Can inherited memories of stress help protect crops from pests and disease?

by

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This thesis is submitted for the degree of PhD

to the Lancaster Environment Centre,

Lancaster University,

December 2024

Abstract

Since their development in the 1940s, synthetic pesticides have been a vital tool in combatting crop losses to pests and pathogens. However, growers are being encouraged to move towards alternative pest control methods due to concerns about the effects of pesticides on the environment. Priming, the potentiation of plants' natural defences, is one such alternative to chemical pesticides. The primed state can also be inherited from parent plants to their offspring. If transgenerational priming were implemented commercially, this may ease the introduction of priming into pest control by reducing the input required by growers. We tested the effects of five parental priming treatments: β-aminobutryic acid (BABA), benzo(1,2,3)thiadiazole-7-carbothioic acid *S*-methyl ester (BTH), methyl jasmonate (MeJA), hexanoic acid (HA), and Fytosave, on the resistance of offspring Micro Tom tomato plants to *Pseudomonas syringae* or *Botrytis cinerea*. We also tested whether any of our treatments could transgenerationally influence the expression of *PATHOGENESIS RELATED PROTEIN-1* (*PR-1*). BABA, BTH, and Fytosave could all provide transgenerational resistance against *P. syringae*. None of our treatments could produce any consistent transgenerational effects on resistance against *B. cinerea*, however they could all transgenerationally potentiate expression of *PR-1* upon treatment with 2,6-dichloroisonicotinic acid (INA). Additionally, we tested if parental treatment with our elicitors would influence growth of the offspring. Each of our treatments could affect growth of offspring plants, however the exact effect of parental treatment differed between elicitors. We conclude that Fytosave may be a suitable candidate for commercial application of transgenerational priming in tomato crops as it appears to be capable of transgenerationally priming the salicylic acid defence pathway against biotrophic pathogens without negatively affecting fruit yield.

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Acknowledgements

I would like to thank Mike Roberts for his continued and invaluable support in every aspect of my degree, as well as his unwavering patience when I have often struggled. I would also like to thank Cesar Ponce, Geoff Holroyd, and Dawn Worrall for technical guidance in the laboratory. I would finally like to thank my parents for their undying love and encouragement in these dark six years.

Author’s Declaration

I declare that this thesis is entirely my own work and has not been submitted, in part or in whole, for any previous application for a degree.

List of Abbreviations

Term	Definition
BABA	β-aminobutryic acid
BTH	Benzo(1,2,3)thiadiazole-7-carbothioic acid *S*-methyl ester
CFU	Colony forming unit
COS-OGA	Chitosan oligogalacturonides
DAMPs	Damage-associated molecular patterns
ET	Ethylene
ETI	Effector-triggered immunity
ETS	Effector-triggered susceptibility
HA	Hexanoic acid
HR	Hypersensitive response
INA	2,6-dichloroisonicotinic acid
IPM	Integrated pest management
ISR	Induced systemic resistance
JA	Jasmonic acid
JA-Ile	Jasmonate isoleucine
JAZ	Jasmonate ZIM domain
LS-LB	Low sodium Luria Bertani
MAPK	Mitogen-activated protein kinase
MeJA	Methyl jasmonate
NLR	Nucleotide-binding leucine-rich/repeat receptor protein
PAMPs	Pathogen-associated molecular patterns
PDA	Potato dextrose agar
P&Ps	Pests and pathogens
PR	Pathogenesis-related
PR-1	Pathogenesis-related protein 1
PRR	Pattern-recognition receptor protein
PTI	Pattern-triggered immunity
RLCK	Receptor-like cytoplasmic kinase
RdDM	RNA-directed DNA methylation
ROS	Reactive oxygen species
SA	Salicylic acid
SAR	Systemic acquired resistance
TF	Transcription factor

1 – Introduction

1.1 Food Security

According to the United Nations Committee on World Food Security, a person or group of people is said to be food secure when they possess the "physical, social, and economic access to sufficient, safe and nutritious food that meet their dietary needs and food preferences for an active and healthy life" (FAO, 1996). An absence of food security can have many consequences for both the short- and long-term wellbeing of a community, including excess mortality, growth stunting, and child wasting (FSIN and Global Network Against Food Crises, 2023). In 2015, the United Nations established a total of 17 Sustainable Development Goals to tackle a variety of issues in both developed and developing countries, including the eradication of hunger and establishment of global food security by 2030 (United Nations, 2015).

However, progress towards meeting this global food security goal has been slowed in recent years due to the COVID-19 pandemic and the conflict in Ukraine. Indeed, there was a sharp increase in global food insecurity from 2019 to 2020 because of economic shocks resulting from the pandemic (FSIN and Global Network Against Food Crises, 2023, FAO, 2023). Food insecurity affected approximately 29.6 % of the global population, or 2.4 billion people, in 2022 (FAO, 2023). Although the global level of food insecurity has plateaued from 2020 to 2022, 319 million more people were food insecure in 2022 than in 2019 (FAO, 2023). The economic recovery from the pandemic has helped to stop the increasing levels of food insecurity but this positive effect has been stymied by repercussions of the war in Ukraine, such as rising prices of fuel and food (FSIN and Global Network Against Food Crises, 2023, FAO, 2023).

Conflicts and economic shocks are currently the biggest drivers of food insecurity in 2024 (FSIN and Global Network Against Food Crises, 2024), however there are a variety of other concerning long-term factors such as climate change. The global mean temperature has already risen by 1 °C between 1901 and 2016, and could be an additional 4.7 °C higher by 2021 (USGCRP, 2017). Carbon dioxide levels in the

atmosphere have also experienced an increase from 284 ppm in 1832 to over 400 ppm in 2017 (USGCRP, 2017, Wheeler and von Braun, 2013). These changes are expected to lead to warmer temperatures, changing patterns of rainfall, and more frequent and severe extreme weather events (Wheeler and von Braun, 2013). There is considerable variance in climate models due to the extreme numbers of factors that can be considered such as possible effects of CO_2 fertilization or future changes in crop management. However, there is a common consensus that the effects of climate change on overall global crop productivity will be negative (Müller and Robertson, 2014, Schleussner et al., 2018, Wheeler and von Braun, 2013). Additionally, the consequences of climate change are predicted to be more severe in tropical regions which often coincide with areas that are already suffering from food insecurity (Schleussner et al., 2018, Wheeler and von Braun, 2013). Indeed, recent La Niña and El Niño weather events have already exacerbated food security situations in economically depressed areas of Africa and Asia (FSIN and Global Network Against Food Crises, 2023).

Water scarcity and soil degradation are also potential dangers to food security in the future. As the world population has grown exponentially over the past century, so too has pressure on food production to feed this expanding population. Indeed, food production will need to increase by as much as 70 % between 2005 and 2050 to provide global food security (Dinar et al., 2019, Kopittke et al., 2019). Much of the increase in food production so far has been accomplished by intensifying agricultural production on pre-existing agricultural soil (Kopittke et al., 2019). However, water scarcity has been increasing and around 52 % of agricultural land is already affected by soil degradation (Dinar et al., 2019, Kopittke et al., 2019). Therefore, there are concerns that there may not be adequate land and water resources to meet demand, if sufficient improvements in crop management and technology are not made.

Pests and pathogens (P&Ps) are another barrier to global food security. However, attempts to quantify the global burden of P&Ps on crops are rare. A 2019 survey investigated worldwide losses due to P&Ps in five major crops: wheat, rice, maize, potato, and soybean. It found that losses ranged on average between 17 and 30 %

for all five crops (Savary et al., 2019). These losses are often lower in wealthier areas that generate more food surplus, such as Northwest Europe and the United States, and much higher in low income regions such as the Indo-Gangetic Plain and Sub-Saharan Africa (Savary et al., 2019).

Pesticides have therefore been an important and universal tool in strengthening global food security by fighting P&Ps in a wide variety of agricultural settings. Usage of pesticides has exploded globally ever since the development of the first synthetic organic pesticide in the 1940s and has been increasing with even greater intensity in recent decades (United Nations Environment Programme et al., 2022a). The total quantity of pesticide active ingredients being used globally has doubled from 2 million to 4.1 million tons between 1990 and 2016 (United Nations Environment Programme et al., 2022a). In 2018, the value of the pesticide market was estimated at 65 billion USD in the United States alone (United Nations Environment Programme et al., 2022a). Indeed, pesticides can provide a range of social benefits in addition to improving crop yields, such as the protection of wooden structures and reducing the prevalence of vector-borne diseases by killing their insect transmitters (United Nations Environment Programme et al., 2022c). Although pesticides are expected to provide economic benefit through reduction of crops losses and greater livestock yields, no recent reviews on the economic benefits of pesticides are available.

Despite the benefits that pesticides have provided, numerous concerns have arisen about the effects that pesticides may have on the health of both humans and the environment. In humans, exposure to pesticides can result in sudden acute poisoning, or chronic health conditions if the exposure is over a long period of time (United Nations Environment Programme et al., 2022b). Acute poisoning typically effects agricultural workers who work directly with pesticides but chronic exposure can effect both workers and also residents living close to agricultural fields through various means, such as spray drift or volatilisation of pesticides beyond a treated area (United Nations Environment Programme et al., 2022b). Global instances of acute poisoning have risen along with the usage of pesticides, to approximately 385 million cases of unintentional poisonings per year, around 11,000 of which are

fatalities (United Nations Environment Programme et al., 2022b). Chronic exposure to pesticides can result in a wide variety of adverse health effects including non-Hodgkin lymphoma, Parkinson's disease, asthma, and various kinds of cancer (United Nations Environment Programme et al., 2022b).

Pesticides and their residues can culminate in several different environmental compartments, such as the atmosphere, both surface and ground water, the soil, and any of the organisms in these spaces (United Nations Environment Programme et al., 2022b). This culmination can occur regardless of whether pesticides are being misused or handled properly. Once deposited in the environment, pesticide residues can remain there for decades after their original use (United Nations Environment Programme et al., 2022b). One of the most significant concerns around pesticides is how they can harm non-target organisms, especially natural predators of pests, which can diminish an ecosystem's natural ability to control pest levels, potentially leading to resurgence of pests or development of secondary pests (United Nations Environment Programme et al., 2022b).

In response to the concerns surrounding synthetic pesticides, many countries have introduced regulatory frameworks to determine if pesticides are sustainable and safe for human and environmental health (Handford et al., 2015). These regulations have resulted in many pesticides no longer being approved for use. Additionally, as a result of the increased regulations on agrochemical products, registration costs for pesticides have doubled between 1995 and 2014 and now constitute 34% of the total development cost (Serazetdinova, 2019). Accordingly, the number of new pesticides being introduced into the EU market per year is decreasing (Serazetdinova, 2019).

Several groups are worried about the potential consequences of banning pesticides without providing adequate replacements. In the UK, many farmers are anxious that the loss of some plant protection products will result in severely reduced crop productivity, leading to loss of jobs, a greater reliance on imports, and an increased cost of produce for consumers (National Farmers' Union, 2014). There are also concerns that some crops may be much more difficult to grow in the UK if sufficient protection products are not available, and that a smaller and less diverse pool of

usable pesticides will result in a larger chance of pests developing resistance (National Farmers' Union, 2014).

Due to the potential sustainability issues of pesticides, such as persistent chemical residues or the development of pesticide resistance, Integrated Pest Management (IPM) strategies have been adopted by many countries as an environmentally friendly alternative method of controlling pests (Deguine et al., 2021). IPM is defined by the UN Food & Agriculture Organization as “the careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations”. Although several variations on the definition of IPM exist, they commonly involve the coordinated use of techniques, drawing from a variety of disciplines, to suppress pest populations in a complementary manner (Parsa et al., 2014). IPM strategies use a combination of agronomic, mechanical, physical, and biological principles to control pests, but still employ the use of traditional pesticides as a last resort when other preventative methods prove inadequate to control a pest population (Barzman et al., 2015). Synthetic pesticides are often applied with reduced dose or frequency when they are used as a part of IPM strategies (Barzman et al., 2015). Additionally, a variety of chemicals with different modes of action may be deployed to minimise the chance of a resistant population emerging (Barzman et al., 2015).

Despite global endorsement by scientists and policymakers, adoption of IPM has been very weak by farmers in developing countries (Parsa et al., 2014). A 2013 study revealed a variety of reasons for this poor uptake, including a lack of economic incentive or favourable government policies, and insufficient technical support to develop an IPM strategy (Parsa et al., 2014). Another noteworthy reason discovered was that many farmers are reluctant to adopt IPM if others in their community are not using it as IPM is most effective when used collaboratively at the regional level (Parsa et al., 2014).

The temporal and spatial diversification of crops is an example of an agronomic practice used to control pests in IPM. To create temporal diversity, a farmer may rotate a field between crop species belonging to different families (Barzman et al., 2015). Spatial diversity may similarly be achieved by growing a variety of cultivars,

intercropping two or more crop species together, or interspacing semi-natural vegetation in their crops (Barzman et al., 2015). By exploiting temporal and spatial diversity, a farmer can disrupt the life cycles of many pests and prevent a single population of pests from becoming dominant whilst only exerting a low selective pressure on them (Barzman et al., 2015).

Before the use of traditional pesticides is necessary, IPM strategies will often utilise various forms of biological protection such as biological control agents or biopesticides (Barzman et al., 2015). A biological control agent is a living means, including viruses, to controlling pests. They can function directly by predating on pests or infecting them, or indirectly by outcompeting the pest for certain resources. Biological control agents currently only represent 7% of the total crop protection market but sales of biological control agents are growing at 15-20% per year which is at a much higher rate than that of synthetic chemical pesticides (United Nations Environment Programme et al., 2022a). The education and training required to effectively employ biological control is still a barrier of entry to many farmers (Barzman et al., 2015).

Biopesticides are naturally occurring compounds or derived from naturally occurring compounds in microorganisms (Kumar et al., 2021). Biopesticides represent a desirable alternative to chemical pesticides due to their host-specific and eco-friendly qualities. However, there are several downsides to using biopesticides. They often have a shorter shelf life than synthetic pesticides as they are sensitive to fluctuations in temperature and humidity and can be confusing to work with for farmers as multiple biopesticides may need to be used simultaneously due to their very high specificity (Kumar et al., 2021). Biopesticides also have a very high variability of effectiveness, ranging from 80% to less than 50% reduction in pest and disease, due to their previously mentioned sensitivities to temperature and humidity (Serazetdinova, 2019). There is also a relatively narrow range of biopesticides products available, as around 90% of biopesticides currently on the market are derived from the *Bacillus thuringiensis* bacterium (Kumar et al., 2021, Serazetdinova, 2019).

Another potential tool available to growers in IPM is the use of defence elicitors. Unlike pesticides which are directly toxic to pests and pathogens, defence elicitors are naturally occurring or synthetic compounds which can be applied to crops in order to augment the natural defence responses of the plant. Initial attempts of elicitors to enter the market and become a mainstream alternative to pesticides failed to materialise however (Yassin et al., 2021). Although elicitors could be applied to crops to activate defence responses and make them more resistant to subsequent invasion, this induced resistance was associated with direct costs to plant fitness, such as reduced growth or seed production, and was therefore undesirable to growers (Yassin et al., 2021). Even so, an interest has remained in the use of defence elicitors in IPM. This is partly due to the continued push away from 'zero tolerance' policy on pests and pathogens, but also because of the discovery that application of elicitors in smaller doses can 'prime' plant defences without directly activating them, resulting in less fitness costs (Yassin et al., 2021). Further research and greater understanding of plant defence systems (**discussed in sections 1.2 and 1.3**) will allow further optimisation of the use of defence elicitors in IPM strategy.

1.2 Plant Defence

1.2.1 The Innate Immune System

Plants form the foundation of many food chains and are therefore a source of nutrients for a huge diversity of organisms. As plants cannot move themselves to escape these dangers, they have developed complex physical and chemical defence systems to protect themselves from a wide variety of threats, including herbivores, pathogens, and numerous abiotic factors. Plant pathogens are often divided into three categories depending on how they extract their nutrients from their hosts: biotrophic, hemibiotrophic, and necrotrophic. Biotrophic pathogens obtain nutrients from living plant cells, and therefore form close, long-term relationships with their hosts in order to keep them alive. Necrotrophic pathogens kill plant tissues as they must get their nutrients from dead plant cells.

Hemibiotrophs are pathogens that are biotrophic in the early stages of infection but then transition into a necrotrophic mode as the disease progresses.

Plants have evolved both constitutive and induced forms of resistance to protect themselves from these pathogens. Constitutive mechanisms of defence are always activated to deter potential attackers, and include preformed structural barriers such as cell walls, waxy epidermal cuticles, and bark. Induced mechanisms of resistance are not activated until the plant encounters and recognises a or pathogen. Initiation of induced resistance leads to activation of the plant's innate immune system and many downstream defence mechanisms, such as production of pathogenesis-related (PR) proteins or synthesis of secondary metabolites that are directly harmful to pests and pathogens.

The plant innate immune system has commonly been visualised in a 'zigzag' model as proposed by Jones and Dangl in 2006 (Jones and Dangl, 2006). In this model, innate immunity is composed of two distinct branches: pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) (Jones and Dangl, 2006). PTI is the first layer of the plant immune system in the zig-zag model and is initiated by pattern-recognition receptor (PRR) proteins (Jones and Dangl, 2006). PRRs are transmembrane proteins that recognise pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) in the apoplast via an extracellular ligand-binding domain (Couto and Zipfel, 2016). PAMPS are conserved molecular structures that are present in a broad range of pathogens, such as bacterial flagellin, elongation factor Tu, peptidoglycan, or fungal chitin (Couto and Zipfel, 2016). DAMPs are host-derived molecules, such as systemin or ATP, that are released into the extracellular space upon pathogen attack or cell damage (Boutrot and Zipfel, 2017).

Upon recognition of PAMPs or DAMPs by PRRs, the PRR intracellular domain then recruits co-receptors, such as CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1) in Arabidopsis, to form an activated heteromeric receptor complex (Couto and Zipfel, 2016, Yuan et al., 2021b). This receptor complex can then phosphorylate receptor-like cytoplasmic kinases (RLCKs) to activate a wide range of downstream processes, such as production of reactive oxygen species (ROS), influx of Ca^{2+} ions into the cell,

or initiation of mitogen-activated protein kinase (MAPK) cascades to ultimately upregulate expression of PAMP-induced genes (Couto and Zipfel, 2016, Yuan et al., 2021b). These processes lead to many eventual defensive outcomes, such as the production of antimicrobial compounds and PR proteins, activation of downstream plant defence hormone pathways, or the reinforcement of plant cell walls and closure of stomata to directly limit pathogen entry and spread within the plant (Couto and Zipfel, 2016, Yuan et al., 2021b).

PTI is a quantitative form of resistance that is active against a wide range of nonspecialised microbes. However, many virulent pathogens have developed virulence factors, known as effectors, to disrupt PTI and make the host susceptible to infection. This is referred to as effector-triggered susceptibility (ETS) (Jones and Dangl, 2006). A typical example of this kind of interaction is the type III secretion system of *Pseudomonas syringae* which is used to directly inject effector proteins into the intracellular space of plant cells and interfere with typical cell activity (Xin et al., 2018). In response, plants have developed a polymorphic, rapidly evolving family of intracellular nucleotide-binding leucine-rich/repeat (NLR) receptor proteins (Jones and Dangl, 2006, Yu et al., 2024). NLR proteins can either recognise effector molecules directly, or indirectly through modification of a host protein that is guarded by the NLR protein (Cui et al., 2015). When an effector molecule is identified by an NLR, it triggers the second layer of immunity within the zig-zag model: effector-triggered immunity (ETI) (Jones and Dangl, 2006, Yu et al., 2024).

Activation of ETI results in many of the same downstream results as PTI, however they are greatly accelerated and amplified in ETI as opposed to PTI (Jones and Dangl, 2006). A typical hallmark of ETI is the hypersensitive response (HR), resulting in localised cell death at the site of infection (Cui et al., 2015). Compared to PTI, the body of knowledge surrounding the early signalling processes of ETI is relatively small. Recent studies suggest that NLRs may function in networks consisting of ‘sensor’ and ‘helper’ NLRs, in which sensors are responsible for detecting the presence of effectors, and helpers mediate this perception into downstream signalling (Yu et al., 2024).

Within the zig-zag model of plant defence, PTI acts as a primary defence against a broad spectrum of nonspecialised pathogens and ETI is a secondary line of defence that has evolved in response to pathogens that attempt to interfere with PTI by inducing ETS via effector molecules. An evolutionary 'arms race' then ensues in which NLR proteins and effector molecules exert selective pressure on each other to rapidly evolve and evade each other's strategies. If a pathogen can successfully induce ETS after both PTI and ETI, the level of protection then remaining available to the plant is referred to as 'basal resistance' (Jones and Dangl, 2006).

Since its inception, many have praised the zig-zag model as being a useful tool for conceptualising the evolution of the plant innate immune system. However, much research published since the creation of the model has highlighted its limitations. Although PTI and ETI may be triggered by different types of ligands and have distinct early signalling mechanisms, they are otherwise very similar and share many downstream processes such as production of ROS, influx of Ca^{2+} ions, and activation of MAPK cascades (Yuan et al., 2021b). Indeed, some works have found components of PTI and ETI signalling interact with or are dependent on each other. For example, a pair of 2021 studies in Arabidopsis found that activation of NLRs caused upregulation of several PTI-related signalling components, and that activation of PRRs is required for a successful ETI response (Ngou et al., 2021, Yuan et al., 2021a). It is therefore of increasingly limited use to researchers to display them as distinct resistance pathways, prompting Yuan et al in 2022 to suggest a new model in which ETI potentiates and reinforces the PTI response when PTI would otherwise be suppressed by ETS or 'endogenous braking mechanisms' (Yuan et al., 2021b).

There are also many interactions between microbes and plants that cannot be cleanly integrated into the zig-zag model, such as exchange of nutrients or symbiotic relationships (Pritchard and Birch, 2014). Neither does the zig-zag model accommodate resistance against necrotrophic pathogens as HR-induced cell death, the typical result of ETI, would likely result in susceptibility rather than immunity to a pathogen that feeds on dead tissue. Indeed, resistance to necrotrophs is largely independent of ETI and reliant on PTI (Liao et al., 2022). Gene-

for-gene interactions are indeed involved in plant resistance against necrotrophic pathogens, but rather than effectors and NLRs as seen in biotrophic resistance, they are based on the neutralisation of toxins produced by host-specific necrotrophs (Liao et al., 2022).

1.2.2 Phytohormone Resistance Pathways

Upon detection of a pathogen, plants initiate a variety of immune responses, including plant hormone signalling pathways. Chief among these phytohormones are jasmonic acid (JA), salicylic acid (SA) and ethylene (ET). Each of these small molecules controls defence pathways to different kinds of biotic stresses, allowing plants to translate external signals into an effective stress response. These pathways can also interact with each other in both synergistic and antagonistic ways so plants can regulate and fine-tune their immune responses depending on the threat they are defending against.

1.2.2.1 Jasmonic Acid Defence Pathway

The jasmonates are a group of fatty acid-derived plant hormones that include JA and its bioactive derivatives, such as methyl jasmonate (MeJA) or jasmonate isoleucine (JA-Ile). Jasmonates play a central role in regulating vital plant growth and development processes, as well as facilitating defence against a variety of abiotic stresses, such as high salinity or heavy metal toxicity (Wang et al., 2021). Jasmonates also regulate plant defence against necrotrophic pathogens. Under normal and non-stressed conditions, jasmonate ZIM domain (JAZ) proteins bind to and repress various transcription factors (TFs) that control JA-responsive genes, preventing expression of these genes in the absence of JA (Campos et al., 2014). Activation of the JA defence pathway by necrotrophs leads to the biosynthesis of JA and subsequent degradation of JAZ proteins, allowing for production of a variety of defence-related secondary metabolites and proteins, as well as development of defence-related structures such as glandular trichomes (Campos et al., 2014).

The starting substrate of JA biosynthesis is α-linolenic acid (α-LeA), which is released from galactolipids in chloroplast membranes by phospholipase A1 (PLA1) upon detection of certain developmental cues, or environmental stimuli, such as

PAMPs or DAMPs (Macioszek et al., 2023, Wang et al., 2021). Within the chloroplast, α-LeA is transformed into 12-oxo-phytodienoic acid (ODPA) through a series of reactions by 13-lipoxygenase (LOX), allene oxide synthase (AOS), and then allene oxide cyclase (AOC) (Wang et al., 2021). OPDA is then transported into the peroxisome from chloroplasts (Wang et al., 2021). Although the mechanism behind this transport is not yet fully understood, the transporter proteins responsible for moving OPDA out of the chloroplast and into the peroxisome, respectively named JASSY and CTS, have been identified (Wang et al., 2021). OPDA is then converted into 3-oxo-2-cyclopentane-1-octanoic acid (OPC-8:0) by OPDA reductase 3 (OPR3) (Wang et al., 2021). OPC-8:0 is then subsequently transformed into JA by a series of three β-oxidation reactions by acyl-CoA oxidase (ACX), multifunctional protein (MEP), and L-3-ketoacyl CoA thiolase (KAT) (Wang et al., 2021). JA is then transported into the cytoplasm where it can undergo a wide variety of potential metabolic conversions, such as methylation by a jasmonate methyltransferase (JMT), or amino acid conjugation by a jasmonate amino acid synthetase (Macioszek et al., 2023, Wang et al., 2021). JA is converted into JA-Ile, which is currently considered the most biologically active jasmonate, by JASMONATE RESISTANT 1 (JAR1) in the cytosol (Macioszek et al., 2023).

The direct receptor of JA-Ile is the F-box protein COI1 (Ruan et al., 2019). Along with the SKP1 and Cullin proteins, COI1 is part of the SCF-type E3 ubiquitin ligase complex (SCF^{COI1}) (Ruan et al., 2019). Binding of JA-Ile to COI1 facilitates the interaction between the COI1 and JAZ proteins, leading to ubiquitination of JAZ, and subsequent transport to the 26S proteasome for degradation (Ruan et al., 2019). Degradation of JAZ proteins releases a range of TFs from the MYC, MYB, and WRKY protein families, causing direct or indirect upregulation of a variety of JA-responsive genes (Ruan et al., 2019). Examples of JA-responsive genes include defence genes such as plant defensin 1.2 (*PDF1.2*), or JA biosynthesis genes such as *LOX2* (Macioszek et al., 2023).

1.2.2.2 Salicylic Acid Defence Pathway

Like JA, SA is a plant hormone that is vital for several plant processes in plant growth and development, as well as defence, but is only present at low basal levels (Peng

et al., 2021). Unlike JA, SA controls plant defence responses to biotrophic and hemibiotrophic pathogens, as opposed to necrotrophs. Pathogen infection triggers a transcriptional cascade, resulting in upregulation of various SA biosynthesis genes and a subsequent increase in SA concentration (Peng et al., 2021). SA can then bind with various receptors to upregulate SA-responsive genes (Peng et al., 2021).

SA can be produced in plants via two different pathways: the isochorismate (IC) pathway or the phenylalanine ammonia-lysase (PAL) pathway (Ding and Ding, 2020). Chorismate is the starting primary metabolite for both pathways (Ding and Ding, 2020). Via the IC pathway, chorismate is converted to IC by ICS1/2 within the chloroplast before being transported to the cytosol by the EDS5 transporter (Peng et al., 2021). Within the cytoplasm, IC is conjugated to glutamate by PBS3 to form isochorismoyl-9-glutmate (IC-9-Glu) (Peng et al., 2021). IC-9-Glu is then broken down, either spontaneously or with the assistance of EPS1, to form SA (Peng et al., 2021). In the PAL pathway, chorismate is first converted to phenylalanine through a series of reactions in the plastid (Ding and Ding, 2020). Phenylalanine is then transported into the cytoplasm and converted to trans-cinnamic acid (t-CA) by PAL (Peng et al., 2021). t-CA is then oxidised to form benzoic acid by ABNORMAL INFLORESCENCE MERISTEM 1 (AIM1), which is then hydroxylated to form SA by a hypothetical BA-2-hydroxylase (BA2H) for which the gene has not yet been identified (Ding and Ding, 2020, Peng et al., 2021). The IC pathway appears to contribute more to pathogen-induced SA biosynthesis than the PAL pathway (Ding and Ding, 2020, Peng et al., 2021).

Detection of pathogens by PRRs leads to a number of early immune signals, including increase in cytosolic Ca^{2+} concentrations, activation of the RLCKs PCRK1 and PCRK2, and activation of the TGA TFs (Couto and Zipfel, 2016, Peng et al., 2021, Yuan et al., 2021b). These signals cause upregulation of *SARD1* and *CBP60g*, two TFs that then upregulate expression of three genes involved in SA biosynthesis: *ICS1*, *PBS3*, and *EDS5* (Peng et al., 2021). Increase in expression of these genes subsequently causes an increase in SA levels. The major receptor of SA is NONEXPRESSOR OF PR GENES 1 (NPR1) (Klessig et al., 2018). Under non-stressed conditions and low levels of cytosolic SA, NPR1 proteins form oligomers that are

joined together by disulfide bonds (Klessig et al., 2018). When SA levels increase, NPR1 binds SA and then dissociates into monomers that can be transported into the nucleus (Klessig et al., 2018). NPR1 monomers then directly associate with TGA TFs to upregulate various SA-responsive defence-associated genes, such as *PATHOGENESIS RELATED PROTEIN-1* (*PR-1*) (Klessig et al., 2018). SA also binds to and inhibits the actions of the NPR3 and NPR4 receptors in the nucleus (Hou and Tsuda, 2022). NPR3 and NPR4 serve as negative regulators of SA-responsive gene expression in the absence of SA (Hou and Tsuda, 2022).

1.2.2.3 Ethylene Defence Pathway

The effect of ET on plants has been the subject of research for over a century. Interest in ET first began in the 1800s when researchers noticed that leaks of illuminating gas, which contained ET as a byproduct, caused leaf yellowing and flower wilting in nearby plants (Bakshi, 2015). These observations eventually led to ET becoming the first discovered biologically active gaseous signalling molecule, and the subsequent realisation that it could indeed be biosynthesised by plants (Bakshi, 2015). Early interest in ET was primarily due to its role in stimulating senescence, growth, and fruit ripening, but later work discovered that ET can also regulate defence responses to abiotic and biotic stresses (Bakshi, 2015). Along with JA, the ET signalling pathway contributes to defence against necrotrophic pathogens.

The starting metabolite of ET biosynthesis is methionine, which is first converted into S-adenosyl methionine (SAM) by a SAM synthetase (Bakshi, 2015, Li N, 2019). SAM is then converted into 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS) (Bakshi, 2015, Li N, 2019). The action of ACS also produces a byproduct called 5'-methylthioadenosine (MTA), which is recycled back into methionine via a series of reactions known as the 'Yang Cycle' (Bakshi, 2015, Li N, 2019). ACC is finally converted into ethylene by the action of an ACC oxidase (ACO) (Bakshi, 2015, Li N, 2019). Regulation of ACS turnover and degradation is a key method of controlling ET biosynthesis (Bakshi, 2015, Broekgaarden et al., 2015). When a pathogen is detected, stress-activated MAPKs phosphorylate ACS enzymes

to stabilise them and prevent rapid degradation by the 26s proteasome, allowing ET to accumulate (Bakshi, 2015, Broekgaarden et al., 2015).

When accumulated, ET binds to ETHYLENE RESISTANT 1 (ETR1), is which is directly associated with the CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1) kinase in the endoplasmic reticulum (Broekgaarden et al., 2015). Under normal conditions, CTR1 phosphorylates the C-terminal domain of ETHYLENE INSENSITIVE 2 (EIN2) and blocks the ET signalling pathway (Broekgaarden et al., 2015). Binding of ET to ETR1 deactivates CTR1, causing the dephosphorylated C-terminus of EIN2 to be cleaved and transported into the nucleus (Broekgaarden et al., 2015). Within the nucleus, the EIN2 C-terminus represses the action of the EIN3-binding F-box 1 and 2 (EBF1/2) proteins (Broekgaarden et al., 2015). EBF1/2 repress the action of EIN3, a TF that directly activates expression of other TFs, such as *ETHYLENE RESPONSE FACTOR 1* (*ERF1*), which themselves activate the expression of ET-responsive genes (Bakshi, 2015, Broekgaarden et al., 2015). When the C-terminus of EIN2 lifts the repression of EIN3 via EBF1/2, expression of ET-responsive genes is activated (Broekgaarden et al., 2015).

1.2.2.4 Crosstalk Between Pathways

In natural environments, plants are often threatened simultaneously by a variety of dangers, including both abiotic and biotic stresses. Defence signalling pathways, initiated by phytohormones, allow plants to effectively respond to any perceived stresses. These pathways can interact with each other, in both synergistic and antagonistic ways, to regulate plant responses. Crosstalk in this manner allows plants to produce a response that is appropriate to the combination and intensity of threats they are facing. As activating defence responses is energetically costly, hormonal crosstalk also allows plants to minimise resource allocation by ensuring only relevant pathways are activated. Knowledge of communication between defence pathways is somewhat limited as dissecting crosstalk between hormones is often extremely difficult due to the complexity of their pathways and the interactions between them.

Observed interactions between the SA and JA pathways are typically antagonistic. JA can inhibit the biosynthesis of SA by activating expression of three NAC TF genes (*ANAC019*, *ANAC055*, and *ANAC072*) via the MYC2 TF, which then directly inhibit the expression of *ICS1* (Hou and Tsuda, 2022, Peng et al., 2021). JA signalling can also stimulate expression of genes involved in the metabolism of SA, such as SA methyltransferase *BSMT1*, to prevent SA accumulation (Hou and Tsuda, 2022, Peng et al., 2021). Some *P. syringae* strains have evolved to manipulate this antagonism by producing the phytotoxin coronatine, which mimics JA-Ile, to suppress the SA pathway through activation of the JA pathway (Peng et al., 2021).

Likewise, SA appears to be capable of suppressing both JA accumulation and expression of JA-responsive genes. SA can interfere with JA biosynthesis by binding to and inhibiting CATALASE2 (CAT2), an enzyme that interacts with and activates ACX2 and 3 (Hou and Tsuda, 2022). NPR1 and two SA-induced WRKY TFs, WRKY70 and WRKY62, have been shown to be involved in inhibition of expression of JA-responsive genes although the mechanisms behind these interactions are not yet fully understood (Hou and Tsuda, 2022, Peng et al., 2021).

The JA and ET pathways interact both synergistically and antagonistically in order to calibrate plant defence responses. Degradation of JAZ by JA leads to promotion of MYC2, and upregulation of *ERF1* as JAZ also inhibits the transcriptional activity of *EIN3* (Yang et al., 2019). ERF1 and MYC2 respectively upregulate the expression of *PDF1.2* and *VEGETATIVE STORAGE PROTEIN 2* (*VSP2*), which correspondingly control defence to necrotrophic pathogens and herbivores (Yang et al., 2019). Interestingly, EIN3 and MYC2 also bind to each other to repress each other's activity (Broekgaarden et al., 2015, Yang et al., 2019). When accumulated ET increases activity of EIN3, it upregulates ERF1 as well as repressing MYC2, pushing the JA pathway towards defence against necrotrophic pathogens via *PDF1.2* (Yang et al., 2019).

1.2.2.5 Systemic Responses

In addition to local defence responses at the point of infection, plants are also capable of activating defence responses at distal, uninfected parts of the plant. By

pre-emptively activating defences across the rest of the plant in this way, systemic responses allow plants to minimise damage and contain infection. Examples of systemic responses include systemic acquired resistance (SAR), which is mediated by the SA pathway in response to pathogens; or induced systemic resistance (ISR), which is initiated by certain species of beneficial rhizobacteria and is controlled by the JA/ET pathways (Choudhary et al., 2007, Singh et al., 2017). Several mechanisms have been explored as potential methods for the propagation of long-distance defence signals, such as electrical/ionic signals, production of volatile compounds, or the movement of hormones and other small molecules via the phloem or xylem (Hilleary and Gilroy, 2018). Indeed, it appears likely that systemic defence signalling involves multiple means of communication between distant plant tissues (Hilleary and Gilroy, 2018). However, many key questions remain behind how these signals may talk to each other or what they each contribute to the overall response.

1.2.2.6 Resistance vs Growth – Defence as a Downside

After the discovery of systemic defence responses, there was natural commercial interest in the exploitation of inducible plant defences as an alternative to pesticides. Exogenous application of various defence 'elicitor' compounds, such as SA and JA and their respective analogues, were found to be capable of directly inducing defence responses across whole plants, thereby increasing resistance to pest and pathogen infections without first requiring an exposure to them (Yassin et al., 2021). The initial deployment of these elicitors in agriculture quickly fell out of favour however, as direct induction of hormone-regulated plant defences was found to have negative effects on plant fitness, and therefore on crop yield (Yassin et al., 2021).

Activation of SA or JA inducible defences has been demonstrated to have a variety of costs to plant fitness (Vos, 2013). For example, treatment with SA causes reduced seed production in Arabidopsis (Cipollini, 2002a). Treatment with benzo(1,2,3)thiadiazole-7-carbothioic acid *S*-methyl ester (BTH), an analogue of SA, also causes reduction of both biomass and seed production in wheat (Heil, 2000). Likewise, application of MeJA leads to a decrease in seed production in *Nicotiana*

attenuata (Baldwin, 1998). JA treatment also causes reduced seed production in tomato, as well as delayed fruit-set and longer ripening times (Redman et al., 2001). These effects are the product of trade-offs between growth and defence and are often attributed to allocation costs or indirect ecological costs. Allocation costs are hypothesised to occur when plants redirect resources into mounting a defence when they would otherwise be used for growth and reproduction (Vos, 2013). As plants have limited natural resources, induced resistance would therefore lead to reduced plant fitness.

Ecological costs arise when induced defence responses interfere with how plants interact with biotic and abiotic factors in their environment. For example, activation of SA defences can restrict the ability of beneficial soil bacteria to colonise plant roots, or induction of the JA defence pathway can reduce the number of visits to a plant by beneficial pollinators (Vos, 2013). Induction of defences via one pathway can also lead to increased vulnerability to pathogens that would be defended against via another pathway due to hormonal crosstalk (discussed in **section 1.2.2.4**).

Another emerging theory is that growth-defence tradeoffs might not occur entirely due to limits on available resources but may in fact be a deliberate strategy on the plants' behalf. In this scenario, slowing down growth would prevent plants from providing new and unprotected tissues as food for pests and pathogens, whilst simultaneously activating systemic defences so future tissues are defended against further attack (He et al., 2022). Indeed, crosstalk can occur at multiple stages of signalling between defence pathways, such as SA and JA, and growth pathways, such as auxin or gibberellin (He et al., 2022, Huot et al., 2014). For example, treatment with BTH downregulates 21 genes involved in various stages of auxin signalling (Huot et al., 2014). JA is also known to affect normal distribution of auxin and downregulate gibberellin biosynthesis (Huot et al., 2014). Deeper understanding of the mechanisms behind growth-defence tradeoffs will allow the development of future plant breeds with further optimised balance between growth and defence.

1.3 Priming

1.3.1 An Introduction to Priming

When plants encounter a stressor such as a pest or pathogen, the response to this encounter can form a 'stress memory' which allows the plant to mount a more rapid and robust response when it encounters a threat of a similar nature in the future (Martinez-Medina et al., 2016). This phenomenon is referred to as 'priming'. Unlike the direct induction of plant defences, priming is a form of induced resistance with relatively low costs to fitness as the plant is not continuously expending energy to maintain its defences but is merely poised for a stronger defence activation when necessary (Martinez-Medina et al., 2016). Allocation costs may still be caused by the establishment and maintenance of the primed state and ecological costs can still occur due to negative hormonal crosstalk (Martinez-Medina et al., 2016). However, primed plants are still expected to have a fitness advantage over unprimed or 'naïve' plants in environments with high disease pressure (Martinez-Medina et al., 2016).

A wide variety of environmental and chemical stimuli have been recorded as capable of inducing priming. For example, inoculation of pepper plants with lipopolysaccharide, a ubiquitous component of cell walls in gram-negative bacteria, results in reduced bacterial growth when challenged with two incompatible strains of *Xanthomonas axonopodis* as well compared to pepper plants that are pretreated with water (Newman et al., 2002). Lipopolysaccharide-treated plants also experience faster induction of *P6* mRNA, a *PR-1* homologue, as well as more rapid accumulation of two antibiotic compounds, coumaroyl-tyramine and feruloyl-tyramine, when they are challenged with the compatible *Xanthomonas campestris* (Newman et al., 2002). Similarly, pretreatment of Arabidopsis plants with oxo-C14-HSL, a bacterial quorum sensing molecule, results in a number of amplified defences when plants are challenged with *P. syringae*, such as enhanced callose deposition and accelerated stomatal closure, as well as enhanced accumulation of SA and ODPA (Schenk et al., 2014).

In addition to bacterial pathogens, plants also appear to be able to prime defences in response to insect attack. In a 2015 study, *N. attenuata* plants were primed by exposure to oviposition by *Spodoptera exigua* moths (Bandoly et al., 2015). When later challenged by *S. exigua* larval feeding, primed plants experienced less feeding damage, slower larval growth, and increased larval mortality (Bandoly et al., 2015). Oviposition-experienced plants also had greater accumulation of caffeoylputrescine and enhanced trypsin protease inhibitor activity, two defensive traits that are controlled by the JA pathway (Bandoly et al., 2015). Interestingly, plants can also produce priming signals to warn their neighbours of insect threats. Indole is a volatile organic compound produced and released by maize plants in response to herbivory (Erb et al., 2015). Erb et al observed that exposure of maize plants to indole caused enhanced induction of defensive green leaf volatiles when plants were elicited with a combination of wounding and *Spodoptera littoralis* regurgitant (Erb et al., 2015). Indole-treated maize plants also experienced enhanced accumulation of JA and JA-Ile, as well as several terpenoid volatile compounds (Erb et al., 2015).

Mutualistic interactions between plants and nonpathogenic soil microorganisms can also induce priming responses in plants. For example, a 2007 study found that treatment of Arabidopsis plants with the *Pseudomonas putida* LSW17S rhizobacterium resulted in enhanced defence against *P. syringae* (Ahn et al., 2007). This form of defence was associated with an increased accumulation of callose and hydrogen peroxide, as well as increased transcription of both *PR-1* and *PDF1.2* genes upon challenge with *P. syringae* (Ahn et al., 2007). A separate study observed that colonisation of Arabidopsis roots with *Trichoderma asperelloides* T203, a beneficial fungus, also caused enhanced resistance against *P. syringae* (Brotman et al., 2012).

A wide variety of chemical treatments have proved capable of eliciting a priming response in plants. These elicitors include SA and JA and their respective analogues, BTH and MeJA, but also a number of other small organic molecules such as β-aminobutryic acid (BABA) or hexanoic acid (HA) (Finiti et al., 2014, Kohler et al., 2002, Thulke and Conrath, 1998, Wang K, Worrall et al., 2012). Notably, it is possible

for plants to establish a primed state without first mounting an associated direct defence response, creating significant commercial interest in priming agents as a means of creating a state of induced resistance in crops without severe fitness costs (De Kesel et al., 2021).

1.3.2 Possible Mechanisms Behind Priming

Priming has been found to potentiate a number of plant defence-related responses, such as gene expression, accumulation of defence hormones and metabolites, induction of volatiles, or physical changes such as callose deposition and stomatal closure (Conrath et al., 2015, Martinez-Medina et al., 2016). Several possible explanations have been found for the molecular mechanisms behind how this primed state is established. These mechanisms are not necessarily mutually exclusive and plants are likely to utilise a combination of them to prime their defences.

1.3.2.1 Accumulation of Defence-Related Proteins

An early hypothesis was that primed plants may accumulate inactive forms of defence-related signalling proteins which, upon activation triggered by a stressor, would greatly enhance the plant's defence response (Prime, 2006). A 2009 study later decided to test this theory by investigating accumulation of the MPK3 and MPK6 MAPK proteins in primed Arabidopsis plants. MAPK proteins are involved in transmitting and amplifying signals in a number of plant processes, including both PTI and ETI responses (discussed in **section 1.2.1**). The authors indeed observed that treatment of Arabidopsis plants with BTH resulted in accumulation of the MPK3 and MPK6 proteins and their respective mRNA transcripts (Beckers et al., 2009). These accumulated proteins were inactive however as they were not phosphorylated on a TEY amino acid motif which is critical for kinase activity (Beckers et al., 2009). MPK3 and MPK6 proteins were more strongly activated via TEY phosphorylation in BTH-treated Arabidopsis when challenged with *P. syringae* pv. *maculicola* (Beckers et al., 2009).

Another study observed that inoculation of cucumber roots with the beneficial fungus *Trichoderma asperellum* induced accumulation of *Trichoderma-induced*

MAPK (*TIPK*), an orthologue of *MPK3* (Shoresh et al., 2006). Cucumber plants overexpressing *TIPK* were more resistant to *P. syringae* pv. *Lachrymans* (Shoresh et al., 2006). Similarly, overexpression of *MK1*, the pepper *MPK3* orthologue, in rice resulted in increased resistance to rice blast disease (Lee et al., 2004).

A 2014 report observed that treatment of Arabidopsis plants with BTH resulted in increased levels of the PRR FLAGELLIN SENSING2 (FLS2) and its signalling partner BRI1-ASSOCIATED RECEPTOR KINASE1 (BAK1) (Tateda et al., 2014). PRRs are transmembrane proteins that recognise molecular markers associated with pests and pathogens in order to initiate a defence response (discussed in **section 1.2.1**). This increase in accumulation of FLS2 and BAK1 was associated with increased ROS production and callose deposition when plants were treated with the flagellin peptide flg22 (Tateda et al., 2014). The authors also observed that BTH treatment increased levels of CHITIN ELICITOR RECEPTOR KINASE (CERK1), a PRR that recognises chitin and peptidoglycan (Tateda et al., 2014). Taken together, these reports provide some evidence that the accumulation of defence signalling proteins, such as MAPKs or PRRs, may contribute to the establishment of priming.

1.3.2.2 Changes to Plant Metabolism

Another potential mechanism for priming is the storage of inactive forms of signalling metabolites which could quickly be activated and released upon encounter with a stress. Accumulation of the glucosylated forms of SA, SA O-β-D-glucoside (SAG) and glucose ester of SA (SGE), has been observed in both tobacco and Arabidopsis after infection with *P. syringae* (Lee and Raskin, 1998, Song, 2006). Pastor et al also demonstrated in 2014 that priming Arabidopsis plants with BABA or avirulent *P. syringae* resulted in higher levels of SAG and SGE (Pastor et al., 2014). It has therefore been hypothesised that plants convert pathogen-induced SA into SAG/SGE via the action of an SA glucosyltransferase so that it can be stored and later rapidly converted back to SA by a β-glucosidase when needed (Pastor, 2013). Indeed, Boachon et al would observe in 2014 that *ugt74f1*, an Arabidopsis mutant impaired in the production of SAG, was more susceptible to *P. syringae* infection than a wild type counterpart (Boachon, 2014).

In addition to the production and storage of phytohormone conjugates, priming can also stimulate changes in the levels of various primary metabolites in plants, such as amino acids, sugars, or intermediate molecules in the tricarboxylic acid cycle. Treatment of Arabidopsis plants with either BABA or avirulent *P. syringae* has been demonstrated to produce distinct profiles of primary metabolites (Pastor et al., 2014). For example, BABA treatment causes an increase in levels of certain carboxylic acids, such as citrate, malate, and fumarate, whilst priming with avirulent *P. syringae* repressed the accumulation of these same compounds (Pastor et al., 2014). Colonisation of Arabidopsis roots by *Trichoderma asperelloides* T203 also results in a significantly different metabolic profile from that of untreated plants (Brotman et al., 2012). By affecting primary metabolism, priming may condition plants to more effectively channel their limited resources into defence.

1.3.2.3 Epigenetic Mechanisms

The term 'epigenetics' refers to mechanisms that can alter the regulation of the genome without causing direct changes to the DNA sequence. Epigenetic changes, such as DNA methylation or chromatin modification, can also survive cell division and can even be heritable from parent to offspring.

Genomic DNA exists in the nucleus in the form of chromatin, which consists of repeating units called nucleosomes (Luger et al., 1997). The core of each nucleosome is composed of 145-147 base pairs of DNA wrapped around an octamer made up of two copies of each histone protein, H2A, H2B, H3, and H4 (Luger et al., 1997). Chromatin can be of various densities according to how tightly or loosely the DNA is wrapped around the histone octamer (Wilkinson et al., 2019). Densely packed chromatin (heterochromatin) is associated with reduced areas of gene expression as the cell's transcription machinery is obstructed from reaching the DNA (Wilkinson et al., 2019). Conversely, loosely packed chromatin (euchromatin) is associated with increased transcription (Wilkinson et al., 2019). The density of chromatin, and therefore the transcriptional activity of that area of the genome, can be adjusted by the methylation or acetylation of histone proteins (Wilkinson et al., 2019).

Various priming elicitors have been identified as capable of causing histone modifications on defence-related genes. Jaskiewicz et al first identified in 2011 that BTH treatment caused trimethylation on lysine 4 of histone 3 (H3K4me3), a histone modification associated with increased gene activity, on the genes of three defence-related transcription factors: WRKY29, WRKY6, and WRKY53 (Jaskiewicz et al., 2011). Indeed, expression of all three of these genes was augmented in BTH-treated plants compared to untreated plants when challenged with *P. syringae maculicola* (Jaskiewicz et al., 2011). Similarly, a 2018 study observed that treatment of Arabidopsis with another elicitor, sulforaphane, resulted in deposition of H3K4me3 and acetylation of lysine 9 of histone 3 (H3K9ac) on the *WRKY6* and *PDF1.2* defence genes (Schillheim et al., 2018). The authors also confirmed that these covalent modifications coincided with chromatin unpacking in the *WRKY6* and *PDF1.2* regions (Schillheim et al., 2018). Another 2018 report also found that BABA treatment of potato resulted in H3K4me2 and H3K27me3 deposition on the *NPR1* and *SUPPRESSOR OF NPR1, INDUCIBLE* (*SNI1*) genes (Meller et al., 2018). Finally, Lopez et al observed in 2011 that *NRPD2*, a constitutively primed Arabidopsis mutant, possesses H3K4me3 and H3K9ac deposits on the *PR-1* gene (Lopez). Expression of *PR-1* was potentiated in *NRPD2* mutants compared to wild type plants when challenged with *Plectosphaerella cucumerina* (Lopez et al., 2011).

In addition to histone modifications, DNA methylation is another form of epigenetic modification that has been proposed to facilitate the establishment of priming. Unmethylated DNA is typically associated with euchromatin, and methylated DNA is typically associated with heterochromatin (Wilkinson et al., 2019). In mammals, DNA methylation primarily occurs in the CG sequence context (Niederhuth and Schmitz, 2017). In plants however, DNA methylation can occur in three different sequence contexts: CG, CHG, and CHH, where H is any base except guanine (Wilkinson et al., 2019, Yang et al., 2022, Niederhuth and Schmitz, 2017). Methylation in each of these sequence contexts in plants is established and maintained through different mechanisms (Niederhuth and Schmitz, 2017). The epigenetic marker of 5-methylcytosine (5-mC) can also be removed by the actions

of DNA glycosylases such as REPRESSOR OF SILENCING 1 (ROS1) or DEMETER (DME) (Yang et al., 2022).

The CG and CHG sequence contexts are both symmetrical and this symmetry is vital to the mechanism that underlies the maintenance of CG methylation following DNA replication (Niederhuth and Schmitz, 2017). Due to the semi-conservative nature of DNA replication, CG sites in newly replicated DNA are hemi-methylated as only the original DNA strand is methylated. These hemi-methylated CG sites are recognised by VARIANT IN METHYLATION 1 (VIM1-5) which then recruits METHYLTRANSFERASE 1 (MET1) to methylate the CG site on the new and unmethylated strand (Niederhuth and Schmitz, 2017, Wilkinson et al., 2019).

Methylation at CHG contexts is maintained by the CHROMOMETHYLASE3 (CMT3) enzyme (Niederhuth and Schmitz, 2017, Wilkinson et al., 2019). CMT3 is a methyltransferase which recognises and binds to the H3K9me2 histone modification and, upon doing so, methylates nearby CHG sites (Niederhuth and Schmitz, 2017, Wilkinson et al., 2019). Methylation of the H3K9 residue is dependent on the histone methyltransferases KRYPTONITE (KYP), Su(var)3-9 homologue 5 (SUVH5) and SUVH6 (Niederhuth and Schmitz, 2017). Each of these methyltransferases recognises and binds to methylated DNA in order to dimethylate H3K9 residues (Niederhuth and Schmitz, 2017). As each of these modifications leads to the establishment of the other, methylated CHG and H3K9me2 can self-replicate and maintain their localisation in the genome (Niederhuth and Schmitz, 2017).

Unlike the CG and CHG contexts, CHH is asymmetrical. Cytosine methylation is therefore not maintained at CHH sites following DNA replication as there is no cytosine on the opposite strand to direct DNA methylation (Niederhuth and Schmitz, 2017). CHH methylation is therefore established *de novo* following each round of replication via two potential mechanisms (Niederhuth and Schmitz, 2017). The first mechanism involves CMT2, another chromomethylase which, like CMT3, binds to H3K9me2 residues on histones to methylate DNA (Niederhuth and Schmitz, 2017). CMT2 differs from CMT3 however in that it prefers methylating CHH sequences over CHG (Niederhuth and Schmitz, 2017). Methylation at CHH

sequences can also be performed by RNA-directed DNA methylation (RdDM) (Wilkinson et al., 2019). RdDM is a process that relies on 24-nucleotide short interfering RNAs (siRNAs) guiding the DOMAINS REARRANGED 2 (DRM2) methyltransferase to perform *de novo* DNA methylation at their target sites (Niederhuth and Schmitz, 2017, Wilkinson et al., 2019). RdDM is not exclusive to CHH methylation and can direct *de novo* DNA methylation in all three sequence contexts (Niederhuth and Schmitz, 2017).

It was Lopez et al in 2011 who first discovered evidence connecting DNA methylation and defence priming. They observed that several constitutively primed Arabidopsis mutants that were deficient in RdDM possessed enhanced resistance to *P. syringae* as well as potentiated induction of *PR-1* upon challenge with *P. syringae* (Lopez et al., 2011). The findings of this report were supported by further studies such as a 2013 paper which observed that elicitation of Arabidopsis defences with flg22 triggered demethylation and transcriptional activation of several transposable elements (Yu et al., 2013). Likewise, a 2016 report found that two different hypo-methylated and hyper-methylated Arabidopsis mutants possessed enhanced resistance and susceptibility, respectively, to the biotrophic *Hyaloperonospora arabidopsidis* (Sánchez et al., 2016).

Connections between DNA methylation and defence priming have also been observed in tomato, wherein the CHH context appears to be of particular importance (Catoni et al., 2022, Stevens et al., 2025). Catoni et al in 2022 observed that treatment of Money Maker tomato with BABA resulted in a long-lasting resistance to *B. cinerea* that was associated with global demethylation (Catoni et al., 2022). This was especially prevalent in CHH differentially methylated regions (DMRs) which were almost exclusively hypomethylated following BABA treatment (Catoni et al., 2022). The majority of BABA-primed genes did not contain a CHH DMR however, suggesting that methylation may regulate primed genes *in-trans* from distant genomic regions (Catoni et al., 2022). Stevens et al in 2025 also noticed that BABA treatment of Micro Tom tomato plants at two weeks old, but not 12 weeks, granted long lasting resistance against *B. cinerea* (Stevens et al., 2025). They did not observe BABA treatment of two-week-old seedlings to induce any changes in global

methylation, unlike Catoni et al's work, perhaps due to differences in perception of BABA between tomato cultivars (Catoni et al., 2022, Stevens et al., 2025). They did however observe that levels of CHH methylation were much lower in the leaf tissue of two-week-old Micro Tom plants than those of 12-week-old plants, leading Stevens et al to speculate that CHH hypomethylation may facilitate a greater plasticity which allows plants to develop long term resistance following BABA treatment (Stevens et al., 2025).

1.3.3 How Long Does Priming Last?

Priming responses described in the literature have been measured over a wide variety of timescales. Many studies will analyse the effects of priming within the first few days after elicitor treatment (Baysal, 2007, Dufour, 2013, Finiti et al., 2014, Wang K, 2015). However, the effects of priming appear to be capable of being much more long lasting and primed defences can endure for a number of weeks after the initial treatment. Indeed, JA treatment of two-week-old Arabidopsis plants creates a resistance against herbivory which lasts for at least five weeks (Wilkinson et al., 2023). Likewise, BABA-induced resistance against *P. syringae* was detectible for up to 4 weeks after treatment in Arabidopsis (Luna et al., 2014). In tomato, treatment of seeds with JA resulted in primed defence when plants were challenged with red spider mites seven weeks later (Worrall et al., 2012). Additionally, BABA-induced priming against *B. cinerea* persists for nine weeks after the initial treatment of two week old tomato plants (Wilkinson, 2018).

Priming also appears to be capable of acting transgenerationally, as several reports have observed that untreated offspring of primed parents can still display enhanced resistance to biotic stressors. In Arabidopsis, priming of JA-dependent defences via MeJA treatment or *Pieris rapae* caterpillar herbivory has been demonstrated to be heritable (Rasmann et al., 2012). Likewise, the offspring of Arabidopsis plants that were treated with BABA or *P. syringae* inoculation display primed activation of SA-dependent defences (Luna et al., 2012, Slaughter et al., 2012). Transgenerational priming has been demonstrated in other plants as well, including crop species, such as potato, tomato, oilseed rape, and rice (Hatzig et al., 2018, Meller et al., 2018, Rasmann et al., 2012, Ye et al., 2017). For example, ratoon rice plants generated

from parent plants which had been exposed to *Cnaphalocrocis medinalis* caterpillars were more resistant to *C. medinalis* herbivory (Ye et al., 2017). The mechanistic basis of transgenerational resistance is thought to be epigenetic as metabolites or signalling proteins are not likely to be heritable. Indeed, Rasmann et al observed that Arabidopsis mutants which were deficient in the production of small interfering RNAs failed to establish transgenerational priming upon challenge with MeJA or *P. rapae* feeding (Rasmann et al., 2012). Small interfering RNAs can alter the transcription of genes by directing changes in DNA methylation (Vaucheret, 2006). Luna et al also reported that *drm1drm2cmt3*, an Arabidopsis mutant that is reduced in non-CG DNA methylation, displays a similar primed phenotype to the offspring of BABA-treated parents (Luna et al., 2012). These results are suggestive that disease resistance is inherited through changes in DNA methylation.

1.3.4 Induced Resistance in Agriculture

There has been a natural commercial interest in the exploitation of induced resistance in crops due to a number of potential advantages that elicitors may have over traditional chemical pesticides. As the defence that elicitors offer is plant based rather than chemical, the chance that off-target organisms, such as predators of pests, are affected is greatly reduced leading to less disruption of natural ecosystems. Moreover, as plant defence systems use a combination of several mechanisms simultaneously to defend against invaders, it is unlikely that a pathogen will manage to evolve resistance to all of a plant's natural defences. This is in stark contrast to traditional pesticides, which often contain a single active ingredient that places a strong selective pressure on pests to acquire resistance. Many elicitors have also been observed to be capable of inducing resistance against a broad spectrum of pathogens, such as BABA, which can stimulate systemic protection against pathogens with both biotrophic and necrotrophic lifestyles (Jeun, 2000, Van der Ent et al., 2009, Zimmerli et al., 2000). Examples of commercially applied elicitors include BTH, which is registered by Syngenta as Bion or Actigard, and probenazole (Walters et al., 2013). Probenazole, registered as Oryzemate, has even seen widespread use in Japan for several decades as a means of controlling rice blast (Walters et al., 2013).

Despite some commercial success stories such as probenazole, many barriers exist that have prevented the wider adoption of elicitors by growers. Elicitors typically cannot provide the high level of protection that is offered by traditional pesticides and instead should be used as part of a larger IPM strategy. These treatment strategies often need to be completely bespoke to each grower as the field is a complex environment with many potential abiotic and biotic factors that can interact with the elicitor agents, therefore requiring a level of expertise far above that required by a typical pesticide spraying timetable (Walters et al., 2013). The effectiveness of a given elicitor treatment can also depend on the species and even cultivar of the host plant (Walters et al., 2013). For example, Sharma et al observed in 2010 that the ability of BABA to induce protection against *Phytophthora infestans* varied depending on the accession of tomato (Sharma, 2010).

1.4 Aims of Thesis

Transgenerational priming against various pathogens has been demonstrated in several plant species, including Arabidopsis, tomato and potato (Luna et al., 2012, Meller et al., 2018, Rasmann et al., 2012, Slaughter et al., 2012). If transgenerational priming could be implemented commercially, this may alleviate some of the issues elicitors are currently facing in agriculture (**discussed in section 1.3.5**). For example, if growers could purchase seeds which had been produced by primed parents and inherited the resistance from them, then they would no longer need to perform the priming treatments themselves. The aim of this thesis is to investigate whether chemical elicitor agents can be used on parental tomato plants to produce primed seed that have characteristics beneficial to growers.

In order to achieve this aim, our experimental objectives were as follows:

Produce groups of seed from plants that have been treated with elicitor agents capable of inducing priming responses against different groups of pathogens.

Test whether offspring from the different parental treatments provide resistance to *B. cinerea* (a necrotrophic fungus) or *P. syringae* (a hemibiotrophic bacteria).

Investigate mechanisms behind observed resistance phenotypes by measuring defence-related gene expression in offspring plants.

Assess the effects of parental priming treatments on the growth and development of the offspring.

2 - Methodology

2.1 Growth of Plants

2.1.1 Preparation of Parental Plants

Tomato (*Solanum lycopersicum*) seeds of the Micro Tom commercial variety were planted in Levington M3 compost (Evergreen Garden Care, United Kingdom). Seeds were sown directly in compost in small pots and then later transplanted to larger pots around five weeks after sowing. We used Micro Tom for our experiments as it takes a short time to produce fruit in comparison to larger commercial cultivars. The small size of Micro Tom also suited our needs as we grew all of our offspring plants on shelves in a controlled environment room and were therefore very limited for space. Plants were grown in the glasshouse and given their initial respective treatments at two weeks after sowing. For each treatment, including the control treatment, 12 parental plants were grown. When fruiting, plants were fed once a week with Tomorite (Evergreen Garden Care, United Kingdom). Fruits were stored in the dark at four °C until their seeds were harvested (**section 2.4.4**).

2.1.2 Preparation of Offspring Plants

Micro Tom seeds from the parental plants were planted in Levington M3 compost and given their initial respective treatments at two weeks after sowing. Plants were grown in controlled environment rooms on lighting racks (Weiss Technik, Germany) with the following conditions: 14 h days at 24 °C, ten h nights at 18 °C, a light intensity of approximately 150 µmol m^{-2} s^{-1}, and ambient levels of relative humidity and CO_2. Plants were bottom-watered three times per week to maintain consistent and adequate soil moisture. Fruiting plants were additionally fed once a week with Tomorite.

2.1.3 Plant Priming Treatments

All plants were treated at two weeks when they were still seedlings in small pots. Root drench treatments were performed with a concentration ten times higher than the final desired concentration by addition of one tenth volume of the estimated soil

volume. For spray treatments, plant leaves were sprayed until the leaves were saturated and the treatment solution started to run off the surface of the leaf.

The control treatment used was a root drench with RO water. A spray with RO water was not included in the control treatment. Final treatments used were as follows: 0.5 mM root drench for HA (Scalschi et al., 2013, Vicedo et al., 2009); 0.05 mM root drench for MeJA (Krol et al., 2015); 0.5 mM root drench for BABA (Wilkinson, 2018); one mM spray for BTH (Ahn et al., 2011); and a 1:500 dilution spray for Fytosave (recommendation by the manufacturer). All treatment solutions were prepared by dissolving the elicitor into RO water. Concentrations shown for the root drench treatments are the final concentrations in soil. Following initial treatment, Fytosave-treated plants were given an additional spray with Fytosave solution once every seven days as per the manufacturer's recommendations. Fytosave was manufactured by FytoFend and supplied by Gowan UK.

2.1.4 Priming Treatments for qPCR Experiments

3-week-old Micro Tom seedlings from various parental priming backgrounds were given either a control treatment of 0.7% ethanol solution or a treatment of 0.7% ethanol solution containing 2.5 mM 2,6-dichloroisonicotinic acid (INA).

2.2 *P. syringae* Resistance Assays

2.2.1 Preparation of LS-LB and Kings B Liquid Medium

For preparation of inoculum, *P. syringae* was cultured in King's B liquid medium. *P. syringae* was added to medium under a sterile hood. The King's B medium contained ten g/L proteose peptone, 1.5 g/L K_2HPO_4, 15 g/L glycerol at pH 7.0, and was autoclaved following preparation. Following autoclaving of medium, five mM $MgSO_4$, 25 µg/mL of rifampicin, and 25 µg/mL of kanamycin were added.

For the culturing of *P. syringae* on solid media, low sodium Luria Bertani (LS-LB) medium was used. *P. syringae* was added to medium under a sterile hood. The LS-LB medium contained ten g/L tryptone, five g/L yeast extract, five g/L NaCl, 2.5%

agar at pH 7.0, and was autoclaved following preparation. Following autoclaving of medium, 25 µg/mL of rifampicin and 25 µg/mL of kanamycin were added.

2.2.2 Preparation of Plant Inoculum

P. syringae pathovar tomato T1 avrPto (Pst T1 avrPto) was plated on LS-LB medium and incubated at 29 °C for two to three days. Following incubation, a loopful of Pst T1 avrPto was used to inoculate 25 ml of Kings B liquid medium in a 50 ml falcon tube. The inoculated Kings B medium was then incubated at 29 °C for approximately 24 h whilst being shaken at 200 rpm. The liquid medium containing the Pst T1 avrPto was then centrifuged at 3500 rpm for eight minutes. The supernatant was then poured off and the bacteria resuspended in 25 ml of ten mM $MgCl_2$. The cells were then centrifuged again and resuspended in ten ml of ten mM $MgCl_2$ after pouring off the supernatant. The OD_{600} of the cell suspension was then measured using a spectrophotometer that was blanked using ten mM $MgCl_2$.

The formula $a = bc/d$ was used to calculate the volume of cells to use in the inoculum: where a is the volume of cells to use in the dilution in ml; b is the final volume of solution in ml; c is the final desired OD_{600}; and d is the OD_{600} of cells after centrifugation and resuspension in ten ml of ten mM $MgCl_2$. If the dipping method of inoculation was being used, 0.05% L-77 Silwet (Momentive Performance Materials, United States) was also included in the inoculum.

2.2.3 Inoculation by Dipping

Twenty-four hours after priming, two-week-old seedlings were inoculated by dipping the whole plants in Pst T1 avrPto solution for approximately 15 seconds. Plants were then returned to the controlled environment room for 72 h before extraction and quantification of Pst T1 avrPto. This method was ineffective at inoculating Micro Tom with *P. syringae* so infiltration was later used as a means of inoculation.

2.2.4 Inoculation by Infiltration

24 hours following their respective priming treatments, two-week-old seedlings were inoculated with Pst T1 avrPto solution by infiltrating the solution through the

underside of the cotyledons using a one ml syringe. After inoculation, plants were returned to the controlled environment room for 72 h before extraction and quantification of Pst T1 avrPto. 12 plants were grown and sampled for each treatment group. 12 results may not have been counted however, if the extractions were too dense to be counted or failed to produce any CFUs at all. All results in **Figure 3.3** were generated using the infiltration method of infection.

2.2.5 Quantification of *P. syringae* Populations

Cotyledons were removed from each seedling and fresh weight recorded. They were then ground in a pestle and mortar using ten ml of ten mM $MgCl_2$ per g of fresh weight of leaf. One ml of the ground leaf material was then transferred to a microcentrifuge tube. Ten µl of the sample was then added to 90 µl of RO water and mixed well. Serial dilutions were performed in this manner until dilutions 10^{-1} through 10^{-7} were prepared. Ten µl of each dilution was then pipetted, under a sterile hood, onto one square of a grid on a Petri dish containing LS-LB medium and incubated at 29 °C for approximately 30 h.

Colony forming units (CFUs) were then counted from the highest dilution that yielded clear single colonies in the ten to 100 CFU range. The bacterial population in CFU g^{-1} of the original extract was estimated by multiplying the CFU count by the dilution factor and then multiplying by 1000. A diagram of an example plate is included in **figure 2.1**.

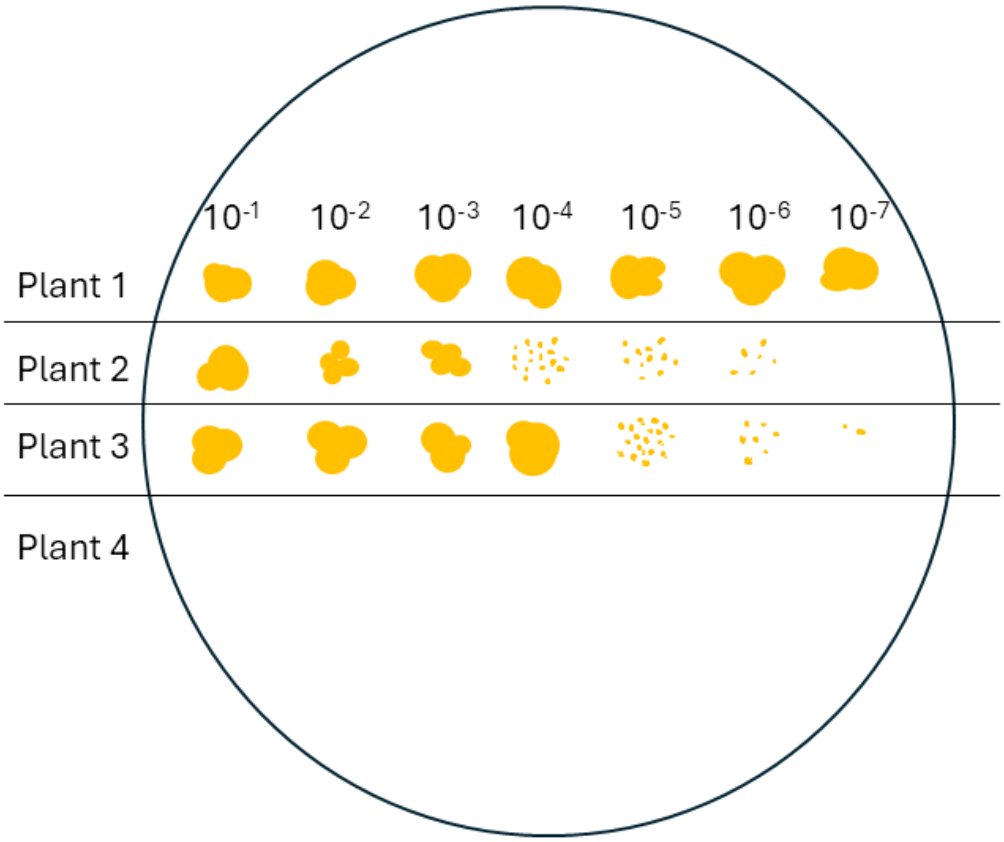

Figure 2.1. Example diagram of a Petri dish containing LS-LB media and serial extraction dilutions from four different plants infected with *P. syringae*. In this example, counts would not be taken from plant 1 as all of the dilutions are too dense to count individual CFUs. Nor would counts be taken from plant 4 as none of the dilutions produced any visible *P. syringae* growth. Counts would be taken from plants 2 and 3 at the 10^{-4} and 10^{-5} dilutions, respectively, as these are the first dilutions to produce individual CFUs.

2.3 *B. cinerea* Resistance Assays

2.3.1 Culture of *B. cinerea*

B. cinerea strain R16 (Faretra and Pollastro, 1991) was grown on potato dextrose agar (PDA) (Sigma-Aldrich, United Kingdom) medium and incubated in a plant growth chamber at a constant 22 °C with a ten h light cycle, ambient levels of relative humidity and CO_2, and Osram fluora lamps (Osram, Germany). The plates were wrapped in tin foil for the first week of incubation to allow the mycelium to grow and cover the plate in the dark. The plates were then unwrapped from the foil and left for an additional week to allow the fungus to sporulate in the light.

2.3.2 Preparation of Infection Solution

A quarter of a PDA medium plate containing sporulated *B. cinerea* was cut up and put into a 50 mL falcon tube containing ten mL of 0.01% tween 80. The tube was shaken thoroughly for a few seconds and then the contents were filtered through a 20 µm mesh held in a small funnel. The filtered solution was then centrifuged for ten minutes at 4000 rpm. After centrifugation, the spore pellet was resuspended in ten mL of RO water and the number of spores was calculated using a haemocytometer.

The final infection solution used was 0.05 M glucose, 0.03 M KH_2PO_4, and 10^5 spores per mL. Infection solution was incubated for 2.5 h at room temperature before use to promote spore germination.

2.3.3 Leaf Infection

For each treatment group, eight plants were grown and the first two true leaves were taken from each plant for infection. Incubations were performed on excised leaves incubated in plastic trays. The plastic trays were prepared by lining the bottom of the tray with approximately four layers of blue tissue paper and wetting the paper with approximately 500 mL of water. Plastic grids were then laid on top of this tissue paper. The petioles of the leaves were wrapped in moist tissue paper and the leaves were laid on top of the grids so the petioles were touching the wet tissue paper and the leaves were supported on the plastic grids. Two five µL drops of infection solution were pipetted onto each leaflet, avoiding the veins. The infection solution was frequently stirred whilst pipetting as the spores can quickly settle. The trays were wrapped in plastic bags and incubated at room temperature for at least three days. In order to facilitate *B. cinerea* spore production, trays were stored in the dark whilst incubating. Infected leaves were photographed with a ruler in the frame and lesion diameters were measured using ImageJ software (software version 1.54h) (Schneider et al., 2012). An example photograph is included in **Figure 2.2**.

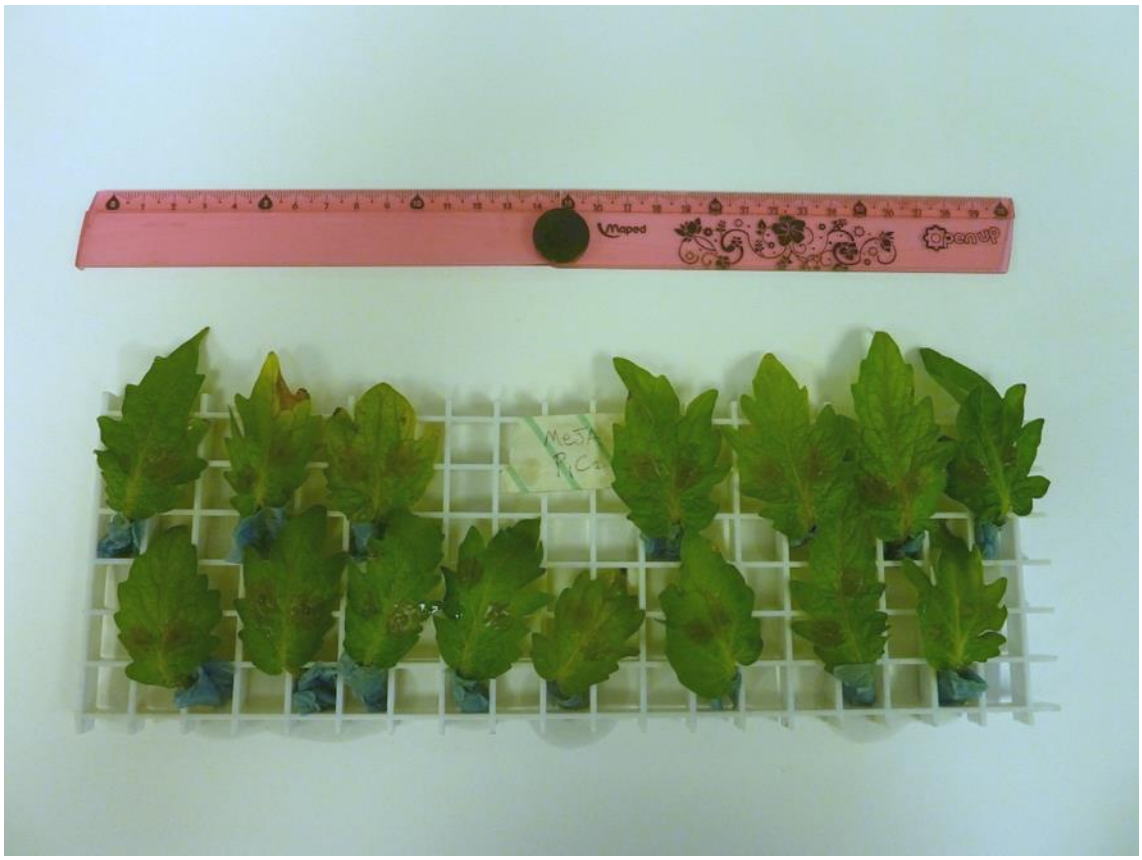

Figure 2.2. Example image of *B. cinerea* leaf infections. Pictured are the leaves from MeJA P_1C_2 plants at three days post infection with *B. cinerea*.

2.4 Growth Assays

2.4.1 Leaf Area and Dry Weight Measuring

The true first leaf was cut from each plant and its leaf area was measured using an LI-3100C Leaf Area Meter (LI-COR, United States). To measure dry weight, plants were cut at the stem just above soil level, placed in a paper bag with their excised first leaf and then dried to a constant mass in an oven at 50 °C for roughly 72 h. Dried samples were then weighed and recorded. For each of our growth assays, 12 seeds were planted and intended to be used for samples. Samples numbers were often lower than 12 however as not every seed would germinate.

2.4.2 Flowering Phenology

Flowering phenology was recorded on all plants. Plants were considered to have reached first flower when a bud had opened enough for yellow petals to be clearly visible.

2.4.3 Measuring of Fresh Fruit Weight and Seed Count

Fruits were collected from all plants when they were approximately 19 weeks old. Collected fruits were stored in paper bags and weighed immediately. Seeds were then isolated from fruits (as described in section 2.4.4), stored in paper bags and weighed. Seed count was determined by dividing the total seed weight of the plant by the hundred seed weight and then multiplying that number by 100.

2.4.4 Tomato Seed Isolation

Fruits were quartered using a scalpel and seeds were separated from flesh into a beaker. 3 N HCl was then added in equal volume to the pulp and seed. After 15 minutes, seed and acid were stirred with a glass rod and then left for another 15 minutes. The beaker was then filled with water to dilute the acid, and seeds were rinsed into a strainer under tap water. Seeds were then drained onto blotting paper and left to dry overnight before being put into paper bags and stored in the dark at four °C.

2.5 Statistical Analysis

One way ANOVA were performed using the univariate generalised linear model function in SPSS software (software version 29) (IBM, United States). This model allowed us to observe if offspring or parental treatment had an effect on any of the characteristics we were interested in. It would also allow us to detect any interactions between parental and offspring treatments. A p-value of 0.05 was used to determine if an interaction was significant or not.

2.6 qPCR Assays

2.6.1 RNA Extraction

Following treatment, three-week old leaves were excised and immediately wrapped in foil packets, flash frozen in liquid nitrogen and stored in a -80 °C freezer. Samples were ground under liquid nitrogen using a pestle and mortar. Each sample consisted of three leaves excised from three individual plants of the same treatment group. RNA extractions were performed using Spectrum Plant Total RNA kits (Sigma-Aldrich, United Kingdom) according to the manufacturer's instructions. RNA concentrations of the samples were measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific, United States) blanked against the elution solution. When not in use, RNA samples were stored at -80 °C.

2.6.2 cDNA Synthesis

cDNA synthesis reactions were performed using ProtoScript II Reverse Transcriptase kits (New England Biolabs, United Kingdom) according to the manufacturer's instructions. cDNA synthesis reactions were performed in a 20 µL total volume and contained one µg of template RNA which was typically approximately two to four µL of RNA. Following synthesis, cDNA samples were stored at -20 °C.

2.6.3 qPCR reactions

Each qPCR reaction consisted of one µL (50 ng) of cDNA template, four µL of EvaGreen qPCR master mix (qARTA Bio, USA), 0.2 µL each of forward and reverse primers (ten pmol/µl), and 14.6 µL of RNAse-free water. The reaction runtime consisted of an initial hot start of three minutes at 95 °C to denature the DNA and then 40 cycles of the following: 15 seconds at 95 °C, 20 seconds at 56 °C, and then 20 seconds at 72 °C. Three reactions were performed for each sample to serve as technical replicates. qPCR reactions were performed using an Agilent AriaMx qPCR System (Agilent, United States) and software (version 1.7) provided by Agilent. Relative gene expression was determined by calculating $2^{(-\Delta Cq)}$, where ΔCq is the Cq of the gene of interest minus the Cq of a control gene. Primer sequences used are contained in **Table 2.1**

Primer	Sequence
PR-1 Forward	CCGTGCAATTGTGGGTGTC
PR-1 Reverse	GAGTTGCGCCAGACTACTTGAGT
UBQ-1 Forward	GCCAAGATCCAGGACAAGGA
UBQ-1 Reverse	GCTGCTTTCAGGCGAAA

Table 2.1. Sequences of primers used in qPCR reactions.

3 - Transgenerational priming

3.1 Introduction

Priming via elicitor treatment has great potential to be an environmentally friendly alternative to chemical pesticides. As elicitors merely augment the existing defence systems of the plant, there is much less risk of off-target organisms being harmed. There is also reduced chance of pests developing resistance to all of a plant's defence mechanisms compared to the evolution of resistance to pesticides with a single mode-of-action. However, there are a number of critical factors that have prevented elicitor agents from being a mainstream alternative to pesticides. Chief among them is that elicitors are not as bluntly effective as traditional pesticides at reducing pest populations (Yassin et al., 2021). Elicitors also need tailored solutions to be implemented as part of a larger IPM strategy that requires very specific knowledge and expertise (Walters et al., 2013).

The use of transgenerational priming to protect crops may alleviate some of the current issues that growers face in adopting the use of elicitors in IPM. If growers could buy primed seed from suppliers, they would not need to treat the crops themselves, thereby reducing the labour needed on the behalf of growers. This approach would also minimise the possibility of phytotoxicity associated with application of elicitors to the growing crop. A wide variety of chemicals have proven capable of eliciting defence responses in plants via the SA, JA, or other defence pathways (Friedrich et al., 1996, Scalschi et al., 2013, Thomma, 2000, Ton and Mauch-Mani, 2004, van Aubel et al., 2016). In this chapter, we tested a range of elicitors for their ability to provide transgenerational resistance in Micro Tom against a necrotrophic pathogen, *B. cinerea*, as well as a hemibiotrophic pathogen, *P. syringae*. The background of each of these elicitors is discussed in **sections 3.1.1 through 3.1.5**. We also performed qPCR assays to observe if expression of *PATHOGENESIS RELATED PROTEIN-1* (*PR-1*), a typical SAR marker gene that is often associated with the SA defence pathway, was affected transgenerationally by any of our parental treatments.

3.1.1 BABA

β-aminobutryic acid (BABA) is a naturally occurring non-protein amino acid that has been demonstrated to rapidly accumulate after exposure to both biotic and abiotic stresses in a variety of plant species (Thevenet et al., 2017). Indeed, BABA has the unusual quality of being able to prime plant defences against a broad spectrum of biotic stresses including the fungus *B. cinerea*, the bacteria *P. syringae*, the oomycetes *Peronospora parasitic* and *Phytophthora infestans*, as well as the tobacco mosaic virus (Cohen et al., 1994, Siegrist, 2000, Slaughter et al., 2012, Zimmerli et al., 2000, Zimmerli et al., 2001). In Arabidopsis, BABA can potentiate the accumulation of *PR-1* mRNA upon infection with *P. syringae* (Zimmerli et al., 2000). This priming effect is absent in SAR-impaired mutants, implying that BABA primes Arabidopsis plants through the SA defence pathway (Zimmerli et al., 2000). However, a separate study observed that BABA priming against salt stress and *Hyaloperonospora parasitica* was compromised in two Arabidopsis mutants impaired in ABA-dependent defences (Ton et al., 2005). BABA therefore seems to act through multiple defence pathways. In 2014, Luna et al provided evidence that BABA binds to the IBI1 aspartyl-tRNA synthase in Arabidopsis (Luna et al., 2014). Luna et al proposed that binding of BABA to IBI1 may trigger BABA-induced resistance, whilst also causing an accumulation of uncharged tRNAs that leads to stunted growth via a separate pathway (Luna et al., 2014). BABA has been observed to produce transgenerational priming before. BABA treatment of Arabidopsis and potato can create progeny which are more resistant to *Hyaloperonospora arabidopsidis* and *P. infestans*, respectively (Meller et al., 2018, Slaughter et al., 2012). *PR-1* expression was potentiated in the progeny of BABA-treated potatoes upon challenge with *P. infestans*, suggesting that at least BABA-induced SA defences are capable of being transferred from parent to offspring (Meller et al., 2018).

3.1.2 BTH

Benzo(1,2,3)thiadiazole-7-carbothioic acid *S*-methyl ester (BTH; also known as acibenzolar-S-methyl) is a synthetic analogue of salicylic acid that was first described in 1996 as being able to induce plant defences in tobacco through the SA

pathway (Friedrich et al., 1996). Since then, BTH has proved effective at priming defences in a number of plant species, including Arabidopsis, tomato, grapevine, and apple (Brisset, 2000, Herman et al., 2008, Iriti et al., 2004, Kohler et al., 2002). Much is still unknown about the mechanisms behind BTH-induced resistance, however BTH treatment has been demonstrated to potentiate callose deposition and expression of several genes that are typically associated with the SAR response, such as *PR-1* (Herman et al., 2008, Kohler et al., 2002).

3.1.3 HA

Like BABA, hexanoic acid (HA) has proved effective at producing broad spectrum priming responses against both biotrophic and necrotrophic pathogens and appears to work through multiple defence pathways. HA can prime defences against *B. cinerea* in both Arabidopsis and tomato via the JA pathway (Kravchuk et al., 2011, Leyva, 2008, Vicedo et al., 2009). The mechanism of HA-induced resistance against *B. cinerea* appears to be somewhat species-specific however, as HA treatment potentiated callose deposition upon challenge with *B. cinerea* in several varieties of tomato but not in Arabidopsis (Kravchuk et al., 2011, Vicedo et al., 2009). Additionally, Finiti et al observed in 2014 that HA priming potentiated expression of several defence genes and reduced the accumulation of reactive oxygen species around *B. cinerea* infection sites on tomato leaves (Finiti et al., 2014). Besides *B. cinerea*, HA priming has also provided resistance against *Alternaria alternata* in Fortune mandarin, and against *P. syringae* in tomato (Llorens et al., 2016, Scalschi et al., 2013). Unusually, HA treatment appears to prime both the JA and SA pathways to protect tomato plants from *P. syringae* infection (Scalschi et al., 2013).

3.1.4 MeJA

Methyl jasmonate (MeJA) is a naturally occurring plant hormone and bioactive derivative of JA. Treatment with MeJA can induce resistance against a variety of necrotrophic pathogens in a number of plant species, including Arabidopsis, wheat, peach, strawberry and tomato (Desmond, 2005, Jin, 2009, Thomma, 2000,

Valenzuela-Riffo et al., 2020, Zhu, 2012). Several studies have observed MeJA treatment to potentiate expression of defence-related genes, as well as the activity of defensive enzymes in response to disease (Jiang, 2015, Motallebi, 2017, Tang, 2013, Valenzuela-Riffo et al., 2020). A 2014 study also noted that MeJA pretreatment caused enhanced production of various phenolic compounds in Chinese bayberry in response to *Penicillium citrinum* infection (Wang, 2014). Treatment with MeJA has previously been shown to be capable of providing transgenerational resistance against herbivory by *Helicoverpa zea* caterpillars in Arabidopsis and tomato (Rasmann et al., 2012). This transgenerational priming required a functioning perception of JA (Rasmann et al., 2012).

3.1.5 Fytosave

Fytosave (Fytofend, Belgium) is a commercially available defence elicitor based on the COS-OGA compound. COS-OGA is a molecular complex consisting of chitosan (COS), a deacetylated form of chitin that is produced by invasive fungi; and oligogalacturonides (OGA), fragments of pectin that are produced by the degradation of plant cell walls (Cabrera et al., 2010). Application of COS-OGA has proved effective at priming defences against various biotrophic pathogens in grapevine, cucumber, tomato, and potato (Clinckemaillie, 2017, van Aubel, 2014, van Aubel et al., 2016). Indeed, COS-OGA can potentiate accumulation of genes related to SA-dependent defence, such as *PR-1* and *PR-2*, suggesting that COS-OGA primes defences through SAR-related mechanisms (Clinckemaillie, 2017, van Aubel et al., 2016). COS-OGA also appears to be a broad-spectrum elicitor however, as it can prime defences against the nematode *Meloidogyne graminicola* in rice and the necrotrophic fungus *B. cinerea* in postharvest grape (Calderone et al., 2022, Singh et al., 2019). Additionally, COS-OGA induced priming against nematode in rice is not dependent on the SA or JA defence pathways (Singh et al., 2019).

3.2 Generation of Parental Plants

To investigate the potential effects of transgenerational priming in tomato, a series of parental generations of control-treated or primed tomato plants was produced.

The Micro Tom cultivar of tomato was chosen for our experiments as it matures and produces fruit quickly and requires less growing space in comparison to larger commercial varieties.

Parental plants were given either a control or priming treatment whilst they were two-week-old seedlings and then allowed to reach maturity so seeds could be collected from them. BABA, HA, MeJA, BTH, and Fytosave were used as our priming treatments. Offspring generation plants were grown from these seeds for use in experiments to test the impact of parental and offspring priming treatments on disease resistance. All offspring plants were categorised according to the treatments they and their parent plants received. For example, an offspring (T_2) plant that had been given a priming treatment but whose parent (T_1) was given a control treatment would be in the C_1P_2 treatment group (Control generation 1, Primed generation 2). The nomenclature for each offspring treatment group is explained in **Fig. 3.1**.

Not only would experimentation with these treatment groups allow us to observe any effects of same-generation or transgenerational priming, but it would also allow us to observe if there are any compound effects of priming over multiple generations within the P_1P_2 treatment group.

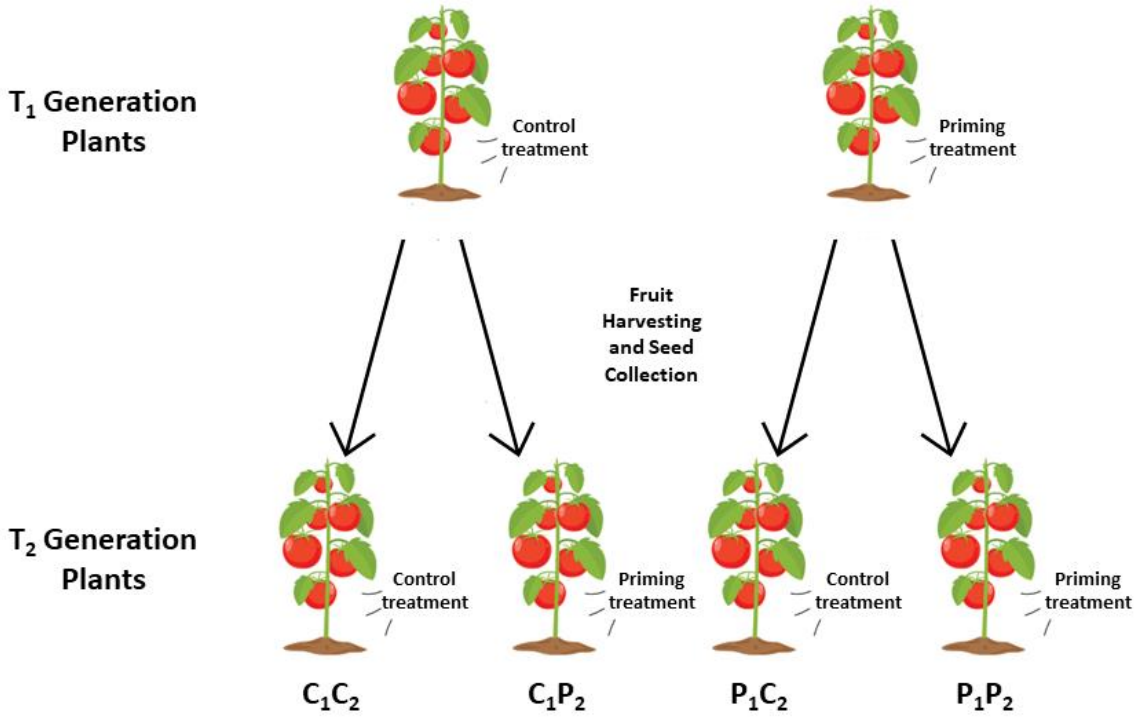

Figure 3.1. Nomenclature of offspring plant treatment groups used in transgenerational priming experiments. C_1 and P_1 refers to offspring plants whose parents were given a control or priming treatment, respectively. C_2 and P_2 refers to offspring plants which were respectively given a control or priming treatment.

3.3 Preparation of a *P. syringae* Inoculation Method in Micro Tom

P. syringae is a common plant pathogen that affects tomato plants and causes bacterial speck disease in the leaves and fruit. Defence against *P. syringae* is mediated through the SA-dependent SAR pathway. We therefore assayed resistance against *P. syringae* infection in our treatment groups to observe whether any of our elicitors can create a transgenerational SAR effect in tomato.

Dipping was originally chosen as the method of inoculating Micro Tom plants with *P. syringae*, however leaf extracts from dip-inoculated Micro Tom plants frequently yielded extremely few or no colony forming units (CFUs) when measured. This observation is in line with a previous study which noted that dipping or spraying with several different strains of *P.* syringae was ineffective at infecting Micro Tom plants (Takahashi, 2005). However, the authors found that direct infiltration was successful at inoculating Micro Tom (Takahashi, 2005). Infiltration was therefore examined as a possible method of inoculation, although this method cannot measure any effects

that priming may have on stomatal resistance against pathogens. Indeed, direct comparisons between dipping in the Money Maker and Micro Tom cultivars revealed that bacterial populations in inoculated Money Maker extracts were two orders of magnitude larger than those from Micro Tom (**Fig. 3.2 A**). Additionally, only 50% of the Micro Tom extracts yielded visible CFUs when plated whilst 88% of Money Maker extracts had clear bacterial growth (data not shown). Inoculation by infiltration yielded greater bacterial growth in Micro Tom (**Fig. 3.2 A**) but several inoculated cotyledons showed clear signs of necrosis as they were wilted with dry dark spots.

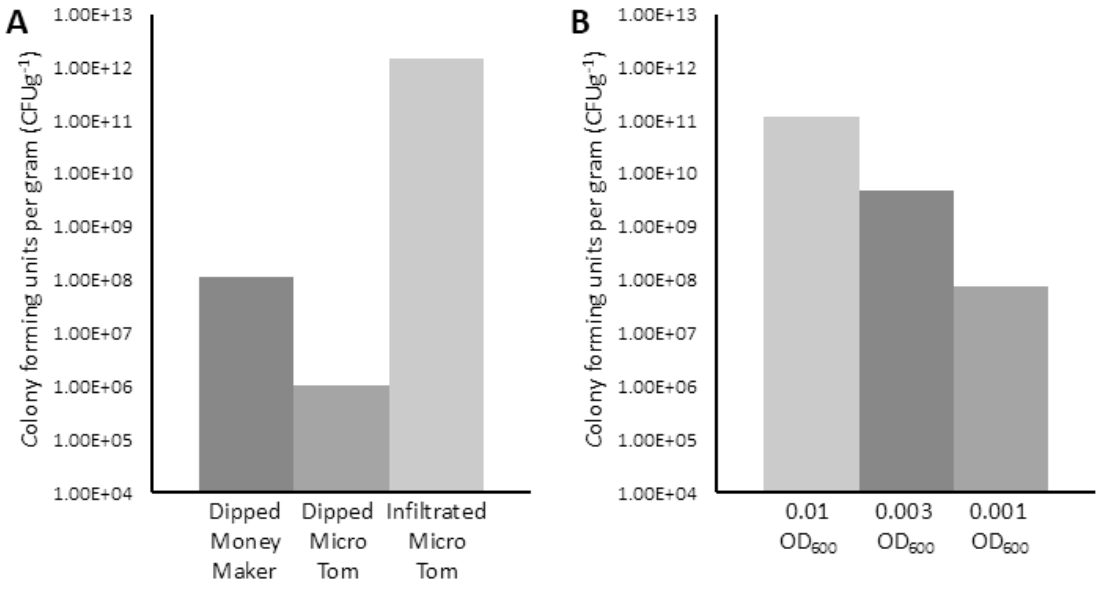

Figure 3.2. Quantification of bacterial populations in extracts from two-week-old tomato seedlings 72 h following inoculation with Pst T1 avrPto. **(A)** Micro Tom or Money Maker plants were inoculated with a 0.10 OD_{600} bacterial solution by dipping or infiltration. **(B)** Micro Tom plants were inoculated by infiltration with various optical densities of bacterial solution. Columns represent bacterial population size in colony forming units per gram.

Since the initial infiltration tests resulted in severe symptoms, several lower optical densities of bacterial solution were tested to find a suitable inoculum for infiltrating Micro Tom seedlings. An optical density of 0.01 OD_{600} yielded strong bacterial growth (**Fig. 3.2 B**), however 63% of the plated extracts were too dense to count (data not shown) and therefore unsuitable for experimentation. 0.003 OD_{600} was chosen as

the optical density to use for experiments going forward as most of the extracts produced bacterial growth with no necrosis and individual CFUs that were plainly visible and countable.

3.4 *P. syringae* Resistance Assays

Resistance to the Pst T1 avrPto strain of *P. syringae* was assayed in our Micro Tom treatment groups to test if the SAR pathway can have a transgenerational effect in tomato. Priming agents used in our treatments were BABA, BTH, HA, MeJA, and Fytosave. Treatments were identical between generations within treatment groups.

Within both the BABA and BTH experiments, the bacterial populations in the C_1P_2 and P_1C_2 groups were lower than their C_1C_2 counterpart, although the reduction in the bacterial population was greater in the C_1P_2 treatment group in both experiments (**Fig. 3.3 A** and **Fig. 3.3 B**). Indeed, two-way ANOVA confirms that BABA and BTH treatments in both the parental and offspring generations had a significant effect on the bacterial population (**Table 3.1**). However, plants in the P_1P_2 group in both experiments did not experience a further reduction in bacterial population after being treated in both generations, as confirmed by the significant interaction (P_2 treatment affected C_1 but not P_1 lines).

P_1C_2 plants that received a parental HA treatment had a larger bacterial population than the C_1C_2 plants in the same experiment (**Fig. 3.3 C**). ANOVA analysis confirmed a significant effect of parental treatment (**Table 3.1**). However, plants which had received HA treatment in both parental and offspring generations had a bacterial population size similar to the C_1C_2 group (**Fig. 3.3 C**), indicating that an offspring HA treatment seems to rescue the increased susceptibility of parentally treated plants to *P. syringae*. Indeed, a significant parental-offspring treatment interaction is observed in the ANOVA analysis (**Table 3.1**).

In the MeJA experiment, bacterial populations were higher in both the C_1P_2 and P_1P_2 treatment groups compared to the C_1C_2 group. However, the effect of offspring generation treatment was not significant in the ANOVA analysis (**Fig. 3.3 D** and **Table 3.1**). The bacterial population was lower in the P_1C_2 group compared to the C_1C_2

group, but ANOVA analysis detected no significant effect of parental priming (**Fig. 3.3 D** and **Table 3.1**).

The results for the Fytosave experiment were similar to those of the BTH and BABA experiments. The bacterial populations in the C_1P_2 and P_1C_2 groups were lower than that of the C_1C_2 group (**Fig. 3.3 E**). However, only offspring treatment was found to have a significant effect on bacterial population size in the ANOVA analysis (**Table 3.1**). Additionally, P_1P_2 plants did not experience an additive effect of priming over two generations.

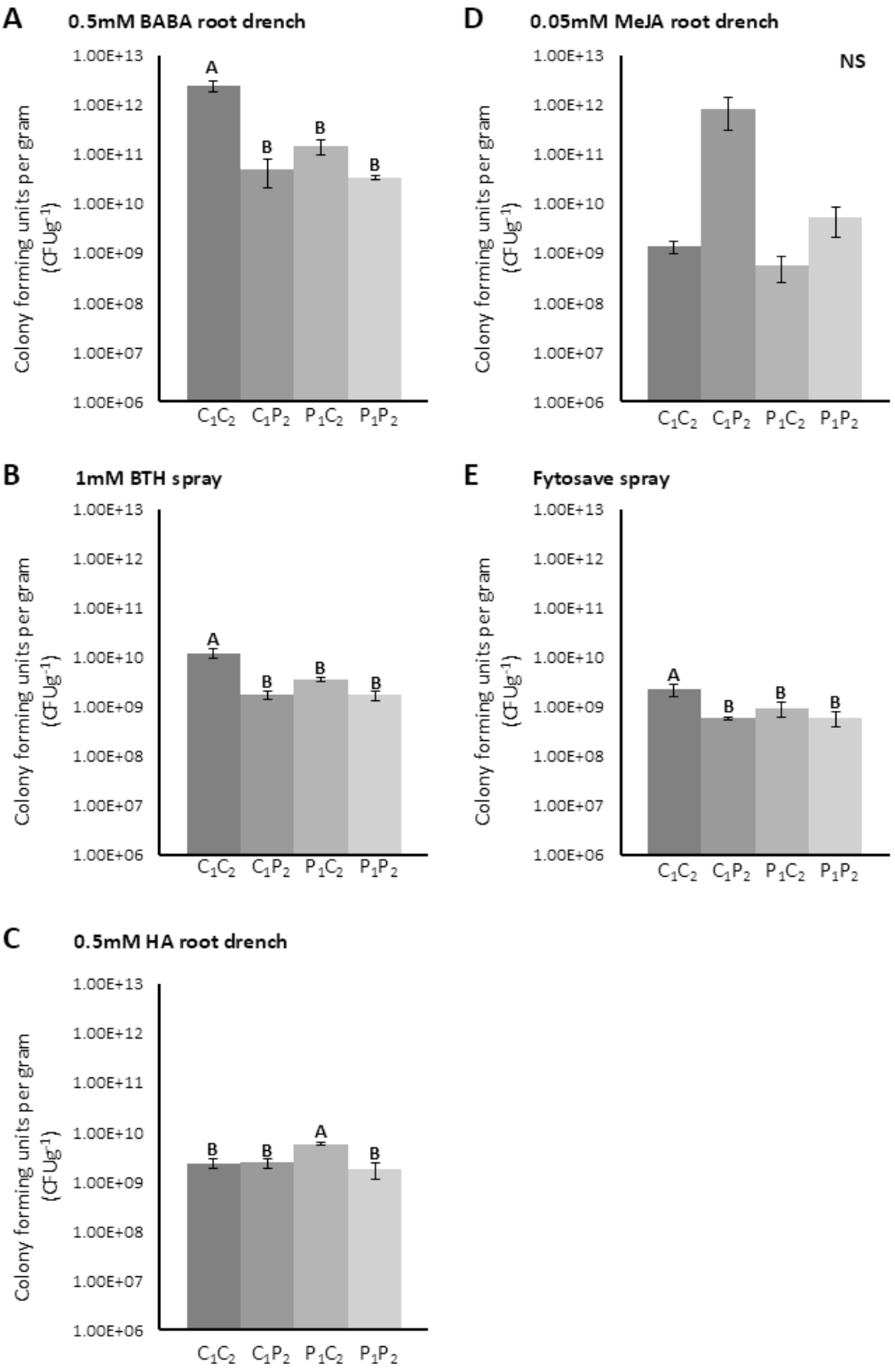

Figure 3.3. Quantification of bacterial populations in extracts from two-week-old Micro Tom seedlings 72 h following inoculation with a 0.003 OD_{600} Pst avrPto solution by infiltration. Plants were treated with a control or priming treatment 24 h before inoculation. Priming treatments used were a 0.5 mM BABA root drench **(A)**, a 1 mM BTH spray **(B)**, a 0.5 mM HA root drench **(C)**, a 0.05 mM MeJA root drench **(D)**, or a FytoSave spray **(E)**. Control treatment used was a root drench with RO water. Columns represent mean bacterial population size in colony forming units per gram ± standard error. n=12 for all groups, except the BABA P1C2 group where n=11. Letters represent significant difference at $P<0.05$. NS represents no significant differences.

	Parental priming treatment	Offspring priming treatment	Parental*Offspring Interaction
BABA	1, 13.07, **7.81×10^{-4}**	1, 15.36, **3.14×10^{-4}**	1, 12.67, **9.21×10^{-4}**
BTH	1, 8,81, **5.00×10^{-3}**	1, 17.47, **1.36×10^{-4}**	1, 8.80, **5.00×10^{-3}**
HA	1, 8.07, **7.00×10^{-3}**	1, 16.97, **1.65×10^{-4}**	1, 17.21, **1.51×10^{-4}**
MeJA	1, 2.49, 0.122	1, 2.54, 0.188	1, 2.48, 0.122
FS	1, 3.67, 0.062	1, 8.46, **6.0×10^{-3}**	1, 3.42, 0.071

Table 3.1. ANOVA analyses (df, F, P) for parental treatments, offspring treatments, and interactions between parental and offspring treatments in the *P. syringae* resistance assays. Significant P values are bold.

3.5 *B. cinerea* Resistance Assays

B. cinerea is a necrotrophic fungus that causes grey mould in many kinds of plant, including tomato, and can result in significant financial losses for growers. Defence priming against necrotrophic pathogens such as *B. cinerea* is facilitated by the JA-dependent signalling pathway. Resistance to *B. cinerea* was therefore assayed in our various treatment groups to assess if the JA-dependent induced resistance pathway can transgenerationally influence resistance an economically-relevant necrotroph. *B. cinerea* resistance was assayed by innoculating the surface of Micro Tom leaves with 5 µL droplets of *B. cinerea* solution and comparing the resulting lesion diameters between treatment groups.

Results from BABA-treated plants were inconsistent between the two experiments performed. In the first experiment, lesions on C_1P_2 plants were of similar size to those of the C_1C_2 group, however the lesions in P_1C_2 and P_1P_2 groups were slightly smaller and a significant effect of parental treatment was observed in the ANOVA analysis (**Fig. 3.4 A** and **Table 3.2**). This effect was absent in the second experiment however, as the lesions of the C_1P_2 and P_1C_2 groups were similar to those in the C_1C_2 group (**Fig. 3.4 B**) and no effect of offspring or parental treatment was present in the ANOVA analysis (**Table 3.2**).

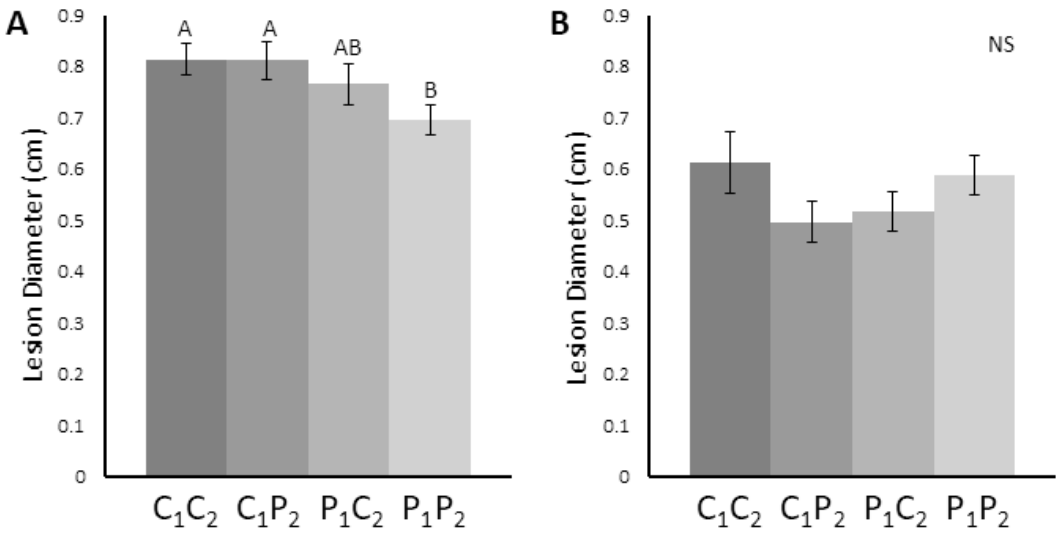

Figure 3.4. Measurements of *B. cinerea* lesion size on the excised first leaves of six-week-old Micro Tom plants. Plants were treated at two weeks old with either a control treatment of an RO water root drench, or a 0.5 mM BABA root drench. Leaves were inoculated with two 5 µL drops of solution containing 10^5 spores per mL and incubated for a minimum of 3 days. Results shown are from the first **(A)** and second **(B)** experiments. Columns represent mean lesion diameter in centimetres ± standard error. n=19-21 for the first experiment; n=12-25 for the second experiment. Letters represent significant difference at P<0.05. NS represents no significant differences.

	Parental priming treatment	**Offspring priming treatment**	**Parental*Offspring Interaction**
Experiment 1	1, 5.474, **0.022**	1, 1.060, 0.307	1, 0.916, 0.342
Experiment 2	1, 1.554×10^{-5}, 0.984	1, 0.270, 0.605	1, 4.395, **0.039**

Table 3.2. ANOVA analyses (df, F, P) for parental treatments, offspring treatments, and interactions between parental and offspring treatments in the *B. cinerea* resistance assays for BABA-treated Micro Tom plants. Significant P values are bold.

For the BTH experiment, P_1 plants had larger lesions on average than their C_1 counterparts and an almost statistically significant effect of parental treatment was noted in the ANOVA analysis (**Fig. 3.5** and **Table 3.2**). Conversely, P_2 plants were found to have significantly smaller lesions than C_2 plants (**Fig. 3.5** and **Table 3.2**).

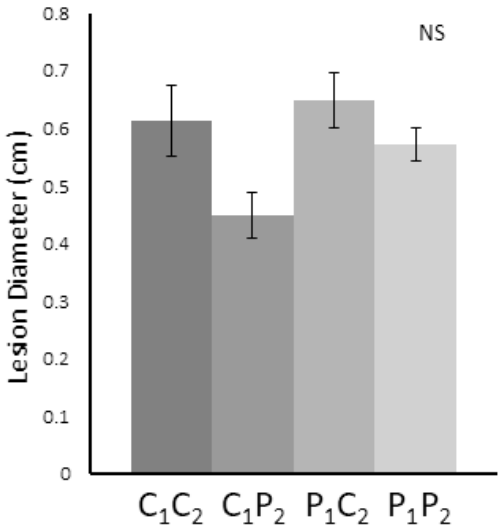

Figure 3.5. Measurements of *B. cinerea* lesion size on the excised first leaves of six-week-old Micro Tom plants. Plants were treated at two weeks old with either a control treatment of an RO water root drench, or a 1 mM BTH spray. Leaves were inoculated with two 5 µL drops of solution containing 10^5 spores per mL and incubated for a minimum of 3 days. Columns represent mean lesion diameter in centimetres ± standard error. n=12-25. Letters represent significant difference at P<0.05. NS represents no significant differences.

	Parental priming treatment	Offspring priming treatment	Parental*Offspring Interaction
Experiment 1	1, 2.999, 0.088	1, 6.846, **0.011**	1, 0.868, 0.355

Table 3.3. ANOVA analysis (df, F, P) for parental treatment, offspring treatment, and interaction between parental and offspring treatment in the *B. cinerea* resistance assay for BTH-treated Micro Tom plants. Significant P values are bold.

In the first experiment with HA-treated plants, neither P_1 or P_2 plants were found to have significantly different lesion sizes from their control group counterparts (**Fig. 3.6** and **Table 3.4**). A statistically significant interaction between parental and offspring treatments was observed however, as priming in the offspring generation reduced lesions size in C_1 lines, but increased it in P_2 lines (**Fig. 3.6** and **Table 3.4**).

Results from the second HA experiment were incongruous with those of the first experiment. P_1 plants appeared to have slightly larger lesions than C_1 plants with an effect close to significance, however offspring treatment had no effect on lesion size (**Fig. 3.6** and **Table 3.4**). No interaction between priming in the parental and offspring treatments was observed.

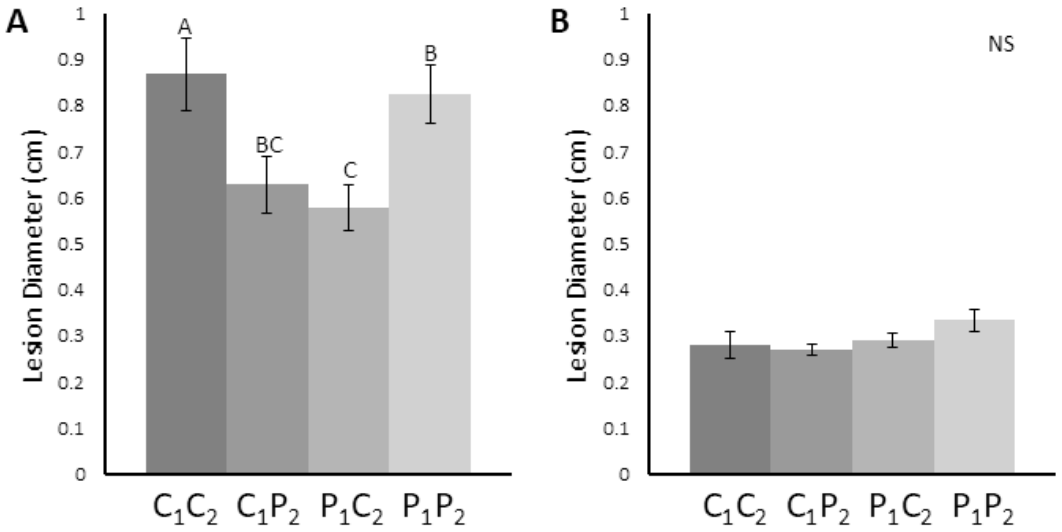

Figure 3.6. Measurements of *B. cinerea* lesion size on the excised first leaves of six-week-old Micro Tom plants. Plants were treated at two weeks old with either a control treatment of an RO water root drench, or a 0.5 mM HA root drench. Leaves were inoculated with two 5 µL drops of solution containing 10^5 spores per mL and incubated for a minimum of 3 days. Results shown are from the first **(A)** and second **(B)** experiments. Columns represent mean lesion diameter in centimetres ± standard error. n=20-26 for the first experiment; n=19-22 for the second experiment. Letters represent significant difference at P<0.05. NS represents no significant differences.

	Parental priming treatment	Offspring priming treatment	Parental*Offspring Interaction
Experiment 1	1, 0.489, 0.486	1, 0.003, 0.954	1, 13.747, **3.633×10^{-4}**
Experiment 2	1, 3.228, 0.076	1, 0.620, 0.434	1, 1.611, 0.208

Table 3.4. ANOVA analyses (df, F, P) for parental treatments, offspring treatments, and interactions between parental and offspring treatments in the *B. cinerea* resistance assays for HA-treated Micro Tom plants. Significant P values are bold.

In the first MeJA experiment, parentally treated plants had significantly larger lesions than C_1 plants (**Fig. 3.7** and **Table 3.5**). However, P_2 plants had significantly smaller lesions than C_2 plants (**Fig. 3.7** and **Table 3.5**). None of these interactions were repeated in the second and third experiments as no significant effects of parental or offspring treatment on lesion size were observed (**Fig. 3.7** and **Table 3.5**).

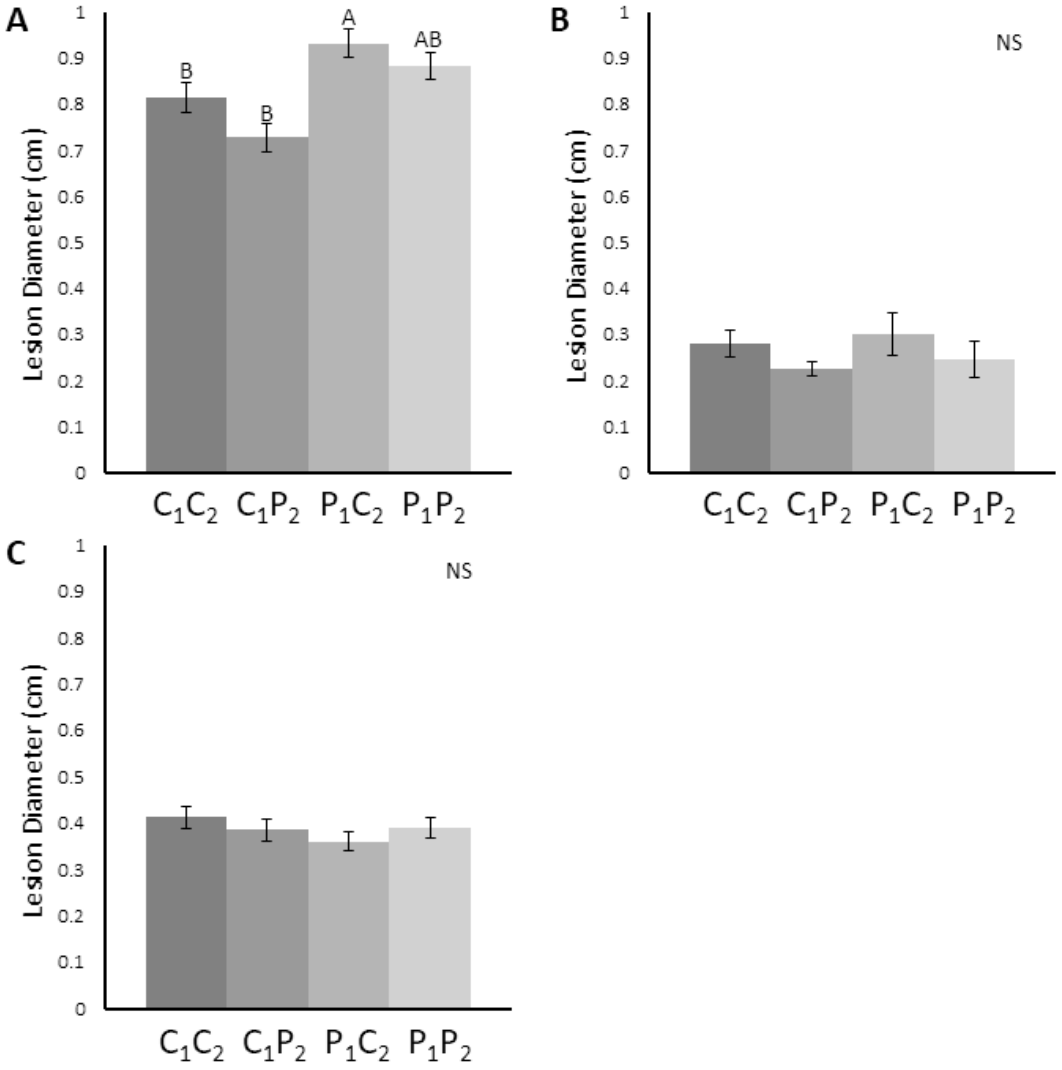

Figure 3.7. Measurements of *B. cinerea* lesion size on the excised first leaves of six-week-old Micro Tom plants. Plants were treated at two weeks old with either a control treatment of an RO water root drench, or a 0.05 mM MeJA root drench. Leaves were inoculated with two 5 µL drops of solution containing 10^5 spores per mL and incubated for a minimum of 3 days. Results shown are from the first **(A),** second **(B)** and third **(C)** experiments. Columns represent mean lesion diameter in centimetres ± standard error. n=19-27 for the first experiment; n=14-19 for the second experiment; n=15-20 for the third experiment. Letters represent significant difference at $P<0.05$. NS represents no significant differences.

	Parental priming treatment	Offspring priming treatment	Parental*Offspring Interaction
Experiment 1	1, 19.342, **3.02 x 10^{-5}**	1, 4.749, **0.032**	1, 0.380, 0.539
Experiment 2	1, 0.394, 0.532	1, 2.716, 0.104	1, 4.312 x 10^{-7}, 0.996
Experiment 3	1, 1.075, 0.304	1, 8.997 x 10^{-6}, 0.975	1, 1.533, 0.220

Table 3.5. ANOVA analyses (df, F, P) for parental treatments, offspring treatments, and interactions between parental and offspring treatments in the *B. cinerea* resistance assays for MeJA-treated Micro Tom plants. Significant P values are bold.

For both the first and second Fytosave experiments, P_2 plants were observed to have significantly smaller lesion size than C_2 plants (**Fig. 3.8** and **Table 3.6**). However, no effect of Fytosave treatment in the parental generation on lesion diameter was observed in either experiment (**Fig. 3.8**).

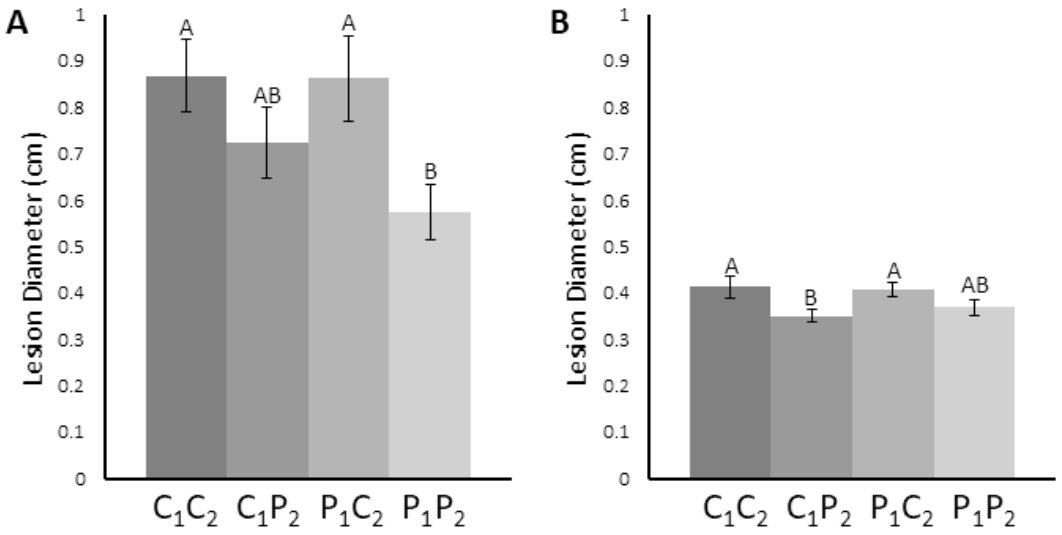

Figure 3.8. Measurements of *B. cinerea* lesion sizes on the excised first leaves of six-week-old Micro Tom plants. Plants were treated at two weeks old with either a control treatment of an RO water root drench, or a Fytosave spray. Leaves were inoculated with two 5 µL drops of solution containing 10^5 spores per mL and incubated for a minimum of 3 days. Results shown are from the first **(A)** and second **(B)** experiments. Columns represent mean lesion diameter in centimetres ± standard error. n=13-26 for the first experiment; n=17-19 for the second experiment. Letters represent significant difference at P<0.05.

	Parental priming treatment	Offspring priming treatment	Parental*Offspring Interaction
Experiment 1	1, 0.913, 0.343	1, 6.888, **0.011**	1, 0.768, 0.384
Experiment 2	1, 0.157, 0.693	1, 8.333, **5.00×10^{-3}**	1, 0.462, 0.499

Table 3.6. ANOVA analyses for parental treatments, offspring treatments, and interactions between parental and offspring treatments in the *B. cinerea* resistance assays for Fytosave-treated Micro Tom plants. Significant P values are bold.

3.6 Analysis of Possible Mechanisms Behind Transgenerational *P. syringae* Resistance

Parental effects of priming with BABA, BTH and Fytosave were observed in our *P. syringae* resistance assays. In order to better understand the molecular mechanisms underpinning this transgenerational response, differences in gene expression between offspring plants from primed or control-treated parents were analysed. Offspring plants were treated with a 2.5 mM INA 0.7% ethanol spray or a 0.7% ethanol spray control treatment. In these experiments, P_2 plants therefore refer to offspring plants that have been treated with INA spray. These treatment groups would allow us to observe if there are any differences in basal levels of *PR-1* mRNA between P_1 and C_1 plants, or if accumulation of *PR-1 mRNA is* potentiated in P_1 plants upon treatment with INA. RNA was extracted at 0, 2, 4, 6, and 24 h after treatment with the INA or control spray. *PR-1* was chosen to be our marker gene as the SAR response triggers upregulation of *PR-1* expression and accumulation of the PR-1 protein.

Relative expression of *PR-1* in the C_1P_2 group was roughly equal to that of the C_1C_2 group for the 0, 2, 4 and 6 h timepoints. At 24 h after INA spray treatment, C_1P_2 *PR-1* relative expression greatly increased to around 11 times greater than C_1C_2. *PR-1* relative expression for the P_1C_2 group of each treatment followed a similar overall trend: P_1C_2 *PR-1* relative expression was higher than C_1P_2 at the 0, 2, 4, and 6 h timepoints, but was then overtaken by C_1P_2 at 24 h (**Fig. 3.7**).

P_1P_2 *PR-1* relative expression also followed an overall trend between each of the treatments: P_1P_2 *PR-1* relative expression was typically higher than both C_1P_2 and P_1C_2 relative expression at the 0, 2, 4, and 6 h timepoints, and then roughly equal to or greater than C_1P_2 relative expression at 24 h after treatment (**Fig. 3.7**). Notable

exceptions to this pattern were at the 0 h timepoint for the BTH and Fytosave treatment experiments, where P_1P_2 relative expression was roughly equal to P_1C_2 (**Fig. 3.7 B** and **E**). Fytosave P_1P_2 relative expression was also roughly equal to P_1C_2 at the 6 and 24 h timepoints, and drastically lower than C_1P_2 at 24 h (**Fig. 3.7 E**).

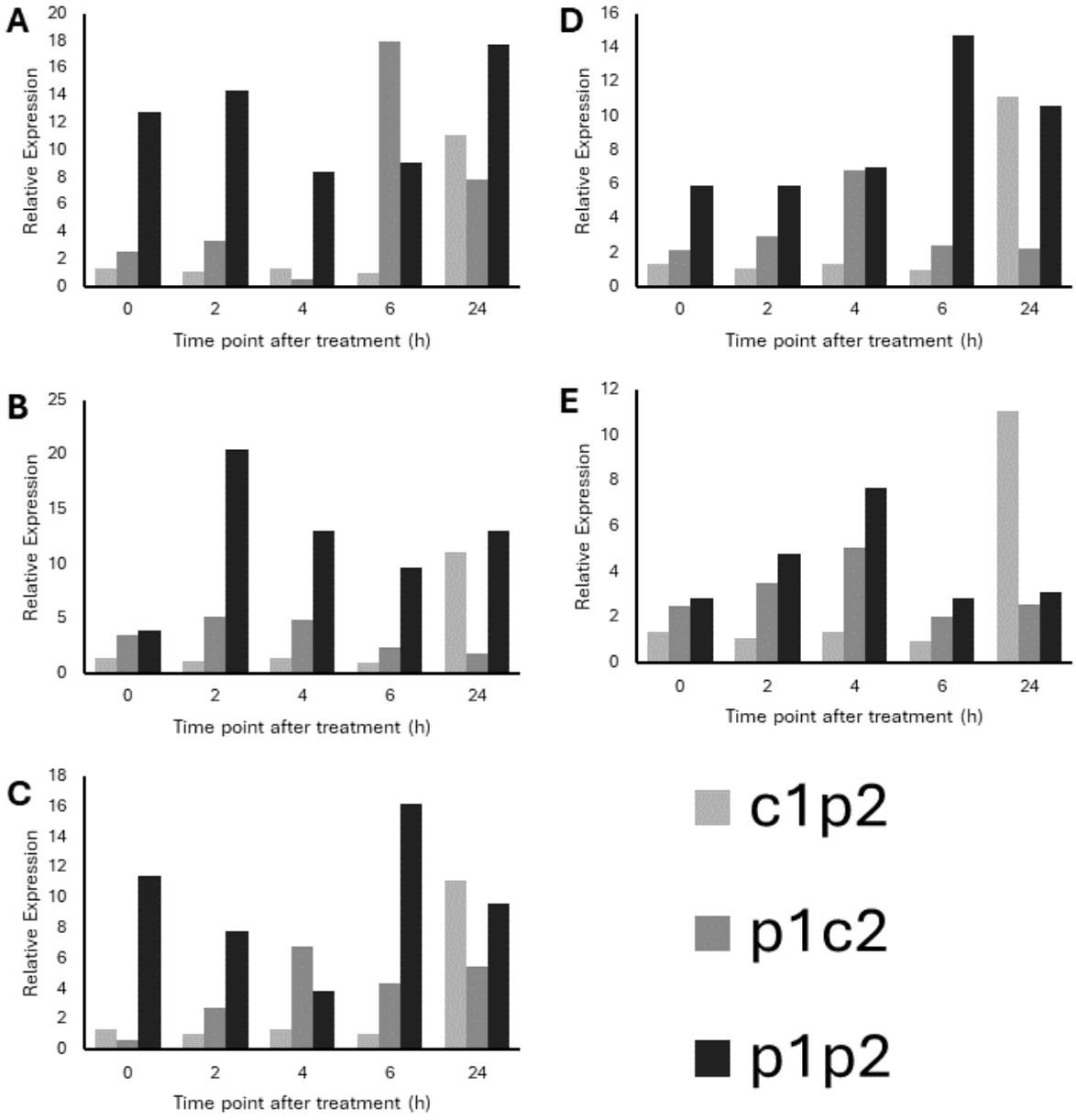

Figure 3.7. Expression of *PR-1* mRNA in three-week-old Micro Tom plants of various treatment groups relative to *PR-1* expression in the C_1C_2 control group. Parental plants were given either a control treatment of an RO water root drench, a 0.5 mM BABA root drench **(A)**, a 1 mM BTH spray **(B)**, a 0.5 mM HA root drench **(C)**, a 0.05 mM MeJA root drench **(D)**, or a Fytosave spray **(E)**. Offspring plants were given either a 0.7% ethanol spray or a 2.5 mM INA 0.7% ethanol spray. RNA was extracted at various time points after treatment. Columns represent relative expression of *PR-1* mRNA. n=3 for all treatment groups.

3.7 Discussion

In this chapter, we tested the ability of several priming agents to induce either same-generation or transgenerational priming against *P. syringae* or *B. cinerea*. *P. syringae* and *B. cinerea* were chosen as the challenge inoculations in our resistance assays as defence priming against these pathogens is chiefly controlled by the SA and JA pathways, respectively. Assaying resistance against these pathogens therefore allows us to examine the possibility of our agents priming both or either of the two major resistance pathways. With regards to our choice of priming agents, BTH and MeJA are both functional analogues of SA and JA, respectively. BABA, HA, and COS-OGA (the active substance of Fytosave) have each been shown to be effective against a broad range of pathogens. All plants were given their priming treatments at either two weeks or three weeks after planting, respectively. Previous work has demonstrated that priming treatments are more effective when tomato plants are young and fail to induce long-lasting resistance if applied when the plants are starting to mature and grow fruit (Wilkinson, 2018).

3.7.1 Summary of Resistance Phenotypes

In our study, we observed that both parental and offspring treatment with BABA, BTH, or Fytosave could induce resistance against *P. syringae* in Micro Tom tomato plants. Offspring treatment was more effective at preventing bacterial population growth than parental treatment in all of these cases and treating with the same agent over two generations did not produce additional defensive effects. We did not observe any effects of MeJA treatment in either generation on *P. syringae* bacterial populations in offspring plants. HA treatment in the offspring generation did not produce any effect on *P. syringae* resistance in the offspring of control-treated parents, however HA treatment in the parental generation produced offspring plants that were more vulnerable to *P. syringae* infection. Curiously, treating the offspring of HA-treated parents with HA restored a level of resistance similar to C_1C_2 plants.

During our challenge inoculations with *B. cinerea* we observed no significant and repeatable effects of treatment with BABA, HA, or MeJA, on lesion size. However, it is worth noting that the biological variations in outcomes both within and between experiments in the *B. cinerea* resistance assays were much greater than those of the

P. syringae assays which makes it more difficult to detect consistent statistically significant treatment effects. For example, experiment 2, and experiments 2 and 3 of the HA and MeJA treatment groups, respectively, produced lesion sizes that were dramatically smaller than those of their respective experiment 1. As the overall extent of disease progression varied greatly between experiments, it is therefore possible that a clearer pattern of priming may have emerged in these treatment groups if the experiments with smaller lesions were left for a few more days post inoculation. We observed that P2 treatment with either BTH or Fytosave could induce resistance against *B. cinerea*, however no transgenerational effects for either of these priming treatments were observed.

3.7.2 Priming for SA-Dependent Resistance is More Likely to Persist to the Next Generation

We observed that treating tomato plants with BABA can induce both same-generation and transgenerational priming against *P. syringae*. However, there was no 'primed to be primed' response over two generations of treatment. Previous studies in both Arabidopsis and the Rio Grande tomato cultivar have observed that BABA treatment can produce same-generation defence against *P. syringae* (Baysal, 2007, Flors, 2008, Zimmerli et al., 2000). Our results are also consistent with previous studies that observed BABA could induce transgenerational defence in Arabidopsis and potato against *P. syringae* and *P. infestans*, another biotrophic pathogen, respectively (Meller et al., 2018, Slaughter et al., 2012). Interestingly, Slaughter et al. also observed in Arabidopsis that the priming effects of BABA priming could indeed compound over two generations of treatment, resulting in further increased resistance to *P. syringae* (Slaughter et al., 2012). The lack of this 'primed to be primed' phenotype in our study may be due to differences in BABA perception or transgenerational defence mechanisms between different species. Taken together with past works, our results suggest that BABA can transgenerationally induce the SA defence pathway in several plants.

BABA treatment of tomato plants was not observed to grant same generation resistance against *B. cinerea* in our study. Our results are in direct contrast with a 2017 study, which observed that a BABA root drench of Micro Tom seedlings resulted

in smaller lesions on the fruits of mature plants upon challenge inoculation with *B. cinerea* (Wilkinson, 2018). However, it is worth noting that our study examined the effects of BABA priming on *B. cinerea* lesion sizes on leaves which are a very different kind of tissue from fruit. Another study has found that BABA root drench could indeed protect leaves against *B. cinerea* in the Money Maker variety of tomato (Luna et al., 2016). The apparent resistance to *B. cinerea* of Micro Tom leaves could be related to the issues we observed in **section 3.3** when trying to inoculate Micro Tom leaves with *P. syringae* via dipping. It may be the case that Micro Tom leaves are naturally resistant to pathogen invasion. We also did not observe any transgenerational impacts of BABA treatment on Micro Tom resistance to *B. cinerea*. No work on the transgenerational interactions between BABA and plant resistance to necrotrophic pathogens presently exists.

BTH and Fytosave were both capable of same-generation and transgenerational priming against *P. syringae* in tomato. Past works have observed that BTH has previously primed plant defences against *P. syringae* in both tobacco and tomato, and COS-OGA has protected against another biotroph, powdery mildew, in tomato (Delaney et al., 1994, Herman et al., 2008, Stout, 1999, Thaler et al., 2002, van Aubel et al., 2016). Although no previous studies exist on possible transgenerational effects of COS-OGA, a 2019 study found that BTH treatment of the common bean could protect their offspring from the *X. axonopodis* bacterium (Akköprü, 2020). Our results align with previous works and suggest that BTH and COS-OGA can transgenerationally prime SA-dependent defences in tomato.

BTH and Fytosave were also both able to induce same-generational priming against *B. cinerea* in our experiments. No transgenerational effects of Fytosave treatment were observed, however, parental BTH treatment led to increased susceptibility to *B. cinerea* in the offspring generation. Previous works have also noticed that BTH and COS-OGA can induce same-generation priming against *B. cinerea*, although BTH has also been demonstrated to antagonise the JA defence pathway and increase susceptibility to herbivores in tomato (Achuo et al., 2002, Calderone et al., 2022, Harel et al., 2014, Iriti et al., 2004, Stout, 1999, Thaler et al., 2002). Though it may seem strange that BTH treatment antagonises the JA pathway but also induces

resistance against *B. cinerea*, a necrotrophic pathogen, BTH has in fact been observed to prime expression of *Pti5* and *PI2*, two genes that are independent of the SA pathway and involved in defence against *B. cinerea* (Harel et al., 2014). It may be the case that BTH treatment activates both SA-induced defences and other potential defence pathways, however only the SA-related defences are transgenerationally induced, leading to antagonism of defence against *B. cinerea* in the offspring generation.

Same-generation treatment with HA did not have an effect on *P. syringae* or *B. cinerea* infection in our study. Parental treatment did not affect inoculation with *B. cinerea*, although it did increase susceptibility to *P. syringae* which could then be rescued by HA treatment in the offspring generation. Our results contrast with existing literature which observed treatment by HA root drench protected Ailsa Craig cultivar tomato plants from both *P. syringae* and *B. cinerea*, although neither of these works examined potential transgenerational effects of HA treatment (Scalschi et al., 2013, Vicedo et al., 2009). These same studies also found evidence that HA can act through both the SA and JA defence pathways (Scalschi et al., 2013, Vicedo et al., 2009). If HA activates both pathways, differential activation of these pathways depending on the generation that treatment is applied may explain why parental HA increases susceptibility to *P. syringae* and this vulnerability is rescued by offspring generation treatment. The exact effects of HA-dependent priming in tomato may therefore be dependent on the cultivar used and the generation the treatment is applied.

MeJA treatment, in both the parental and offspring generations, was not observed to have any effect on infection by either *P. syringae* or *B. cinerea*. The lack of impact on *P. syringae* infection is not entirely unexpected, considering that the JA and SA signalling pathways are often reported to antagonise each other (El Oirdi et al., 2011, Scalschi et al., 2020, Zhao et al., 2003). Consistent with this antagonistic relationship, plants treated with MeJA supported notably higher populations of Pst than controls (**Fig. 3.3D**), although this was not statistically significant in our analysis. As *P. syringae* is a hemibiotrophic pathogen, it may be the case that MeJA treatment may have enhanced defence during the later stages of *P. syringae*

infection when the pathogen switched to a necrotrophic mode. This may partly explain why MeJA treatment did not significantly exacerbate *P. syringae* infection as might be expected. The results of the *B. cinerea* assays, however, do contrast with previous studies which found that activation of the JA signalling pathway leads to greater resistance to *B. cinerea* in tomato and other species (Jia et al., 2016, Liu et al., 2019, Worrall et al., 2012). Application of MeJA treatment has also been reported to transgenerationally potentiate JA-dependent defences in Arabidopsis and tomato, although that study analysed resistance to herbivory by caterpillar as opposed to infection by *B. cinerea* (Rasmann et al., 2012). It may be the case that the effect of MeJA on plant defences is dependent on the genotype of the host as has previously been observed for BABA and HA in tomato (Sharma, 2010, Vicedo et al., 2009).

3.7.3 Molecular Responses to Elicitor Treatments

In order to further understand the mechanisms behind the observed transgenerational effects in our resistance assays, we also used qPCR to test if parental treatment with our priming agents could potentiate the accumulation of *PR-1* mRNA upon treatment of offspring plants with INA. *PR-1* is a defence-associated gene that is upregulated by SA-dependent defence responses and therefore often used as a marker gene for SAR priming (Durrant and Dong, 2004). INA is a synthetic analogue of SA which can directly activate SA-dependent defences when applied in large doses (Ward et al., 1991). If any of our agents can transgenerationally prime SA-dependent defences, then expression of *PR*-1 should be potentiated in P1 plants upon treatment with INA. Parental treatment with each of our priming agents could, to different extents, raise both basal *PR-1* expression and INA-induced *PR-1* expression in Micro Tom.

In the qPCR experiments, we observed that the P_1C_2 plants of each treatment group typically had higher *PR-1* relative expression than the C_1P_2 plants for at least the first 6 hours after INA treatment, until they were overtaken by C_1P_2 at the 24 h timepoint. Additionally, INA treatment of P_1 plants typically resulted in a further increase for the first 6 hours and was equal to or greater than C_1P_2 at the 24 h timepoint. A notable exception to these trends was the Fytosave treatment group which experienced a

drastic drop in P_1C_2 and P_1P_2 relative expression at the 6 and 24 h timepoints. Our results potentially indicate that parental treatment with BABA, BTH, MeJA, and HA results in an increased basal expression of *PR-1* mRNA in their offspring, and a possible potentiated induction of *PR-1* mRNA accumulation in response to priming treatments such as INA. Unusually, however, all of our treatment groups barring BTH had a P_1P_2 relative expression that was higher than P_1C_2 at the 0 h timepoint, even though INA could not have had an effect on *PR-1* accumulation yet.

Interestingly, parental treatment with MeJA led to an increase of *PR-1* mRNA accumulation in offspring plants. Despite the aforementioned antagonism between the SA and JA pathways, MeJA treatment has indeed been reported to lead to a same-generation increase in *PR-1* expression in wheat plants (Liu et al., 2016). However, no previous studies have examined the potential transgenerational effects of the JA pathway on *PR-1* expression. Our results here may be indicative that interactions between JA and SA pathways are species-specific.

3.7.4 Mechanisms for Transgenerational Priming Memory

Parental treatment with each of our priming agents could, to varying degrees, increase basal *PR-1* mRNA levels and potentiate *PR-1* mRNA accumulation in response to INA treatment in offspring plants. There is indeed a precedent in the literature for elicitor treatments to influence gene expression in the offspring of treated plants. Treatments with BABA and INA, an SA analogue, have both been demonstrated to transgenerationally influence *PR-1* expression in potato and common bean (Martinez-Aguilar et al., 2021, Meller et al., 2018).

A prevailing theory regarding transgenerational priming effects is that they may be due to heritable changes in methylation of the plant genome. Methylated DNA is typically associated with tightly packed heterochromatin which is inaccessible to the transcription machinery. As DNA methylation can be maintained through meiosis, it represents a possible mechanism for plants to form stress memories that can transgenerationally regulate gene expression. Luna and colleagues observed in 2012 that the offspring of *P. syringae*-inoculated Arabidopsis plants possessed primed activation of SA-dependent defence genes (Luna et al., 2012). This

transgenerational SAR phenotype was mimicked by *drm1drm2crt3*, a mutant affected in DNA methylation, indicating SAR priming may be directed by hypomethylated genes (Luna et al., 2012). Similarly, Rasmann et al also documented in 2012 that caterpillar herbivory and MeJA treatment can induce transgenerational resistance against insect herbivory (Rasmann et al., 2012). Arabidopsis mutants that were deficient in siRNA biogenesis were unable to establish transgenerational priming (Rasmann et al., 2012). As siRNAs are involved in directing changes to DNA methylation, this was further evidence that stress memories may be stored in DNA methylation patterns which can be transmitted from parent to offspring. A 2019 study also noted that BABA treatment caused heritable adjustments to the methylome in potato (Kuznicki et al., 2019). This study also observed that the methylation status of several SA-dependent defence genes was not linked to their transcription status, indicating transcription changes in genes may be due to changes in methylation of the wider transcriptional network (Kuznicki et al., 2019). The transgenerational effects observed in our study may be due to heritable reprogramming in the methylation status of *PR-1* or the network regulating *PR-1*.

4 - Effects of Transgenerational Priming on Plant Fitness

4.1 Introduction

Despite the potential benefits of elicitors in agriculture, early attempts to commercially exploit the chemical activation of plant defences were hampered by the discovery that direct activation of plant defences also resulted in severe costs to plant fitness (Yassin et al., 2021). Indeed, direct activation of both SA and JA defences with elicitors has been observed to cause several negative effects on plant growth in a variety of species, such as reduced biomass, seed production, delayed fruit-set and longer ripening times (Heil, 2000, Baldwin, 1998, Redman et al., 2001). These fitness costs can be attributed to allocation costs that occur when plants redirect their resources from growth to induced resistance, or ecological costs that occur when a plant's defence response interferes with how the plant interacts with the abiotic and biotic factors in its environment (Vos, 2013).

Priming has also been demonstrated to cause fitness costs however these costs are lower than those caused by direct activation of induced resistance (van Hulten et al., 2006, Walters, 2008). Primed plants actually appear to have better fitness than naïve plants under high disease pressure, although this is obviously undesirable for growers (van Hulten et al., 2006, Walters, 2008). In this chapter, using the same elicitors as were previously used in chapter 4, we observed if priming treatment of Micro Tom plants in the parental generation affected fitness in the offspring generation.

Experimental structure for the growth assays was identical to the resistance assays performed in chapter 3. Parental plants were given their respective treatments at 2 weeks old and then allowed to mature so seed could be collected from them. Offspring plants were again categorised into treatment groups as described in **Fig. 3.1**. To understand how priming affects the early stages of plant growth, both area of the first leaf and plant dry weight were recorded for all treatment groups when they were 3 weeks, 4 weeks, and 5 weeks old. All plants were given their respective

priming treatments at 2 weeks old. The total time in days for plants to reach their first flower was also recorded.

Fruits were collected from all plants when they were approximately 19 weeks old. Total fresh fruit weight, total seed weight, and the ratio of seed to fruit weight were recorded for each individual Micro Tom plant. These metrics would allow us to observe how our priming treatments affect not only the yield of a plant, but also the maturity of those fruits.

4.2 Fitness Assays for BABA-Treated Plants

For all 3 timepoints, P_2 plants had significantly smaller leaf area and dry weight than C_2 plants, although this negative effect of offspring treatment on plant fitness decreased over time (**Fig. 4.1 and Table 4.1**). Parental treatment alone had no significant effect on leaf area or dry weight at any timepoint (**Fig. 4.1** and **Table 4.1**) except for week 5 when parental treatment with BABA resulted in an almost significant reduction in dry weight compared to C_1 plants (**Fig. 4.1 F** and **Table 4.1**). A significant interaction between parental and offspring treatment was not observed for leaf area or dry weight at any time point except for the leaf area in week 4 when the negative effect of offspring BABA treatment was slightly reduced in P_1P_2 plants compared to C_1P_2 plants (**Fig. 4.1 B** and **Table 4.1**).

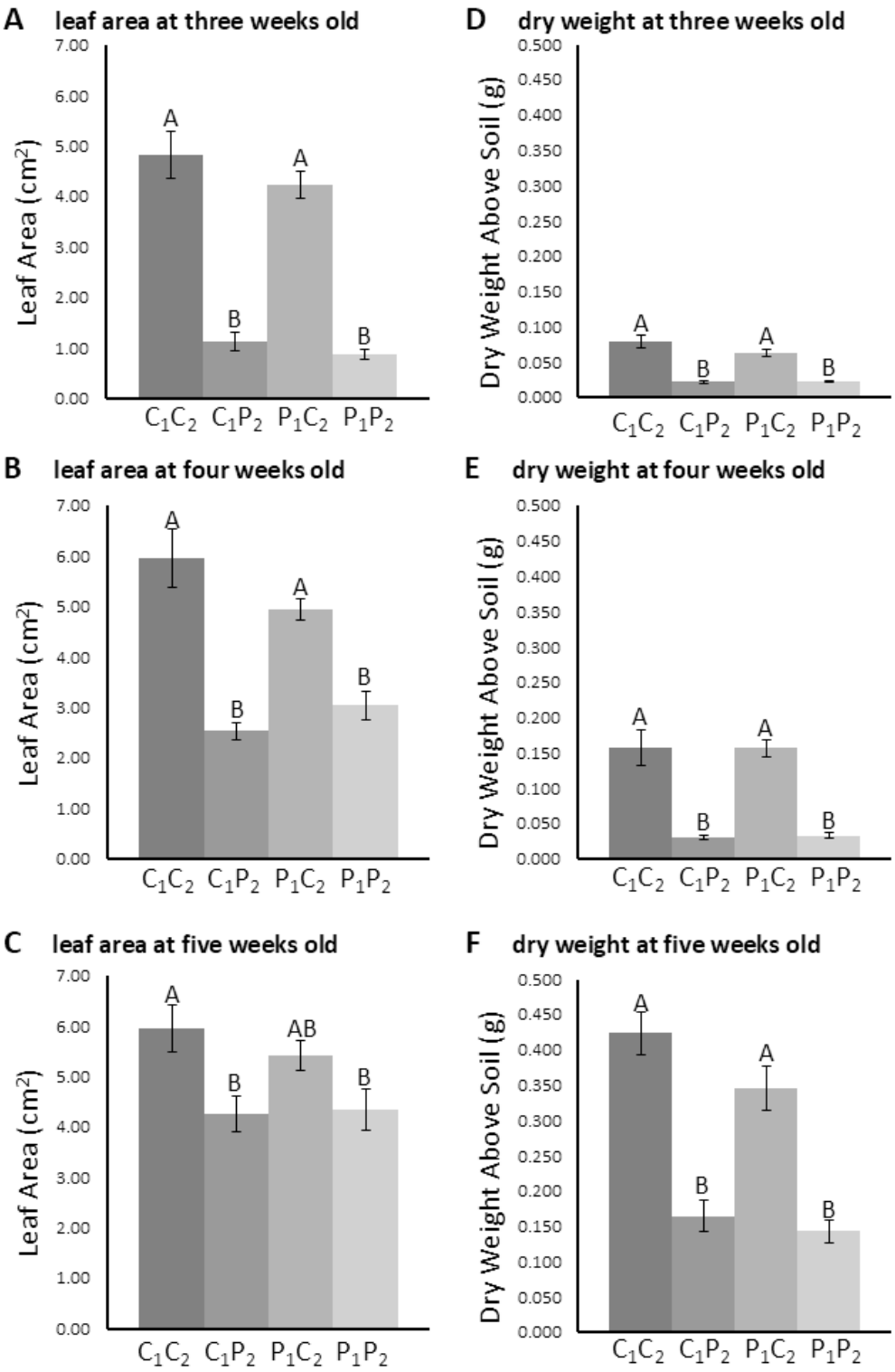

Figure 4.1. Effects of BABA priming on plant growth in young Micro Tom plants. Plants were treated at two-weeks-old with either a control treatment of an RO water root drench or a 0.5 mM BABA root drench. Measurements were taken at three-weeks-old (**A** and **D**), four-weeks-old (**B** and **E**), and five-weeks-old (**C** and **F**). Columns in graphs **A**, **B**, and **C** represent mean area of excised first leaves (cm^2) ± standard error. Columns in graphs **D**, **E**, and **F** represent mean above ground dry weight of plants in g ± standard error. n=7-9 for all experiments. Letters represent significant differences at $P<0.05$.

	Parental priming treatment	Offspring priming treatment	Parental*Offspring Interaction
Leaf Area Week 3	1, 1.97, 0.171	1, 138, **2.52×10^{-12}**	1, 0.316, 0.579
Leaf Area Week 4	1, 0.52, 0.476	1, 59.1, **2.24×10^{-8}**	1, 4.75, **0.038**
Leaf Area Week 5	1, 0.351, 0.558	1, 12.7, **1.27×10^{-3}**	1, 0.690, 0.413
Dry Weight Week 3	1, 1.58, 0.220	1, 69.0, **4.91×10^{-9}**	1, 1.86, 0.183
Dry Weight Week 4	1, 0.004, 0.949	1, 73.4, **2.41×10^{-9}**	1, 0.007, 0.932
Dry Weight Week 5	1, 3.31, 0.079	1, 70.8, **2.82×10^{-9}**	1, 1.05, 0.313

Table 4.1. ANOVA analyses (df, F, P) for parental treatments, offspring treatments, and interactions between parental and offspring treatments in growth assays in plants of the BABA treatment groups. Significant P values are bold.

Flowering time was significantly reduced in BABA P_2 plants compared to C_2 plants, but only by 2-3 days (**Fig. 4.2 A** and **Table 4.2**). Offspring treatment also significantly increased fresh fruit weight compared to C_2 plants (**Fig. 4.2 B** and **Table 4.2**). On the other hand, parental treatment caused significantly reduced fresh fruit weight compared to C_1 plants (**Fig. 4.2 A** and **Table 4.2**).

There was no effect of offspring treatment on the total seed weight, however parentally treated plants experienced an almost statistically significant overall reduction in seed weight compared to C_1 plants (**Fig. 4.2 C** and **Table 4.2**). A significant interaction between parental and offspring treatment was observed for seed weight, as offspring treatment appeared to rescue seed weight in P_1 plants and restore a seed weight closer to C_1 plants (**Fig. 4.2 C** and **Table 4.2**). Offspring treatment had a negative effect on the seed-to-fruit ratio as it was slightly lower in P_2 plants than the C_2 plants (**Fig. 4.2 D** and **Table 4.2**). However, the significant interaction which was observed for seed weight is also observed for the seed-to-fruit ratio, as parental treatment with BABA appears to rescue the seed-to-fruit ratio in P_2 plants (**Fig. 4.2 D** and **Table 4.2**).

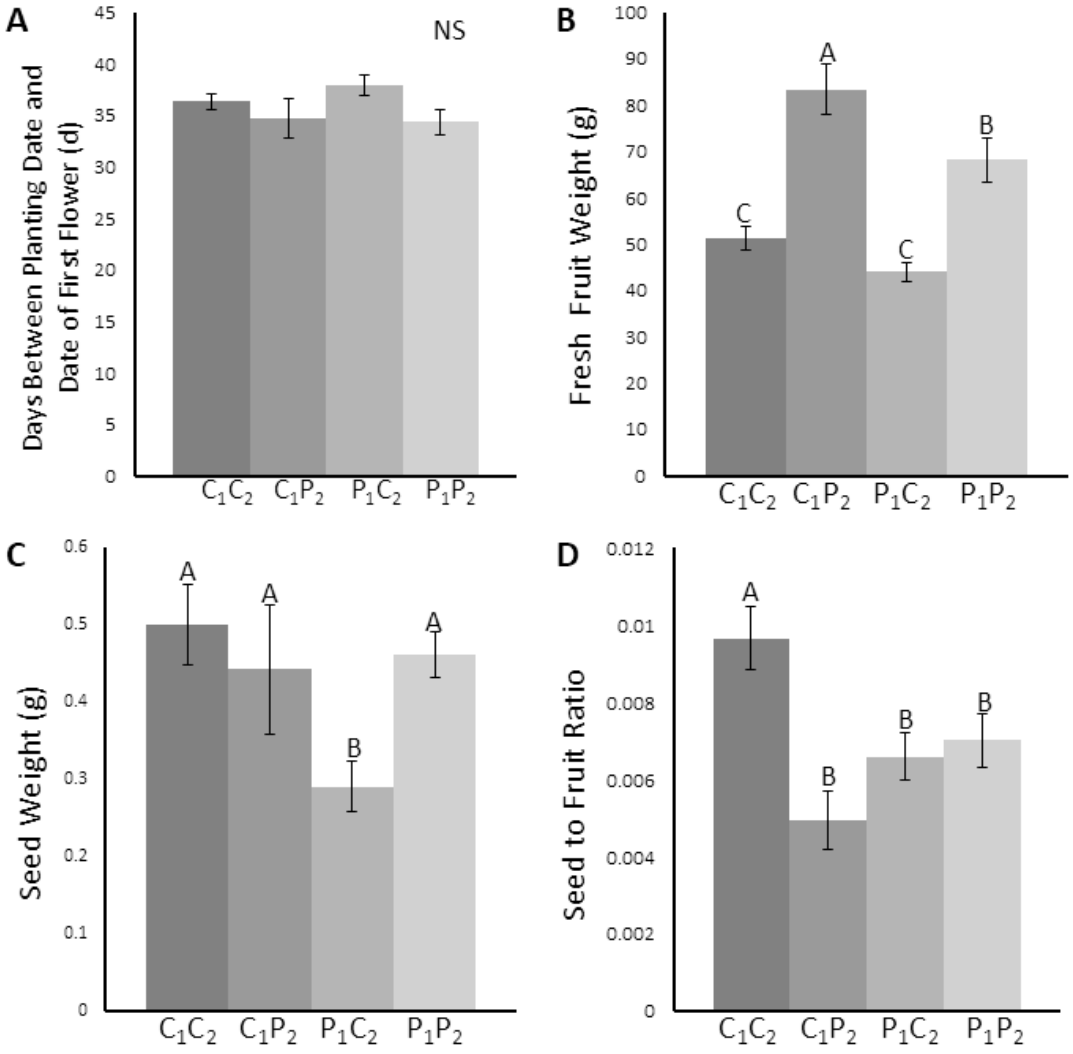

Figure 4.2. Effects of BABA priming on maturation of Micro Tom plants. Plants were treated at two-weeks-old with either a control treatment of an RO water root drench or a 0.05 mM BABA root drench. Fruit was harvested from plants at 19-weeks-old. (**A**) Time in days between date of planting and date of first flower ± standard error. (**B** and **C**) Mean weight in grams of fresh fruit or seed collected from each plant, respectively, ± standard error. (**D**) Ratio of seed weight to fresh fruit weight for each plant ± standard error. n=8-12 for all experiments. Letters represent significant differences at P<0.05. NS represents no significant differences.

	Parental priming treatment	Offspring priming treatment	Parental*Offspring Interaction
Flowering Time	1, 0.266, 0.609	1, 4.37, **0.044**	1, 0.563, 0.458
Fresh Fruit Weight	1, 9.78, **3.42 x 10^{-3}**	1, 62.4, **1.86 x 10^{-9}**	1, 1.24, 0.273
Seed Weight	1, 1.11, 0.080	1, 3.23, 0.297	1, 4.64, **0.038**
Seed to Fruit Ratio	1, 0.425, 0.518	1, 8.08, **7.26 x 10^{-3}**	1, 11.6, **1.61 x 10^{-3}**

Table 4.2. ANOVA analyses (df, F, P) for parental treatments, offspring treatments, and interactions between parental and offspring treatments in fitness assays in plants of the BABA treatment groups. Significant P values are bold.

4.3 Fitness Assays for BTH-Treated Plants

Unlike the BABA experiments, effects of BTH on dry weight and leaf area were inconsistent between the timepoints measured. In the third week of growth, parental and offspring BTH treatment both had a significant negative effect on the leaf area and dry weight of plants (**Fig. 4.3 A** and **D**, and **Table 4.3**). The leaf area of P_1 and C_1 plants responded similarly to offspring treatment, resulting in the negative effect on leaf area being intensified over two generations of priming (**Fig. 4.3 A** and **Table 4.3**). However, this intensification was not observed for dry weight as P_1P_2 plants had similar dry weight to P_1C_2 and C_1P_2 plants and a significant interaction between parental and offspring was observed in the ANOVA analysis (**Fig. 4.3 D** and **Table 4.3**). During the fourth week of growth, P_1 plants had significantly lower dry weights and leaf areas than C_1 plants (**Fig. 4.3 B** and **E**, and **Table 4.3**).

At the fifth week timepoint, P_1 plants have a significantly reduced dry weight, and an almost significantly lower leaf area compared to C_1 plants (**Fig. 4.3 C** and **F**, and **Table 4.3**). A statistically significant interaction between parental and offspring treatment was observed for both leaf area and dry weight, as offspring treatment in P_1 plants resulted in a partially rescued phenotype (**Fig. 4.3 C** and **F**, and **Table 4.3**).

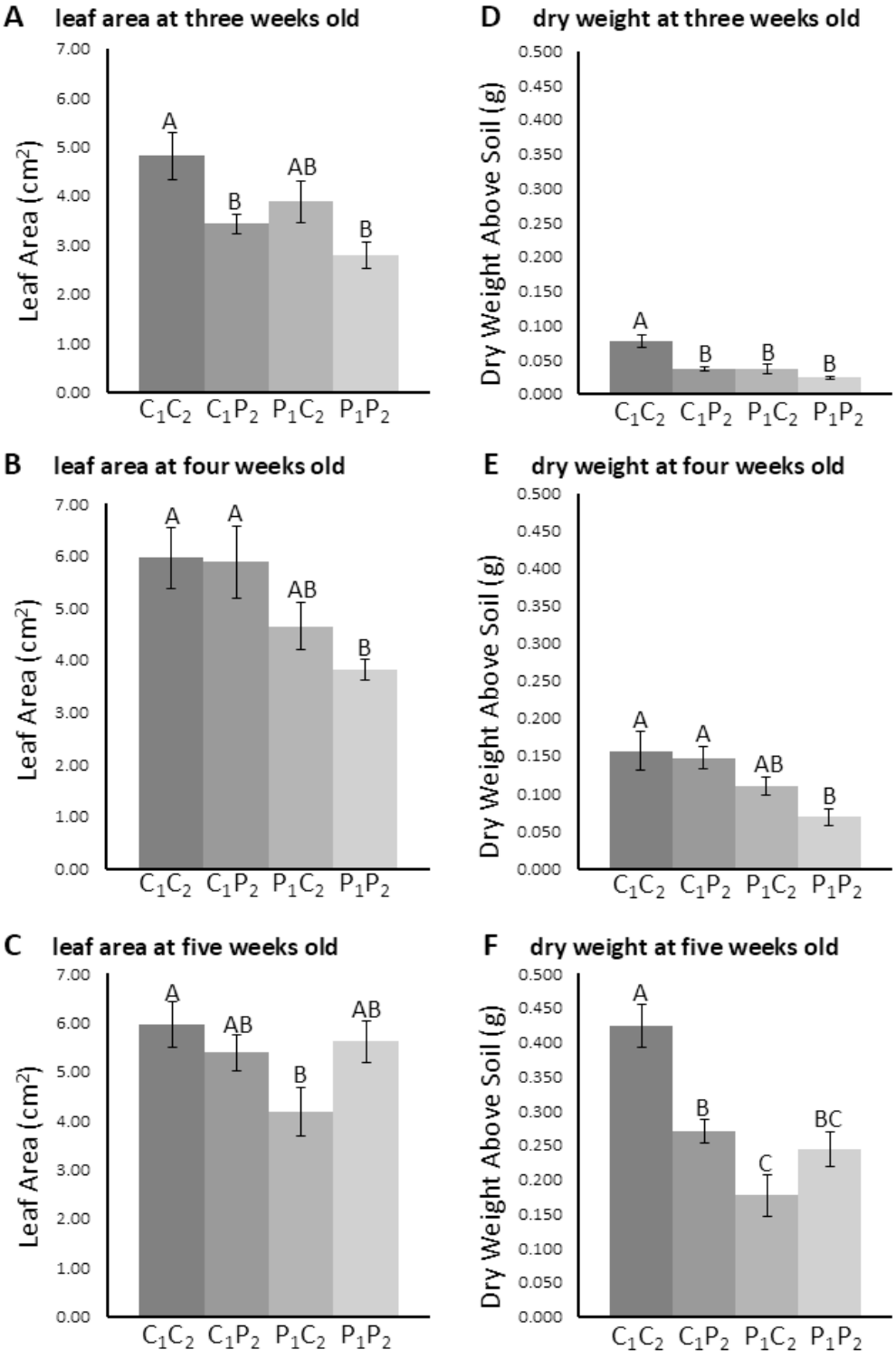

Figure 4.3. Effects of BTH priming on plant growth in young Micro Tom plants. Plants were treated at two-weeks-old with either a control treatment of an RO water root drench or a 1 mM BTH spray. Measurements were taken at three-weeks-old (**A** and **D**), four-weeks-old (**B** and **E**), and five-weeks-old (**C** and **F**). Columns in graphs **A**, **B**, and **C** represent mean area of excised first leaves in cm^2 ± standard error. Columns in graphs **D**, **E**, and **F** represent mean above ground dry weight of plants in g ± standard error. n=7-8 for **A**, **D**, **B** and **E**; n=8-9 for **C** and **F**. Letters represent significant differences at $P<0.05$.

	Parental priming treatment	Offspring priming treatment	Parental*Offspring Interaction
Leaf Area Week 3	1, 4.72, **0.039**	1, 11.6, **2.15×10^{-3}**	1, 0.173, 0.681
Leaf Area Week 4	1, 9.94, **4.05×10^{-3}**	1, 0.733,0.400	1, 0.513, 0.480
Leaf Area Week 5	1, 3.09, 0.090	1, 0.956, 0.336	1, 5.28, **0.029**
Dry Weight Week 3	1, 19.2, **1.73×10^{-4}**	1, 20.0, **1.36×10^{-4}**	1, 5.57, **0.026**
Dry Weight Week 4	1, 12.8, **1.40×10^{-3}**	1, 2.06, 0.163	1, 0.829, 0.371
Dry Weight Week 5	1, 21.6, **6.85×10^{-5}**	1, 2.12, 0.156	1, 14.0, **8.18×10^{-4}**

Table 4.3. ANOVA analyses (df, F, P) for parental treatments, offspring treatments, and interactions between parental and offspring treatments in growth assays in plants of the BTH treatment groups. Significant P values are bold.

Neither parental nor offspring BTH treatment was found to have a significant effect on flowering time (**Fig. 4.4 A** and **Table 4.4**). They were also not found to have a significant effect on the total seed weight or seed to fruit ratio of treated plants (**Fig. 4.4 C** and **D**, **Table 4.4**). Offspring treatment caused a significant increase in fresh fruit weight however this increase was significantly reduced in P_1 plants (**Fig. 4.4 B**).

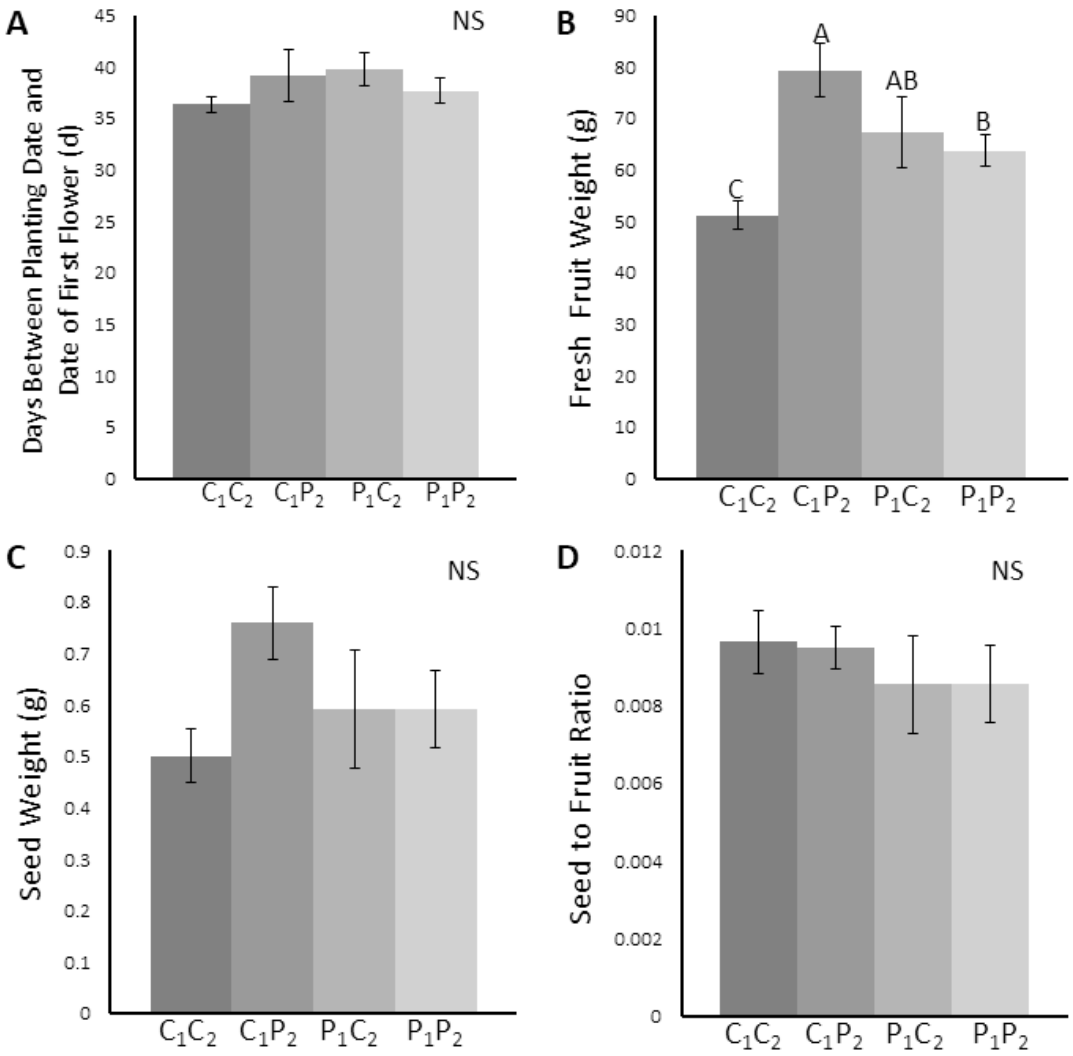

Figure 4.4. Effects of BTH priming on maturation of Micro Tom plants. Plants were treated at two-weeks-old with either a control treatment of an RO water root drench or a 1 mM BTH spray. Fruit was harvested from plants at 19-weeks-old. (**A**) Time in days between date of planting and date of first flower ± standard error. (**B** and **C**) Mean weight in grams of fresh fruit or seed collected from each plant, respectively, ± standard error. (**D**) Ratio of seed weight to fresh fruit weight for each plant ± standard error. n=7-14 for **A**; n=7-12 for **B**, **C** and **D**. Letters represent significant differences at $P<0.05$. NS represents no significant differences.

	Parental priming treatment	Offspring priming treatment	Parental*Offspring Interaction
Flowering Time	1, 0.381, 0.541	1, 0.46, 0.830	1, 2.37, 0.132
Fresh Fruit Weight	1, 3.05×10^{-3}, 0.956	1, 7.80, $\mathbf{8.76 \times 10^{-3}}$	1, 13.0, $\mathbf{1.05 \times 10^{-3}}$
Seed Weight	1, 0.220, 0.642	1, 2.57, 0.119	1, 2.57, 0.119
Seed to Fruit Ratio	1, 0.551, 0.463	1, 0.045, 0.832	1, 0.131, 0.720

Table 4.4. ANOVA analyses (df, F, P) for parental treatments, offspring treatments, and interactions between parental and offspring treatments in fitness assays in plants of the BTH treatment groups. Significant P values are bold.

4.4 Fitness Assays for HA-Treated Plants

During the third week of growth, P_2 HA-treated plants had both significantly lower dry weight and leaf area than their C_2 counterparts (**Fig. 4.5 A** and **D**, **Table 4.5**). Parental treatment had no effect on leaf area or dry weight (**Fig. 4.5 A** and **D**, **Table 4.5**). However, a significant interaction between parental and offspring treatment was observed, as the reduction in growth caused by offspring HA treatment was much lower in P_1 plants than C_1 plants (**Fig. 4.5 A** and **D**, **Table 4.5**).

At the week 4 timepoint, offspring treatment was not found to have a significant effect on leaf area, although parental treatment had an almost statistically significant effect (**Fig. 4.5 B** and **Table 4.5**). Conversely, parental treatment had no effect on the dry weight of plants, but offspring treatment was observed to have an almost significant effect on dry weight (**Fig. 4.5 E** and **Table 4.5**).

During week 5, no effects of parental treatment on leaf area were observed, however P_2 plants had significantly lower leaf area than C_2 plants (**Fig. 4.5 E** and **Table 4.5**). Both parental and offspring HA treatment were observed to have significantly lower dry weights than their control counterparts (**Fig. 4.5 F** and **Table 4.5**). A significant interaction between parental and offspring treatment was also observed for dry weight in week 5 however, as parental treatment appeared to inhibit growth less when offspring plants were also treated with HA (**Fig. 4.5 F** and **Table 4.5**).

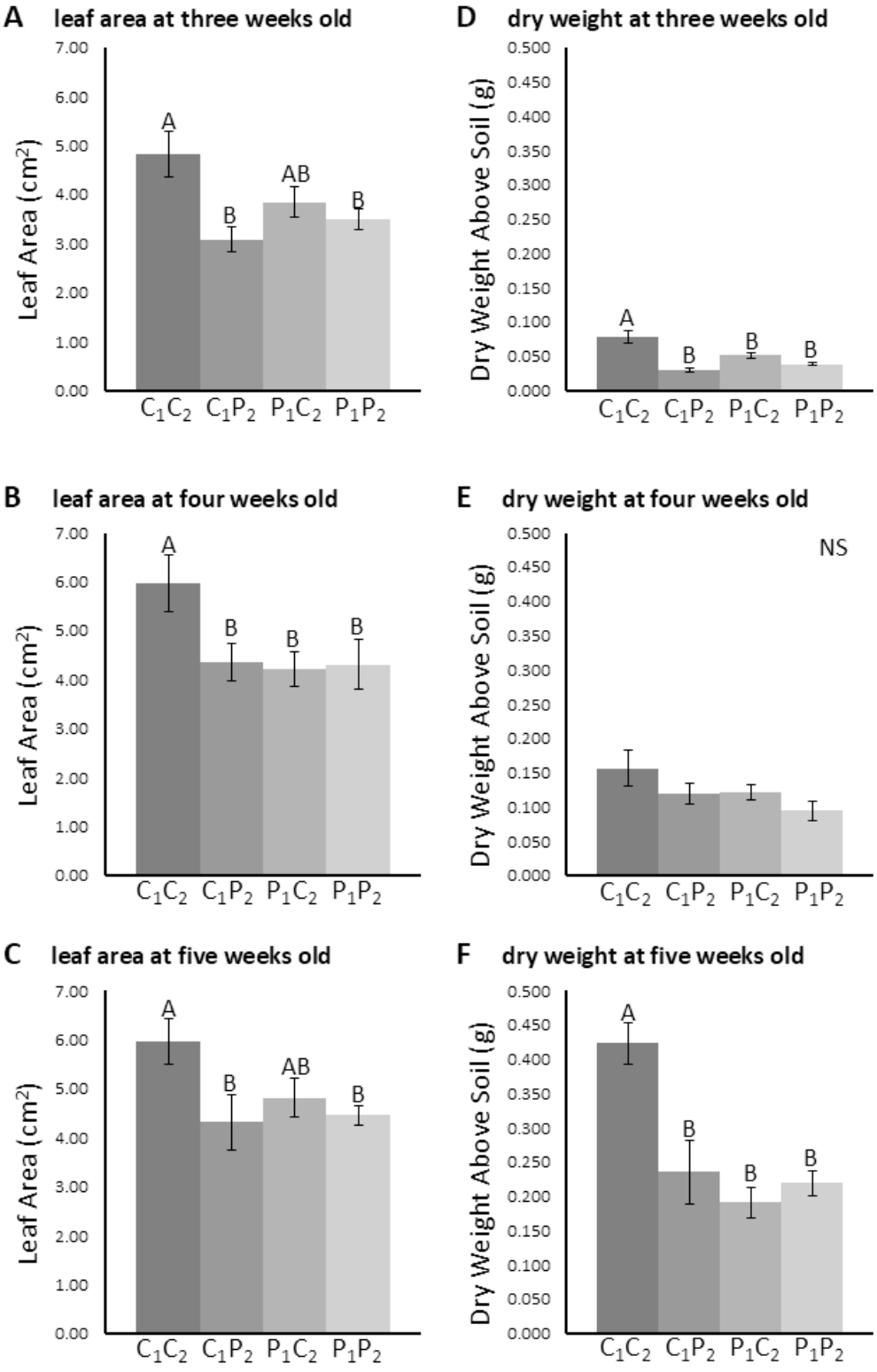

Figure 4.5. Effects of HA priming on plant growth in young Micro Tom plants. Plants were treated at two-weeks-old with either a control treatment of an RO water root drench or a 0.5 mM HA root drench. Measurements were taken at three-weeks-old (**A** and **D**), four-weeks-old (**B** and **E**), and five-weeks-old (**C** and **F**). Columns in graphs **A**, **B**, and **C** represent mean area of excised first leaves in cm^2 ± standard error. Columns in graphs **D**, **E**, and **F** represent mean above ground dry weight of plants in g ± standard error. n=6-8 for **A** and **D**; n=7-9 for **B**, **C**, **E** and **F**. Letters represent significant differences at $P<0.05$. NS represents no significant differences.

	Parental priming treatment	Offspring priming treatment	Parental*Offspring Interaction
Leaf Area Week 3	1, 0.672, 0.420	1, 9.33, **5.00×10^{-3}**	1, 4.13, 0.053
Leaf Area Week 4	1, 3.72, 0.064	1, 2.57, 0.120	1, 3.28, 0.081
Leaf Area Week 5	1, 1.33, 0.259	1, 5.23, **0.030**	1, 2.18, 0.151
Dry Weight Week 3	1, 2.41, 0.133	1, 28.4, **1.58×10^{-5}**	1, 9.81, **4.00×10^{-3}**
Dry Weight Week 4	1, 2.76, 0.108	1, 3.25, 0.082	1, 0.079, 0.781
Dry Weight Week 5	1, 15.8, **4.71×10^{-4}**	1, 6.54, **0.016**	1, 12.1, **1.76×10^{-3}**

Table 4.5. ANOVA analyses (df, F, P) for parental treatments, offspring treatments, and interactions between parental and offspring treatments in growth assays in plants of the HA treatment groups. Significant P values are bold.

No effect of offspring HA treatment was observed on flowering time, however P_1 plants took significantly longer to flower than C_1 plants (**Fig. 4.6 A** and **Table 4.6**). Similarly, P_1 plants had significantly higher total fresh fruit weights than C_1 plants, whilst offspring treatment had no effect on fresh fruit weight (**Fig. 4.6 B** and **Table 4.6**).

Neither parental nor offspring HA treatment had any significant effect on total seed weight or the ratio of seed to fruit (**Fig. 4.6 C** and **D**, **Table 4.6**).

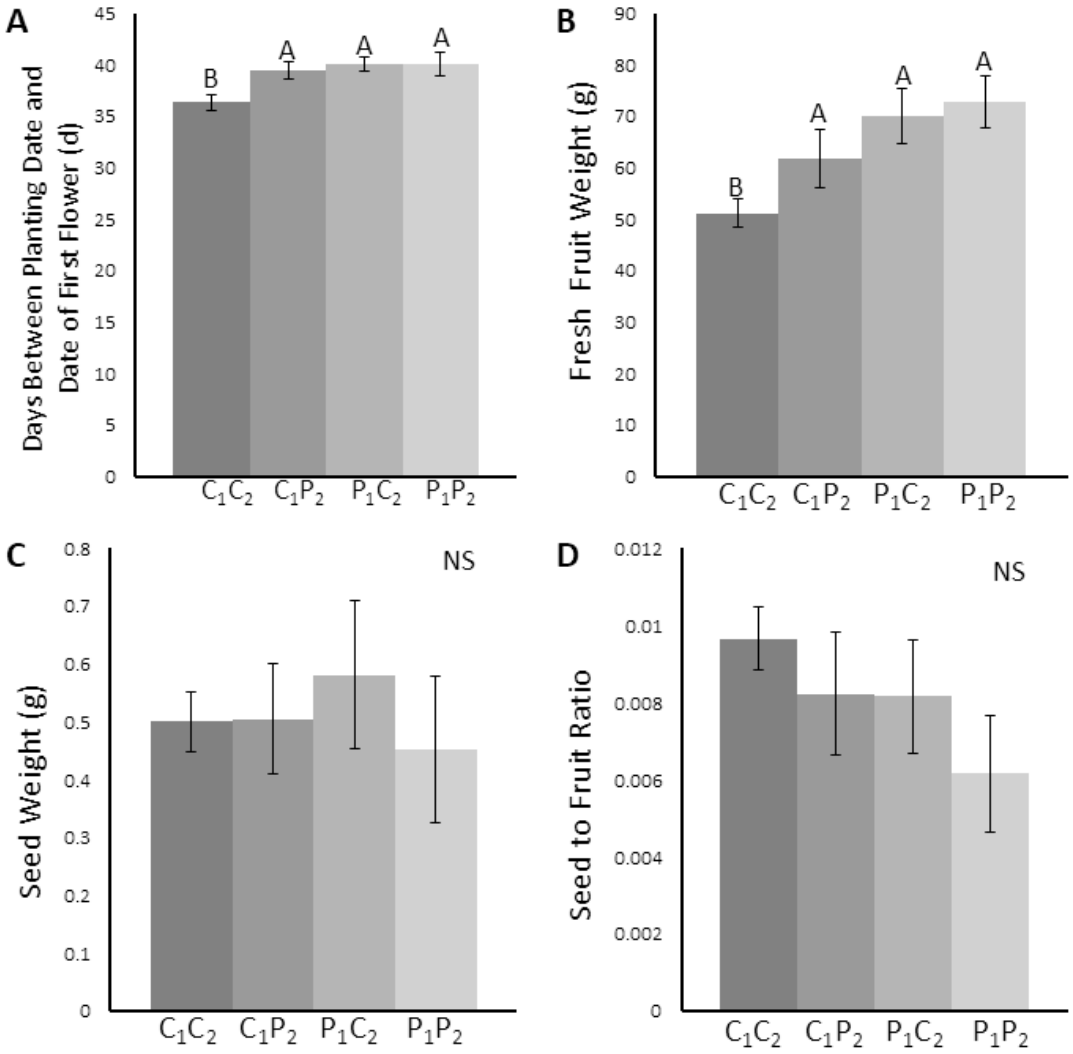

Figure 4.6. Effects of HA priming on maturation of Micro Tom plants. Plants were treated at two-weeks-old with either a control treatment of an RO water root drench or a 0.5 mM HA root drench. Fruit was harvested from plants at 19-weeks-old. (**A**) Time in days between date of planting and date of first flower ± standard error. (**B** and **C**) Mean weight in grams of fresh fruit or seed collected from each plant, respectively, ± standard error. (**D**) Ratio of seed weight to fresh fruit weight for each plant ± standard error. n=6-12 for all experiments. Letters represent significant differences at $P<0.05$. NS represents no significant differences.

	Parental priming treatment	Offspring priming treatment	Parental*Offspring Interaction
Flowering Time	1, 5.04, **0.033**	1, 2.69, 0.112	1, 2.69, 0.112
Fresh Fruit Weight	1, 10.9, **2.52×10^{-3}**	1, 2.21, 0.148	1, 0.739, 0.397
Seed Weight	1, 0.022, 0.883	1, 0.418, 0.523	1, 0.471, 0.498
Seed to Fruit Ratio	1, 1.84, 0.185	1, 1.69, 0.203	1, 0.50, 0.824

Table 4.6. ANOVA analyses (df, F, P) for parental treatments, offspring treatments, and interactions between parental and offspring treatments in fitness assays in plants of the HA treatment groups. Significant P values are bold.

4.5 Fitness Assays for MeJA-Treated Plants

Effects of MeJA treatment on young plant growth were consistent between all three of the timepoints measured. Parental treatment was not observed to have a significant effect on leaf area or dry weight, however offspring treatment consistently caused significant reductions in leaf area and dry weight (**Fig. 4.7** and **Table 4.7**). Additionally, a significant interaction between parental and offspring treatment was observed for both leaf area and dry weight at the week 3 timepoint, parental priming appeared to rescue leaf area and dry weight in P_2 plants (**Fig. 4.7 A** and **D**, **Table 4.7**).

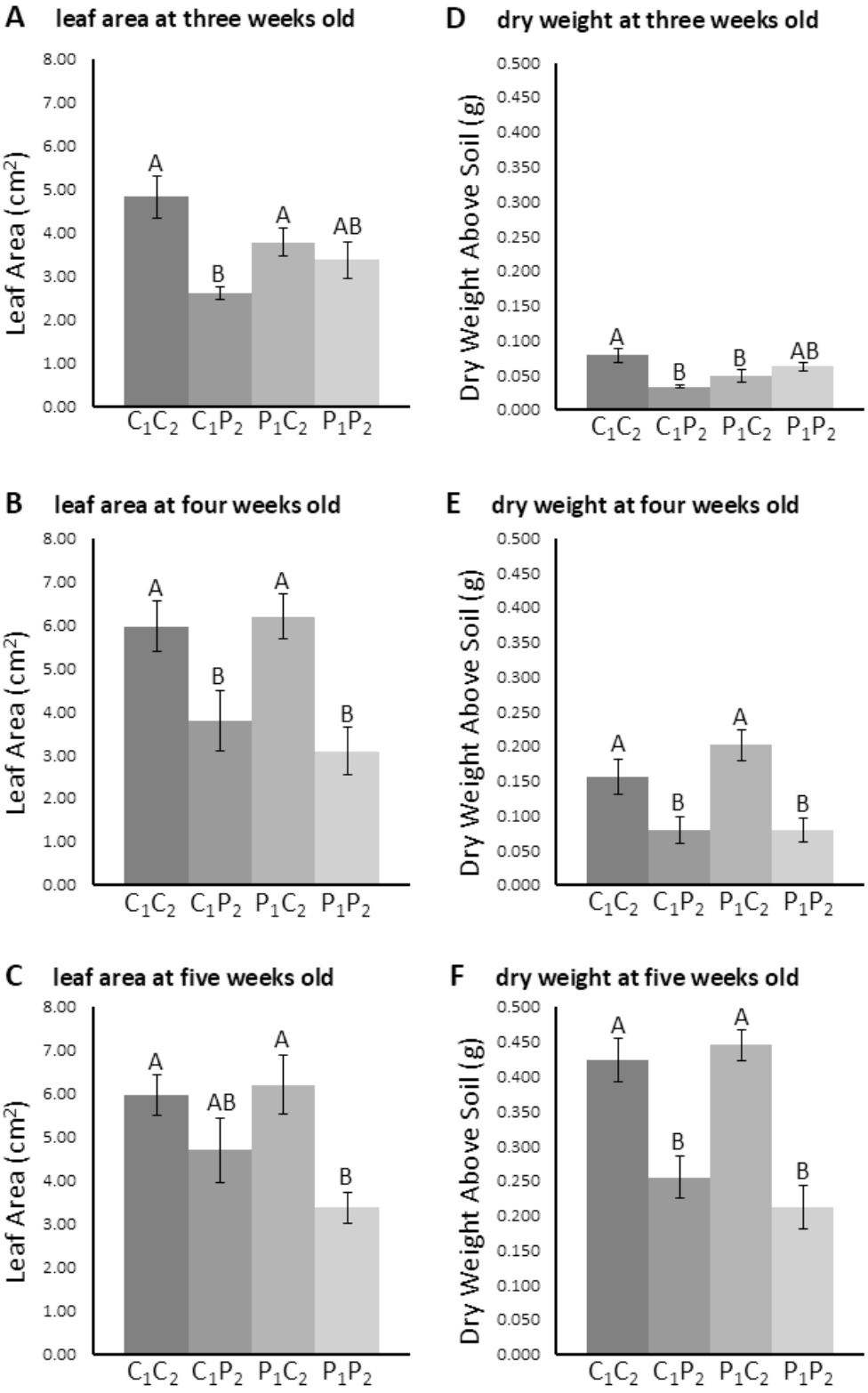

Figure 4.7. Effects of MeJA priming on plant growth in young Micro Tom plants. Plants were treated at two-weeks-old with either a control treatment of an RO water root drench or a 0.05 mM MeJA root drench. Measurements were taken at three-weeks-old (**A** and **D**), four-weeks-old (**B** and **E**), and five-weeks-old (**C** and **F**). Columns in graphs **A**, **B**, and **C** represent mean area of excised first leaves in cm^2 ± standard error. Columns in graphs **D**, **E**, and **F** represent mean above ground dry weight of plants in g ± standard error. n=7-8 for **A** and **D**; n=8-9 for **B** and **E**; n=7-9 for **C** and **F**. Letters represent significant differences at $P<0.05$.

	Parental priming treatment	Offspring priming treatment	Parental*Offspring Interaction
Leaf Area Week 3	1, 0.143, 0.708	1, 12.6, **1.50×10^{-3}**	1, 5.88, **0.023**
Leaf Area Week 4	1, 0.165, 0.687	1, 20.6, **8.57×10^{-5}**	1, 0.633, 0.432
Leaf Area Week 5	1, 0.904, 0.350	1, 13.2, **1.08×10^{-3}**	1, 1.93, 0.175
Dry Weight Week 3	1, 6.83×10^{-4}, 0.979	1, 4.25, **0.049**	1, 14.9, **6.65×10^{-4}**
Dry Weight Week 4	1, 1.10, 0.304	1, 21.9, **5.71×10^{-5}**	1, 1.14, 0.293
Dry Weight Week 5	1,0.129, 0.722	1, 45.1, **2.29×10^{-7}**	1, 1.18, 0.287

Table 4.7. ANOVA analyses (df, F, P) for parental treatments, offspring treatments, and interactions between parental and offspring treatments in fitness assays in plants of the HA treatment groups. Significant P values are bold.

Offspring MeJA treatment caused a significant increase in the flowering time of Micro Tom plants (**Fig. 4.8 A** and **Table 4.8**). However, parental treatment significantly reduced this effect, and lowered it closer to that in C_2 plants (**Fig. 4.8 A** and **Table 4.8**). Parental treatment caused significant reductions in fresh fruit weight, total seed weight, and the ratio of seed weight to fruit weight (**Fig. 4.8 B, C**, and **D,** and **Table 4.8**). Offspring MeJA treatment had no effect on any of these metrics in C_1 plants, however it did rescue seed weight and seed to fruit ratio in P_1 plants (**Fig. 4.8 B, C**, and **D,** and **Table 4.8**).

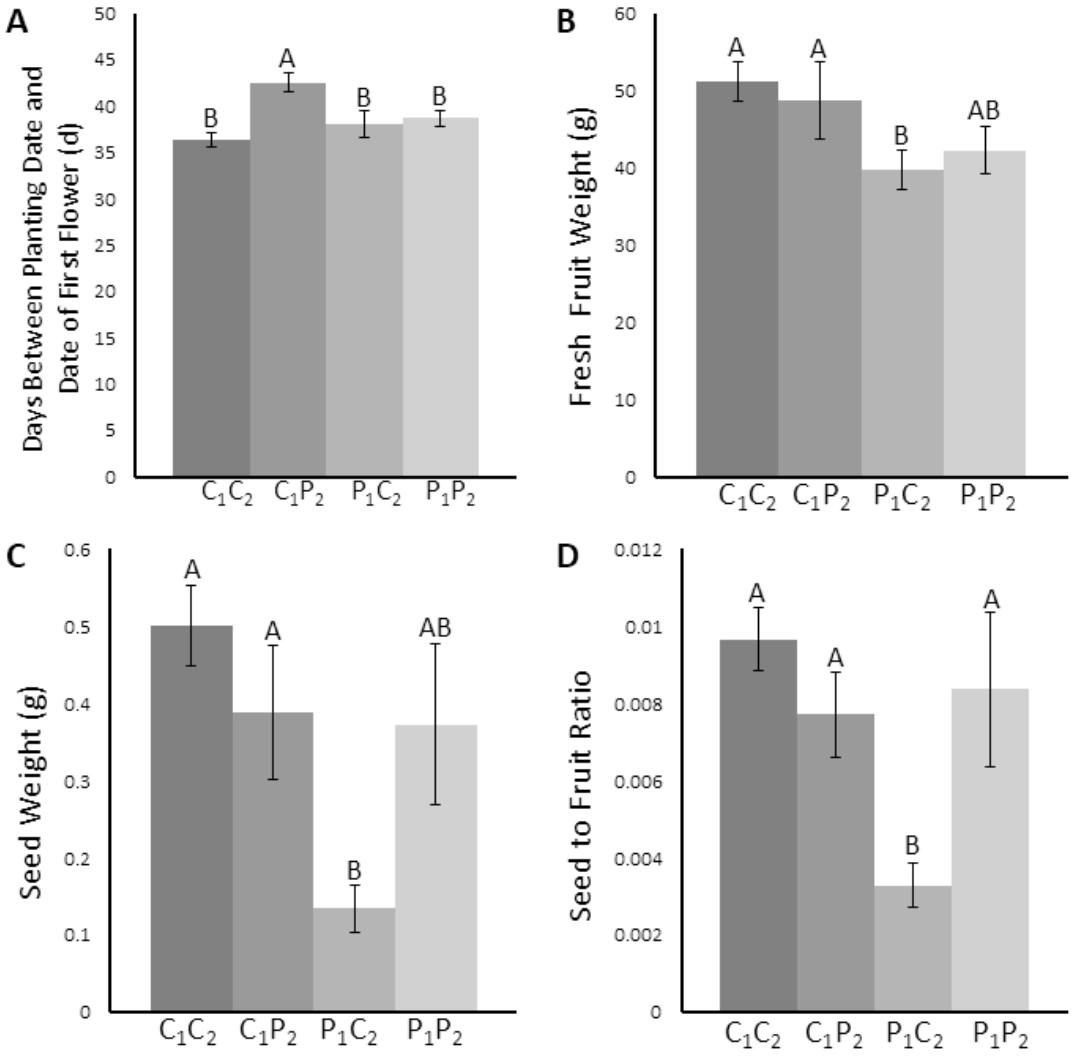

Figure 4.8. Effects of MeJA priming on maturation of Micro Tom plants. Plants were treated at two-weeks-old with either a control treatment of an RO water root drench or a 0.05 mM MeJA root drench. Fruit was harvested from plants at 19-weeks-old. (**A**) Time in days between date of planting and date of first flower ± standard error. (**B** and **C**) Mean weight in grams of fresh fruit or seed collected from each plant, respectively, ± standard error. (**D**) Ratio of seed weight to fresh fruit weight for each plant ± standard error. n=8-12 for all experiments. Letters represent significant differences at $P<0.05$.

	Parental priming treatment	Offspring priming treatment	Parental*Offspring Interaction
Flowering Time	1, 0.958, 0.335	1, 9.21, **4.59×10^{-3}**	1,6.00, **0.020**
Fresh Fruit Weight	1, 6.73, **0.014**	1, 4.07×10^{-8}, 1.000	1, 0.535, 0.469
Seed Weight	1, 6.65, **0.014**	1, 0.724, 0.401	1, 5.65, **0.023**
Seed to Fruit Ratio	1, 5.39, **0.026**	1, 1.64, 0.209	1, 8.15, **7.29×10^{-3}**

Table 4.8. ANOVA analyses (df, F, P) for parental treatments, offspring treatments, and interactions between parental and offspring treatments in fitness assays in plants of the MeJA treatment groups. Significant P values are bold.

4.6 Fitness Assays for Fytosave-Treated Plants

At the week 3 growth timepoint, no effect of offspring treatment was observed on leaf area, however, P_1 plants had significantly reduced leaf area compared to C_1 plants (**Fig. 4.9 A** and **Table 4.9**). Both P_1 and P_2 plants had reduced dry weight compared to their respective controls (**Fig. 4.9 D** and **Table 4.9**).

During the fourth week of growth, P_1 and P_2 plants both experienced significantly reduced leaf area (**Fig. 4.9 B** and **Table 4.9**). However, only P_1 plants experienced a significant reduction in dry weight, and offspring treatment partially rescued dry weight in parentally treated plants (**Fig. 4.9 E** and **Table 4.9**).

By week 5, no significant effects of either parental or offspring Fytosave treatment were observed (**Fig. 4.9 C** and **Table 4.9**). However, both parental and offspring treatment still resulted in a significant reduction in dry weight (**Fig. 4.9 F** and **Table 4.9**).

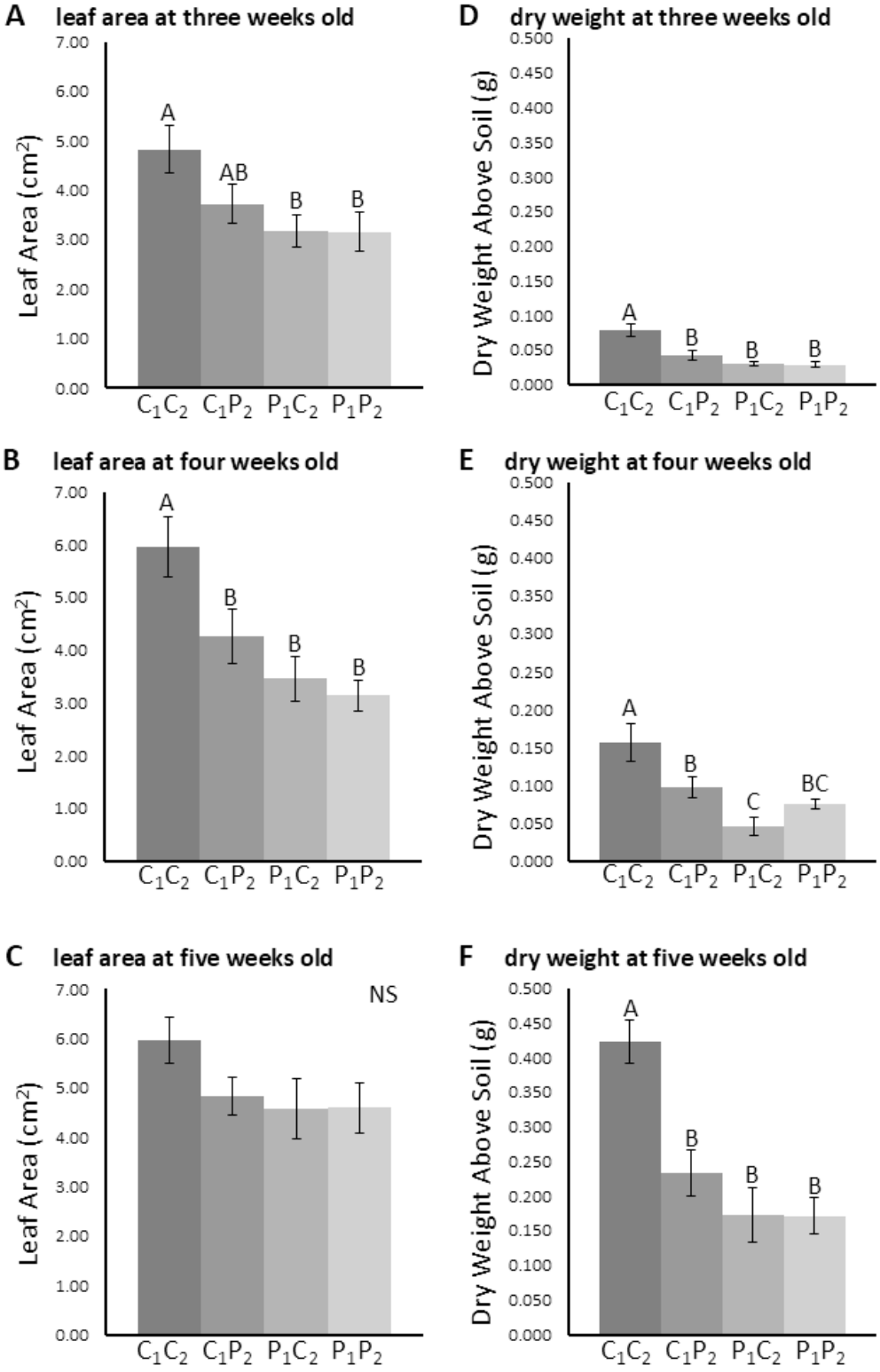

Figure 4.9. Effects of Fytosave priming on plant growth in young Micro Tom plants. Plants were treated at two-weeks-old with either a control treatment of an RO water root drench or a Fytosave spray. Measurements were taken at three-weeks-old (**A** and **D**), four-weeks-old (**B** and **E**), and five-weeks-old (**C** and **F**). Columns in graphs **A**, **B**, and **C** represent mean area of excised first leaves in cm^2 ± standard error. Columns in graphs **D**, **E**, and **F** represent mean above ground dry weight of plants in g ± standard error. n=7-8 for **A**, **B**, **D** and **E**; n=7-9 for **C** and **F**. Letters represent significant differences at $P<0.05$. NS represents no significant differences.

	Parental priming treatment	Offspring priming treatment	Parental*Offspring Interaction
Leaf Area Week 3	1, 7.71, **9.88×10^{-3}**	1, 2.00, 0.173	1, 1.83, 0.187
Leaf Area Week 4	1, 14.2, **8.49×10^{-4}**	1, 4.39, **0.046**	1, 2.08, 0.161
Leaf Area Week 5	1, 2.77, 0.108	1, 1.26, 0.272	1, 1.40, 0.248
Dry Weight Week 3	1, 23.1, **5.14×10^{-5}**	1, 8.69, **6.53×10^{-3}**	1, 7.00, **0.013**
Dry Weight Week 4	1, 16.2, **4.40×10^{-4}**	1, 0.850, 0.365	1, 6.92, **0.014**
Dry Weight Week 5	1, 22.6, **5.83×10^{-5}**	1, 8.50, **7.05×10^{-3}**	1, 8.25, **7.83×10^{-3}**

Table 4.9. ANOVA analyses (df, F, P) for parental treatments, offspring treatments, and interactions between parental and offspring treatments in fitness assays in plants of the Fytosave treatment groups. Significant P values are bold.

Offspring Fytosave treatment significantly increased the time taken for Micro Tom plants to flower (**Fig. 4.10 A** and **Table 4.10**). Parental treatment by itself had no significant effect, however, the addition of parental treatment partially rescued flowering time in P_2 plants (**Fig. 4.10 A** and **Table 4.10**).

Conversely, parental treatment, but not offspring treatment, significantly increased fresh fruit weight overall (**Fig. 4.10 B** and **Table 4.10**). However, there was also a significant interaction (Table 4.10). The data suggests that offspring treatment had opposing effects depending on parent treatment, increasing fresh fruit weight in C_1 plants but reducing it in P_1 (**Fig. 4.10 B**).

There was also a significant interaction between treatments on total seed weights. Parental and offspring treatments both incresed seed weight over C_1C_2 controls, but there was no additive effect, such that offspring treatment increased seed yield in C_1 but not P_1 plants (**Fig. 4.10 C** and **Table 4.10**). Offspring priming treatment also caused a significant increase in the ratio of seed weight to fruit weight (**Fig. 4.10 D** and **Table 4.10**). Parental treatment by itself had no significant effect on seed to fruit ratio, however parental treatment of P_2 plants reduced the seed to fruit ratio closer that of control plants (**Fig. 4.10 D** and **Table 4.10**).

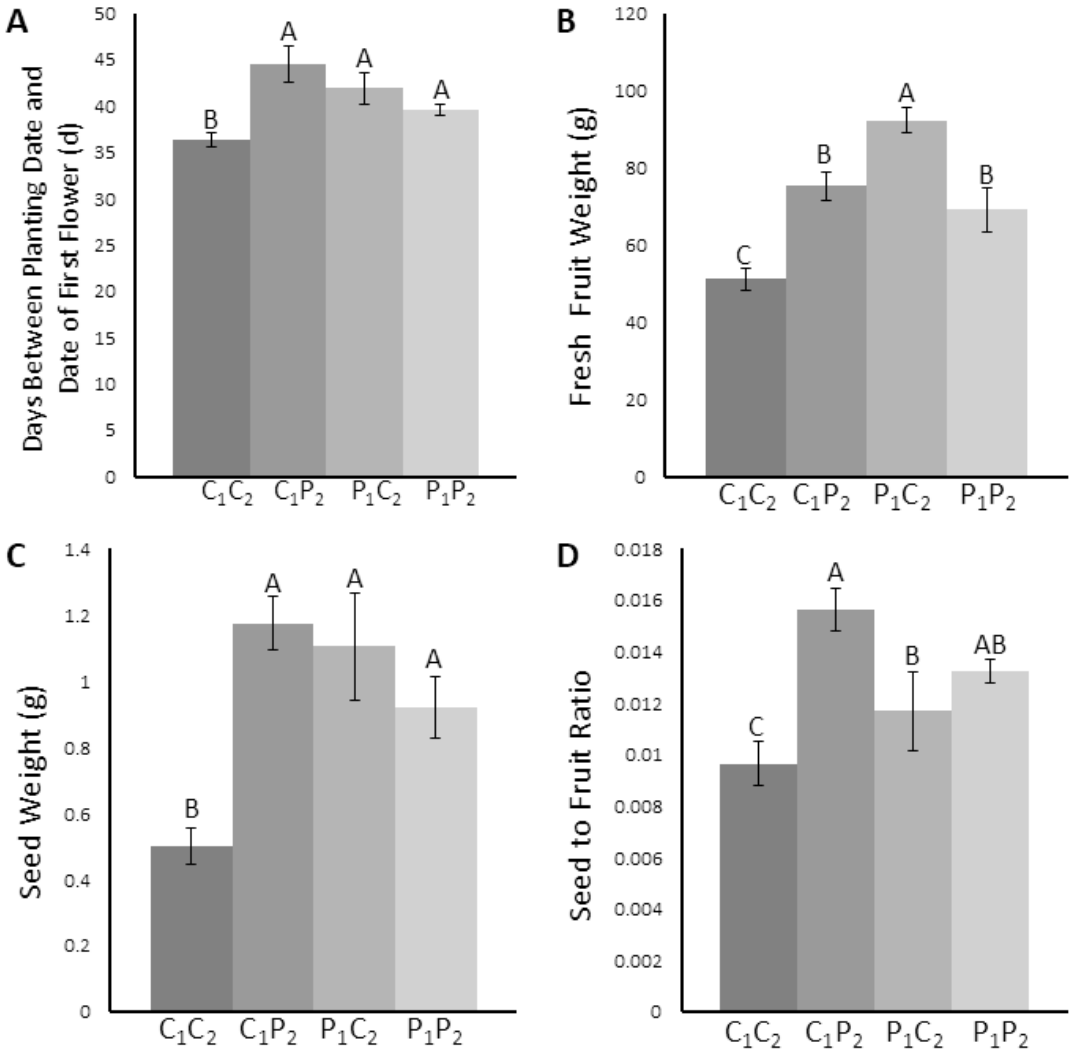

Figure 4.10. Effects of Fytosave priming on maturation of Micro Tom plants. Plants were treated at two-weeks-old with either a control treatment of an RO water root drench or a Fytosave spray. Fruit was harvested from plants at 19-weeks-old. (**A**) Time in days between date of planting and date of first flower ± standard error. (**B** and **C**) Mean weight in grams of fresh fruit or seed collected from each plant, respectively, ± standard error. (**D**) Ratio of seed weight to fresh fruit weight for each plant ± standard error. n=7-12 for **A**; n=8-12 for **B**, **C** and **D**. Lettering represents significant differences at P<0.05.

	Parental priming treatment	Offspring priming treatment	Parental*Offspring Interaction
Flowering Time	1, 0.054, 0.818	1, 4.49, **0.042**	1, 14.5, **5.77×10^{-4}**
Fresh Fruit Weight	1, 20.9, **6.97×10^{-5}**	1, 0.015, 0.903	1, 38.2, **6.40×10^{-7}**
Seed Weight	1, 3.18, 0.084	1, 6.23, **0.018**	1, 19.0, **1.26×10^{-4}**
Seed to Fruit Ratio	1, 0.034, 0.855	1, 14.5, **6.08×10^{-4}**	1, 5.03, **0.032**

Table 4.10. ANOVA analyses (df, F, P) for parental treatments, offspring treatments, and interactions between parental and offspring treatments in fitness assays in plants of the Fytosave treatment groups. Significant P values are bold.

4.7 Discussion

In this chapter, we analysed the effects of both parental and offspring generation treatment with each of our elicitors on the early vegetative growth and fruit production of Micro Tom plants. In order to analyse the effects of priming treatment on the early vegetative growth, both above ground dry weight and area of the first leaf were measured for each plant. These measurements were taken once a week starting at the third week after planting, as this was a full week after initial priming treatment, and continued until 5 weeks after planting, as many Micro Tom plants at this age start to mature and produce their first flowers.

Aside from effects on vegetative growth, we were also interested in the effects of elicitor treatment on the generative growth and reproductive maturation of Micro Tom plants. Therefore, we measured the time taken for Micro Tom plants in each of our treatment groups to reach first flower, as well as the total weight of their fresh fruit and seed at approximately 19 weeks after planting. Additionally, the ratio of seed weight to fruit weight was recorded for each plant, as some nuanced observations are not possible from just the individual seed weights and fruit weights. For example, a treatment group may have a relatively high fresh fruit weight but also have a high proportion of green fruits, indicating a delay in the maturation of its fruit. This insight would not be available when looking at just the fruit weight or seed weight.

Despite initial attempts to exploit plants's natural defences for crop protection, initial attempts at commercialising elicitors were unsuccessful due to the fitness costs incurred when directly activating plant defences (Yassin et al., 2021). Priming has attracted commercial interest because it incurs less costs to plant fitness and can even provide significant fitness benefits under disease pressure (Baldwin, 1998, van Hulten et al., 2006). If these costs are mainly incurred in the generation that received elicitor treatment, we would expect the fitness costs of parental priming in our experiment to be less than offspring priming.

As with the *P. syringae* and *B. cinerea* resistance assays in chapter 3, all plants in these growth assays were given their respective priming treatments at two weeks

after planting. In order to enable easy visualisation and permit comparisons of our growth assay data, results for all growth assay experiments are summarised in **Table 4.11**.

	Leaf Area		Dry Weight		Flowering Time		Fresh Fruit Weight		Seed Weight		Seed:Fruit Weight Ratio	
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
BABA	N	Y	N	Y	N	Y	Y	Y	Y	N	N	Y
MeJA	N	Y	N	Y	N	Y	Y	N	Y	N	N	N
HA	N	Y	N	Y	Y	N	Y	N	N	N	N	N
BTH	Y	N	Y	N	N	N	N	Y	N	N	Y	N
Fytosave	Y	N	Y	Y	N	Y	Y	N	N	Y	N	Y

Table 4.11. Summary of outcomes of parental (P_1) or offspring (P_2) treatment with each priming agent on the first leaf area, dry weight, flowering time, fresh fruit weight, seed weight, and seed to fruit weight ratio of offspring plants. 'N' denotes no significant effect and 'Y' denotes a significant effect of treatment. Blue and red highlighting respectively denote a positive or negative effect of treatment (i.e. larger or smaller than C_1C_2 plants).

We observed that treatment with each elicitor delayed vegetative growth in the first 3 weeks of growth, however this occurred only when applied either to parent plants or to offspring, but not both. For example, leaf area was decreased following offspring, but not parental treatment with BABA, MeJA and HA, and following parental, but not offspring treatment for BTH and Fytosave. The exception to this trend was the Fytosave treatment, which reduced biomass with both parental and offspring treatment. Effects on leaf area and dry weight in parental and offspring generations were almost exactly the same for each treatment.

Priming treatment in either the parental or offspring generation was also capable of causing reductions or increases in flowering time of Micro Tom plants, although these changes did not necessarily correlate with changes in leaf area and dry weight. For example, treatment in the offspring generation with BABA and MeJA both caused reductions in early vegetative growth, but respectively caused an increase and decrease in flowering time. We also observed that changes in flowering time do not also necessarily correlate with changes in fruit or seed production. For example,

BABA and MeJA P_2 treatments respectively caused a decrease and increase in flowering time, but both decreased fresh fruit and seed weight.

Although treatment with each of our priming agents resulted in stunted growth in young plants, these treatments could also, with the exception of MeJA, cause an increase in fruit production later on in the plant's life. This may perhaps be due to some sort of compensatory response that allocates resources to reproductive growth at the cost of early vegetative growth. The generation of treatment that caused increased fresh fruit weight did not necessarily correlate with the generation that experience delayed growth. For example, parental BTH treatment resulted in reduced early vegetative growth, whilst offspring BTH treatment resulted in increased fruit production. Surprisingly, there was also no correlation between effects on fruit weight and seed weight, and the seed to fruit weight ratio was not always affected. Offspring and parental generation treatment with BABA and MeJA, respectively, caused decreases in the seed to fruit ratio, indicating less mature fruits. Offspring treatment with Fytosave resulted in an increased seed to fruit ratio.

Same-generation treatment with almost all of our elicitors, apart from BTH, resulted in reduced vegetative growth during the early stages of development in Micro Tom. Whilst no prior works exist studying the priming allocation costs of HA or COS-OGA treatment, our results do resemble those of previous investigations which noted that BABA and JA treatment reduced the relative growth rate of Arabidopsis plants (Cipollini, 2002b, van Hulten et al., 2006). A 2018 study observed that treatment of two-week-old Micro Tom seedlings with BABA resulted in reduced number of fruits at six weeks of age, but this difference was no longer present at seven and eight weeks of age (Wilkinson, 2018). A 2020 report also observed that treatment of Micro Tom seedlings with BABA resulted in initial delayed fruit ripening but a faster rate of fruit production after the number of ripened fruits had equalised between control and BABA-treated plants (Luna et al., 2020). Our results seem to depict a similar positive tradeoff in which treatment with BABA results in impaired early fitness but greater fruit production later on. Overall, our results here may be further proof demonstrating that some allocation costs are associated with priming.

Given that same-generation treatment with several of our elicitors resulted in reduced vegetative growth, one may also expect that parental treatment with that elicitor would cause a similar allocation cost in the offspring generation when transgenerational priming is observed. Indeed, parental treatment with BTH or Fytosave was also observed to result in a reduction of early vegetative growth. However, this cost reduction was not observed in the offspring of BABA-treated plants even though all 3 of these treatments could induce transgenerational priming against *P. syringae* in our chapter 3 experiments. A potential explanation for this may be differences in the epigenetic memories formed by these priming agents. For example, BABA epigenetic priming may simply prepare offspring for a stress response, whilst BTH and COS-OGA-treated parents may give much more complex instructions to their offspring, including specifically diverting resources away from growth. It may also be possible that the offspring of BABA-treated plants are experiencing allocation costs but they are too subtle to detect in our study. Alternatively, it could be the case that the main allocation costs of transgenerational BABA priming are experienced in the parental generation and offspring plants receive an epigenetic memory that does not impose a significant growth cost on them. Past investigations have shown that some costs of priming can only be detected when primed plants are in competition with other plants or are experiencing other stresses (Van Dam, 1998). Therefore, allocation costs of parental treatment with BABA may be visible if we repeated our experiment in a more complex environment.

5 – General Discussion

With as many as 2.4 billion people being food insecure in 2022, food security remains a pressing global issue (FAO, 2023). However a number of barriers remain to achieving global food security. Chief among these barriers are conflict and economic shocks, but a number of other long term issues exist such as climate change, water scarcity, and soil degradation. Pests and pathogens are yet another potential barrier to food security. Pesticides have therefore been a key tool in reducing losses due to pests and disease in food production. Due to a number of sustainability issues regarding the effects of pesticides on both people and the environment, many countries have introduced regulatory frameworks to control pesticides and growers are being encouraged to move towards alternative ways of controlling pests and pathogens.

To adhere to these new regulations, there has been commercial interest in using priming elicitors as 'plant vaccines' to potentiate the natural defences of plants. Priming has a number of potential upsides over traditional pesticides, such as a reduced risk of harming off-target organisms. Priming cannot offer the same high level of protection that pesticides can however, and often requires a bespoke IPM strategy to be implemented effectively (Walters et al., 2013). Elicitors are also classified as biopesticides under UK and EU law, despite often having no direct pesticidal or fungicidal activity, and are subject to many of the same regulations controlling synthetic pesticides. Although in the UK, the registration cost for biopesticides is lower than that of synthetic pesticides due to the 2006 'Biopesticide Scheme'. Regardless, interest in elicitors has persisted and the global market for 'biostimulant' products is projected to reach 4.5 billion USD by 2027 (Yassin et al., 2021).

We observed that treating parental plants with BABA, BTH and Fytosave could all provide resistance against *P. syringae* in the offspring of Micro Tom tomato plants. These results possibly indicate succesful transgenerational priming of the SA defence pathway. Although we did not observe any apparent priming of the JA defence pathway by any of our elicitor treatments, none of them appeared to

antagonise the JA pathway either as none of them had a consistent effect on *B. cinerea* infection. BABA, BTH or Fytosave parental treatments may therefore be suitable for protecting crops against hemi/biotrophic pathogens without increasing susceptility to herbivores and necrotrophic pathogens.

In addition to biotrophic pathogens, SA also controls resistance against a number of abiotic stresses such as salinity, heat, and drought (Khan et al., 2015, Rhaman et al., 2020). It may therefore be possible that these treatments can transgenerationally prime against these abiotic stresses as well. Parental treatment caused fitness costs in the offspring of all treated Micro Tom plants, however the exact nature of these costs differed between treatments. Fytosave may be the most appropriate choice out of the treatments studied for commercial application of transgenerational priming, as although parental Fytosave treatment stunted early vegetative growth, it did not have any deleterious effects on fruit production and in fact appeared to cause an increase in fruit mass. Fytosave also has the additional advantage of already being registered for commercial use in the UK.

A significant caveat of our research is the absence of an RO spray in the control treatment. It may be possible that treatment via spraying could have had an impact on the growth or defence of our BTH and Fytosave treatment groups that we would not have been able to observe in our experimental design. Another potential downside lies in the Micro Tom cultivar used in our experiments. Micro Tom is characterised by several mutations that cause reduced brassinosteroid synthesis and a determinate growth habit (Gasparini et al., 2025). These mutations result in a dwarf plant with a short generation time, making it favourable for many researchers. However these same mutations may cause Micro Tom to react differently to our priming treatments than a more commercially relevant cultivar would.

In the UK, tomato is a financially significant crop, with a market worth approximately 1 billion GBP per annum. Indeed, approximately 200 hectares of glasshouse-grown tomatoes presently exist in the UK. Serious pests and pathogens of tomato include glasshouse whitefly, spider mites, *Agrobacterium tumefaciens*, *B. cinerea*, and pepino mosaic virus. As our successful priming treatments appeared to prime the SA pathway and defend against *P. syringae*, they may be effective at controlling other

biotrophic tomato pathogens such as *A*. tumefaciens or pepino mosaic virus. Tomato growers in the UK already use IPM strategies to control pests and pathogens, many of which use biological control to combat pests (Koppert, 2025). As priming has a reduced risk of harming off-target organisms, it could be integrated into tomato growers' exisiting IPM strategies without disrupting current biological control methods. Additionally, treatment of tomato plants to produce transgenerationally primed seed would be an easy matter for seed producers and require no input on behalf of growers. Future works may wish to investigate if our priming treatments can provide transgenerational protection against abiotic threats such as heat stress or drought. This would be of particular value to many tropical countries where preexisting food insecurity is being exacerbated by climate change (Schleussner et al., 2018, Wheeler and von Braun, 2013). Overall, this thesis presents a tractable solution to protect tomato crop yields via transgenerational priming.

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