INVESTIGATING DISEASE RESISTANCE IN *EUTREMA SALSUGINEUM*

INVESTIGATING DISEASE RESISTANCE IN *EUTREMA SALSUGINEUM* & THE ESTABLISHMENT OF A *EUTREMA-P. SYRINGAE* PLANT PATHOSYSTEM

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TITLE: Investigating disease resistance in *Eutrema salsugineum* & the establishment of a *Eutrema-P. syringae* plant pathosystem

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Abstract

Eutrema salsugineum is an extremophile plant native to the Yukon Territory and coastal China. As an extremophile, Yukon *Eutrema* plants are tolerant to highly saline, drought conditions and cold temperatures while Shandong *Eutrema* plants can survive in highly saline conditions (Griffith et al., 2007; Guevara et al., 2012; Inan et al., 2004). The disease resistance responses of the Yukon and Shandong accessions of *Eutrema* were investigated to understand how an abiotic stress-tolerant plant responds to biotic stress. A pathosystem was first developed using *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*) to examine *Eutrema* defense responses. Compared to *Arabidopsis* (Col-0), both *Eutrema* accessions exhibited resistance to *Pst,* with the Shandong accession displaying greater resistance than Yukon *Eutrema*. Resistance to *P. syringae* pv. *maculicola* (*Psm*) was observed in both accessions with Yukon *Eutrema* exhibiting greater resistance to this *P. syringae* strain compared to *Pst*. Both *Eutrema* accessions displayed a differential capacity to resist to different avirulent *Pst* strains *(Pst(avrRpt2)* and *Pst(avrRps4))*. *Pst* growth monitored over several weeks revealed that older Yukon and Shandong plants were more resistant compared to younger plants suggesting that age-related resistance (ARR) occurs in both *Eutrema* accessions. RNA-Seq data of uninoculated Shandong *Eutrema* vs. Yukon *Eutrema* revealed an overrepresentation of defense genes including *PR1* (*pathogenesis-related1*; Champigny et al., 2013). Expression of the *Eutrema PR1* ortholog in uninoculated Shandong leaves combined with enhanced resistance to *Pst* compared to Yukon *Eutrema* or Col-0 *Arabidopsis* suggests that Shandong plants exist in a defense-primed state. Resistance to other pathogens such as *Pectobacterium carotovorum* ssp. *wasabiae* (*Pcw*) further supported the hypothesis that Shandong *Eutrema* is primed for pathogen tolerance. Pathogen studies and abiotic stress studies in a lab setting often do not replicate the concurrent, multiple and simultaneous stresses experienced by plants in their natural habitat. The *Eutrema-P. syringae* pathosystem will facilitate future studies to understand how *Eutrema* deals with multiple or concurrent stresses and this knowledge will contribute to efforts to improve tolerance to both abiotic and biotic stress in crop plants.

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List of Abbreviations

µg	Microgram
µl	Microlitre
µmol	Micromole
ABA	Abscisic acid
ACT	Actin
ARR	Age-related resistance
avr	Avirulence gene
BABA	ß-aminobutyric acid
BTH	Benzothiadiazole
CC	Coiled-coil
cDNA	Complementary DNA
cfu	Colony forming unit
Col-0	Columbia
cor	Coronatine
dpg	Days post-germination
dpi	Days post-inoculation
EST	Expressed sequence tag
ETI	Effector-triggered immunity
ETS	Effector-triggered susceptibility
fw	Fresh weight
GC-MS	Gas chromatography-mass spectrometry
GO	Gene Ontology
GUS	ß-glucuronidase
H3	Histone 3
H4	Histone 4
hpi	Hours post-inoculation
HR	Hypersensitive response
INA	2,6-dichloroisonicotinic acid
ISR	Induced systemic resistance
ITC	Isothiocyanate
JA	Jasmonic acid
JGI	Joint Genome Institute
KB	King's Broth
LB	Luria Bertani
ld	Leaf disc
LRR	Leucine-rich repeat
MAMP	Microbe-associated molecular pattern
MAPK	Mitogen-activated protein kinase
MEK (MAPKK)	MAPK activator
MEKK (MAPKKK)	MEK activator
$MgCl_2$	Magnesium chloride

ml	Millilitre
mM	Millimolar
MMLV	Moloney Murine Leukemia Virus
mRNA	Messenger RNA
MS	Murashige and Skoog
NaCl	Sodium chloride
NBS	Nucleotide binding sequence
NPR	Nonexpresser of PR genes
NYG	Nutrient yeast glycerol
OD	Optical density
OR	Osmotically regulated
PAMP	Pathogen-associated molecular pattern
PCR	Polymerase chain reaction
Pcw	*Pectobacterium carotovorum* subspecies *wasabiae*
PR	Pathogenesis-related
PRR	Pathogen recognition receptor
Psm	*Pseudomonas syringae* pathovar *maculicola*
Pst	*Pseudomonas syringae* pathovar *tomato*
PTI	Pathogen-triggered immunity
pv	Pathovar
RNA-Seq	RNA sequencing
rpm	Rotations per minute
RT-PCR	Reverse transcriptase polymerase chain reaction
SA	Salicylic acid
SAR	Systemic acquired resistance
sid2	*Salicylic acid-induced deficient2*
SNP	Single nucleotide polymorphism
ssp	Subspecies
T3SS	Type Three Secretion System
TEY	Threonine-Glutamatic Acid-Tyrosine
TGA	TGACG Motif-Binding Factor
TIR	Toll-like receptor
TMV	Tobacco mosaic virus
UNT	Untreated
wpg	Weeks post-germination
Ws	Wassilewskija
Xcc	*Xanthomonas campestris* pathovar *campestris*

Chapter 1 – Introduction

In order to maintain food production for 7 billion humans and an estimated 20 billion livestock, agricultural scientists must overcome current and future challenges caused by climate change and loss of arable land (FAOSTAT, 2013). The world population doubled in the last five decades and is expected to increase by another one billion in the next twelve years alone, reaching a total of 8.2 billion people by 2025. Most of this population growth will occur in developing regions where people live without food security (United Nations Department of Economic and Social Affairs, 2004). In terms of climate change, most of the focus has been placed on the effects of increasing global temperature, however decreasing temperature trends must also be considered. From 2001-2010, parts of North America, Europe, Asia and Australia experienced cold waves caused by Arctic and North Atlantic Oscillations (The Global Climate 2001-2010 – World Meteorological Organization, 2013). To continue providing food to the human population in the face of changing climate and population increases, developing solutions to maintain crop production is required. The study of extremophile plants that are able to withstand severe environmental stress may reveal novel mechanisms that can be transferred to crops to increase food production.

1.1 *Eutrema salsugineum*

Eutrema salsugineum (monophyletic name *Thellungiella salsuginea*) is also known as saltwater cress and is found in suboptimal growing environments including the Yukon Territory of Canada and the Shandong province in China. *Eutrema's* growing season in the Yukon lasts about three months from early May to late July (Guevara et al., 2012). The average temperature for the Yukon growing season is 20°C but can reach low nighttime temperatures from 7-15°C. The Yukon also experiences low levels of precipitation, averaging 86.9 mm over the growing season (Guevara et al., 2012), compared to temperate regions like Hamilton, which receives 245.4 mm of precipitation on average between May and July. The Yukon accession of *Eutrema* grows on salt flats,

demonstrating its ability to tolerate not only drought, but also salt stress (Wong et al., 2006). The Shandong accession hails from the northeast coastal Shandong province in China and unlike the Yukon, experiences monsoonal amounts of precipitation (Champigny et al., 2013) and temperate climate conditions. In this area, Shandong plants can be found growing on highly saline soils along the inland of the Yellow River flood plain (Inan et al., 2004).

Eutrema shares a common ancestry with the well-established model plant *Arabidopsis thaliana,* and is a sister taxa to the crop plant *Brassica rapa.* Similar to *Arabidopsis, Eutrema* has a relatively short life cycle of about eight weeks in length, a short stature that facilitates growth in controlled experimental settings and produces large numbers of seed (Guevara et al., 2012). Due to the close relationship between the two species, many experimental protocols for *Arabidopsis* are transferable to *Eutrema*. *Eutrema* contains ~27,000 genes divided into seven chromosomes, with a genome size that is approximately twice the size of *Arabidopsis* (Wu et al., 2012). Interestingly, many genes that are differentially expressed between Yukon and Shandong *Eutrema* are involved in defense and stress responses (Champigny et al., 2013) suggesting that these accessions may defend themselves from disease using different strategies.

1.2 Basal Resistance

To protect themselves from microbial disease, plants have developed sophisticated defense pathways to respond to pathogen invasion. The plant immune system is composed of several layers that have evolved as a result of the evolutionary arms race between plants and pathogens. The primary layer of defense employed by plants is basal resistance. It can be divided into constitutive and induced responses and provides resistance to many types of pathogens (bacteria, fungi, viruses). Constitutive responses include preformed physical and chemical barriers such as the cell wall, cuticle layer and constitutive production of antimicrobial compounds (Ahmad et al., 2010; da Cunha et al., 2006). However, constitutive defenses are effective against most pathogens. The

recognition of microbial- or pathogen-associated molecular patterns (MAMPs/PAMPs) by plant pattern recognition receptors (PRRs) activates induced basal responses and leads to PAMP-triggered immunity (PTI; Jones & Dangl, 2006). PAMPs are common pathogen components that can be recognized by a range of plant species. The Flg22 peptide from the N-terminus of bacterial flagellin is one such PAMP that is recognized in *Arabidopsis* by the FLS2 PRR (Felix et al., 1999). The interaction between the plant PRR and the PAMP initiates a calcium-dependent MAPK signaling cascade resulting in the transcriptional activation of defense genes, the production and secretion of antimicrobial agents and strengthening of the cell wall by callose deposition (Xin & He, 2013). The secondary metabolite salicylic acid (SA) plays an important role as a signaling compound to activate defense pathways including PTI. Evidence for the importance of SA during defense resulted in part from studies using SA accumulation-deficient transgenic tobacco and *Arabidopsis* plants that express the bacterial salicylate hydroxylase gene *NahG*. *NahG* is derived from *Pseudomonas putida* and its enzymatic activity converts SA to a biologically inactive compound called catechol (You et al., 1991; Lawton et al., 1995). These transgenic *NahG* tobacco and *Arabidopsis* plants are unable to accumulate SA during defense and display enhanced susceptibility to *P. syringae* pv. *tabaci* and pv. *tomato*, respectively (Delaney et al., 1994). SA signaling is important for SA-dependent *pathogenesis-related (PR)* expression through the master regulator Nonexpresser of PR1 (NPR1). In the presence of SA, cytosolic NPR1 oligomers monomerize and translocate to the nucleus. Monomeric NPR1 in the nucleus activates TGA transcription factors to induce the expression of defense genes such as *PR1* and a number of *WRKY* transcription factors (Fu et al., 2012).

1.3 R Gene-Mediated Resistance

To counteract pathogen virulence effectors, plants have evolved resistance (R) receptors. R receptors are classified based on their structural composition, generally consisting of a coiled-coil (CC) or Toll-like interleukin (TIR) domain at the N-terminus, a

nucleotide-binding site (NBS) and leucine-rich repeats (LRRs) at the C-terminus (Belkhadir et al., 2004). The interaction between the effector and the receptor can be direct or indirect. Physical contact between the receptor and effector represents a direct interaction while indirect interactions involve another protein. An example of an indirect interaction involves the modification of a host molecule by the effector. Modification of the host molecule is perceived by the R receptor that guards the host molecule. This modification and perception by the R receptor triggers effector-triggered immunity (ETI) (Dodds & Rathjen, 2010). Many features of ETI overlap with PTI and include rapid calcium influx, the production of reactive oxygen species and similar expression of defense genes through mitogen-activated protein kinase (MAPK) signaling. However, ETI is characterized by the hypersensitive response (HR), a programmed form of cell death at the site of infection to limit pathogen growth. Although they share similar immune response outputs, ETI is considered to be a more effective version of PTI (Jones & Dangl, 2006).

1.4 The Zigzag Model of Plant Immunity

The plant immune system and the interactions between plant and pathogen are best described in the zigzag model of plant immunity and pathogen virulence (reviewed in Jones & Dangl, 2006 and illustrated in Figure 1). The model highlights the evolutionary interactions between plant receptors and PAMPs/MAMPs. The four stages of the model represent the key interactions that occur between the plant and the pathogen. The first stage is the recognition of PAMPs or MAMPs by PRRs, which initiates PTI. In the second stage, successful pathogens translocate effectors into the plant cell to promote pathogen growth. This interaction interferes with PTI and is known as effector-triggered susceptibility (ETS). The third stage is characterized by the specific recognition of an effector by a cognate plant R receptor. This interaction results in ETI and initiates a signaling cascade leading to an effective resistance response that includes the hypersensitive response (HR) at the site of infection. In the fourth stage, the pathogen

adapts by evolving new effectors to suppress ETI signaling or by eliminating the effector(s) that are recognized by plant R receptors, allowing the remaining effectors to promote pathogen virulence (ETS). The third and fourth stages alternate throughout the evolution of the plant and pathogen as each organism evolves to enhance their fitness and impair growth of the other organism.

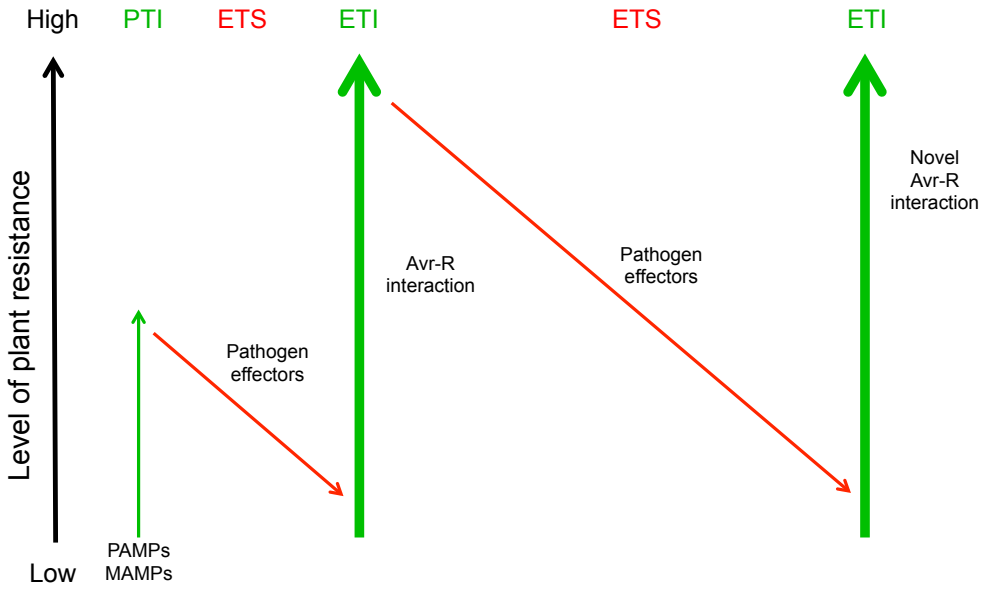


Figure 1. Zigzag model of plant immunity. This schematic is modified from Jones & Dangl, 2006 and highlights the evolutionary arms race between plant and pathogen. PAMP-triggered immunity (PTI) occurs in response to recognition of pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs) by plant pattern recognition receptors (PRRs). Pathogen effectors are then injected into the plant host and suppress resistance to cause effector-triggered susceptibility (ETS). The plant evolves receptors that recognize effectors and triggers effector-triggered immunity (ETI). Selective pressure drives the evolution of novel pathogen effectors to suppress resistance and the evolution of novel plant receptors to improve resistance, leading to an evolutionary arms race between plant and pathogen. The size of the arrow indicates the strength of the resistance response.

1.5 *Pseudomonas syringae* pv. *tomato* (*Pst*) as a model pathogen to study disease

The best-studied plant-pathogen interaction to date is the *A. thaliana* and *Pseudomonas syringae* pv. *tomato* (*Pst*) pathosystem. *P. syringae* was the first pathogen shown to cause disease on susceptible *A. thaliana* accessions such as Columbia-0 (Col-0) in a lab setting (Katagiri et al., 2002). *Pseudomonas syringae* is a rod-shaped, hemibiotrophic, Gram-negative bacterium that causes disease on a wide range of species. *P. syringae* survives on the surface of the leaves as an epiphyte but takes advantage of natural openings in the leaf such as stomata and wounds to enter the plant apoplast, where it transitions from its epiphytic lifestyle to an endophytic lifestyle (Xin & He, 2013). Within the apoplast, its first mode of survival is biotrophic, where the pathogen obtains nutrients from the plant host without causing cell death. In opportunistic and optimal conditions, such as elevated moisture and moderate temperatures, *P. syringae* growth occurs rapidly in the apoplast. *P. syringae* changes its mode of pathogenesis to a necrotrophic phase and causes host cell death which appears as necrotic lesions on the surface of the infected leaves. This dual lifestyle classifies *P. syringae* as a hemibiotrophic pathogen (Melotto et al., 2008).

P. syringae employs different strategies to disrupt plant immunity by targeting PTI and ETI. Effectors target multiple nodes in these pathways, interfering with the transcriptional activation of defense genes, vesicular trafficking of antimicrobial compounds to the extracellular environment and callose deposition at the cell wall (reviewed in Xin & He, 2013). The phytotoxin coronatine is produced by *Pst* DC3000 and has been shown to negatively affect many components of plant defense, such as reopening stomata to allow entry of bacteria into the apoplast and activation of the jasmonic acid (JA) pathway which is antagonistic to the SA signaling pathway required for successful PTI and ETI to *Pst* infection.

1.6 Age-Related Resistance

Age-related resistance (ARR) is a developmentally regulated defense response in which mature plants display enhanced resistance to normally virulent pathogens. In short-day conditions, young *Arabidopsis* plants (3-4 wpg) exhibit high *in planta Pst* bacterial growth and disease symptoms indicative of a susceptible phenotype. In contrast, mature plants (>5 wpg) support 10- to 100-fold less growth compared to young plants and often appear symptomless (Kus et al., 2002). This developmentally regulated response is observed not only in *Arabidopsis* to *Pst* (and to *Hyaloperonospora arabidopsidis*) but in other plant species including in *Nicotiana tabacum* to *Phytophtora parasitica* and in *Oryza sativa* to *Xanthomonas oryzae* pv. *orzyae* (Rusterucci et al., 2005; Hugot et al., 1999; Mazzola et al., 1994). How plants acquire competence for ARR is still unknown however SA and the floral transition have been associated with ARR (reviewed in Panter & Jones, 2002). SA-deficient *Arabidopsis* such as *NahG* and *sid2 (salicylic acid induction deficient2)* are ARR-defective suggesting that SA accumulation is required for ARR. Furthermore Cameron & Zaton (2004) demonstrated that plants undergoing ARR accumulate intercellular SA. The floral transition was correlated with the onset of ARR as measured by the number of inflorescence bolts present on plants undergoing ARR (Rusterucci et al., 2005). However, Wilson et al. (2013) determined that the transition to flowering was not responsible for the initiation of ARR competence. Their results showed that the early-flowering mutant *svp-31* produced inflorescence stems at 3 wpg in the absence of ARR. The separation of the floral transition from ARR demonstrated that the floral transition was not the cue for ARR competence (Wilson et al., 2013).

1.7 Systemic Acquired Resistance

Defense responses against pathogens can be transmitted from the primary site of infection to the entire plant to induce whole plant resistance. One example of this type of response is systemic acquired resistance (SAR). This defense pathway induces resistance in distant leaf tissue after a primary exposure to a necrotizing pathogen (Kuc, 1982;

Champigny & Cameron, 2009). There are four main stages of SAR that ultimately lead to a reduction in symptoms and resistance to a broad spectrum of pathogens in a number of plant species that include *Arabidopsis*, cucumber and tobacco (Champigny & Cameron, 2009; Shah & Zeier, 2013). Induction is the first stage and requires an initial necrotizing infection that leads to the production of a mobile signal. The second stage is the movement of a long distance signal(s) from the initial site of infection to distant tissues and this is thought to occur through the phloem. By stage three, SAR signals are perceived in distant leaves. At this stage, the plant is primed for an enhanced response to pathogen infection and modest *PR1* expression is observed in primed leaves (Champigny & Cameron, 2009). The fourth and final stage is the manifestation of the SAR response. During a secondary infection the plant is effectively able to limit pathogen growth by a rapid resistance response to normally virulent pathogens.

1.8 Priming

As discussed in the previous section, systemic acquired resistance is a classic example of biological priming. Priming is defined as a physiological state in which primed plants display a heightened response to a secondary stimulus that is rapid and more effective compared to unprimed plants (Conrath et al., 2002). Priming stimuli include several pathogens as well as chemical compounds such as beta-aminobutyric acid (BABA) and the phytosanitary product Brotomax. BABA-primed plants enhance salt and drought tolerance in *Arabidopsis* while Brotomax-treated fruits are associated with an increase in the accumulation of the phytoalexin scoparone and enhanced resistance to *Hyaloperonospora arabidopsidis* (Jakab et al., 2005; Ortuno et al. 1997). Since the early 1990s, priming has been shown to be an important component of many types of systemic plant immunity including SAR, induced systemic resistance (ISR) and (BABA)-induced resistance (Ton et al., 2009; Po-Wen et al., 2012). Priming provides broad-spectrum resistance against many pathogens and more recently has been shown to protect plants against abiotic stresses such as drought and salt (Jakab et al., 2005). These important

features of priming suggest that priming is likely a large contributor to plant responses to stress in crop fields and the natural environment where plants are exposed to multiple external stimuli.

Many chemicals have also been shown to induce priming responses and include functional analogs of SA such as 2,6-dichloroisonicotinic acid (INA) and benzothiadaizole (BTH), both of which prime plants for enhanced resistance (Dempsey et al., 1999). Other chemicals are also effective in priming for resistance to a variety of stresses. One example is the non-protein amino acid, beta-aminobutyric acid (BABA). Early studies using BABA application examined BABA's ability to prime *Arabidopsis* against the oomycete *Hyaloperonospora parasitica*. BABA-treatment primed plants to respond to *H. parasitica* infection by accumulating callose in the cell wall leading to reduction of *H. parasitica* hyphal penetration and sporulation (Zimmerli et al., 2000). BABA treatment also protects against osmotic stress as demonstrated by increased tolerance to salt stress (300 mM NaCl treatment) compared to plants that did not receive a BABA treatment prior to the stress (Jakab et al., 2005). After the application of 300 mM NaCl, a smaller proportion of BABA-treated plants (40%) wilted compared to control, well-watered plants (80%). BABA-induced tolerance to salt stress was diminished in the abscisic acid (ABA) biosynthesis mutant *aba1* and the ABA-signaling mutant *abi4*, demonstrating that BABA-induced salt tolerance is based on ABA-dependent signaling (Jakab et al., 2005). The ability of BABA to induce tolerance to *H. parasitica* and salt stress suggests that BABA activates a common signal for both abiotic and biotic stress tolerance.

Until recently, the molecular mechanisms for priming were unknown. A working hypothesis for the molecular basis of priming includes the accumulation of inactive molecules that are required for cellular signal amplification in response to the subsequent stress (Conrath et al., 2006). These inactive molecules become activated rapidly after exposure to a subsequent infection and initiate a signaling cascade leading to rapid and efficient activation of defense responses and plant immunity. The potential signaling molecules involved in priming for defense were not known until Beckers et al. (2009)

discovered that two mitogen-activated protein kinases (MPK), MPK3 and MPK6 are involved in defense priming. MPK3/6 are part of MPK cascades that transmit extracellular signals into intracellular responses while amplifying the signals at each step of the cascade (Widmann et al., 1999; Suarez Rodriguez et al., 2010). *MPK3*/6 mRNA and protein are thought to be important for priming as demonstrated by accumulation of *MPK3*/6 mRNAs and inactive, unphosphorylated MPK3/6 proteins in *Arabidopsis* plants primed for resistance by BTH treatment (Beckers et al., 2009). Activation of inactive MPK3 was observed in BTH-treated plants compared to untreated plants after dip inoculation with *Psm* where the activated form of MPK3, TEY-phosphorylated MPK3 protein (and TEY-phosphorylated MPK6 to a lesser extent) was detected by western blot. The TEY amino acid motif (threonine-glutamic acid-tyrosine) is located within the activation loop of MPK3 and its phosphorylation is required for MPK3 kinase activity (Ray & Sturgill, 1988). The role of MPK3 and MPK6 in positively regulating plant immunity is further supported by their dephosphorylation and subsequent inactivation by the *Pst* effector HopAI1 (Li et al., 2007). Consequently the upregulation of *MPK3* and *MPK6* levels enhance the *Arabidopsis* immune response.

Chromatin modification of defense promoters has been implicated as important during priming. Studies on the role of histones in regulating gene expression have demonstrated that acetylation and methylation of specific lysine residues in H3 and H4 histones relaxes the interaction between genomic DNA and histones producing an open chromatin configuration that promotes gene activation (Eberharter & Becker, 2002). Changes in histone modifications were examined during priming with BTH or *Psm* in the promoters of the defense genes *WRKY6*, *WRKY29* and *WRKY53*. An increase in H3K and H4K tri- and di-methylation and acetylation of the *WRKY* promoters was observed after priming with BTH or *Psm* prior to the challenging stress. Although the expression of these genes did not increase after priming with BTH or *Psm*, *WRKY* gene expression increased after challenge with water infiltration and was associated with an increase in H3 and H4 histone modifications (Jaskiewicz et al., 2011). These findings suggest that *Psm* and BTH priming induced the production of an endogenous signal that was converted and

stored as specific histone modifications on the promoters of defense genes and in turn, these marks serve as the memory of priming allowing the primed plants to respond rapidly to subsequent infections by expressing the appropriate defense genes.

The priming literature presented provides examples in which plants display enhanced resistance to biotic and abiotic stress after an initial treatment with a pathogen or a chemical. Priming has been observed in many plant species, such as *Arabidopsis*, cucumber, rice and pepper (Conrath, 2011). In *Eutrema*, priming for enhanced freezing tolerance and drought tolerance was assessed in two separate studies. Griffith et al. (2007) demonstrated that Yukon plants exposed to a combination of drought and cold stress displayed greater freezing tolerance compared to Yukon plants exposed to only one type of stress. Drought-training studies showed that Yukon plants exposed to an initial episode of drought took longer to wilt in a second drought episode compared to well-watered plants suggesting that prior drought exposure enhanced drought tolerance in Yukon plants (Dedrick, 2007). These two studies demonstrate that *Eutrema* can be primed for enhanced tolerance to abiotic stress.

1.9 Why study *Eutrema*?

Research on *Arabidopsis* has advanced the field of plant biology in terms of the development of molecular tools and the advancement of plant functional genomics (Bressan et al., 2001). In terms of salt tolerance, several studies have identified *Arabidopsis* genes known as osmotic stress-regulated (OR) genes that are induced in response to salt. These OR genes encode a wide variety of proteins involved in osmolyte biosynthesis and ion transporters (Zhu et al., 1997). They function to compartmentalize Na^+ into vacuoles as well as reduce cytosolic osmotic potential through the production of compatible solutes. However, the regulation of these genes and the pathways they are involved in may differ between *Arabidopsis* and halophytes. Furthermore halophytes may have novel genes that allow them to tolerate extreme salt stress that may be absent in *Arabidopsis*. Therefore there is a need to study and identify genes from diverse plant

species, not just from a few model plants, as this will enhance the discovery of unique pathways or novel functions for already characterized genes. There are several benefits to study *Eutrema* as a plant model; *Eutrema* is amenable to genetic manipulation, shares sequence similarity with *Arabidopsis* and its genome has recently been sequenced. Furthermore, if *Eutrema* is also tolerant to biotic stress, investigating abiotic and biotic stress pathways may reveal common signaling components that are shared between both stress responses.

1.10 *Eutrema* Genomics

The draft genome of Shandong *Eutrema* became available in 2011 and was assembled using 1.6 million expressed sequence tags (ESTs) generated from 454 sequencing of 5 cDNA libraries (www.phytozome.net). Based on this whole genome data, the Shandong *Eutrema* genome was estimated to be 243.1 Mb in size on seven chromosomes. Further sequencing efforts of the Shandong *Eutrema* genome were independently performed by two other groups utilizing Illumina and Roche next generation sequencing platforms. Wu et al. (2012) identified 28,457 protein-coding regions that encoded for a wide range of Gene Ontology (GO) terms, including the "response to stimulus" which was further broken down to more specific terms such as "response to salt" and "response to osmotic stress". The expression of genes in these subcategories was significantly different between *Arabidopsis* and *Eutrema* and is consistent with the abiotic tolerance features of *Eutrema salsugineum*. Champigny et al. (2013) sequenced several cDNA libraries generated from cabinet- and field-grown Yukon plants and cabinet-grown Shandong plants using the 454 Roche sequencing platform. Over 10 million high quality reads were aligned against the JGI reference genome, resulting in the identification of 26,351 protein-coding loci. Combined with 665 loci that were already annotated, this RNA-Seq study predicted a total of 27,016 unique loci, a value comparable to the total number obtained by Wu et al. (2012). Champigny et al.'s (2013) analysis also examined polymorphic differences in the transcriptomes of cabinet-

and field-grown Yukon *Eutrema* as well as cabinet-grown Shandong *Eutrema*. Over 39,000 SNPs were identified when Yukon cabinet plants were compared with Shandong cabinet plants. As expected, the number of SNPs decreased in comparisons between Yukon cabinet and field plants to 4,475. GO classification of the differentially expressed genes (DEGs) between Shandong and Yukon cabinet plants yielded results such as "responses to stress" as well as "defense responses". Examples of genes found within these categories include the defense marker *PR1* and R receptor *RPS4*, which were both upregulated in cabinet-grown Shandong plants relative to cabinet-grown Yukon plants. This data suggested that Yukon and Shandong *Eutrema* displayed differential defense responses and that disease resistance should be examined in both *Eutrema* accessions.

1.11 Research Hypotheses and Objectives

The main goal of this thesis is to establish a *Eutrema-P. syringae* pathosystem to investigate disease resistance in the Yukon and Shandong accessions of *Eutrema salsugineum*.

Hypothesis 1: Yukon and Shandong *Eutrema* are resistant to biotic stress due to the expression of defense genes in healthy, uninoculated plants.
Objective 1: Examine the response to *Pst* and other *P. syringae* strains in Yukon and Shandong *Eutrema*.
Objective 2: Examine the expression of defense-related genes (*PR1*) in untreated Yukon and Shandong leaves and leaves inoculated with *Pst*.

Hypothesis 2: If Shandong *Eutrema* exists in a defense-primed state, then Shandong *Eutrema* should be resistant to other pathogens.
Objective 3: Investigate Shandong *Eutrema's* response to *Xanthomonas campestris* pv. *campestris* and *Pectobacterium carotovorum* ssp. *wasabiae*.

Hypothesis 3: If *Eutrema* employs similar strategies to tolerate abiotic and biotic stress then an abiotic stress should enhance *Pst* resistance.
Objective 4: Determine if salt treatment of Yukon and Shandong plants enhances resistance to *Pst*.

Chapter 2 – Methods

2.1 Plant Growth Conditions

Eutrema salsugineum (also known as *Thellungiella salsuginea*) accessions Yukon and Shandong were used throughout this project. In addition, *Arabidopsis thaliana* ecotypes Columbia-0 (Col-0) and the *salicylic acid induction deficient2* (*sid2*) mutant were used. For both species, seeds were surface sterilized and stratified at 4°C for two days. Seeds were sown on Murashige and Skoog (MS, Caisson Labs) agar plates and germinated under continuous light (60 µmol/m^2s) for 7 days at 22°C. Seedlings were transplanted onto soil (Sunshine Mix #1, Jack Van Klaveren) hydrated with 1 g/L 20-20-20 (N-P-K) fertilizer. To provide high humidity to the new seedlings, a clear, plastic dome was placed over the flats for two days. Plants were fertilized again at 2 wpg with the same fertilizer mix. Plants were grown at 22°C in 75-85% relative humidity with either a 9 hour photoperiod (short-day) or 16 hour photoperiod (long-day) with a 150 µmol/m^2s light intensity.

2.2 *Pseudomonas* growth, inoculation and *in planta Pst* quantification

The following *Pseudomonas syringae* strains were used: *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 (containing pVSP61), *Pst* DC3000 (pVSP61 + *avrRpt2*), *Pst* DC3000 (*avrRps4*), *Pst* DC3000 (*cor*$^-$), *Pst* DC3000 (*hrpA*$^-$) , *Pst* DC3000 (HopW1-1), *Pst* DC3000 (HopZ1c), and *P. syringae* pv. *maculicola* (*Psm*) ES4326. Bacteria were cultured in King's B (KB) medium supplemented with 100 µg/µl rifampicin (Biobasic) and 50 µg/µl kanamycin (Sigma). Tubes were shaken at 200 rpm at room temperature until the exponential growth phase was reached (OD_{600} = 0.1 to 0.6). The culture was diluted to either 10^5, 10^6 or 10^7 colony-forming units (cfu)/ml in 10 mM $MgCl_2$. The *Psm* strain was cultured in the same manner at 28°C. The bacterial inoculum was pressure-infiltrated into the abaxial side of plant leaves using a 1 ml needleless syringe. *In planta* bacterial density was quantified at various time points after inoculation. Leaf discs (three replicates each consisting of eight discs) were collected with a No. 2 cork borer (4 mm

diameter) and shaken at 200 rpm in a 0.02% Silwet L-77 solution in 10 mM $MgCl_2$ for 1 hour. The bacterial solution was serially diluted, then plated onto KB agar containing 100 µg/µl rifampicin for *Psm* or a combination of 100 µg/µl rifampicin and 50 µg/µl kanamycin for *Pst*.

2.3 *Xanthomonas* culture and infection

Xanthomonas campestris pv. *campestris* (*Xcc*) strain 8004 was cultured in NYG medium supplemented with 50 µg/ml rifampicin at 200 rpm at 30°C until the exponential growth phase was reached (OD_{600} = 0.1 to 0.6; minimum of 16 hours). The culture was adjusted to 10^8 cfu/ml in 10 mM $MgCl_2$. Four wpg long-day-grown plants were pre-treated by placing a plastic dome over the plants for one hour prior to infection to increase humidity. A 10 µl pipette tip was used to puncture the mid-vein of leaves three times in close proximity. The tip was dipped into the suspension in between each puncture. Mock-treated plants were punctured after dipping into a 10 mM $MgCl_2$ solution. High humidity was maintained for 24 hours after infection with clear plastic domes. Wounding surrounding the punctures was monitored for several days after infection and *in planta* bacterial density was quantified 7 dpi as described in Section 2.2 but NYG agar plates containing 50 µg/ml rifampicin were used instead of KB agar plates.

2.4 *Pectobacterium* culture and inoculation

Pectobacterium carotovorum ssp. *wasabiae* (*Pcw*; formerly *Erwinia*) was cultured in LB medium at 200 rpm at 28ºC until the exponential growth phase was reached (OD_{600} = 0.1 to 0.6). The culture was diluted to either 10^6 or 5 x 10^6 cfu/ml in 10 mM $MgSO_4$. Silwet L-77 was added to a final concentration of 0.01% and poured into shallow petri dish plates. Plants were turned upside-down and leaves were submerged in the bacterial solution for 15 minutes. Mock-treated plants were dipped in a 0.01% Silwet solution. The plants were kept at high humidity overnight using a clear, plastic dome. The percentage of rotted leaves was measured one and two days post-infection. The abaxial side of leaves

was also pressure-infiltrated with 10^6 cfu/ml *Pcw* as described in Rusterucci *et al.* (2005) and the percentage of rotted leaves was measured 1 day post-inoculation (dpi).

2.5 *Leptosphaeria* culture and infection

Leptosphaeria maculans strain GL11 containing the *Escherichia coli* GUS reporter gene was obtained from G. Seguin-Swartz (AAFC). Filter disks containing pycnidiospores were placed on solid V8-juice agar plates (20% vol/vol V8 juice, 0.75% $CaCO_3$, 1.5% agar, 100 µg/ml Streptomycin, 50 µg/ml Hygromycin and 40 µg/ml Rose Bengal (Sigma)) and grown in black light (300 – 400 nm) at room temperature until visible hyphae covered the entire plate. Spores were harvested by washing the plate with milliQ water. A sterile glass hockey stick was used to scrape the surface of the plate to release the pycnidia. The solution was filtered through a layer of Miracloth to remove large hyphae structures and purify spores. A haemocytometer was used to determine spore concentration of the solution. The spore suspension was diluted to a final concentration of 10^7 spores/ml in milliQ water. The abaxial surface of long-day 4 wpg leaves was punctured with a 10 µl pipette tip before 10 µl of the spore solution was dripped onto the wound. To maintain high humidity, a large clear plastic bag was placed over the inoculated plants for 24 hpi.

2.6 Age-Related Resistance (ARR) assay

Three wpg plants were inoculated with 10^6 cfu/ml *Pst* DC3000 as described in Section 2.2 and *in planta* bacterial density was quantified 3 dpi. This was repeated in a new set of plants at 4, 5, 6 and 7 wpg to determine if ARR occurred. ARR was performed in both short-day and long-day-grown plants.

2.7 Systemic Acquired Resistance (SAR) assay

The lower leaves of 3.5 to 4 wpg short-day-grown plants were induced with 10^6 cfu/ml *Pst* (*avrRpt2*). The age of the plants at the time of induction was dependent on the plant size at the time of the experiment; plants that were small in size at 3.5 wpg were

inoculated later at 4 wpg to encourage further growth. Two days later, upper leaves were challenged with 10^5 cfu/ml *Pst* DC3000 and *in planta* bacterial density was collected 3 days post-challenge.

2.8 Leaf collapse assay

To induce a macroscopic hypersensitive response (HR), half-leaves of 4 wpg short-day-grown plants were challenged with a high dose (10^8 cfu/ml) of three *Pst* strains: *Pst* DC3000, *Pst* DC3000 (*avrRpt2*) and *Pst* DC3000 (*avrRps4*) according to Lewis et al. (2010). High humidity was maintained with a clear, plastic dome and leaves were examined for leaf collapse at 24 hpi.

2.9 Electrolyte leakage assay

Four wpg short-day-grown plants were inoculated either with 10 mM $MgCl_2$, 10^6 cfu/ml *Pst* DC3000, *Pst* DC3000 (*avrRpt2*) or *Pst* DC3000 (*avrRps4*). Leaves were dried and leaf disks were collected after one hour to allow inoculated water to evaporate out of the leaves. Thirty to forty leaf disks were collected per treatment and divided into triplicates (10 disks/vial). The leaf disks were gently shaken at 50 rpm for 1 hour in a petri dish filled with milliQ water before they were transferred to glass vials containing milliQ water. The first conductance measurement was made approximately 3-4 hpi, followed by measurements every three hours up to 29 hpi. A conductivity meter was used to measure conductance; the probe was rinsed in milliQ water between measurements. Total electrolyte levels in leaves was measured by autoclaving the leaf disks and determining total conductance. Percent conductance was calculated by dividing conductance over total conductance x 100%.

2.10 RNA Extraction, cDNA synthesis and RT-PCR

Leaf samples were harvested at desired time points and flash-frozen in liquid N_2 and transferred to the -80°C freezer in biological triplicates. Frozen tissue (<100 mg) was ground in dry ice and total RNA was extracted using the Norgen Plant/Fungi Total RNA

Purification Kit. Residual genomic DNA was eliminated using the Sigma On-Column DNase I Digestion Set after binding the RNA to the Binding column. First-strand cDNA was synthesized with 2 µg of template total RNA using Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase (Life Technologies) and oligo-dT as the primer. *PR1* expression was analyzed in both *Arabidopsis* and *Eutrema* with species-specific primers for both *PR1* and *ACTIN1* (Table 1). *EsPR1* and *EsACT1* primers were obtained the from JGI Shandong *Eutrema* genome to amplify both Yukon and Shandong *PR1*. Initial experiments were performed to identity PCR cycling conditions resulting in exponential amplification of all target genes. The following conditions were used to measure *PR1* expression in Figure 15: PCR cycles were used for both *AtPR1* and *AtACT1* in Col-0; 25 cycles were used for *EsPR1* and *EsACT1* amplification in Yukon and Shandong *Eutrema*. For Figure 16, 26 cycles were used for both *EsACT1* and *EsPR1* in Yukon and Shandong *Eutrema*.

Table 1: RT-PCR primers for *PR1* and *ACTIN1* expression

Target Gene	Locus	Primers
EsPR1	Thhalv10022890	F 5'CGTCAAAGGCTCAAGACAGC R 5'CACCTCGCCTTACCACATCCG
EsACTIN1	Thhalv10016809	F 5'GGCGATGAAGCTCAATCGAAACG R 5'GGTCACGACCCGCAAGATCAAGACG
AtPR1	AT2G14610	F 5'TCTAAGGGTTCACAACCAGGC R 5'TTGGCACATCCGAGTCTCAC
AtACTIN1	AT2G37620	F 5'GGCGATGAAGCTCAATCCAAACG R 5'GGTCACGACCAGCAAGATCAAGACG

2.11 Free and total salicylic acid quantification

To measure total salicylic acid content in *Eutrema*, uninoculated, 12 and 24 h leaves inoculated with *Pst* were flash frozen in liquid N_2 and stored at -80°C. Leaf tissue was ground in liquid N_2 and weighed into 0.1 g replicates. Two-hundred and fifty µl 0.1 M (pH 5.6) sodium acetate buffer was added to thawed samples and homogenized for 1 minute. Samples were centrifuged at 16000 g for 15 minutes at 4°C. The samples were split in half to measure free SA (without β-glucosidase) and total SA (with β-glucosidase). β-glucosidase solution was prepared by dissolving 4 units of β-glucosidase powder in sodium acetate. Samples were incubated for one hour before they were spun down for 5 minutes. To measure SA-dependent luminescence, 20 µl of crude extract (or *sid2* leaf extract), 60 µl LB and 50 µl of *Acinetobacter* sp. ADPWH_lux culture (OD_{600} = 0.4) were added to each well in a 96-well plate. A standard curve was generated by first dissolving 0.01 g SA in 1000 µl acetate buffer. Serial dilutions were performed to obtain SA standards with a range of SA concentrations (0.8 – 200 ng/ml SA; Figure 2). 10 µl of each SA standard was added to 100 µl of *sid2* leaf extract. *sid2* leaf extracts were prepared the same way as crude leaf extract but 500 µl 0.1 M (pH 5.6) sodium acetate buffer was added to 0.2 g of tissue. SA-dependent luminescence was detected after the plates were incubated for 1 hour at 37°C in a Biotek plate reader at 490 nm. Luminescence was converted to SA concentration by plotting luminescence against known SA concentrations using the standard curve.

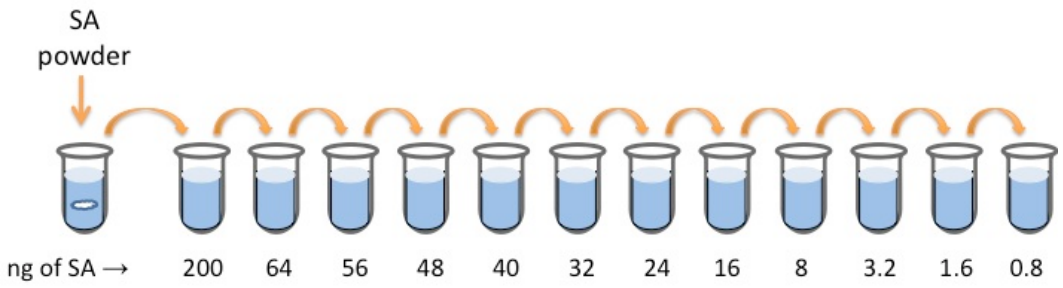


Figure 2. Salicylic acid dilution series. 0.01 g of SA was initially dissolved in 1000 μl NaOAc buffer and diluted to obtain a range of SA concentrations from 0.8 to 200 ng/ml SA.

2.12 Seedling exposure to salt

Seedlings were germinated on MS plates and transferred to MS plates supplemented with 250 mM NaCl at 4 dpg. Col-0 seedlings were transferred to 100 mM NaCl plates. Seedlings were also transferred to regular MS plates without NaCl as a negative control. After 5 days of exposure to NaCl, seedlings were transplanted to soil. At 4 wpg, plants were inoculated with 10^6 cfu/ml *Pst* DC3000 and bacterial density was quantified at 3 dpi. A schematic of the experimental outline is presented in Figure 3.

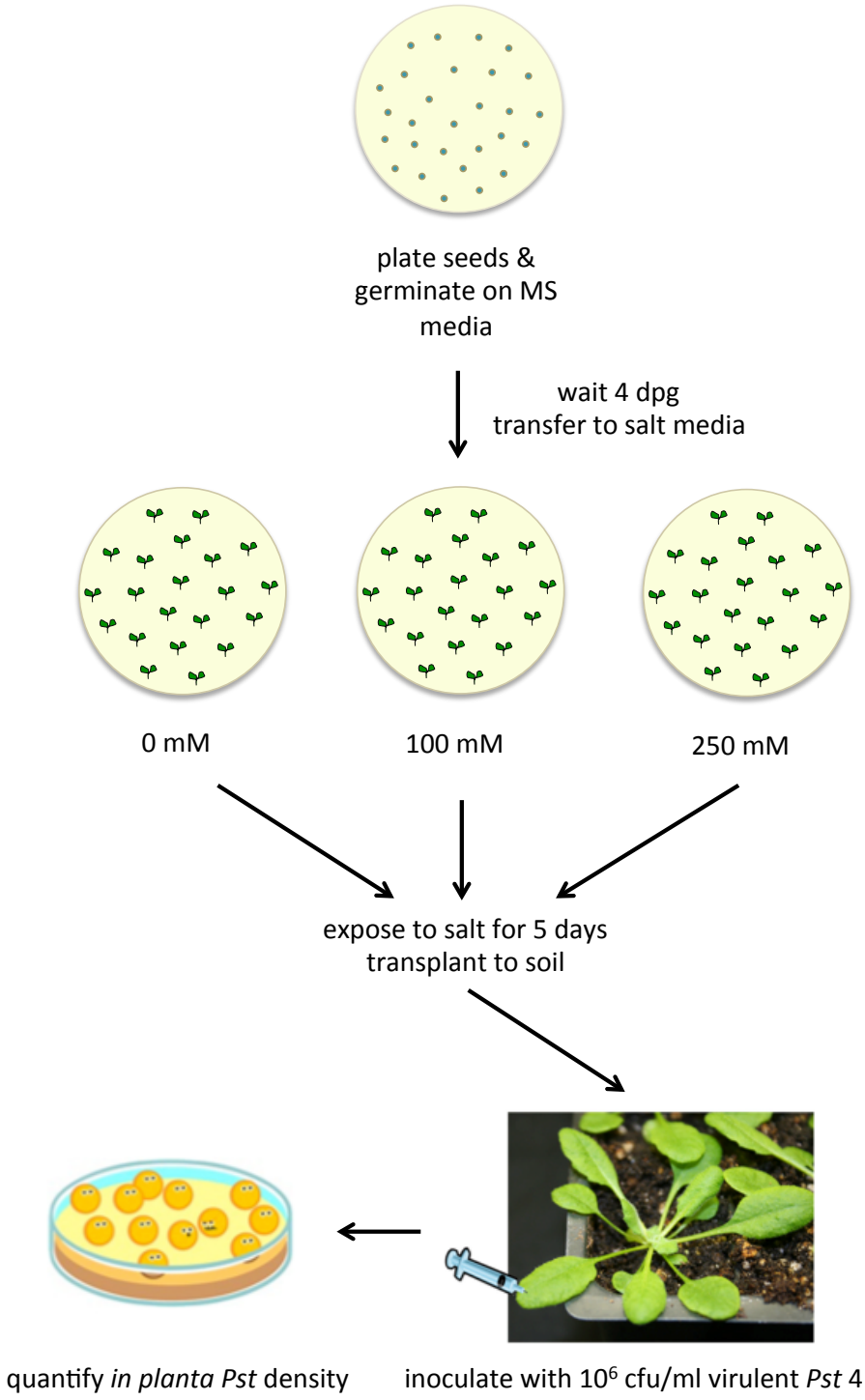


Figure 3. Schematic representation of seedling exposure to NaCl. Seedlings germinated on MS plates were transferred to NaCl plates. 5 days post-exposure, seedlings were transplanted to soil and challenged with *Pst* at 4 wpg.

2.13 NaCl irrigation

Three wpg short-day-grown plants were irrigated once with 50 ml 200 mM NaCl. Col-0 plants were also watered with 100 mM NaCl. Control plants were watered with H_2O. Watering was withheld prior to NaCl treatment in both series to ensure that the soil would take up the saline solution. At 4 wpg, plants were inoculated with 10^6 cfu/ml *Pst* DC3000 and bacterial density was quantified at 3 dpi.

2.14 Statistical analysis

Statistically significant differences in bacterial levels *in planta* were determined by ANOVA when comparing multiple treatments followed by Tukey's honestly significant differences (HSD) post-hoc test. The Games-Howell post-hoc test was used in cases of unequal variance of the bacterial numbers. Tabular outputs of the post-hoc tests (Multiple Comparisons and Homogenous Subset Tables) are provided in the Appendix. Independent Student's *t*-tests were used for pair-wise comparisons of control and treatment means. IBM SPSS Statistics 21 was used to perform all statistical analyses.

Chapter 3 – Results

3.1 Examining the *Eutrema* response to *Pst* and *Psm*

3.1.1 Yukon and Shandong *Eutrema* display differential resistance to *Pst* DC3000

To determine if abiotically stress-tolerant *Eutrema* was also tolerant of biotic infections, both *Eutrema* accessions were inoculated with 10^6 cfu/ml *Pst* DC3000 and bacterial growth was measured over three days (Figure 4A). To ensure that similar numbers of bacteria were inoculated into the intercellular space, bacterial density was measured two hours post inoculation. *In planta* bacterial density was similar in Yukon and Shandong *Eutrema* as well as Col-0 ranging from 3.0 x 10^2 to 7.0 x 10^2 cfu/ld. Differences in bacterial density were observed at 1 dpi, with *Pst* levels in Col-0 and Yukon reaching 3.9 x 10^5 and 2.5 x 10^5 cfu/ld, respectively while *in planta Pst* levels reached 2.2 x 10^4 cfu/ld in Shandong. *Pst* levels in Shandong *Eutrema* plateaued from 1 dpi onward, remaining at 2.2 x 10^4 cfu/ld over the 3-day experiment. *Pst* levels in Yukon *Eutrema* reached 1.4 x 10^6 cfu/ld 2 dpi and dipped to 6.3 x 10^5 cfu/ld by 3 dpi. *Pst* levels in Col-0 increased throughout the assay and reached 1.1 x 10^7 cfu/ld by 3 dpi, indicative of a typical Col-0 response to *Pst*. Compared to *Pst,* levels in Col-0 at 3 dpi, *Pst* levels in Yukon and Shandong *Eutrema* were 17- and 493-fold lower. Compared to growth at 0 dpi, *Pst* levels increased 1200 and 69-fold by 3 dpi in Yukon and Shandong *Eutrema*, respectively.

These data suggest that both *Eutrema* accessions suppress *Pst* growth and are more resistant to *Pst* compared to Col-0. Leaf symptoms were also assessed throughout the assay (Figure 4B). These leaves represent typical symptoms observed in several experimental replicates. Inoculated Col-0 leaves displayed zones of chlorosis present throughout the leaves by 2 dpi and these chlorotic regions increased in size by 3 dpi. Inoculated Yukon leaves displayed patches of necrosis with mild chlorosis at 2 dpi and occasional wilting by 3 dpi. Shandong leaves appeared symptomless throughout the entire

assay. These symptoms indicate that in comparison to Col-0 *Arabidopsis*, Yukon *Eutrema* is resistant to *Pst*, while Shandong *Eutrema* is highly resistant to *Pst*.

Over the course of this thesis, *Pst* inoculation experiments were performed numerous times. These experiments were compiled and compared to examine the variability over 29 months. It has been demonstrated that low humidity levels in the winter (October to March) contribute to reduced growth of *Pst* compared to the summer (April to September) where humidity is higher and is associated with higher *Pst* growth in *Arabidopsis*. Variation in *Pst* growth in Yukon and Shandong *Eutrema* did occur between experiments (Figure 5) however these differences were not associated with the summer or winter. Overall, these experimental replicates of *Pst* levels in Yukon and Shandong *Eutrema* demonstrate that both Yukon and Shandong display resistance phenotypes to *Pst* DC3000 in the summer and winter.

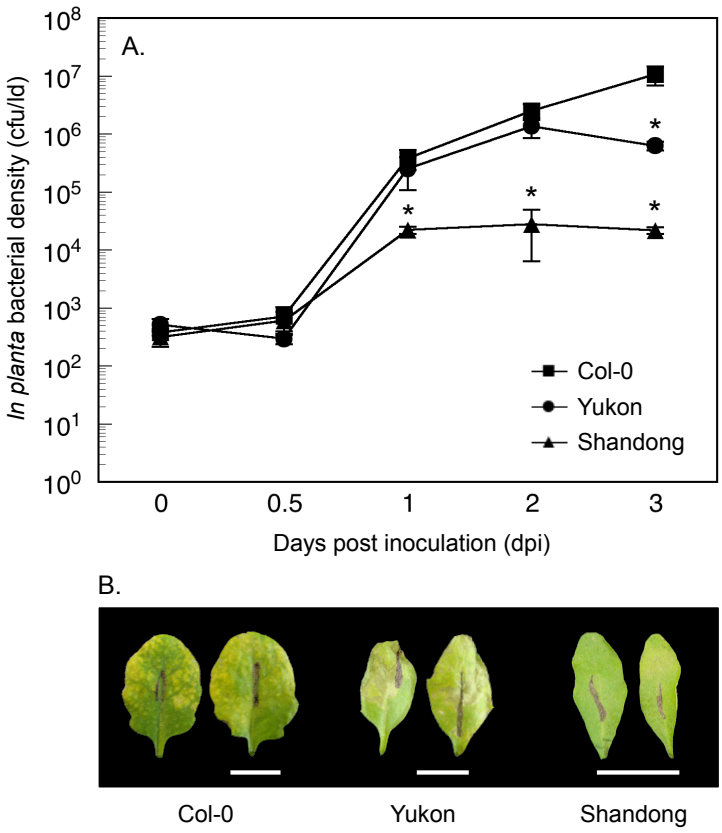


Figure 4. *In planta* bacterial growth of *Pseudomonas syringae* pv. *tomato* (*Pst*) in *Eutrema* with accompanying disease symptoms. (A) Four-week-old Col-0, Yukon and Shandong plants were inoculated with 10^6 cfu/ml. Bacterial levels were quantified at the indicated time points and are presented as the mean ± standard deviation of three biological replicates. Asterisks (*) denote significant differences relative to Col-0 at the same time point according to Tukey's HSD ($p < 0.01$). Corresponding images of inoculated leaves 3 dpi are presented in (B). Bar = 1 cm. This experiment was repeated three times with similar results.

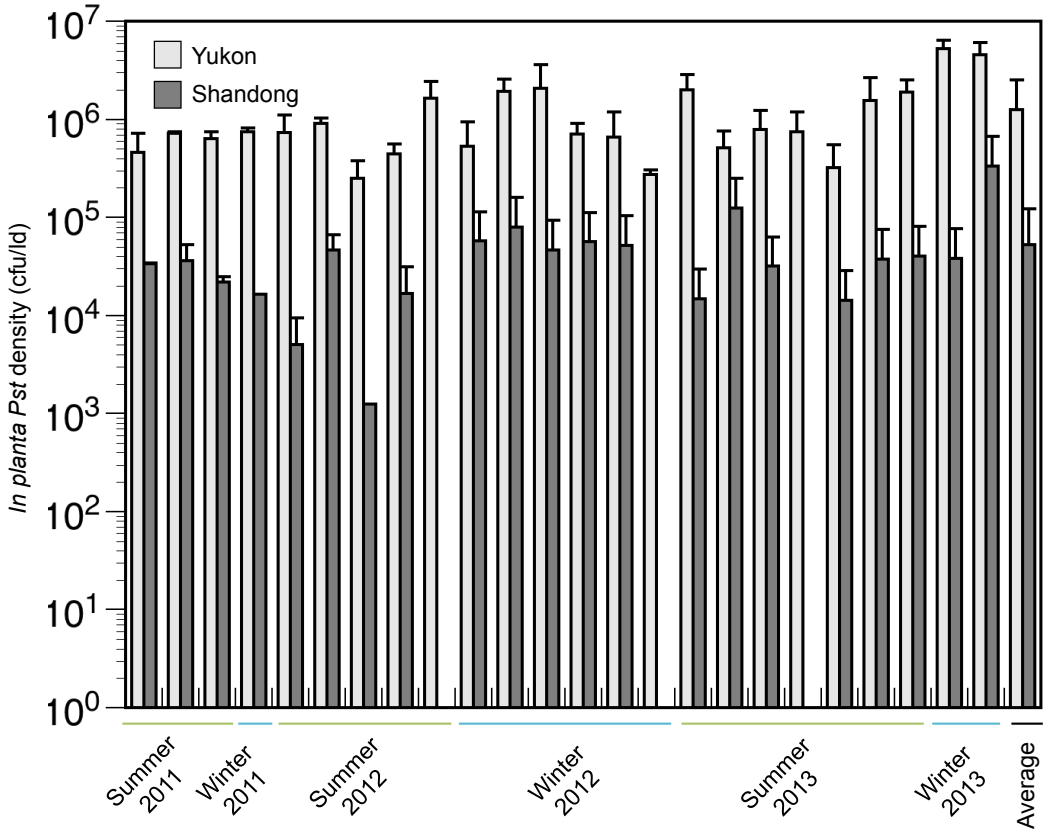


Figure 5. *In planta* bacterial growth of *Pst* DC3000 in Yukon and Shandong *Eutrema* over multiple seasons (summer = April to September; winter = October to March). Bacterial levels at 3 dpi were determined for different experiments over the course of 29 months and a final calculated average is also presented. Each bar represents the mean ± standard deviation of three biological replicates.

3.1.2 Does the phytotoxin coronatine contribute to *Pst*'s ability to grow in *Eutrema?*

Coronatine is an important bacterial toxin produced by *Pst* that contributes to pathogen virulence in *Arabidopsis*. As a structural and functional mimic of JA, coronatine suppresses SA-mediated defense responses by upregulating JA-responsive genes that antagonize the SA pathway. Consequently, coronatine-deficient *Pst* strains show reduced growth compared to wildtype *Pst*, since SA-mediated defenses are suppressed. To determine if coronatine also suppresses *Eutrema* disease resistance, plants were inoculated with a coronatine-deficient *Pst* strain (*Pst* DC3118 *cor*$^{-}$). A higher dose of *Pst* DC3118 *cor*$^{-}$ was used because the absence of coronatine reduces this strain's ability to grow *in planta*. The *Pst* DC3118 *cor*$^{-}$ strain should show reduced growth compared to wildtype *Pst* in Col-0, however this was not observed in three experimental replicates (Figure 6). Although the difference between wildtype *Pst* and *Pst* DC3118 *cor*$^{-}$ was statistically significant, its biological relevance was not significant as it was expected that the *Pst* DC3118 *cor*$^{-}$ strain would have reduced growth compared to wildtype *Pst*. Biological differences in growth between both strains were not observed in either *Eutrema* accession, which may suggest that coronatine does not contribute to *Pst*'s ability to grow in *Eutrema*. However, the coronatine-deficient strain must display typical reduced growth in Col-0 to make conclusions about coronatine's affect on *Eutrema*.

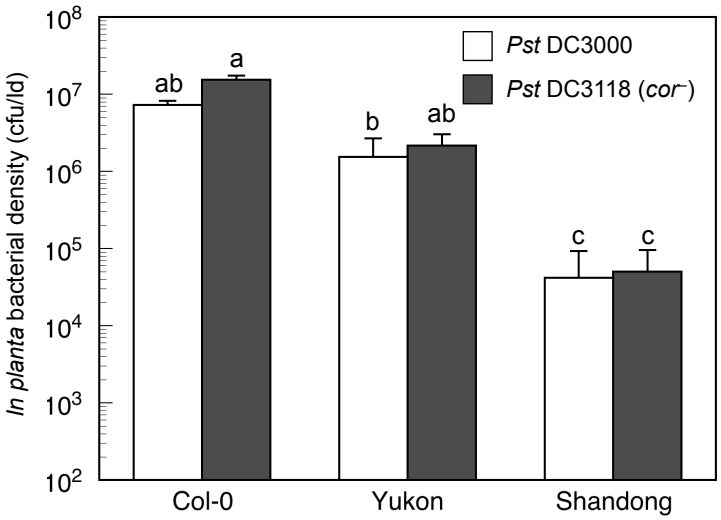


Figure 6. Examining the effect of coronatine on *Pst* growth in *Eutrema* using a coronatine-deficient *Pst* strain. Four-week-old plants were inoculated with 10^7 cfu/ml *Pst* DC3118 (*cor*$^-$) or 10^6 cfu/ml *Pst* DC3000 and *in planta* bacterial growth was measured 3 dpi. Each bar represents the mean ± standard deviation of three biological replicates. Different letters denote significant differences according to Tukey's HSD ($p < 0.05$). This experiment was repeated three times with similar results.

3.1.3 Loss of *HrpA* enhances resistance in *Eutrema*

Pst requires a functional Hrp pilus to inject virulence effectors into the plant to suppress basal defense and induce effector-mediated suppression. The *hrpA*- mutant of *Pst* is unable to form a functional Hrp pilus resulting in reduced virulence and disease on *Arabidopsis*. To determine if the Hrp pilus and injected effectors contribute to *Pst* virulence in *Eutrema*, 10^7 cfu/ml *Pst* DC3000 (*hrpA*$^-$) or 10^6 cfu/ml *Pst* Dc3000 was inoculated into plants. A higher dose of the *hrp*-deficient strain was required because it doesn't grow well as *Arabidopsis* is highly resistant to this mutant strain; furthermore this will induce *PR1* expression in sufficient cells to allow detection of *PR1*. As expected, a 865-fold difference was observed between *Pst* DC3000 and *Pst* DC3000 *(hrpA*$^-$*)* in Col-0 (Figure 7). A 1060-fold and 44-fold difference was observed between *Pst* DC3000 and *Pst* DC3000 *(hrpA*$^-$*)* in Yukon and Shandong *Eutrema*, respectively. These results demonstrate that the Hrp pilus and injected effectors contribute to virulence of *Pst* in Yukon *Eutrema*.

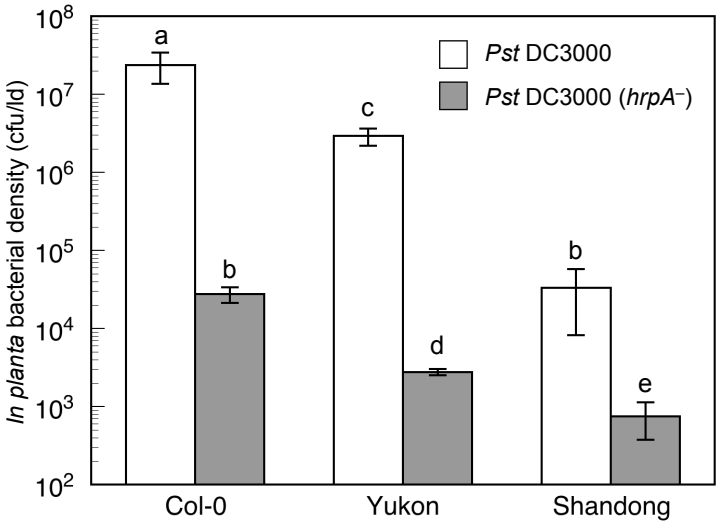


Figure 7. *In planta* bacterial growth of HrpA-deficient *Pst*. Four-week-old plants were inoculated with 10^7 cfu/ml *Pst* DC3000 (*hrpA*⁻) or 10^6 cfu/ml *Pst* DC3000 and *in planta* bacterial growth was measured 3 dpi. Each bar represents the mean ± standard deviation of three biological replicates. Different letters indicate significant differences according to Tukey's HSD ($p < 0.05$). This experiment was repeated twice with similar results.

3.1.4 Responses to two avirulent strains, *Pst(avrRpt2)* & *(avrRps4)*, in *Eutrema*

3.1.4.1 Examining the growth of *Pst(avrRpt2)* and *Pst(avrRps4*) in *Eutrema*

Transcriptome analysis of cabinet-grown, untreated Yukon and Shandong tissue identified a suite of defense genes that were differentially expressed between the two accessions (Champigny et al., 2013). Among these were genes orthologous to R genes found in *Arabidopsis* that act as receptors for pathogen effectors to initiate downstream signaling for R gene-mediated defense. Two of these R genes were RPS2 and RPS4 that recognize the *Pst* effectors AvrRpt2 and AvrRps4, respectively, in Col-0. To determine if the RPS2 and RPS4 *Eutrema* orthologs were functional R receptors for AvrRpt2 and AvrRps4, Yukon and Shandong plants were inoculated with two *Pst* strains expressing these effectors, plus *Pst* DC3000, which does not express any avirulence proteins. As a positive control, Col-0 plants were also inoculated with these three strains and growth was compared to *Pst* DC3000. Comparisons of bacterial growth were made between *Pst* DC3000 and the avirulent strain for each accession and statistically significant differences were reported relative to *Pst* DC3000 at each time point. Statistically significant differences between *Pst* DC3000 and *Pst(avrRpt2)* and *Pst(avrRps4)* were observed at 2 dpi, with a 11- and 31-fold difference in bacterial levels compared to *Pst* DC3000, respectively in Col-0 (Figure 8A). These differences increased by 3 dpi and were also statistically significant, as *Pst* levels continued to increase while both avirulent strains plateaued or decreased in Col-0. Reduced growth of both avirulent strains in Col-0 compared to *Pst* DC3000 is indicative of R gene-mediated resistance. In Yukon, *Pst*(*avrRpt2*) levels increased by 1 dpi and plateaued at 2 dpi eventually reaching 1.5 x 10^5 cfu/ld by 3 dpi (Figure 8B). The maximum difference between *Pst* and *Pst(avrRpt2)* growth in Yukon was observed at 1 dpi with a 23-fold statistically significant difference. Although a significant 19-fold difference was observed 1 dpi between *Pst* and *Pst(avrRps4)*, this difference leveled off by 2 and 3 dpi. This data suggests that an ETI response is observed in Yukon *Eutrema* in response to *Pst* expressing AvrRpt2. *Pst(avrRpt2)* and *Pst* levels were similar in Shandong plants until 2 dpi such that

Pst(avrRpt2) levels dropped to 6.7 x 10^3 cfu/ld and remained the same even at 3 dpi with a significant 5-fold difference in growth (Figure 8C). *Pst(avrRps4)* levels were lower compared to *Pst* levels, reaching 2.6 x 10^3 cfu/ld at 3 dpi. This statistically significant 13-fold difference between *Pst* and *Pst(avrRps4)* at 3 dpi suggests that Shandong *Eutrema* exhibits AvrRps4-triggered immunity to *Pst(avrRps4)*.

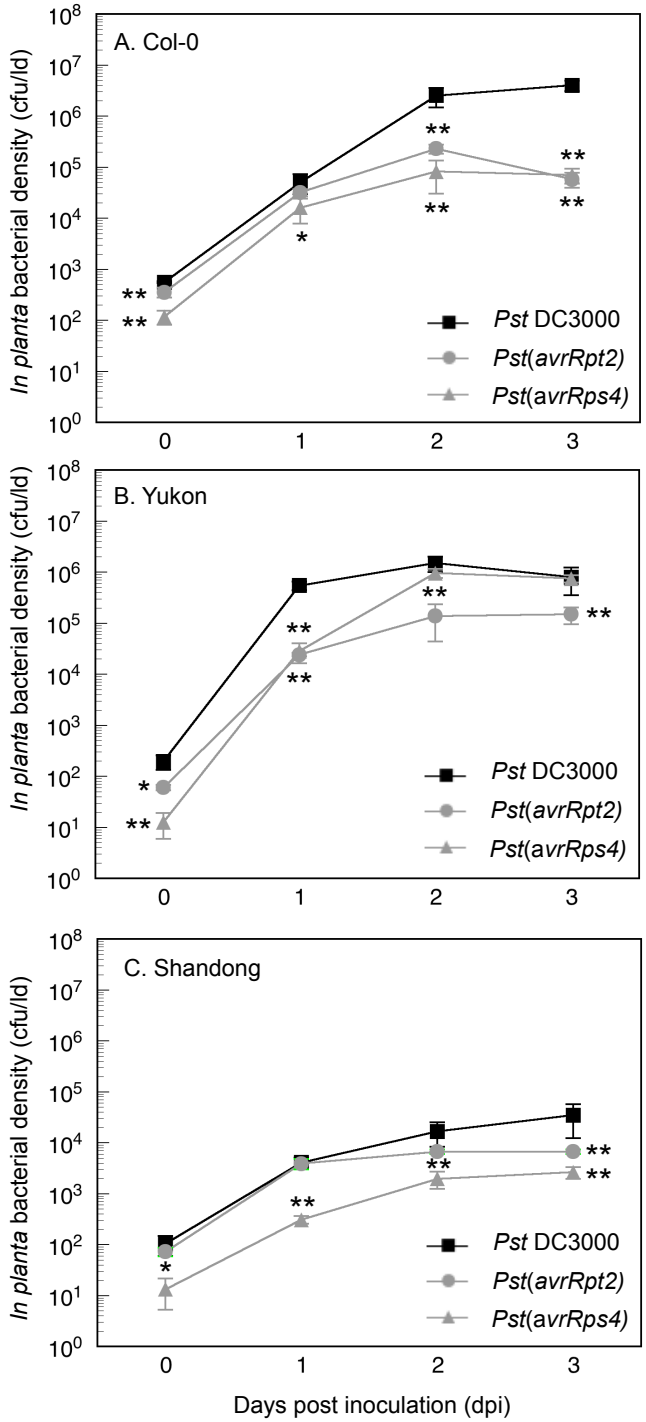

A. Col-0
B. Yukon
C. Shandong
In planta bacterial density (cfu/ld)
Days post inoculation (dpi)
Pst DC3000
Pst(avrRpt2)
Pst(avrRps4)

Figure 8. *In planta* bacterial growth of *Pst* DC3000 (black squares), *Pst(avrRpt2)* (grey circles) and *Pst(avrRps4)* (grey triangles) over a period of 0-3 days after inoculation. Col-0 (A), Yukon (B) and Shandong (C) plants were inoculated with an inoculum concentration of 10^6 cfu/ml. Each data point represents the mean ± standard deviation of three biological replicates. Asterisks (*) denote significant differences between levels of *Pst* and *Pst* expressing either of the avirulence proteins at each time point according to Tukey's HSD (* = $p < 0.05$ and ** = $p < 0.01$). In some cases the error bar does not extend beyond the symbol. Each experiment was repeated twice with similar results.

3.1.4.2 Electrolyte leakage assay to monitor cell death during *Pst* DC3000 and *Pst*(*avrRpt2*) interactions with Yukon and Shandong *Eutrema*

The previous section suggests that R gene-mediated resistance occurs in Yukon and Shandong *Eutrema*. In *Arabidopsis*, HR cell death symptoms occur during the ETI response elicited by AvrRpt2 and AvrRps4. *Arabidopsis* leaves that are inoculated with a high dose of an avirulent *Pst* strain show an extreme HR response and collapse (Lewis et al., 2010). To examine macroscopic HR in *Eutrema*, half leaves of Yukon, Shandong and Col-0 were inoculated with a high dose of *Pst*(*avrRpt2*), *Pst* DC3000 or $MgCl_2$ as a negative control and leaf collapse was measured 24 hpi. If the half-leaf that was inoculated appeared wilted and caved in, this was defined as leaf collapse. As expected, leaves inoculated with $MgCl_2$ or *Pst* DC3000 did not collapse in Col-0 (Figure 9A). Leaf collapse was observed in all Col-0 leaves inoculated with *Pst*(*avrRpt2*). Leaf collapse was not observed in Yukon leaves inoculated with *Pst* DC3000 but was present in all leaves inoculated with *Pst*(*avrRpt2*) (Figure 9B). Three Shandong leaves inoculated with *Pst* DC3000 did collapse but the majority did not collapse (Figure 9C). The collapse of Shandong leaves was not as severe as the collapse observed in Col-0 or Yukon leaves.

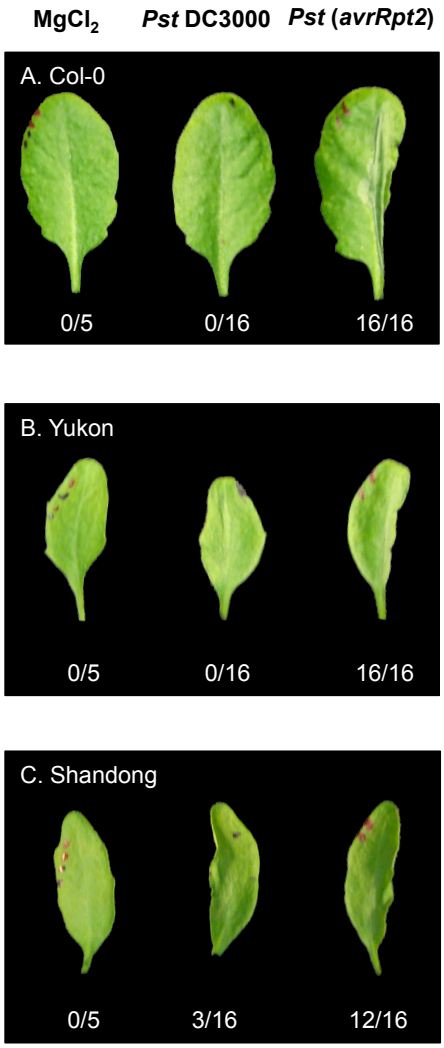


Figure 9. Representative leaves from a leaf collapse assay. Half-leaves of Col-0 (A), Yukon (B) and Shandong (C) plants were infiltrated with 10 mM $MgCl_2$ or 10^8 cfu/ml *Pst* DC3000 or *Pst*(*avrRpt2*). Photographs were taken 24 hour after inoculation. The numbers of leaves showing leaf collapse are indicated below each representative leaf.

3.1.4.3 Conductivity measurements in *Eutrema* inoculated with different *Pst* strains

R gene-mediated HR can be quantified by detecting HR cell death-associated ion leakage at the site of inoculation. As the cells at the site of HR die, they release electrolytes into the extracellular environment. The conductance of these electrolytes from damaged and dying cells can be measured and used as a proxy for HR cell death. To determine if cell death is occurring in *Eutrema* after inoculation with HR-inducing strains (avirulent *Pst* strains), Col-0, Yukon and Shandong *Eutrema* were inoculated with *Pst* DC3000, *Pst* DC3000 (*avrRpt2*) and *Pst* DC3000 (*avrRps4*). As a negative and wounding control, plants were also inoculated with $MgCl_2$. These plants displayed little cell death or electrolyte leakage (Figure 10A, B, C). Leaf disks were collected and electrolyte leakage into sterile water was measured over time. Electrolyte leakage from Col-0 leaves inoculated with *Pst*(*avrRpt2*) increased substantially between 5 and 17 hpi, leveling off from 20 to 29 hpi (Figure 10A). Electrolyte leakage from Col-0 leaves inoculated with *Pst* DC3000 and *Pst(avrRps4)* was similar to the $MgCl_2$ control. Electrolyte leakage was similar in Yukon and Shandong leaves inoculated with all three *Pst* strains and $MgCl_2$ (Figure 10B and C). Bacterial density of leaves inoculated with *Pst, Pst(avrRpt2)* and *Pst(avrRps4)* were also collected 3 dpi and is presented in Figure 10D. Taken together, these 2 cell death assays suggest that Yukon exhibits AvrRpt2-mediated resistance and Shandong exhibits AvrRps4-mediated resistance in the absence of cell death.

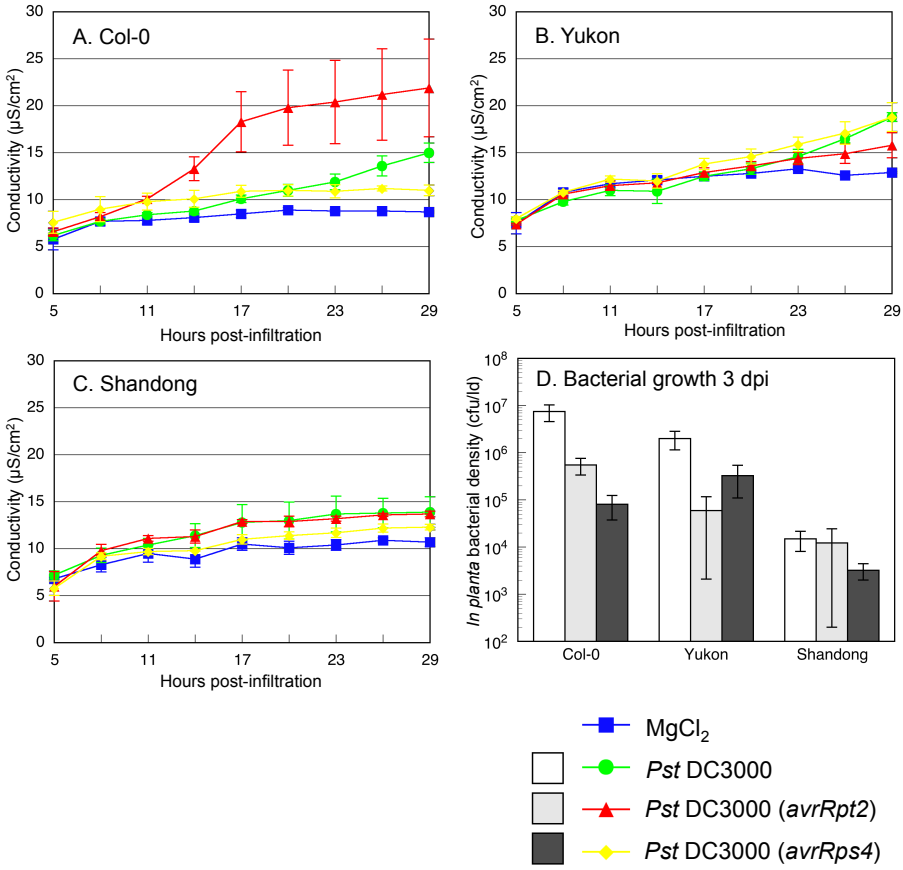


Figure 10. Electrolyte leakage assay of (A) Col-0, (B) Yukon and (C) Shandong leaf disks after inoculation with *Pst* DC3000, *Pst*(*avrRpt2*) and *Pst*(*avrRps4*). Leaves were inoculated with 10^6 cfu/ml and conductivity was measured at the indicated time points. $MgCl_2$ was inoculated into leaves as a negative and wound control. *In planta* bacterial growth was measured at 3 dpi for all bacterial strains in Col-0, Yukon and Shandong plants (D). Each data point represents the mean ± standard deviation of three biological replicates.

3.1.5 Examination of SAR in Yukon and Shandong *Eutrema*

Priming plants by infection with a necrotizing pathogen can lead to enhanced disease resistance or SAR in the form of reduced *in planta* bacterial growth and symptoms. To determine if Yukon and Shandong *Eutrema* display this response, Yukon and Shandong *Eutrema* were induced for SAR with *Pst(avrRpt2)* or mock-inoculated with 10 mM $MgCl_2$. Col-0 was also included as a positive control for the SAR response. Two days later, distant leaves were challenged with virulent *Pst* DC3000 and leaf disks were collected 3 dpi to quantify *in planta Pst* growth in distant tissue. As expected, a 4.6-fold lower *Pst* levels was observed in SAR-induced compared to mock-inoculated Col-0, indicative of a SAR response (Figure 11). A statistically insignificant 3.2-fold difference was observed between mock- and SAR-induced Yukon *Eutrema*. Although it was not statistically significant, a 3-fold difference between mock- and SAR-induced *Pst* growth suggests that SAR occurs in this *Eutrema* accession. In other Yukon SAR replicates, the SAR response was weaker (Appendix Figure 24) however in those replicates, the SAR response in Col-0 was also weak. SAR-induced Shandong *Eutrema* is not more resistant to infection compared to mock-inoculated Shandong *Eutrema*. Other Shandong SAR replicates (Appendix Figure 24) displayed a similar response. Taken together, this data suggests that SAR does not occur in Shandong, but may occur in Yukon *Eutrema*, however further replicate experiments are required to validate these results.

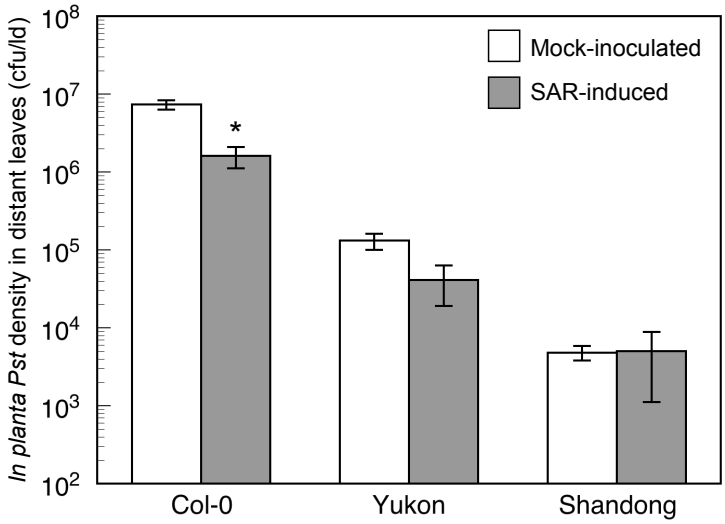


Figure 11. *Pst* DC3000 levels in distant leaves induced for SAR. SAR assays were conducted by mock-inoculating with 10 mM $MgCl_2$ or inducing for SAR with *Pst*(*avrRpt2*), followed by challenge inoculation with *Pst* DC3000 in distant leaves 2 days later. *In planta* bacterial levels were quantified 3 dpi. Asterisks (*) denote significant differences between challenged distant leaves of mock- and SAR-induced plants according to the Student's *t*-test ($p < 0.01$). This experiment was repeated twice with similar results.

3.1.6 Examination of ARR in Yukon and Shandong *Eutrema*

3.1.6.1 Yukon *Eutrema* displays ARR in short-day conditions

The developmental age of a plant can affect the level of resistance observed whereby some plants exhibit ARR at later stages of development. To determine if enhanced resistance was observed as plants aged, resistance to *Pst* was monitored in Yukon and Shandong *Eutrema* each week, starting at 3 wpg and ending at 7 wpg. Each week, Shandong and Yukon *Eutrema* were inoculated with 10^6 cfu/ml *Pst* and bacterial levels were determined at 3 dpi. Col-0 served as a positive control while *sid2-1* was a negative control for the ARR response to *Pst*. *In planta* bacterial levels were determined 3 dpi. As expected, ARR was observed in Col-0 beginning at 5 wpg, with a 6-fold reduction in bacterial levels compared to 4 wpg plants and a 14-fold reduction in *Pst* levels between young (3 wpg) and mature plants (6 or 7 wpg) (Figure 12). Although bacterial levels did decrease in *sid2* plants from 3 to 7 wpg, bacterial levels remained above 10^7 cfu/ld throughout the experiment. In Yukon *Eutrema*, a 5-fold reduction in *Pst* levels was observed between 4 and 5 wpg, with a 21-fold reduction observed in 7 compared to 6 wpg plants. An overall 62-fold decrease in *Pst* levels between young (3 wpg) and mature (7 wpg) in Yukon *Eutrema* suggests a strong ARR response occurred. In contrast, the age of Shandong *Eutrema* did not appear to affect growth of *Pst in planta,* with similar levels of *Pst* in young and mature plants. These results suggest that in short-day conditions (9 hours light) Shandong *Eutrema* does not display ARR while Yukon *Eutrema* does display a strong ARR response.

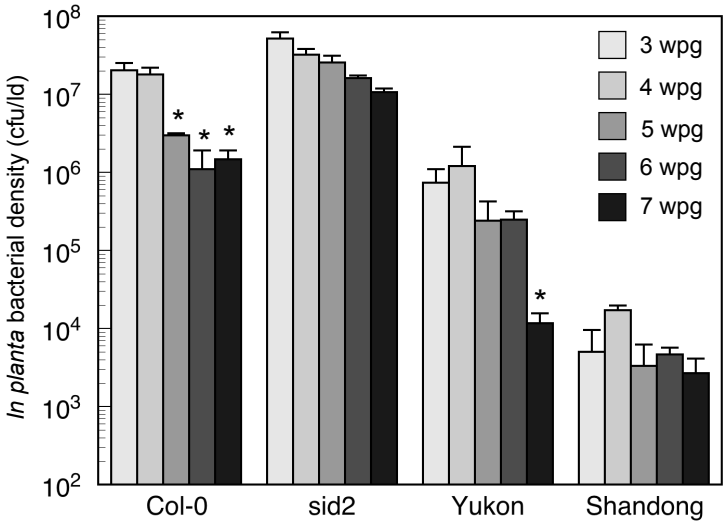


Figure 12. Response of Yukon and Shandong *Eutrema* to *Pst* from 3-7 weeks of age. Plants were grown in a short-day photoperiod (9h light) and inoculated with 10^6 cfu/ml *Pst* DC3000 each week. Bacterial levels were quantified 3 dpi. Each data point represents the mean ± standard deviation of three biological replicates. Asterisks (*) denote significant differences in bacterial growth relative to the levels observed in plants of the same accession at three weeks of age according to Tukey's HSD ($p < 0.01$). This experiment was repeated three times with similar results.

3.1.6.2 The ARR response occurs in Shandong *Eutrema* grown in long days

Initial pathogen studies with *Eutrema* were performed in short-days (9 hours of light) to allow comparisons to the highly characterized *Arabidopsis-Pseudomonas* pathosystem. To delay flowering and increase leaf size and number for pathogen inoculation, *Arabidopsis* is grown in short-day conditions (Dong et al., 1991; Whalen et al., 1991; Katagiri et al., 2002). These short-day conditions however produced small Yukon and Shandong plants, making it difficult to collect sufficient tissue for biochemical studies. In addition, the RNA-Seq transcriptome study was conducted on plants grown in long-day conditions to mimic the conditions present in the Yukon. For these two reasons, disease resistance was examined in long-days (16 hours of light) from 3 to 7 wpg. Both accessions appeared larger and morphologically different compared to their short-day counterparts. These differences included shorter petioles and larger leaves in long-day-grown Yukon and Shandong *Eutrema* (Appendix Figure 31). In two replicate experiments, Yukon *Eutrema* supported *Pst* levels similar to those observed in short-day-grown plants. An ARR response was also observed with a 12-fold less *Pst* in 5 compared to 4 wpg plants and 7-fold in 6 compared to 5 wpg (Figure 13). Long-day-grown Shandong *Eutrema* also supported *Pst* levels similar to short-day Shandong. However *Pst* resistance was enhanced between 5 and 7 wpg, suggesting that Shandong *Eutrema* displays ARR when grown in long days.

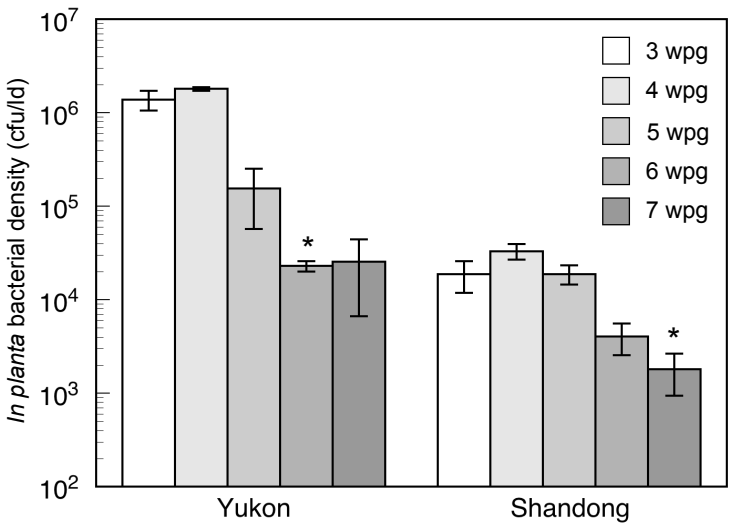


Figure 13. Yukon and Shandong *Eutrema* grown in long-day conditions and inoculated with 10^6 cfu/ml *Pst* DC3000 each week between 3 and 7 weeks post germination. *In planta* bacteria were quantified 3 dpi. Each data point represents the mean ± standard deviation of three biological replicates. Asterisks (*) denote significant differences in bacterial levels relative to plants of the same accession at three weeks of age according to the Games-Howell post-hoc test ($p < 0.05$). This experiment was repeated twice with similar results.

3.1.7 Does *Eutrema* contain salicylic acid, a key molecule for defense?

Salicylic acid is required for the activation of defense gene expression in *Arabidopsis* (Shah & Zeier, 2013). To determine if SA was produced in *Eutrema* in response to *Pst*, free and total SA levels were measured in total leaf extracts. Total SA represents the amount of free SA plus conjugated SA (salicylic acid glucoside). Typical background levels of free SA were detected in untreated and mock-inoculated Col-0 leaves (Figure 14A). Free SA levels rose at 12 and 24 hpi with *Pst* in Col-0 leaves. A similar trend was observed in total SA levels in Col-0. Negative SA values were observed for free and total SA in both *Eutrema* accessions (Figure 14B, C). These results may be due to the use of *sid2 Arabidopsis* leaves to construct the SA standard curve. Differences in the composition of leaf extracts between *Arabidopsis* and *Eutrema* may be responsible for the negative values observed. Using *sid2* leaf extracts for the standard curve does not appear to be suitable for quantifying SA levels in *Eutrema*.

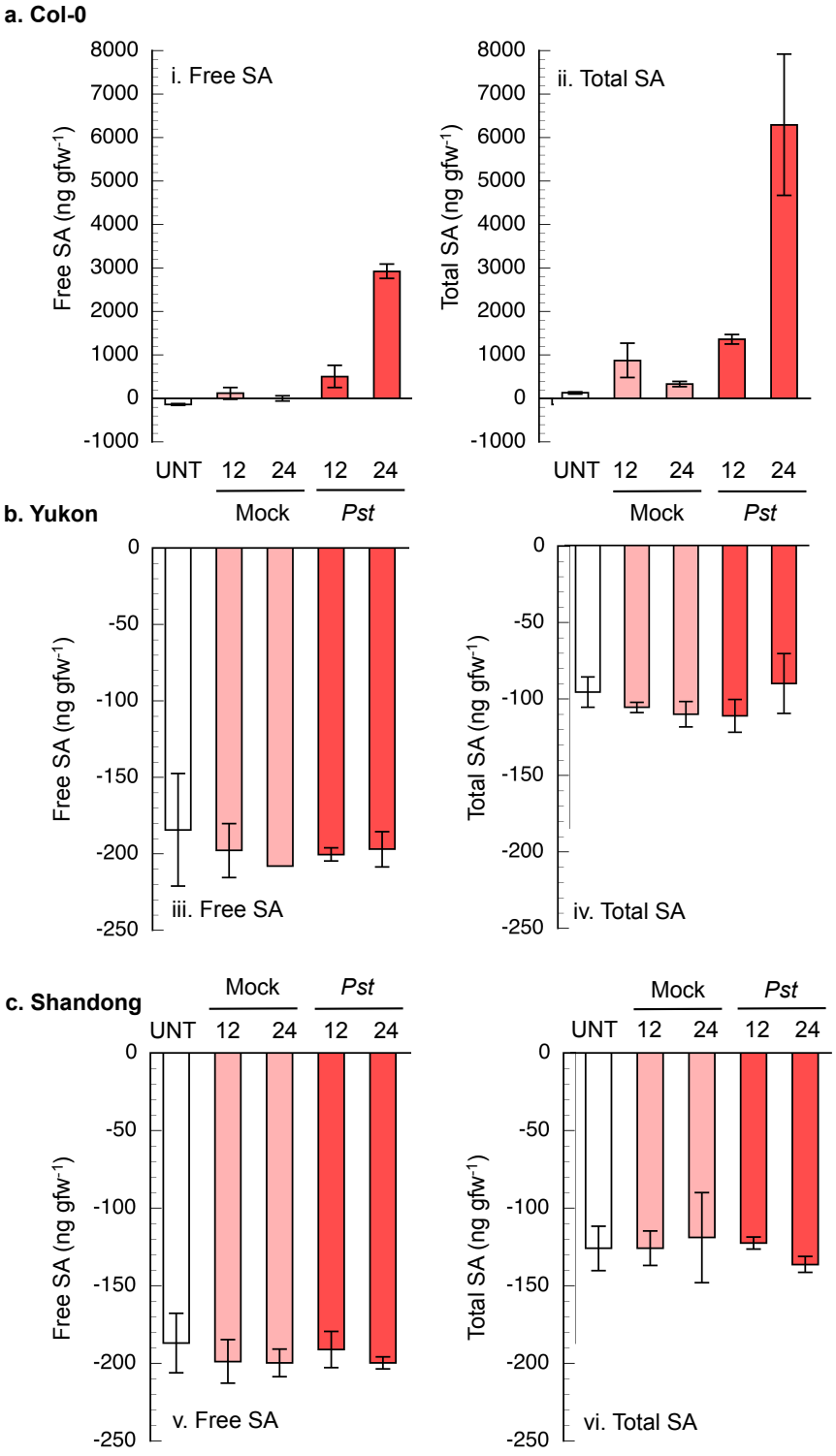

a. Col-0
i. Free SA
ii. Total SA
Free SA (ng gfw-1)
Total SA (ng gfw-1)
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
UNT
12
24
12
24
Mock
Pst
b. Yukon
iii. Free SA
iv. Total SA
0
-50
-100
-150
-200
-250
c. Shandong
v. Free SA
vi. Total SA

Figure 14. Quantification of total and free SA levels in (a) Col-0, (b) Yukon and (c) Shandong. 4 week-old plants were inoculated with 10^6 cfu/ml *Pst* DC3000 or mock-inoculated with 10 mM $MgCl_2$. Leaves were collected at 12 and 24 hpi and untreated leaves was collected for each accession. Free and total SA levels were determined and presented as ng/g·fw for Col-0 (a. i. & ii.), Yukon (b. iii. & iv.) and Shandong (c. v. & vi.). Each bar represents the mean ± standard deviation of three biological replicates. UNT = untreated.

3.1.8 *PR1* expression in Yukon and Shandong *Eutrema* after pathogen inoculation

3.1.8.1 *PR1* expression in Yukon and Shandong *Eutrema* inoculated with *Pst*

Expression of *PR1* is used as a molecular marker for the activation of defense responses in infected leaves (Van Loon et al., 2006). In addition, *PR1* expression serves as a priming marker to monitor the rapid and enhanced expression of defense genes observed in defense-primed plants (Ryals et al., 1996; Kohler et al., 2002; Gruner et al., 2013). RNA-Seq transcriptome data derived from uninoculated *Eutrema* plants demonstrated that healthy Shandong plants expressed *EsPR1* at a level five-fold higher than in Yukon plants (Yeo et al., in submission; Champigny et al., 2013), suggesting that these plants exist in a primed-like state for enhanced pathogen defense. If Shandong exists in this highly alert, primed state, then we expected to observe enhanced *EsPR1* expression after inoculation with *Pst* in Shandong, but not in Yukon plants. To test this hypothesis, *PR1* expression was examined using RT-PCR in *Eutrema* and *Arabidopsis* (Col-0) leaves inoculated with *Pst* (Figure 15). *Actin1* expression was used as an RNA loading control in these experiments. *PR1* expression in Col-0 served as a positive control for a typical, unprimed *Arabidopsis PR1* response to *Pst*. Unexpectedly, very faint *PR1* was expressed in untreated Col-0 leaves. Modest *PR1* expression was observed at 4 hpi with expression rising at 12, 24 and 48 hpi in Col-0. Little *EsPR1* expression was observed in Yukon plants until 48 hpi with *Pst*. In contrast to the Yukon accession, *EsPR1* was expressed in untreated Shandong leaves, with enhanced expression observed at 12, 24 and 48 hpi compared to untreated Shandong leaves. *EsPR1* expression was observed at earlier time points after inoculation (4, 12 and 24 hpi) in Shandong compared to Yukon *Eutrema.* Early and enhanced *EsPR1* expression in response to *Pst* compared to modest expression in untreated tissue supports the idea that Shandong *Eutrema* exists in a primed-like state allowing it to respond effectively to *P. syringae.*

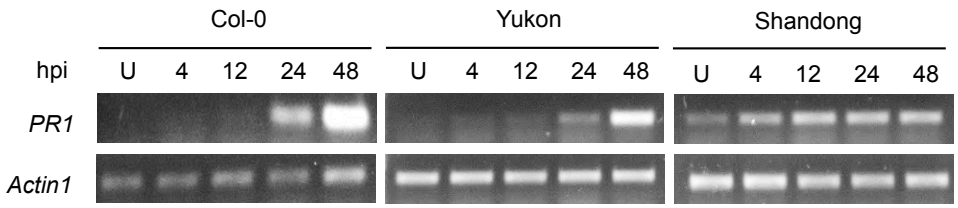


Figure 15. *PR1* expression in leaves inoculated with 10^6 cfu/ml *Pst* DC3000. *PR1* and *Actin1* expression were measured by RT-PCR of cDNA generated from four-week-old Col-0, Yukon and Shandong leaf tissue collected at the indicated hours post infection (hpi) with *Pst*. U = uninoculated leaf tissue. This experiment was repeated twice with similar results.

3.1.8.2 Yukon and Shandong *PR1* expression in a *hrp*-deficient *Pst* strain

PR1 expression was also examined in Yukon and Shandong leaves infected with a *Pst* strain unable to produce a functional Hrp pilus. In the absence of a functional pilus, *Pst* is unable to mount effector-mediated suppression and limit *PR1* expression and this has been demonstrated by Nobuta et al. 2007, demonstrating higher *PR1* expression 24 hpi in *hrcC*$^-$-inoculated plants compared to *Pst*-inoculated plants. If *Pst* virulence effectors are responsible for suppressing *PR1* expression, then an increase in *PR1* expression should be observed in Yukon and Shandong plants inoculated with the *hrpA*$^-$ mutant. To test this hypothesis, *PR1* expression was monitored using RT-PCR in *Eutrema* leaves inoculated with *Pst* DC3000 and *Pst* DC3000 *(hrpA*$^-$*)*. Again, *Actin1* expression was used as an internal control in these experiments. In Yukon plants inoculated with *Pst* DC300, *PR1* expression was not observed until 24 hpi and increased at 48 hpi (Figure 16A). Faint *PR1* expression was observed at 12 hpi and strongly at 48 hpi in Yukon plants inoculated with the *hrp*-deficient strain. In Shandong plants inoculated with *Pst*, *PR1* was expressed at all time points (Figure 16B). Expression of *PR1* was absent at 4 hpi and observed at 12, 24 and 48 hpi in plants inoculated with the *hrp*-deficient strain. Overall, these findings suggest that the T3SS and injected effectors somewhat suppressed Yukon *PR1* expression while it does not appear to affect Shandong *PR1* expression, except immediately after inoculation with *Pst*.

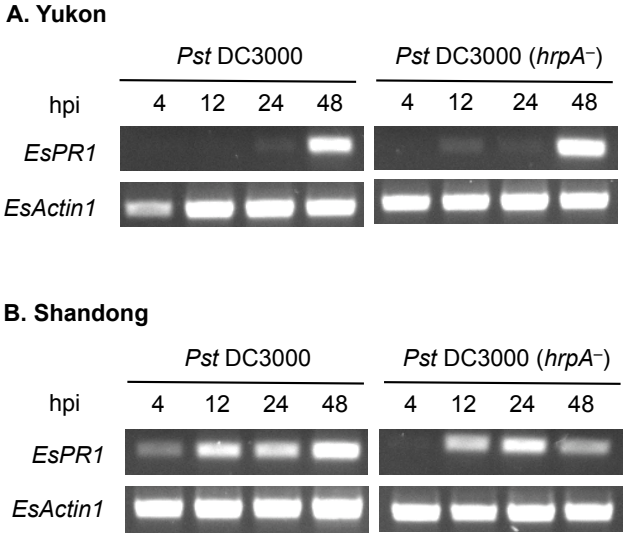


Figure 16. *PR1* expression in leaves inoculated with 10^6 cfu/ml *Pst* DC3000 or *Pst* DC3000 (*hrpA*⁻). *PR1* and *Actin1* expression were measured by RT-PCR of cDNA generated from four-week-old Yukon (A) and Shandong (B) leaf tissue collected at the indicated hours post infection (hpi) with *Pst*. This experiment was repeated twice with similar results.

Disclaimer: Philip Carella performed the initial *Psm* replicate and I performed subsequent replicates (Section 3.1.9).

3.1.9 Yukon and Shandong *Eutrema* exhibit enhanced resistance to *P. syringae* pv. *maculicola* (*Psm*)

The previous sections examined *Eutrema's* responses to virulent and avirulent *Pst* strains and demonstrated that Yukon and Shandong *Eutrema* are highly resistant to *Pst* DC3000 and exhibit ETI-mediated responses. These resistance responses could indicate that *Pst* is not adapted for growth on *Eutrema*. It is possible that both accessions deploy ETI-mediated resistance against *Pst* because they possess resistance receptors that specifically recognize *Pst* effectors other than AvrRpt2 and AvrRps4. To determine if *Eutrema* resistance to *Pst* is limited to one particular *P. syringae* strain, a second *P. syringae* pathovar, *P. syringae* pv. *maculicola (Psm),* which is virulent on many *Arabidopsis* accessions, was tested on *Eutrema*. Yukon and Shandong *Eutrema* were inoculated with 10^6 cfu/ml *Psm* and growth was monitored for three days after inoculation. The response to *Pst* DC3000 was compared to *Psm* in both *Eutrema* accessions. In Col-0, *Psm* growth was similar to *Pst,* which was an anticipated response since both strains are virulent on Col-0 (Appendix Figure 27). In Yukon plants inoculated with *Psm*, *Psm* levels were reduced compared to plants inoculated with *Pst* such that *Psm* levels were 31-fold lower by 3 dpi (Fig. 17A) indicating that Yukon *Eutrema* is more resistant to *Psm* than to *Pst.* In contrast, *Psm* growth in Shandong *Eutrema* closely matched *Pst* growth throughout the experiment, reaching 2.4 x 10^4 cfu/ld by 3 dpi (Figure 17B). Leaves inoculated with *Psm* were also examined 3 dpi to determine if differences existed in disease symptoms compared to *Pst*. In Yukon leaves inoculated with *Psm*, small areas of chlorosis were observed consistent with lower levels of *Psm* growth (Figure 17C). In Shandong leaves inoculated with *Psm*, symptoms were absent, identical to the symptomless leaves observed in Shandong *Eutrema* leaves inoculated with *Pst*. Taken together, this data supports the hypothesis that Yukon *Eutrema* is more resistant to *Psm* compared to *Pst* in terms of its ability to suppress *in planta* growth and disease symptoms. Lack of differences in symptoms and bacterial growth in Shandong *Eutrema*

inoculated with either *P. syringae* pathovar suggests that Shandong *Eutrema* is equally resistant to *Psm* as it is to *Pst*.

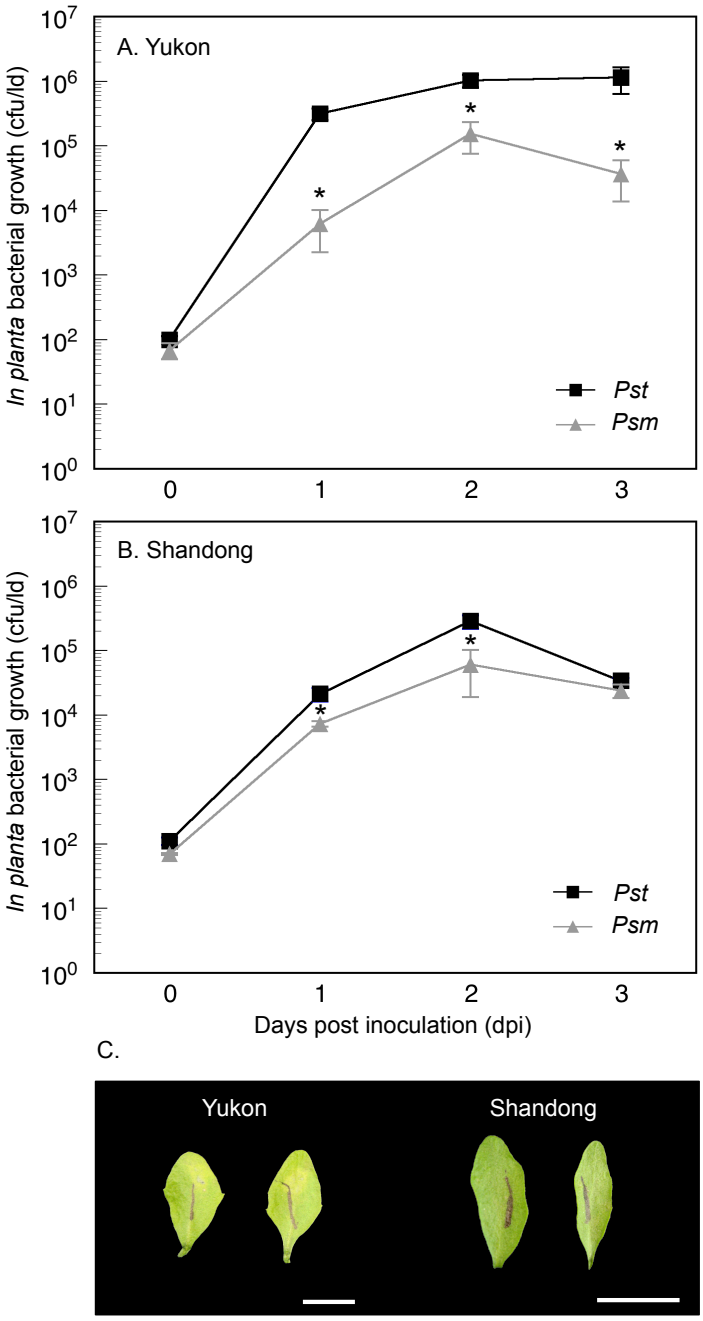


Figure 17. Comparison of *in planta* bacterial growth of *Psm* and *Pst*. Four-week-old Yukon (A) and Shandong (B) plants were inoculated with 10^6 cfu/ml *Psm* or *Pst*. Bacterial growth was quantified at the indicated time points and presented as the mean ± standard deviation of three biological replicates. Asterisks (*) denote significant differences between *Psm* and *Pst* levels at each time point according to the Student's *t*-test ($p < 0.05$). Corresponding images of inoculated leaves 3 dpi are presented in (C). Bar = 1 cm. This experiment was repeated twice with similar results.

3.1.10 Responses to a *Pst* strain expressing *Psm*-specific effectors in Yukon and Shandong *Eutrema*

Comparing *Psm* and *Pst* growth in both *Eutrema* accessions revealed that Yukon is 31-fold more resistant to *Psm* compared to *Pst*. To understand why Yukon *Eutrema* responded differently to *Pst* compared to *Psm*, we took advantage of a study done by Baltrus et al. (2011) who examined effector differences between *Pst* and *Psm*. HopW1-1 is a *Psm*-specific effector that initiates ETI in the Ws accession but not in other accessions (Lee et al., 2008). To determine if Yukon *Eutrema* also recognizes HopW1-1 to initiate ETI and limit *Psm* growth, a *Pst* DC3000 strain expressing HopW1-1 was used to inoculate Yukon *Eutrema*, Shandong *Eutrema* and Col-0. In hindsight, the Ws accession should have been used instead of Col-0 to serve as a better positive control to observe HopW1-1-mediated ETI resistance. In Col-0, HopW1-1 expression actually conferred a growth advantage compared to *Pst* DC3000 that lacks its expression (Lee et al., 2008) and this result was observed in Figure 18A. In Shandong plants, no difference was observed in bacterial levels for either strain. In Yukon plants, there was also no difference in *in planta* levels of *Pst* DC3000 or *Pst* DC3000 expressing HopW1-1. This data suggests that HopW1-1 does not affect the resistance response observed in Yukon *Eutrema to Psm*.

HopZ1c is another *Psm*-specific effector and its expression in a *Pst* DC3000 background was used to determine if Yukon *Eutrema* also recognizes HopZ1c to initiate ETI and limit *Psm* growth. Increased *in planta* bacterial density was observed in Yukon plants inoculated with *Pst* DC3000 (HopZ1c) compared to *Pst* DC3000 suggesting that it does not affect the resistance response observed in Yukon *Eutrema* to *Psm* (Figure 18B). In fact, the presence of HopZ1c actually enhanced *Pst* DC3000's ability to cause disease in both *Eutrema* accessions as well as Col-0.

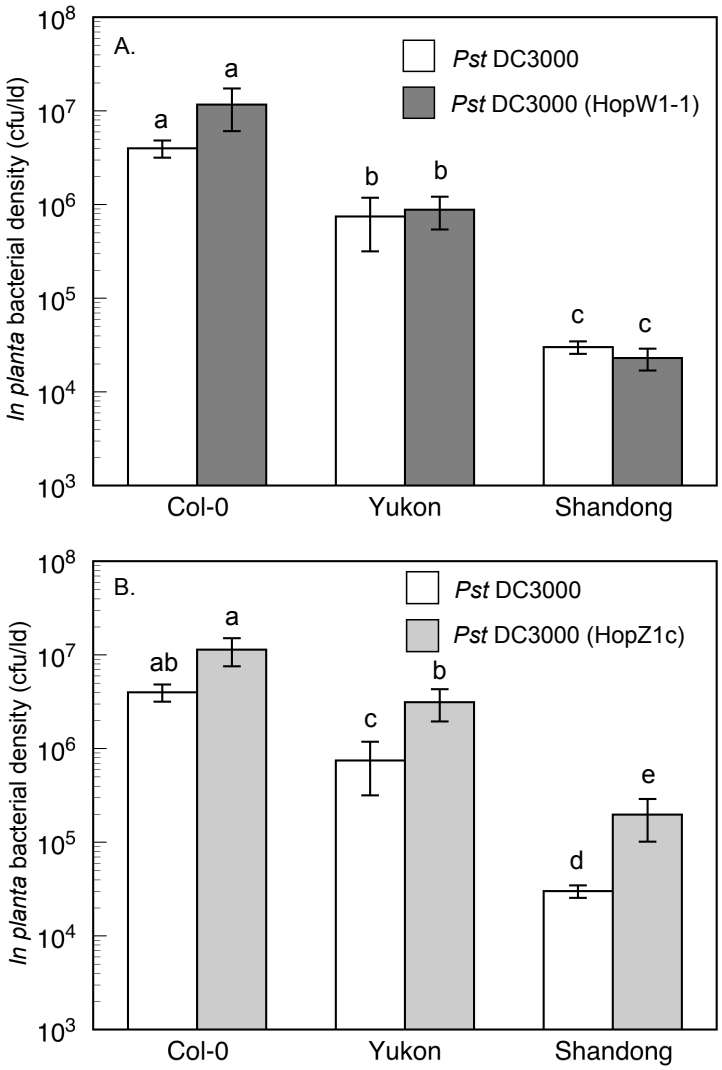


Figure 18. *In planta* growth of *Pst* DC3000, *Pst* DC3000 expressing HopW1-1 and *Pst* DC3000 expressing HopZ1c. Four-week-old plants were inoculated with 10^6 cfu/ml of *Pst* DC3000, *Pst* DC3000 (HopW1-1) (A) or *Pst* DC3000 (HopZ1c) (B). Leaf discs were collected 3 dpi. Different letters denote significant differences according to Tukey's HSD ($p < 0.05$). This experiment was repeated twice with similar results.

3.2 Responses to other pathogens

3.2.1 Examination of Shandong *Eutrema*'s response to *Pectobacterium carotovorum* ssp. *wasabiae (Pcw)*

The *Pst* resistant phenotype in combination with *PR1* expression in untreated Shandong leaves suggests that it may exist in a primed-like state of defense-preparedness. To test this hypothesis, another pathogen called *Pectobacterium carotovorum* sp. *wasabiae* (*Pcw*) was examined. The first inoculation assay involved dipping Shandong leaves in a *Pcw* suspension with 0.01% Silwet, followed by scoring the number of rotted leaves at 1 and 2 dpi. The percentage of rotted leaves in the positive control Col-0 was 38.3% and 58.3% at 1 and 2 dpi, respectively, while the percentage of rotted Shandong leaves was lower at 7.59% and 16.5% at 1 and 2 dpi (Figure 19A). Though no signs of disease symptoms were observed on Shandong leaves, mock-dipped leaves were speckled and had a sparkly appearance. Concerned that the Silwet solution was adversely affecting the leaves and perhaps the course of the disease, another method of inoculation was used. The second method involved inoculating leaves, followed by scoring susceptibility similar to the dipping protocol. As a negative control, a different set of Col-0 and Shandong plants were inoculated with a 0.9% NaCl solution. NaCl was used as a buffer to resuspend *Pcw* strain 3193 as per Brader et al. 2001. 41.7% of Col-0 leaves and 16.7% of Shandong leaves were rotted 1 dpi (Figure 19B). These values are very similar to the 1 dpi percentages of dipped plants. However, a large percentage of Col-0 leaves mock inoculated with the saline solution rotted as well (37.5%), while the number of saline-inoculated Shandong leaves remained low at 3.7%. Older Col-0 leaves dipped with *Pcw* collapsed and rotted while Shandong leaves did not rot or collapse (Figure 19C). These preliminary results suggest that the primed-like state of Shandong *Eutrema* provided resistance against *Pcw* infection, however further replicate experiments are required to confirm these findings.

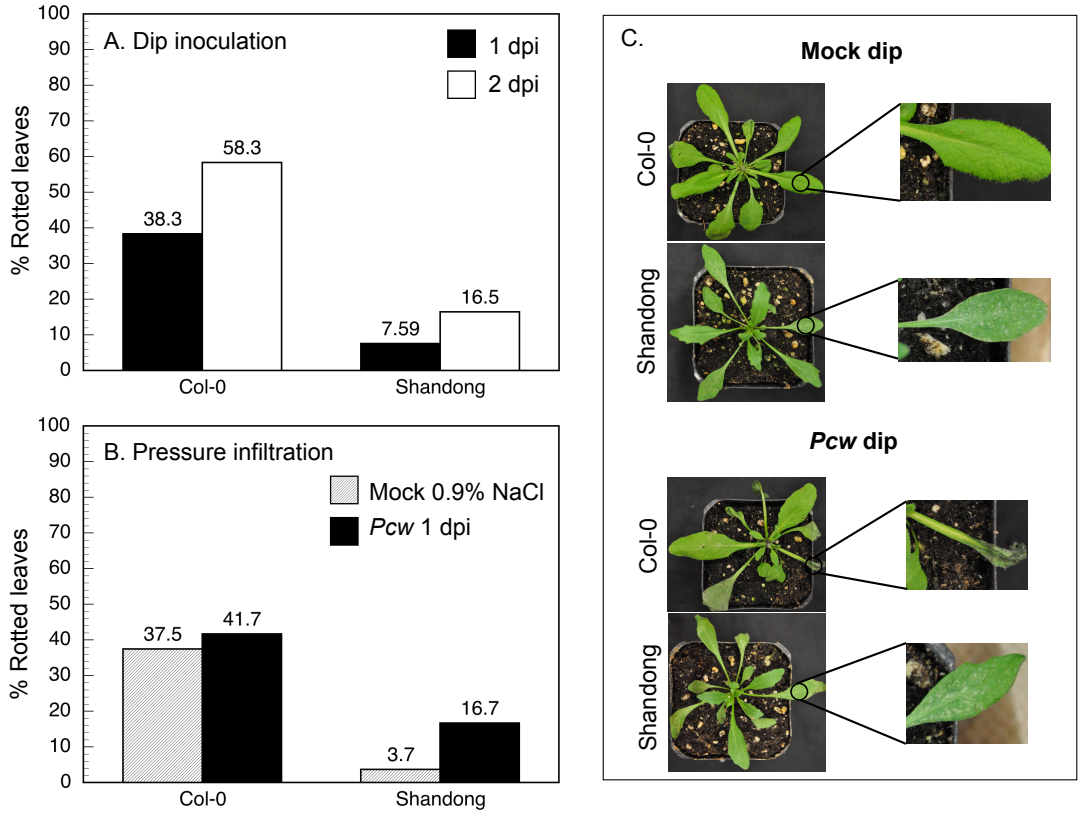


Figure 19. *Pcw* SCC3193 dip and inoculation assays in Shandong *Eutrema*. Four-week-old long-day-grown plants were either dipped with a 10^6 cfu/ml *Pcw* SCC3193 suspension for 15 minutes (A) or inoculated with 10^6 cfu per ml *Pcw* SCC3193 (B). Mock treatments consisted of infiltrating with a 0.9% NaCl solution. The number of rotted leaves was counted 1 and 2 dpi for the dip method or 1 dpi for the pressure infiltration method. Symptoms of mock- and *Pcw*-dipped leaves are shown in (C). This experiment has been repeated twice for the dip method and once for the pressure infiltration method.

3.2.2 Examination of *Eutrema*'s response to *Xcc* inoculation

To continue testing the primed-like state of Shandong *Eutrema*, another pathogen was assayed for its ability to cause disease on Shandong as well as Yukon *Eutrema*. *Xanthomonas* species are known to infect many *Brassica* species including *B. oleracea* and cause vascular wilts through infection of the xylem tissue. To determine if this pathogen causes similar symptoms and disease in *Eutrema*, the mid vein of leaves was punctured and inoculated with a high dose of an *Xcc* strain known to be pathogenic in Col-0. High *in planta Xcc* levels in Col-0 were obtained in one replicate experiment (Appendix Figure 29) while the other two replicate experiments supported lower than expected levels in Col-0. One of these replicates is presented in Figure 20. V-shaped disease lesions were present in Col-0 leaves that supported lower levels of *Xcc* growth compared to Shandong *Eutrema* (Figure 20B). In all replicates, infected Yukon leaves supported an average of 10^4 cfu/ld (Figure 20A) and did not display V-shaped lesions. Instead limited chlorosis surrounding the wounds was observed. Inoculated Shandong plants consistently supported high levels of growth at 10^6 cfu/ld and V-shaped lesions were present in one experiment. Taken together, the presence of V-shaped lesions and high *in planta Xcc* growth indicates that *Xcc* was able to successfully colonize Shandong *Eutrema*.

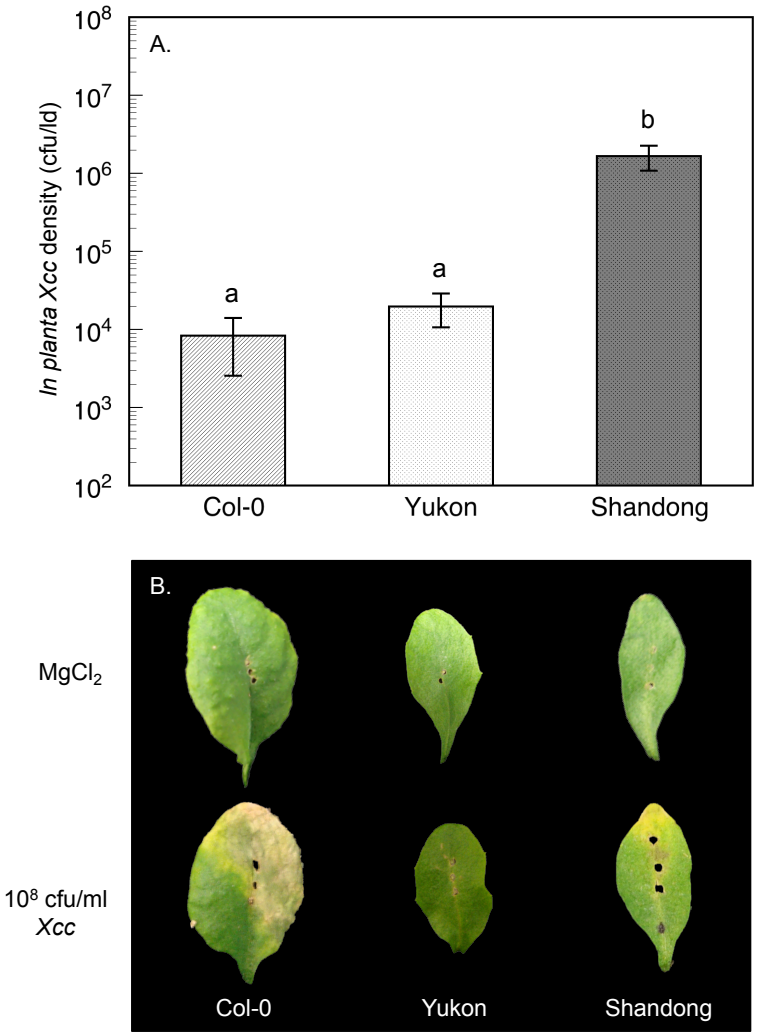


Figure 20. Inoculation of *Eutrema* with *Xcc*. Four-week-old long-day-grown Col-0, Yukon and Shandong plants were inoculated with 10^8 cfu/ml *Xcc* 8004 or mock inoculated with $MgCl_2$. At 7 dpi, *in planta* bacterial growth was measured (A) and photos of infected leaves were captured (B). Each bar represents the mean ± standard deviation of three biological replicates. Different letters denote significant differences according to Tukey's HSD ($p < 0.05$). This experiment was repeated three times with similar results.

3.3 Abiotic priming in *Eutrema*

3.3.1 Salt priming of *Eutrema* produces variable responses to *Pst*

Griffith et al. (2007) and Dedrick (2007) both demonstrated that Yukon *Eutrema* could be primed for enhanced tolerance to cold and drought, respectively, through prior exposure to cold and drought. M. Champigny (unpublished data) was the first to demonstrate that an abiotic stress (drought) was able to enhance tolerance to a biotic stress (*Pst*) in Yukon *Eutrema*. To determine if another abiotic stress could prime for enhanced resistance, plants were treated with NaCl and then challenged with *Pst*. Initial salt priming experiments exposed seedlings to 250 mM NaCl (or 100 mM for Col-0) for 5 days. Control seedlings were transferred to new media that did not contain NaCl. Seedlings were then transferred to soil and inoculated with 10^6 cfu/ml *Pst* at 4 wpg. The first replicate of three showed a 18-fold reduction in *Pst* levels in salt-treated compared to untreated Yukon plants (Figure 21A). Salt treatment of Shandong plants actually induced a 21-fold increase in *Pst* levels compared to untreated plants. However, this significant difference between *in planta Pst* levels in salt-treated and untreated Yukon plants could not be replicated in other experiments (Appendix Figure 30).

Since these seedling exposure studies could not be replicated, a different method of salt application was carried out. Three wpg Col-0, Yukon and Shandong *Eutrema* were irrigated once with a 100 mM (Col-0) or 200 mM saline solution and challenged with 10^6 cfu/ml *Pst* at 4 wpg. Col-0 plants irrigated with a low dose of NaCl (100 mM) did not differ in their ability to support pathogen growth in comparison to plants watered with H_2O (Figure 21B). Watering Col-0, Yukon and Shandong plants with 200 mM NaCl also did not have an effect on *Pst* growth compared to control plants watered with H_2O. Taken together, irrigation of 3 wpg plants and exposure to NaCl at the seedling stage did not enhance Yukon and Shandong *Eutrema's* resistance to *Pst*.

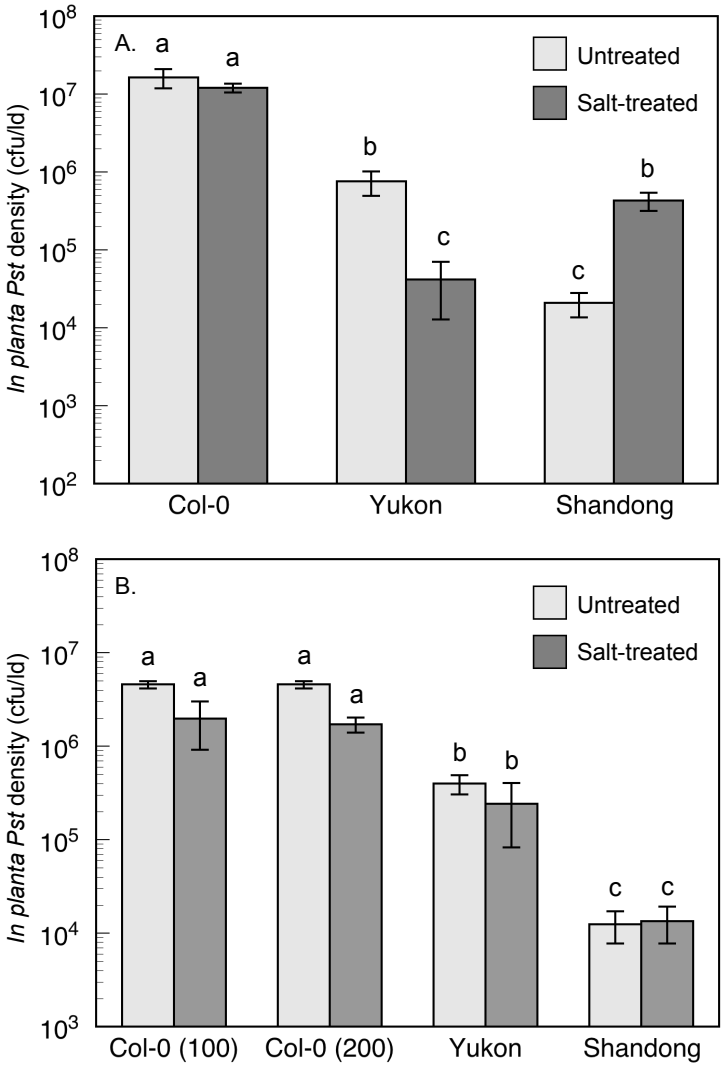


Figure 21. Assessing *Pst* resistance after treatment with NaCl. Seedlings were exposed to 250 mM NaCl (or 100 mM for Col-0) for 5 days or 3 wpg plants were irrigated with 200 mM NaCl (and 100 mM for Col-0). At 4 wpg, salt-treated plants were inoculated with 10^6 cfu/ml *Pst* and bacterial levels were quantified 3 dpi. *In planta Pst* levels in adult plants that were salt-treated as seedlings are presented in (A) and of saline-irrigated plants are presented in (B). Each bar represents the mean ± standard deviation of three biological replicates. Different letters denote significant differences according to Tukey's HSD ($p < 0.05$). The plate exposure assay was repeated three times while the irrigation assay was performed once.

Chapter 4 – Discussion

4.1 Development of the *Eutrema-P. syringae* pathosystem

The main objective of this thesis was to develop a pathosystem for the abiotic stress-tolerant model *Eutrema* and *P. syringae* to improve our ability to investigate plant responses to both abiotic and biotic stress. I established a viable and reproducible model pathosystem and demonstrated that both Yukon and Shandong *Eutrema* are more resistant to *Pst* than Col-0. Moreover, the two accessions of *Eutrema* displayed different levels of resistance to *Pst* such that *Pst* growth increased 1200-fold and 70-fold from 0 to 3 dpi in Yukon and Shandong plants, respectively, demonstrating Shandong *Eutrema's* highly resistant phenotype. The response to other *Pseudomonas* strains was also examined in *Eutrema* to further develop the pathosystem and explore the *Eutrema-P. syringae* interaction. These included strains expressing avirulence proteins (*Pst(avrRpt2)* and *Pst(avrRps4)),* strains lacking a functional T3SS (*hrpA*$^-$ mutant) or the phytotoxin coronatine (*cor*$^-$). Studies with avirulent *Pst* strains suggest that Yukon and Shandong plants exhibit differential capacities for ETI. The absence of the *Pst* T3SS compromised *Pst* growth in *Eutrema*. To determine if resistance was limited to one *P. syringae* strain, the *Eutrema* response to *Psm* was tested and demonstrated that Yukon plants displayed enhanced resistance to *Psm* relative to *Pst*. I monitored resistance at different developmental stages and found that Yukon displayed ARR in long and short-days while Shandong was ARR-competent in long-day conditions only. Evaluating *Eutrema's* responses to *P. syringae* and examining disease resistance pathways have demonstrated that this abiotic stress-tolerant plant species also exhibits resistance to *Pst*. This *Eutrema-P. syringae* pathosystem will make it possible to investigate both disease resistance and abiotic stress to understand how plants handle multiple stresses in their natural environment.

4.2 Does an agonistic relationship exist between biotic and abiotic stress pathways in *Eutrema*?

The stress response pathways activated by abiotic and biotic stress is largely mediated by plant hormones such as ABA, SA and JA. SA signaling pathways contribute to biotrophic pathogen resistance while JA/ethylene signaling contributes to herbivore resistance. ABA-mediated signaling contributes to drought and salt tolerance and stomatal closure during PTI. These pathways can also act synergistically or antagonistically to enhance or decrease tolerance. For example, SA-dependent defense such as callose deposition was compromised in plants treated with ABA. One-hundred μm ABA-treated *Arabidopsis* plants inoculated with *Pst* developed larger necrotic patches as measured by lactophenol-trypan blue staining and had decreased lignin accumulation compared to plants that did not receive ABA prior to *Pst* (Mohr & Cahill, 2003). In *Arabidopsis*, ABA treatment inhibited the induction of SAR by two chemical inducers that act upstream and downstream of SA signaling and suppressed *PR1* expression (Yasuda et al., 2008). Conversely, SAR activation suppressed the expression of ABA-biosynthesis and ABA-responsive genes (Yasuda et al., 2008). Although the role of ABA and SA in *Eutrema* are unknown, *Eutrema's* enhanced capacity to tolerate abiotic stress and *P. syringae* resistance suggests that an antagonistic relationship may not exist between abiotic tolerance and pathogen resistance if both hormones play similar signaling roles in *Eutrema*.

MAPK signaling is responsible for transmitting the perception of external stimuli into intercellular responses. Many stress response pathways rely on MAPK cascades and different cascades are activated in response to different external stimuli. For example, in *Arabidopsis*, the MAPK cascade downstream of perception of salt, cold and drought is composed of MEKK1, MKK2, MPK4 and/or MPK6 while the cascade downstream of the PRR *flg22* is composed of an unknown MEKK, MKK4/5 and MPK3/6 (Suarez Rodriguez et al., 2010). It is possible that MAPK cascades downstream of abiotic stimuli overlap with cascades downstream of biotic stimuli in *Eutrema*, therefore integrating these two pathways may activate both pathogen resistance and abiotic stress tolerance. For example,

as mentioned above, *MPK3* and *MPK6* are involved in both biotic and abiotic stress in *Arabidopsis* and are also involved in ABA-mediated stomatal closure in response to drought (Gudesblat et al., 2006). If *MPK3* and *MPK6* are upregulated in *Eutrema* and contribute to drought tolerance by closing stomata, their presence may also enhance pathogen resistance since *MPK3* and *MPK6* were activated in response to *flg22* in *Arabidopsis*. If abiotic and biotic stress signals converge at *MPK3* and *MPK6* in *Eutrema*, then they may contribute to enhanced pathogen resistance as well as abiotic stress tolerance. Interestingly, *MPK3* and *MPK6* transcript levels were detected in untreated Shandong and Yukon tissue (Champigny et al., 2013) and suggest that abiotic and biotic stress signals may converge on these MAPK proteins.

4.3 Does *Eutrema* produce more antimicrobial compounds to combat pathogenesis?

The production of secondary metabolites such as camalexin and glucosinolates is activated by MPK signaling cascades (Ren et al., 2008; Clay et al., 2009). Both compounds are secondary metabolites and function as antimicrobial compounds in response to pathogen infection. Camalexin induction by the upstream MAPKK protein MEK2 was reduced and delayed in *mpk3* and *mpk6 Arabidopsis* mutants demonstrating that MPK3 and MPK6 signaling was required for camalexin biosynthesis (Ren et al., 2008). The detection of *MPK3* and *MPK6* transcripts in untreated *Eutrema* leads to the idea that secondary metabolites with potential antimicrobial activity are produced in the absence of pathogen infection and contribute to basal defense. Crucifers constitutively synthesize glucosinolates that are hydrolyzed by myrosinases into functional compounds called isothiocyanates (ITCs). The toxicity of ITCs is responsible for deterring herbivore feeding (Halkier & Gershenzon, 2006). ITCs are also thought to have antimicrobial activity (Pedras et al., 2007). Two novel ITCs called rapalexins A and B, isolated from *Brassica rapa* infected with *Albugo candida* inhibited *A. candida* zoospore germination, demonstrating their antimicrobial activity (Pedras et al., 2007). Furthermore, flg22-induced callose deposition in Arabidopsis was mediated through glucosinolate biosynthesis and its degradation to a biologically active isothiocyanate derivative (Clay et

al., 2009). Increased glucosinolate biosynthesis in *Eutrema* plants compared to other *Brassica* species may contribute to enhancing *Eutrema* disease resistance pathways by supporting callose deposition and producing glucosinolate-derived antimicrobial compounds. Metabolite profiling of Shandong *Eutrema* infected with *Leptosphaeria maculans* and *A. candida* demonstrated that several compounds accumulated in *Eutrema*, including rapalexin A, two wasalexins (A and B) and 3-methylsulfanylpropylisothiocyanate (Pedras & Zheng, 2010). Both wasalexins A and B have antimicrobial activity based on an *L. maculans* spore germination inhibition assay (Pedras et al., 1999). The antimicrobial activity of 3-methylsulfanylpropylisothiocyanate has yet to be verified though its isothiocyanate structure suggests that it is antimicrobial. These phytoalexins in addition to others may be upregulated in *Eutrema* in response to *P. syringae* infections.

4.4 Is there a relationship between SA levels and *Pst* resistance in *Eutrema*?

The central role of salicylic acid as a signaling molecule has been demonstrated in many disease resistance pathways in *Arabidopsis* and other species (Dempsey et al., 1999). Therefore, salicylic acid levels were measured in Yukon and Shandong plants after inoculation with *Pst* to determine if SA accumulates in response to *Pst*. In the Cameron lab, SA levels in *Arabidopsis* are quantified using the ADPWH_*lux* bacterial biosensor that emits luciferase proportional to the amount of SA present (Huang et al., 2006). Negative SA concentration values were observed using the SA biosensor assay in both *Eutrema* accessions. It is possible that a molecule(s) in Yukon and Shandong leaf extracts interferes with the assay by quenching SA-induced luciferase-produced luminescence. Alternatively, an antimicrobial compound in *Eutrema* leaf extracts may also destroy the bacterial biosensor, preventing SA detection. The SA-deficient *Arabidopsis sid2-1* mutant was used to create a standard curve of known SA amounts to convert luminescence to SA concentration. In hindsight, a standard curve using untreated Yukon and Shandong leaf tissue should have been prepared for each accession to account for possible differences in the composition of the leaf extracts. Using untreated *Eutrema* leaf extracts or a transgenic

Eutrema 35S:NahG line that does not accumulate SA for standard curve preparation may allow us to detect SA in *Eutrema* using the SA biosensor assay. If these experiments are still unsuccessful, we may need to perform GC-MS to quantify SA in *Eutrema*.

4.5 Does coronatine suppress disease resistance in *Eutrema*?

Coronatine is a phytotoxin produced by *Pst* and is a structural and functional mimic of JA (Bender et al., 1999). Coronatine has been shown to suppress SA-mediated defense signaling by inducing jasmonate signaling, which has been shown to be mutually antagonistic with the SA signaling pathway (Kunkel and Brooks, 2002). Coronatine-deficient *Pst* exhibits a reduced ability to grow in *Arabidopsis* when inoculated on the leaf surface. If coronatine behaves in a similar manner to suppress defense signaling in *Eutrema*, then a coronatine-deficient *Pst* strain will not grow as well *in planta* compared to a wild-type *Pst* strain. However the coronatine-deficient strain grew to wild-type *Pst* levels in Col-0 and indicates that the mutant strain was unable to produce a reliable phenotype in the Col-0 line to serve as a positive control. Therefore, conclusions made about coronatine's involvement in *Eutrema* defense cannot be made until the coronatine-deficient strain shows reduced growth relative to wild-type *Pst* in Col-0. If coronatine-deficient *Pst* grew to similar levels as wild-type *Pst* in both Yukon and Shandong plants (given that an appropriate reduction in *cor*$^{-}$ growth was observed in Col-0), this would suggest that coronatine does not suppress defense in Yukon or Shandong *Eutrema*. In *Arabidopsis*, coronatine acts through the JA-Ile receptor COI1 to up-regulate JA responsive genes, some of which act to suppress SA signaling and biosynthesis. Furthermore, in *Arabidopsis*, coronatine promotes the physical interaction between COI1 and the JAZ repressor and causes the degradation of the JAZ repressor, which is normally bound to MYC transcription factors to block the expression of JA-responsive genes (Reviewed in Xin & He, 2013). It is possible that the *Eutrema* COI1 receptor may be insensitive to coronatine, which could explain why loss of coronatine does not have an impact on the growth capacity of coronatine-deficient *Pst*.

4.6 Shandong *Eutrema* exists in a primed-like state

Based on the *Eutrema* disease resistance assays, it was evident that in comparison to Col-0 and Yukon, Shandong *Eutrema* exhibited the highest level of resistance to *Pst* and *Psm*. In *Arabidopsis*, differences in resistance between accessions also occur and may be a consequence of the pathogen load experienced by each accession (Ahmad et al., 2010). A stressful environmental could select for plants that have adapted best to the stress and developed enhanced abiotic or biotic stress responses. Although the Yukon Territory is known for its unfavourable climate, the growing season for this accession occurs over a short window from May to July during the Yukon summer in which conditions are comparable to more temperate climates. The exact growing conditions of Shandong plants in their native surroundings have yet to be reported. However, the average daily July temperature of the Shandong province (~26°C) is typically higher than that of the Yukon Territory (19°C) and could favour pathogenicity compared to the Yukon climate (www.eldoradocountyweather.com;www.climate.weather.gc.ca). High temperatures have been associated with compromised defense to *Pst* in *Arabidopsis*. Plants grown at a lower temperature (22°C) prior to *Pst* inoculation showed reduced *in planta Pst* growth compared to plants grown at a higher temperature of 28°C (Wang et al., 2009). Elevated temperatures are associated with higher humidity due to the fact that at higher temperatures, the air has a greater capacity to hold more moisture. The Shandong province is more humid and therefore may promote higher pathogen pressures such that Shandong plants became adapted to higher pathogen pressure and evolved enhanced defense responses.

The zigzag model is frequently used to describe how plants and their pathogens co-evolve in terms of PTI and ETI, however there are other defense pathways such as defense priming employed by plants. The enhanced resistance and constitutive expression of defense-related genes observed in Shandong *Eutrema* suggests that this accession exists in a state of immune preparedness similar to defense-primed plants. Additionally, uninoculated Shandong *Eutrema* expressed many defense-related genes at higher levels in comparison to uninoculated Yukon *Eutrema* plants (Champigny et al., 2013). These

included PTI and ETI genes such as *RPS4, RIN4, PAD4* and several *PR* proteins including *PR1*. RT-PCR amplification of *PR1* in untreated Shandong plants validated the RNA-Seq data and the hypothesis that Shandong *Eutrema* exists in a defense-primed state. In SAR-primed *Arabidopsis* plants, *PR1* expression is rapidly enhanced after a secondary infection in the distant leaves (Maldonado et al., 2002). Accordingly, enhanced *PR1* expression in Shandong plants after inoculation with *Pst* strongly suggests that defense priming is occurring and may contribute to *Pst* resistance in Shandong *Eutrema*.

Cameron lab members also queried the *Eutrema* transcriptomes against a list of known defense-related genes in *Arabidopsis* (Champigny et al., 2013; Appendix Figure 32). This strategy was employed to identify potential defense-related genes that may not have been identified using the non-targeted search (GO term search). This search yielded the flagellin receptor FLS2 at the start of the PTI pathway. The expression of pathogen sensory receptors such as FLS2 in uninoculated tissue may also contribute to the enhanced resistance phenotype of Shandong *Eutrema*. *MPK3* and *MPK6* were also found to be upregulated in Shandong in this targeted list. Accumulation of *MPK3* and *MPK6* mRNA and inactive protein levels in BTH-induced primed *Arabidopsis* plants suggest that they may function as signaling molecules for priming (Beckers et al., 2009). If both *MPKs* function similarly in *Eutrema*, then detection of these transcripts in uninoculated Shandong *Eutrema* further supports the hypothesis that Shandong plants exists in a defense-primed state. The increased expression of R genes may also contribute to Shandong *Eutrema's* defense-primed state (Appendix Figure 33). Similar to *FLS2*, overexpression of R receptors may improve the sensitivity of Shandong to perceive cognate avirulence proteins in *Pst* leading to enhanced activation of defense even at the beginning of an infection when few pathogen cells are present. For example, the AvrRps4-mediated ETI observed in Shandong *Eutrema* is likely a result of an AvrRps4 interaction with the TIR-type RPS4 ortholog. Compared to the number of R genes that are upregulated in inoculated Yukon tissue (Appendix Figure 34), Shandong expresses three times as many R genes at higher levels and further supports the idea that Shandong *Eutrema* is more sensitive at recognizing avirulence *Pst* proteins. The expression of these

putative R proteins in uninoculated Shandong likely arose from selective pressure in its natural environment and contributed to the development of the defense-primed state in Shandong *Eutrema*. Overall, the transcriptome data suggests that Shandong *Eutrema's* defense- primed state includes the enhanced ability to perceive the presence of pathogens using highly expressed ETI and PTI receptors.

4.7 Shandong *Eutrema's* tolerance to *Pcw* may be mediated through PTI-responsive genes

Resistance to two other pathogens, *Pcw* and *Xcc* was examined in Shandong *Eutrema* to determine if its putative defense-primed state provides broad-spectrum resistance to other pathogens, which is observed in other priming pathways such as SAR (Kuc, 1982). Of the two pathogens tested, Shandong *Eutrema* displayed resistant to *Pcw* and susceptibility to *Xcc* (Section 3.2). Resistance to *Pcw* has been observed in BABA-primed plants through the expression of the PTI-responsive genes *PR1, FRK1* and *NDR1* (Po-Wen et al., 2012). Transcriptome analysis (Champigny et al., 2013) indicated that *NDR1* and *PR1* were expressed in uninoculated Shandong plants, suggesting that *Pcw* resistance in Shandong could be mediated through heightened PTI. The expression of another PTI-responsive gene, *CYP81F2*, was also enhanced in BABA-primed *Arabidopsis*. Interestingly, *CYP81F2* encodes a monooxygenase that is essential for 4-methyloxyindol-3-lymethylglucosinolate accumulation and its corresponding isothiocyanate is important for pathogen defense and is activated during PTI (Bednarek et al., 2009). *CYP81F2* transcripts were not detected in uninoculated Shandong *Eutrema*, however pathogen-induced *CYP81F2* expression may contribute to enhanced pathogen resistance through glucosinolate biosynthesis. Comparison of the defense-primed Shandong *Eutrema* and BABA-primed *Arabidopsis* suggest that resistance to *Pcw* in Shandong plants may occur through similar signaling pathways mediated by PTI responsive genes.

Xcc infects the vascular tissue and spreads to systemic tissue, producing blackened veins along the way. Typical V-shaped lesions and high *in planta* growth was observed in

Shandong plants wound-inoculated with *Xcc* (Section 3.2). This pathogen was the first to cause disease symptoms and virulence in Shandong *Eutrema* in our hands. The tissue in which *Xcc* colonizes is vascular while *Pst* prefers to inhabit the intercellular space (Vincente & Holub, 2013; Katagiri et al., 2002). This difference in tissue-specificity of the pathogen may explain why *Xcc* is virulent on Shandong *Eutrema*. Antimicrobial compounds present in the intercellular space of Shandong *Eutrema* may create a hostile environment for intercellular pathogen growth while the xylem may be more suited for infection by *Xcc*. To further examine intercellular versus vascular pathogen resistance, other pathogens that colonize vascular structures should be tested in Shandong *Eutrema*. The fungal pathogen, *Leptosphaeria maculans* that is the causal agent of blackleg in the *Brassica* was tested for its ability to cause disease n Shandong *Eutrema*. *Leptosphaeria's* life cycle include a biotrophic phase where it reproduces within the intercellular space before switching to a necrotrophic phase and spreads to the xylem to produce visible disease symptoms (Howlett et al., 2001). GUS staining of *L. maculans* expressing the *Escherichia coli GUS* gene was not detected in Shandong leaves infected with *L. maculans* but was detected in susceptible *Brassica napus* cultivars (data not shown). This may suggest that Shandong *Eutrema* is resistant to *L. maculans*. This pathogen however does not represent a pathogen whose initial mode of infection is through the vasculature. Therefore other pathogens that primarily infect the vasculature should be examined for their ability to cause disease in Shandong *Eutrema* to determine if Shandong is susceptible to only vascular-colonizing pathogens. Examples of vascular-colonizing pathogens include *Ralstonia solanacearum* and the fungal pathogen *Fusarium oxysporum*. Both have wide host ranges and may cause disease in Shandong *Eutrema* by infecting its vascular system, evading the potentially hostile intercellular space (Peeters et al., 2013; Michielse & Rep, 2009).

4.8 Abiotic stress tolerance

Recent evidence suggests that plants subjected to an initial non-lethal stress may be primed for enhanced tolerance to a wide variety of biotic and abiotic stresses (Po-Wen et al., 2012; Jakab et al., 2005; Zimmerli et al, 2000). Priming by exposing plants to a salt stress to enhance resistance to *Pst* in Yukon was observed only once in three experimental replicates in Yukon *Eutrema*. The irreproducibility of this response could be a result of experimental error, insufficient exposure to salt or minor variations in experimental outlines between replicates. In the first replicate, it is possible that an inaccurate number of leaf disks were counted to produce a significant difference in *in planta Pst* density between salt-treated and control plants. Variations in salt exposure time could have also affected the outcome of these replicates. The first replicate exposed seedlings to salt for four days compared to five for the other replicates. If salt treatment did enhance *Pst* resistance in the first replicate, the additional day of salt stress in the other replicates could have overwhelmed the plant's ability to induce a priming response. In addition, the seedlings may not have taken up the NaCl on the media plates as well in the second and third replicates and therefore did not induce a priming response. The inconclusive experimental results obtained from these replicates require further replicates and optimization of the protocol to determine if salt priming enhances *Pst* tolerance.

Evidence from unpublished transcriptome data suggests however that salt treatment may prime Yukon *Eutrema* for enhanced pathogen resistance. The comparison of salt-treated Yukon cabinet plants versus cabinet control plants revealed an upregulation of defense genes (Macleod et al., unpublished). These genes included *PDF1.2, JAZ10, PR3, PR4* and *PAL*. The expression of these defense-related genes in salt-treated plants may contribute to the ten-fold reduction in *Pst* growth of salt-primed seedlings observed in the first replicate. Enhanced expression of defense-related genes, specifically *NDR1*, *FRK1* and *PR1* has also been observed in BABA-primed *Arabidopsis* plants inoculated with *Pcw* compared to H_2O-treated plants (Po-Wen et al., 2012). In this study, the promoters of *NDR1* and *FRK1* were enriched for H3K4me2 and H3K9K14ac histone marks associated with open chromatin configurations in BABA-primed plants, likely

favouring their expression after inoculation with *Pcw* (Po-Wen et al., 2012). In addition, salt treatment of *Arabidopsis* plants enhanced genome-wide histone modifications compared to unprimed plants (Sani et al., 2013). Taken together, these findings indicate that salt treatment induces histone modifications associated with open chromatin confirmations and may enhance the expression of defense-related genes after pathogen challenge in primed plants. This mechanism of tolerance may be similarly utilized in salt-treated Yukon *Eutrema* based on the overexpression of defense genes in the transcriptome data.

4.9 Future Directions and Conclusions

To gain a better understanding of the Yukon and Shandong *Eutrema* defense response to *Pst*, the transcriptomes of Yukon and Shandong plants inoculated with *Pst* should be generated. Comparison of genes differentially expressed between the two accessions will identify common genes required for defense while genes that are differentially expressed will provide clues about defense pathway differences used by Shandong and Yukon *Eutrema*. Additionally, a forward genetics approach could be used to elucidate genes important for *Pst* resistance in *Eutrema*. Mutant populations of plants (T-DNA insertions, or Fast Neutron-generated insertion/deletions) could be screened for individuals that are highly susceptible to *Pst*. A collection of T-DNA mutants will first have to be generated in *Eutrema*. These individuals can be characterized to isolate the genes that are required for *Pst* resistance in *Eutrema*. Mutants that are isolated from mutagenized populations of both Yukon and Shandong plants may reveal common defense pathways shared between both accessions as well as novel mechanisms.

To determine if glucosinolates or their degradation products contribute to pathogen resistance in *Eutrema*, RNAi or CRISPR-Cas technology could be used to reduce the expression of glucosinolate biosynthesis and degradation genes. Metabolite profiling using GC-MS of plants inoculated with *Pst* could also reveal antimicrobial compounds that are involved in defense such as salicylic acid and phytoalexins. The activity of *Eutrema*-specific putative antimicrobials that are identified through GC-MS

can be tested using in vitro *Pst* growth inhibition assays. The successful quantification of SA levels in untreated plants and plants inoculated with *Pst* using the ADPWH_*lux* SA biosensor (or GC-MS) would allow us to determine if SA-mediated defense also occurs in *Eutrema*. Furthermore, the creation of *35S:NahG Eutrema* that overexpress the bacterial salicylate hydroxylase should be considered to determine how the loss of SA affects *Eutrema* disease resistance. The analysis of glucosinolate biosynthesis pathways and the identification of antimicrobial compounds such as SA will elucidate their role in mediating *Eutrema* disease resistance.

Previous studies by Dedrick et al. (2010) have demonstrated that drought exposure can prime plants for enhanced drought tolerance. Furthermore, preliminary data by Marc Champigny suggested that exposing plants to an episode of drought enhanced resistance to *Pst*. The salt stress experiments performed in this thesis for the most part did not prime Yukon plants for enhanced resistance. It may be that the methods used to irrigate plants with saline solutions or exposing seedlings to media supplemented with NaCl was insufficient to prime for pathogen resistance. In future studies, *Salt Overly Sensitive1* (*SOS1*) gene expression should be measured to indicate if salt uptake is occurring in these plants as it functions as an Na^+/H^+ antiporter to shuttle Na^+ out of the cytosol during salt uptake. Although *SOS1* is expressed in untreated Shandong plants, it has been used as a marker for salt uptake as its transcript abundance has been demonstrated to increase with an increase in NaCl concentration (Oh et al., 2009). Additionally, NaCl uptake can be visualized using the CoRoNa Green fluorescent sodium indicator. The most direct way to measure salt uptake would be to perform physiological measurements such as solute and water potential before and after salt treatment. Further optimization of the salt stress assays is required to determine the ideal salt exposure time and concentration to induce a priming response. It would also be interesting to determine if a biotic stress such as *Pst* could prime for abiotic stress tolerance in *Eutrema*. If inoculating with *Pst* enhances tolerance to salt stress, this would suggest that signaling pathways between abiotic and biotic stress pathways exist in *Eutrema*.

The investigation of disease resistance responses in the extremophile model plant *Eutrema salsugineum* to the hemibiotrophic plant pathogen *P. syringae* has demonstrated that both accessions are resistant to *Pst*, with Shandong plants exhibiting greater resistance compared to Yukon plants. Moreover, differences in the effectiveness of ETI and ARR were observed in both accessions. The expression of the defense marker *PR1* in uninoculated healthy Shandong plants suggest that this *Eutrema* accession exists in a defense-primed state. This is further supported by an overrepresentation of other defense genes such as *FLS2* and *RPS2* in uninoculated Shandong tissue, which may contribute to the enhanced detection of PAMPs and effectors and subsequent defense response. The generation of transcriptomes of inoculated plants will provide insight into how *Eutrema* responds to *Pst* and potentially uncover novel defense genes and pathways that are important for disease resistance. The *Eutrema-P. syringae* pathosystem will facilitate future studies to understand how *Eutrema* tolerates abiotic and biotic stress in order to improve stress tolerance in crops that are exposed to multiple, concurrent and simultaneous stresses.

Appendix

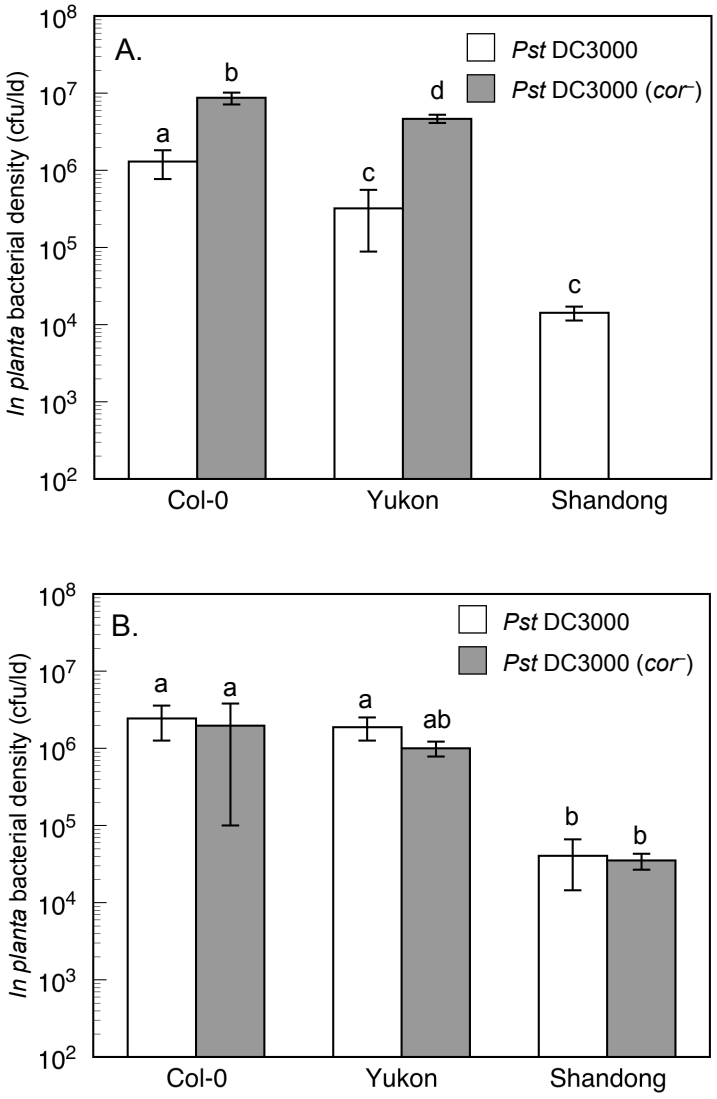


Figure 22. Additional replicates of examining the effect of coronatine on *Pst* growth in *Eutrema* using a coronatine-deficient *Pst* strain. Four-week-old plants were inoculated with 10^7 cfu/ml *Pst* DC3000 (*cor*⁻) or 10^6 cfu/ml *Pst* DC3000 and *in planta* bacterial growth was measured 3 dpi. Each bar represents the mean ± standard deviation of three biological replicates. Different letters denote significant differences according to Tukey's HSD ($p < 0.05$). (A) July 2013 (B) Sept 2013.

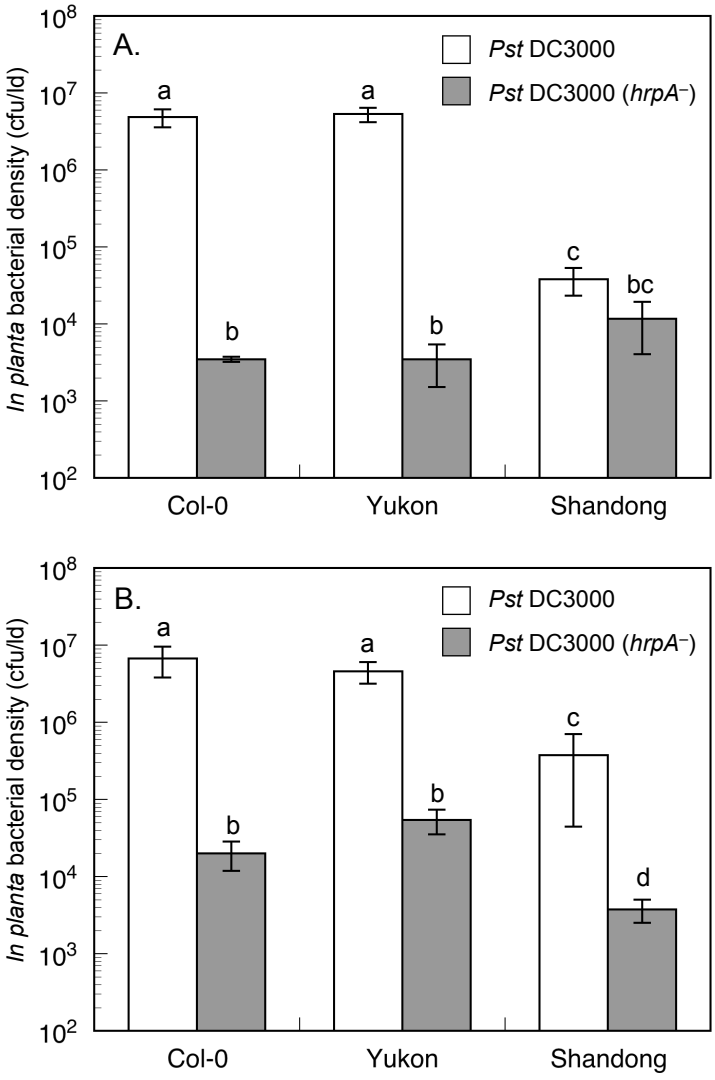
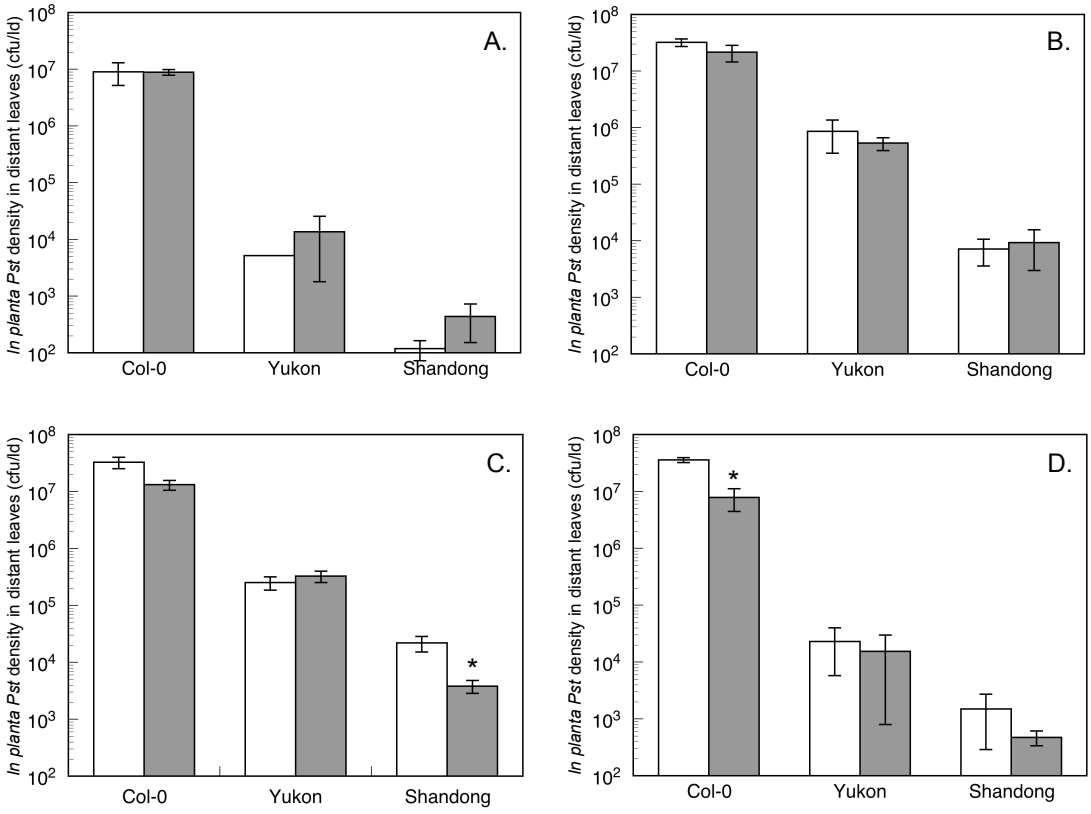


Figure 23. Additional replicates of *in planta* bacterial growth of HrpA-deficient *Pst*. Four-week-old plants were inoculated with 10^7 cfu/ml *Pst* DC3000 (*hrpA*⁻) or 10^6 cfu/ml *Pst* DC3000 and *in planta* bacterial growth was measured 3 dpi. Each bar represents the mean ± standard deviation of three biological replicates. Different letters indicate significant differences according to Tukey's HSD ($p < 0.05$). The replicates were repeated in (A) October 2013 and (B) November 2013.



E. SAR Response (Mock-inoculated/SAR-induced)

	A	Fig. 11	B	C	D
Col-0	1.02	4.6	1.5	2.5	4.59
Yukon	0.38	3.18	1.6	0.77	1.5
Shandong	0.27	0.96	0.76	5.75	3.16

Figure 24. Additional replicates of *Pst* DC3000 levels in distant leaves induced for SAR. SAR assays were conducted by mock-inoculating with 10 mM $MgCl_2$ or inducing for SAR with *Pst*(*avrRpt2*), followed by challenge inoculation with *Pst* DC3000 in distant leaves 2 days later. *In planta* bacterial levels were quantified 3 dpi. Asterisks (*) denote significant differences between challenged distant leaves of mock- and SAR-induced plants according to the Student's *t*-test ($p < 0.01$). The replicates were repeated in (A) July 2012, (B) August 2012 and (C) & (D) September 2012. Replicates (A)-(C) were performed by Salima Chatur. (E) Table of SAR response observed from (A)-(D).

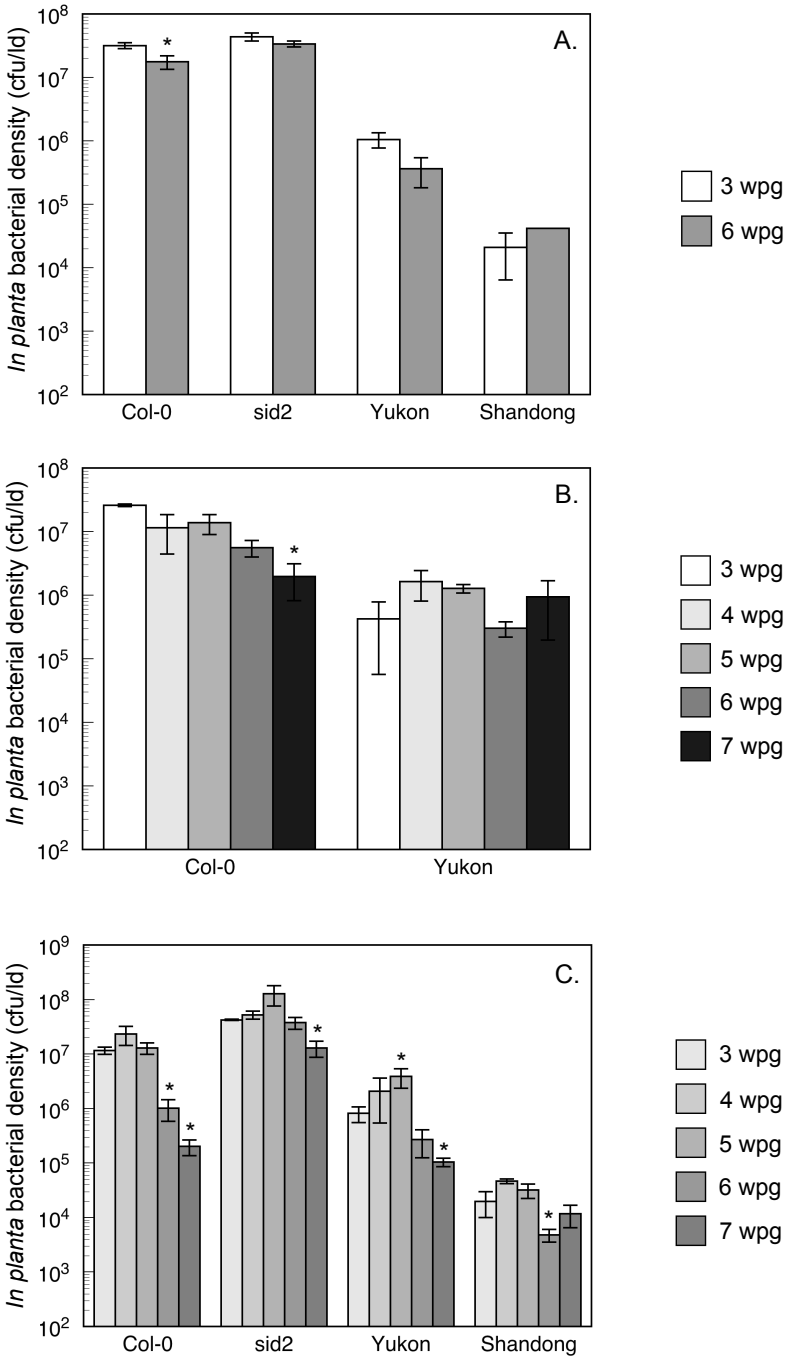

A.
B.
C.
In planta bacterial density (cfu/ld)
Col-0
sid2
Yukon
Shandong
3 wpg
4 wpg
5 wpg
6 wpg
7 wpg

Figure 25. Additional replicates of Yukon and Shandong *Eutrema's* response to *Pst* from 3-7 weeks of age. Plants were grown in a short-day photoperiod (9h light) and inoculated with 10^6 cfu/ml *Pst* DC3000 each week. Bacterial levels were quantified 3 dpi. Each data point represents the mean ± standard deviation of three biological replicates. Asterisks (*) denote significant differences in bacterial growth relative to the levels observed in plants of the same accession at three weeks of age according to Tukey's HSD ($p < 0.05$). The replicates were repeated in (A) December 2011, (B) October 2012 and (C) February 2013.

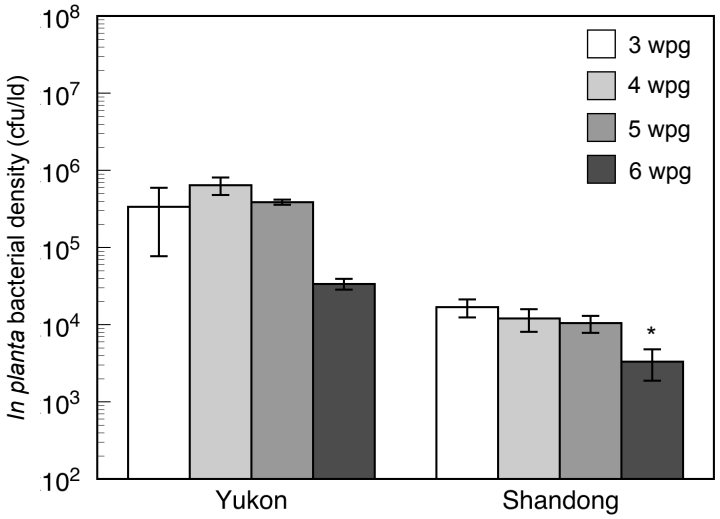
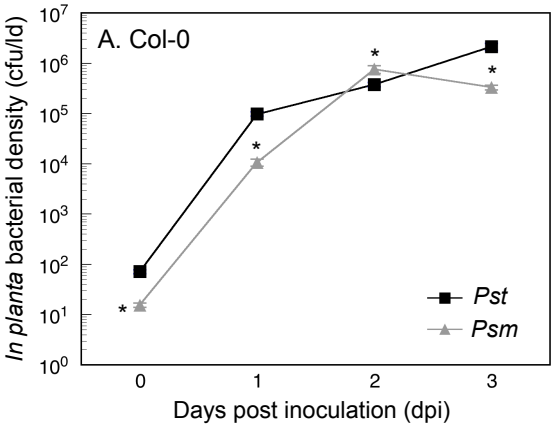
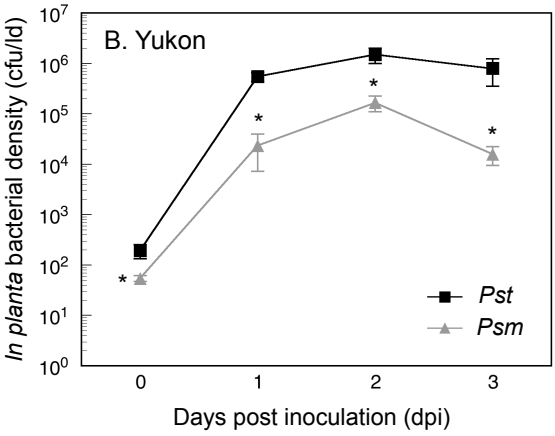


Figure 26. Additional replicate of Yukon and Shandong *Eutrema* grown in long-day conditions and inoculated with 10^6 cfu/ml *Pst* DC3000 each week between 3 and 7 weeks post germination. *In planta* bacteria were quantified 3 dpi. Each data point represents the mean ± standard deviation of three biological replicates. Asterisks (*) denote significant differences in bacterial levels relative to plants of the same accession at three weeks of age according to the Games-Howell post-hoc test ($p < 0.05$). This replicate was performed in April 2013.


A. Col-0
In planta bacterial density (cfu/ld)
Days post inoculation (dpi)
Pst
Psm


B. Yukon
In planta bacterial density (cfu/ld)
Days post inoculation (dpi)
Pst
Psm

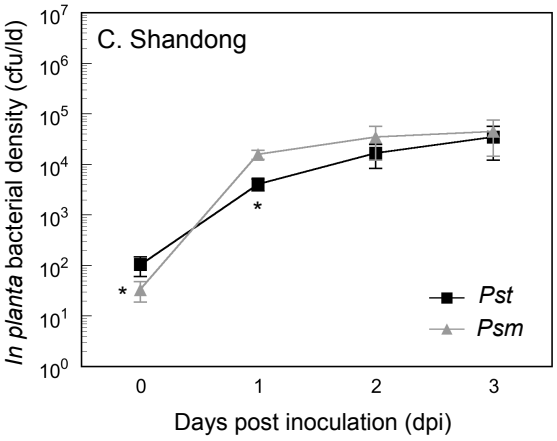

C. Shandong
In planta bacterial density (cfu/ld)
Days post inoculation (dpi)
Pst
Psm

Figure 27. Additional replicates comparing *in planta* bacterial growth of *Psm* and *Pst*. Four-week-old Col-0 (A), Yukon (B) and Shandong (C) plants were inoculated with 10^6 cfu/ml *Psm* or *Pst*. Bacterial growth was quantified at the indicated time points and presented as the mean ± standard deviation of three biological replicates. Asterisks (*) denote significant differences between *Psm* and *Pst* levels at each time point according to the Student's *t*-test ($p < 0.05$). The replicates were repeated in (A) January 2013 and (B)-(C) May 2013.

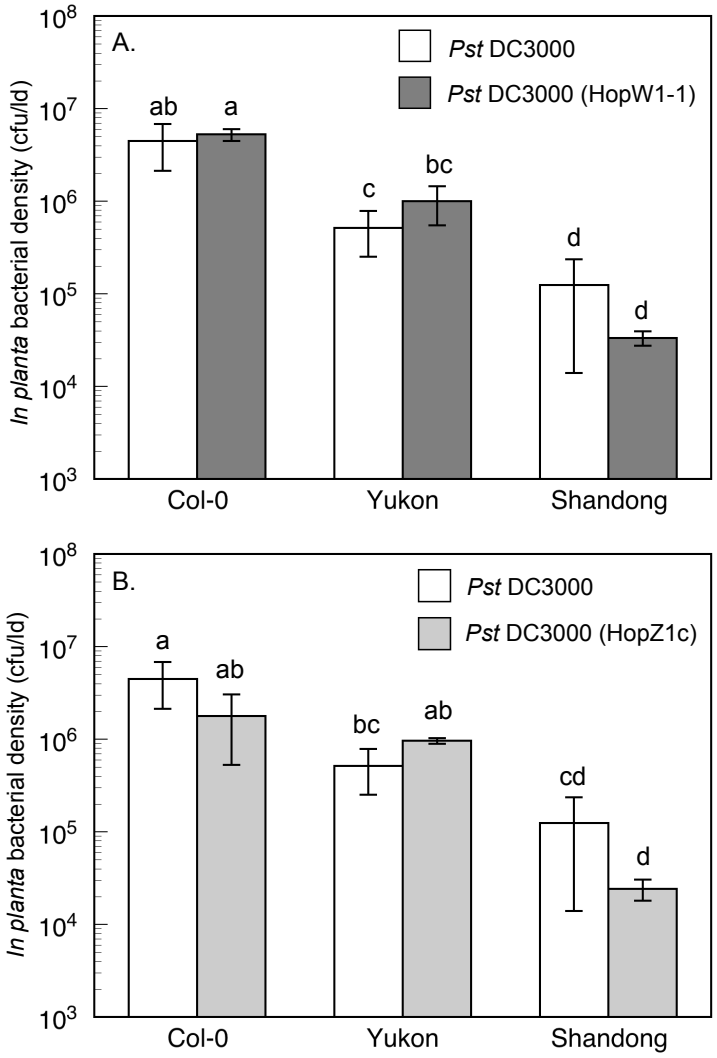


Figure 28. Additional replicates comparing *in planta* growth of *Pst* DC3000, *Pst* DC3000 expressing HopW1-1 and *Pst* DC3000 expressing HopZ1c. Four-week-old plants were inoculated with 10^6 cfu/ml of *Pst* DC3000, *Pst* DC3000 (HopW1-1) (A) or *Pst* DC3000 (HopZ1c) (B). Leaf discs were collected 3 dpi. Different letters indicate significant differences according to Tukey's HSD ($p < 0.05$). The replicates were repeated in May 2013.

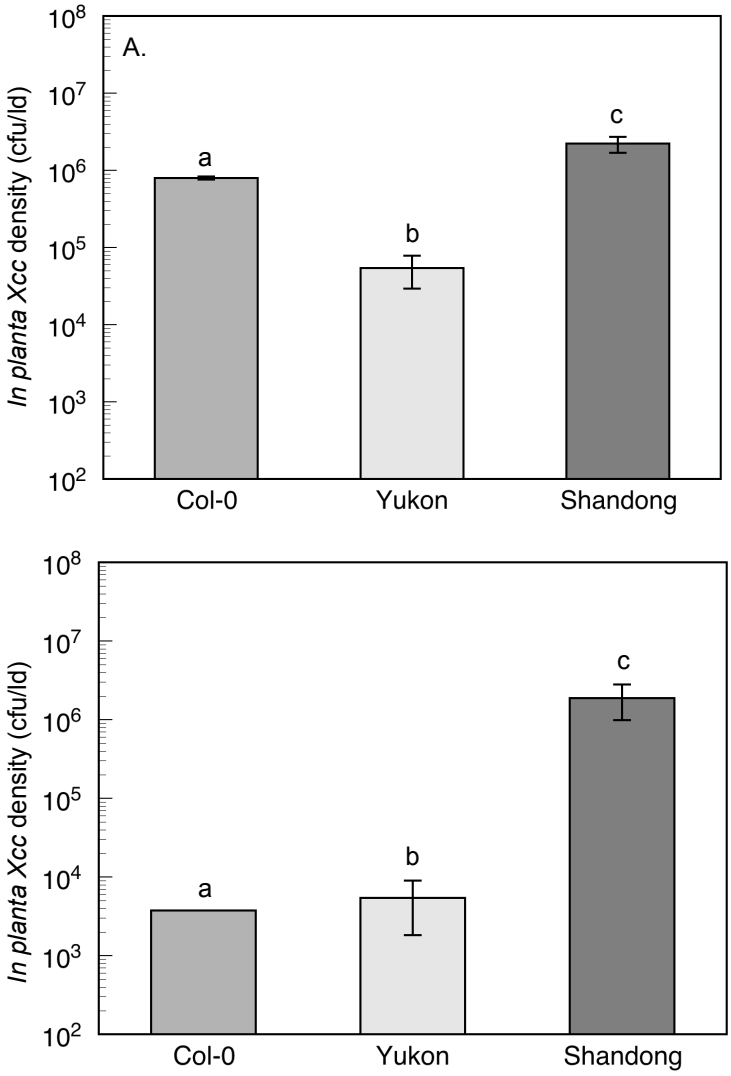


Figure 29. Additional replicates of *Eutrema* inoculated with *Xcc*. Four-week-old long-day-grown Col-0, Yukon and Shandong plants were inoculated with 10^8 cfu/ml *Xcc* 8004 or mock inoculated with $MgCl_2$. At 7 dpi, *in planta* bacterial growth was measured. Each bar represents the mean ± standard deviation of three biological replicates. Different letters denote significant differences according to Tukey's HSD ($p < 0.05$). The replicates were repeated in September 2013.

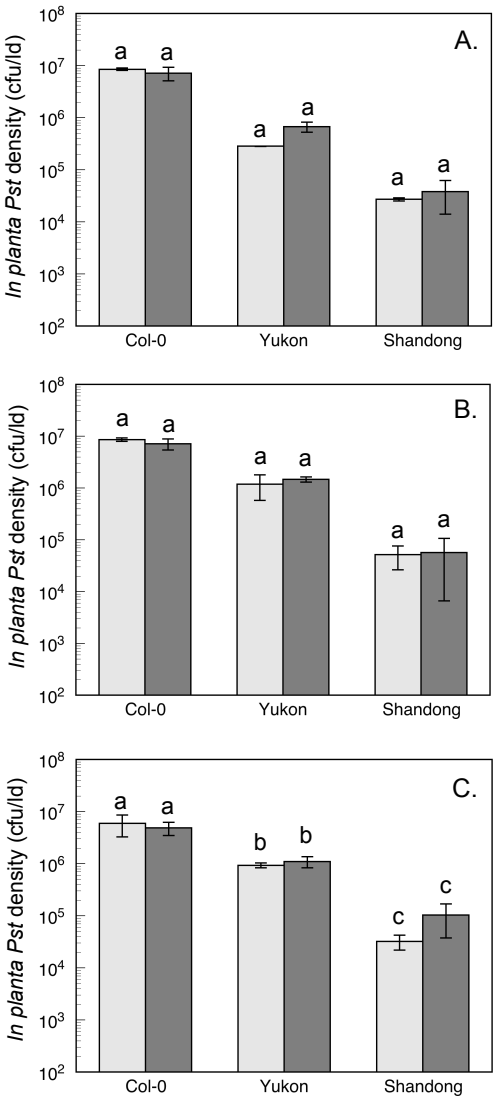


Figure 30. Additional replicates of assessing *Pst* resistance after treatment with NaCl. Seedlings were exposed to NaCl (100 mM for Col-0, 250 mM for Yukon and Shandong). At 4 wpg, salt-treated plants were inoculated with 10^6 cfu/ml *Pst* and bacterial levels were quantified 3 dpi. *In planta Pst* levels in adult plants that were salt-treated as seedlings are presented in (A)-(C). In (A), seedlings were exposed to 5 days of NaCl with Shandong receiving 300 mM NaCl. In (B), seedlings were exposed for 4 days to NaCl. In (C), seedlings were exposed for 5 days of NaCl. Each bar represents the mean ± standard deviation of three biological replicates. Different letters denote significant differences according to Tukey's HSD ($p < 0.05$).

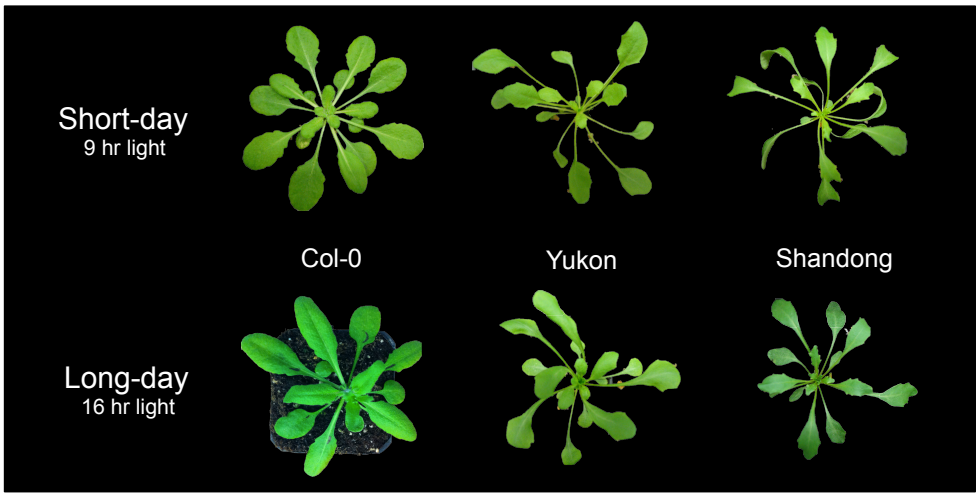


Figure 31. Representative images of *Arabidopsis* and *Eutrema* plants. The top row of plants represent 4 wpg plants grown under short-day conditions (9 hours of light) while the bottom row are 4 wpg plants grown under long-day conditions (16 hours of light).

Eutrema Locus	Fold Difference in Transcript Abundance (Shandong vs. Yukon)	Best annotated match in *Arabidopsis*	Function
Thhalv10004581m.g	159.9	AtPRX71	Cell wall bound peroxidase
Thhalv10000750m.g	73.5	RPS4	R gene
Thhalv10014149m.g	73.5	DND2	HR signaling component
Thhalv10029036m.g	26.9	AT4G12490.1 (LTP)	Response to fungus
Thhalv10021478m.g	19.7	RIN4	R gene signaling
Thhalv10019053m.g	6.6	AT1G75050.1	Pathogenesis-related
Thhalv10022890m.g	5.0	PR1	Pathogenesis-related
Thhalv10011112m.g	2.7	PAD4	R gene signaling

Figure 32. Expression of defense-related transcripts in *Eutrema*. A list of defense-related transcripts that are differentially expressed in uninoculated leaves of Shandong plants relative to Yukon was manually curated from RNA-Seq data generated by Champigny *et al*. 2013. The *Eutrema* locus identifier (Phytozome 9.1 release), fold-difference in transcript abundance in Shandong vs. Yukon *Eutrema*, best hit to an *Arabidopsis* locus, and predicted function of the *Arabidopsis* hit are presented.

Eutrema Locus	Fold Difference in Transcript Abundance (Shandong vs. Yukon)	Best annotated match in *Arabidopsis*	Function
Thhalv10000770m.g	Inf.	AT5G38350.1	NBS-LRR
Thhalv10001165m.g	Inf.	TTR1	TIR-NBS-LRR
Thhalv10000765m.g	Inf.	AT4G36150.1	TIR-NBS-LRR
Thhalv10026484m.g	Inf.	AT5G51630.1	TIR-NBS-LRR
Thhalv10006759m.g	Inf.	RPS5	CC-NBS-LRR
Thhalv10000750m.g	74.7	RPS4	TIR-NBS-LRR
Thhalv10023246m.g	54.8	AT1G63350.1	CC-NBS-LRR
Thhalv10023263m.g	28.6	N-term myrisolated	CC-NBS-LRR
Thhalv10026964m.g	17.6	AT5G17680.1	TIR-NBS-LRR
Thhalv10023237m.g	16.6	CW9	CC-NBS-LRR
Thhalv10027844m.g	11.5	AT5G41540.1	TIR-NBS-LRR
Thhalv10028092m.g	10.9	AT5G41550.1	TIR-NBS-LRR
Thhalv10027752m.g	10.3	AT5G41550.1	TIR-NBS-LRR
Thhalv10024211m.g	9.2	AT1G69550.1	TIR-NBS-LRR
Thhalv10022909m.g	3.5	AT5G38340.1	TIR-NBS-LRR
Thhalv10024295m.g	2.9	RPP1	TIR-NBS-LRR

Figure 33. Putative *Eutrema* R genes that are more abundantly expressed in uninoculated Shandong leaf tissue relative to Yukon. A list of R gene transcripts that are differentially expressed in uninoculated Shandong compared to Yukon leaf tissue was manually curated from RNA-Seq data generated by Champigny et al., 2013. The *Eutrema* locus identifier (Phytozome 9.1 release), fold difference in transcript abundance in Shandong vs. Yukon *Eutrema*, best hit to an *Arabidopsis* locus, and predicted function of the *Arabidopsis* hit are presented. Inf = no detectable transcript in Yukon *Eutrema*.

Eutrema Locus	Fold Difference in Transcript Abundance (Shandong vs. Yukon)	Best annotated match in *Arabidopsis*	Function
Thhalv10026756m.g	Inf.	AT4G19520.1	TIR-NBS-LRR
Thhalv10027412m.g	Inf.	AT4G19530.1	TIR-NBS-LRR
Thhalv10023235m.g	20.6	AT1G58807.1	CC-NBS-LRR
Thhalv10023248m.g	9.5	AT1G61180.2	LRR AND NB-ARC
Thhalv10023301m.g	5.3	AT1G61180.2	LRR AND NB-ARC

Figure 34. Putative *Eutrema* R genes that are more abundantly expressed in uninoculated Yukon leaf tissue relative to Shandong. A list of R gene transcripts that are differentially expressed in uninoculated Yukon compared to Shandong leaf tissue was manually curated from RNA-Seq data generated by Champigny et al., 2013. The *Eutrema* locus identifier (Phytozome 9.1 release), fold difference in transcript abundance in Yukon vs. Shandong *Eutrema*, best hit to an *Arabidopsis* locus, and predicted function of the *Arabidopsis* hit are presented. Inf = no detectable transcript in Shandong *Eutrema*.

Multiple Comparisons

Dependent Variable: lg10bac_den

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CC	CP	.3249	.26993	.827	-.5818	1.2316
	SC	2.6288*	.26993	.000	1.7221	3.5355
	SP	2.7879*	.26993	.000	1.8812	3.6946
	YC	.8754	.26993	.061	-.0313	1.7821
	YP	1.0936*	.26993	.016	.1869	2.0003
CP	CC	-.3249	.26993	.827	-1.2316	.5818
	SC	2.3039*	.26993	.000	1.3973	3.2106
	SP	2.4630*	.26993	.000	1.5563	3.3697
	YC	.5505	.26993	.376	-.3562	1.4572
	YP	.7687	.26993	.116	-.1380	1.6754
SC	CC	-2.6288*	.26993	.000	-3.5355	-1.7221
	CP	-2.3039*	.26993	.000	-3.2106	-1.3973
	SP	.1590	.26993	.990	-.7476	1.0657
	YC	-1.7534*	.26993	.000	-2.6601	-.8467
	YP	-1.5352*	.26993	.001	-2.4419	-.6285
SP	CC	-2.7879*	.26993	.000	-3.6946	-1.8812
	CP	-2.4630*	.26993	.000	-3.3697	-1.5563
	SC	-.1590	.26993	.990	-1.0657	.7476
	YC	-1.9125*	.26993	.000	-2.8191	-1.0058
	YP	-1.6943*	.26993	.000	-2.6009	-.7876
YC	CC	-.8754	.26993	.061	-1.7821	.0313
	CP	-.5505	.26993	.376	-1.4572	.3562
	SC	1.7534*	.26993	.000	.8467	2.6601
	SP	1.9125*	.26993	.000	1.0058	2.8191
	YP	.2182	.26993	.960	-.6885	1.1249
YP	CC	-1.0936*	.26993	.016	-2.0003	-.1869
	CP	-.7687	.26993	.116	-1.6754	.1380
	SC	1.5352*	.26993	.001	.6285	2.4419
	SP	1.6943*	.26993	.000	.7876	2.6009
	YC	-.2182	.26993	.960	-1.1249	.6885

Homogeneous Subsets

Dependent variable: lg10bac_den

Tukey HSD

treatment	N	Subset		
		1	2	3
SP	3	4.3979		
SC	3	4.5570		
YP	3		6.0922	
YC	3		6.3104	6.3104
CP	3		6.8609	6.8609
CC	3			7.1858
Sig.		.990	.116	.061

Tables 2 and 3. Multiple Comparisons and Homogenous Subsets Tables. These tables indicate the results of the Tukey's post-hoc test that performed multiple pairwise comparisons of the following treatments: Col-0 inoculated with *Pst* DC3000 (CP), Col-0 inoculated with *Pst* DC3118 (*cor*$^-$) (CC), Yukon inoculated with *Pst* DC3000 (YP), Yukon inoculated with *Pst* DC3118 (*cor*$^-$) (YC), Shandong inoculated with *Pst* DC3000 (SP) and Shandong inoculated with *Pst* DC3118 (*cor*$^-$) (SC). In Table 2, the treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. Homogenous subsets are presented in Table 3. These statistical analyses correspond to Figure 6.

Multiple Comparisons

Dependent Variable: lg10bac_den

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CH	CP	-2.9175*	.15177	.000	-3.4273	-2.4077
	SH	1.5982*	.15177	.000	1.0885	2.1080
	SP	-.0101	.15177	1.000	-.5199	.4997
	YH	.9935*	.15177	.000	.4838	1.5033
	YP	-2.0250*	.15177	.000	-2.5348	-1.5152
CP	CH	2.9175*	.15177	.000	2.4077	3.4273
	SH	4.5158*	.15177	.000	4.0060	5.0255
	SP	2.9074*	.15177	.000	2.3976	3.4172
	YH	3.9110*	.15177	.000	3.4013	4.4208
	YP	.8925*	.15177	.001	.3828	1.4023
SH	CH	-1.5982*	.15177	.000	-2.1080	-1.0885
	CP	-4.5158*	.15177	.000	-5.0255	-4.0060
	SP	-1.6084*	.15177	.000	-2.1181	-1.0986
	YH	-.6047*	.15177	.017	-1.1145	-.0949
	YP	-3.6232*	.15177	.000	-4.1330	-3.1135
SP	CH	.0101	.15177	1.000	-.4997	.5199
	CP	-2.9074*	.15177	.000	-3.4172	-2.3976
	SH	1.6084*	.15177	.000	1.0986	2.1181
	YH	1.0036*	.15177	.000	.4939	1.5134
	YP	-2.0149*	.15177	.000	-2.5246	-1.5051
YH	CH	-.9935*	.15177	.000	-1.5033	-.4838
	CP	-3.9110*	.15177	.000	-4.4208	-3.4013
	SH	.6047*	.15177	.017	.0949	1.1145
	SP	-1.0036*	.15177	.000	-1.5134	-.4939
	YP	-3.0185*	.15177	.000	-3.5283	-2.5087
YP	CH	2.0250*	.15177	.000	1.5152	2.5348
	CP	-.8925*	.15177	.001	-1.4023	-.3828
	SH	3.6232*	.15177	.000	3.1135	4.1330
	SP	2.0149*	.15177	.000	1.5051	2.5246
	YH	3.0185*	.15177	.000	2.5087	3.5283

Homogeneous Subsets

Dependent Variable: lg10bac_den

Tukey HSD

treatment	N	Subset				
		1	2	3	4	5
SH	3	2.8334				
YH	3		3.4381			
CH	3			4.4317		
SP	3			4.4418		
YP	3				6.4566	
CP	3					7.3492
Sig.		1.000	1.000	1.000	1.000	1.000

Tables 4 and 5. Multiple Comparisons and Homogenous Subsets Tables. These tables indicate the results of the Tukey's post-hoc test that performed multiple pairwise comparisons of the following treatments: Col-0 inoculated with *Pst* DC3000 (CP), Col-0 inoculated with *Pst* DC3000 (*hrpA*$^{-}$) (CH), Yukon inoculated with *Pst* DC3000 (YP), Yukon inoculated with *Pst* DC3000 (*hrpA*$^{-}$) (YH), Shandong inoculated with *Pst* DC3000 (SP) and Shandong inoculated with *Pst* DC3000 (*hrpA*$^{-}$) (SH). In Table 4, the treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. Homogenous subsets are presented in Table 5. These statistical analyses correspond to Figure 7.

Multiple Comparisons

Dependent Variable: bac_den

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
avrRps4-0	avrRpt2-0	-236.6667*	42.13350	.003	-365.9438	-107.3895
	Pst-0	-437.0833*	42.13350	.000	-566.3605	-307.8062
avrRpt2-0	avrRps4-0	236.6667*	42.13350	.003	107.3895	365.9438
	Pst-0	-200.4167*	42.13350	.007	-329.6938	-71.1395
Pst-0	avrRps4-0	437.0833*	42.13350	.000	307.8062	566.3605
	avrRpt2-0	200.4167*	42.13350	.007	71.1395	329.6938

Homogeneous Subsets

Dependent Variable: bac_den

Tukey HSD

treatment	N	Subset		
		1	2	3
avrRps4-0	3	117.9167		
avrRpt2-0	3		354.5833	
Pst-0	3			555.0000
Sig.		1.000	1.000	1.000

Tables 6 and 7. Multiple Comparisons and Homogenous Subsets Tables. These tables indicate the results of the Tukey's post-hoc test that performed multiple pairwise comparisons of the following treatments at 0 dpi: Col-0 inoculated with *Pst* DC3000 (Pst-0), Col-0 inoculated with *Pst*(*avrRpt2*) (avrRpt2-0) and Col-0 inoculated with *Pst*(*avrRps4*) (avrRps4-0). In Table 6, the treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. Homogenous subsets are presented in Table 7. These statistical analyses correspond to Figure 8.

Multiple Comparisons

Dependent Variable: bac_den

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
avrRps4-1	avrRpt2-1	-16083.3333	10992.00888	.371	-49809.8351	17643.1684
	Pst-1	-34816.6667*	10992.00888	.044	-68543.1684	-1090.1649
avrRpt2-1	avrRps4-1	16083.3333	10992.00888	.371	-17643.1684	49809.8351
	Pst-1	-18733.3333	10992.00888	.279	-52459.8351	14993.1684
Pst-1	avrRps4-1	34816.6667*	10992.00888	.044	1090.1649	68543.1684
	avrRpt2-1	18733.3333	10992.00888	.279	-14993.1684	52459.8351

Homogeneous Subsets

Dependent Variable: bac_den

Tukey HSD

treatment	N	Subset	
		1	2
avrRps4-1	3	16050.0000	
avrRpt2-1	3	32133.3333	32133.3333
Pst-1	3		50866.6667
Sig.		.371	.279

Tables 8 and 9. Multiple Comparisons and Homogenous Subsets Tables. These tables indicate the results of the Tukey's post-hoc test that performed multiple pairwise comparisons of the following treatments at 1 dpi: Col-0 inoculated with *Pst* DC3000 (Pst-1), Col-0 inoculated with *Pst*(*avrRpt2*) (avrRpt2-1) and Col-0 inoculated with *Pst*(*avrRps4*) (avrRps4-1). In Table 8, the treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. Homogenous subsets are presented in Table 9. These statistical analyses correspond to Figure 8.

Multiple Comparisons

Dependent Variable: lg10bac_den

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
avrRps4-2	avrRpt2-2	-.5029	.17258	.061	-1.0324	.0266
	Pst-2	-1.5183*	.17258	.000	-2.0478	-.9888
avrRpt2-2	avrRps4-2	.5029	.17258	.061	-.0266	1.0324
	Pst-2	-1.0154*	.17258	.003	-1.5449	-.4859
Pst-2	avrRps4-2	1.5183*	.17258	.000	.9888	2.0478
	avrRpt2-2	1.0154*	.17258	.003	.4859	1.5449

Homogeneous Subsets

Dependent Variable: lg10bac_den

Tukey HSD

treatment	N	Subset	
		1	2
avrRps4-2	3	4.8538	
avrRpt2-2	3	5.3567	
Pst-2	3		6.3721
Sig.		.061	1.000

Tables 10 and 11. Multiple Comparisons and Homogenous Subsets Tables. These tables indicate the results of the Tukey's post-hoc test that performed multiple pairwise comparisons of the following treatments at 2 dpi: Col-0 inoculated with *Pst* DC3000 (Pst-2), Col-0 inoculated with *Pst*(*avrRpt2*) (avrRpt2-2) and Col-0 inoculated with *Pst*(*avrRps4*) (avrRps4-2). In Table 10, the treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. Homogenous subsets are presented in Table 11. These statistical analyses correspond to Figure 8.

Multiple Comparisons

Dependent Variable: lnbac_den

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
avrRps4-3	avrRpt2-3	.1796	.26745	.788	-.6410	1.0002
	Pst-3	-4.0685*	.26745	.000	-4.8891	-3.2479
avrRpt2-3	avrRps4-3	-.1796	.26745	.788	-1.0002	.6410
	Pst-3	-4.2481*	.26745	.000	-5.0688	-3.4275
Pst-3	avrRps4-3	4.0685*	.26745	.000	3.2479	4.8891
	avrRpt2-3	4.2481*	.26745	.000	3.4275	5.0688

Homogeneous Subsets

Dependent Variable: lnbac_den

Tukey HSD

treatment	N	Subset	
		1	2
avrRpt2-3	3	10.9334	
avrRps4-3	3	11.1130	
Pst-3	3		15.1816
Sig.		.788	1.000

Tables 12 and 13. Multiple Comparisons and Homogenous Subsets Tables. These tables indicate the results of the Tukey's post-hoc test that performed multiple pairwise comparisons of the following treatments at 3 dpi: Col-0 inoculated with *Pst* DC3000 (Pst-3), Col-0 inoculated with *Pst*(*avrRpt2*) (avrRpt2-3) and Col-0 inoculated with *Pst*(*avrRps4*) (avrRps4-3). In Table 12, the treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. Homogenous subsets are presented in Table 13. These statistical analyses correspond to Figure 8.

Multiple Comparisons

Dependent Variable: lg10bac_den

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
avrRps4-0	avrRpt2-0	-.7205*	.12779	.003	-1.1126	-.3284
	Pst-0	-1.2121*	.12779	.000	-1.6042	-.8200
avrRpt2-0	avrRps4-0	.7205*	.12779	.003	.3284	1.1126
	Pst-0	-.4917*	.12779	.020	-.8838	-.0996
Pst-0	avrRps4-0	1.2121*	.12779	.000	.8200	1.6042
	avrRpt2-0	.4917*	.12779	.020	.0996	.8838

Homogeneous Subsets

Dependent Variable: lg10bac_den

Tukey HSD

treatment	N	Subset		
		1	2	3
avrRps4-0	3	1.0587		
avrRpt2-0	3		1.7792	
Pst-0	3			2.2708
Sig.		1.000	1.000	1.000

Tables 14 and 15. Multiple Comparisons and Homogenous Subsets Tables. These tables indicate the results of the Tukey's post-hoc test that performed multiple pairwise comparisons of the following treatments at 0 dpi: Yukon inoculated with *Pst* DC3000 (Pst-0), Yukon inoculated with *Pst*(*avrRpt2*) (avrRpt2-0) and Yukon inoculated with *Pst*(*avrRps4*) (avrRps4-0). In Table 14, the treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. Homogenous subsets are presented in Table 15. These statistical analyses correspond to Figure 8.

Multiple Comparisons

Dependent Variable: sqrtbac_den

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
avrRps4-1	avrRpt2-1	10.0908	38.57149	.963	-108.2571	128.4387
	Pst-1	-570.9763*	38.57149	.000	-689.3243	-452.6284
avrRpt2-1	avrRps4-1	-10.0908	38.57149	.963	-128.4387	108.2571
	Pst-1	-581.0671*	38.57149	.000	-699.4150	-462.7192
Pst-1	avrRps4-1	570.9763*	38.57149	.000	452.6284	689.3243
	avrRpt2-1	581.0671*	38.57149	.000	462.7192	699.4150

Homogeneous Subsets

Dependent Variable: sqrtbac_den

Tukey HSD

treatment	N	Subset	
		1	2
avrRpt2-1	3	155.3763	
avrRps4-1	3	165.4671	
Pst-1	3		736.4434
Sig.		.963	1.000

Tables 16 and 17. Multiple Comparisons and Homogenous Subsets Tables. These tables indicate the results of the Tukey's post-hoc test that performed multiple pairwise comparisons of the following treatments at 1 dpi: Yukon inoculated with *Pst* DC3000 (Pst-1), Yukon inoculated with *Pst*(*avrRpt2*) (avrRpt2-1) and Yukon inoculated with *Pst*(*avrRps4*) (avrRps4-1). In Table 15, the treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. Homogenous subsets are presented in Table 17. These statistical analyses correspond to Figure 8.

Multiple Comparisons

Dependent Variable: bac_den

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
avrRps4-2	avrRpt2-2	820833.3333*	256196.35843	.042	34752.5520	1606914.1147
	Pst-2	-541666.6667	256196.35843	.167	-1327747.4480	244414.1147
avrRpt2-2	avrRps4-2	-820833.3333*	256196.35843	.042	-1606914.1147	-34752.5520
	Pst-2	-1362500.0000*	256196.35843	.004	-2148580.7814	-576419.2186
Pst-2	avrRps4-2	541666.6667	256196.35843	.167	-244414.1147	1327747.4480
	avrRpt2-2	1362500.0000*	256196.35843	.004	576419.2186	2148580.7814

Homogeneous Subsets

Dependent Variable: bac_den

Tukey HSD

treatment	N	Subset	
		1	2
avrRpt2-2	3	137500.0000	
avrRps4-2	3		958333.3333
Pst-2	3		1500000.0000
Sig.		1.000	.167

Tables 18 and 19. Multiple Comparisons and Homogenous Subsets Tables. These tables indicate the results of the Tukey's post-hoc test that performed multiple pairwise comparisons of the following treatments at 2 dpi: Yukon inoculated with *Pst* DC3000 (Pst-2), Yukon inoculated with *Pst*(*avrRpt2*) (avrRpt2-2) and Yukon inoculated with *Pst*(*avrRps4*) (avrRps4-2). In Table 18, the treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. Homogenous subsets are presented in Table 19. These statistical analyses correspond to Figure 8.

Multiple Comparisons

Dependent Variable: lg10bac_den

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
avrRps4-3	avrRpt2-3	.7183*	.15473	.008	.2435	1.1931
	Pst-3	.0223	.15473	.989	-.4524	.4971
avrRpt2-3	avrRps4-3	-.7183*	.15473	.008	-1.1931	-.2435
	Pst-3	-.6960*	.15473	.010	-1.1707	-.2212
Pst-3	avrRps4-3	-.0223	.15473	.989	-.4971	.4524
	avrRpt2-3	.6960*	.15473	.010	.2212	1.1707

Homogeneous Subsets

Dependent Variable: lg10bac_den

Tukey HSD

treatment	N	Subset	
		1	2
avrRpt2-3	3	5.1527	
Pst-3	3		5.8487
avrRps4-3	3		5.8710
Sig.		1.000	.989

Tables 20 and 21. Multiple Comparisons and Homogenous Subsets Tables. These tables indicate the results of the Tukey's post-hoc test that performed multiple pairwise comparisons of the following treatments at 3 dpi: Yukon inoculated with *Pst* DC3000 (Pst-3), Yukon inoculated with *Pst*(*avrRpt2*) (avrRpt2-3) and Yukon inoculated with *Pst*(*avrRps4*) (avrRps4-3). In Table 20, the treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. Homogenous subsets are presented in Table 21. These statistical analyses correspond to Figure 8.

Multiple Comparisons

Dependent Variable: bac_den

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
avrRps4-0	avrRpt2-0	-59.1667	22.03743	.081	-126.7835	8.4502
	Pst-0	-90.8333*	22.03743	.015	-158.4502	-23.2165
avrRpt2-0	avrRps4-0	59.1667	22.03743	.081	-8.4502	126.7835
	Pst-0	-31.6667	22.03743	.382	-99.2835	35.9502
Pst-0	avrRps4-0	90.8333*	22.03743	.015	23.2165	158.4502
	avrRpt2-0	31.6667	22.03743	.382	-35.9502	99.2835

Homogeneous Subsets

Dependent Variable: bac_den

Tukey HSD

treatment	N	Subset	
		1	2
avrRps4-0	3	13.3333	
avrRpt2-0	3	72.5000	72.5000
Pst-0	3		104.1667
Sig.		.081	.382

Tables 22 and 23. Multiple Comparisons and Homogenous Subsets Tables. These tables indicate the results of the Tukey's post-hoc test that performed multiple pairwise comparisons of the following treatments at 0 dpi: Shandong inoculated with *Pst* DC3000 (Pst-0), Shandong inoculated with *Pst*(*avrRpt2*) (avrRpt2-0) and Shandong inoculated with *Pst*(*avrRps4*) (avrRps4-0). In Table 22, the treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. Homogenous subsets are presented in Table 23. These statistical analyses correspond to Figure 8.

Multiple Comparisons

Dependent Variable: bac_den

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
avrRps4-1	avrRpt2-1	-3566.6667*	657.50651	.004	-5584.0772	-1549.2561
	Pst-1	-3775.0000*	657.50651	.003	-5792.4105	-1757.5895
avrRpt2-1	avrRps4-1	3566.6667*	657.50651	.004	1549.2561	5584.0772
	Pst-1	-208.3333	657.50651	.947	-2225.7439	1809.0772
Pst-1	avrRps4-1	3775.0000*	657.50651	.003	1757.5895	5792.4105
	avrRpt2-1	208.3333	657.50651	.947	-1809.0772	2225.7439

Homogeneous Subsets

Dependent Variable: bac_den

Tukey HSD

treatment	N	Subset	
		1	2
avrRps4-1	3	308.3333	
avrRpt2-1	3		3875.0000
Pst-1	3		4083.3333
Sig.		1.000	.947

Tables 24 and 25. Multiple Comparisons and Homogenous Subsets Tables. These tables indicate the results of the Tukey's post-hoc test that performed multiple pairwise comparisons of the following treatments at 1 dpi: Shandong inoculated with *Pst* DC3000 (Pst-1), Shandong inoculated with *Pst*(*avrRpt2*) (avrRpt2-1) and Shandong inoculated with *Pst*(*avrRps4*) (avrRps4-1). In Table 24, the treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. Homogenous subsets are presented in Table 25. These statistical analyses correspond to Figure 8.

Multiple Comparisons

Dependent Variable: lg10bac_den

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
avrRps4-2	avrRpt2-2	-.5552*	.13149	.013	-.9586	-.1517
	Pst-2	-.9220*	.13149	.001	-1.3254	-.5185
avrRpt2-2	avrRps4-2	.5552*	.13149	.013	.1517	.9586
	Pst-2	-.3668	.13149	.071	-.7702	.0367
Pst-2	avrRps4-2	.9220*	.13149	.001	.5185	1.3254
	avrRpt2-2	.3668	.13149	.071	-.0367	.7702

Homogeneous Subsets

Dependent Variable: lg10bac_den

Tukey HSD

treatment	N	Subset	
		1	2
avrRps4-2	3	3.2671	
avrRpt2-2	3		3.8223
Pst-2	3		4.1891
Sig.		1.000	.071

Tables 26 and 27. Multiple Comparisons and Homogenous Subsets Tables. These tables indicate the results of the Tukey's post-hoc test that performed multiple pairwise comparisons of the following treatments at 2 dpi: Shandong inoculated with *Pst* DC3000 (Pst-2), Shandong inoculated with *Pst*(*avrRpt2*) (avrRpt2-2) and Shandong inoculated with *Pst*(*avrRps4*) (avrRps4-2). In Table 26, the treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. Homogenous subsets are presented in Table 27. These statistical analyses correspond to Figure 8.

Multiple Comparisons

Dependent Variable: lg10bac_den

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
avrRps4-3	avrRpt2-3	-.4132	.14100	.059	-.8458	.0194
	Pst-3	-1.0710*	.14100	.001	-1.5037	-.6384
avrRpt2-3	avrRps4-3	.4132	.14100	.059	-.0194	.8458
	Pst-3	-.6578*	.14100	.008	-1.0905	-.2252
Pst-3	avrRps4-3	1.0710*	.14100	.001	.6384	1.5037
	avrRpt2-3	.6578*	.14100	.008	.2252	1.0905

Homogeneous Subsets

Dependent Variable: lg10bac_den

Tukey HSD

treatment	N	Subset	
		1	2
avrRps4-3	3	3.4091	
avrRpt2-3	3	3.8223	
Pst-3	3		4.4801
Sig.		.059	1.000

Tables 28 and 29. Multiple Comparisons and Homogenous Subsets Tables. These tables indicate the results of the Tukey's post-hoc test that performed multiple pairwise comparisons of the following treatments at 3 dpi: Shandong inoculated with *Pst* DC3000 (Pst-3), Shandong inoculated with *Pst*(*avrRpt2*) (avrRpt2-3) and Shandong inoculated with *Pst*(*avrRps4*) (avrRps4-3). In Table 28, the treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. Homogenous subsets are presented in Table 29. These statistical analyses correspond to Figure 8.

Multiple Comparisons

Dependent Variable: lg10bacden

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
C3	C4	.0516	.13819	.995	-.4032	.5064
	C5	.8292*	.13819	.001	.3744	1.2840
	C6	1.3352*	.13819	.000	.8804	1.7900
	C7	1.1520*	.13819	.000	.6972	1.6068
C4	C3	-.0516	.13819	.995	-.5064	.4032
	C5	.7776*	.13819	.002	.3228	1.2324
	C6	1.2835*	.13819	.000	.8287	1.7383
	C7	1.1004*	.13819	.000	.6456	1.5552
C5	C3	-.8292*	.13819	.001	-1.2840	-.3744
	C4	-.7776*	.13819	.002	-1.2324	-.3228
	C6	.5060*	.13819	.028	.0512	.9608
	C7	.3228	.13819	.211	-.1320	.7776
C6	C3	-1.3352*	.13819	.000	-1.7900	-.8804
	C4	-1.2835*	.13819	.000	-1.7383	-.8287
	C5	-.5060*	.13819	.028	-.9608	-.0512
	C7	-.1831	.13819	.683	-.6379	.2717
C7	C3	-1.1520*	.13819	.000	-1.6068	-.6972
	C4	-1.1004*	.13819	.000	-1.5552	-.6456
	C5	-.3228	.13819	.211	-.7776	.1320
	C6	.1831	.13819	.683	-.2717	.6379

Homogeneous Subsets

Dependent Variable: lg10bacden

Tukey HSD

treatment	N	Subset		
		1	2	3
C6	3	5.9640		
C7	3	6.1471	6.1471	
C5	3		6.4700	
C4	3			7.2475
C3	3			7.2992
Sig.		.683	.211	.995

Tables 30 and 31. Multiple Comparisons and Homogenous Subsets Tables. These tables indicate the results of the Tukey's post-hoc test that performed multiple pairwise comparisons of the following treatments: 3 wpg Col-0 plants (C3), 4 wpg Col-0 plants (C4), 5 wpg Col-0 plants (C5), 6 wpg Col-0 plants (C6) and 7 wpg Col-0 plants (C7). In Table 30, the treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. Homogenous subsets are presented in Table 31. These statistical analyses correspond to Figure 12.

Multiple Comparisons

Dependent Variable: bac_den

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
si3	si4	19500000.0000*	4911475.90400	.017	3335929.3686	35664070.6314
	si5	26133333.3333*	4911475.90400	.002	9969262.7020	42297403.9647
	si6	35733333.3333*	4911475.90400	.000	19569262.7020	51897403.9647
	si7	41080000.0000*	4911475.90400	.000	24915929.3686	57244070.6314
si4	si3	-19500000.0000*	4911475.90400	.017	-35664070.6314	-3335929.3686
	si5	6633333.3333	4911475.90400	.669	-9530737.2980	22797403.9647
	si6	16233333.3333*	4911475.90400	.049	69262.7020	32397403.9647
	si7	21580000.0000*	4911475.90400	.009	5415929.3686	37744070.6314
si5	si3	-26133333.3333*	4911475.90400	.002	-42297403.9647	-9969262.7020
	si4	-6633333.3333	4911475.90400	.669	-22797403.9647	9530737.2980
	si6	9600000.0000	4911475.90400	.351	-6564070.6314	25764070.6314
	si7	14946666.6667	4911475.90400	.073	-1217403.9647	31110737.2980
si6	si3	-35733333.3333*	4911475.90400	.000	-51897403.9647	-19569262.7020
	si4	-16233333.3333*	4911475.90400	.049	-32397403.9647	-69262.7020
	si5	-9600000.0000	4911475.90400	.351	-25764070.6314	6564070.6314
	si7	5346666.6667	4911475.90400	.809	-10817403.9647	21510737.2980

si7	si3	-41080000.0000*	4911475.90400	.000	-57244070.6314	-24915929.3686
	si4	-21580000.0000*	4911475.90400	.009	-37744070.6314	-5415929.3686
	si5	-14946666.6667	4911475.90400	.073	-31110737.2980	1217403.9647
	si6	-5346666.6667	4911475.90400	.809	-21510737.2980	10817403.9647

Homogeneous Subsets

Dependent Variable: bac_den

Tukey HSD

treatment	N	Subset		
		1	2	3
si7	3	10620000.0000		
si6	3	15966666.6667		
si5	3	25566666.6667	25566666.6667	
si4	3		32200000.0000	
si3	3			51700000.0000
Sig.		.073	.669	1.000

Tables 32 and 33. Multiple Comparisons and Homogenous Subsets Tables. These tables indicate the results of the Tukey's post-hoc test that performed multiple pairwise comparisons of the following treatments: 3 wpg *sid2* plants (si3), 4 wpg *sid2* plants (si4), 5 wpg *sid2* plants (si5), 6 wpg *sid2* plants (si6) and 7 wpg *sid2* plants (si7). In Table 32, the treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. Homogenous subsets are presented in Table 33. These statistical analyses correspond to Figure 12.

Multiple Comparisons

Dependent Variable: lg10bacden

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Y3	Y4	-.1491	.21302	.952	-.8502	.5519
	Y5	.5537	.21302	.144	-.1473	1.2548
	Y6	.4559	.21302	.276	-.2451	1.1570
	Y7	1.7863*	.21302	.000	1.0852	2.4874
Y4	Y3	.1491	.21302	.952	-.5519	.8502
	Y5	.7029*	.21302	.049	.0018	1.4040
	Y6	.6051	.21302	.100	-.0960	1.3062
	Y7	1.9355*	.21302	.000	1.2344	2.6365
Y5	Y3	-.5537	.21302	.144	-1.2548	.1473
	Y4	-.7029*	.21302	.049	-1.4040	-.0018
	Y6	-.0978	.21302	.989	-.7989	.6033
	Y7	1.2326*	.21302	.001	.5315	1.9336
Y6	Y3	-.4559	.21302	.276	-1.1570	.2451
	Y4	-.6051	.21302	.100	-1.3062	.0960
	Y5	.0978	.21302	.989	-.6033	.7989
	Y7	1.3304*	.21302	.001	.6293	2.0314
Y7	Y3	-1.7863*	.21302	.000	-2.4874	-1.0852
	Y4	-1.9355*	.21302	.000	-2.6365	-1.2344
	Y5	-1.2326*	.21302	.001	-1.9336	-.5315
	Y6	-1.3304*	.21302	.001	-2.0314	-.6293

Homogeneous Subsets

Dependent Variable: lg10bacden

Tukey HSD

treatment	N	Subset		
		1	2	3
Y7	3	4.0494		
Y5	3		5.2819	
Y6	3		5.3797	5.3797
Y3	3		5.8357	5.8357
Y4	3			5.9848
Sig.		1.000	.144	.100

Tables 34 and 35. Multiple Comparisons and Homogenous Subsets Tables. These tables indicate the results of the Tukey's post-hoc test that performed multiple pairwise comparisons of the following treatments: 3 wpg Yukon plants (Y3), 4 wpg Yukon plants (Y4), 5 wpg Yukon plants (Y5), 6 wpg Yukon plants (Y6) and 7 wpg Yukon plants (Y7). In Table 34, the treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. Homogenous subsets are presented in Table 35. These statistical analyses correspond to Figure 12.

Multiple Comparisons

Dependent Variable: bac_den

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
S3	S4	-12100.0000*	2264.88950	.002	-19553.9374	-4646.0626
	S5	1666.6667	2264.88950	.943	-5787.2707	9120.6040
	S6	330.0000	2264.88950	1.000	-7123.9374	7783.9374
	S7	2330.0000	2264.88950	.837	-5123.9374	9783.9374
S4	S3	12100.0000*	2264.88950	.002	4646.0626	19553.9374
	S5	13766.6667*	2264.88950	.001	6312.7293	21220.6040
	S6	12430.0000*	2264.88950	.002	4976.0626	19883.9374
	S7	14430.0000*	2264.88950	.001	6976.0626	21883.9374
S5	S3	-1666.6667	2264.88950	.943	-9120.6040	5787.2707
	S4	-13766.6667*	2264.88950	.001	-21220.6040	-6312.7293
	S6	-1336.6667	2264.88950	.974	-8790.6040	6117.2707

	S7	663.3333	2264.88950	.998	-6790.6040	8117.2707
S6	S3	-330.0000	2264.88950	1.000	-7783.9374	7123.9374
	S4	-12430.0000*	2264.88950	.002	-19883.9374	-4976.0626
	S5	1336.6667	2264.88950	.974	-6117.2707	8790.6040
	S7	2000.0000	2264.88950	.897	-5453.9374	9453.9374
S7	S3	-2330.0000	2264.88950	.837	-9783.9374	5123.9374
	S4	-14430.0000*	2264.88950	.001	-21883.9374	-6976.0626
	S5	-663.3333	2264.88950	.998	-8117.2707	6790.6040
	S6	-2000.0000	2264.88950	.897	-9453.9374	5453.9374

Homogeneous Subsets

Dependent Variable: bac_den

Tukey HSD

treatment	N	Subset	
		1	2
S7	3	2670.0000	
S5	3	3333.3333	
S6	3	4670.0000	
S3	3	5000.0000	
S4	3		17100.0000
Sig.		.837	1.000

Tables 36 and 37. Multiple Comparisons and Homogenous Subsets Tables. These tables indicate the results of the Tukey's post-hoc test that performed multiple pairwise comparisons of the following treatments: 3 wpg Shandong plants (S3), 4 wpg Shandong plants (S4), 5 wpg Shandong plants (S5), 6 wpg Shandong plants (S6) and 7 wpg Shandong plants (S7). In Table 36, the treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. Homogenous subsets are presented in Table 37. These statistical analyses correspond to Figure 12.

Multiple Comparisons

Dependent Variable: sqrtbac_den

Games-Howell

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Y3	Y4	-171.8527	85.89824	.468	-786.9429	443.2376
	Y5	786.5792*	109.78353	.010	289.7790	1283.3794
	Y6	1015.3112*	84.66578	.021	369.9535	1660.6690
	Y7	1019.1768*	94.84334	.007	512.9319	1525.4218
Y4	Y3	171.8527	85.89824	.468	-443.2376	786.9429
	Y5	958.4318*	71.78430	.012	459.2565	1457.6072
	Y6	1187.1639*	16.39683	.000	1085.8870	1288.4407
	Y7	1191.0295*	45.78014	.001	910.8016	1471.2573
Y5	Y3	-786.5792*	109.78353	.010	-1283.3794	-289.7790
	Y4	-958.4318*	71.78430	.012	-1457.6072	-459.2565
	Y6	228.7320	70.30485	.235	-304.9223	762.3863
	Y7	232.5976	82.27841	.214	-174.6044	639.7996
Y6	Y3	-1015.3112*	84.66578	.021	-1660.6690	-369.9535
	Y4	-1187.1639*	16.39683	.000	-1288.4407	-1085.8870
	Y5	-228.7320	70.30485	.235	-762.3863	304.9223
	Y7	3.8656	43.42358	1.000	-318.7123	326.4435
Y7	Y3	-1019.1768*	94.84334	.007	-1525.4218	-512.9319
	Y4	-1191.0295*	45.78014	.001	-1471.2573	-910.8016
	Y5	-232.5976	82.27841	.214	-639.7996	174.6044
	Y6	-3.8656	43.42358	1.000	-326.4435	318.7123

Table 38. Multiple Comparisons Table. This table indicates the results of the Games-Howell post-hoc test that performed multiple pairwise comparisons of the following treatments: 3 wpg Yukon plants (Y3), 4 wpg Yukon plants (Y4), 5 wpg Yukon plants (Y5), 6 wpg Yukon plants (Y6) and 7 wpg Yukon plants (Y7). The treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. These statistical analyses correspond to Figure 13.

Multiple Comparisons

Dependent Variable: bac_den

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
S3	S4	-14166.6667*	3798.02580	.025	-26666.2816	-1667.0518
	S5	.0000	3798.02580	1.000	-12499.6149	12499.6149
	S6	14708.3333*	3798.02580	.020	2208.7184	27207.9482
	S7	16958.3333*	3798.02580	.008	4458.7184	29457.9482
S4	S3	14166.6667*	3798.02580	.025	1667.0518	26666.2816
	S5	14166.6667*	3798.02580	.025	1667.0518	26666.2816
	S6	28875.0000*	3798.02580	.000	16375.3851	41374.6149
	S7	31125.0000*	3798.02580	.000	18625.3851	43624.6149
S5	S3	.0000	3798.02580	1.000	-12499.6149	12499.6149
	S4	-14166.6667*	3798.02580	.025	-26666.2816	-1667.0518
	S6	14708.3333*	3798.02580	.020	2208.7184	27207.9482
	S7	16958.3333*	3798.02580	.008	4458.7184	29457.9482
S6	S3	-14708.3333*	3798.02580	.020	-27207.9482	-2208.7184
	S4	-28875.0000*	3798.02580	.000	-41374.6149	-16375.3851
	S5	-14708.3333*	3798.02580	.020	-27207.9482	-2208.7184
	S7	2250.0000	3798.02580	.973	-10249.6149	14749.6149
S7	S3	-16958.3333*	3798.02580	.008	-29457.9482	-4458.7184
	S4	-31125.0000*	3798.02580	.000	-43624.6149	-18625.3851
	S5	-16958.3333*	3798.02580	.008	-29457.9482	-4458.7184
	S6	-2250.0000	3798.02580	.973	-14749.6149	10249.6149

Homogeneous Subsets

Dependent Variable: bac_den

Tukey HSD

treatment	N	Subset		
		1	2	3
S7	3	1791.6667		
S6	3	4041.6667		
S3	3		18750.0000	
S5	3		18750.0000	
S4	3			32916.6667
Sig.		.973	1.000	1.000

Tables 39 and 40. Multiple Comparisons and Homogenous Subsets Table. These tables indicate the results of the Tukey's post-hoc test that performed multiple pairwise comparisons of the following treatments: 3 wpg Shandong plants (S3), 4 wpg Shandong plants (S4), 5 wpg Shandong plants (S5), 6 wpg Shandong plants (S6) and 7 wpg Shandong plants (S7). In Table 39, the treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. Homogenous subsets are presented in Table 40. These statistical analyses correspond to Figure 13.

Multiple Comparisons

Dependent Variable: lg10bac_den

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CP	CW	-.4340	.13109	.054	-.8743	.0063
	SP	2.1224*	.13109	.000	1.6820	2.5627
	SW	2.2467*	.13109	.000	1.8064	2.6870
	YP	.7644*	.13109	.001	.3241	1.2047
	YW	.6734*	.13109	.003	.2331	1.1137
CW	CP	.4340	.13109	.054	-.0063	.8743
	SP	2.5563*	.13109	.000	2.1160	2.9967
	SW	2.6806*	.13109	.000	2.2403	3.1210
	YP	1.1984*	.13109	.000	.7581	1.6387
	YW	1.1074*	.13109	.000	.6671	1.5477
SP	CP	-2.1224*	.13109	.000	-2.5627	-1.6820

	CW	-2.5563*	.13109	.000	-2.9967	-2.1160
	SW	.1243	.13109	.926	-.3160	.5646
	YP	-1.3579*	.13109	.000	-1.7982	-.9176
	YW	-1.4489*	.13109	.000	-1.8892	-1.0086
SW	CP	-2.2467*	.13109	.000	-2.6870	-1.8064
	CW	-2.6806*	.13109	.000	-3.1210	-2.2403
	SP	-.1243	.13109	.926	-.5646	.3160
	YP	-1.4822*	.13109	.000	-1.9226	-1.0419
	YW	-1.5732*	.13109	.000	-2.0136	-1.1329
YP	CP	-.7644*	.13109	.001	-1.2047	-.3241
	CW	-1.1984*	.13109	.000	-1.6387	-.7581
	SP	1.3579*	.13109	.000	.9176	1.7982
	SW	1.4822*	.13109	.000	1.0419	1.9226
	YW	-.0910	.13109	.979	-.5313	.3493
YW	CP	-.6734*	.13109	.003	-1.1137	-.2331
	CW	-1.1074*	.13109	.000	-1.5477	-.6671
	SP	1.4489*	.13109	.000	1.0086	1.8892
	SW	1.5732*	.13109	.000	1.1329	2.0136
	YP	.0910	.13109	.979	-.3493	.5313

Homogeneous Subsets

Dependent Variable: lg10bac_den

Tukey HSD

treatment	N	Subset		
		1	2	3
SW	3	4.3494		
SP	3	4.4737		
YP	3		5.8316	
YW	3		5.9226	
CP	3			6.5960
CW	3			7.0300
Sig.		.926	.979	.054

Tables 41 and 42. Multiple Comparisons and Homogenous Subsets Tables. These tables indicate the results of the Tukey's post-hoc test that performed multiple pairwise comparisons of the following treatments: Col-0 inoculated with *Pst* DC3000 (CP), Col-0 inoculated with *Pst* DC3000 (HopW1-1) (CW), Yukon inoculated with *Pst* DC3000 (YP), Yukon inoculated with *Pst* DC3000 (HopW1-1) (YW), Shandong inoculated with *Pst* DC3000 (SP) and Shandong inoculated with *Pst* DC3000 (HopW1-1) (SW). In Table 41, the treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. Homogenous subsets are presented in Table 42. These statistical analyses correspond to Figure 18.

Multiple Comparisons

Dependent Variable: lg10bac_den

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CP	CZ	-.4381	.14750	.095	-.9335	.0574
	SP	2.1224*	.14750	.000	1.6269	2.6178
	SZ	1.3504*	.14750	.000	.8550	1.8459
	YP	.7644*	.14750	.002	.2690	1.2599
	YZ	.1276	.14750	.948	-.3678	.6230
CZ	CP	.4381	.14750	.095	-.0574	.9335
	SP	2.5604*	.14750	.000	2.0650	3.0558
	SZ	1.7885*	.14750	.000	1.2931	2.2839
	YP	1.2025*	.14750	.000	.7071	1.6979
	YZ	.5657*	.14750	.022	.0702	1.0611
SP	CP	-2.1224*	.14750	.000	-2.6178	-1.6269
	CZ	-2.5604*	.14750	.000	-3.0558	-2.0650
	SZ	-.7719*	.14750	.002	-1.2673	-.2765
	YP	-1.3579*	.14750	.000	-1.8534	-.8625
	YZ	-1.9948*	.14750	.000	-2.4902	-1.4993
SZ	CP	-1.3504*	.14750	.000	-1.8459	-.8550
	CZ	-1.7885*	.14750	.000	-2.2839	-1.2931
	SP	.7719*	.14750	.002	.2765	1.2673
	YP	-.5860*	.14750	.018	-1.0814	-.0906
	YZ	-1.2228*	.14750	.000	-1.7183	-.7274

YP	CP	-.7644*	.14750	.002	-1.2599	-.2690
	CZ	-1.2025*	.14750	.000	-1.6979	-.7071
	SP	1.3579*	.14750	.000	.8625	1.8534
	SZ	.5860*	.14750	.018	.0906	1.0814
	YZ	-.6368*	.14750	.010	-1.1323	-.1414
YZ	CP	-.1276	.14750	.948	-.6230	.3678
	CZ	-.5657*	.14750	.022	-1.0611	-.0702
	SP	1.9948*	.14750	.000	1.4993	2.4902
	SZ	1.2228*	.14750	.000	.7274	1.7183
	YP	.6368*	.14750	.010	.1414	1.1323

Homogeneous Subsets

Dependent Variable: lg10bac_den

Tukey HSD

treatment	N	Subset				
		1	2	3	4	5
SP	3	4.4737				
SZ	3		5.2456			
YP	3			5.8316		
YZ	3				6.4684	
CP	3				6.5960	6.5960
CZ	3					7.0341
Sig.		1.000	1.000	1.000	.948	.095

Tables 43 and 44. Multiple Comparisons and Homogenous Subsets Tables. These tables indicate the results of the Tukey's post-hoc test that performed multiple pairwise comparisons of the following treatments: Col-0 inoculated with *Pst* DC3000 (CP), Col-0 inoculated with *Pst* DC3000 (HopZ1c) (CZ), Yukon inoculated with *Pst* DC3000 (YP), Yukon inoculated with *Pst* DC3000 (HopZ1c) (YZ), Shandong inoculated with *Pst* DC3000 (SP) and Shandong inoculated with *Pst* DC3000 (HopZ1c) (SZ). In Table 43, the treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. Homogenous subsets are presented in Table 44. These statistical analyses correspond to Figure 18.

Multiple Comparisons

Dependent Variable: lg10bac_den

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
C	S	-2.3421*	.17709	.000	-2.8855	-1.7987
	Y	-.4058	.17709	.133	-.9492	.1375
S	C	2.3421*	.17709	.000	1.7987	2.8855
	Y	1.9363*	.17709	.000	1.3929	2.4797
Y	C	.4058	.17709	.133	-.1375	.9492
	S	-1.9363*	.17709	.000	-2.4797	-1.3929

Homogeneous Subsets

Dependent Variable: lg10bac_den

Tukey HSD

treatment	N	Subset	
		1	2
C	3	3.8580	
Y	3	4.2638	
S	3		6.2001
Sig.		.133	1.000

Tables 45 and 46. Multiple Comparisons and Homogenous Subsets Tables. These tables indicate the results of the Tukey's post-hoc test that performed multiple pairwise comparisons of the following treatments: Col-0 inoculated with *Xcc* (C), Yukon inoculated with *Xcc* and Shandong inoculated with *Xcc*. In Table 45, the treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. Homogenous subsets are presented in Table 46. These statistical analyses correspond to Figure 20.

Multiple Comparisons

Dependent Variable: lg10bac_den

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CM	CS	.1068	.15491	.980	-.4136	.6271
	SM	2.8994*	.15491	.000	2.3791	3.4198
	SS	1.5593*	.15491	.000	1.0390	2.0796
	YM	1.3379*	.15491	.000	.8175	1.8582
	YS	2.6429*	.15491	.000	2.1226	3.1633
CS	CM	-.1068	.15491	.980	-.6271	.4136
	SM	2.7927*	.15491	.000	2.2723	3.3130
	SS	1.4525*	.15491	.000	.9322	1.9729
	YM	1.2311*	.15491	.000	.7108	1.7515
	YS	2.5362*	.15491	.000	2.0158	3.0565
SM	CM	-2.8994*	.15491	.000	-3.4198	-2.3791
	CS	-2.7927*	.15491	.000	-3.3130	-2.2723
	SS	-1.3401*	.15491	.000	-1.8605	-.8198
	YM	-1.5616*	.15491	.000	-2.0819	-1.0412
	YS	-.2565	.15491	.581	-.7769	.2638
SS	CM	-1.5593*	.15491	.000	-2.0796	-1.0390
	CS	-1.4525*	.15491	.000	-1.9729	-.9322
	SM	1.3401*	.15491	.000	.8198	1.8605
	YM	-.2214	.15491	.711	-.7418	.2989
	YS	1.0836*	.15491	.000	.5633	1.6040
YM	CM	-1.3379*	.15491	.000	-1.8582	-.8175
	CS	-1.2311*	.15491	.000	-1.7515	-.7108
	SM	1.5616*	.15491	.000	1.0412	2.0819
	SS	.2214	.15491	.711	-.2989	.7418
	YS	1.3050*	.15491	.000	.7847	1.8254

YS	CM	-2.6429*	.15491	.000	-3.1633	-2.1226
	CS	-2.5362*	.15491	.000	-3.0565	-2.0158
	SM	.2565	.15491	.581	-.2638	.7769
	SS	-1.0836*	.15491	.000	-1.6040	-.5633
	YM	-1.3050*	.15491	.000	-1.8254	-.7847

Homogeneous Subsets

Dependent Variable: lg10bac_den

Tukey HSD

treatment	N	Subset		
		1	2	3
SM	3	4.3005		
YS	3	4.5570		
SS	3		5.6406	
YM	3		5.8620	
CS	3			7.0931
CM	3			7.1999
Sig.		.581	.711	.980

Tables 47 and 48. Multiple Comparisons and Homogenous Subsets Tables. These tables indicate the results of the Tukey's post-hoc test that performed multiple pairwise comparisons of the following treatments: mock-treated Col-0 inoculated with *Pst* DC3000 (CM), salt-treated Col-0 inoculated with *Pst* DC3000 (CS), mock-treated Yukon inoculated with *Pst* DC3000 (YM), salt-treated Yukon inoculated with *Pst* DC3000 (YS), mock-treated Shandong inoculated with *Pst* DC3000 (SM) and salt-treated Shandong inoculated with *Pst* DC3000 (SS). In Table 47, the treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. Homogenous subsets are presented in Table 48. These statistical analyses correspond to Figure 21A.

Multiple Comparisons

Dependent Variable: lg10bac_den

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
C1	C2	.0113	.15954	1.000	-.5335	.5561
	CM	-.4193	.15954	.189	-.9640	.1255
	SM	2.1661*	.15954	.000	1.6213	2.7108
	SS	2.1337*	.15954	.000	1.5890	2.6785
	YM	.6483*	.15954	.015	.1035	1.1930
	YS	.9295*	.15954	.001	.3848	1.4743
C2	C1	-.0113	.15954	1.000	-.5561	.5335
	CM	-.4306	.15954	.169	-.9753	.1142
	SM	2.1548*	.15954	.000	1.6100	2.6995
	SS	2.1224*	.15954	.000	1.5777	2.6672
	YM	.6370*	.15954	.018	.0922	1.1817
	YS	.9182*	.15954	.001	.3735	1.4630
CM	C1	.4193	.15954	.189	-.1255	.9640
	C2	.4306	.15954	.169	-.1142	.9753
	SM	2.5853*	.15954	.000	2.0406	3.1301
	SS	2.5530*	.15954	.000	2.0083	3.0978
	YM	1.0676*	.15954	.000	.5228	1.6123
	YS	1.3488*	.15954	.000	.8040	1.8935
SM	C1	-2.1661*	.15954	.000	-2.7108	-1.6213
	C2	-2.1548*	.15954	.000	-2.6995	-1.6100
	CM	-2.5853*	.15954	.000	-3.1301	-2.0406
	SS	-.0323	.15954	1.000	-.5771	.5124
	YM	-1.5178*	.15954	.000	-2.0625	-.9730
	YS	-1.2365*	.15954	.000	-1.7813	-.6918
SS	C1	-2.1337*	.15954	.000	-2.6785	-1.5890
	C2	-2.1224*	.15954	.000	-2.6672	-1.5777
	CM	-2.5530*	.15954	.000	-3.0978	-2.0083
	SM	.0323	.15954	1.000	-.5124	.5771
	YM	-1.4855*	.15954	.000	-2.0302	-.9407
	YS	-1.2042*	.15954	.000	-1.7490	-.6595
YM	C1	-.6483*	.15954	.015	-1.1930	-.1035

	C2	-.6370*	.15954	.018	-1.1817	-.0922
	CM	-1.0676*	.15954	.000	-1.6123	-.5228
	SM	1.5178*	.15954	.000	.9730	2.0625
	SS	1.4855*	.15954	.000	.9407	2.0302
	YS	.2812	.15954	.590	-.2635	.8260
YS	C1	-.9295*	.15954	.001	-1.4743	-.3848
	C2	-.9182*	.15954	.001	-1.4630	-.3735
	CM	-1.3488*	.15954	.000	-1.8935	-.8040
	SM	1.2365*	.15954	.000	.6918	1.7813
	SS	1.2042*	.15954	.000	.6595	1.7490
	YM	-.2812	.15954	.590	-.8260	.2635

Homogeneous Subsets

Dependent Variable: lg10bac_den

Tukey HSD

treatment	N	Subset		
		1	2	3
SM	3	4.0727		
SS	3	4.1050		
YS	3		5.3092	
YM	3		5.5904	
C2	3			6.2274
C1	3			6.2387
CM	3			6.6580
Sig.		1.000	.590	.169

Tables 49 and 50. Multiple Comparisons and Homogenous Subsets Tables. These tables indicate the results of the Tukey's post-hoc test that performed multiple pairwise comparisons of the following treatments: mock-treated Col-0 inoculated with *Pst* DC3000 (CM), 100 mM NaCl-treated Col-0 inoculated with *Pst* DC3000 (C1), 250 mM NaCl-treated Col-0 inoculated with *Pst* DC3000 (C2), mock-treated Yukon inoculated with *Pst* DC3000 (YM), salt-treated Yukon inoculated with *Pst* DC3000 (YS), mock-treated Shandong inoculated with *Pst* DC3000 (SM) and salt-treated Shandong inoculated with *Pst* DC3000 (SS). In Table 49, the treatments that were compared are listed in the first two columns and its corresponding p-value is indicated in the fourth column (Sig.). The confidence interval is located in the last column. Homogenous subsets are presented in Table 50. These statistical analyses correspond to Figure 21B.

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