Does genotypic variation in ethylene sensitivity affect crop response to soil compaction?

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Declaration

I declare that the work contained within this thesis, unless cited or otherwise stated, has been originally produced by myself and has not been previously submitted for the award of a higher degree at any other institution.

Lisandra Hancke

Lancaster, UK, September 2024

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Abstract

Soil compaction limits crop growth and yields and is an increasing problem in modern agriculture as farming machinery has become heavier. Compacted soils have less pore space causing ethylene to accumulate and limit crop growth. This research aimed to identify whether (wheat, potato, and tomato) crop sensitivity to soil compaction in different cultivars depended on their sensitivity to ethylene. Ethylene sensitivity was assessed in wheat and tomato by placing pre-germinated seeds in Petri-dishes with 100 µmol of ethephon and allowed to grow until the roots were measured and the percentage reduction in root length calculated. In sprouted potato, minitubers (<20 mm) were placed into containers with 20 ppm of ethylene and root length measured to calculate percentage reduction in root length. Plants were grown in soils of varying levels of compaction, and a range of root and shoot morphological variables measured to identify whether varieties with higher sensitivity to ethylene responded more to compaction. Greater growth inhibition with compaction was positively related to ethylene sensitivity in most of the cultivars studied. Nevertheless, there were some unexpected results with an ethylene sensitive wheat cultivar showing increased growth under high compaction in an ethylene sensitive cultivar of wheat and also in an ethylene sensitive mutant of tomato. Overall, this research has demonstrated the importance of compaction effects on crop shoot and root growth and that these responses rely on ethylene sensitivity, and also that utilising less ethylene sensitive cultivars/genotypes may allow adaptation to increasing compaction.

Glossary

Acronym	Meaning
ABA	Abscisic Acid
AdoMet/ SAM	S-adenosyl-L-methionine
AC	Ailsa Craig
ACC	1-aminocyclopropane 1-carboxylic acid
ACO	ACC Oxidase
ACS	ACC Synthase
BD	Bulk Density
BRs	Brassinosteroids
Br8	Breaker + 8 days
CTF	Controlled Traffic Farming
CTR1	CONSTITUTIVE TRIPLE RESPONSE 1
EBF1	EIN3 BINDING F-BOX PROTEIN 1
EBF2	EIN3 BINDING F-BOX PROTEIN 2
ERS1	ETHYLENE RESPONSE SENSOR 1
EIL1	EIN3-LIKE 1
EIN2-CEND	EIN2-C-terminal
EIN4	ETHYLENE INSENSITIVE 4
ERF	ETHYLENE RESPONSE FACTOR
ERS2	ETHYLENE RESPONSE SENSOR 2
ETRs	Ethylene Receptors
ETR1	Ethylene Receptor 1
ETR2	ETHYLENE RESPONSE 2
Fe	Iron
GFP	Green Fluorescence Protein
IAA	Indole-3-acetic acid

Met	L-methionine
MG	Mature Green
MT	Micro-Tom
MTA	5’-methylthioadenosine
N	Nitrogen
Nr	Never-ripe
OMC	Optimum Moisture Content
PGRs	Plant Growth Regulators
PLP	Pyridoxal-5’-Phosphate
POD	Peroxide
PPFD	Photosynthetic Photon Flux Density
PRM	Parallel Reaction Monitoring
ROS	Reactive Oxygen Species
RSA	Root System Architecture
RTF	Random Traffic Farming
SOD	Superoxide Dismutase
SOM	Soil Organic Matter
SR	Soil Resistance
TAA1/TAR	TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS-1/ AMINOTRANSFERASE-
TIR1/AFB	TRANSPORT INHIBITOR RESPONSE1/ AUXIN SIGNALLING F-BOX
WT	Wild-type
YUC	YUCCA

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1. General Introduction

1.1. Introduction to soil compaction

Soil compaction is a form of physical soil degradation (Nawaz et al., 2013) and indicates that soil particles are pressed together and thus pore spaces between them are reduced (Correa et al., 2019). Soil compaction results in the formation of dense soil layers in the subsoil and in the topsoil (Nortjé et al., 2012), and is influenced by a variety of factors including: vehicular traffic, soil water content, soil structure, soil texture, and soil organic matter (SOM) (Nawaz et al., 2013). Soil compaction is a global problem, affecting 68 million ha of soil worldwide (Nawaz et al., 2013), and costs England and Wales £468 million a year (Graves et al., 2015). Compaction causes late and low germination, stunts root and shoot growth, and decreases soil biodiversity which is damaging to soil health and productivity (Mueller et al., 2010), and therefore crop performance.

Vehicular traffic is a large contributor to soil compaction as heavy farming machinery (e.g., tractors that pull ploughs, harrows, fertiliser spreaders, balers, trailers, sprayers, cultivators) used in planting and growing a field of crop, and harvesters to collect the crop, all exert pressure on the soil. All of this machinery has gradually become larger in size and therefore impacts soil compaction to a greater extent (Raper, 2005) by decreasing soil pore size which limits the diffusion of available oxygen, water, and nutrients. While tillage seems to solve this problem, mechanically loosening the soil means it no longer has the structural stability it needs to withstand traffic (Raper, 2005). Alternative mitigation methods to reduce the soil area that is being compacted include tram lines/ designated lanes which are tracks used by vehicles that pass through crop fields multiple times during growth (such as sprayers), axle load reduction (Raper, 2005; Botta et al., 2022), and different types of wheels/ tyres, for example ‘zero-wheel’ harvesters with continuous tracks and weight distribution alterations through modified intake unit placement (Kolchin et al., 2019; Zhaoguo et al., 2021).

Soil compaction changes with soil water as soils with higher water content show higher levels of compaction and are closer to their plastic limit (To and Kay, 2005; Hu et al., 2020), possibly due to the lubricating effects water has on soil particles which allows them to move past one another and increase soil density (Rahmat and Ismail, 2018). Decreased pore space also diminishes soil water holding capacity and the unsaturated conductivity compared to non-compacted soil (Tubeileh et al., 2003; Correa et al., 2019). Unsaturated soil conductivity is a function of water content (Gallage et al., 2013) and refers to the ability of soil to transmit water when the pores are partially filled with both air and water and is influenced by soil texture, moisture content, soil particle size and soil structure (Ramli et al., 2021). Thus, soil compaction can be induced by a variety of factors and is negative to plant growth by reducing pore spaces and increasing hypoxic conditions.

Soil compaction is measured in both bulk density, which refers to the weight of dry soil to volume of soil and is measured in g cm^{-3} (Correa et al., 2019), and penetrometer resistance (measured with a penetrometer fitted with cones of various sizes) which shows the level of soil resistance or strength faced by roots (Kolb et al., 2017; Correa et al., 2019) and is often measured in MPa. Both are referred to in the literature, however it may be beneficial to report both values as they can vary depending on the type of growth medium that is used, for example soils containing more clay are often more compaction susceptible than soils containing more sand.

Soil structure and texture influences compaction in that particle size and behaviour of those particles can cause alterations in soil strength at the same soil density as other soils. For example, research has identified that soils with higher clay content can have a higher soil strength than soils with less clay at the same density (Atwell, 1993). This outcome is observed because soils with higher clay contents have larger contact areas between particles compared to soils with high sand content (Khan et al., 2012; Correa et al., 2019). Clay soils are more restrictive than sand soils, for example, in clay soils root growth is inhibited at 1.47 g cm^{-3} and 1.85 g cm^{-3} in sandy soils (Correa et al., 2019). Although other research concluded that clay soils remain mechanically weaker over a longer period of time as they dry (Whalley et al., 2006) . Some research does suggest that light-to-moderate levels of compaction may benefit crop growth because this can increase the level of contact between roots and the soil, making water and nutrient uptake easier for plants (Sojka, 1993; Tracy et al., 2012).

Soil organic matter (SOM) improves soil aggregation, increases the number of failure zones, reduces strength, and increases the formation of micro-cracks (Correa et al., 2019). For example, increased application of compost and manure decreases soil compaction with the addition of 8% (~ 200 t/ha) organic matter decreasing soil compaction by 10% and is especially effective in clay loam soils compared to loam soils (Shahgholi and Janatkhah, 2018).

The introduction of heavy machinery in the 20th Century, with giant tractors becoming popular around 1955 (Smith, 2020), had a large impact on compaction sparking a need for research on the mechanisms involved (Håkansson and Medvedev, 1995; Håkansson and Voorhees, 2020). An example is the weight of farming machinery, such as harvesters, as technologies become more complex and sophisticated, the weight of machinery increases (Andersen et al., 2013) and thus the level of compaction they cause increases. The benefit of developing new technology (e.g., harvesters that can harvest and sort crops by size at the same time) is that crop growth becomes more efficient and requires less processes, and farming equipment can carry out almost every task without significant human intervention (e.g., most modern harvesters work using GPS, have various sensors to ensure optimal harvest, and can grade produce all at once (Astanakulov et al., 2021)), although there can be negative consequences. Since increase in yield is not progressing at the level required to meet global demand and mechanical methods to overcome soil compaction have not been entirely successful, it is important to understand plant response to help us understand compaction to a greater degree.

1.2. The importance and impact of soil compaction on crop growth

Wheat (*Triticum aestivum*) is a major UK arable crop and has been grown on the British mainland for millennia, with it becoming a staple in many households in the 19th century. The UK produces around 2% of global wheat (Harkness et al., 2020) with 13,988,000 t produced in 2021 (FAO, 2021). The advantage of growing wheat in the UK is the favourable weather conditions (Harkness et al., 2020) and, as such, high average yields can be obtained; in 2021 an average yield of 7.37 t ha^{-1} was achieved, compared to a world average of 3.42 t ha^{-1} (FAOSTAT, 2021). The yield of wheat has gradually increased by around 100 kg ha^{-1} yr^{-1} from the 1950s and a 217 grains m^{-2} yr^{-1} increase occurred between 1972 to 1995 (Shearman et al., 2005); this was most likely due to the improvement of plant growth technology such as selective breeding and genetic engineering (Mackay et al., 2011). Unfortunately yields have plateaued at around 8 t ha^{-1} and the necessary 2.4% increase per annum in yield to meet demand is not being met (current yield increase is 0.9% per annum) (Hall et al., 2023). Researching possible reasons for low yield increases with a link to early growth, such as factors like soil compaction and ethylene (C_2H_4) sensitivity, are highly useful and necessary to aid in meeting demand whilst also remaining aware of the impact farming has on the environment.

Potatoes (*Solanum tuberosum*) were first grown in the UK towards the end of the 16th century with production peaking in England and Wales between the 18th and 19th centuries. Potatoes are a staple in many UK households with an average consumption of ~100 g/day for adults (Weichselbaum, 2010) and sit just behind wheat, corn, and rice in worldwide consumption (Soare and Chiurciu, 2021). Potato, like wheat, has increased in yield owing to improved farming practices and fertilisers due to the "Green Revolution" (Stokstad, 2019). Even though the field area allocated to potato growth has more than halved between 1961 to 2021, the yield of potato has increased from 22 t ha^{-1} in 1961 to 38.7 t ha^{-1} in 2021 (FAOSTAT, 2021). The problem is that the same varieties are often grown continuously and have remained unchanged for a long time, this is due to the slow process of producing new varieties of potato, but recent technology has provided innovative and time-saving methods to produce new potato cultivars (Stokstad, 2019) that perform better. This is a large reason for a need to investigate ethylene sensitivity and soil compaction in various potato varieties as these traits can be selected for or against to increase yields even more and to meet demand.

Tomatoes (*Solanum lycopersicum*) are not a widely grown crop in the UK with only 195 ha of land harvested for tomato in 2021 (FAOSTAT, 2021), the largest consumer and producer of fresh and processing tomatoes is China who represent one-third of global production (Subramanian, 2016). Tomato yield has increased globally due to increased disease resistance, improved nutrient content, and utilisation of CRISPR systems for genetic alterations (Hanson, 2016; Wang et al., 2019). Much like wheat and potato the demand for tomato is increasing at steep rates depending on global location, for example, India experienced a 30% increase in tomato consumption annually around 2016 (Subramanian, 2016; Costa and Heuvelink, 2018). Growing demand requires increased growth/ yield and with research focusing on areas such as soil compaction and the role ethylene plays in root response, and thus yield (as the roots of any plant influence growth, health, and yield), the gap between supply and demand can be reduced.

Root system architecture (RSA) is the spatial arrangement of roots (Correa et al., 2019) within the area of soil the plant inhabits and influences the exploration of roots in soil. RSA plasticity is a result of both plant environment and genetics and is dependent on root curving, elongation, and branching (Rich and Watt, 2013; Correa et al., 2019) which are influenced by hormones such as auxins, cytokinins, and ethylene (López-Bucio et al., 2003). RSA plasticity, or phenotypic plasticity, is a process used by plants to allow them to adapt to the ever-changing environment (El-Soda et al., 2014). Soil compaction limits water and nutrient uptake by changing RSA, in most cases higher compaction leads to shallow roots (**Figure 1.2.1**) that are unable to reach deeper layers with high water and nutrient content (Correa et al., 2019), and soil strength and density significantly influence RSA wherein higher soil density leads to a greater soil volume within a confined space, consequently increasing the strength and resistance of the soil.

RSA is highly important in water uptake of crops and influences water accessibility, which is the ability of roots to reach areas of water in deeper layers of soil (Colombi et al., 2018). Water accessibility is mainly influenced by soil penetration resistance because this factor can alter the rate of elongation (Colombi et al., 2018) of roots by providing restriction, whether this be mechanical or not, and increases the force that is required to penetrate layers of increased compaction. The increased resistance leads to shallower, thicker, and less roots (Colombi et al., 2018; Patel et al., 2020), as well as reduced growth of the shoots (**Figure 1.2.1**) (Colombi et al., 2018; Gürsoy, 2021; Shaheb et al., 2021). These shallow roots are therefore forced to obtain their water from layers closer to the surface, which is often revealed by increased topsoil drying in areas where plants are growing in soils of higher resistance (Colombi et al., 2018).

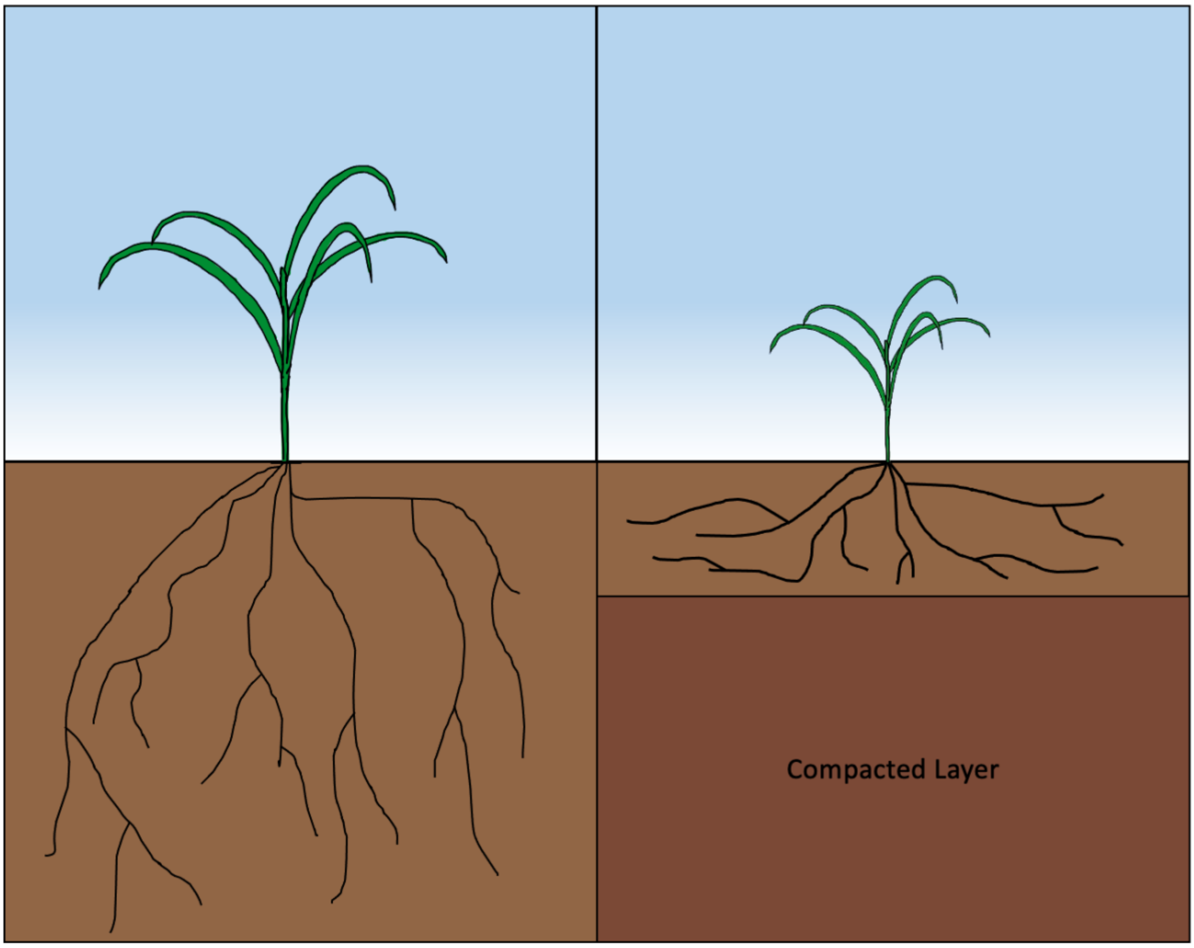

Figure 1.2.1 graphic representation of the difference often seen in RSA in uncompacted soil (left) compared to compacted soil (right). Under compacted soils the roots are shallower and thicker due to the effects of increased soil strength and density, and the shoots show reduced growth as a result of various factors caused by high compaction, e.g., root-to-shoot signalling between ACC and gibberellins.

RSA is not only important for water uptake, but also for nutrient uptake, such as Nitrogen (N), which is taken up as nitrate, ammonium, and organic N (Jia and von Wirén, 2020). Nitrogen can impact RSA in that it increases lateral root branching and elongation by influencing signalling pathways controlling auxin and meristematic activity (Jia and von Wirén, 2020). This also could be a factor influencing the growth of roots in soils of high compaction by limiting root ability to reach nutrients such as N. High compaction often results in increased root diameter (Chen et al., 2014; Lynch et al., 2022; Vanhees et al., 2022) in order to increase the strength and ability of roots to explore their surroundings and absorb water and nutrients, however, some research suggests that thinner roots have a higher surface area than thicker roots due to interactions between phosphorus (P), iron (Fe), and root hairs (Wang et al., 2006).

RSA not only influences root growth and distribution but also has an effect on shoot growth and other above-ground aspects, such as seed number, offspring survival, fruit growth rate, biomass, and early shoot growth (Younginger et al., 2017). Phenotypic changes in this context can be highly complex and result from an array of factors, however, adaptive plasticity through changes in phenotype must also have a genetic component and result in a beneficial change that increases the fitness of the plant (Correa et al., 2019) (such as being larger, which results in higher seed production, and, consequently, greater offspring production).

Adaptive plasticity confers tolerance/ resistance, which can be defined as the ability of a plant to maintain fitness while experiencing environmental stress (Correa et al., 2019), but what is environmental stress? Environmental plant stress can be described as any disadvantageous condition, or substance, that has an impact on plant growth, metabolism, or development (i.e., any adverse event that leads to cell or plant death) (Lichtenthaler, 1996); however, it is important to note that plant stress is defined differently throughout the literature and varies depending on whether the definition focuses on cause or effect (Wallace, 1986; Souza and Cardoso, 2003; Blum, 2015).

Stress causes strain which is the phenotypic expression of stress (Correa et al., 2019), for example, the main strain under compaction stress is reduced root and shoot growth. Another term used when discussing plasticity is resilience, which is the ability of a plant to reverse the damage caused due to stress (Correa et al., 2019) and can be referred to as an elastic response (Blum, 2015). When growth occurs in environments of low nutrient supply (such as in a highly compacted soil environment) up to 52% of the carbohydrates produced through photosynthesis during the day are used by the roots and continues to increase as growth rate falls (Lambers et al., 2002), this is an example of a negative consequence that may occur due to adaptive plasticity.

In some cases adaptive plasticity may be a result of ontogeny/ allometric growth or ‘apparent plasticity’, for example, increased root to shoot ratio occurs in highly compacted soils, but is also observed during early plant growth and could indicate that plants grown in soils of higher compaction are simply growing slower, and not less, than those in soils of lower compaction (Correa et al., 2019). This is important to distinguish from true plasticity because it could result in inaccurate assumptions linked to phenotypic changes to the environment, and could be unfavourable when looking at methods of mitigation to alter plant growth or yield in specific ways (Xie et al., 2015). Apparent plasticity does not increase the fitness of a plant through environment interactions, it is simply as a result of the environment impacting the behaviour of a plant in some way; in other words, the plant is reacting to the environment but not altering its growth in any way to reduce the impact of these environmental factors. An example of this is the change in plant mass and height, which shows a curvilinear relationship, as a plant becomes larger the mass of it increases more than the height does (Weiner, 2004).

1.3. Methods of mitigation and soil compaction avoidance

Soil compaction impacts crop growth, crop yield, and crop health to a significant scale, for example, soil compaction can reduce yield by up to 50% depending on soil properties and crop type (Shaheb et al., 2021). This means that methods to mitigate or methods to avoid these effects are needed, especially with global food supply struggling to meet the growing demand (see **chapter 1.2**). Some examples of methods of mitigation include: the use of simplified tillage techniques over conventional tillage (depending on crop type and soil properties), the use of lighter machinery, addition of SOM, and subsoiling (especially in areas that are prone to soil compaction, e.g., in the nine provinces of South Africa (Laker and Nortjé, 2020)) (Chamen et al., 2015; Orzech et al., 2021). Some methods of avoidance include: low ground pressure tyres, controlled traffic farming (CTF), and tracked tractors (Chamen et al., 2015). Both processes are effective in various ways but may have some costs that need to be analysed on an individual basis when deciding which strategies to use, and factors such as soil texture and composition must be considered as these techniques can be specific to certain types of soil.

Conventional tillage includes techniques that require multiple passes through a field with harrows and ploughs that result in soil that is very loose and smooth, however, conventional tillage results in soils with decreased organic matter, soil erosion, and poor soil structure (Raper, 2005) which subsequently increases compaction in later growth stages (Orzech et al., 2021). Simplified tillage often focuses on reduced tillage by either direct drilling or tilling shallow layers where seeds are to be sowed, this results in reduced soil disturbance and lower levels of compaction (Orzech et al., 2021). Simplified tillage increases soil fertility by exerting less of a detrimental effect on SOM (Szostek et al., 2022), soil carbon (Aziz et al., 2013) and hydraulic conductivity, and may also be beneficial in reducing greenhouse gas emissions by reducing the volume of fossil fuels used to till a field (Dayou et al., 2017). A consequence of simplified tillage or reduced tillage is the formation of a plough pan/ no-till pan/ tillage pan just below the reach of simplified tillage machinery (Schlüter et al., 2018), this area of highly compacted soil may have negative effects on crop growth, however, these may be offset by the beneficial effects of simplified tillage.

Theoretically the use of lighter machinery should reduce the level of compaction caused by machinery used in agriculture, which is true, but only to some extent. Lighter tractors do indeed cause less compaction (increase bulk density to a lesser extent) than heavier tractors between depth ranges of 300 – 600mm (Jorajuria et al., 1997; Chamen et al., 2015) when passed over a field the same number of times. However, it was also discovered that the lighter tractor of 2.3 Mg increased bulk density to the same extent as the heavier tractor of 4.2 Mg with more passes, and could increase bulk density more than the heavier tractor (Jorajuria et al., 1997). Therefore, with increasing numbers of passes, regardless of tractor weight, compaction increases (Botta et al., 2006). This means that care must be taken when using lighter machinery to reduce compaction, especially if the lighter machinery would require more passes due to, e.g., the use of smaller ploughs or harrows as a consequence of lighter machinery with reduced power.

SOM is essential in altering soil aggregation, nutrient exchange, soil compaction, soil moisture and water retention, and soil conductivity (Gurmu, 2019). The addition of organic matter has been identified as an effective technique to reduce compaction (Kumar et al., 2009; Shahgholi and Janatkhah, 2018; Bashir et al., 2021). Practices that increase SOM are able to increase soil aeration, reduce waterlogging, and increase root penetration by reducing mechanical impedance (King et al., 2020). The addition of SOM is most effective in soils that are highly susceptible to compaction, such as soils with high clay content and in soils that are below or close to their optimum moisture content (OMC) (Zhang et al., 1997). Although the effectiveness of some soil organic matter addition techniques are argued, such as the application of straw (Powlson et al., 2011; Chamen et al., 2015), research generally suggests that adding organic matter is beneficial and identifying any techniques to increase crop performance are essential to keep up with demand (Chamen et al., 2015; Bashir et al., 2021).

Subsoiling a field can cost between £49/ha - £56.10/ha in the UK (Chamen et al., 2015) but has been identified as a reasonably successful strategy to reduce compaction, for example, plant height, root density, bulk density, and yield were all found to be improved in subsoiled compacted areas when compared to compacted areas that were not subsoiled in clay loam soils (Abu-Hamdeh, 2003). Some research has found that subsoiling is sometimes only beneficial when looking at root penetration and does not have any significant effect on yield, such as a study in Sweden in which the addition of SOM (straw pellets) and subsoiling did not increase yield but did show improved root penetration (using pilot equipment, however, that is still under development) (Piccoli et al., 2022). However, in the same study in a different location (Romania) subsoiling was effective at alleviating soil compaction and also crop yield, this could have been due to different soil types or the presence of land ice in Sweden and not in Romania (Piccoli et al., 2022). In potato the effects of soil compaction are greatest 3-4 weeks after emergence with higher soil resistance reducing tuber yield and size and a 2-4 week delay in root elongation (Ghosh and Daigh, 2020). Subsoiling is effective at reducing compaction and improving potato production, especially in fields that regularly experience drought, but only in soils that have sufficient strength and should be avoided in weak soils such as high silt soils (Ghosh and Daigh, 2020).

The use of low ground pressure tyres works by ensuring a more optimal load distribution in farming machinery, but the research has suggested that only the stress applied to topsoil layers is reduced using this avoidance technique, whereas subsoil layers are more influenced by load (Botta et al., 2008; Söhne, 2014; Shaheb et al., 2021). Other research found that soil stress was reduced to a depth of 0.6 m in a fine-textured soil when the tyre-soil contact patch was increased and tyre inflation decreased, with the tyre with the largest tyre-soil contact patch increasing air permeability up to 0.3 m (ten Damme et al., 2019). Traffic system experiments on a field scale often do show a benefit of increased yield when using low ground pressure techniques (Vermeulen and Klooster, 1992).

CTF is a technique used to reduce compaction by confining farm traffic to designated lanes to carry out necessary tasks to grow crop, CTF may also utilise GNSS or GPS technology to ensure that farm traffic remains within the designated traffic lanes. The effectiveness of CTF, again, depends on the soil type with a £18/ha^{-1} yield reduction observed in sandy loam soil and a £25/ha^{-1} yield increase found in clay loam soil (Chamen et al., 2015). In contrast to this, other research has found that CTF compared to random traffic farming (RTF) in Denmark increased crop yield of potato, winter squash, beetroot, and white cabbage by 70%, 43%, 42%, and 27% in sandy loam soils respectively (Hefner et al., 2019). Some research has suggested that CTF on average can increase yields by 22% in loam soil, 19% in clay soil, and 8% in silt soils for root crops (Chamen et al., 2015). CTF is not only useful at increasing yield due to the confinement of farming machinery to specific tracks and therefore compaction, but also reduces the use of fossil fuels, increases productivity and efficiency, reduces waterlogging, increases drainage, improves SOM levels (Vermeulen et al., 2010), reduces soil nitrous oxide, soil ammonia, and soil methane emissions (Gasso et al., 2013).

Tracked tractors/ track tractors/ crawler tractors are vehicles that are fitted with continuous tracks rather than individual wheels that are used conventionally, this method would theoretically increase the area of contact between the machinery and the soil, creating optimal load distribution and decrease the level of compaction caused by the equipment. When looking at tracks versus tyres the pressure exerted on the ground by a parked tracked tractor is 4-8 psi compared to radial tyres which exert a pressure of 0.5 – 1 kg higher than the proper inflation pressure (Kumari et al., 2020). Another benefit of tracks is increased traction and decreased slip which may be beneficial in wet conditions (Arvidsson et al., 2011; Andreev and Vantsevich, 2017; Boland et al., 2022). Research looking at a comparison between standard tyres, water-ballasted tyres, half-tracked, and fully-tracked tractors identified that full rubber tracks had less of an impact on soil compaction and also had increased tractive efficiency when compared to the other tyre configurations (Molari et al., 2012).

1.4. Introduction to Ethylene

Ethylene is a gaseous plant hormone that plays a role in many plant processes such as seed germination, fruit ripening, senescence, root growth and development, flowering, and abiotic stress responses (Schaller and Kieber, 2002), ethylene is also a hormone that regulates growth and development processes under both optimal and stressful conditions as it can be considered a stress hormone (Fatma et al., 2022). Ethylene has been suggested as the main cause for the changes observed in RSA in highly compacted soils, due to the reduced diffusion of ethylene in less aerated soils that sends signals to the roots to reduce their growth, thus it is not the mechanical impedance that changes root growth but the presence of root signals (Pandey et al., 2021).

Ethylene was discovered during the 19th and 20th Centuries almost by accident, illuminating gas from coal was used for lighting and leaks from these pipelines carrying illuminating gas caused premature abscission and senescence in plants within the vicinity. Dimitry Neljubov concluded that ethylene was causing this response using the triple response in 1901 (Schaller and Kieber, 2002; Kieber and Schaller, 2019), and in 1904 Richards and MacDougal found that carbon monoxide results a similar response (Sisler, 2018), then in the 1930s Gane and colleagues identified ethylene as an endogenous product of plant metabolism (Schaller and Kieber, 2002).

The effects of ethylene on plant growth and development were recorded around 1980 as well as the biosynthesis of ethylene (Kende, 1993). Much of early ethylene research was conducted in *Arabidopsis* due to the ease of genetic dissection of transduction pathways (Schaller and Kieber, 2002). The most commonly used method to identify the effect of ethylene on plant growth and development is the triple response which involves shortening and thickening of roots and hypocotyls and exaggerated apical hooks (this is what should be observed when seeds are grown in the dark) (Merchante and Stepanova, 2017) after seeds are administered the ethylene precursor 1-Aminocyclopropane 1-carboxylic acid (ACC). Ethylene mutants are identified by observing dicot seedlings that do not show the triple response and usually fall into two categories, mutants that are insensitive to ethylene and mutants with a constitutive ethylene response (Schaller and Kieber, 2002).

Ethephon ($C_2H_6ClO_3P$) is a synthetic plant growth regulator (2-chloroethylphosphonic acid) often used in agricultural practices to control lodging (bending over of the stems near ground level, which can drastically reduce harvest yield), fruit ripening, leaf shedding, flower induction and plant height while increasing yield (Tripathi et al., 2004; Mutlu and Öktem, 2017). Ethephon was used in this research to investigate ethylene sensitivity as the ethephon is converted into ethylene by the plant and can be used in liquid form instead of as a gas, which can make some assays simpler.

1.5. Ethylene production and signalling

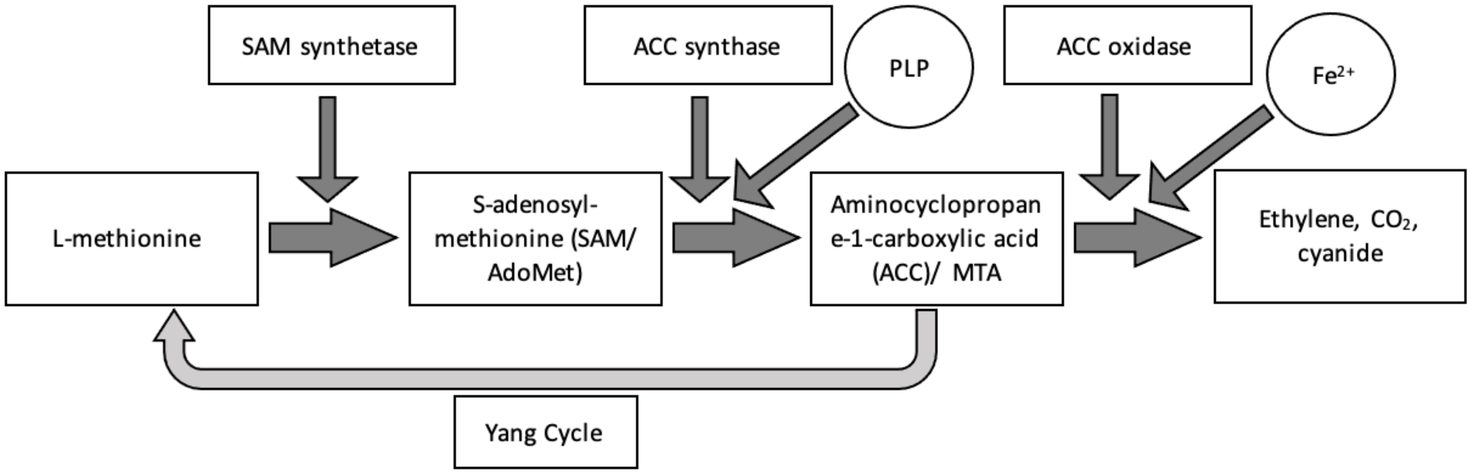

Figure 1.5.1 ethylene biosynthesis in a diagrammatic format, this figure is adapted from (Hegelund et al., 2017).

The gaseous hormone ethylene is derived from L-methionine (L-Met) (Hu et al., 2017) (see **Figure *1.5.1*** for a diagram of ethylene synthesis) which is converted into S-adenosyl-L-methionine (AdoMet or SAM) by AdoMet/SAM synthetase using ATP (Hu et al., 2017) and then into ACC by ACC synthase (ACS) (Pattyn et al., 2021), this step uses pyridoxal-5'-phosphate (PLP) cofactor (Hu et al., 2017). An alternative route that can be followed is called the Yang Cycle in which a recycling process is completed where SAM is converted into 5'-methylthioadenosine (MTA) by ACS and then recycled into Met (Yang and Hoffman, 1984; Hu et al., 2017), the Yang Cycle occurs at higher proportions during fruit ripening, leaf abscission, flower senescence, heat stress, water stress, and as a result of hormonal cues (Yang and Hoffman, 1984). ACC is then oxidatively cleaved into ethylene, CO_2, and cyanide by ACC oxidase (Harkness et al.) (Hu et al., 2017) through the involvement of a ferrous ion (Fe^{2+}), the toxicity of cyanide is eliminated through the conversion of cyanide into β-cyanoalanine by β-cyanoalanine synthases (Hatzfeld et al., 2000). ACS and ACO control ethylene production through changes in their transcription, translation, and stability and are the main two enzymes that regulate ethylene biosynthesis (Pattyn et al., 2021).

Root growth is affected by the binding of ethylene to the endoplasmic reticulum to activate or repress downstream signalling pathways (Hua and Meyerowitz, 1998; Qin and Huang, 2018). As mentioned in **chapter 1.1**, high soil compaction reduces the available air spaces in soil through increased soil particle density, this causes a build-up of ethylene and, as a consequence, also leads to auxin accumulation (Zhang et al., 2018) in the elongation zone which further inhibits root elongation (Qin and Huang, 2018) with abscisic acid (ABA) controlling radial cell expansion (Huang et al., 2022) (**Figure** *1.5.2*). Ethylene mutants of varying types would theoretically show differential sensitivity to soil compaction compared to plants without mutations, these could involve less available ethylene receptors for binding, downregulation of ethylene biosynthesis as a result of decreased binding of upstream signalling molecules, or reduced levels of molecules involved in ethylene biosynthesis (Stepanova and Ecker, 2000). Research using the rice mutants *oseil1* which has a mutation in the ETHYLENE-INSENSITIVE 2 gene that is involved in the ethylene signalling cascade and *osein2* which has a mutation in another gene (dependent on rice cultivar) involved in ethylene signalling/ response pathways has showed that mutants with ethylene insensitivity are able to penetrate soils of high compaction (Pandey et al., 2021). A mutation in ethylene receptor 1 (ETR1) by overexpression of *TaETR1-1* (a gain-of-function mutant of *TaETR1*) revealed that wheat developed an insensitivity to ethylene after administering ACC (Wei et al., 2023).

Auxin is a hormone involved in determining the direction of root and shoot growth through the control of cell differentiation, cell elongation, and cell division. Auxin controls direction of growth by accumulating along one side of the shoot or the root and can activate or inhibit cell elongation to alter growth towards factors such as light or nutrients or away from harmful chemicals or highly compacted soil layers. Ethylene, ABA, and auxin are all involved in the response to soil compaction, ethylene has antagonising effects on ABA,

ABA recruits auxin downstream to regulate root elongation and radial expansion (Qin et al., 2022), and ethylene modulates auxin biosynthesis, transport, and signalling (Qin and Huang, 2018). Auxin biosynthesis is less well understood than ethylene biosynthesis, however, it has been elucidated that TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS-1/ AMINOTRANSFERASE-RELATED (TAA1/ TAR) YUCCA (YUC) flavin monooxygenase-like proteins are required for indole-3-acetic acid (IAA) biosynthesis (Mashiguchi et al., 2011; Stepanova et al., 2011). The TAA family produces IPyA through Tryptophan (Trp) conversion into IPyA and the YUC family plays a role in the conversion of IPyA, using flavin monooxygenase-like proteins that catalyse rate-limiting steps in IAA biosynthesis into IAA (Mashiguchi et al., 2011). Auxin levels rapidly control gene expression through changes in transcription and brings together F-box family proteins called TRANSPORT INHIBITOR RESPONSE1/ AUXIN SIGNALLING F-BOX (TIR1/AFB), members of the Aux/IAA transcriptional repressor family, which can be considered coreceptors for auxin (Leyser, 2017), and the AUXIN RESPONSE FACTOR (ARF). E3 ubiquitin ligases catalyse the conjugation of substrate proteins and ubiquitin, these substrates (Aux/IAA) are then targeted by the 26S proteasome to be degraded (Quint and Gray, 2006) and ARF repression is released (Lavy and Estelle, 2016).

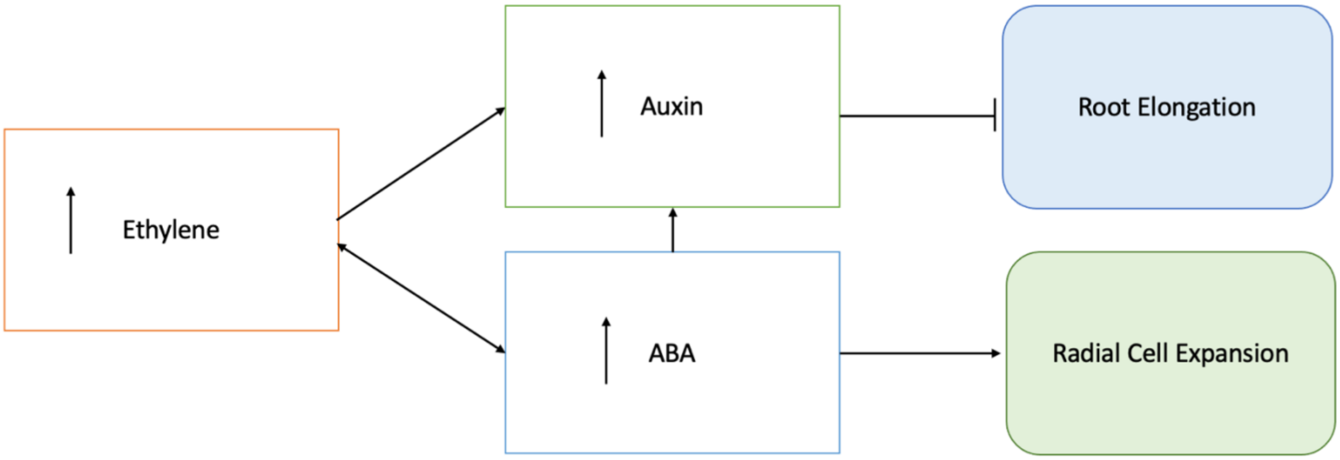

Figure 1.5.2 the interactions observed between ethylene, ABA, and auxin, ethylene has antagonising effects on ABA and vice versa, ABA recruits auxin downstream to regulate root elongation and radial cell expansion and ethylene modulates auxin biosynthesis, transport, and signalling (Qin and Huang, 2018; Qin et al., 2022).

ABA controls many plant activities such as seed dormancy promotion, adaptive responses to biotic and abiotic stresses, stomatal control, and growth and development in non-stressed conditions (Brookbank et al., 2021). Above ground activities that are controlled by ABA include the opening and closing of stomata, recent studies have found that ethylene reduces the sensitivity of stomata to ABA and so acts as an antagonist (as mentioned above). Some research has also found that ABA employs auxin downstream to act as a regulator of root elongation and radial cell expansion, which can cause the roots to become short and swollen and impair the root ability to penetrate compacted soil (Qin et al., 2022).

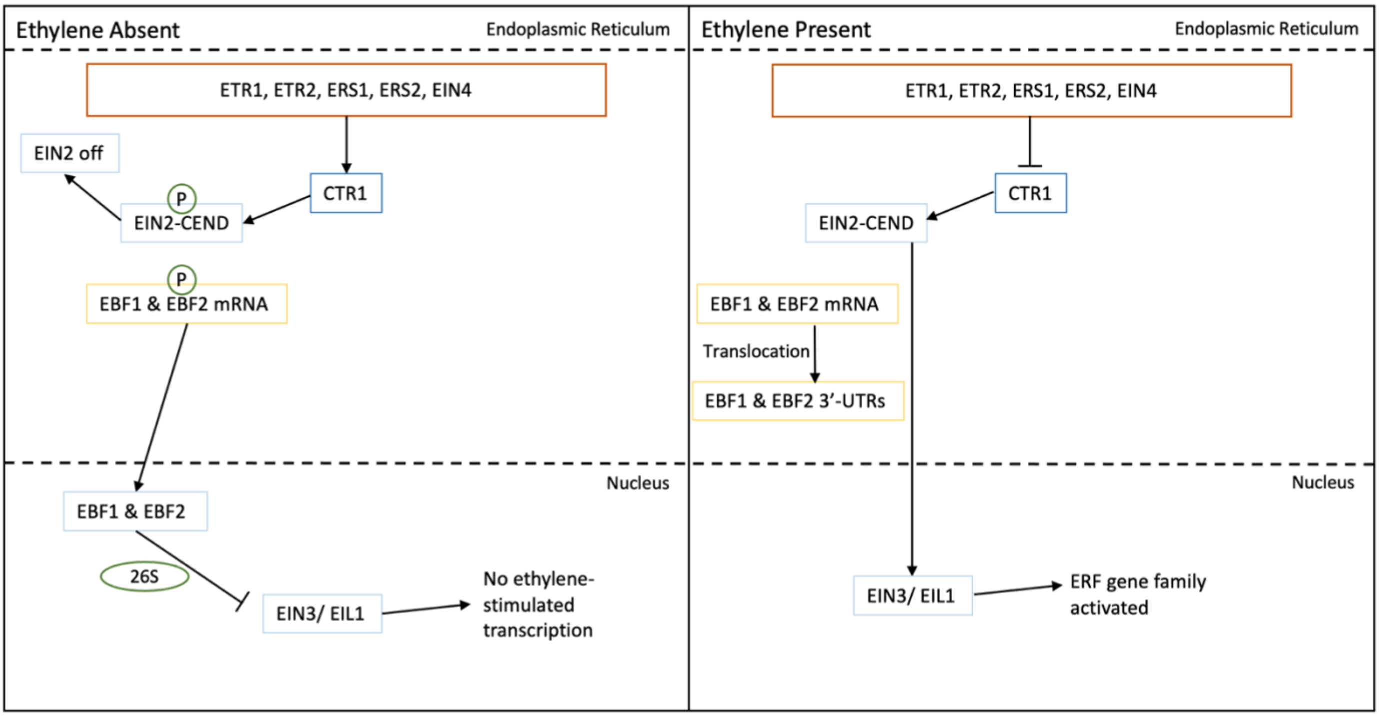

Figure 1.5.3 the signalling pathway involved in ethylene signalling with a comparison between the process with ethylene absent and with ethylene present, diagram is adapted from Fatma et al. (2022).

Ethylene signalling was elucidated in *Arabidopsis thaliana* and is applied to other plant species (Binder, 2020; Fatma et al., 2022). Due to ethylene being a gaseous hormone a plasma membrane-localised receptor is not required for detection so ethylene is detected by ETHYLENE RESPONSE1 (ETR1), ETHYLENE RESPONSE2 (ETR2), ETHYLENE RESOPONSE SENSOR1 (ERS1), ETHYLENE RESPONSE SENSOR2 (ERS2), AND ETHYLENE INSENSITIVITE4 (EIN4) family receptors (**Figure** *1.5.3*) that act negatively on ethylene signalling, these receptors are located on the Golgi and endoplasmic reticulum membranes as two-component histidine kinase receptors (Fatma et al., 2022). Once ethylene is produced it can diffuse throughout the plant freely and bind to receptors on the endoplasmic reticulum membrane (Fatma et al., 2022).

If ethylene is not detected the receptors activate the CONSTITUTIVE TRIPLE RESPONSE1 (CTR1), which is a Raf-like Ser/Thr protein kinase (Guo and Ecker, 2004), and phosphorylates the C-terminal of EIN2 and turns it off, F-box protein mRNAs EIN3 BINDING F-BOX PROTEIN1 (EBF1) and EIN3 BINDING F-BOX PROTEIN2 (EBF2) are then translated and the proteasome targets EIN3 and EIN3-LIKE1 (EIL1) for degradation (Fatma et al., 2022). When ethylene is present CTR1 is inactivated and EIN2 is dephosphorylated and cleaved to release the EIN2 C-terminal (EIN2-CEND) and moved to the cytosol and nucleus, the C-terminal of EIN2 then represses the translation of EBF1 and EBF2 and are transcribed by directly or indirectly binding to their 3’-untranslated regions (3’-UTRs) and move to the nucleus, the EIN2 C-terminus then directly or indirectly promotes the activity of EIN3 and EIL1, and the EIN3 and EIL1 transcription factors are regulated by ethylene and control the transcription of ethylene-responsive genes like ETHYLENE RESPONSE FACTOR (ERF) (Fatma et al., 2022).

1.6. How Ethylene is involved in the response to soil compaction

Seminal research on ethylene sensitivity, soil compaction, and crop growth together has been conducted but recent research is limited and often focuses on either soil compaction and crop growth or ethylene and soil compaction, not all three factors in one. So, from the limited research that has been performed, this is what we know.

1.6.1. The impact of ethylene and soil compaction on the growth of wheat

Research using wheat (*Triticum aestivum*) has shown that there is a significant decrease in ethylene diffusivity in highly compacted soils due to the increase in bulk density and soils with a density of 1.7 Mg m^{-3} resulted in significant reductions in wheat seedling root length in sandy loam and sandy clay loam soils (Yu et al., 2024). Some research identified that there is a balance between ethylene levels and the regulation of stress responses in crops with low ethylene concentrations/ low ethylene sensitivity facilitating the activation of defence signalling and inhibition of defence signalling at high ethylene concentrations/ plants with high sensitivity to ethylene, suggesting that this could be involved in the response to soil compaction (an environmental stress) (Fatma et al., 2022). For example, defence responses such as increasing root diameter may improve the ability to grow in compacted soil by increasing root strength.

1.6.2. Ethylene levels and soil characteristics caused by compaction and the effects these have on potato growth

Field trials with potato (*Solanum tuberosum*) have shown that high ethylene concentrations, found in partially compacted plots, and low oxygen concentrations were associated with low tuber quality, reduced shoot growth, leaf injury, increased tuber respiration (ethylene and ABA interactions), and 20% reduction in tuber yield (Campbell and Moreau, 1979). Exposure to high concentrations of ethylene during sprouting caused enlarged lenticles, swollen sprouts, and retarded root development after 6 days of exposure, these sprouts failed to grow even after ethylene was removed (Timm et al., 1986). When potatoes were subjected to drought and subsoiling in one paper, the yields were significantly increased due to the reduction in soil strength and bulk density and an increase in soil porosity, but this effect was not seen at near optimum levels of irrigation (Ibrahim, 1985). This result was likely observed because of an increase in soil strength as a result of drying which would have a more significant effect on growth in soils of high compaction (not subsoiled) due to decreased porosity of dense soil, therefore, subsoiling could help reduce the strength of the soil and facilitate increased yield.

1.6.3. Manipulation of ethylene production using ethephon and silver and the impact this has on tomato growth

Research on tomato (*Solanum lycopersicum*) has showed that growth varies in different levels of compaction depending on ethylene levels, one study used wild-type Ailsa Craig and transgenic $ACO1_{AS}$ genotypes in which ethephon (an ethylene precursor) and silver ions were used to increase or decrease ethylene production, the tomatoes in split pot-treatments (1.1/ 1.5 g cm^{-3}) showed significant differences depending on genotype (Hussain et al., 1999a). Ailsa Craig showed significant reductions in growth compared to $ACO1_{AS}$ plants which have a reduced capacity to produce ethylene, this was concluded as being due to ethylene because treatment with silver restored shoot growth in Ailsa Craig and treatment with ethephon reduced shoot growth in $ACO1_{AS}$ (Hussain et al., 1999a). Research was conducted looking at the potential for ABA to be the signal causing the changes in leaf expansion of barley in compacted soils, however, it was concluded that ABA was not the root-sourced molecule responsible for the inhibition of leaf expansion and subsequent studies found that it was the second-sourced root signal ethylene that was responsible due to increased ethylene production under high compaction (Hussain et al., 1999b).

1.7. How Ethylene sensitivity is important to crop growth

Ethylene sensitivity is very important in crop growth, especially in soils that are susceptible to compaction or drought as the diffusion of ethylene around the roots determines their growth and RSA (see **chapter 1.4**). This means that plants with varying levels of ethylene sensitivity/ production show altered responses to high compaction (see **chapters 1.4**, **1.5**, and **1.6.3**). *Arabidopsis* grown in soils with high mechanical impedance show a characteristic ethylene response in which primary root growth is reduced, root diameter is increased, and root hairs grow closer to the root tip, along with this, mutants of ethylene and auxin demonstrated that intact responses to these endogenous hormones is required for a mechanical impedance response (Jacobsen and Gillian, 2016).

Ethylene is also involved in the response to environmental stressors, acting as a stress hormone, for example when wild-type and mutant *Arabidopsis* were exposed to osmotic stress changes in compatible solutes and gene transcription were identified, with the authors concluding that ethylene signalling is involved in increasing the upregulation of soluble sugar and proline to aid in adjustment to osmotic stress (Cui et al., 2015). Ethylene defective mutants also exhibited higher oxidative stress, had increased activity of reactive oxygen species (ROS) superoxide dismutase (El-Soda et al.) and peroxidase (Dayou et al.) when compared to wild-type plants, suggesting increased oxidative stress under drought conditions (Cui et al., 2015). Some research has suggested that crop nutritional value, yield, and stress tolerance can be altered by controlling the timing of senescence, after plants are exposed to stress there is a small peak of ethylene at stress onset (presumably due to the consumption of all available ACC) and then a much larger peak later on (from the synthesis of more ACC) which precedes senescence, abscission, and chlorosis which worsen the effects of stress, so limiting this spike in ethylene could reduce the consequences observed on plant growth or yield (Gepstein and Glick, 2013). These results suggest that modulating ethylene sensitivity or even ethylene production can result in benefits for crop performance which is essential for the future as supply struggles to meet demand.

1.8. Ethylene reporter lines

The hypothesis that ethylene concentrations in the surrounding area of the root increase due to a decrease in pore space of highly compacted soils was tested in a study that used the *Arabidopsis* ethylene reporter EIN3-GFP (green fluorescent protein), results showed that covering root tips in a gas-impermeable cover lead to an increase in EIN3-GFP in root elongation cell nuclei (**Figure 1.8.1**) that was not due to hypoxia (Pandey et al., 2021). This finding highlights that the hypotheses on the impact of a reduction in soil porosity due to increased soil strength and the accumulation of ethylene around the roots in strong soils as robust. Another experiment was carried out looking at whether ethylene signalling increased in root tissues in soils of high compaction, *EIN3* and *OsEIL1* sequences fused with GFP were used, in uncompacted soils the reporters *35S:EIN3-GFP* and pro*OsEIL1:OsEIL1-GFP* were not detectable but in compacted soil the reporters were detected in nuclei in the root elongation zones (Pandey et al., 2021).

Similar research in wheat, potato, and tomato is very limited and reporter lines in wheat have not yet been developed, however GUS and GFP have been used in potato and tomato (Lee et al., 2007; Zhong et al., 2008; Zhang et al., 2009) studies using ethylene reporters for research not related to soil compaction. This is a considerable gap in the literature and in scientific knowledge when looking to understand ethylene signalling, production, and localisation when focusing on soil compaction in crop species other than *Arabidopsis*. The application of research conducted in *Arabidopsis* to other species (chapter 1.5) is logical as plants do indeed, for example, show similar responses when grown in soils of low versus high compaction (see chapters 1.2 and 1.6) but, as demonstrated by the lack of research using ethylene reporter lines in soil compaction research on potato and tomato, this may not yield optimal results and conducting research and studies developing these reporter lines would be beneficial to crop science.

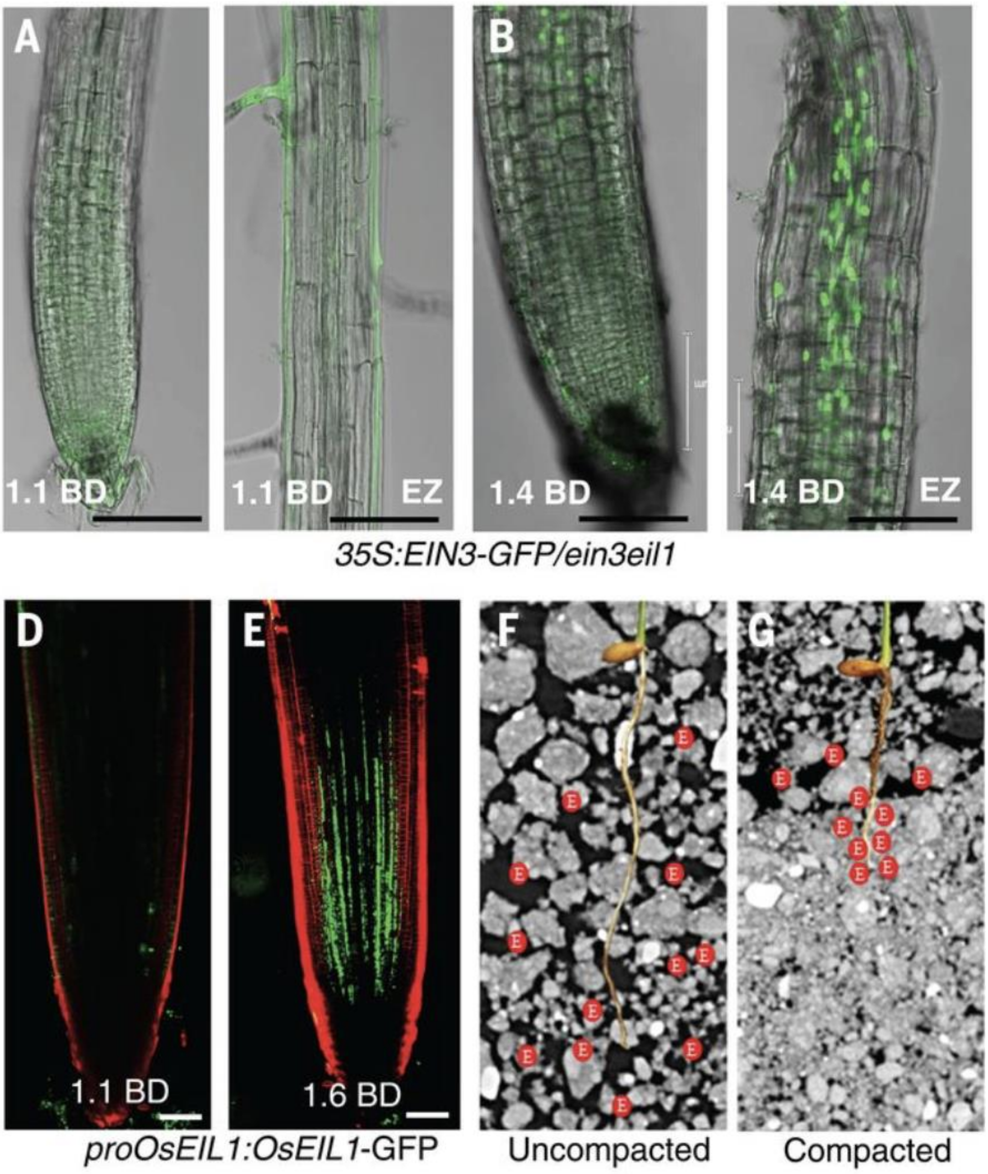

Figure 1.8.1 taken from (Pandey et al., 2021) showing fluorescent images of ethylene reporter lines. (A) *Arabidopsis* EIN3-GFP showing no GFP signal when grown in uncompacted soils, (B) *Arabidopsis* EIN3-GFP fluorescing when grown in compacted soils demonstrating increased ethylene signalling. (E) rice OsEIL1-GFP-based ethylene translational reporter shows elevated levels in compacted soils when compared to uncompacted soils (D). (F and G) demonstrate the diffusion of ethylene (in red) around the roots in compacted and uncompacted soils.

1.9. Thesis Structure

This thesis aims to identify whether variation in ethylene sensitivity alters responses to soil compaction and evaluates whether certain varieties are less sensitive to compaction.

Chapter 2 investigated the role of ethylene sensitivity in the response of six wheat cultivars to low (0.4 MPa), intermediate (1.2 MPa), and high compaction (2.4 MPa) (Chewink, Kutz, Ladham, Chilham, Nissaba, and Talisker) putatively differing in ethylene sensitivity (R. Fusi, University of Nottingham, pers. comm.). The sensitivity to ethylene was measured by providing ethephon to seeds grown on Petri-dishes and reduction in root length measured. Plants were harvested after around 4 weeks and measurements taken, then the data were analysed statistically using two-way ANOVAs.

Chapter 3 looked at the response of four common UK potato cultivars to compaction (Marfona, Cara, Pentland Javelin, and Hermes) which have not been researched as extensively as the most commonly grown Maris Piper to soil compaction (**Figure** *1.9.1*). Ethylene sensitivity was measured by administering ethylene to minitubers in airtight containers, with the change in root length relative to controls (no ethylene) indicating ethylene sensitivity. Potatoes were also grown under low (0.4 MPa) compaction, intermediate (1.2 MPa) compaction, and high (2.4 MPa) compaction. Plants were harvested after around 6 weeks and measurements taken, then the data were analysed statistically using two-way ANOVAs.

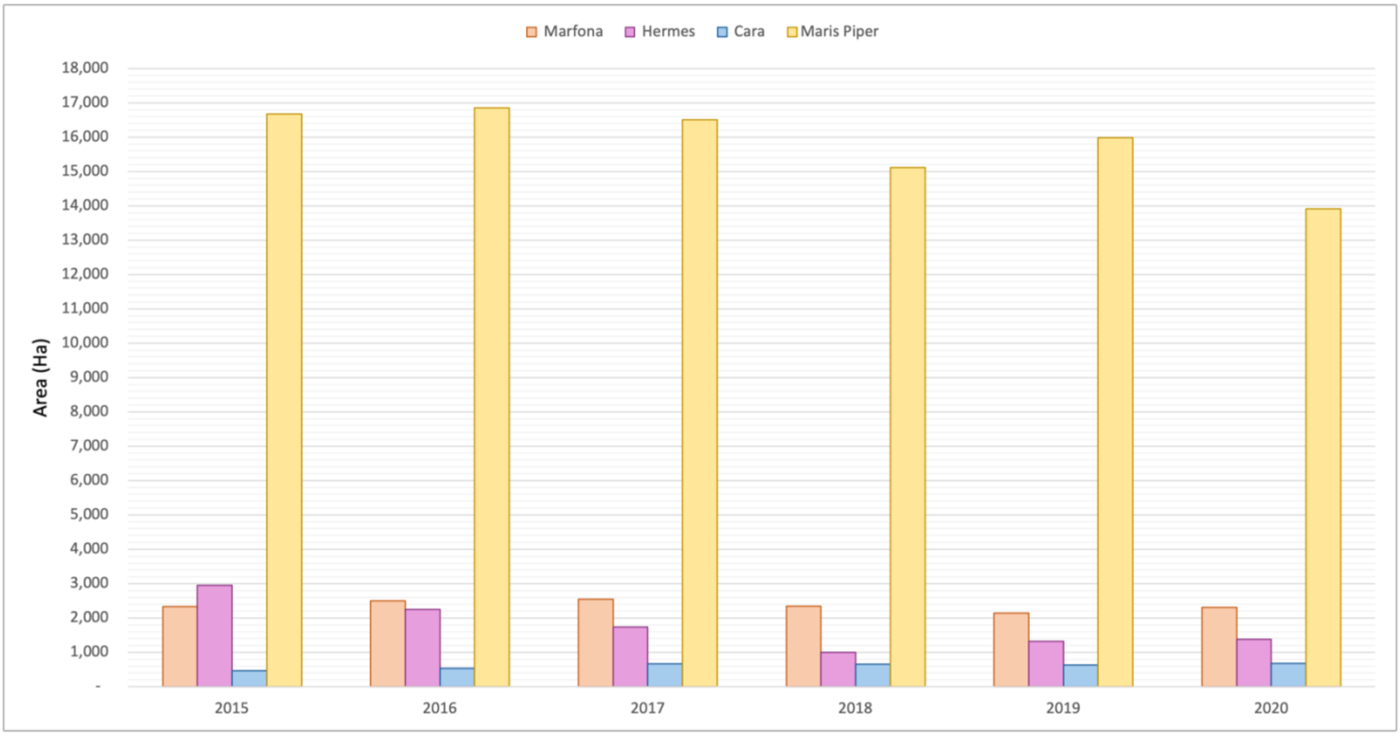

Figure 1.9.1 Cultivation by cultivar of potatoes grown in the UK from 2015 – 2020 obtained from (AHDB, 2021). Cultivars Marfona, Hermes, and Cara are shown along with Maris Piper to act as a comparison with Maris Piper being the most commonly grown potato variety in the UK. No data was available for Pentland Javelin and so could not be included.

Chapter 4 researched two tomato cultivars (Ailsa Craig and Micro-Tom) each with two genotypes (wild-type, WT and Never-ripe, Nr). The original seeds had been stored for some time, so plants were first grown in the same greenhouse until seeds could be harvested, to confirm the genotypes were correct. Pre-germinated seeds were then either grown on Petri-dishes with ethephon to assess ethylene sensitivity or pre-germinated on Petri-dishes with water to be planted into soil columns containing low (0.4 MPa), intermediate (1.2 MPa), and high (2.4 MPa) compaction. Plants were harvested after around 5 weeks and measurements taken, then the data were analysed statistically using two-way ANOVAs.

2. The role of ethylene sensitivity in compaction responses of six wheat cultivars

2.1. Introduction

Identifying the impacts of soil compaction on crops such as wheat are highly important, mainly due to our reliance on wheat for food products in our diet and the cost of soil degradation (£1.2 billion in 2015) (Graves et al., 2015), but also for the expansion of scientific knowledge and understanding. With UK national wheat yields remaining at 8 t ha^{-1} from 2013 to 2023 (**Figure** ***2.1.1***) and increased consumer demand not being met globally (Hall et al., 2023), scientists need to identify why yield is not increasing and propose solutions to this problem. Soil compaction may be one of the causes of steady wheat yields because of the impact of farming equipment, loss of SOM (**chapter 1.1**), grazing, and poor crop diversification (Shah et al., 2017). High compaction increases bulk density and soil strength and decreases soil health by decreasing soil porosity, nutrient availability, hydraulic conductivity, soil biodiversity, and aggregate stability (Nawaz et al., 2013; Shah et al., 2017) which lowers crop performance.

With environmental changes expected to occur and those that have already taken place such as shifts in growing seasons and increased soil erosion in areas with heavy rainfall, limiting the impacts of certain factors (like soil compaction) are important because environmental conditions are not easily regulated around broadacre crops such as wheat. Simple practices, such as applying organic matter to farmlands can somewhat alleviate the compaction of soil by improving the structure of the soil thereby increasing porosity and water infiltration (Bashir et al., 2021).

Research on the impact of soil compaction on wheat is reasonably extensive and demonstrates that high compaction (high soil strength and low porosity) decreases crop growth and yield (Arvidsson and Håkansson, 2014). Interestingly, some crops tend to perform better if soil is recompacted after loosening (Reichert et al., 2009), with moderate compaction (around 1.4 g/cm^3) improving the yield of wheat compared to loosened soil (around 1.2 g/cm^3) (Arvidsson and Håkansson, 2014). In three winter wheat cultivars tested in loamy sand, researchers found that higher compaction, when compared to a no compaction reference treatment, had decreased effective rooting depths (between 10 – 50 cm according to the cultivars), decreased available soil water in the rootzone (by 90 mm), and decreased total dry matter (possibly due to decreased conversion of light energy into biomass) and grain yield by 8% and 9% respectively (Andersen et al., 2013). Compaction at moderate (BD ~ 1.6 Mg/m^{-3}), and high (BD ~ 1.8 Mg/m^{-3}) compaction levels significantly limits root growth compared to the "no" (BD ~ 1.4 Mg/m^{-3}) compaction treatment, but this effect was diminished by adding N (Mondal, 2022). One wheat genotype (HD-2967) was better able to regulate the expression of N-assimilating genes under high compaction than another (Choti Lerma) (Mondal, 2022). In sandy loam (control BD = 1.19 Mg/m and high BD = 1.40 Mg/m) and sandy clay loam soils (control BD = 1.24 Mg/m and high BD = 1.73 Mg/m) the shoot dry weight of wheat was significantly reduced in soils with high bulk densities (a 48% reduction) as well as root dry weight, and despite texture-dependent impacts on root growth, shoot growth and grain yield reduction were similar (Khan et al., 2012). Therefore, high soil compaction is expected to restrict growth of the chosen UK spring wheat cultivars.

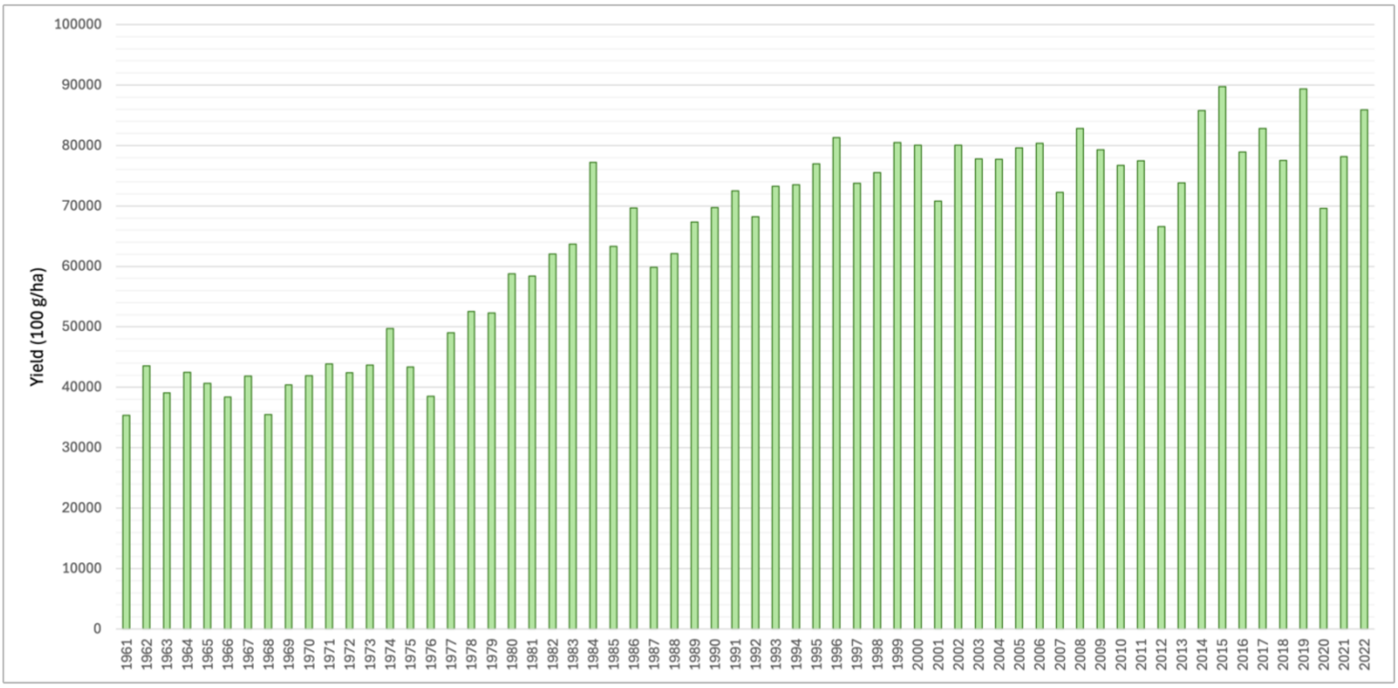

Figure 2.1.1 data obtained from (FAOSTAT, 2021) showing UK wheat yields over the years from 1961 – 2021 (the most recent year available). Wheat yields have remained stable between 1997-2022.

Ethylene has been researched in relation to drought tolerance and stomatal conductance of wheat. Stomata of older wheat leaves were more sensitive to ethylene than younger leaves, irrespective of their endogenous ACC concentrations or ethylene emission (Chen et al., 2013). Plant growth regulators (PGRs) like ethephon decreased main shoot development (weight per main shoot) by 20% in wheat, enhanced tiller production, reduced CO_2 exchange rate, and shortened the straw (Rajala and Peltonen-Sainio, 2001). Applying ethephon as a foliar spray (0, 0.038, 0.38, 3.8, and 19 g/L^{-1}) to wheat plants only slightly retarded root elongation in wheat compared to oat and barley and the differences were attributed to varying sensitivities to ethylene (Rajala et al., 2002). Acetylene (C_2H_2) and ethylene (C_2H_4) are gradually released by calcium carbide (CaC_2) and application of CaC_2 at 30 mg/kg^{-1} increased tillers, root weight, straw, grain yield, and N-use efficiency of wheat (Yaseen et al., 2006). Therefore, with reductions in root growth there should be reductions in shoot growth also.

To date, the interaction between compaction levels and ethylene sensitivity in wheat have not been investigated. While high soil compaction should significantly decrease root and shoot dry weight, leaf growth, leaf area, and coleoptile height in ethylene-sensitive wheat varieties, those that are less sensitive to ethylene are hypothesised to grow better in compacted soil.

2.2. Materials and Methods

Plant and Soil Method

Six wheat varieties, putatively differing in ethylene sensitivity, were obtained from ADAS, and planted in pairs over a 3-month period, the first pair being Chewink and Kutz, the second Ladum and Chilham, and the third Nissaba and Talisker. Soil used was Norfolk topsoil from Bailey's of Norfolk (Hevingham, Norfolk, UK), and nutrient analysis was performed on the soil (results can be seen in Table *2.2.1*).

The pots were manufactured from Polyvinyl chloride pipe (Keyline, Northampton, UK) to have an interior diameter of 6.8 cm and a height of 25 cm. Plants that were not required to grow for more than two weeks were grown in pots of 12.5 cm in height; according to Hooke's law and spring constants, using a pot with a smaller volume should not change the overall level of compaction (Thompson, 1926). Stainless steel wire mesh from Mesh Direct (Stoke-On-Trent, UK) was melted onto the pipe at one end to produce a pot.

Soil compaction levels were created by allowing the soil to air dry until completely dry and then re-wetted with specific volumes of water to produce three levels of compaction: low (0 g/kg), intermediate (50 g/kg), and high (160 g/kg). Soil was compacted using an Arbour Press (PK3000, Jack Sealy Ltd., Bury St Edmunds, UK) with a metal disk fitted to match the interior diameter of the pots in order to compact soil. Force was applied using a torque wrench and soil was compacted in ~ 2cm intervals to ensure homogenous compaction with the soil surface being disturbed to prevent boundary effects. Soil was compacted until it was ~2cm below the rim of the pot and 2 wheat seeds placed on-top, then covered with loose topsoil. Once the seeds were established and shoots were visible the seedlings were thinned with only the most homogenous subset remaining. For growth in compacted soils the plants were not given any fungicides, pesticides, or fertilisers. Soil resistance was measured using the Van Welt hand penetrometer (Haslemere, UK); low compaction soil had a resistance of 0.4 ± 0.12 MPa, intermediate compaction a resistance of 1.2 ± 0.16 MPa, and high compaction a resistance of 2.4 ± 0.18 MPa (with ~ 10 replicates per compaction level and SE indicated). Soil bulk density was also calculated to observe whether there was a relationship between soil resistance and bulk density, low compaction had a bulk density of 1.14 ± 0.17 g/cm^3, intermediate compaction a bulk density of 1.38 ± 0.13 g/cm^3, and high compaction a bulk density of 1.59 ± 0.14 g/cm^3 (**Figure** *2.2.1*).

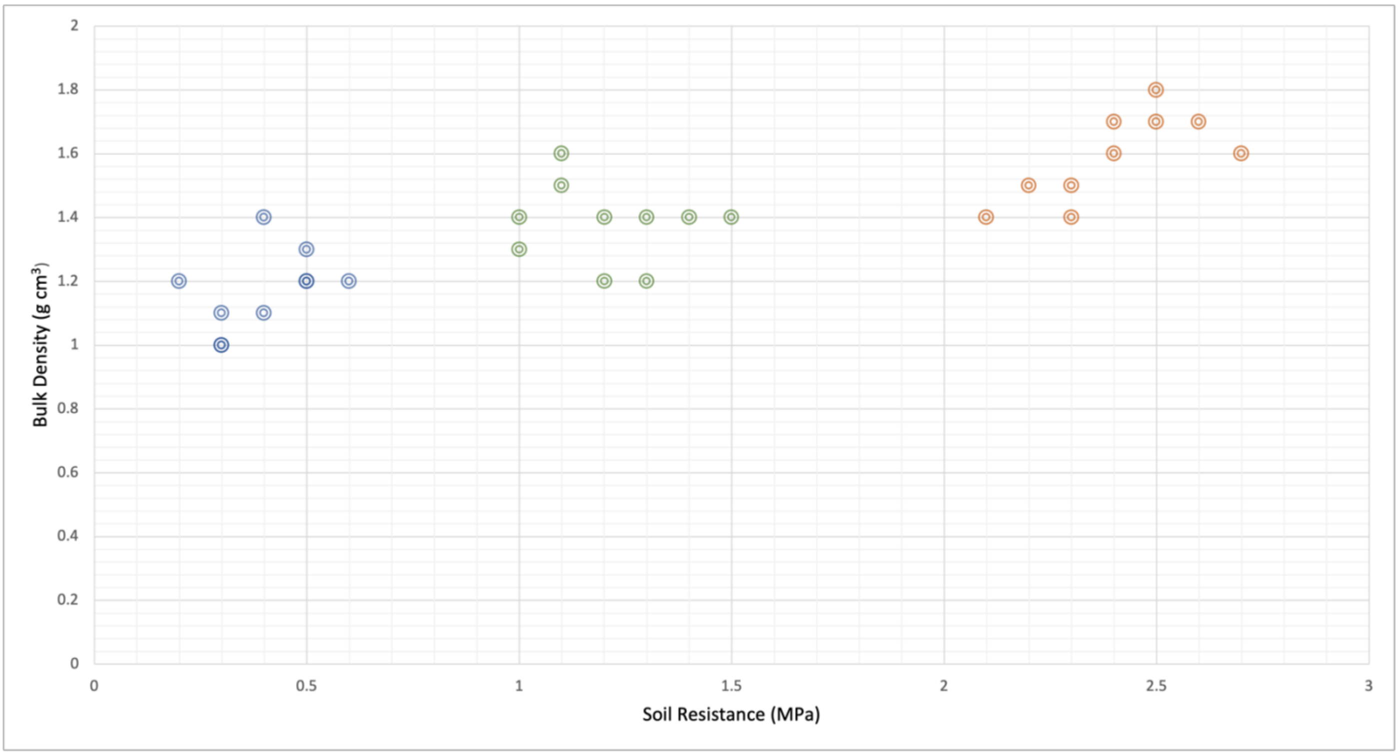

Figure 2.2.1 soil resistance vs bulk density of low (blue), intermediate (green), and high (orange) compaction showing the relationship between the two measurements of soil compaction levels with 10 replicates for each.

Table 2.2.1 soil nutrient analysis of Bailey's of Norfolk topsoil obtained by NRM (Cawood, Bracknell, UK) excluding particle size and texture. Analysis was carried out on one sample.

Soil Parameter	Value
pH	*7.4*
Available P (mg/l)	*45.2*
Available K (mg/l)	*337.0*
Available Mg (mg/l)	*67.0*
Available N (kgN/ha)	*216.1*
Nitrate Nitrogen (mg/kg)	*50.5*
Ammonium Nitrogen (mg/kg)	*3.5*
Soil Organic Matter (LOI%)	*2.9*
Copper (mg/l)	*6.5*
Boron (mg/l)	*1.3*
Sodium (mg/l)	*45.0*
Zinc (mg/l)	*8.2*
Calcium (mg/l)	*1750.0*
Iron (mg/l)	*86.4*
Sulphate (mg/l)	*119.0*
Manganese (mg/l)	*5.8*

Wheat was grown using a randomised block design in a controlled environment room for four weeks with illumination provided by metal halide lamps (Osram Powerstar HQI-T, Munich, Germany) with a PPFD at bench height of 450 µmol/m^2/s. and a daytime temperature maintained between 24-25°C for sixteen hours a day and a night-time temperature of 15-16°C.

Ethylene Assay Method

Wheat seeds were pre-germinated in Petri-dishes on filter paper for 5 days or until sufficient roots had formed to uptake the administered solutions. 100 µM of ethephon was used. After seeds had produced roots the seeds were moved into new Petri-dishes that were either soaked in deionised water or 100 µM of ethephon. The Petri-dishes were taped shut to prevent any gases from escaping and placed in a dark area to grow for 7 days, with the seeds being checked once a day to ensure the filter paper remained saturated. After this period had passed the Petri-dishes were removed and the most uniform roots measured against a ruler.

Measurements

Coleoptile height was measured 6-7 days after seedling emergence, and percentage emergence calculated, to find if soil compaction levels impacted the likelihood of wheat varieties emerging and the height that could be reached in the early stages of growth.

Soil water content was monitored by weighing pots daily at 10am after water holding capacity (WHC) was determined and maintained at this weight. WHC was obtained by allowing the pots to take up water through capillary action until fully saturated, they were then removed from the water and allowed to drain and then weighed. This data was also collected to assess if there was a significant difference between water loss and compaction levels. Leaf expansion of the second and third leaves were measured daily at 10 am using a ruler from the ligule to the tip of the leaf, and expansion plotted against time.

At harvest second and third leaf areas were measured using a leaf area meter (LiCor's LI-3100C, Cambridge, UK) and total leaf area was collected additionally. After leaf area had been collected for all plants the leaves and shoots were placed in an oven for 96 hours until completely dry and weighed to obtain dry shoot biomass. Unfortunately, root scanning analysis was not possible as wheat roots were too fine for reliable imaging at 800 dpi (the maximum resolution that could be used due to software restrictions), therefore, root dry weight was obtained instead of root length and diameter classes.

Roots were harvested and carefully washed using a sieve after leaf area was obtained and placed into paper bags and stored in a drying oven (50°C) until completely dry, leaves were dried along with roots to obtain root and shoot biomass.

Statistical Analysis

Two-way ANOVAs discriminated effects of soil compaction, wheat cultivar and their interaction (VassarStats) on all measured variables, along with post-hoc tests including Tukey and Games-Howell using SPSS with the decision of which one to be used was determined using Levene's test to find if the data were normally distributed. If the data were normally distributed the Tukey post-hoc test was used, if they were not normally distributed, Games-Howell was used. T-tests were also carried out for measurements against variety due to variable numbers being below 2. Statistics were completed on all parameters measured: ethylene assay root length, water loss, coleoptile height, leaf expansion, leaf area, dry shoot biomass, and dry root biomass. The data were also analysed for outliers by calculating quartiles 1 and 3, inter-quartile range, and the upper and lower bounds, and any data points outside this range were removed for statistical analysis.

2.3. Results

Soil compaction x genotype had no significant effect on second and third leaf expansion, total leaf area, root dry weight, and water loss. This was expected for the results from Ladham and Chilham due to their very similar sensitivity to ethylene, and Nissaba and Talisker perhaps also because of the results seen from the ethephon assay (**Figure 2.3.1**). Therefore, Kutz and Chewink will be the main focus of this section due to the greater differences observed between the two cultivars in some of the measurements taken.

The ethephon assay used to investigate the sensitivity of each variety to ethylene showed that the most sensitive out of the six was Chewink with Kutz being much less sensitive, Ladham and Chilham were similarly sensitive to ethylene, and Nissaba and Talisker also had very similar sensitivity levels, however both may still have been slightly more sensitive than Ladham and Chilham (**Figure 2.3.1**), but not significantly. This would indicate that Chewink responds to ethylene much more than Kutz and may be more sensitive to compaction.

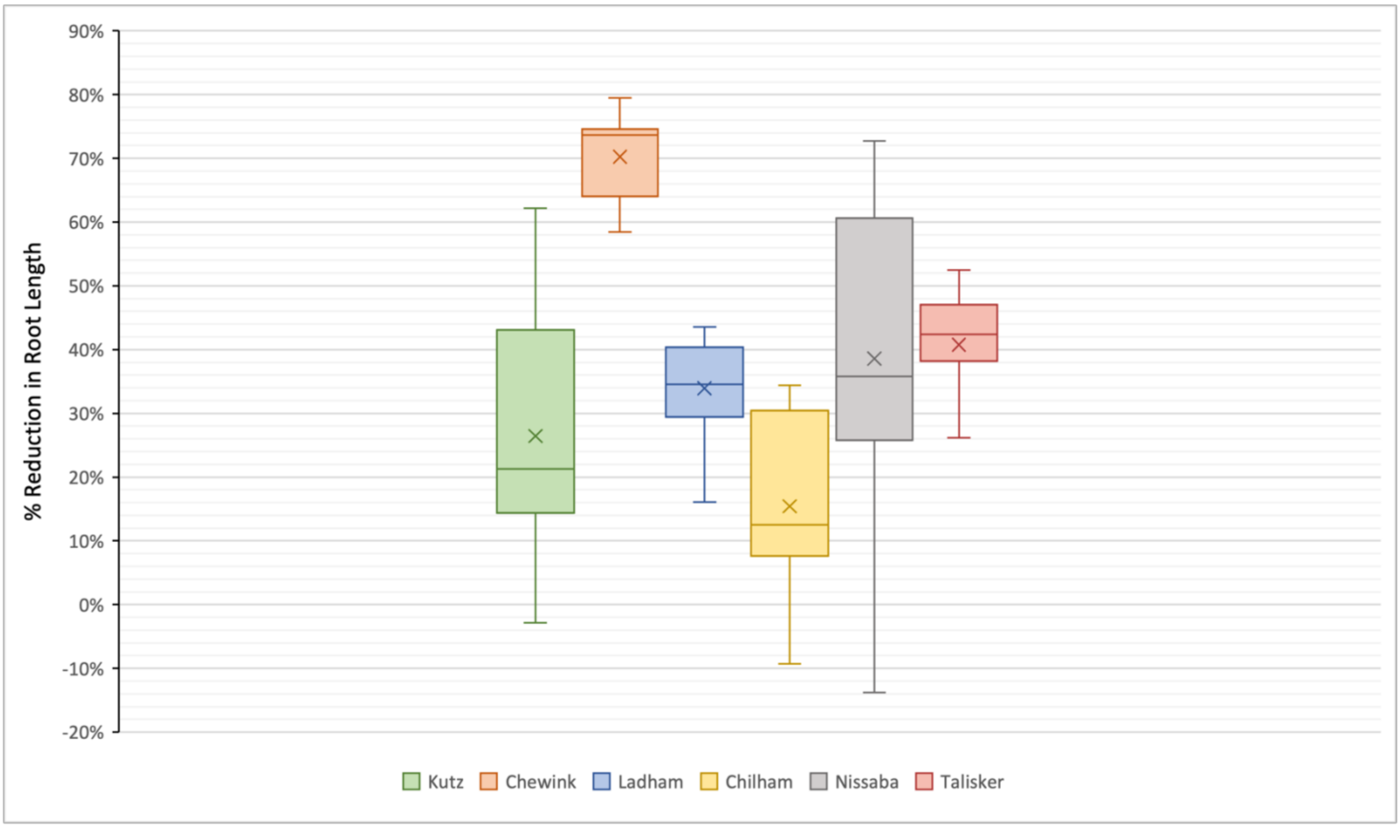

Figure 2.3.1: Ethylene sensitivity assay plotted as % reduction in root length, n = 20 for all varieties. A two-tailed t-test was conducted on the results of this assay and the difference between responses to ethephon were found to be significant with p = <.0001 for Kutz and Chewink. This result suggests that the wheat variety Kutz is significantly less sensitive to ethephon (and therefore ethylene) than Chewink. Another significant difference was calculated between Ladham and Chilham with p = 0.004 which, with details given by the graph, means that Ladham is significantly more sensitive to ethylene than Chilham. A non-significant difference was observed between Nissaba and Talisker (p = 0.80), meaning that these two varieties likely have a very similar level of sensitivity to ethylene. Two-way ANOVA across all 6 genotypes found that p = 0.0001 for cultivar, p = 0.0365 for treatment, and p = <.0001 for genotype x treatment (the raw data were analysed, therefore, a two-way ANOVA was used to compare the control vs ethephon and variety 1 vs variety 2).

Table 2.3.1: Average results of the ethephon assay with % inhibition and significance values calculated using two-way ANOVA, significance at the 0.05 level is indicated by an asterisk (*). NaN means that there is either perfect collinearity between the data, or, due to small sample sizes, there is a division by zero in the calculation, or finally it could be due to zero total variance in which the sum of all the squares is zero. ANOVAs were calculated based on absolute root length values and % inhibition calculated separately.

Cultivar	Control (mm)	Ethephon (mm)	% Inhibition
Kutz	60.7 ± 5.3	44.9 ± 4.2	26 ± 6.9
Chewink	85.5 ± 5.3	25.6 ± 1.6	70 ± 2.3
Ladham	69 ± 2.4	45.5 ± 1.4	34 ± 2.7
Chilham	52.3 ± 2.4	44.4 ± 1.9	15 ± 5
Nissaba	36.5 ± 2.2	22.3 ± 2.3	39 ± 8.1
Talisker	74.4 ± 1.6	43.9 ± 1.5	41 ± 2.9
Cultivar			0.0001 *
Ethephon			0.0365
Interaction			< 0.0001 *

Table 2.3.2: The significance variables calculated for all of the data collected from each variety. Significance at the 0.05 level is indicated by an asterisk (*).

Cultivar	Variable Measured	Compaction Significance (p)	Variety Significance (p)	Interaction Significance (p)
Kutz and Chewink	*Coleoptile Height*	0.025*	0.044*	0.121
	2nd Leaf Growth	0.606	0.231	0.292
	3rd Leaf Growth	0.143	0.508	0.470
	2nd Leaf Area	0.018*	0.836	0.275
	3rd Leaf Area	0.042*	0.014*	0.641
	Total Leaf Area	0.627	0.002*	0.041*
	Shoot Dry Weight	1	0.002*	<.0001*
	Root Dry Weight	1	1	1
Ladham and Chilham	*Coleoptile Height*	0.242	0.100	0.688
	2nd Leaf Growth	1	0.155	0.905
	3rd Leaf Growth	1	0.624	0.227
	2nd Leaf Area	0.532	0.367	0.861
	3rd Leaf Area	0.743	0.016*	0.788
	Total Leaf Area	0.711	0.066	0.124
	Shoot Dry Weight	1	0.085	0.011*
	Root Dry Weight	0.126	1	0.853
Nissaba and Talisker	*Coleoptile Height*	0.452	0.014*	0.157
	2nd Leaf Growth	<.0001*	0.721	0.0003*
	3rd Leaf Growth	0.155	0.232	0.844
	2nd Leaf Area	0.0007*	0.370	0.318
	3rd Leaf Area	0.009*	0.571	0.554
	Total Leaf Area	0.031	0.090	0.182
	Shoot Dry Weight	1	0.007*	0.007*
	Root Dry Weight			

All varieties responded similarly in terms of coleoptile height with no significant variety x compaction interaction (**Figure** *2.3.2*, **Table** 2.3.2). However, compaction significantly changed coleoptile height which was reduced by 52.8% between low and high compaction in Kutz, whereas Chewink height only reduced by 11.6%. However, contrary to the original hypothesis stated previously. Chewink seemed to be unaffected by the compaction level when looking at coleoptile height, this could perhaps be due to the method of planting the seeds in that they were placed on top of the compacted layer and then covered in loose soil and so may not have reached the compacted layer yet, or Chewink is simply more vigorous in the beginning compared to Kutz. Germination rates were very low for seeds planted in pots under high compaction with 4% germination of Kutz and 6% of Chewink under high compaction, 19% of Kutz and 21% of Chewink under intermediate compaction, and lastly 35% of Kutz and 50% of Chewink under low compaction.

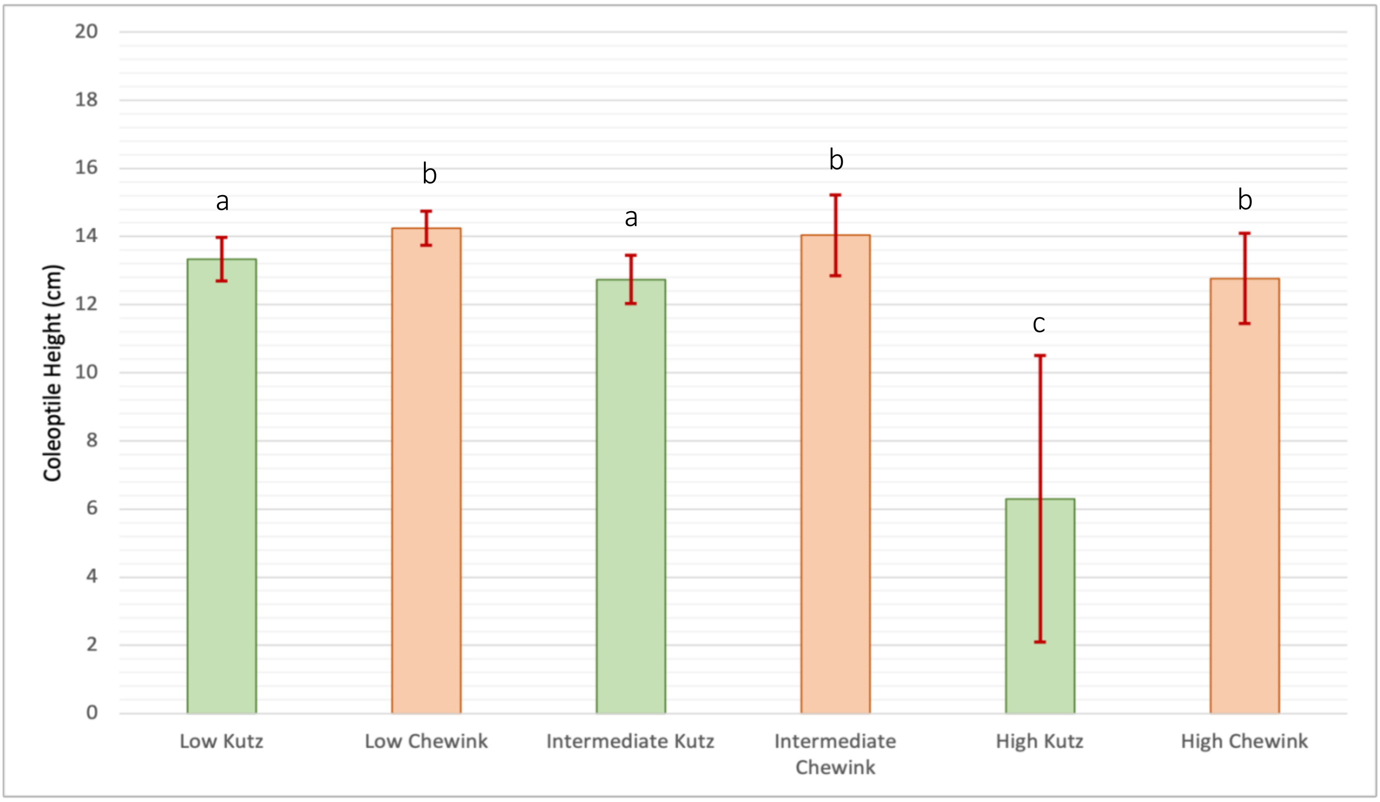

Figure 2.3.2: Coleoptile height of different wheat cultivars at different levels of soil compaction. Bars are means ± SE, and letters of mean discrimination (columns with different letters indicate that their means are significantly different at p = 0.05). Two-way ANOVAs were completed with p = 0.0253 between varieties, p = 0.0436 between compaction levels, and p = 0.1208 between both (interaction). Low Kutz n = 17, Low Chewink n = 24, Intermediate Kutz n = 10, Intermediate Chewink n = 10, High Kutz n = 2, and High Chewink n = 3.

Very interestingly, tillering begins earlier in Chewink than it does in Kutz, with 8 Chewink plants tillering ~ 2 weeks after planting and only 3 Kutz plants tillering, it is highly likely that there would be a significant difference between these two numbers. This would mean that Chewink is potentially more vigorous than Kutz, which could make it look as though it performs better under high compaction, even with a higher sensitivity to ethylene.

Soil compaction significantly alters total leaf area (**Figure** *2.3.3*) with Kutz and Chewink behaving differently (indicated by a significant cultivar x compaction interaction). High compaction decreased Kutz leaf area by 43.5% but decreased Chewink leaf area by only 20.7%. Intermediate soil compaction decreased leaf area similarly in Chewink (30.3% decrease) and Kutz (34.9% decrease). This shows that both intermediate and high compaction significantly impact leaf area and to somewhat similar levels, however, perhaps there is some type of adaptation occurring in Chewink under high compaction.

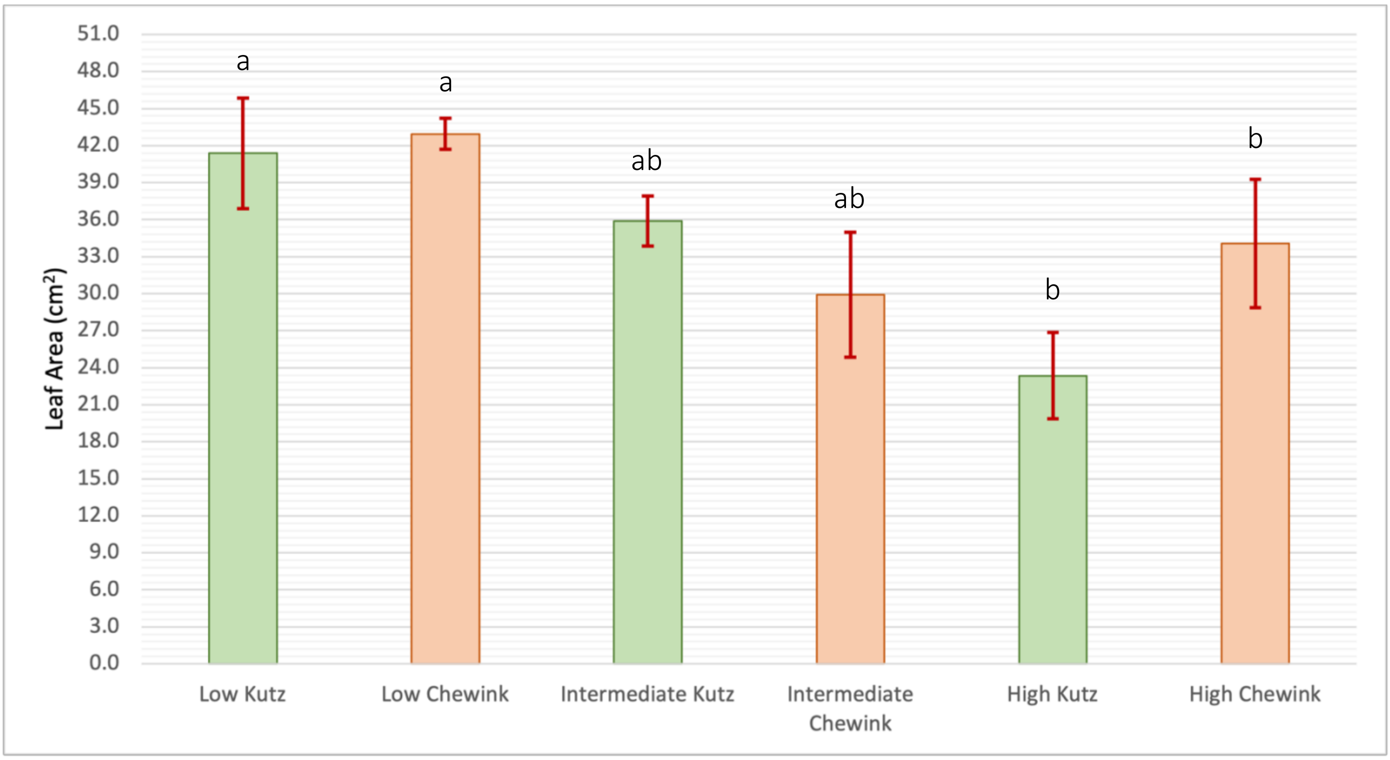

Figure 2.3.3: Total leaf area of different wheat cultivars at different levels of soil compaction (n = 6). Bars are means ± SE, and letters of mean discrimination (columns with different letters indicate that their means are significantly different at p = 0.05). Two-way ANOVA calculated that p = 0.6274 between varieties, p = 0.002 between compaction levels, and p = 0.0405 for the interaction. These results suggest that compaction levels are statistically significant from one another as well as the interaction effects. As can be seen in **figure 2.3.1** there is a pattern that the results follow

with Chewink showing greater leaf area in low and high compaction but not intermediate compaction, this is likely due to the sensitivity of Kutz versus Chewink to ethylene.

Compaction significantly decreased shoot dry weight of Kutz and Chewink (**Figure** *2.3.4*), Ladham and Chilham (**Figure** *2.3.5*), and Nissaba and Talisker (**Figure** *2.3.6*) were all significantly reduced by compaction. Kutz and Chewink responded differently to soil compaction (significant cultivar x compaction interaction) with intermediate and high compaction decreasing Kutz shoot dry weight by 8.5% and 42.1% respectively, and Chewink shoot dry weight by 30.8% and 14.8% respectively. Ladham and Chilham also responded differently to soil compaction (significant cultivar x compaction interaction) with intermediate and high compaction decreasing Ladham shoot dry weight by 25.3% and 40.1% respectively, and Chilham shoot dry weight by 33.3% and 16.4% respectively. Lastly, Nissaba and Talisker showed significance at the interaction level with a 11.7% increase and 15.5% decrease in shoot dry weight of Nissaba respectively, and a 6.3% and 21.8% decrease in Talisker respectively. There seems to be a non-linear response to compaction in terms of shoot dry weight at high compaction for Chewink, and at low versus high compaction in Nissaba and Talisker.

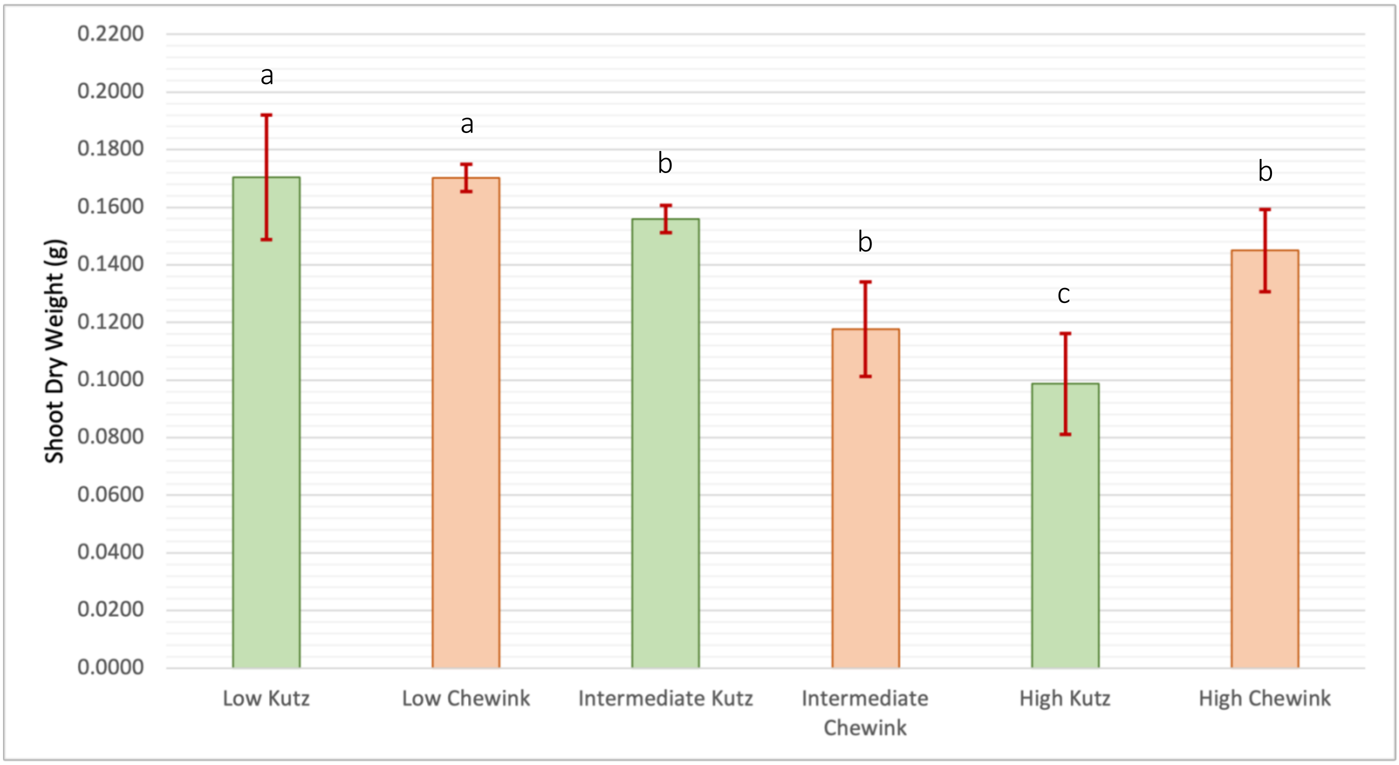

Figure 2.3.4: Shoot dry weight of different wheat cultivars at different levels of soil compaction (n = 5). Bars are means ± SE, and letters of mean discrimination (columns with different letters indicate that their means are significantly different at $p = 0.05$). Two-way ANOVA results show that $p = 1.0$ between varieties, $p = 0.0023$ between compaction levels, and $p = <0.0001$ for the interaction between variety and compaction levels. Specifically, the interaction is highly significant and there is a large difference between group means compared to within-group variation.

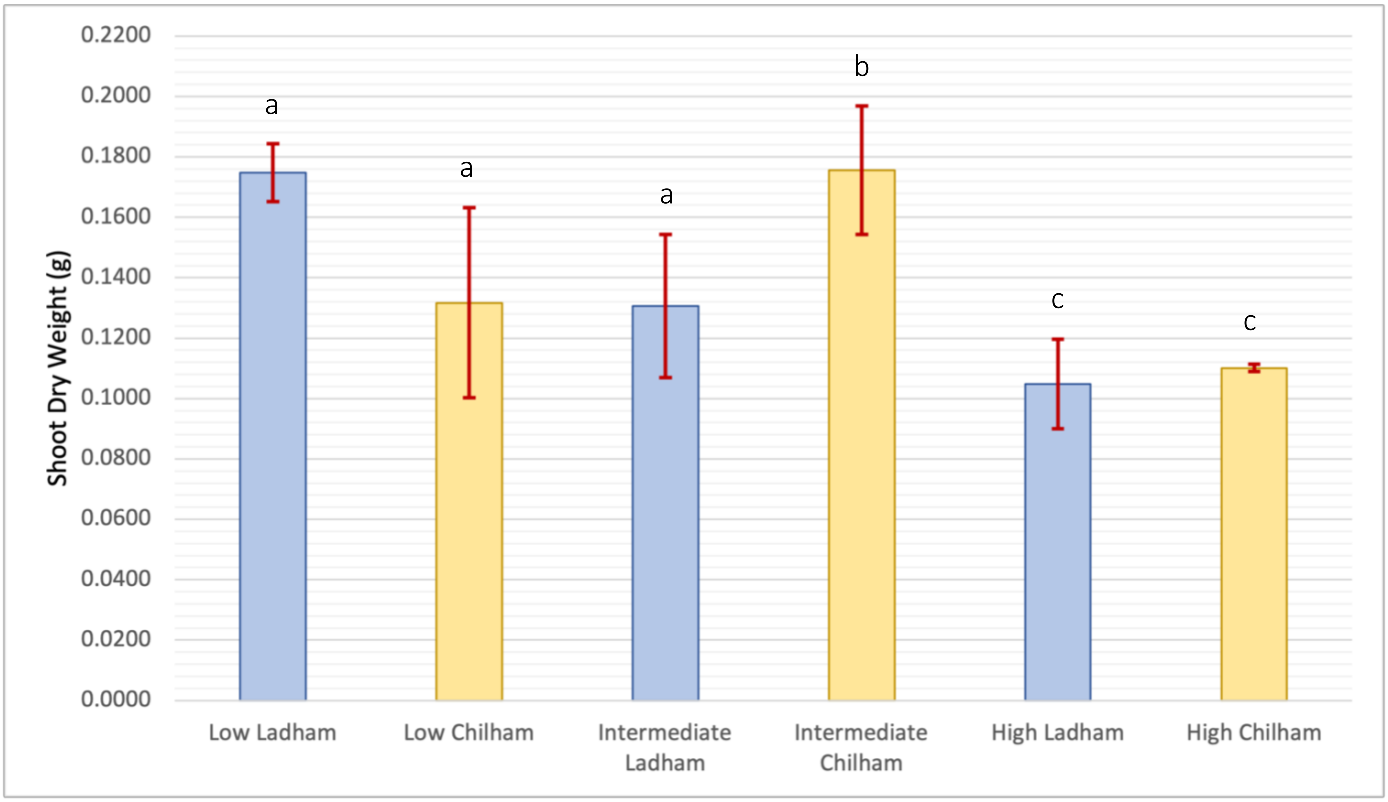

Figure 2.3.5: Shoot dry weight of different wheat cultivars at different levels of soil compaction (n = 6). Bars are means ± SE, and letters of mean discrimination (columns with different letters indicate that their means are significantly different at p = 0.05). Two-way ANOVAs show p = 1.0 between varieties, p = 0.0853 between compaction levels, and p = 0.0106 for interaction effects.

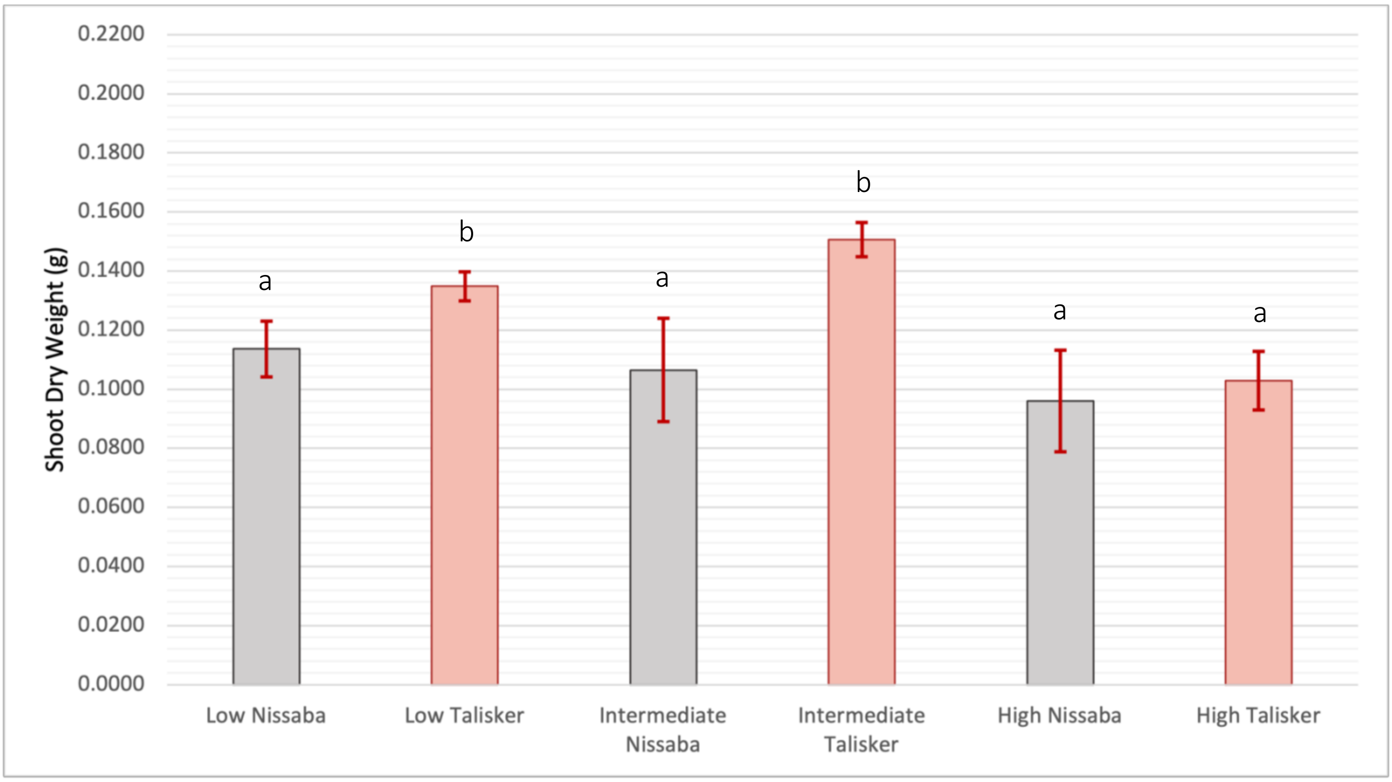

Figure 2.3.6: Shoot dry weight of different wheat cultivars at different levels of soil compaction (n = 7). Bars are means ± SE, and letters of mean discrimination (columns with different letters indicate that their means are significantly different at $p = 0.05$). Two-way ANOVAs showed that $p = 1.0$ between varieties, $p = 0.0065$ between compaction levels, and $p = 0.0065$ for the interaction between variety and compaction level. This shows that compaction and variety have statistically significant effects on shoot dry weight at the 0.05 level.

2.4. Discussion

Differential Response to Soil Compaction in Chewink and Kutz

The pattern observed for leaf area was interesting because Chewink would theoretically have a much higher reduction in leaf area than Kutz under high compaction (**Figure 2.3.1**), however, this was not the case which suggests that the data does not support the hypothesis that varieties with higher ethylene sensitivity would show greater reductions in growth. Due to early tillering of Chewink, the plants may simply be larger than Kutz from the beginning and, again, appear more vigorous than Kutz, with this cultivar potentially adapting in some way to the higher compaction, differential expression of N-assimilating genes perhaps? This would be interesting to investigate in the future. Or could higher ethylene sensitivity result in lower compaction sensitivity?

High compaction is limiting, as already discussed, because of a disruption to soil structure which leads to reduced gas exchange, nutrient and water uptake, and reduced microbial growth (Pandey and Bennett, 2023). The reduced growth observed under high compaction was likely due to roots sensing ethylene that accumulates and is unable to escape under high compaction (Pandey and Bennett, 2023). This growth pattern is true for intermediate compaction in Kutz and Chewink, but not high compaction, and was observed under high compaction in Ladham, Chilham, Nissaba, and Talisker when looking at above-ground traits (**Figure** *2.3.2*, **Figure** *2.3.3*, **Figure** *2.3.4*, **Figure** *2.3.5*, and **Figure** *2.3.6*). As unusual as this may be, this is still an interesting result and would be interesting to look into further. From all of the parameters measured to ascertain which varieties show the greatest response to soil compaction, the largest differences were observed in Kutz and Chewink, more specifically the above-ground responses were significant. A more detailed root analysis may prove more fruitful and should be chosen over dry weight in the future, but based on past research there is a significant impact of soil compaction on root development and growth, e.g., rooting depth and root number (Andersen et al., 2013; Colombi and Walter, 2017).

Interestingly, shoot dry weight was the only variable that consistently showed lower values under high compaction compared with low compaction across all six cultivars, and perhaps plants grown under high compaction grow at a slower rate than those under low compaction (Liu et al., 2022). An interesting point to discuss is the pattern of growth observed in Chewink plants under high compaction, from **Figure** *2.3.2*, **Figure** *2.3.3*, and **Figure** *2.3.4* a pattern emerges in which Chewink shows increased growth compared to Kutz, which is unusual because the ethylene sensitivity assays suggest that Chewink should be much more sensitive than Kutz under high compaction and thus should be less vigorous. However, this is not the case, this could be due to varying sensitivities to compaction under increasing compaction and Chewink may simply be less susceptible to reduced growth under high compaction but still remain more sensitive to ethylene than Kutz, so perhaps intermediate compaction is a better discriminate of compaction effects (due to additional limitation at high compaction being seen only in some cultivars). Although, ethylene sensitivity was assessed based on root elongation, not shoot biomass, therefore shoot and root ethylene sensitivities may differ. From research on soil compaction and the accumulation of ethylene around root tissues due to less macropores for ethylene to diffuse into and away from the roots, the results from this research do somewhat show a similar response in that growth was generally reduced under high compaction with effects varying depending on ethylene sensitivity (Pandey and Bennett, 2023).

Unrelated to ethylene, a suggestion of why this may be is that Chewink is better equipped to assimilate nutrients, such as Nitrogen, under high compaction compared to Kutz, this is speculation as no chlorophyll data is available. This was investigated in a study that found gene expressions of major nitrogen-assimilating enzymes may act as compaction indicators, and compaction causes the up-regulation of N-assimilating genes in HD-2967 but only upregulates expression at moderate compaction in Choti Lerma (which is moderately sensitive to compaction), which then becomes down-regulated at high compaction (Mondal et al., 2022). Perhaps it could be interesting to investigate this in Kutz versus Chewink.

Compaction Response is Genotype Dependent

The ethylene sensitivity assays completed using ethephon can be used to conclude why certain varieties responded to soil compaction in various ways, for example Nissaba and Talisker have very similar levels of ethylene sensitivity, and this may explain why so few of the results were significant (Nissaba and Talisker responded similarly to soil compaction) (Table *2.3.2*) and perhaps shoot dry weight significance was as a result of something other than compaction, perhaps Talisker embryos were larger than those of Nissaba, and possibly seed weight could also contribute. Although when planting, only seeds of a similar size were placed in pots but the seed weights were not recorded. Ladham and Chilham have differing levels of sensitivity to ethylene (Ladham was more sensitive than Chilham) and with this being significant Ladham does show reduced growth compared to Chilham under intermediate and high compaction when looking at shoot dry weight, reinforcing their significant differences in ethylene sensitivity. Although Chewink root elongation was much more sensitive to ethylene than Kutz (Figure 2.3.1), shoot growth sensitivity to soil compaction had the opposite response in that coleoptile height, leaf area, and shoot dry weight were significantly higher in Chewink than Kutz under high compaction (Figure *2.3.2*, Figure *2.3.3*, and Figure *2.3.4*) except for total leaf area under intermediate compaction. Chewink also tillers earlier than Kutz making Chewink appear more vigorous than Kutz. This raises the question of whether soil compaction response is solely an ethylene-mediated response or whether there are there other factors that impact plant response.

Ethephon significantly increased total plant nitrogen content in soft red winter wheat which is often sprayed with ethephon due to their lodging tendencies (Van Sanford et al., 1989). Ethephon mimics the effects of ethylene because it breaks down into ethylene, phosphoric acid, and chloride ions once administered to plants. Appropriate application of ethephon has beneficial effects for farming overall and is very useful for experiments instead of using ethylene or ACC but perhaps there are different signalling pathways involved within plants compared to using ACC. For example, ethephon is broken down into ethylene due to the instability of ethephon in the acidic environments within plant cells, whereas ACC is broken down through an enzymatic pathway (see chapter 1.5).

2.5. Conclusion

Overall, the increased performance of Chewink under high compaction compared to Kutz (**Figure** *2.3.2*, **Figure** *2.3.3*, and **Figure** *2.3.4*) was contrary to its greater ethylene sensitivity than Kutz. While ethylene accumulation around the roots seems the most likely cause of root growth inhibition when crops were grown in soils with high strengths/ densities (Pandey et al., 2021), shoots seemingly responded differently. As well as assays like the ethephon assay completed above there should be other assays completed on shoot ethylene sensitivity rather than root ethylene sensitivity because there is a need to understand possible root to shoot ACC/ ethylene signalling.

However, the varieties were sensitive to different levels of compaction, so one may have been more sensitive to intermediate compaction than high compaction, for example, Chewink may have appeared more vigorous than Kutz due to the early tillering, higher shoot dry weight, increased coleoptile height, and larger leaf area and may have seemed as though Chewink began life with increased growth compared to Kutz under high compaction, but could the early tillering simply make it seem as though Chewink was performing better early-on even though this variety was more sensitive to ethylene. This would be interesting to investigate by looking at the ethylene sensitivity of tillering and whether tillering is more or less sensitive to compaction than shoot biomass. This was why collecting data like leaf area was important as it allowed one to discriminate differential compaction effects at different developmental stages, so from the data collected, Chewink continued to outperform Kutz under high compaction. Perhaps the early tillering of Chewink resulted in larger plants that were still reacting to soil compaction more than Kutz, but the fact that they may be larger in the first place made it look as though there was improved growth.

Finally, ethylene sensitivity, in the case of wheat, impacts crop emergence in an expected pattern for Kutz and Chewink under intermediate compaction, with Chewink plants having smaller coleoptiles than Kutz plants. This pattern did switch under high compaction with Chewink having had significantly larger coleoptiles than Kutz. This could be for multiple mentioned reasons and would be interesting to research what was causing this, e.g., look at gene up/down-regulation under more compaction levels, perhaps five levels of compaction (low, low-intermediate, intermediate, intermediate-high, high) instead of three to create a clearer picture of where this behaviour from Chewink was stemming from. Crop establishment was similarly impacted with Chewink performance improving under high compaction and being below that of

Kutz under intermediate compaction (shoot dry weight and total leaf area). Again, researching why Chewink was behaving this way could help provide an answer to these results.

3. An analysis of the impact of ethylene sensitivity on the response of different varieties of potato to soil compaction

3.1. Introduction

Potatoes are a heavily relied on crop in the UK and even with farm area allocation decreasing (see **chapter 1.6.2**), the yield has increased nevertheless (see **chapter 1.1**) (**Figure** *3.1.1*). The largest yields of potatoes were reported by New Zealand in 2022 with over 50 t/Ha (FAOSTAT, 2021), even with a lower area harvested than many other countries. This is likely because of a favourable climate, fertile soils from volcanic activity, and cultural significance (the latter determining the allocation of area, not yield). Possible reasons for yield stagnation from 2016-2022 (**Figure** *3.1.1*) could be that there are higher levels of compaction experienced in soils from heavier machinery, climate change like dryer summers and more wet winters, loss of soil organic matter from lack of crop rotation, inefficient fertiliser use, and increasing pressure from pests and diseases (like potato cyst nematodes and blight).

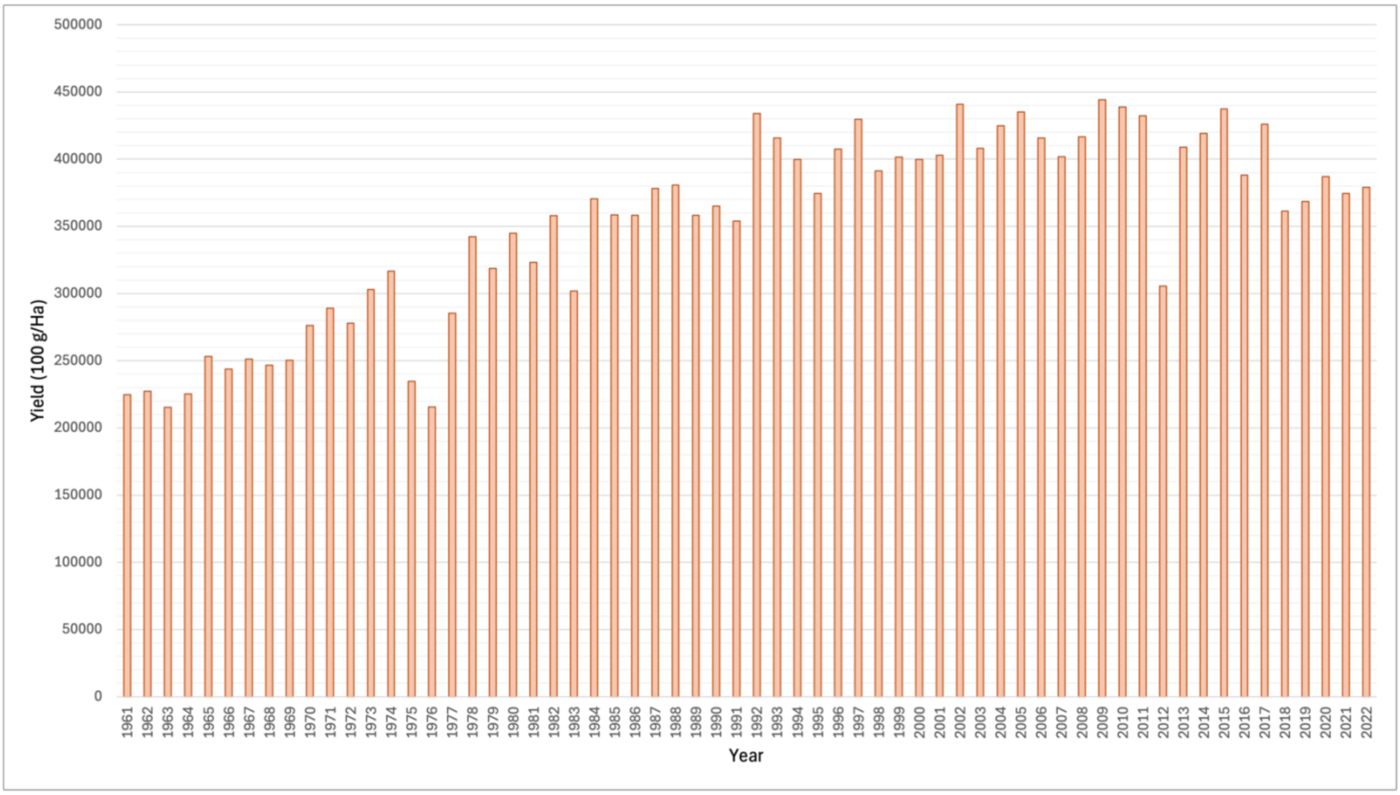

Figure 3.1.1 UK potato yields obtained from (FAOSTAT, 2021) showing the yield of potato every year commencing from 1961 to 2022.

Ethylene often accumulates in compacted soils (Pandey et al., 2021) and aids in warning the plant of highly compacted soil layers nearby the root system, and so the roots can respond accordingly to this. This is a stress-response, the roots of the tubers grow to avoid these layers because they pose issues like reduced nutrient and water uptake or root damage from waterlogging. Ethylene, therefore, can induce acclimation processes in which the roots may respond in particular ways (e.g., through hormone signalling to maintain growth in compact soil) to obtain some level of resistance (Druege, 2006) to specific abiotic stresses. Thus, potatoes have the ability to adapt to high compaction to some extent in order to maintain growth.

Potatoes generally grow a relatively shallow root system (Khan et al., 2016; Gervais et al., 2021) compared to crops with deeper root systems such as Alfalfa and Chickpea, which makes potatoes more susceptible to the effects of compaction due to most of the root system occurring within the upper layers of the soil. The decrease in available oxygen, nutrients, and water seen in highly compacted soils can delay emergence, reduce ground cover expansion, restrict light interception, and reduce tuber expansion, which is undesirable for yields and tuber size (Stalham, 2007). Not only does soil compaction reduce the availability of vital resources, but it also intensifies waterlogging (Griffiths and Philippot, 2013) in high rainfall areas. This waterlogging induces an anaerobic environment in which the roots are deprived of oxygen that is needed to respire, and increases disease risk for pathogens (e.g., potato blight) that have a motile infective stage. Although compaction effects on potato growth have been reasonably well described (Arvidsson and Håkansson, 2014; Skilleter, 2023), interactions of potato growth with ethylene signalling is less explored. Providing information on this topic could improve the sustainability of farming and could reduce the use of farming practices like excessive tilling, which may do more harm than good when looking at compaction, and with global demand increasing it is paramount that we provide food security to the world for the foreseeable future.

There are various management strategies in use, such as using wider tyres, tracked machinery, tram lines or restricted traffic farming, SOM addition, and subsoiling (Hamza and Anderson, 2005). However, research into the significance of the impact compaction has on plant health/ growth and potential existing varieties that are maybe more resistant or resilient to compaction is important to optimise agricultural practices (Skilleter, 2023) and increase yield, especially in areas where potatoes are heavily relied on. Ethylene's production as a result of stress responses can help in understanding how different potato varieties respond to compaction and thus can contribute to selective breeding strategies to produce more resilient varieties that are able to maintain high yields in areas with highly compacted soils.

Based on the improved growth of ethylene-insensitive plants in compacted soil compared to wild-type plants (Pandey et al., 2021), it is hypothesised here that potato varieties with higher sensitivity to ethylene will grow less than those with lower sensitivity in highly compacted soils.

3.2. Materials and Methods

Plant and Soil Method

Four potato varieties were grown over a 3-month period and planted in Bailey's of Norfolk topsoil by being placed on top of the compacted layer and then being covered in loose soil (Hevingham, Norfolk, UK), the varieties used were Cara, Marfona, Pentland Javelin, and Hermes. These were obtained from Strathmore Potatoes (Forfar, UK) and varieties picked were subject to availability.

Pot manufacture and soil compaction was performed in the same way as stated in **chapter 2.2**, with 2 minitubers placed on top of the compacted layer (~ 2cm from the rim) and covered in loose topsoil. Potatoes were grown in a controlled environment room with illumination provided by metal halide lamps (Osram Powerstar HQI-T, Munich, Germany) with a PPFD at bench height of 450 µM/m^2/s. and a daytime temperature maintained between 24-25°C for sixteen hours a day and a night-time temperature of 15-16°C. For growth in compacted soils the plants were not given any fungicides, pesticides, or fertilisers.

Ethylene Assay Method

Minitubers of < 20mm were picked based on the length of roots that had sprouted after being in a dark and warm environment, the most homogenous subset was chosen and placed into 7.8 L plastic containers on top of an acrylic grid (**Figure** *3.2.1*) suspended above ~5cm of water. The grid prevented the minitubers from being submerged in the water while allowing the water to create a humid environment. The containers were duct-taped shut and self-healing ports (**Figure** *3.2.1*) were used to inject ethylene gas into the containers to achieve 20 ppm of ethylene. Once the ethylene was added the containers were covered to keep them in the dark and placed in a warm (~ 24°C) place. These minitubers were left for ~4 weeks to grow and when harvested the root lengths were measured using a ruler, the data collected, and sensitivity calculated using a control.

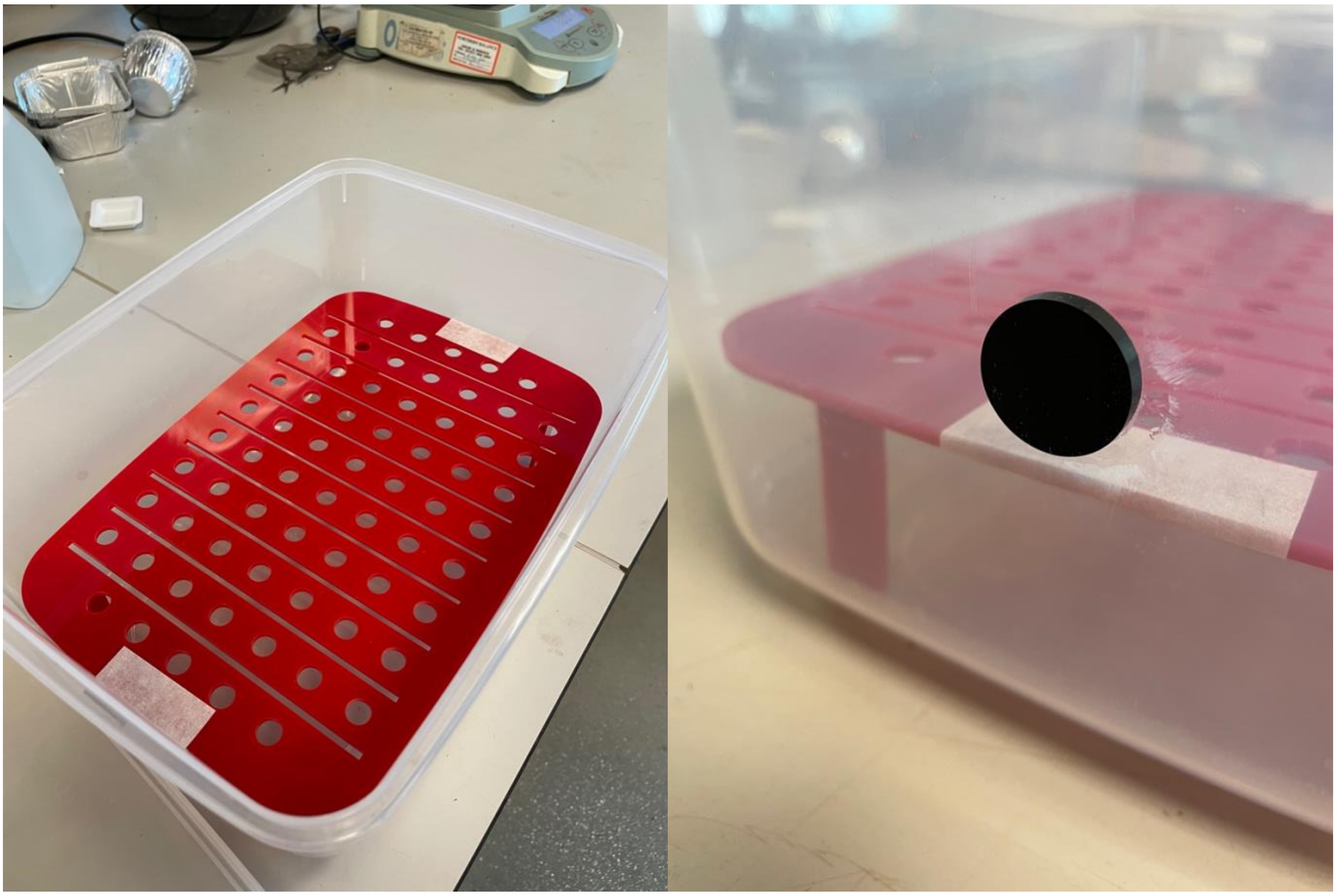

Figure 3.2.1 7.8 L plastic containers used for the ethylene assay on minitubers, each potato was placed on top of one hole to prevent them from moving excessively, the slits allowed the ethylene to move around the minitubers evenly, and the self-healing port used to inject 20 ppm of ethylene gas.

Measurements

Shoot height was measured ~7 days after emergence to assess whether soil compaction had an impact on the emergence of the potato varieties, and whether this relied on ethylene sensitivity.

Soil water content was monitored by weighing pots daily at 10am after water holding capacity (WHC) was determined and maintained at this weight. WHC was obtained by allowing the pots to soak up water through capillary action until fully saturated, they were then removed from the water and allowed to drain and then weighed.

Throughout the entire duration of growth, the plants were photographed every other day from above with a background and scale to calculate increase in total leaf area using ImageJ, with the ImageJ values being used to estimate progress. This growth data could be used to monitor the rate of growth depending on the compaction level and the variety. Relative leaf expansion rate was calculated by taking the values obtained from ImageJ and turning them into a Log graph.

At harvest total leaf area was measured using a leaf area meter (LiCor's LI-3100C, Cambridge, UK), this data was then compared to the leaf area obtained at harvest using ImageJ (**Figure 3.2.2** and **Figure 3.2.3**). Plant height was measured from the soil layer to the tip of the plant after gathering all the branches together holding them upwards. After leaf area had been collected for all plants the leaves and shoots were placed in an oven for 96 hours until completely dry and weighed to obtain dry shoot biomass. Roots were harvested and carefully washed using a sieve after leaf area was obtained and shoots placed in a drying oven, they were then stored in 50% ethanol in a fridge (4°C) until root scanning could be completed. To scan the roots, they were removed from ethanol and placed in a glass tray containing ~1cm water to prevent the roots from sticking together or to the tray and the tray placed in an Epson Expression 12000XL scanner (Suwa, Nagano, Japan). Roots were separated using pipette tips and scanned at a resolution of 800 dpi. Root scans were analysed using Rhizovision, from which total root length, average root diameter, root surface area, and root volume were collected and analysed.

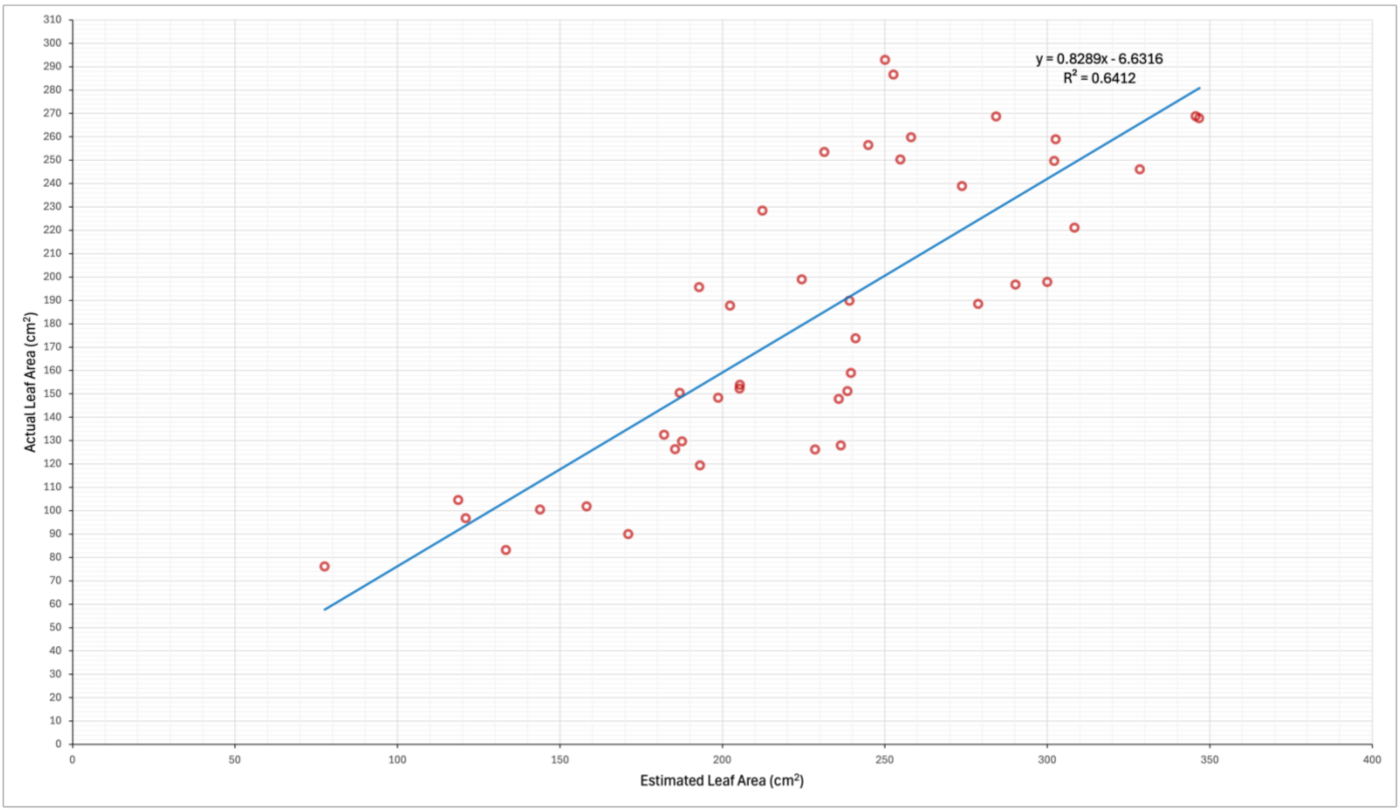

Figure 3.2.2 Estimated leaf area obtained from imaging plants every other day and using ImageJ to obtain leaf area plotted against actual leaf area taken from a leaf area meter (LiCor's LI-3100C, Cambridge, UK) to assess the accuracy of imaging (n = 8). This graph is showing the results from Cara and Marfona. $R^2 = 0.6412$ which shows a good positive relationship between the two parameters which would indicate that imaged leaf area is accurate when compared to actual leaf area. In this case ImageJ seemed to overestimate leaf area using ImageJ compared to actual leaf area.

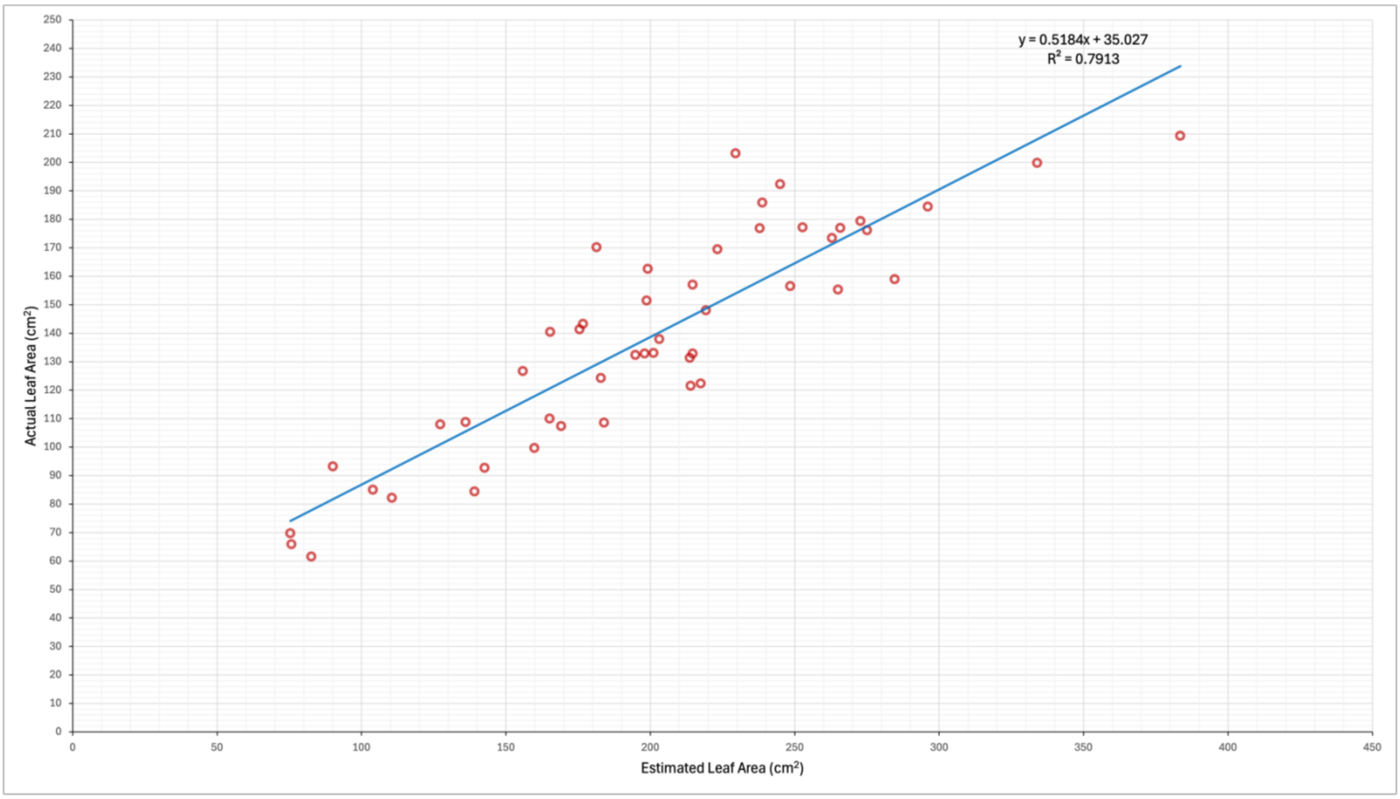

Figure 3.2.3 Estimated leaf area of Pentland Javelin and Hermes obtained from imaging plants every other day and using ImageJ to obtain leaf area plotted against actual leaf area taken from a leaf area meter (LiCor's LI-3100C, Cambridge, UK) to assess the accuracy of imaging (n = 8). R^2 = 0.7913 which shows a strong positive relationship between the two parameters which would indicate that imaged leaf area is accurate when compared to actual leaf area. In this case ImageJ seemed to be some overestimate leaf area using ImageJ compared to actual leaf area.

Statistical Analysis

See **chapter 2.2** for information the statistical analysis completed on the potato data.

3.3. Results

The results obtained throughout growth of the potatoes as well as at harvest mainly followed the hypothesis that varieties with higher ethylene sensitivity would show reduced growth at higher levels of compaction, as can be seen by comparing the results from the ethylene assay (**Figure 3.3.1**) with the measurements taken at harvest (**Table 3.3.1**).

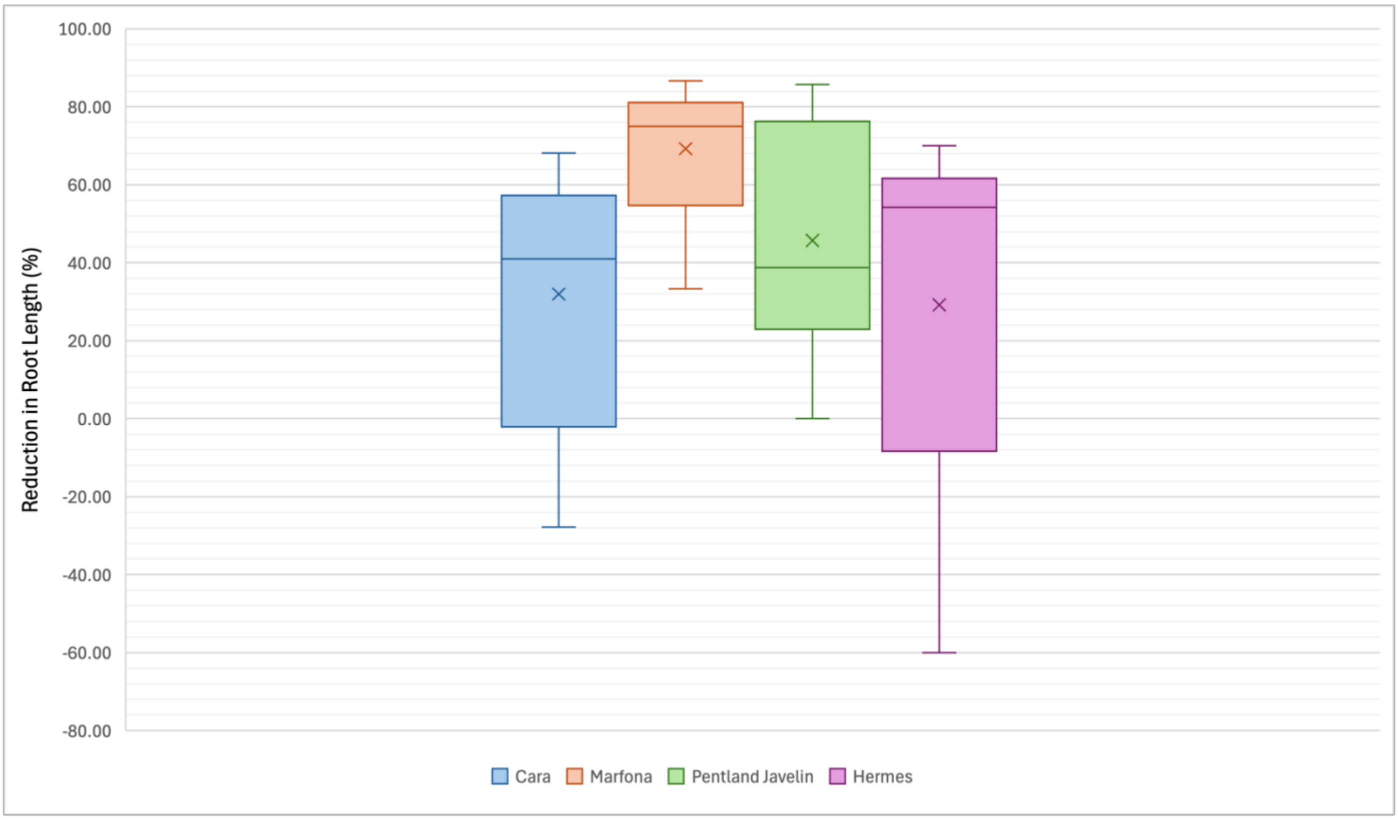

Figure 3.3.1 Ethylene assay completed on minitubers (n = 20). Root lengths were measured, and two-way ANOVAs completed with the results for Cara and Marfona being p = <0.0001 for variety, p = 0.7008 for treatment, and p = 0.0136 for variety x treatment which means that the root lengths of minitubers were significantly dependent on variety and treatment and Cara and Marfona show differences in ethylene sensitivity. For Pentland Javelin and Hermes p = <0.0001 for variety, p = 0.3413 for treatment, and p = 0.4884 for variety x treatment, this means that variety significantly impacted root length depending on variety, but root length did not depend significantly on ethylene or an interaction between the two. Two separate ANOVAs were conducted here because the control growths were more similar.

Table 3.3.1 Average results of the ethylene assay (root length in cm) with % inhibition and significance values calculated using two-way ANOVA, significance at the 0.05 level is indicated by an asterisk (*) and SE included.

Variety	Control	Ethylene	% Inhibition
Cara	8.8 ± 0.8	5.6 ± 0.8	32 ± 10.4
Marfona	10.8 ± 1.4	2.9 ± 0.3	69 ± 5.4
Pentland Javelin	4.2 ± 0.4	2.0 ± 0.3	46 ± 9
Hermes	4.3 ± 0.4	2.7 ± 0.4	29 ± 14.5
Variety			<0.0001 *
Ethylene			0.039 *
Interaction			0.120

Table 3.3.2 ANOVA results (P values reported) for the potato varieties Cara and Marfona, and independently Pentland Javelin and Hermes. An asterisk (*) indicates significance.

Variety	Variable Measured	Compaction Significance (p)	Variety Significance (p)	Interaction Significance (p)
Cara and Marfona	*Seedling Height*	0.498	0.117	0.787
	Shoot Dry Weight	<.0001*	<.0001*	1
	Plant Height	<.0001*	0.049*	1
	Total Leaf Area	<.0001*	0.056	0.914
	Total Root Length	<.0001*	<.0001*	0.458
	Average Root Diameter	0.0002*	0.006*	1
	Root Surface Area	<.0001*	<.0001*	1
	Root Volume	<.0001*	<.0001*	0.879
Pentland Javelin and Hermes	*Seedling Height*	0.009*	0.920	0.844
	Shoot Dry Weight	<.0001*	0.030*	0.093
	Plant Height	<.0001*	0.843	0.024*
	Total Leaf Area	<.0001*	0.003*	0.391
	Total Root Length	<.0001*	0.315	1
	Average Root Diameter	0.004*	0.0001*	1
	Root Surface Area	<.0001*	0.793	0.887
	Root Volume	<.0001*	0.766	0.796

Looking at the response of the root system to soil compaction in terms of root length, all varieties showed significant responses to high compaction with total length being much lower in high compaction than low compaction (**Figure** *3.3.2* and **Figure** *3.3.3*) with a 68% reduction in Cara and 76% reduction in Marfona, and 70% decrease in Pentland Javelin and 69% decrease in Hermes. The non-significant response of Cara and Marfona to compaction was interesting, with Marfona not seeming to be more sensitive to compaction than Cara as would be expected from the ethylene assay, and it seems as though Marfona was sensitive to intermediate compaction to a similar degree as high compaction. Pentland Javelin and Hermes did not show significant differences in root length between intermediate and high compaction levels, but root length was significantly lower between low and intermediate/ high compaction with 56% and 70% reduction in Pentland Javelin respectively and 50% and 69% decrease in Hermes respectively.

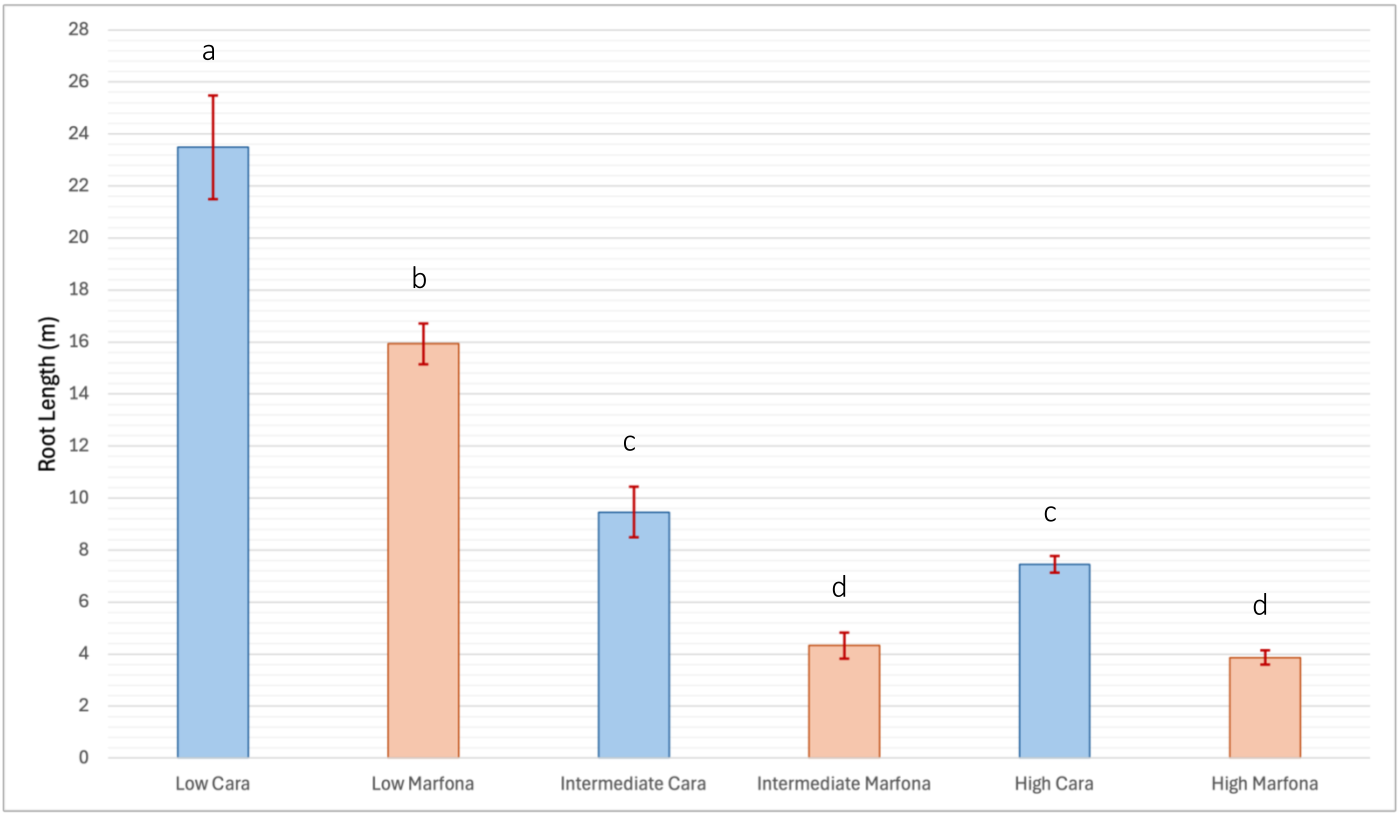

Figure 3.3.2 Root length of different potato varieties at different levels of soil compaction (n = 7). Bars are means ± SE, and letters of mean discrimination (columns with different letters indicate that their means are significantly different at $p = 0.05$). Two-way ANOVAs on the data found that significance for compaction was $p = <.0001$, for variety it was $p = <.0001$, and the interaction was $p = 0.4576$. These results show that both compaction and variety had significant effects on the root length of the potatoes, but compaction response was not dependent on variety.

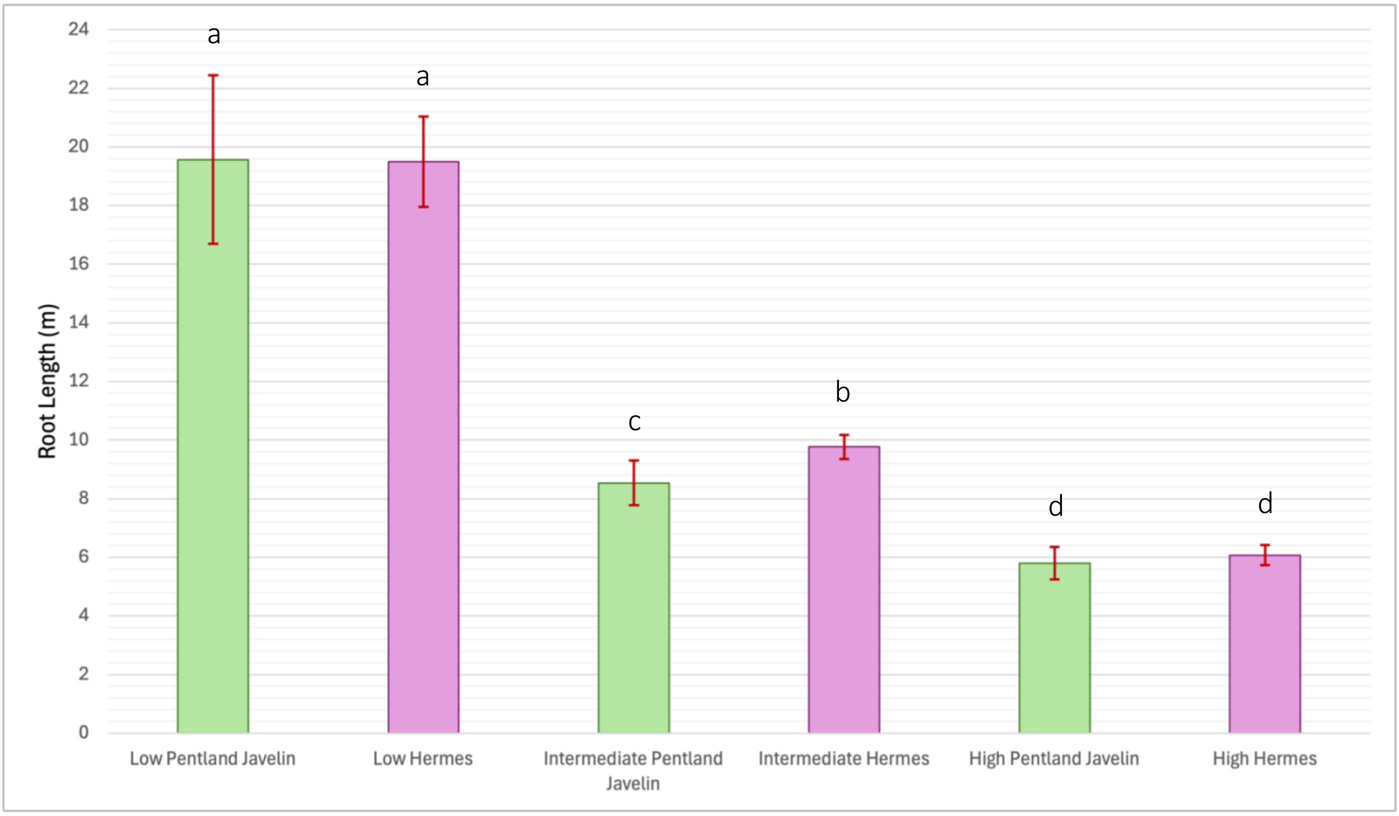

Figure 3.3.3 Root length of different potato varieties at different levels of soil compaction (n = 5). Bars are means ± SE, and letters of mean discrimination (columns with different letters indicate that their means are significantly different at $p = 0.05$). Two-way ANOVAs were conducted and found $p = <.0001$ for compaction, $p = 0.3152$ for variety, and $p = 1$ for interaction effect. In this case root length was only significantly impacted by the compaction level and the varieties did not show significant differences.

Average root diameter was significantly affected by compaction for all potato varieties studied with 27% increase and 12% increase under intermediate and high compaction in Cara and 17% and 14% increase in Marfona respectively, and 0.7% decrease and 6% increase in Pentland Javelin respectively, and 0.3% decrease and 7% increase in Hermes. Again, Marfona was not significantly more sensitive to the higher compaction than Cara, and the average root diameter increased as compaction increased. As has already been discussed this response is often seen in crops grown under high compaction when compared to those grown under low compaction as the plants attempt to adapt to the loss in root length high compaction causes. Hermes was significantly more sensitive to the high compaction than Pentland Javelin with root diameter significantly increasing for both varieties under high compaction, although diameter response to compaction did not depend on variety (**Figure** *3.3.4* and **Figure** *3.3.5*).

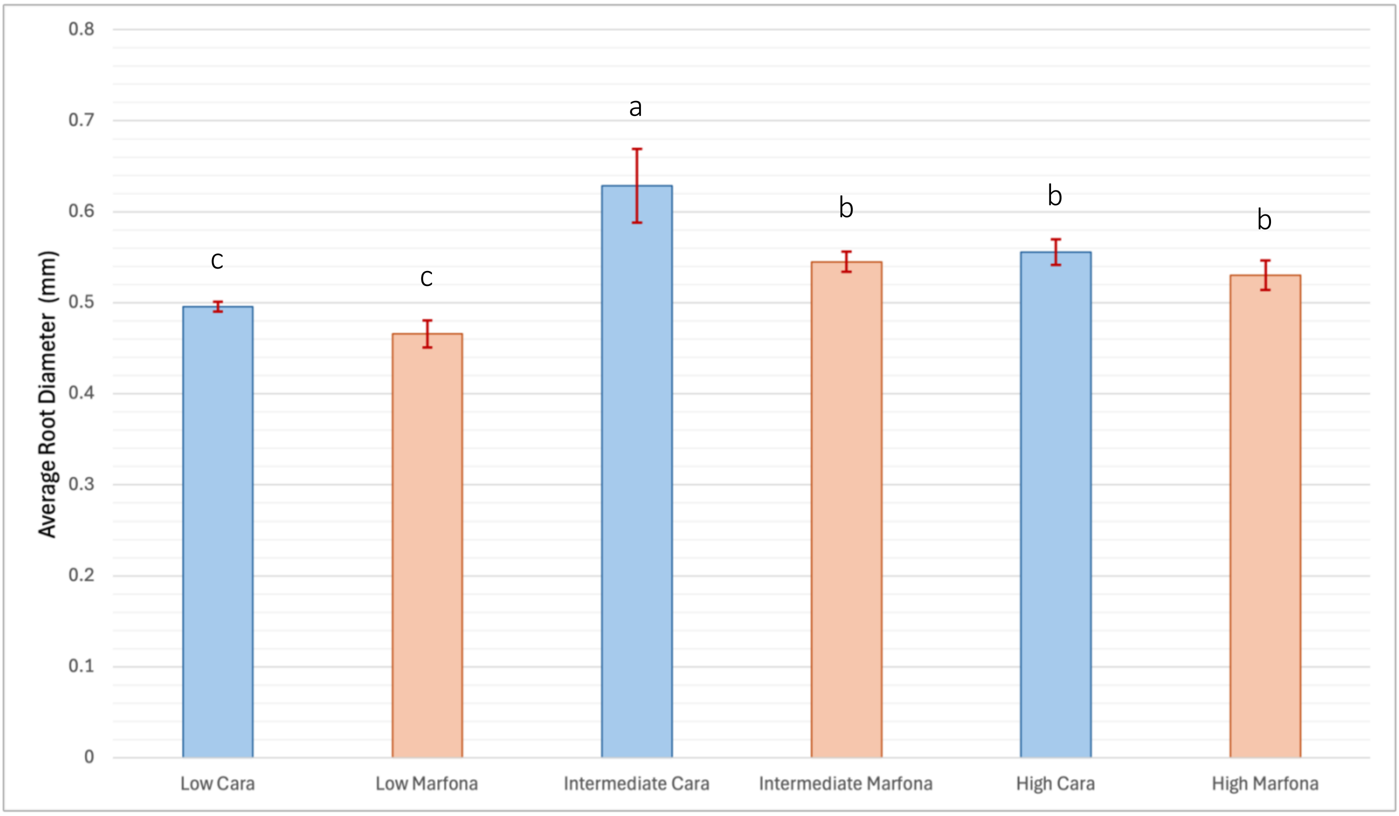

Figure 3.3.4 Average root diameter of different potato varieties at different levels of soil compaction (n = 7). Bars are means ± SE, and letters of mean discrimination (columns with different letters indicate that their means are significantly different at $p = 0.05$). Two-way ANOVAs were conducted, and the results were: $p = 0.0002$ for compaction, $p = 0.0062$ for variety, and $p = 1$ for an interaction between compaction and variety.

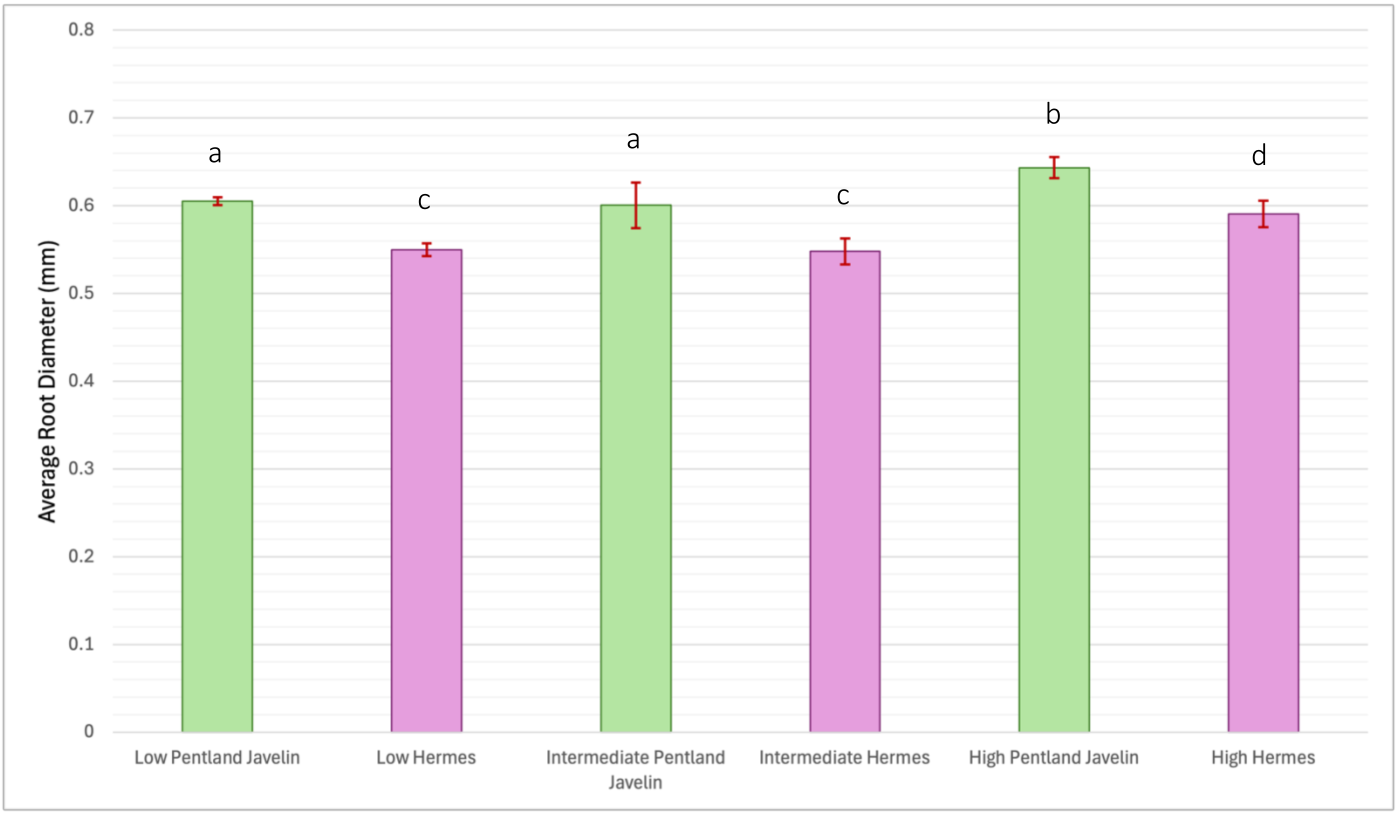

Figure 3.3.5 Average root diameter of different potato varieties at different levels of soil compaction (n = 5). Bars are means ± SE, and letters of mean discrimination (columns with different letters indicate that their means are significantly different at $p = 0.05$). Two-way ANOVAs on the data found that $p = 0.0039$ for compaction, $p = 0.0001$ for variety, and $p = 1$ for interaction effects.

As expected the roots with much larger lengths (those grown in low compaction soil) had higher surface areas compared to those grown in highly compacted soil with 45% and 63% decrease in Cara under intermediate and high compaction and 68% and 73% decrease in Marfona respectively (**Figure** *3.3.6* and 55% and 67% decrease in Pentland Javelin respectively and 51% and 65% reduction in Hermes **Figure** *3.3.7*). Root volume was also significantly affected by compaction by intermediate and high compaction with 23% and 6% decrease in Cara and 68% and 73% decrease in Marfona respectively, and 58% and 68% decrease in Pentland Javelin and 60% and 68% decrease in Hermes respectively. For both volume and surface area Marfona seemed not to be more sensitive to the effects of growing in highly compacted soils when compared to Cara, which was the less sensitive of the two varieties according to the ethylene assay. On top of this, Marfona seemed to react very similarly, again, to intermediate and high compaction meaning that the intermediate bulk density/ resistance chosen in this experiment was high enough to significantly alter the growth of Marfona but not Cara as much. Also as expected was the similar response of Pentland Javelin and Hermes to the highly compacted soils when looking at surface area and volume as the ethylene assay suggested the two had very similar levels of ethylene sensitivity.

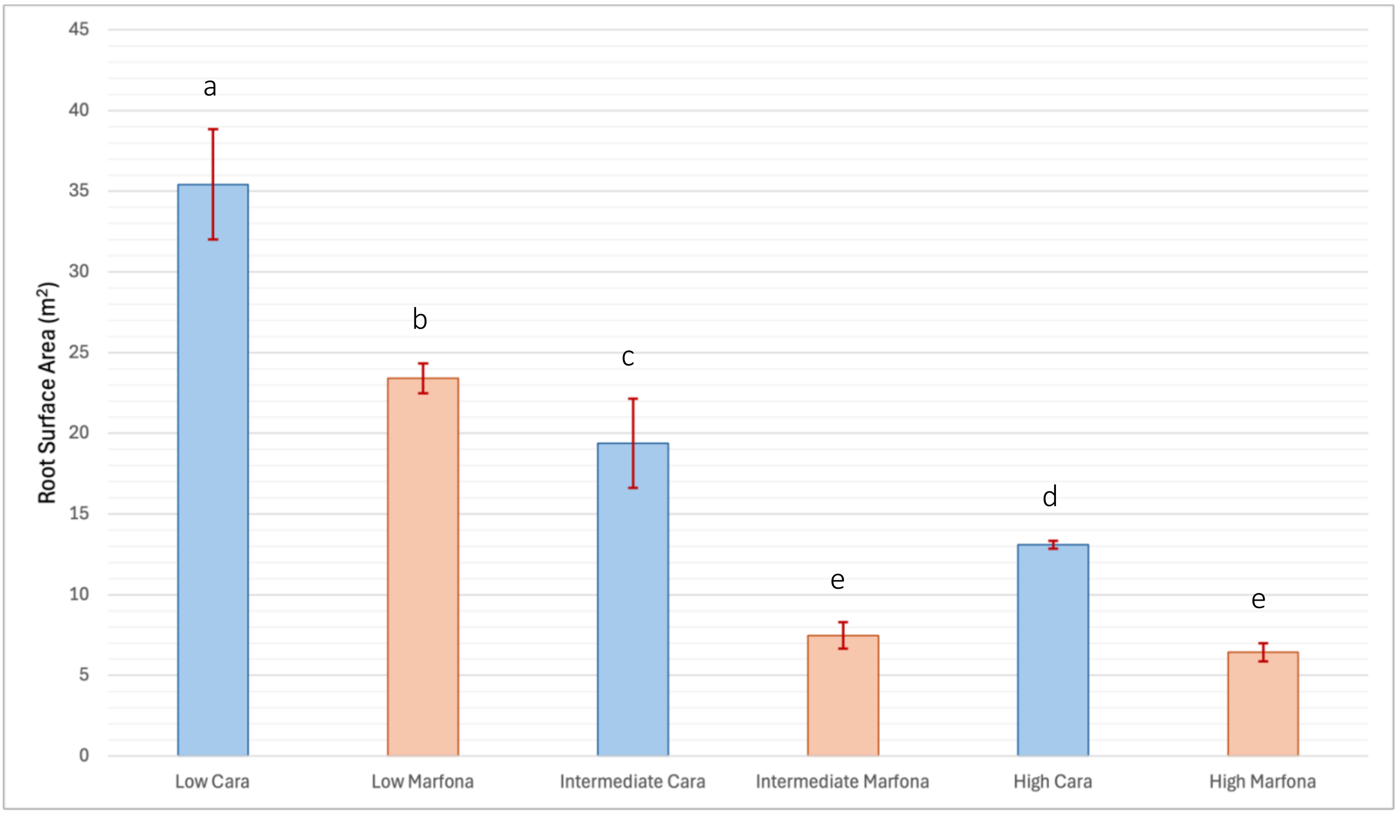

Figure 3.3.6 Root surface area of different potato varieties at different levels of soil compaction (n = 7). Bars are means ± SE, and letters of mean discrimination (columns with different letters indicate that their means are significantly

different at p = 0.05). Two-way ANOVAs on the data calculated that p = <.0001 for compaction, p = <.0001 for variety, and p = 1 for compaction x variety. Therefore, compaction and variety had significant effects on surface area of the roots, but compaction response did not depend on variety.

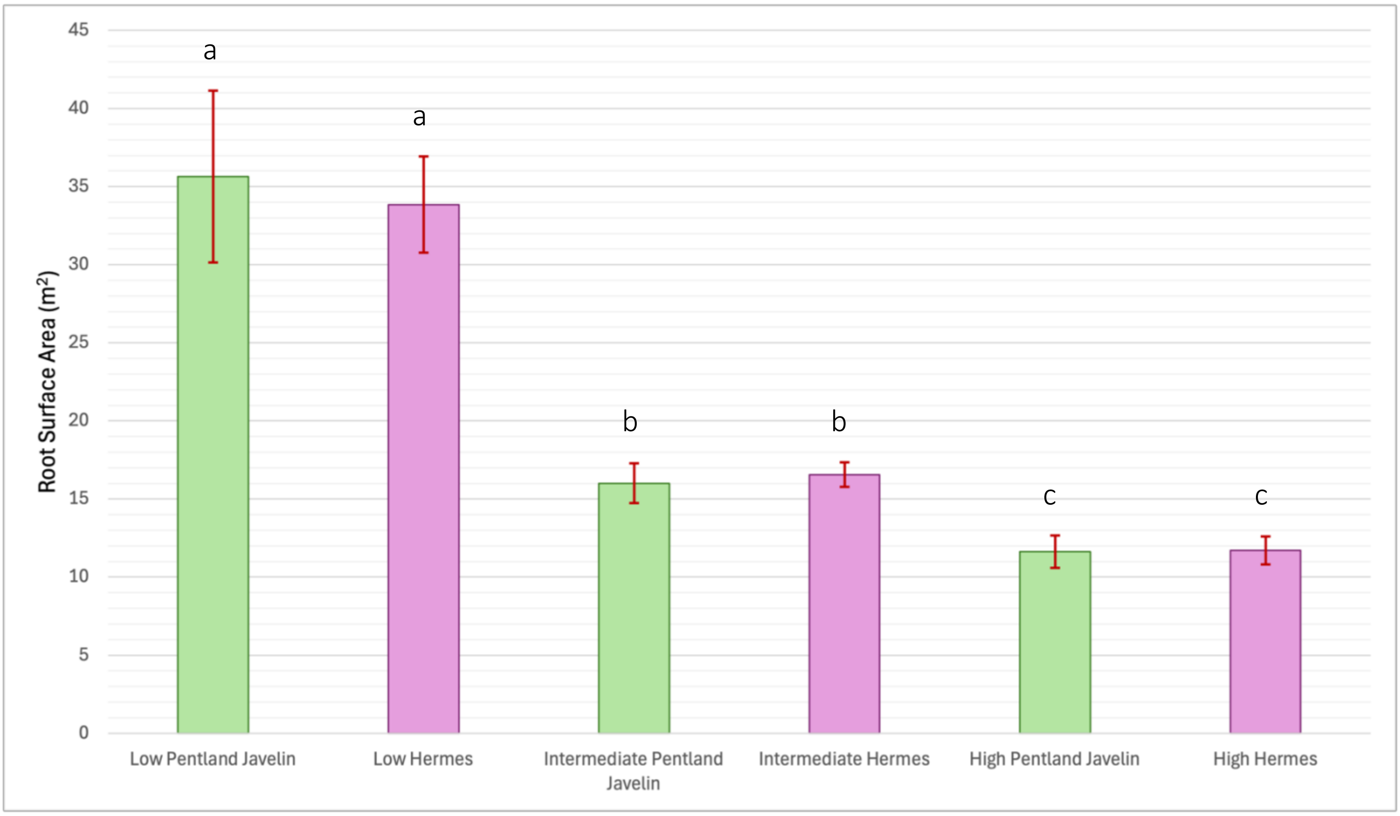

Figure 3.3.7 Root surface area of different potato varieties at different levels of soil compaction (n = 5). Bars are means ± SE, and letters of mean discrimination (columns with different letters indicate that their means are significantly different at p = 0.05). Two-way ANOVAs indicated that p = <.0001 for compaction, p = 0.7930 for variety, and p = 0.8873 for the interaction effect. This means that only compaction level significantly impacted root surface area, with variety and interaction effects showing non-significant results.

Next, looking at the above-ground responses, seedling height in Cara and Marfona did not depend on either compaction or variety. Seedling height in Pentland Javelin and Hermes was significantly lower in high compaction compared to low compaction with a 25% and 18% decrease in Pentland Javelin and Hermes respectively (**Figure** *3.3.8* and **Figure** *3.3.9*), but seedling height did not depend on variety or an interaction between variety and compaction. Cara, Marfona, Pentland Javelin, and Hermes have reasonably similar seedling heights at high compaction, although this early growth may not have been impacted by compaction because the minitubers were placed on top of the compacted layer and covered with loose soil, so until the tubers had established themselves they would have been growing in loose soil.

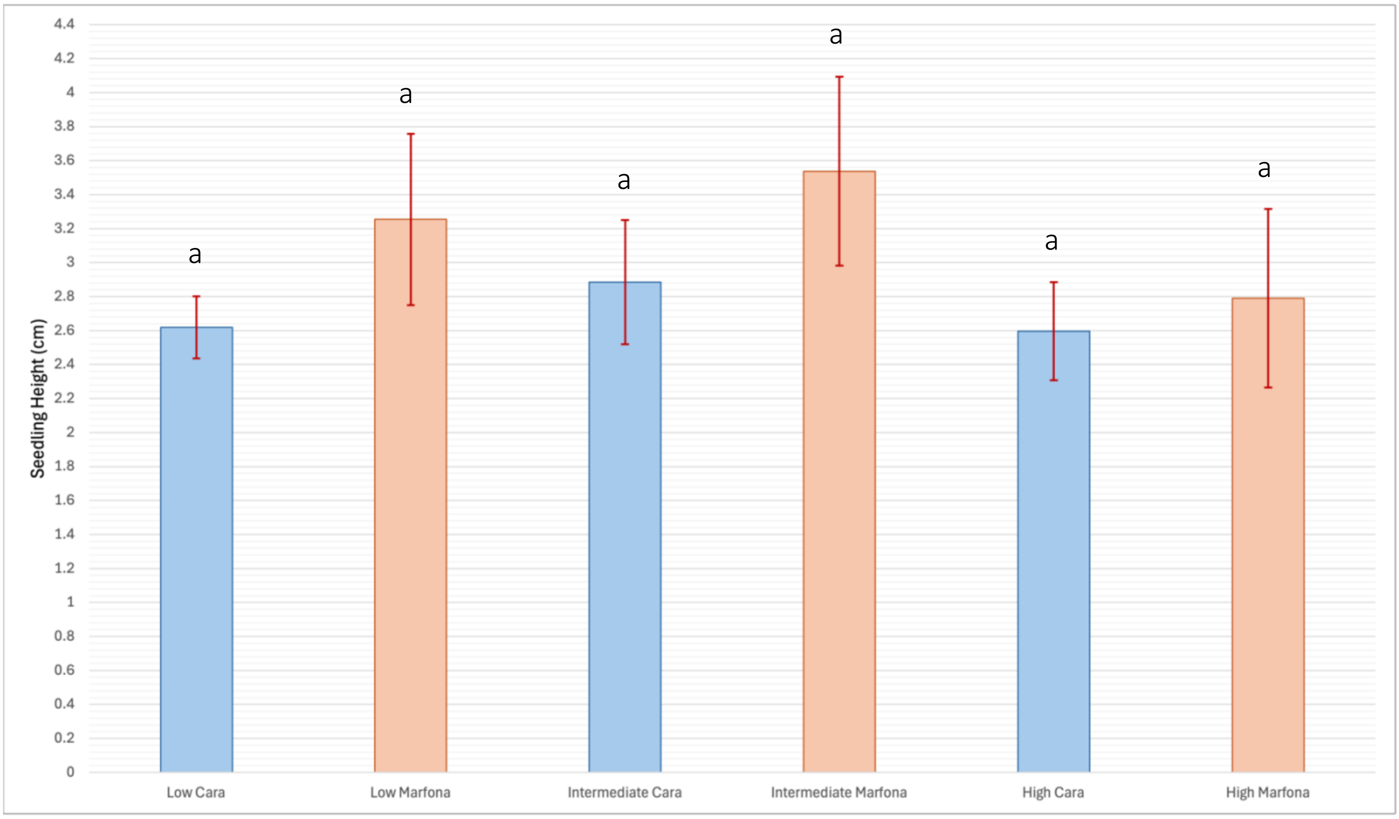

Figure 3.3.8 Seedling height of different potato varieties 7 days after planting at different levels of soil compaction. Bars are means ± SE, and letters of mean discrimination (columns with different letters indicate that their means are significantly different at $p = 0.05$). Two-way ANOVAs completed on the data did not show any significance of the data with $p = 0.4981$ for compaction, $p = 0.1173$ for variety, and $p = 0.7869$ for interaction. Low Cara n = 36, Low Marfona n = 26, Intermediate Cara n = 32, Intermediate Marfona n = 32, High Cara n = 28, High Marfona n = 21.

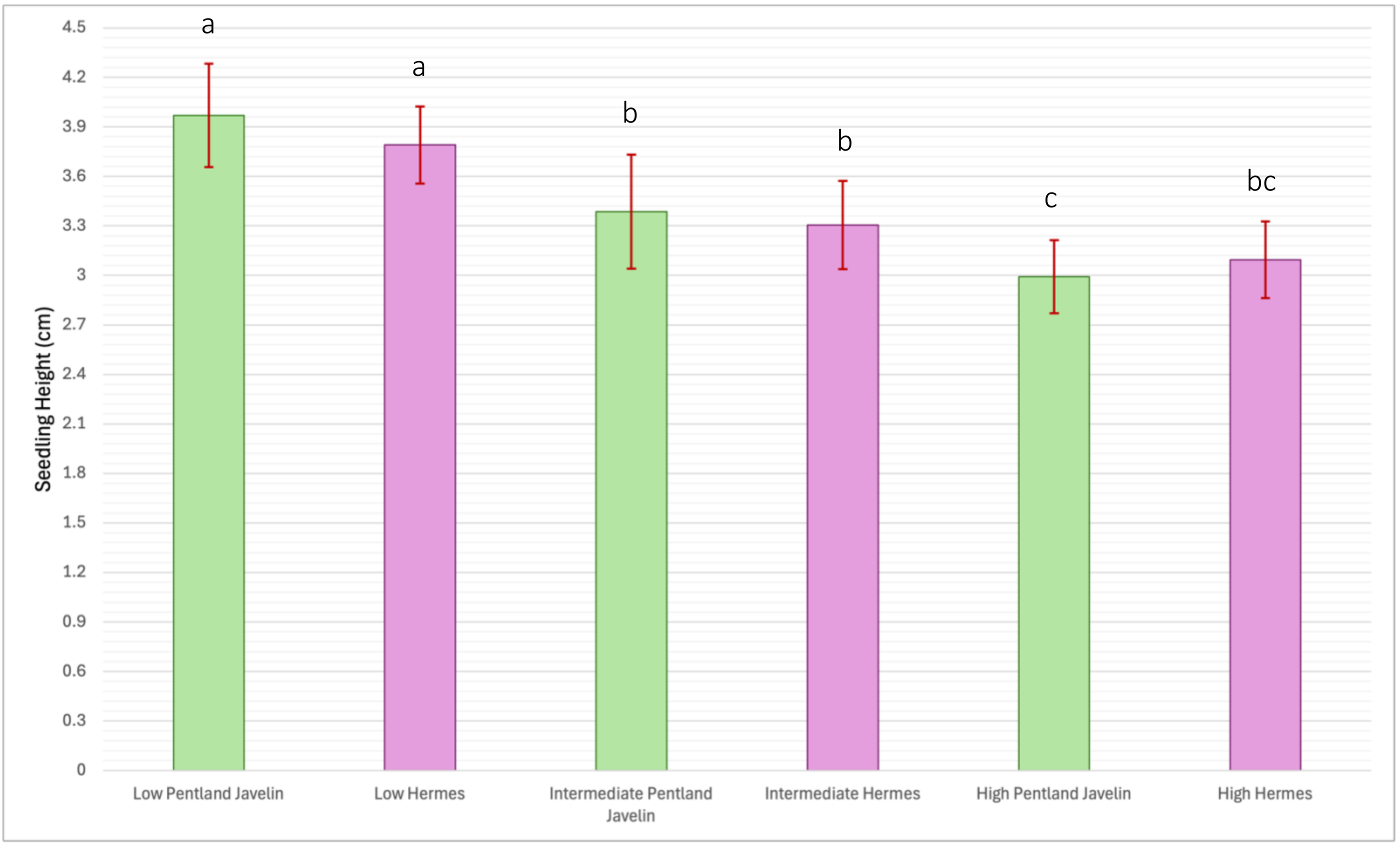

Figure 3.3.9 Seedling height of different potato varieties at different levels of soil compaction. Bars are means ± SE, and letters of mean discrimination (columns with different letters indicate that their means are significantly different at p = 0.05). Two-way ANOVAs were performed on the data with p = 0.0087 for compaction response, p = 0.9204 for variety response, and p = 0.8438 for an interaction response between the two. This indicated that compaction had a significant impact on seedling height, but variety did not, and height under different levels of compaction did not depend on the variety. Low Pentland Javelin n = 29, Low Hermes n = 41, Intermediate Pentland Javelin n = 29, Intermediate Hermes n = 40, High Pentland Javelin n = 33, High Hermes n = 36.

Shoot dry weight was significantly reduced at high compaction compared to low compaction (**Figure** *3.3.10* and **Figure** *3.3.11*) for both pairs of varieties. Marfona also seemed to be significantly impacted to a similar level at intermediate compaction as it was at high compaction. At intermediate and high compaction shoot dry weight was reduced by 12% and 35% in Pentland Javelin respectively and increased by 0.18% and decreased by 40% in Hermes respectively. In Cara shoot dry weight decreased by 26% and 49% and in Marfona the reduction was 42% and 52%. Pentland Javelin and Hermes seem to have reacted to high compaction similarly which was supported by their very similar sensitivity to ethylene as identified by the assay. At low and intermediate compaction Pentland Javelin had a lower shoot biomass than Hermes, but Hermes may simply be a larger variety.

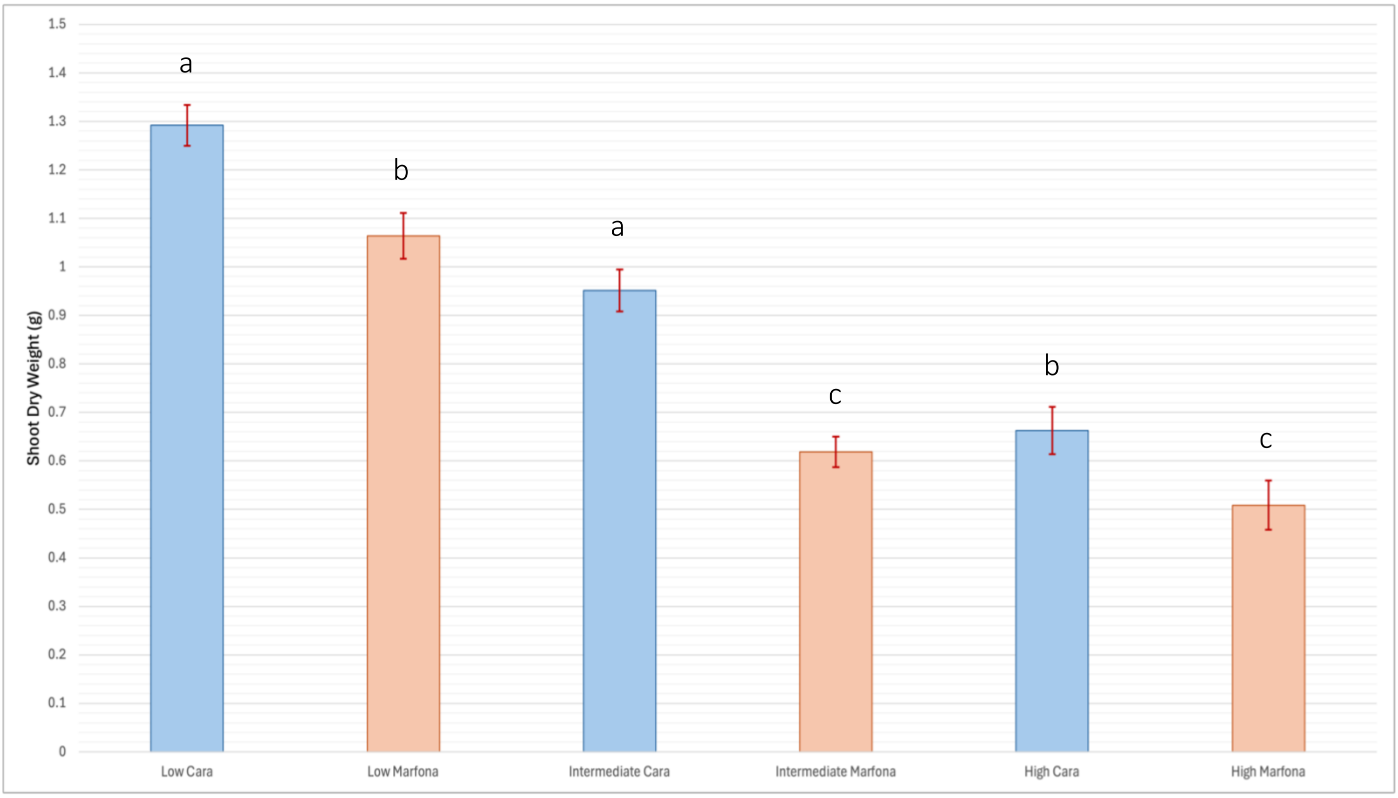

Figure 3.3.10 Shoot dry weight of different potato varieties at different levels of soil compaction (n = 7). Bars are means ± SE, and letters of mean discrimination (columns with different letters indicate that their means are significantly different at $p = 0.05$). Two-way ANOVAs completed on the data showed that $p = <0.0001$ for variety, $p = <0.0001$ for compaction, and $p = 1$ for variety x compaction. This shows that both variety and compaction significantly impact shoot dry weight, but variety response does not depend on the compaction level.

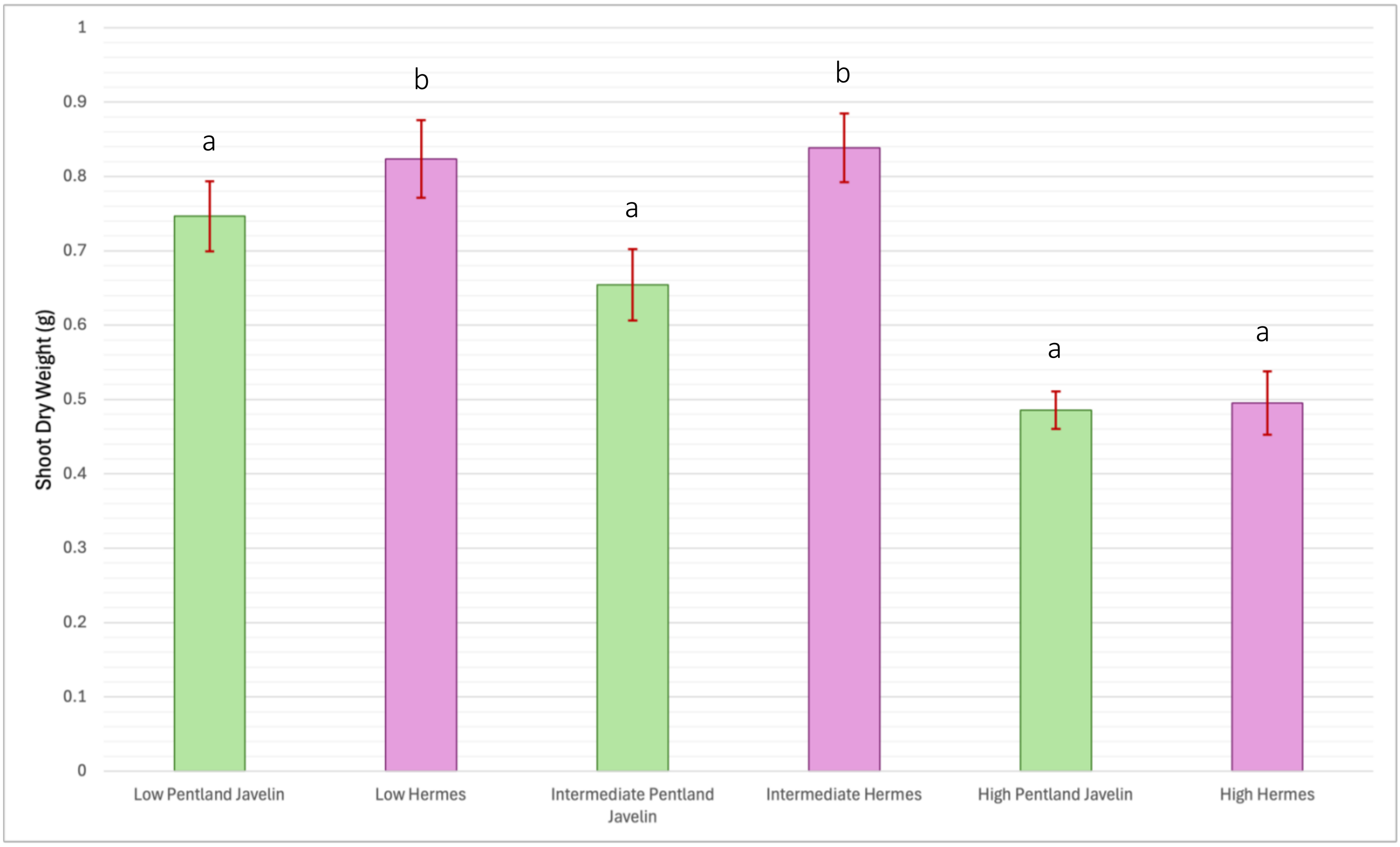

Figure 3.3.11 Shoot dry weight of different potato varieties at different levels of soil compaction (n = 5). Bars are means ± SE, and letters of mean discrimination (columns with different letters indicate that their means are significantly different at $p = 0.05$). Two-way ANOVAs showed that $p = <0.0001$ for variety, $p = 0.0301$ for compaction, and $p = 0.0929$ for an interaction. This shows that variety and compaction significantly impact shoot dry weight but not an interaction between the two.

Plant height of Cara and Marfona was significant at the variety and compaction level but not on the interaction level (**Figure** *3.3.12*) with Marfona having significantly lower plant heights than Cara at high compaction (33% reduction and 26% reduction respectively), this shows that Marfona had significantly different heights between low and high compaction but there was not an interaction effect so the response to compaction did not depend on variety. Plant height of Pentland Javelin and Hermes (**Figure** *3.3.13*), however, was impacted by variety and dependent on compaction with lower plant heights being observed at high compaction with that of Hermes being lower than Pentland Javelin at high compaction compared to low compaction (28% and 20% reductions respectively). Hermes did seem to react slightly more to high compaction here which is somewhat supported by the ethylene assay.

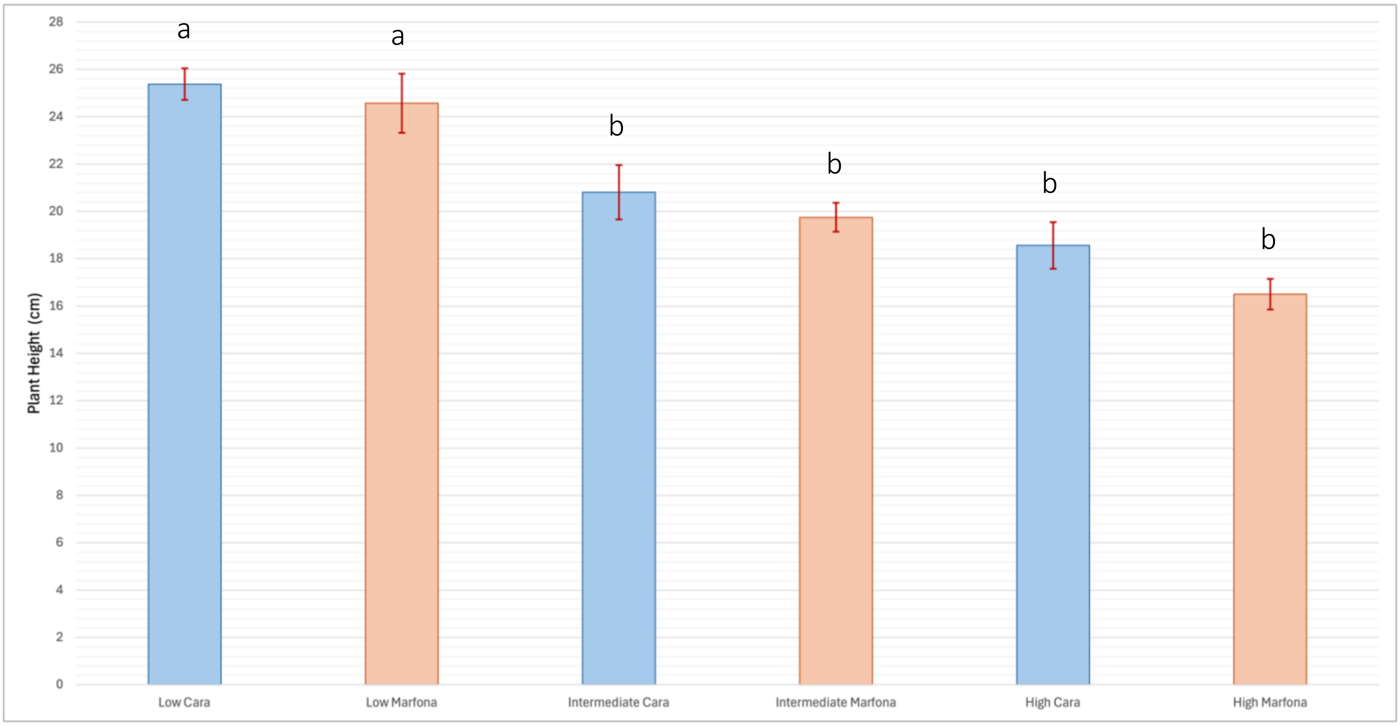

Figure 3.3.12 Plant height at harvest of different potato varieties at different levels of soil compaction (n = 7). Bars are means ± SE, and letters of mean discrimination (columns with different letters indicate that their means are significantly different at p = 0.05). Two-way ANOVAs revealed that p = <0.0001 for variety, p = 0.0492 for compaction, and p = 1 for an interaction effect. This reveals that variety and compaction significantly affect plant height but not an interaction between the two.

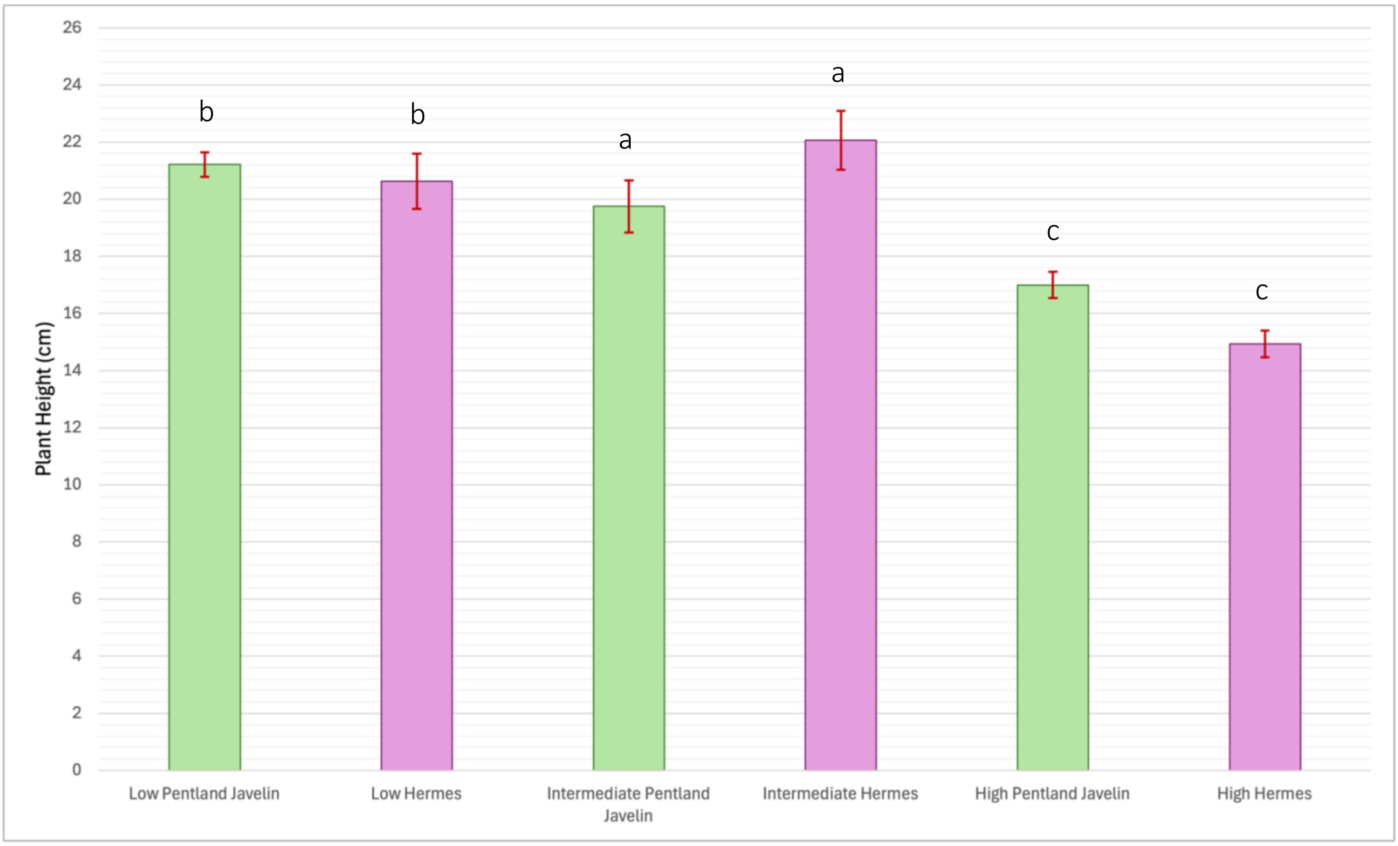

Figure 3.3.13 Plant height at harvest of different potato varieties at different levels of soil compaction (n = 5). Bars are means ± SE, and letters of mean discrimination (columns with different letters indicate that their means are significantly different at $p = 0.05$). Two-way ANOVAs showed that $p = <0.0001$ for variety, $p = 0.8425$ for compaction, and $p = 0.024$ for variety x compaction. This indicates that variety significantly impacts plant height and is dependent on compaction level, but compaction response is not significant.

Total leaf area was significantly lower at high compaction than low compaction in Cara and Marfona with a 33% and 45% reduction at intermediate and high compaction in Cara and 44% and 52% in Marfona (**Figure *3.3.14***) with compaction significantly impacting total leaf area, although the areas were very similar at both intermediate and high compaction. Leaf area of Pentland Javelin and Hermes (**Figure *3.3.15***) was significantly affected by variety and compaction but not both interacting with 6% and 34% reduction in Pentland Javelin at intermediate and high compaction respectively and 0.5% increase and 38% decrease in Hermes respectively. Therefore, leaf area was much lower at high compaction than low compaction (38% decrease) with Hermes having very similar leaf areas at intermediate and low compaction (0.5% increase).

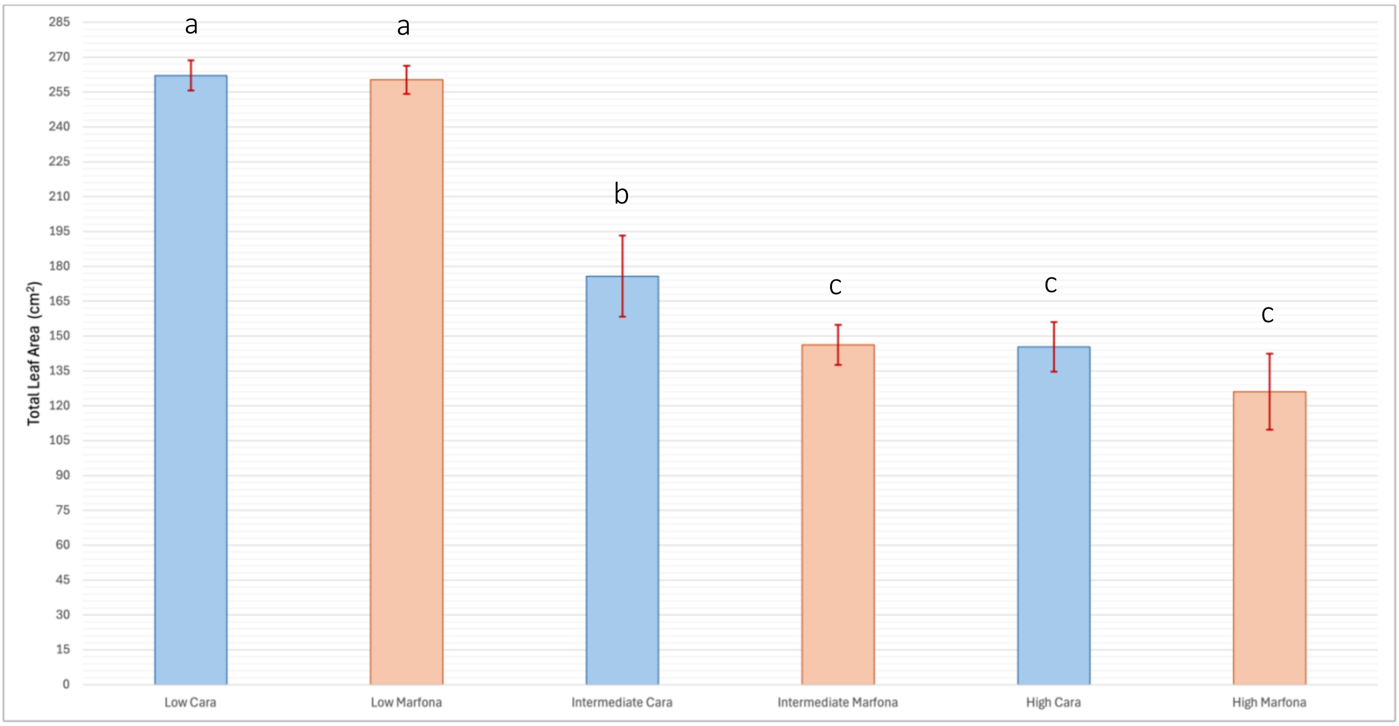

Figure 3.3.14 Total leaf area of different potato varieties at different levels of soil compaction (n = 7). Bars are means ± SE, and letters of mean discrimination discrimination (columns with different letters indicate that their means are significantly different at p = 0.05). Two-way ANOVAs on the data showed that p = 0.0562 for variety, p = <0.0001 for compaction, and p = 0.9141 for interaction effects. This shows that variety response was significantly impacted by compaction.

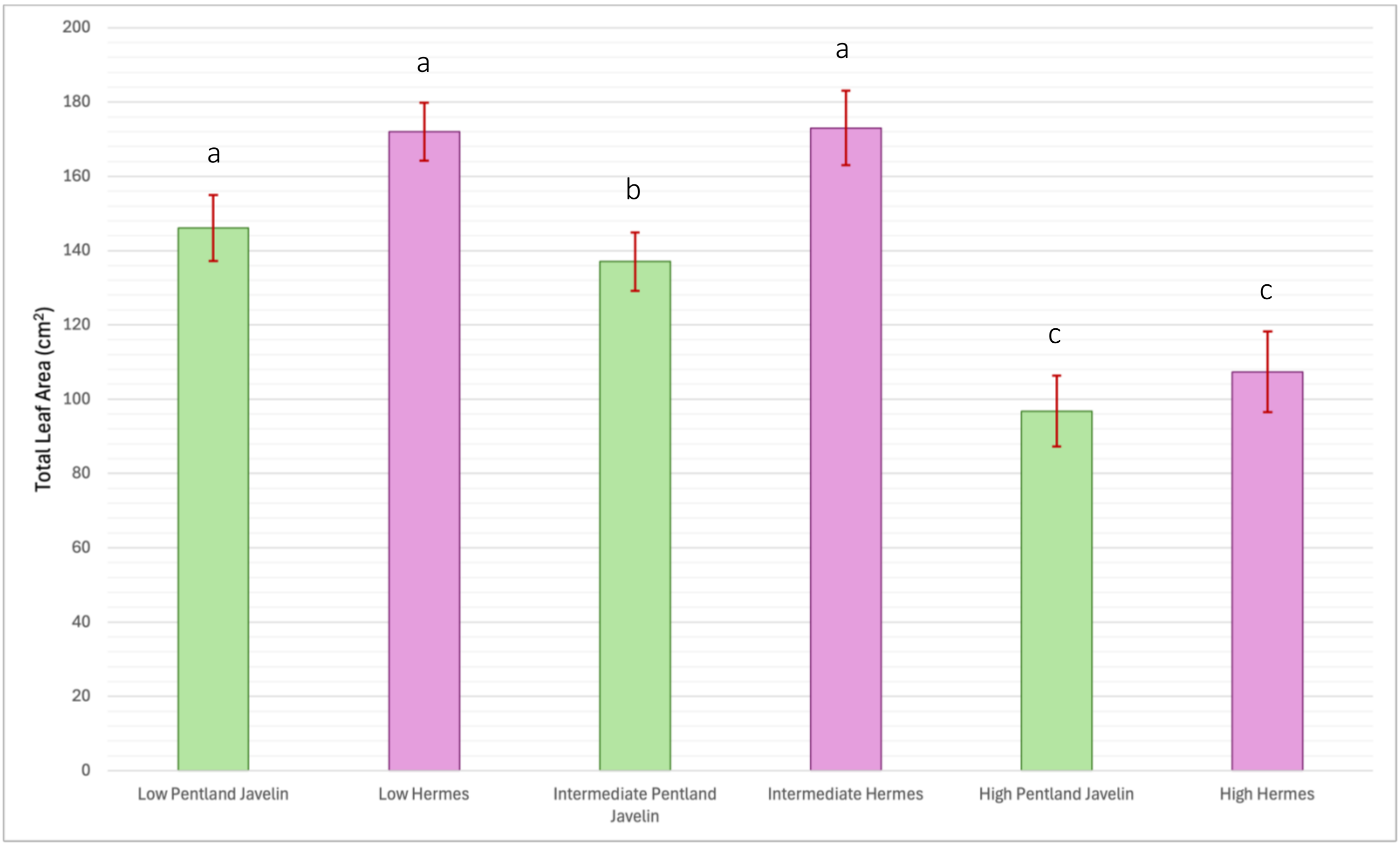

Figure 3.3.15 Total leaf area of different potato varieties at different levels of soil compaction (n = 5). Bars are means ± SE, and letters of mean discrimination discrimination (columns with different letters indicate that their means are significantly different at $p = 0.05$). Two-way ANOVAs calculated that $p = <0.0001$ for variety, $p = 0.0026$ for compaction, and $p = 0.3911$ for compaction x variety. This means that variety and compaction are significant but not an interaction between the two.

The relative leaf expansion rate (RLER) graphs (**Figure** *3.3.16* and **Figure** *3.3.17*) show that Cara's leaves were expanding at a slightly higher rate than Marfona, but Low Marfona started with a higher RLER than Cara. Intermediate Marfona leaves were expanding at a significantly higher rate than Intermediate Cara even though Cara started off with a larger RLER. At high compaction Marfona expanded at a higher rate than Cara even with Cara having a higher initial RLER. This suggests that Marfona's leaves expanded at a higher rate than Cara. Intermediate Cara and Marfona slopes are steepest at intermediate compaction which was the condition in which they would have been expanding at the highest rate, although for both the highest initial value was seen in low compaction. Low Hermes grew at a slightly higher rate than Pentland Javelin and had a higher starting value. Intermediate Pentland Javelin had a higher expansion rate than Hermes even with intermediate Hermes having a higher initial value. High Pentland Javelin grew at a faster rate than high Hermes which had a higher starting value. High Pentland Javelin grew the fastest within the variety and Low Hermes grew the fastest within the variety.

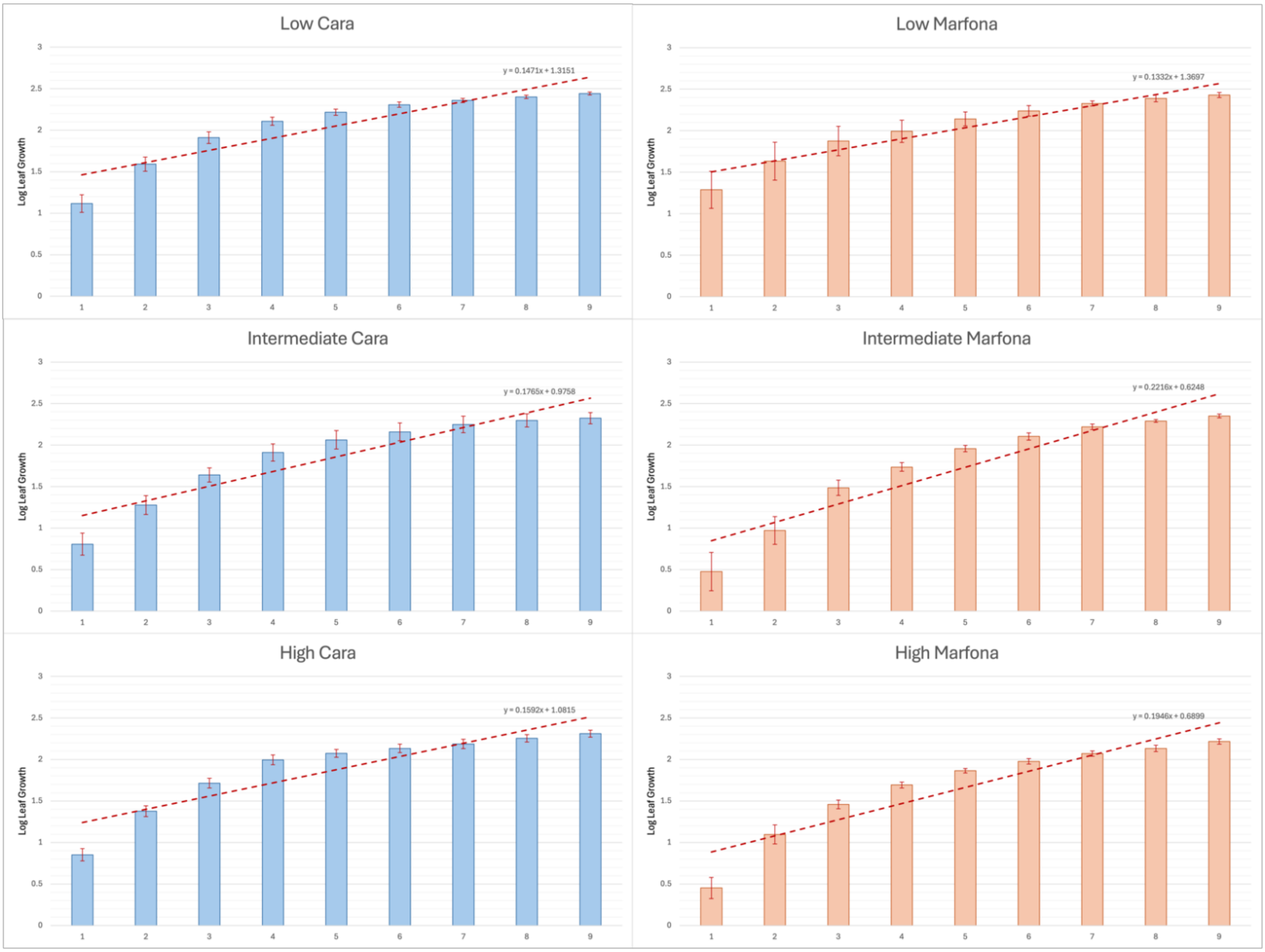

Figure 3.3.16 Relative leaf expansion rate of different potato varieties at different levels of soil compaction. Graphs each contain the equation of the line.

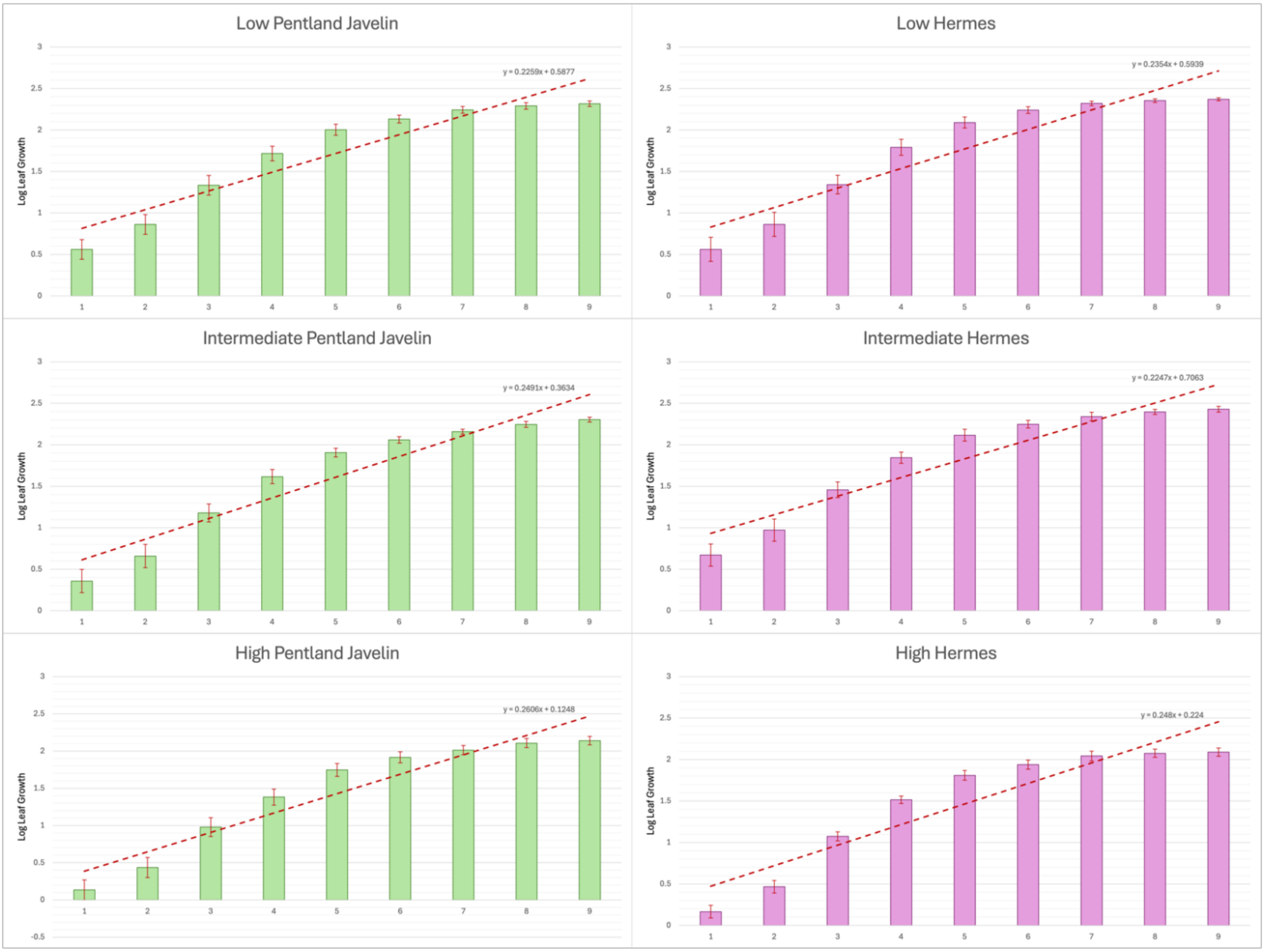

Figure 3.3.17 Relative leaf expansion rate of different potato varieties at different levels of soil compaction. Graphs each contain the equation of the line.

3.4. Discussion

Impact of Soil Compaction on Potato Growth

Highly compacted soils limit plant growth both above- and below-ground. Interestingly, varieties that were more ethylene sensitive like Marfona were not more sensitive to high compaction than less ethylene sensitive varieties like Cara, with a similar impact seen in Marfona and Cara (e.g., **Figure** *3.3.2*, **Figure** *3.3.6*, and **Figure** *3.3.10*).

The only parameter that increased under high compaction was root diameter (**Figure** *3.3.4* and **Figure** *3.3.5*) which has been suggested as being a response to higher levels of compaction through reduced root length but increased root diameter.

Ethylene Sensitivity and its Role in Potato Response to Compaction

The sensitivity of potatoes to ethylene impacts their growth because the accumulation of ethylene around the roots stimulates roots to alter direction and rate of growth (Pandey et al., 2021). This is also why the loss of soil structure is a major contributor to highly compacted soils because the soil is less able to maintain a stable structure and ends up compacting easily. The importance of ethylene sensitivity was demonstrated here through the differential responses of the varieties to soil compaction which mostly followed the hypothesis formed from the results of the ethylene assay (that those varieties with higher ethylene sensitivity would show greater responses to high compaction than those that had lower sensitivities), as was seen with reduced plant height, shoot dry weight, total leaf area, root length, and root volume (**Figure** *3.3.2*, **Figure** *3.3.10*, **Figure** *3.3.12*, and **Figure** *3.3.14*) in Marfona compared to Cara.

The ethylene assay was not very informative in suggesting which varieties would respond more to increasing soil compaction and which would perhaps show less of an effect. From highest to lowest ethylene sensitivity, Marfona showed a similar decrease in growth in the high compaction treatment compared to Cara with, next could be Pentland Javelin, Cara, and then Hermes according to the ethylene assay (**Figure** *3.3.1*), however these last three seemed to show reasonably similar responses to intermediate and high compaction (for example if we look at root length in **Figure** *3.4.1*).

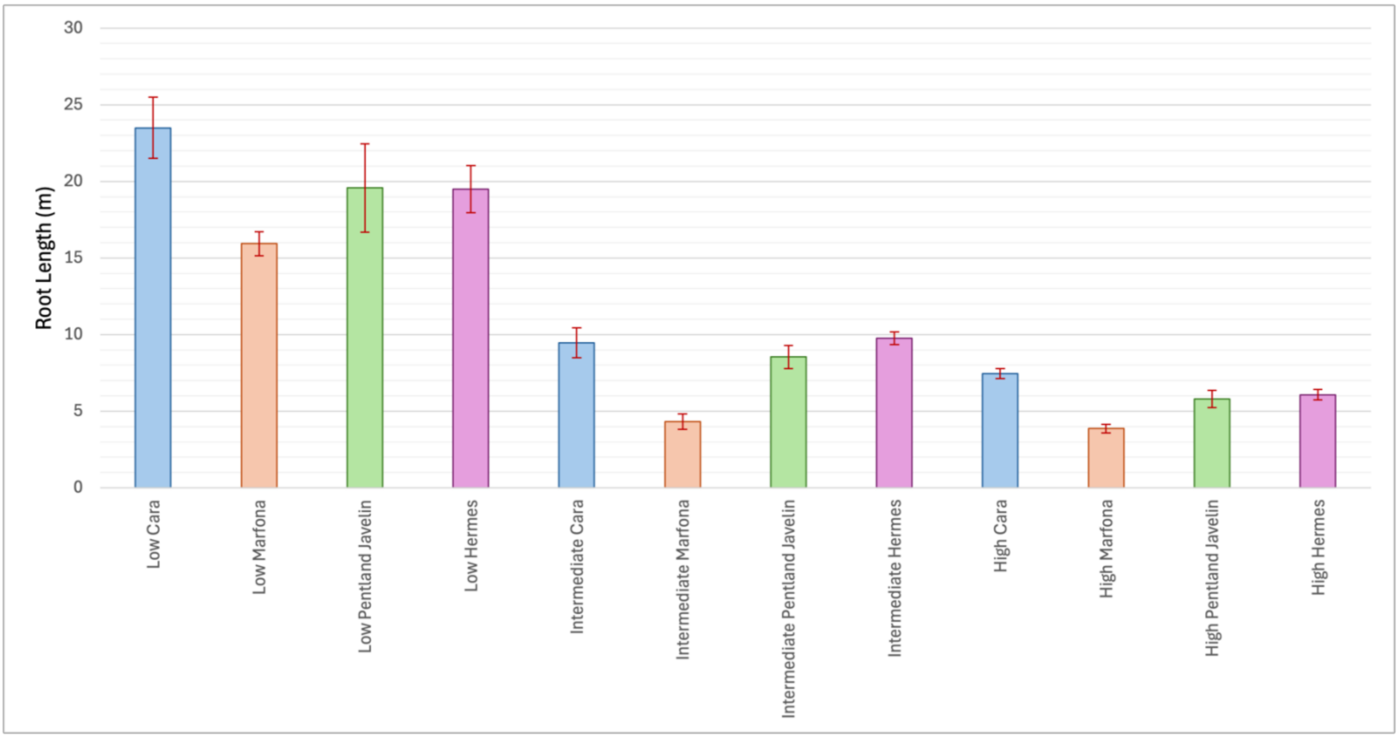

Figure 3.4.1 Root length of different varieties of potato at different levels of compaction with bars indicating SE.

The Impact of Soil Compaction on Root System Architecture

Roots of plants grown in low compaction were much longer and thinner (**Figure** *3.3.2*, **Figure** *3.3.3*, **Figure** *3.3.4*, and **Figure** *3.3.5*) than those of plants grown in high compaction because the plants were better able to explore the soil and take up the required resources effectively. In this research this was found to be true and was also dependent on varietal ethylene sensitivity. In the high compaction treatment, the plants had significantly shorter roots, less root surface area, and lower root volume compared to those grown under low compaction. As well as reductions in root parameters, the average diameter of the roots significantly increased under high compaction compared to low compaction, which was another expected adaptive response, and further supported the impacts high compaction has not only on the shoots but on the roots of crops. The shoot growth may have been limited by high compaction as a result of decreased root growth which caused ACC accumulation and signalling to the shoots, or increased allocation of resources to roots in order to facilitate the larger diameters. However, this did not depend on variety due to the non significant effects seen from the ANOVAs that showed compaction often significantly altered growth, but this was not dependent on variety.

Environmental and Agricultural Implications

Potatoes grown in the UK are mostly grown in soils that are close to their plastic limit for cultivation and perhaps shortening the growing season to allow soil to dry would be more effective than using cultivators (Stalham et al., 2007) . With the significant effects of high compaction on both the above ground and below ground growth of potatoes it can definitively be stated that high compaction was not beneficial to potato growth, and should be avoided through management strategies, such as the addition of soil organic matter, controlled traffic farming, and tracked machinery. But also, the results could be used to aid in breeding varieties that are not impacted as greatly by high compaction, for example, using Marfona would not be wise due to the high sensitivity to ethylene, but using Cara or Pentland Javelin could be beneficial. Environmentally, these results could be used to reflect on sustainable practices and more environmentally friendly methods to reduce the compaction caused by things like machinery, this opens interesting avenues for research to follow.

Suggestions for Future Directions

For future work on the topic area, it may be beneficial to use a much larger set of potato varieties with increased compaction levels to potentially find if levels between those used here are negative for growth, to further optimise growth and possibly even yield. Also looking at the signalling pathways and ethylene production in specific shoot tissues in relation to root ethylene accumulation using markers such as GFP fused to ethylene receptors or similar proteins could be interesting to understand the mechanisms involved in the response to compaction from root-to-shoot in varieties with differing ethylene sensitivity. There is also the potential to perform studies that harvest the potatoes as they grow, which may provide some data on how the RSA develops throughout growth in the varieties with differing ethylene sensitivity. Not only is harvesting at one certain point deemed as the end of the growth period useful in understanding stressors like soil compaction, but it also highlights the need for further research.

3.5. Conclusion

Compaction significantly decreases leaf area, plant height, shoot dry weight, root length, root surface area, and root volume. The growth over time of the leaves of the potatoes did show some interesting results with some large differences seen between the growth of the cultivars under the three levels of compaction, such as the higher rate of growth seen in Marfona at high compaction than Cara. In the case of crop emergence there were significant impacts on the emergence of Pentland Javelin and Hermes with much lower emergence seen at high compaction (which only showed that there were fewer replicates and did not translate to lower growth), while this was not true for Cara or Marfona, suggesting that emergence was not ethylene mediated, the later growth of these two varieties was significantly impacted.

4. A mutational analysis of ethylene sensitivity impacts on soil compaction responses of tomato

4.1. Introduction

Tomato is not a major UK-grown crop and is grown mainly in China. Studies using x-ray micro-computed tomography looked at the roots of tomatoes from the background Ailsa Craig under BDs of 1.2 and 1.6 g cm^{-3} in loamy sand and clay loam soils found that plants grown at higher BDs occupied smaller soil volumes, had reduced total root volume and root length but had larger mean root diameters than at low BDs (Tracy et al., 2015). Comparatively, tomato is more sensitive to compaction than other crops like wheat as the root system is impacted significantly more by high bulk densities, for example, under high compaction wheat roots were somewhat able to adapt by increasing root volume whereas tomato RSA was decreased to a more drastic extent with a 10-fold reduction in root volume (Tracy, 2015; Tracy, 2012). Therefore, we can assume here that the tomato would be more sensitive to compaction than the wheat.

A study looking to determine the impact of ethylene insensitivity on normal and adventitious root formation in Nr (which is partially ethylene insensitive) and Wt (Pearson) tomatoes by treating stem cuttings with auxin and ACC and growing tomatoes in soils with low or high mechanical impedance concluded that ethylene insensitivity reduced adventitious root formation, ACC partially restores root formation, root mass increased in ethylene-insensitive plants, and Nr seedlings were less able to penetrate soils with high impedance compared to Wt (Clark, 1999). Ethylene sensitivity is important in adventitious root formation and affects tomato root growth under mechanical impedance by reducing increase in root length under higher levels of compaction. Altered responses to soil compaction demonstrated ethylene's role in root adaptation to environmental conditions. Shoot growth between WtAC and ACO1$_{AS}$ (antisense suppression of plant ethylene synthesis), which had diminished stress-induced ethylene production, was very similar in uncompacted and compacted soils for both genotypes, however, significant differences in growth were observed in split-pot conditions where AC growth was reduced under compaction but ACO1$_{AS}$ growth was comparable to the uncompacted controls (Hussain, 1999). Wt plants had reduced growth which was linked to increased ethylene production in plants exposed to both high and low soil compaction (1.1 g/cm^{-3} and 1.5 g/cm^{-3}) and ACO1$_{AS}$ exhibited normal growth, therefore lower ethylene levels allowed ACO1$_{AS}$ to have improve growth under high compaction, which was further supported when ethylene-blocking restored growth in Wt plants and ethylene application reduced growth in ACO1$_{AS}$ (Hussain, 1999). From this work it can be assumed that

Nr, being ethylene-insensitive should show better shoot growth than Wt tomatoes but perhaps would have lower root growth due to less adventitious root formation.

When ACC levels are high in plants, for example in highly compacted soils through root-to-shoot signalling, chlorophyll degradation is increased which results in leaves containing less chlorophyll than those grown in soils with lower levels of compaction. This process occurs through ethylene signalling in which chlorophyllase is activated and removes the phytol tail from the chlorophyll molecule, turning chlorophyll into chlorophyllide (which is degraded more easily than chlorophyll), Mg-dechelatase removes the central magnesium ion from chlorophyll, producing pheophytin, and pheophorbide a oxygenase (PAO) catalyses the cleavage of pheophorbide a which leads to the production of non-fluorescent chlorophyll catabolites (NCCs) which are stored in the plant vacuole, removing the green pigment from the chloroplasts (Guyer et al., 2014). During senescence, which ethylene is largely involved with, the first sign of programmed cell degradation is chlorosis, and ethylene accelerates this process while also preventing the synthesis of new chlorophyll molecules (Guyer et al., 2014). In ACS mutants the loss of *ZmACS6* expression delayed leaf senescence in normal growth conditions, inhibited drought-induced senescence, and was also found to cause increased foliar chlorophyll (cosmetic staygreen) in young and fully-expanded leaves (Young et al., 2004). Also, the inhibition of ethylene synthesis reduced drought-induced loss of chlorophyll so ethylene may be involved in determining the onset of leaf senescence (Young et al., 2004). Thus, plants grown in highly compacted soils are expected to have lower chlorophyll content in the oldest leaves.

This chapter investigated the ethylene sensitivity of two tomato cultivars: Ailsa Craig and Micro-Tom. Each comprised a wild-type (in which fruit ripens completely) and the partially ethylene insensitive mutant Never-ripe (which does not fully ripen). Nr is less sensitive to ethylene due to a gain-of-function mutation in ETR3 found by parallel reaction monitoring (PRM) in which the seven ETRs were measured in a series of ripening tomatoes, and the accumulation of ETR3 in Nr fruits between the mature green (MG) stage and Breaker + 8 days stage (Br8) was found to delimit the ripening phase (Chen et al., 2019). The Micro-Tom Never-ripe tomatoes used in this research were accession number: H9 (LA4477), gene ID: Solyc09g075440, map position: chromosome 9 (long arm), gene function: ethylene receptor LeETR3. These tomatoes do not show the classic triple response when exposed to ethylene with shortened, swollen hypocotyls, exaggerated apical hooks, and shortened roots.

MT is a dwarf cultivar that has a short life cycle (Carvalho et al., 2011), this cultivar has several mutations that impact hormone synthesis and signalling pathways compared to WT tomatoes. The most obvious mutation is in the *DWARF* gene which alters brassinosteroid (BR) synthesis which causes the reduced plant size and increased sensitivity to environmental stressors, like drought (likely caused by an inability to regulate stomatal closure effectively) (Carvalho et al., 2011). Applying exogenous BR or complementation with a SlDWARF gene can completely recover these deficits (Lee et al., 2018).

Wild-type tomatoes (cv. Ailsa Craig and Micro-Tom) were hypothesised to be more sensitive to ethylene and soil compaction than their Never-ripe mutants.

4.2. Materials and Methods

Plant and Soil Method

Seed of each tomato cultivar Ailsa Craig (AC) which is a determinate, tall cultivar, and Micro-Tom (MT) which is a semi-determinate dwarf cultivar, comprised the wild-type (WT) and the ethylene-insensitive *Never-ripe* mutant (Nr). Since seeds were originally obtained from storage and supplied by different sources (Tomato Genetic Resources Centre for AC and Prof. Lazaro Peres for Nr), to avoid maternal effects on the resultant progeny, all 4 genotypes were grown in the same conditions for 7 months to obtain fresh seed. The seeds were planted into John Innes No. 3 compost in seed trays and grown until large enough to be transplanted into ~10 cm pots, and then later the AC plants were moved into ~25 cm pots, with plants given Miracle-Gro once a week. AC and MT were grown until they produced ripe fruit, which time the colour of the fruit once soft was used to determine whether the plants were indeed still WT or Nr which were harvested and the seeds collected (see **Figure** *4.2.1*). During growth the presence of Thrips was identified and so the plants were sprayed appropriately with pesticides.

After seeds had been collected, pots were prepared in the same manner as **chapter 2.2** using the same polyvinyl pots and the soil compacted using the same method, however 3 seeds were placed in each column. For growth in compacted soils the plants were not given any fungicides, pesticides, or fertilisers.

At harvest the shoots of the plants were cut at the soil level and the measurements collected that were required. After all plants had their shoots removed, the roots were collected by removing the column of soil that had formed within the pots and the roots washed with cold water until completely clean. The shoots and roots were placed in a drying oven after harvest.

Ethylene Assay Method

Ethephon was used to investigate the sensitivity of the genotypes to ethylene. Seeds collected from the fruits were placed onto filter paper inside of Petri-dishes and soaked with 100 µM of ethephon, sealed shut, and left to grow for 7 days in a dark area (if at any point during this time the filter paper began showing signs of drying out, they were opened and re-soaked). After this period the seeds were removed and the most uniform roots measured using a ruler to aid in identifying which seeds were ethylene sensitive or insensitive.

Measurements

Seedling height was measured after 6-7 days, and germination percentage calculated along with this as seeds planted in high compaction pots could germinate less than those in low compaction pots.

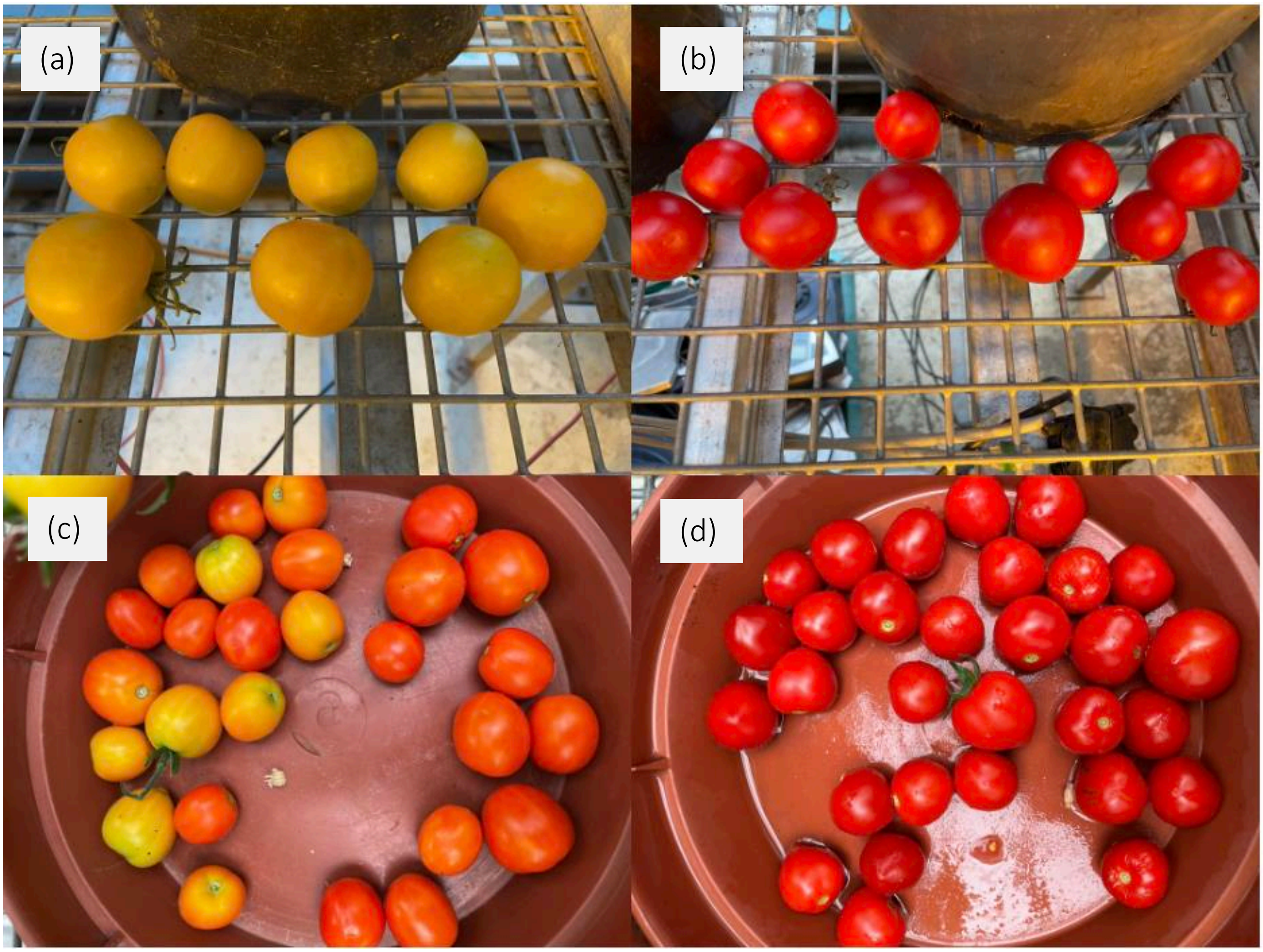

Figure 4.2.1 the tomatoes harvested from (a) NrAC, (b) WTAC, (c) NrMT, and (d) WTMT for collection of seeds to confirm the genotypic difference characteristically observed between WT and Nr fruits. With WT fruits displaying a bright red colour once ripe and Nr fruits having a yellow/ orange colouration once ripe.

From germination until harvesting (around 5 weeks) images were taken from a bird's-eye view using a background containing a scale of each plant every other day to measure estimated leaf area. Estimated leaf area was obtained from the images using ImageJ with the background removed and using the scale to calibrate the software, with the ImageJ values being used to estimate progress. This estimated leaf area (ELA) was then compared to actual leaf area (ALA) at harvest, which was collected using a leaf area meter (LiCor's LI-3100C, Cambridge, UK). ELA at harvest and ALA were plotted against one another and the R^2 value collected to indicate whether the method used to estimate leaf area was accurate.

Plant height was measured at harvest for potential compaction effects on the final height of plants (measured from the soil layer to the tip of the plant after gathering all the branches together holding them upwards), as well as a note made of which plants had started producing flowers, which would suggest that the plants are further along in their development than those not producing flowers. Oldest leaf chlorophyll was measured at harvest in µmol m^{-2} as well using a chlorophyll meter (Chlorophyll Content Meter Apogee Instruments, Logan, UT, USA).

Shoot and root dry biomass were also collected after plant samples had been left in the drying oven for at least 72 hours, root scanning was not completed because of limitations in software, so root dry weight was used to inform root system size differences.

Statistical Analysis

Please see **chapter 2.2** for information on the statistical testing performed on all of the data collected.

4.3. Results

Root growth of WTAC and WTMT was more sensitive to ethephon application than NrAC and NrMT, which confirmed the presence of the Nr mutation (**Figure *4.3.1***). The differences in root length were significant between WTAC and NrAC, as well as between WTMT and NrMT. There were also some interesting results seen in this section as well as many significant impacts of compaction (**Table 4.3.2**).

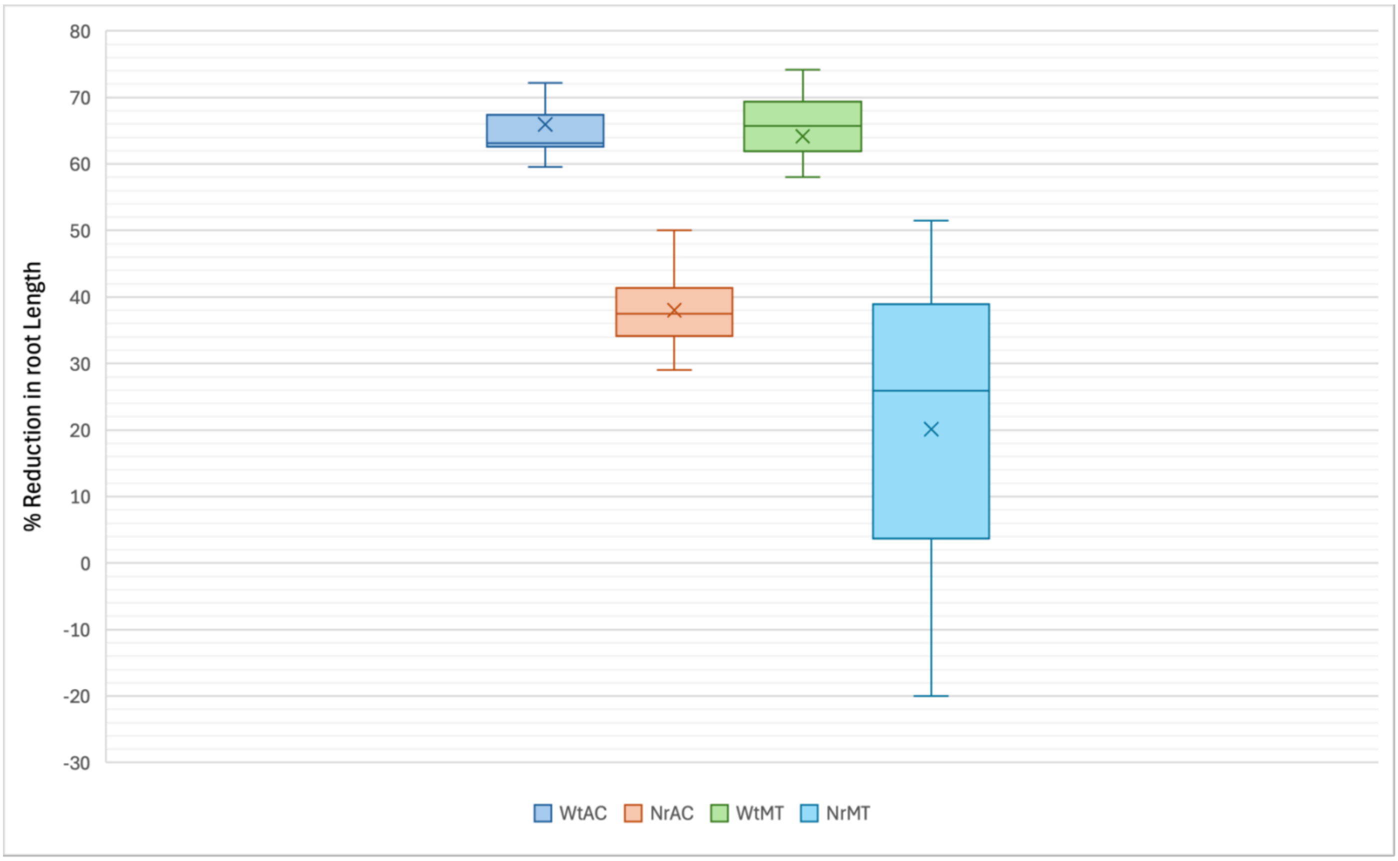

Figure 4.3.1: Ethephon assay results indicated as % reduction in root length (n = 20). Two-tailed t-tests were conducted on the results from each background comparing the responses of the genotypes compared to one another and were found to be statistically significant with p = <.0001 between WTAC and NrAC, and p = <.0001 between WTMT and NrMT, which all suggests that WT genotypes should respond more to soil compaction than the Nr genotypes due to their sensitivity to ethylene.

Table 4.3.1: Average results of the ethephon assay with % inhibition and significance values calculated using two-way ANOVA, significance at the 0.05 level is indicated by an asterisk (*) and SE indicated.

Background x Genotype	Control (mm)	Ethephon (mm)	% Inhibition
WTAC	44.6 ± 2.4	15 ± 1	66 ± 2.1
NrAC	52.9 ± 2.7	32.8 ± 2.3	38 ± 2.9
WTMT	32 ± 1.3	11.3 ± 0.7	64 ± 2.9
NrMT	49.1 ± 3.9	37.1 ± 2.1	20 ± 7.7
Genotype			1.08×10^{-15}*
Ethephon			1.99×10^{-19}*
Ethephon x Genotype			0.0078*

Table 4.3.2: The significance variables calculated for all of the data collected from each background x variety. Significance at the 0.05 level is indicated by an asterisk (*).

Background x Genotype	Variable Measured	Compaction Significance (p)	Genotype Significance (p)	Interaction Significance (p)
WTAC and NrAC	*Seedling Height*	0.005*	0.177	0.561
	Estimated Total Leaf Area	<.0001*	0.170	0.933
	Chlorophyll	0.0002*	0.309	0.309
	Total Leaf Area	<.0001*	0.443	0.216
	Plant Height	<.0001*	0.048*	0.027*
	Shoot Dry Weight	<.0001*	0.436	0.034*
	Root Dry Weight	<.0001*	1	0.039*
WTMT and NrMT	*Seedling Height*	0.079	0.177	0.062
	Estimated Total Leaf Area	0.001*	0.213	0.016*
	Chlorophyll	0.002*	0.026*	0.009*
	Total Leaf Area	0.0004*	0.013*	0.049*
	Plant Height	0.001*	0.001*	0.021*
	Shoot Dry Weight	0.0004*	0.008*	0.052
	Root Dry Weight	0.0009*	0.472	0.127

The impact of genotype and compaction on height of AC plants was statistically significant (**Figure** *4.3.2*) with low WTAC being significantly taller at harvest than high WTAC, and plant height being less impacted in NrAC plants. Plant height of MT plants was also statistically significant (**Figure** *4.3.3*) at the interaction level which indicates that the harvest height of background and genotype tomatoes was significantly altered by compaction and suggests that plant height is significantly lower under increasing compaction, however low NrMT and high NrMT have similar heights which follows the hypothesis, but plant height of WTMT under high compaction is higher than plant height at low and intermediate compaction. Of course, AC plants are much larger than MT plants, this is just because of the nature of their backgrounds, and plant height at harvest was measured to assess consistency across backgrounds.

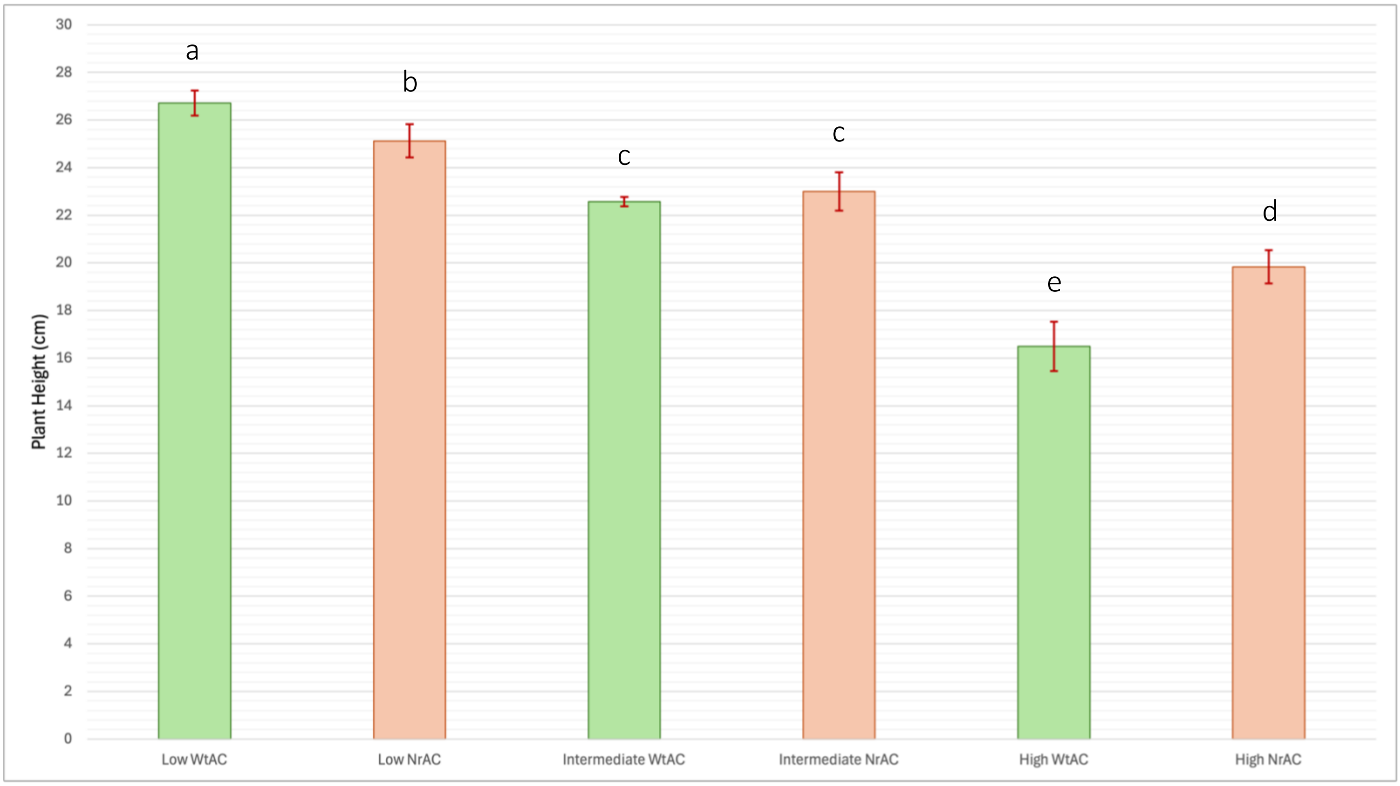

Figure 4.3.2: Plant height of different tomato backgrounds x genotypes at different levels of compaction, with bars of means ± SE, and letters of mean discrimination (n = 6) (columns with different letters indicate that their means are significantly different at p = 0.05). Two-way ANOVAs conducted on the data returned p = <0.0001 for compaction, p = 0.0481 for genotype, and p = 0.0267 for compaction x genotype. This suggests that there is significant effects of genotype and compaction on plant height of Ailsa Craig plants, with WT plants being more sensitive than Nr.

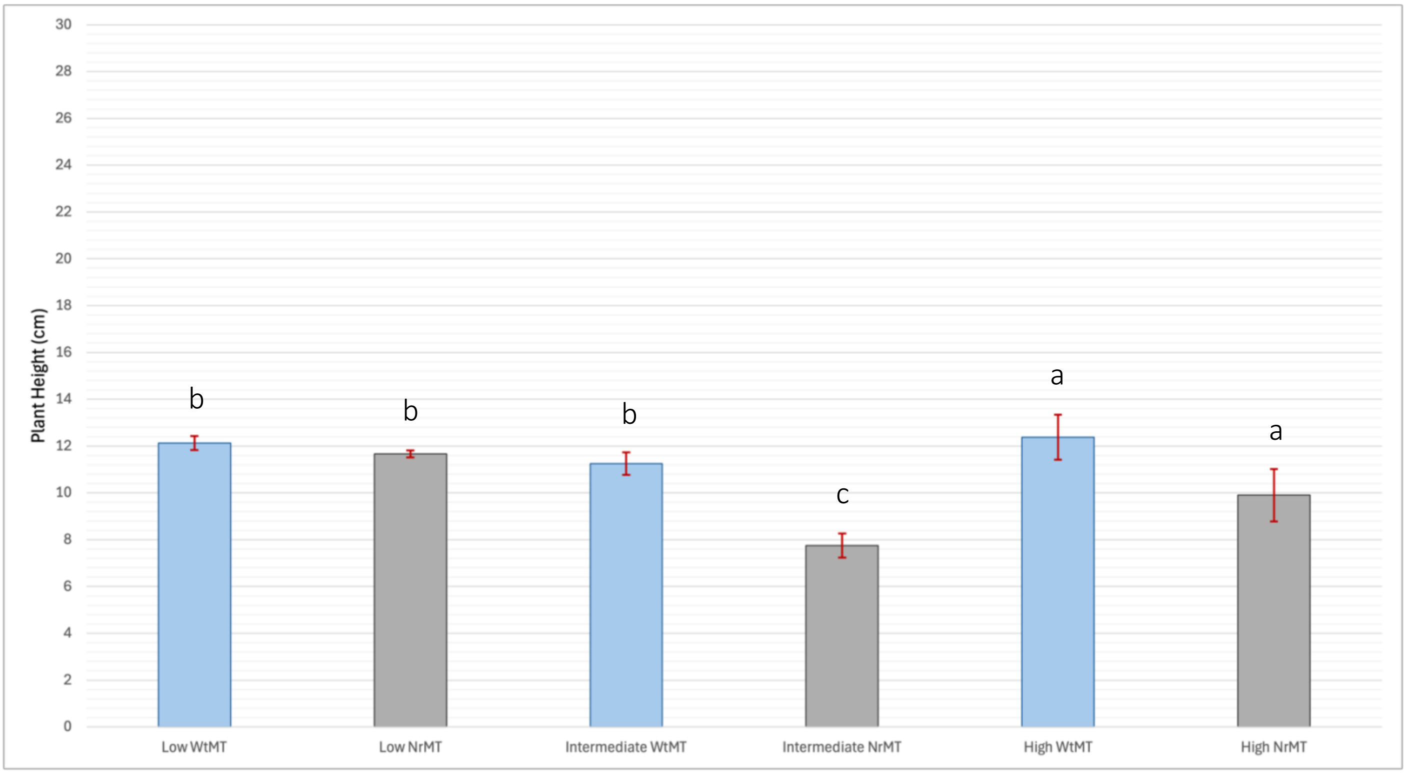

Figure 4.3.3: Plant height of different tomato backgrounds x genotype at different levels of compaction with bars of means ± SE, and letters of mean discrimination (n = 6) (columns with different letters indicate that their means are significantly different at p = 0.05). Two-way ANOVAs were conducted on the data and the results were: p = 0.0010 for compaction, p = 0.0010 for genotype, and p = 0.0207 for interaction. This shows that compaction significantly impacts the plant height of WTMT and NrMT plants with a significant effect of genotype as well.

Total leaf area of WtAC under intermediate and high compaction was reduced by 32% and 76% respectively, and in NrAC leaf area was reduced by 19% and 54% respectively. Total leaf area in WtMT under intermediate and high compaction was reduced by 17% and 11% respectively and was reduced by 56% and 63% in NrMT respectively. The total leaf area was significantly different with compaction significantly impacting leaf area of AC and MT (**Figure** *4.3.4* and **Figure** *4.3.5*). WtAC leaf area decreases with increasing compaction and is impacted more than leaf area of NrAC at intermediate and high compaction. NrMT seems to react more to intermediate and high compaction than WtMT and total leaf area of WtMT does still decrease under intermediate and high compaction. A similar pattern as plant height was seen with leaf area in WtMT with plants having improved performance under high compaction than intermediate compaction. Perhaps these plants show enhanced growth under intermediate compaction as it increases soil-root contact and potentially nutrient/ water uptake.

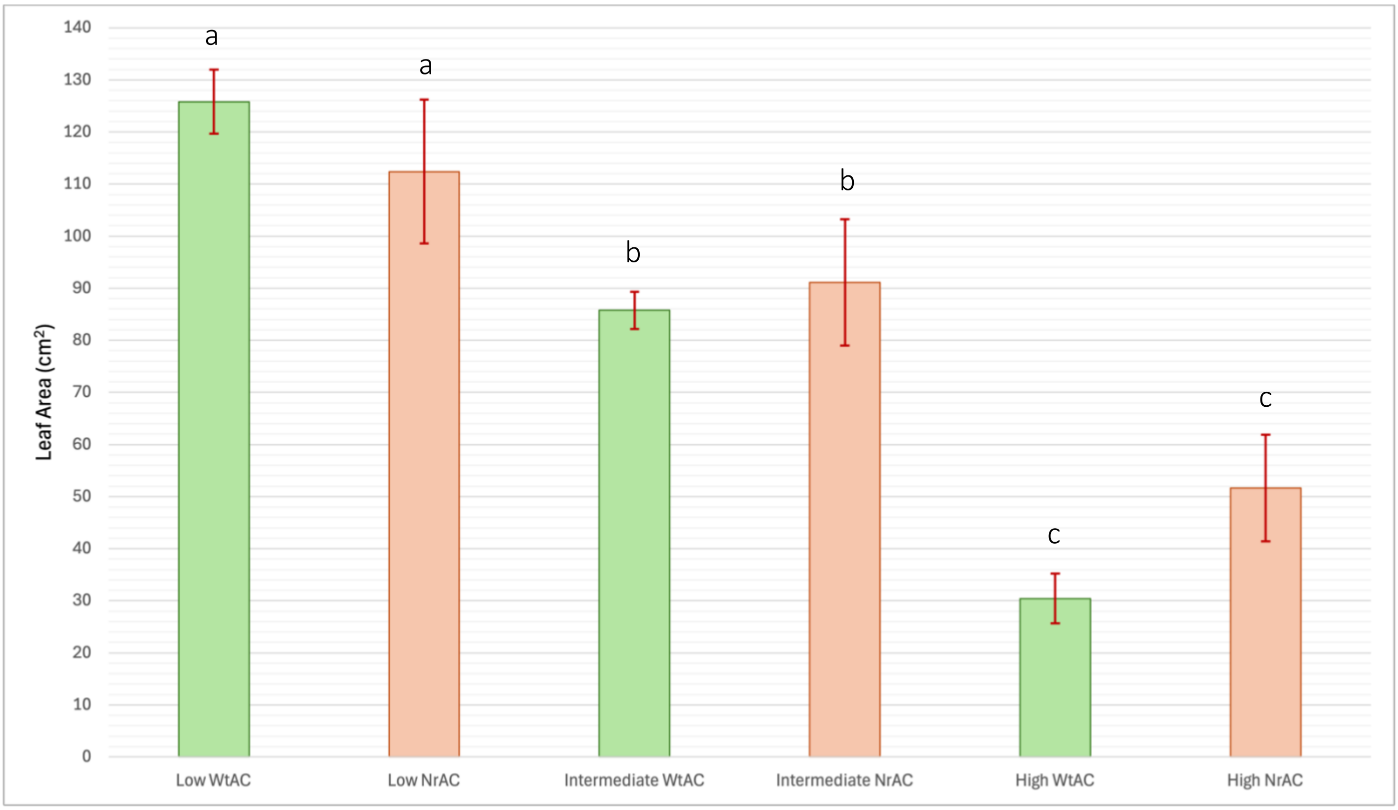

Figure 4.3.4: Total leaf area using a leaf area meter of different tomato genotypes at different levels of compaction with bars of means ± SE, and letters of mean discrimination (n = 6) (columns with different letters indicate that their means are significantly different at p = 0.05). Two-way ANOVAs found that p = <0.0001 for compaction, p = 0.4430 for genotype, and p = 0.2162 for interaction. This suggests that compaction has a significant impact on plant height, but not genotype.

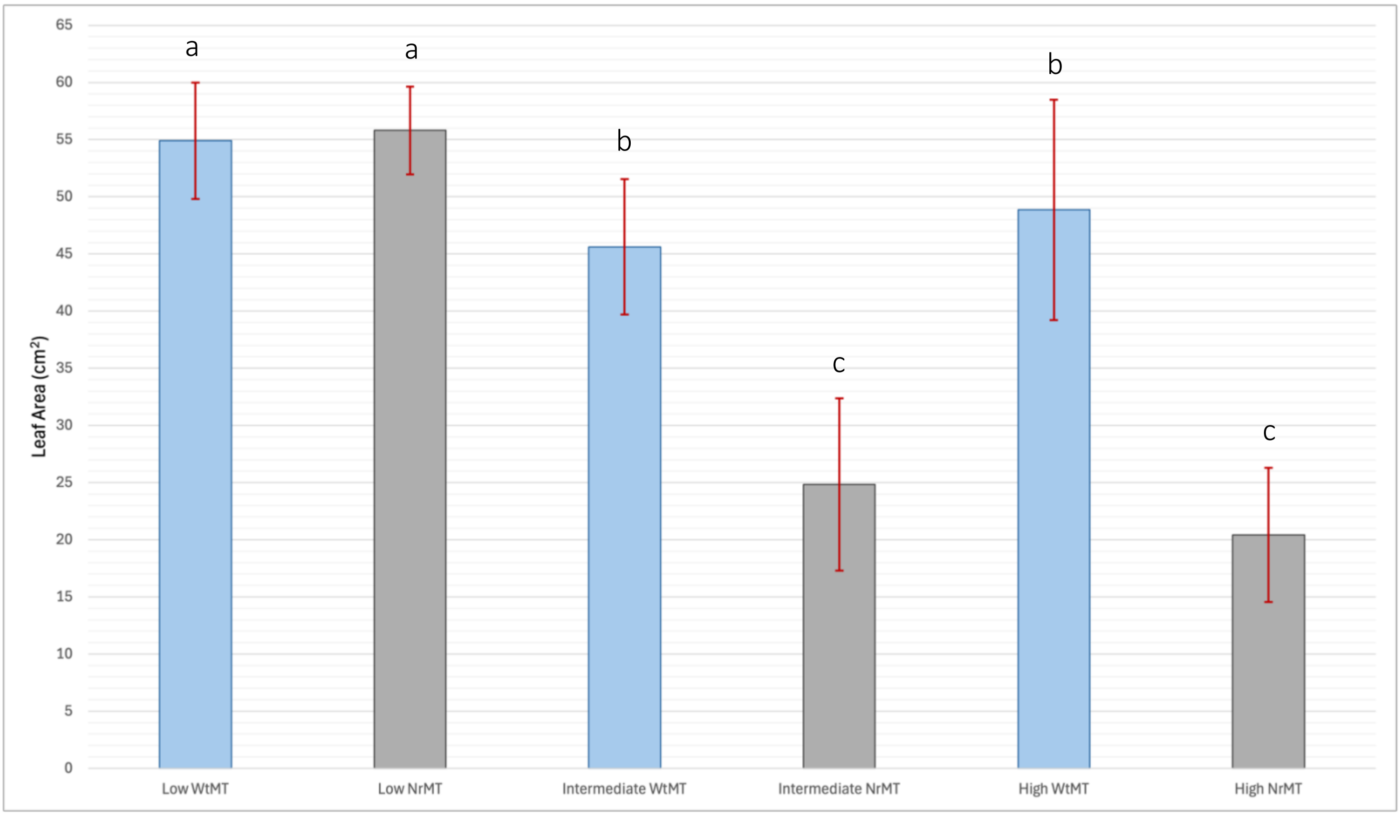

Figure 4.3.5: Total leaf area using a leaf area meter of different tomato genotypes at different levels of compaction with bars of means ± SE, and letters of mean discrimination (n = 6) (columns with different letters indicate that their means are significantly different at $p = 0.05$). Two-way ANOVAs found that $p = <0.0004$ for compaction, $p = 0.0133$ for genotype, and $p = 0.0496$ for interaction. This suggests that compaction and genotype have significant effects on plant height, including an interaction effect.

Leaf growth measured using photos taken every other day showed that AC and MT were impacted by high compaction with smaller leaves seen under high compaction than low compaction (**Figure** ***4.3.6*** and **Figure** ***4.3.7***) but the pattern of increased growth of WTMT compared to NrMT under high compaction can be observed in tiles c and f of **Figure** ***4.3.7***. The AC background looked as though there were more pronounced effects at high compaction when comparing tiles a and d to c and f in **figure 4.3.6**. This shows that both backgrounds were impacted by high compaction with lower leaf growth observed.

Actual leaf area was measured at harvest with a leaf area meter and was measured every other day by taking images of plants and analysing using ImageJ, as mentioned in **chapter 4.2**. The final leaf area measured using the photographs was plotted against the leaf area that was obtained from the LICOR to find if the imaging was reasonably accurate and representative throughout the growth of the tomato plants. **Figure** ***4.3.8*** and

Figure *4.3.9* show these results and for both plants the photo-collection and analysis were very accurate considering that towards the end of growth, some leaves were overlapping due to the size of the tomato plants and the pattern of leaf growth observed in tomato plants.

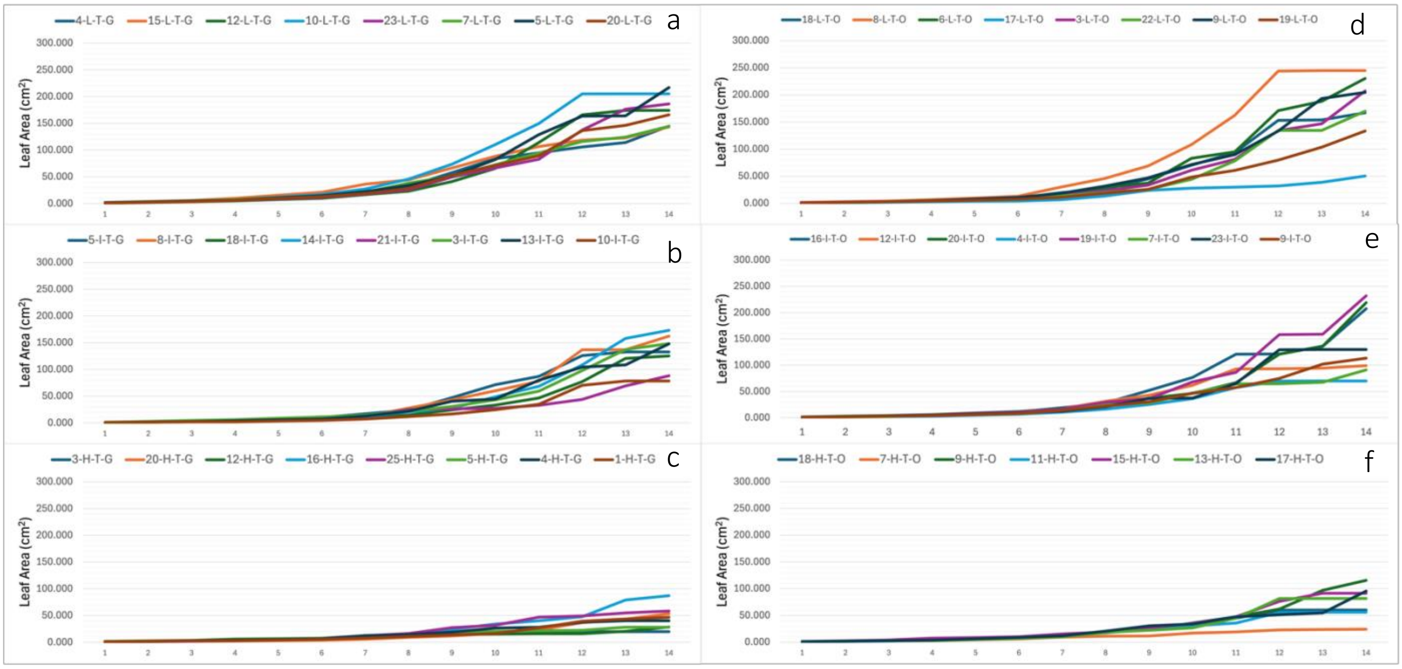

Figure 4.3.6 Leaf growth from sprouting until harvest of different tomato backgrounds x genotypes. This was the data obtained from imaging plants and analysing using ImageJ, (a) Low WTAC, (b) Intermediate WTAC, (c) High WTAC, (d) Low NrAC, (e) Intermediate NrAC, (f) High NrAC. Each line shows an individual replicate.

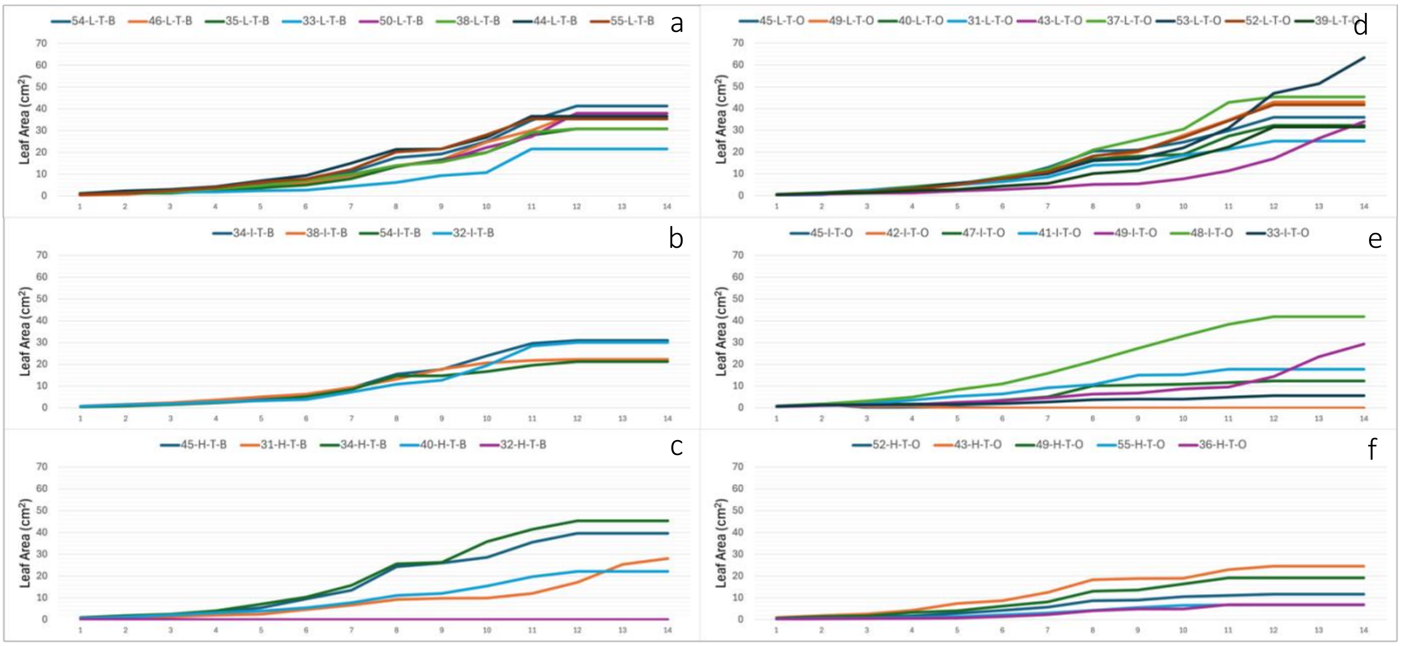

Figure 4.3.7 Leaf growth from sprouting until harvest of different tomato backgrounds x genotypes. This was the data obtained from imaging plants and analysing using ImageJ, (a) Low WtMT, (b) Intermediate WtMT, (c) High WtMT, (d) Low NrMT, (e) Intermediate NrMT, (f) High NrMT. Each line shows an individual replicate.

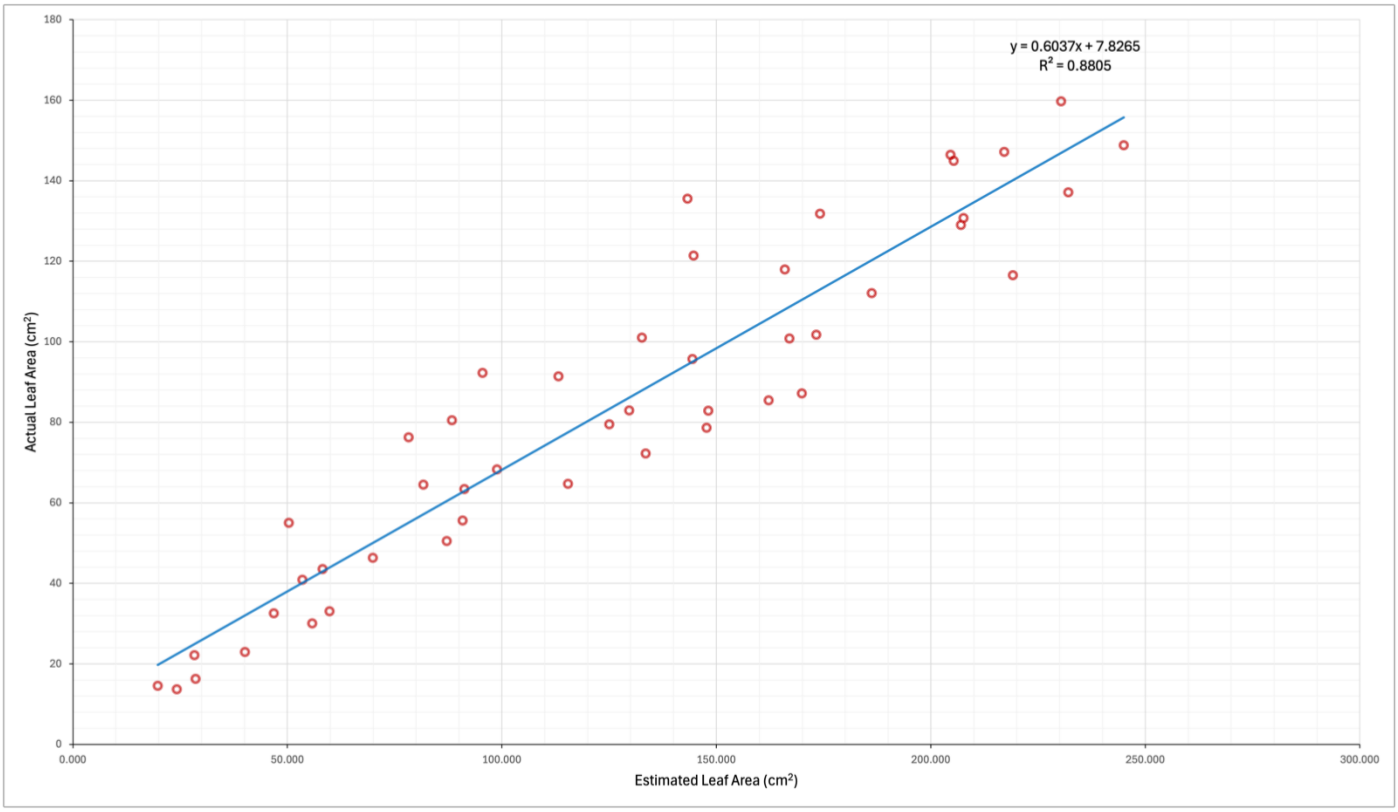

Figure 4.3.8: Actual leaf area measured using a leaf area meter plotted against estimated leaf area of Ailsa Craig plants measured using ImageJ (n = 6). The $R^2 = 0.8805$ which suggests that there is a strong relationship between the two measurements, which means that the method used to estimate leaf area throughout growth was accurate. In this case there seemed to be some overestimation of leaf area using ImageJ compared to actual leaf area.

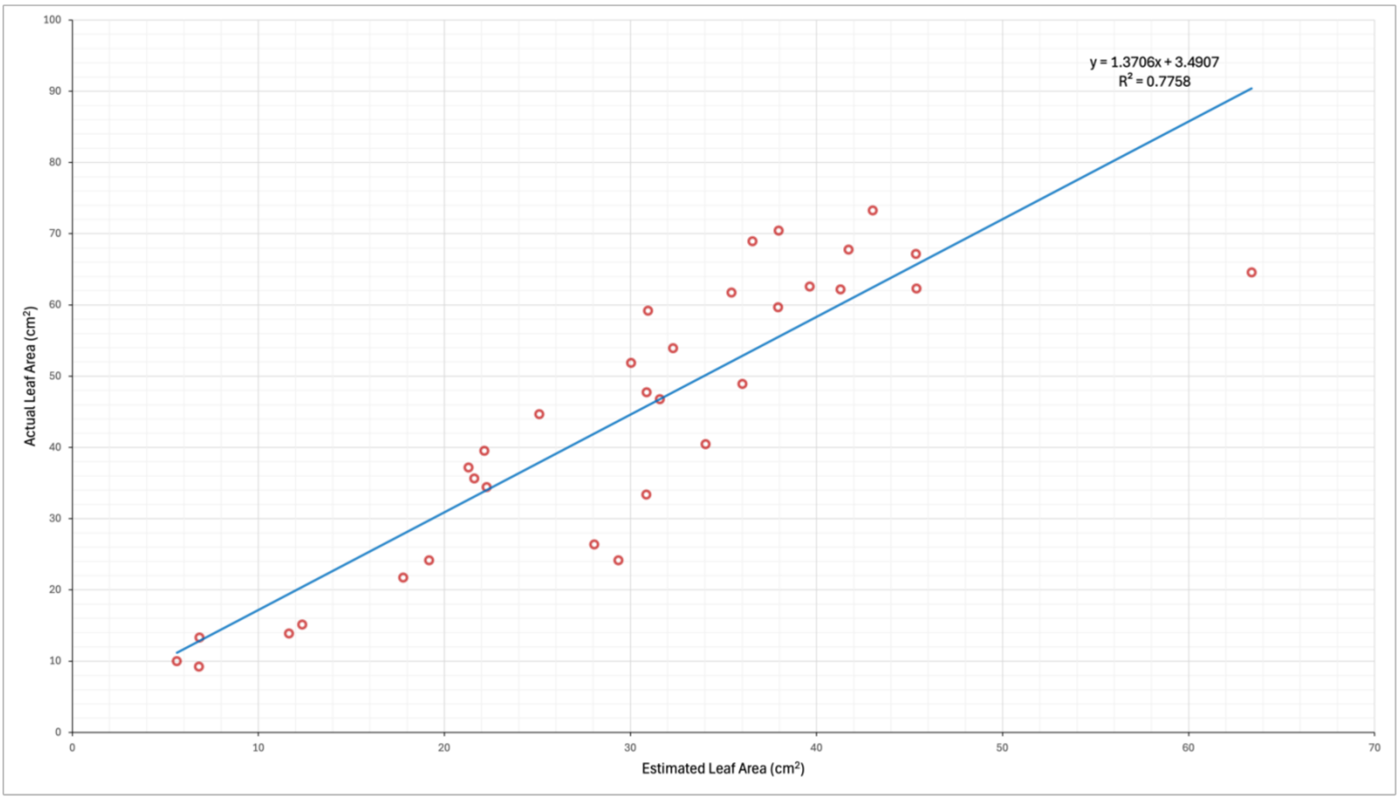

Figure 4.3.9: Actual leaf area measured using a leaf area meter plotted against estimated leaf area of Micro-Tom plants measured using ImageJ (n = 6). The R^2 = 0.7758 which suggests that there is a strong relationship between the two measurements, which means that the method used to estimate leaf area throughout growth was accurate. In this case there seemed to be some underestimation of leaf area using ImageJ compared to actual leaf area.

Shoot dry weight in AC was statistically significant at the compaction and interaction level (**Figure** ***4.3.10***), therefore shoot dry weight at different levels of compaction was significantly different, especially for WtAC with the largest difference observed between low and high compaction (heavier plants at low compaction and the lightest plants at high compaction). There was a 36% reduction and 80% reduction under intermediate and high compaction in WTAC, and a 17% reduction and 54% reduction in NrAC. In MT under intermediate and high compaction there was a 17% and 16% decrease in WtMT respectively, and a 57% and 59% reduction in NrMT. NrAC shoot dry weight is similar at low and intermediate compaction, but is decreased significantly at high compaction, although less so than WtAC. Interaction between genotype and compaction was also present so compaction significantly altered shoot dry weight, with the response also varying depending on genotype. Shoot dry weight of MT was significantly different at the compaction level and genotype level (**Figure** ***4.3.11***), therefore both genotype and compaction significantly altered shoot dry weight of MT, but the change in shoot dry weight under high compaction did not depend on the genotype.

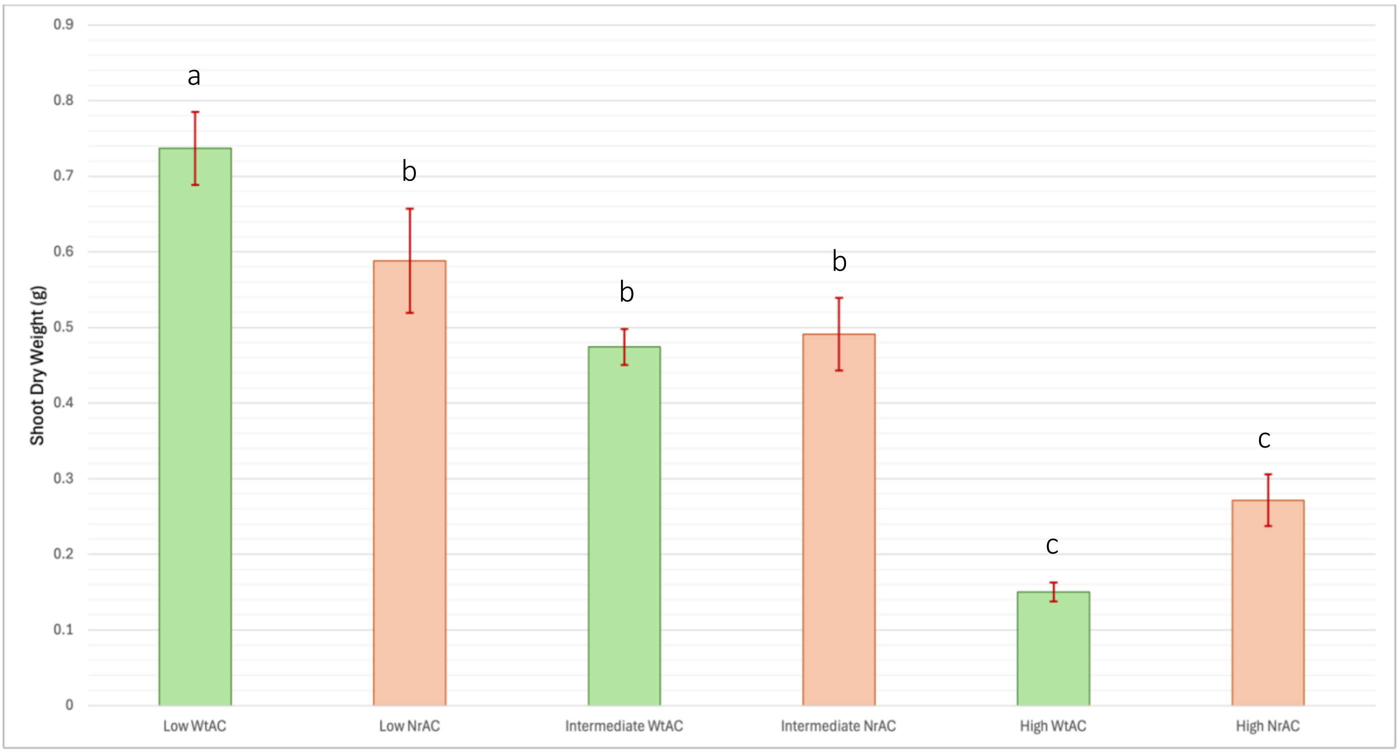

Figure 4.3.10: Shoot dry weight of different tomato genotypes under different levels of compaction with bars of means ± SE, and letters of mean discrimination (n = 6) (columns with different letters indicate that their means are significantly different at p = 0.05). Two-way ANOVAs found that p = <0.0001 for compaction, p = 0.4361 for genotype, and p = 0.0343 for interaction. This shows that compaction significantly impacts shoot dry weight, and both interacting (genotype x compaction) significantly impact shoot dry weight.

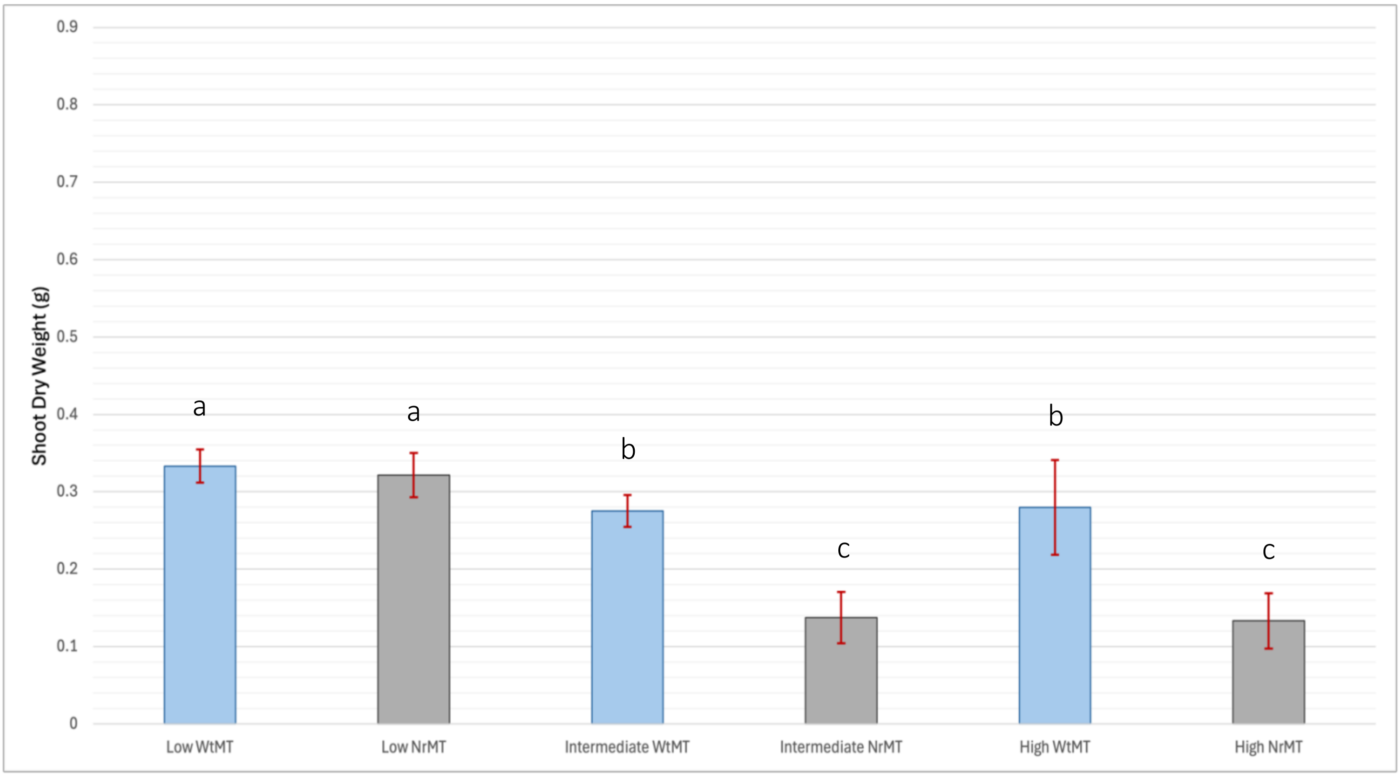

Figure 4.3.11: Shoot dry weight of different tomato genotypes under different levels of compaction with bars of means ± SE, and letters of mean discrimination (n = 6) (columns with different letters indicate that their means are significantly different at p = 0.05). Two-way ANOVAs were conducted on all of the data with the results showing p = 0.0004 for compaction, p = 0.0077 for genotype, and p = 0.0521 for interaction. These results show that compaction and genotype significantly impact shoot dry weight, and the interaction between the two is also significant.

Root dry weight was significantly different in AC at the interaction level with a 25% reduction and 54% reduction under intermediate and high compaction in WtAC, and a 12% increase and 40% reduction in NrAC (**Figure** *4.3.12*). Under intermediate and high compaction there was a 42% and 42% reduction in WtMT respectively, and a 75% and 83% reduction in NrMT respectively, which was found to be statistically significant at the compaction level and the interaction level. Root dry weight decreased with increasing compaction in WtAC and is lower at high compaction than low compaction in NrAC but is increased at intermediate compaction (root dry weight results have not been presented for MT due to no significant effects).

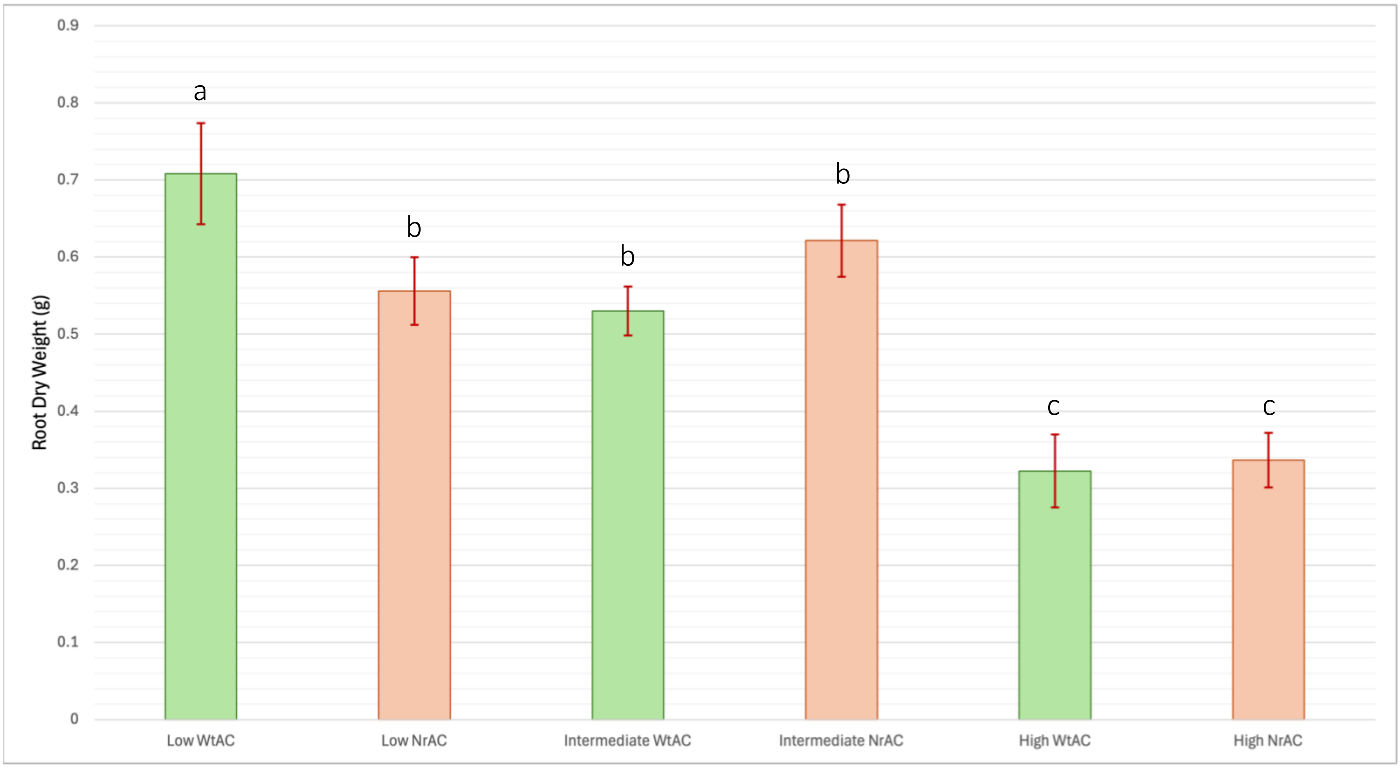

Figure 4.3.12: Root dry weight of different tomato genotypes under different levels of compaction with bars of means ± SE, and letters of mean discrimination (n = 6) (columns with different letters indicate that their means are significantly different at $p = 0.05$). Two-way ANOVAs were conducted on all of the data with the results showing $p = <0.0001$ for compaction, $p = 1$ for genotype, and $p = 0.0387$ for interaction. This shows that compaction and an interaction between genotype and compaction have significant effects on root dry weight.

Chlorophyll content was statistically significant at compaction, genotype, and interaction levels in MT plants with a 10% and a 0.6% reduction at intermediate and high compaction in WtMT respectively, and a 17% and 39% reduction in NrMT respectively (**Figure** *4.3.13*), but only on the compaction level in AC plants. The unusual pattern of WtMT performing better under high compaction than intermediate compaction continues into chlorophyll content of the oldest leaf. Here WtMT grown under intermediate compaction had lower levels of chlorophyll in the oldest leaf, but this then increased under high compaction. Perhaps MT plants are less sensitive to high levels of ethylene through some mechanism of their genetics, but not intermediate levels.

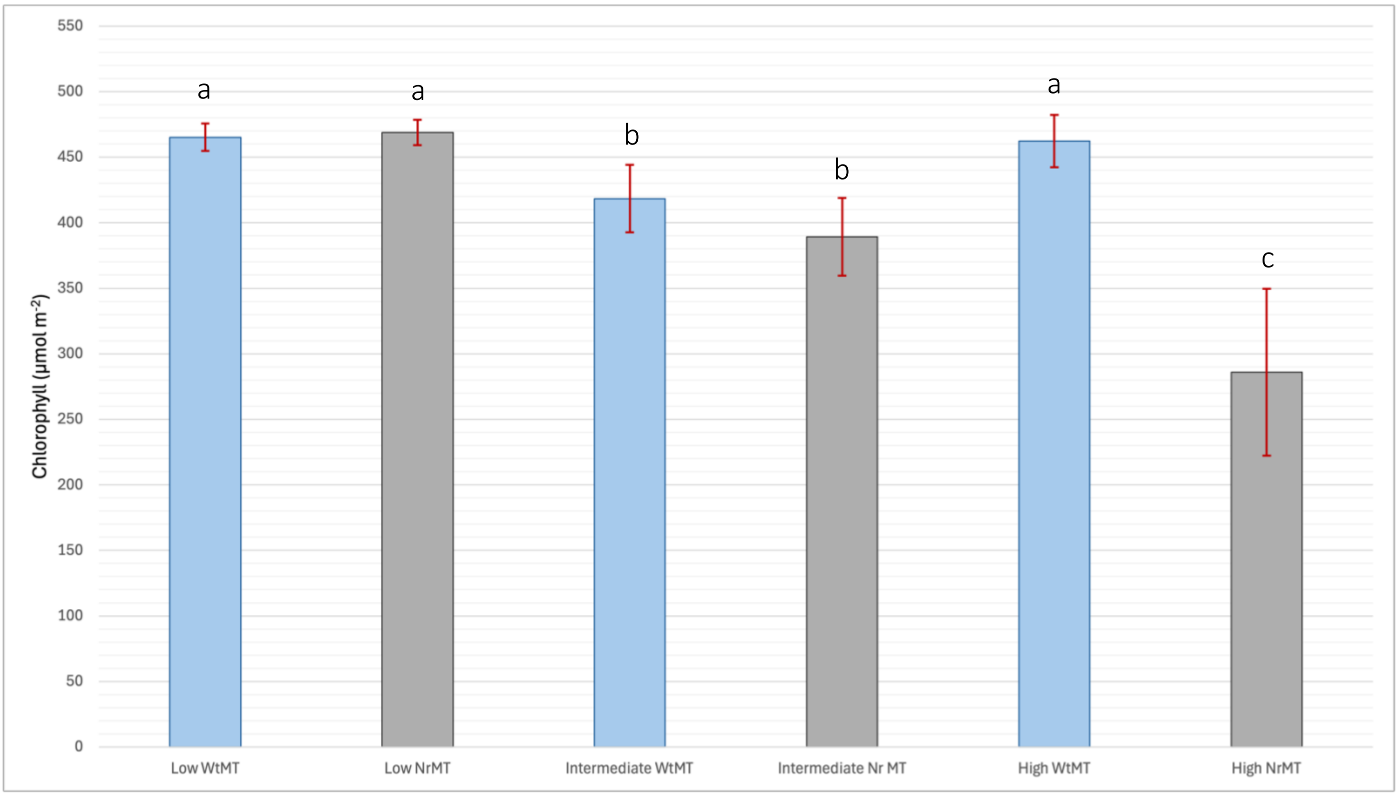

Figure 4.3.13: Chlorophyll content of the oldest leaf of different tomato genotypes at different compaction levels with bars of means ± SE, and letters of mean discrimination (n = 6) (columns with different letters indicate that their means are significantly different at $p = 0.05$). Two-way ANOVAs found that $p = 0.0018$ for compaction, $p = 0.0258$ for genotype, and $p = 0.0085$ for interaction. This means that compaction, genotype, and an interaction between the two impacts chlorophyll content significantly in MT.

Shoot dry weight vs root dry weight of both AC and MT backgrounds (**Figure** *4.3.14* and **Figure** *4.3.15*) do not seem to have strong relationships to one another with 42.7% of variance in one explaining variance in the other in AC, and only 28.7% in MT, so shoot size may not be the most accurate representation of what is occurring below-ground, especially with root dry weight being significant in AC and not MT, also reflected in the higher R^2 value of shoot vs root dry weight in AC plants. However, there still seems to be an upward trend with higher shoot dry weight somewhat correlating with higher root dry weight, more convincingly in AC but this cannot be concluded definitively due to the low coefficient of determination.

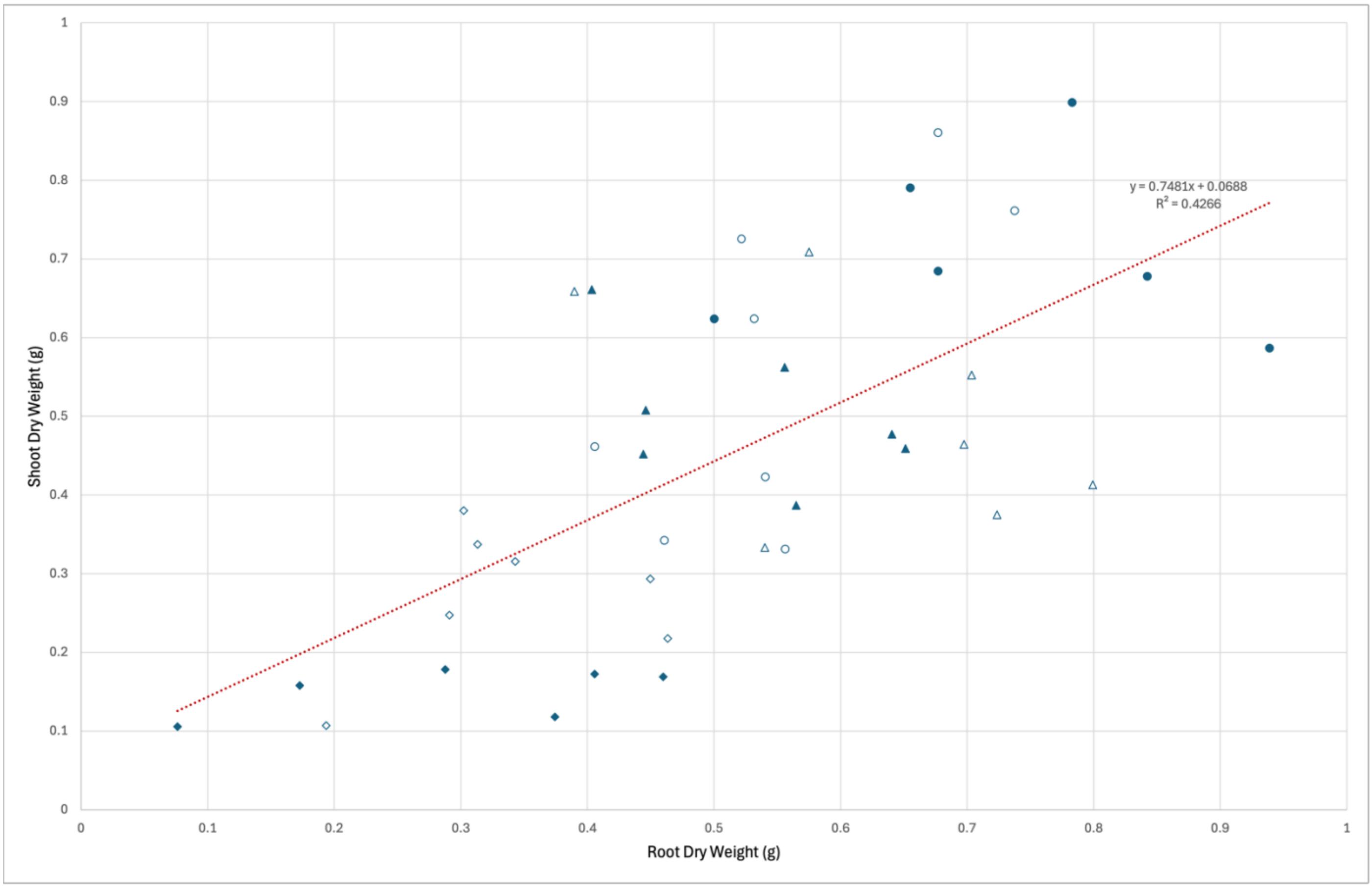

Figure 4.3.14: Shoot dry weight plotted against root dry weight of AC plants. Filled circles = Low WtAC, empty circles = low NrAC, filled triangles = intermediate WtAC, empty triangles = intermediate NrAC, filled diamonds = high WtAC, and empty diamonds = high NrAC. $R^2 = 0.4266$ which means that there is not a strong relationship between shoot dry weight and root dry weight, so the two are likely not impacting one another greatly.

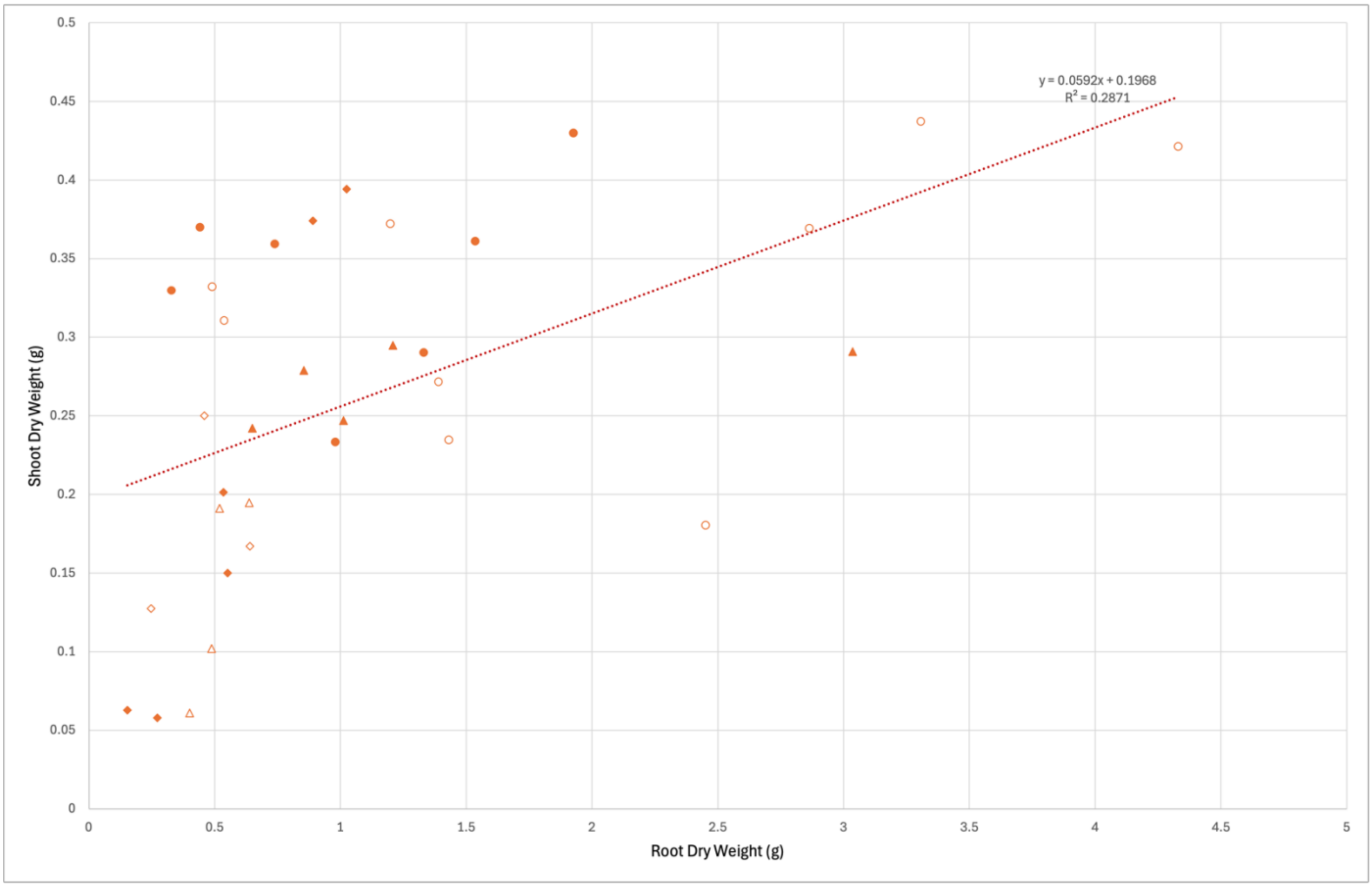

Figure 4.3.15: Shoot dry weight plotted against root dry weight of MT plants. Filled circles = Low WtMT, empty circles = low NrMT, filled triangles = intermediate WtMT, empty triangles = intermediate NrMT, filled diamonds = high WtMT, and empty diamonds = high NrMT. R^2 = 0.2871 which means that there is not a strong relationship between shoot dry weight and root dry weight, so the two are likely not impacting one another greatly.

4.4. Discussion

Impacts of Soil Compaction depend on Ethylene Sensitivity

Consistent with the hypothesis, the Wt genotype in AC responded significantly to higher soil compaction with less growth than the Nr genotype, as evidenced by plant height, leaf area, shoot dry weight, and chlorophyll content. Therefore, the Wt genotype responded more dramatically to soil compaction, which have higher ethylene levels (Pandey et al., 2021). The Nr genotype in the AC background was less significantly affected by high compaction with somewhat reduced growth demonstrated by lower plant height, total leaf area, and shoot dry weight (**Figure** *4.3.2*, **Figure** *4.3.4*, **Figure** *4.3.10*, and **Figure** *4.3.12*). This lower sensitivity was most evident as shoot dry weight, which was decreased less than WTAC (**Figure** *4.3.10*).

These results reinforce the role of ethylene as a key mediator of plant response to soil compaction, with greater growth inhibition in the Wt genotype than the Nr genotype.

Micro-Tom showed a more complex interaction with soil compaction with NrMT showing an expected trend, this being decreased growth with increasing compaction. But WtMT started with an expected trend and then had increased plant height, total leaf area, shoot dry weight, and chlorophyll levels at high compaction than intermediate compaction. Therefore, wild-type Micro-Tom may have a more unique genetic mechanism that allowed this background to overcome increased inhibition at high ethylene levels (high compaction levels) through some regulatory pathway or perhaps a threshold effect where high compaction increased root-soil contact without stressing the plant to a higher level than it could tolerate. However, this was more likely to be caused by less of an ability to distinguish significant effects between Wt and Nr due to the small, or dwarf, size of the plants.

Chlorophyll Degradation at High Compactions

MT chlorophyll content measurements from this study and the role ethylene plays in the chlorophyll content of leaves aligned with the literature, for example one study on the role ethylene played in the effectiveness of photosynthesis identified that the final phase of dose-dependent inhibition of photosynthesis involved chlorophyll breakdown after ethylene was administered (56 hours prior) and that ethylene downregulated chlorophyll biosynthesis and upregulated chlorophyll degradation (Mohorović et al., 2023). Stomatal closure could also have immediately inhibited photosynthesis by reduced CO_2 uptake and O_2 release through the accumulation of root-to-shoot signals like ABA and ACC. Therefore, elevated compaction levels may have resulted in lower chlorophyll levels in both AC and MT, but especially in NrMT due to greater significance. This was consistent with the literature which stated that ethylene accelerated chlorophyll breakdown by activating PAO and other related enzymes to produce NCCs and chlorosis (see **chapter 4.1**).

The greater significance of chlorophyll content differences in MT plants compared to AC (not Nr vs Wt) suggested that there could have been other mechanisms related to chlorophyll degradation in MT compared to AC, such as the increase of leaf chlorophyll content by brassinosteroids by activating or inducing chlorophyll enzyme biosynthesis (Siddiqui et al., 2018). Especially with the significant interaction effects calculated from ANOVAs in MT with lower chlorophyll in Nr than Wt, the complexity of chlorophyll regulation by ethylene is outlined, especially in the Nr genotype.

The findings of this research could show that selecting tomato varieties with more resilience to soil compaction (such as NrAC) is important in areas that grow tomatoes in soil systems due to increasing soil compaction caused by, for example, heavier farming machinery. Reduced sensitivity of WtMT could indicate more suitability for growth in highly compacted soils or poorly drained soils, which may be beneficial when deploying alleles in other crops. On-the-other hand this research also shows that more traditional varieties like AC may need more careful growth management strategies to avoid poor growth/ yields. Perhaps breeding strategies can be identified from this work also to combine desirable traits from both AC and MT backgrounds to produce low ethylene sensitivity mutants with desirable characteristics, like improved RSA under high compaction.

4.5. Conclusion

With Nr and Wt genotypes seemingly responding differently in AC and MT (e.g., Nr seemed to be more sensitive to compaction in MT but less sensitive in AC compared to Wt), there is further research needed on the different genetic backgrounds. The importance of soil management strategies and careful crop background selection has been highlighted by this study as well as the significant role of ethylene in mediating tomato growth responses. The differences observed between AC and MT provide valuable insights into the interaction between ethylene sensitivity and response to compaction for future research and breeding programs to improve tomato resilience to abiotic stresses. Crop emergence was not significantly impacted by compaction (Table *4.3.2*); however, crop establishment and later growth were significantly impacted by soil compaction, especially in WtAC and somewhat in WtMT although still with the unforeseen similarity in growth under intermediate and high compaction. This may be unusual as it would have been expected that the tomatoes show reduced growth under high compaction, but as this was not the case for all of the parameters measured it may have been that intermediate compaction was enough to significantly alter growth and the additional compaction provided by the high condition simply did not increase the effect anymore.

5. General Discussion

Root Biomass Can Be Used To Assess Soil Compaction Sensitivity in Wheat and Tomato

Root dry weight can be used to compare the response of wheat and tomato to soil compaction (**Figure** *4.5.1*) while also considering the overall size of the crop root systems. Chewink was more sensitive to ethylene than Kutz (**Figure 2.3.1**) and we can see that at intermediate compaction this pattern was followed, however, at high compaction there was a reversal with Chewink having a higher root biomass than Kutz (and this pattern was observed in many of the measured parameters such as total leaf area and shoot biomass in **Figure** *2.3.3* and **Figure** *2.3.4*). The response of root dry weight in AC followed that expected from the ethephon assay (**Figure** *4.3.1*) with the wild-type genotype being more compaction sensitive than the Never-ripe genotype. However, in MT the response to increasing compaction was the opposite of what the assay suggested with Never-ripe being more compaction sensitive than wild-type (**Figure** *4.5.1*). Both crops showed some unexpected results, however, this does suggest further research is needed into the potential mechanisms behind these growth responses. We can also see that the root systems of the tomatoes are much larger than that of the wheat because tomatoes tend to have more fibrous roots that explore deeper soil layers than wheat, or this could be due to the presence of a tap root in tomato. In wheat there was also much less variation within samples compared to the tomato which may have been due to the smaller root systems but could also show a more uniform response to compaction. Thus, tomato seems to show more sensitivity to compaction in terms of root biomass with higher levels of significance than wheat (**Figure** *4.3.12*).

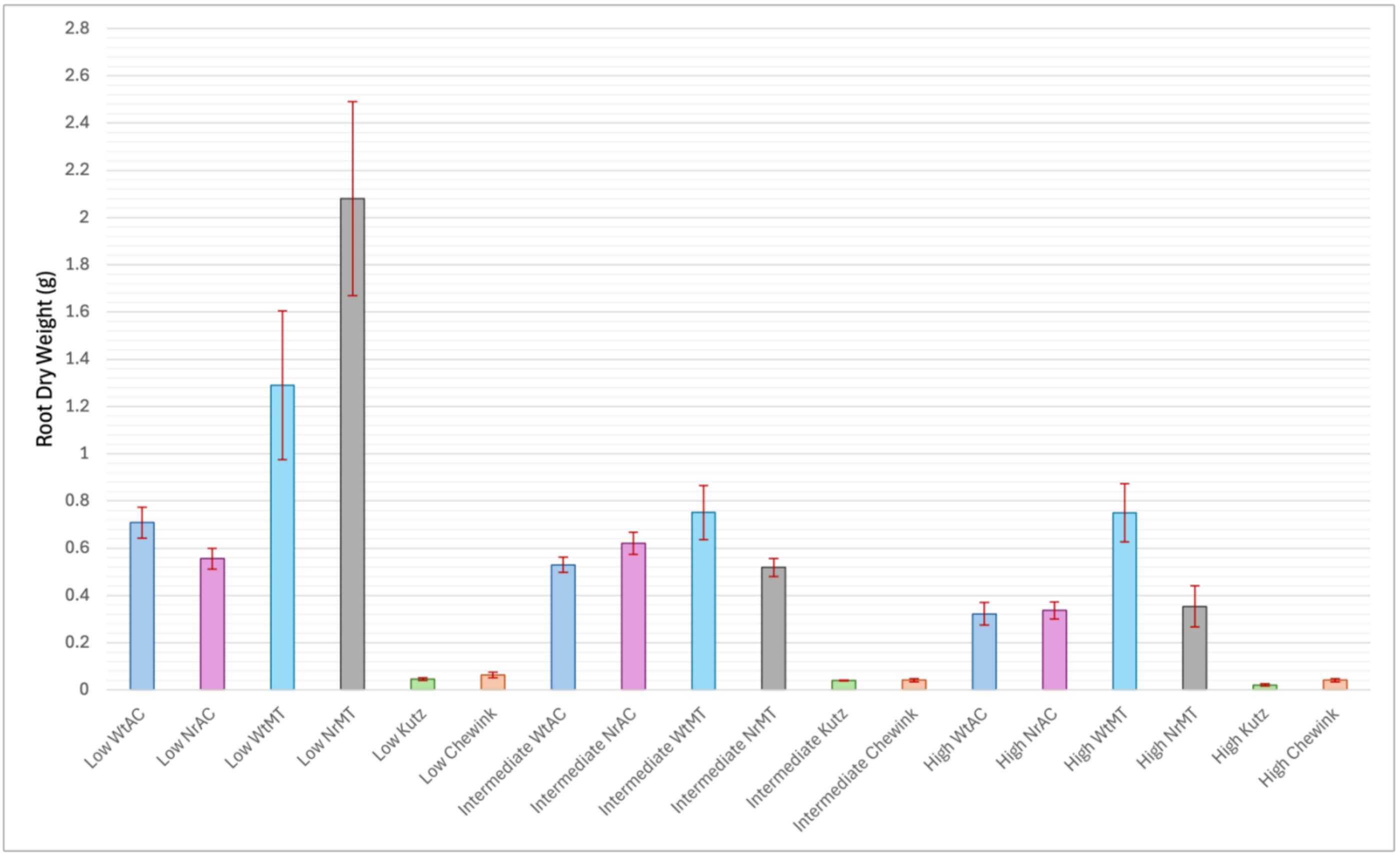

Figure 4.5.1 Root dry weight of different varieties of tomato and wheat with ± SE bars.

Ethylene Sensitivity Compared to Average Root Biomass/ Average Root Length

In wheat, ethephon-induced root length reduction decreased as root biomass increased (**Figure** *4.5.2*), which showed that as root biomass increased the root length increased. Comparatively, Ladham and Chilham were better able to maintain root length at low biomass. Lastly, root length reduction was higher at high ethephon levels, irrespective of biomass.

For potato there was less of an impact with no real correlation between percentage reduction in root length and root length (**Figure** *4.5.3*). This means that longer roots in potato experienced increased root length reduction at high ethephon levels. Therefore, larger root systems were more susceptible to compaction than smaller root systems. This contrasted with what was found for wheat and tomato in which longer roots were more resilient to compaction than shorter roots.

Similar to the wheat, tomato showed that larger root systems were more resilient to compaction. At low root biomass there were reductions of up to 65% in root length for NrAC and WtMT, so smaller root systems in highly compacted soil were more susceptible to losses in root length. NrMT and WtMT showed increased resilience at higher root biomass with smaller reductions in root length. There seemed to be a higher level of sensitivity in the tomato compared to the wheat shown by steep declines in root length at lower biomasses, so the root system may have been more easily impacted by higher levels of compaction than wheat.

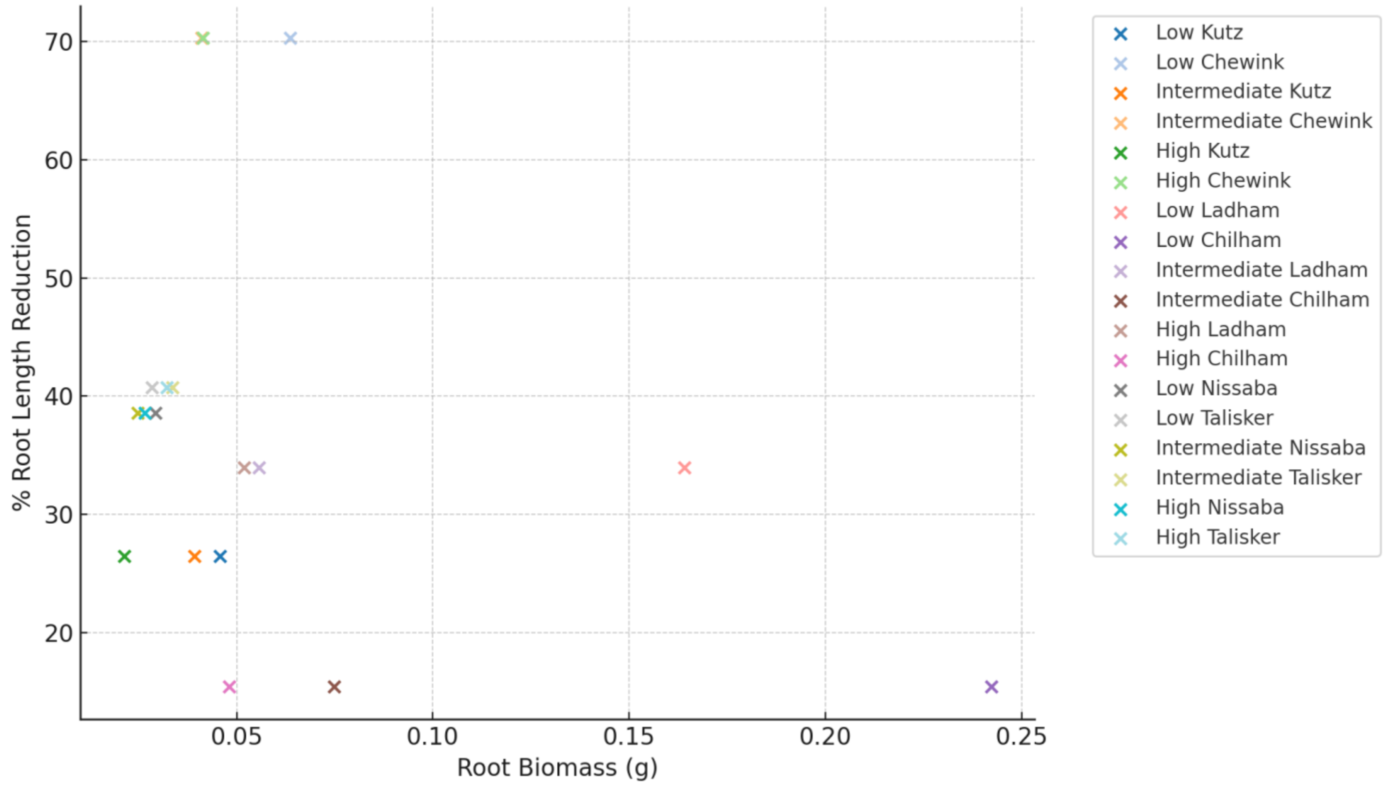

Figure 4.5.2 Average reduction in root length (%) as identified by the ethephon assay (**section 2.2**) of different wheat varieties against root biomass (g) at various compaction levels.

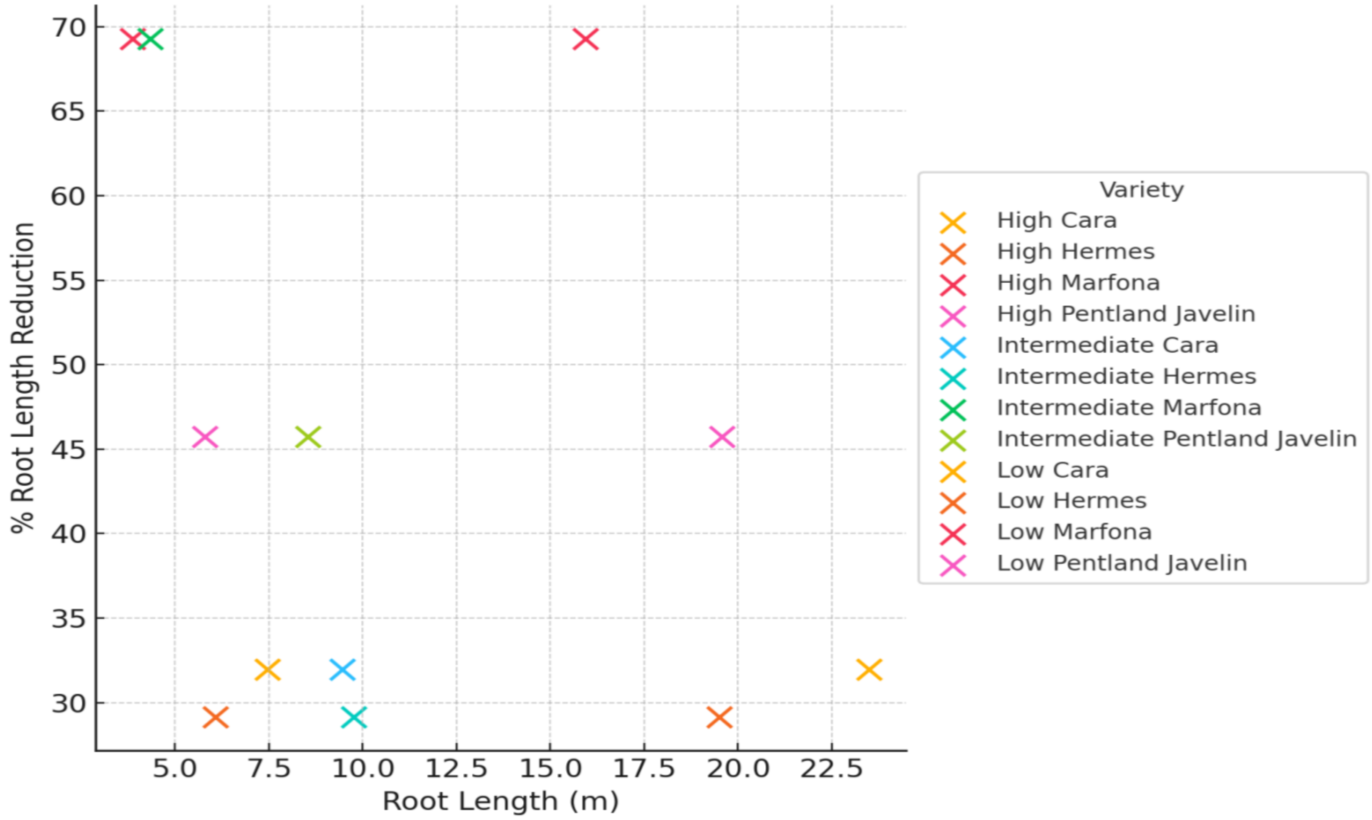

Figure 4.5.3 Average root length reduction (%) from the ethylene assay (**section 3.2**) against root length (m) of different potato varieties at different levels of compaction.

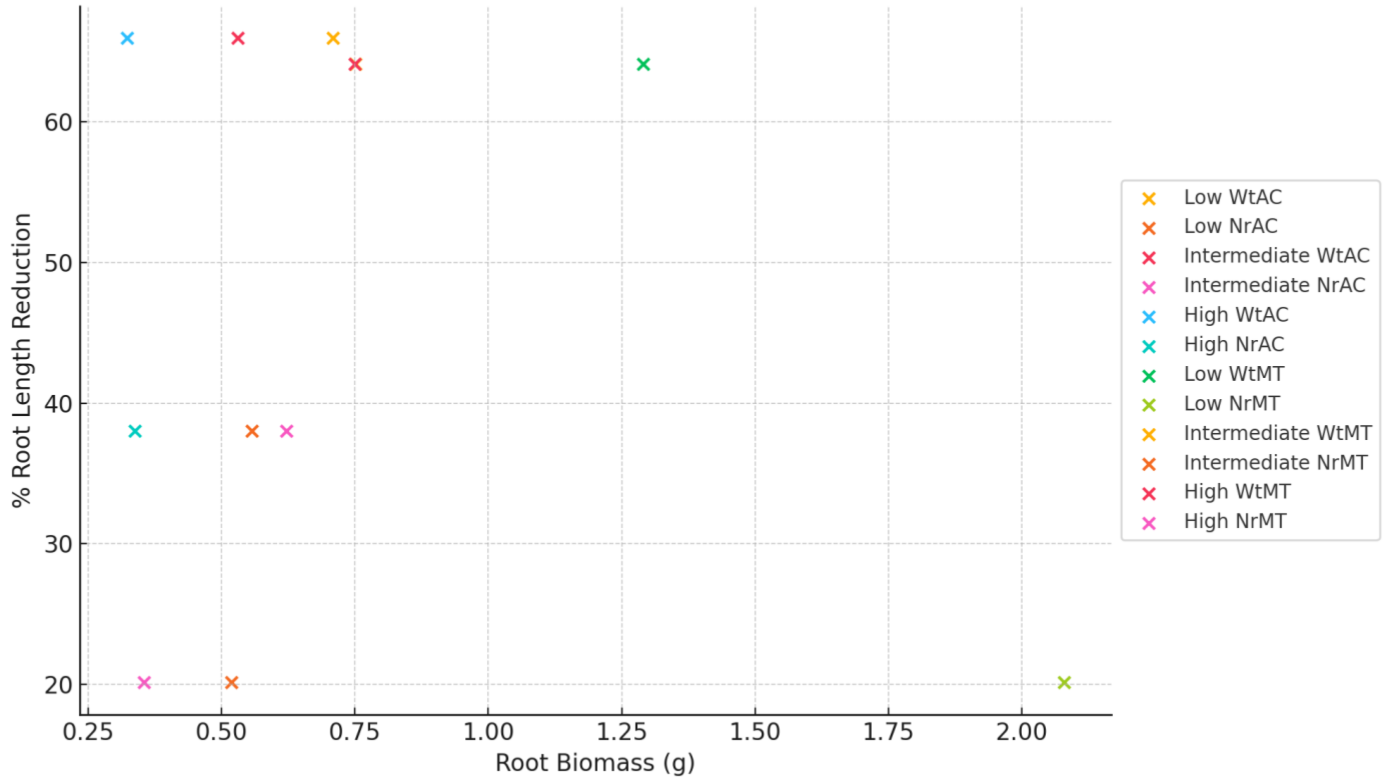

Figure 4.5.4 Root length reduction (%) measured from the ethephon assay (**section 4.2**) against root biomass (g) of different tomato varieties at different levels of compaction.

Root Response to Compaction in Potato

In the potato varieties studied the root length decreased and diameter increased as compaction increased (Munoz-Arboleda et al., 2006), which was associated with enlargement of radial root cells and axial tightening of cell walls and could have been an adaptive response to mechanical impedance (Lipiec et al., 2012). Root thickening was found to occur in potato through aerenchyma formation that was stimulated by saturated soil conditions caused by poor irrigation management and compaction (Munoz-Arboleda et al., 2006), and this thickening may have been to compensate for a loss of longitudinal growth (Bennie and Krynauw, 1985).

Response to Compaction was Linked to Ethylene Sensitivity in Potato

The main aim of this thesis and research was to identify whether there was a correlation between ethylene sensitivity and compaction response in three crops. Cara and Marfona were the most significantly different varieties in their response to compaction with exaggerated compaction response from Marfona (reduced growth at higher compaction) in parameters such as shoot dry weight, plant height, total leaf area, root length, average root diameter, root surface area, and root volume compared to Cara. Not only did Marfona respond more to increasing compaction than Cara (less growth at higher compaction), but Marfona was also found to be significantly more sensitive to ethylene than Cara.

Since this research did not look at the response of the shoots to ethylene and whether root ethylene sensitivity and shoot ethylene sensitivity followed the same pattern, it can only be assumed that shoots may show similar responses as the roots. Although, this could be true in that ethylene regulates downstream signals like auxin and ABA (Huang et al., 2022), which could be used as root-sourced stress signals that are transmitted to the leaves (Skilleter, 2023) and result in altered growth responses. Again, as auxin and ABA were not measured this is speculative but could potentially be a good theory as shoot physiological processes could be mediated by the supply of signalling molecules from the root system, such as potentially controlling gibberellins that alter height (Dodd, 2005). ACC is converted into ethylene by ACO primarily in the shoots (**chapter 1**), but can also be converted in the roots, with production increasing under stress (such as high compaction) (Li, 2023) and so increases transportation via the xylem from roots to shoots.

Adaptive potential of plants relies on efficient communication between the roots and the shoots for resource allocation and communication modes include electric and hydraulic signals (Shabala et al., 2015) to relay information on, for example, soil water availability (Huntenburg et al., 2022) and P availability (Torres et al., 2021). Under high compaction leaves exhibited lower leaf water potential and stomatal closure through ABA accumulation (Sojka, 1992) which decreased water use efficiency and compaction also decreased P availability (Nadian et al., 2005) through decreased root exploration which limited access to P. With ethylene and ABA being long-distance signals it was likely that the physical and hypoxic stresses inflicted on the roots resulted in signalling to the shoots in order to alter growth (Aphale et al., 2005) especially with research finding that ABA-deficient mutants of barley showed poor leaf expansion under high compaction (1.6 g/cm^{-3}) (Mulholland et al., 1996) and that ACO1$_{AS}$ tomato mutants with reduced ethylene production had similar growth in compacted and uncompacted soils (1.1 and 1.5 g/cm^{-3}). From this research we can see that roots and shoots would most likely respond very similarly to compaction, as indicated by growth parameters such as shoot biomass and root length in potato (**Figure** *4.5.5*) in which the shoots responded in a similar pattern as the roots with Marfona being the most compaction and ethylene sensitive.

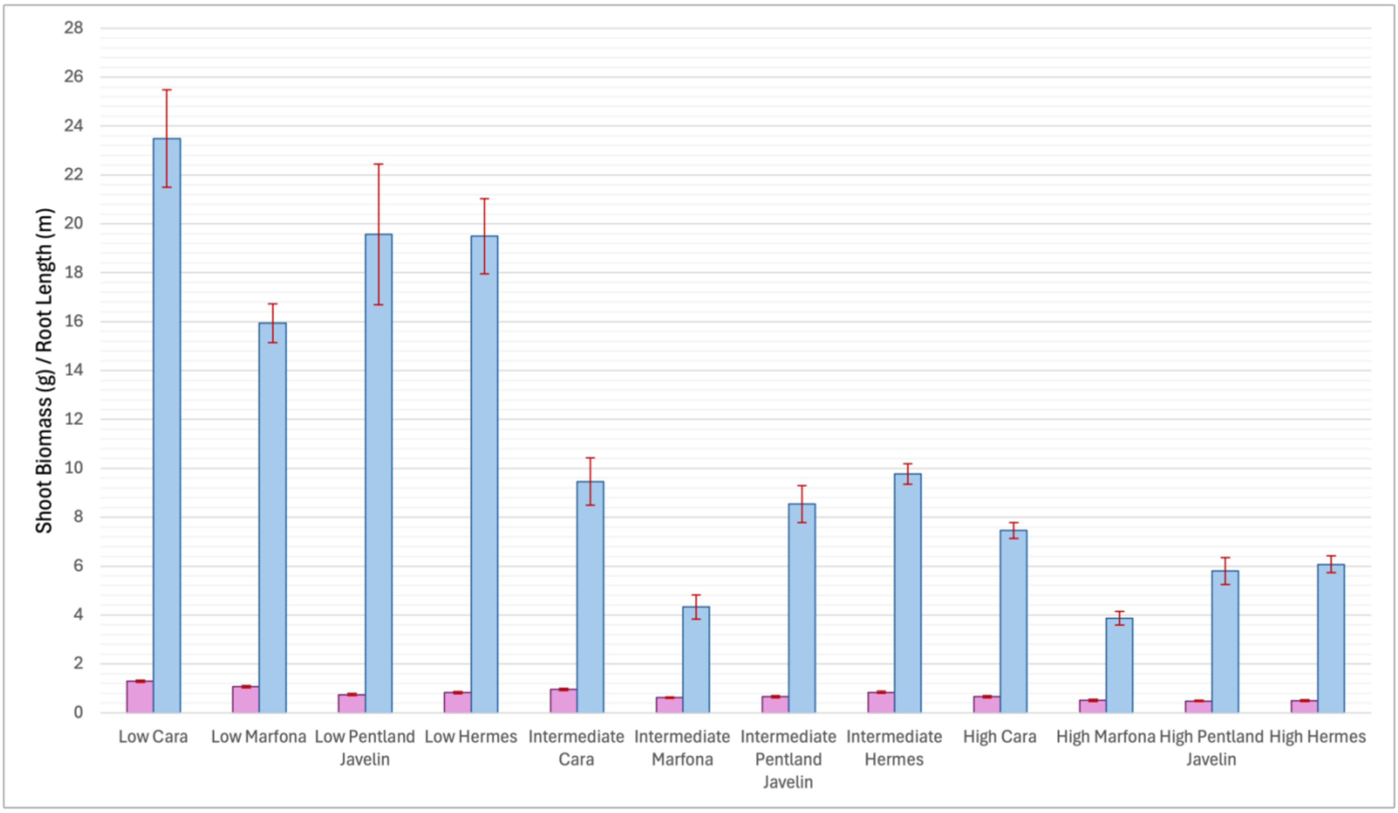

Figure 4.5.5 Shoot biomass (purple) compared to root length (blue) of different potato varieties at different levels of compaction with error bars indicating ± SE.

Ethylene Sensitivity Somewhat Contributed to Compaction Response in Tomato

In tomato the ethephon assay found that Wt tomatoes were significantly more sensitive to ethylene than the Nr tomatoes, consistent with past research (Fiebig, 2014). Looking at the growth experiments this was true depending on the background of the tomatoes studied in this thesis, AC seemed to follow this pattern as shown by plant height, total leaf area, shoot dry weight, and root dry weight but MT displayed the opposite with improved growth in the Wt genotype than the Nr genotype (e.g., **figure 4.3.11**), even though WtMT was more sensitive to ethephon than NrMT (**figure 4.3.1**). Perhaps there were differences in auxin-ethylene crosstalk or ethylene receptor sensitivity in MT that could explain improved growth in WtMT. Mutations in brassinosteroids (BRs) can influence ethylene sensitivity because both regulate similar developmental processes like fruit ripening (Jiroutova et al., 2018), BRs can also regulate the expression of ethylene biosynthetic signalling genes, and have intersecting signalling pathways (Bishop and Koncz, 2002). This would be interesting to research as there does not seem to be research available on possible genetic factors that could cause this response in MT compared to AC. However, some research on Nr from the Ailsa Craig background may explain why this occurred: a paper that used Nr mutants showed that there was growth enhancement during both the vegetative and reproductive stages with higher leaf shoot biomass, root biomass, and fruit biomass as well as decreased carbon assimilation, carbohydrate turnover, and metabolite levels (Nascimento et al., 2021). Nr was also found to have higher photosynthetic rates and fruit yield so the Nr mutation lead to increased growth rates (Nascimento et al., 2021). Although this was not found to be the case in another paper in which tomato was over-watered and Nr was found to be less sensitive to over-irrigation which was linked to ethylene emission (Fiebig, 2014). With this research it can be assumed that the increased growth in Nr may have been more obvious in AC due to the small stature of MT compared to AC which may have required more resources to sustain growth and so were more prone to stress.

Considering the unusual response observed in MT, could this have been different had the experiment ended earlier or later? For the intermediate compaction treatment, the results may have been different had the experiment ended later as more of the NrMT leaves were still expanding when the experiment ended than the WtMT leaves (possibly because WtMT had started producing flowers). Under high compaction the results may have been different had the experiment ended on day 15 or 17 because growth was slightly lower at high compaction than low compaction (4 and 5 on graph tiles c and f, **Figure** *4.3.7*), but the growth was still very similar at this point. Therefore, even if the experiments had ended later or earlier, it is likely that the results found for MT would have been the same. For AC (**Figure** *4.3.6*) the results also would most likely have remained similar as the Nr mutants seemed to continue growing for longer than the WT mutants under intermediate and high compaction. Therefore, even though the results may have been different had the experiment ended earlier it is still quite unlikely because the pattern of growth was reasonably consistent throughout.

Response to Compaction Depending on Ethylene Sensitivity May Show Threshold Effects in Wheat

In wheat there was a somewhat unexpected response to high compaction as the more ethylene sensitive Chewink exhibited increased growth under high compaction when looking at coleoptile height, total leaf area, and shoot dry weight (**Figure** *2.3.2*, **Figure** *2.3.3*, and **Figure** *2.3.4*). Research that mentioned improved contact between the roots and the soil showed that it was moderate compaction that improved growth (Bouwman and Arts, 2000) with the moderate compaction treatment being equal to a 4.5 t load, compaction was not given in terms of BD or SR. It would make sense for the intermediate compaction treatment of Chewink to show higher growth, but it was the high compaction level that seemed to improve growth, although this increase at intermediate compaction was seen in Chilham shoot dry weight (**Figure** *2.3.5*) compared to low compaction, therefore it may be some other mechanism that was causing this. Perhaps the earlier tillering, which was ethylene sensitive in rice (Yin et al., 2017), of Chewink confounded the sensitivity to ethylene and causes Chewink to appear as though it was more vigorous under high compaction, or intermediate compaction may simply be a better discriminate of ethylene-mediated effects.

6. Concluding Remarks

This thesis has demonstrated that there was interspecific and intraspecific variation in soil compaction responses depending on the ethylene sensitivity of the crop cultivars tested. Generally, the more ethylene sensitive cultivars were more negatively impacted by increasing compaction with some reversed effects seen in MT and Chewink, however this could have been due to a variety of factors such as differences in MT versus AC growth in general. There have also been some suggestions made as to how this research could contribute to knowledge and farming, such as identifying varieties with lower ethylene sensitivity in order to produce crops that have enhanced growth under high compaction. This is especially important as compaction is a formidable obstacle and is becoming more of an issue as a result of heavier machinery, which can be avoided through effective management practices (Pandey and Bennett, 2023) such as CTF or SOM addition. It may be best to combine such soil management processes with producing more compaction/ ethylene tolerant crop cultivars to ensure food security as time progresses. For example, Cara was much more compaction tolerant than Marfona and could be selected when considering which potato cultivars to plant. Possibly carrying out field trials in compacted and uncompacted soils with different management practices using the potato varieties used here would provide an ability to definitively conclude that these potatoes would show the same responses to compaction (such as Cara performing more desirably under increasing compaction than Marfona) when grown for food production and not just for research. Overall, it has been shown that high compaction significantly impacts growth in the crops investigated with large effects on both above- and below-ground measurements and this research can be used to inform future work and provide information on the impact of compaction, depending on ethylene sensitivity, on wheat, potato, and tomato.

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Appendix

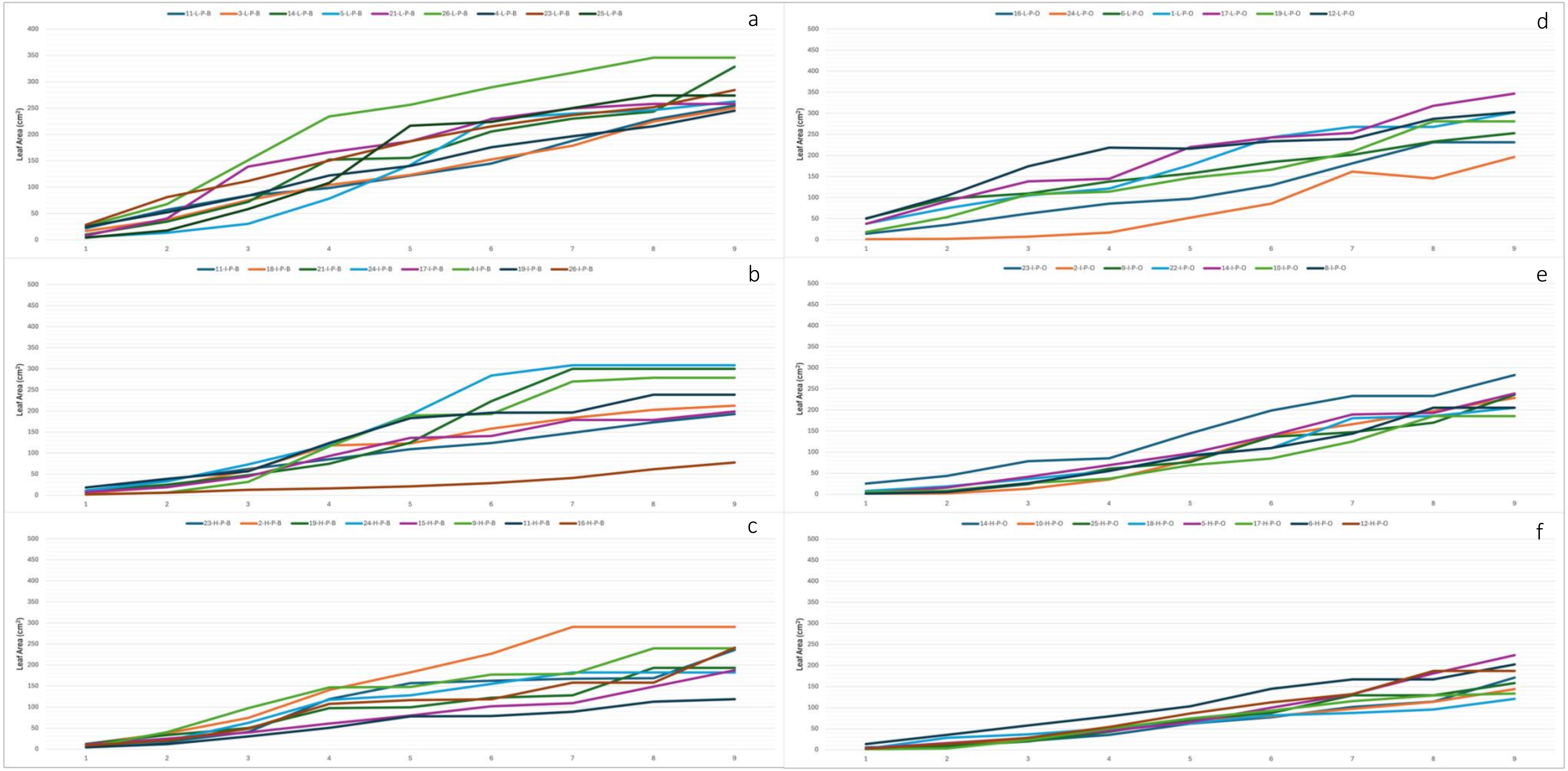

Figure appendix.1 Leaf area growth from germination to harvest of different potato cultivars (a) Low Cara, (b) Intermediate Cara, (c) High Cara, (d) Low Marfona, (e) Intermediate Marfona, (f) High Marfona.

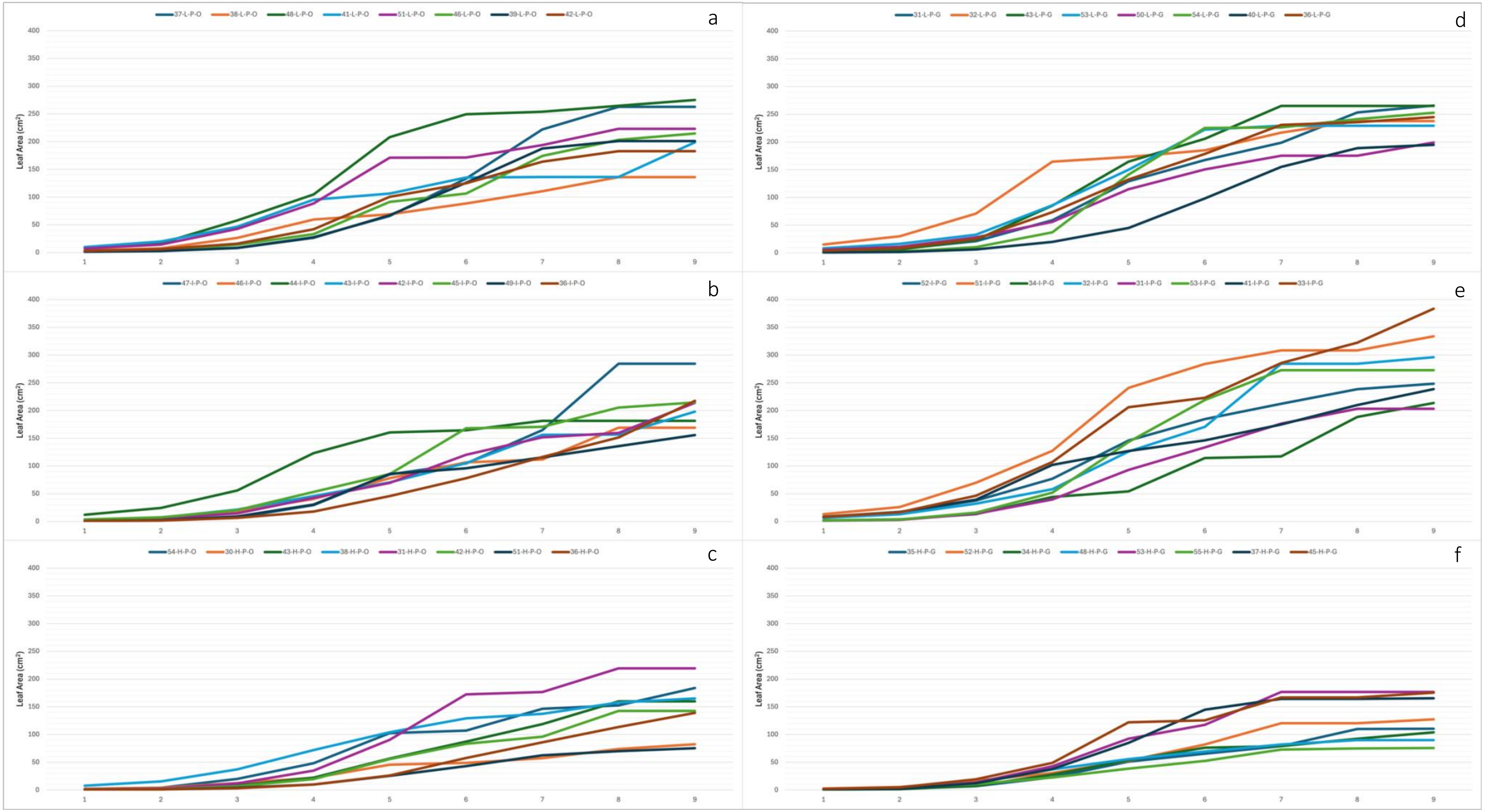

Figure appendix.2 Leaf area growth from germination to harvest of different potato cultivars (a) Low Pentland Javelin, (b) Intermediate Pentland Javelin, (c) High Pentland Javelin, (d) Low Hermes, (e) Intermediate Hermes, (f) High Hermes.