on root development. *Soil and Tillage Research*. **19** (2-3), 111-119.

THEIVASIGAMANI, P., EPHRATH, J., LAZAROVITCH, N., (2021). Grafting of tomato (*Solanum lycopersicum* L.) onto potato (*Solanum tuberosum* L.) to improve salinity tolerance. *Scientai Horticulturae*. **282** (37), 110050.

THOMAS, H., FRANK, M., (2019). Connecting the pieces: uncovering the molecular basis for long-distance communication through plant grafting. *New Phytologist*. **223** (2), 582-589.

TIMM, H., FLOCKER, W., (1966). Responses of potato plants to fertilization and soil moisture tension under induced soil compaction. *Agronomy Journal.* **58**, 153-157.

TORBERT, H., WOOD, C., (1992). Effects of soil compaction and water-filled pore space on soil microbial activity and N losses. *Communications in Soil Science and Plant Analysis*. **23** (11&12), 1321-1331.

TRACY, S., BLACK, C., ROBERTS, J., *et al*., (2012). Quantifying the impact of soil compaction on root system architecture in tomato (*Solanum lycopersicum*) by X-ray micro-computed tomography. *Annals of Botany*. **110** (2), 511-519.

TSEGAYE, T., MULLINS, C., (1994). Effect of mechanical impedance on root growth and morphology of two varieties of pea (*Pisum sativum* L.). *New Phytologist*. **126** (4), 707-713.

UNKOVICH, M., BALDOCK, J., FORBES, M., (2010). Chapter 5 - Variability in Harvest Index of Grain Crops and Potential Significance for Carbon Accounting: Examples from Australian Agriculture. *Advances in Agronomy*. **105**, 173-219.

UNKOVICH, M., MCBEATH, T., MOODIE, M., MACDONALD, L., (2023). High soil strength and cereal crop responses to deeper tillage on sandy soils in a semi-arid environment. *Field Crops Research*. **291**, 108792.

VAN LOON, C., DE SMET, L., BOONE, F., (1985). The effect of a ploughpan in marine loam soils on potato growth. 2. Potato plants responses. *Potato Research*. **28,** 315-330.

Frontiers in Plant Science. **8** (38).

VANHEES, D., SCHNEIDER, H., SIDHU, J., *et al*., (2022). Soil penetration by maize roots is negatively related to ethylene-induced thickening. *Plant, Cell and Environment*. **45**, 789-804.

VISSER, E., NABBEN, R., BLOM, C., VOESENEK, L., (1997). Elongation by primary lateral roots and adventitious roots during conditions of hypoxia and high ethylene concentrations. *Plant, Cell and Environment*. **20**, 647-653.

VORHEES, W., (1987). Assessment of soil susceptibility to compaction using soil and climatic data bases. *Soil and Tillage Research*. **10** (1), 29-38.

VOS, J., OYARZUN, P., (1987). Photosynthesis and stomatal conductance of potato leaves -- effects of leaf age, irradiance, and leaf water potential. *Photosynthesis Research*. **11**, 253-264.
WANG, K., LI, H., ECKER, J., (2002). Ethylene Biosynthesis and Signaling Networks. *The Plant Cell*. **14** (suppl_1), S131-S151.
WHALLEY, W., CLARK, L., GOWING, D., COPE, R., LODGE, R., LEEDS-HARRISON, P., (2006). Does soil strength play a role in wheat yield losses caused by soil drying? *Plant and Soil*. 280, 279-290.
WHALLEY, W., ZHANG, X., WHALLEY, P., *et al*., (2020). A comparison between water uptake and root length density in winter wheat: effects of root density and rhizosphere properties. *Plant and Soil*. **451**, 345-356.
WHITE, P., BROADLY, M., HAMMOND, J., THOMPSON, A., (2005). Optimising the potato root system for phosphorus and water acquisition in low-input growing systems. *Aspects of Applied Biology*. **73**, 111-118.
WHITE, P., WHEATLEY, R., HAMMOND, J., ZHANG, K., (2007). Potato Biology and Biotechnology: Advances and Perspectives Chapter 34: Minerals, Soils and Roots. Elsevier Science.
WISHART, J., GEORGE, T., BROWN, L., RAMSAY, G., BRADSHAW, J., WHITE, P., GREGORY, P., (2013). Measuring variation in potato roots in both field and glasshouse: the search for useful yield predictors and a simple screen for root traits. *Plant and Soil*. **368** (1-2), 231-249.
WOLFE, D., TOPOLESKI, D., GUNDERSHEIM, N., INGALL, B., (1995). Growth and Yield Sensitivity of Four Vegetable Crops to Soil Compaction. *Journal of the American Society for Horticultural Science*. **120** (6), 956-963.
WU, Q., PAGES, L., WU, J., (2016). Relationships between root diameter, root length and root branching along lateral roots in adult, field-grown maize. *Annals of Botany*. **117** (3), 379-390.
XUE, R., SHEN, Y., MARSCHNER, P., (2017). Soil water content during and after plant growth influence nutrient availability and microbial biomass. *Journal of Soil Science and Plant Nutrition*. **17** (3), 702-715.
YAMAGUCHI, J., TANAKA, A., (1990). Quantitative observation on the root system of various crops growing in the field. *Soil Science and Plant Nutrition*. **36** (3), 483-493.
ZHU, C., GAN, L., SHEN, Z., XIA, K., (2006). Interactions between jasmonates and ethylene in the regulation of root hair development in *Arabidopsis*. *Journal of Experimental Botany*. **57** (6), 1299-1308.

Appendix – Chapter 4 weather data

Calendar Date	*Trial 1 Precipitation (mm)*	*Trial 1 Mean Temperature (°C)*	*Trial 2 Precipitation (mm)*	*Trial 2 Mean Temperature (°C)*
17/05	2	10.025	0	17.19
18/05	16	10.654	4.2	16.16
19/05	4	11.425	4.8	14.41
20/05	16	8.9042	0.2	13.36
21/05	9	10.529	0	13.47
22/05	0	8.5875	0	15.1
23/05	8	9.1417	2	14.53
24/05	14	9.0417	4.6	12.27
25/05	4	9.6708	0	13.28
26/05	1	9.7	3.6	13.51
27/05	0	11.546	0	12.47
28/05	0	13.35	0	10.42
29/05	0	15.642	0.4	8.44
30/05	0	11.692	2	9.24
31/05	0	13.813	10.2	9.9
01/06	0	15.029	0	11.15
02/06	0	16.471	0	11.62
03/06	0	17.492	0	12.75
04/06	0	14.788	0	12.07
05/06	0	16.838	14.8	10.53
06/06	0	15.713	5.6	11.72
07/06	19	17.092	0	13.04
08/06	0	18.021	1.4	16.15
09/06	0	18.971	0	15
10/06	0	19.179	0	16.75
11/06	0	18.492	0	16.15
12/06	0	16.483	0	14.86
13/06	0	18.896	0	14.25
14/06	0	18.258	0	15.19
15/06	0	15.6	4.2	17.03
16/06	0	20.467	3.8	19.36
17/06	23	16.225	0	21.83
18/06	36	12.533	4.4	13.8
19/06	1	13.196	0.2	12.58
20/06	2	12.492	0	13.63
21/06	0	12.254	0	16.21
22/06	0	12.496	0	18.17
23/06	0	14.175	0	17.67
24/06	0	16.521	0	16.87
25/06	9	13.663	0.4	16.65
26/06	0	13.929	0	15.57

27/06	0	14.525	0.4	13.69
28/06	1	13.208	0.4	14.51
29/06	1	14.563	1.4	16.7
30/06	0	12.754	0	15.23
01/07	0	13.671	0	13.91
02/07	0	16.792	0.8	14.01
03/07	5	17.475	0.6	14.01
04/07	5	18.192	0	14.35
05/07	1	16.617	0	14.92
06/07	26	15.688	0	17.1
07/07	8	16.975	0	17.91
08/07	0	18.733	0	18.81
09/07	0	18.654	0	17.89
10/07	1	18.15	0	18.47
11/07	1	16.771	0	20.21
12/07	3	16.667	0	21.19
13/07	0	16.588	0	18.92
14/07	0	17.554	0	14.62
15/07	0	16.446	0	16.19
16/07	0	17.867	0	17.22
17/07	0	21.525	0	20.83
18/07	0	21.458	0	24.37
19/07	0	20.1	0	26.63
20/07	0	21.479	0.4	21.61
21/07	0	20.079	0	18.47
22/07	0	19.971	0	16.01
23/07	0	15.908	0	19.95
24/07	0	16.642	1.6	22.09
25/07	0	17.146	0.6	18.78
26/07	0	19.471	0	15.07
27/07	8	17.083	0.6	16.8
28/07	11	15.683	1.8	16.77
29/07	0	15.617	0	16.33
30/07	40	13.808	0.2	19.06
31/07	0	16.579	10.4	18.56
01/08	1	14.338	0	18.81
02/08	0	15.325	1.6	22.37
03/08	0	14.767	0	21.8
04/08	0	17.092	0	17.3
05/08	13	16.167	0	14.29
06/08	1	16.996	0	14.37
07/08	5	16.233	0	16.51
08/08	9	15.692	0	17.46
09/08	0	16.358	0	18.46
10/08	0	16.438	0	18.02
11/08	0	18.183	0	19.17

12/08	0	18.767	0	19.23
13/08	0	18.454	0	20.18
14/08	0	17.571	0	20.59
15/08	0	17.738	0	19.18
16/08	0	15.004	40.2	17.76
17/08	0	15.708	4.6	16.52
18/08	0	16.996	0	17.41
19/08	1	17.108	0	11.19
20/08	0	18.704	0	10.36
21/08	2	17.054	0	10.91
22/08	1	16.742	0	11.11
23/08	0	15.938	0.8	11.81
24/08	0	15.8	0	12.01
25/08	0	15.833	22.2	10.54
26/08	0	14.95	0.2	9.66
27/08	0	13.383	0	10.04
28/08	0	14.429	0	9.99
29/08	0	13.304	0	10.81
30/08	0	14.338	0.8	9.78
31/08	1	13.683	0	10
01/09	1	13.983	0	10.33
02/09	0	15.008	0	10.8
03/09	0	14.479	0	11.58
04/09	0	14.838	0	11.36
05/09	0	15.875	0	14.71
06/09	0	19.633	7	20.08
07/09	0	20.321	2.2	19.22
08/09	0	20.113	3	18.31
09/09	0	20.35	1.2	16.53
10/09	0	19.992	1.4	18.85
11/09	0	19.133	0	19.41
12/09	0	14.863	1.6	18.91
13/09	0	15.096	0	18.72
14/09	0	13.508	0	16.8
15/09	0	15.708	0	13.71
16/09	0	15.85	0	11.79
17/09	0	14.996	0	11.47
18/09	0	17.504	0	12.23
19/09	0	16.133	0	11.53
20/09	0	14.671	0	11.94
21/09	0	15.975	0	14.11
22/09	0	16.008	0	15.02
23/09	0	16.883	3	13.84
24/09	0	17.088	1	12.51
25/09	0	18.246	0	12.1
26/09	0	17.079	4.2	10.97