The role of genotypic diversity in stabilizing plant productivity in variable environments

HENRY EDWARD CREISSEN

A thesis submitted to the University of East Anglia
for the degree of Doctor of Philosophy

Crop Genetics Department
John Innes Centre

September 2013

Abstract

Crop varietal mixtures have the potential to increase yield stability compared to monocultures in highly variable and unpredictable environments, yet knowledge of the specific mechanisms underlying enhanced yield stability has been limited. Field studies are constrained by environmental conditions that cannot be fully controlled and thus reproduced. This thesis tested the suitability of *Arabidopsis thaliana* as a model system to allow for reproducible experiments on ecological processes operating within crop genetic mixtures. Knowledge of the ecological processes occurring within varietal mixtures may improve the exploitation of mixtures in both conventional and subsistence agriculture.

Genotypic diversity among accessions of *A. thaliana* buffered against abiotic stress, specifically nutrient and heat stress, and increased yield stability through compensation. The role of compensatory interactions in genotypic mixtures was supported by experiments investigating the ability of *A. thaliana* genotypic diversity to buffer against biotic stress, specifically the oomycete pathogen *Hyaloperonospora arabidopsidis* and viral pathogen *Turnip yellows virus*.

Findings from research on plant phenotypic traits involved in competition and compensation in *A. thaliana,* were translated into the crop plant winter barley in field experiments. Mixtures achieved high and stable yields despite being subjected to multiple abiotic and biotic stresses, some of which were not anticipated. Unexpectedly, facilitation was identified as an important ecological process occurring within mixtures. This indicates that crop varietal mixtures have the capacity to stabilise productivity even when environmental conditions and stresses are not predicted in advance.

Acknowledgements

This project was supported by a Biotechnology and Biological Sciences Research Council Targeted Priority Studentship.

I am extremely grateful to my supervisors Prof. James Brown and Dr. Tove Jorgensen not only for their support, guidance and advice but also for giving me the freedom to take this project in my own direction.

I would like to thank members of James Brown's and Paul Nicholson's groups at the John Innes Centre; Paul Nicholson, Christopher Burt, Andrew Steed, Anuradha Bansal, Cléa Lachaux and Margaret Corbitt, for their support throughout this project. I would like to give a special thanks to Alison Hinchliffe and Dean Cole for their assistance with the harvesting, threshing, and sorting vast amounts of seed.

I would especially like to thank Dr. Graham McGrann for his continued support, encouragement and interest in both me and this empirical project.

Outside of work I would like to thank my brothers Alfie and Charlie, and my parents Deirdre and Terry for their encouragement and admiration of all that I do. Many thanks to my parents-in-law Peter and Christine Blenkinsop for their support and gratitude for finally having another scientist in the family. I would especially like to thank Christine for her rigorous proof reading effort which was very much appreciated.

Most of all I would like to my darling wife Elizabeth, whose continued love and support is unrivalled. Liz has been as committed to the success of this project as she has been to me for the last nine years. I am eternally grateful for her contributions to this project, which has included everything from proof reading papers to spending days harvesting plants in the glasshouse. She has provided a loving, stimulating environment for me to both work and play.

Finally, I would like to dedicate this thesis to my unborn niece/nephew affectionately known as Bean Crocker.

Abbreviations

°C	degrees Celsius
ANOVA	Analysis of Variance
cm	centimetre(s)
d.d.f.	denominator degrees of freedom.
ELISA	enzyme linked immunosorbent assay
F	F-statistic
g	grams
h	hour(s)
GS	growth stage
JIC	John Innes Centre
L	Litre
m	metre(s)
mg	milligram(s)
min	minute(s)
mL	millilitre(s)
µmol	micromole(s)
MS	Murashige and Skoog
N	number of observations
n.d.f.	numerator degrees of freedom
P	probability
pH	$-\log_{10}$ hydrogen ion concentration
%	percentage
s	second(s)
SD	standard deviation
v/v	volume to volume

List of Tables

List of Figures

Contents

Chapter 1
General Introduction

1.1 Plant biodiversity affects stability and productivity of natural systems

How plant populations and communities respond to environmental stresses and natural selection pressures is a question central to plant ecology. Plant diversity is known to affect ecosystem stability, productivity and function (Tilman 1996; Hooper 1998; Hector *et al.* 2002), yet the roles of specific ecological processes and mechanisms underlying such relationships remain poorly understood. Detailed knowledge of such mechanisms will facilitate the exploitation of plant diversity in sustainable agroecosystems contributing to an increase in food and financial security of the worlds' poorest people. The following section explores the ecological processes proposed to be responsible for increased stability of diverse ecosystems and outlines the most widely accepted, yet still heavily debated hypotheses in this area of plant ecology. The section begins by examining the positive relationship between plant diversity, stability and productivity, followed by a discussion of the key ecological mechanisms that may underlie these relationships. Research showing nonpositive relationships between diversity, stability and productivity are then presented to highlight neutral or negative effects of diversity in natural systems. Plant phenotypic traits thought to be responsible for increased stability and productivity in genotypically diverse systems are examined, and their relevance to agriculture briefly discussed.

1.1.1 The diversity-stability hypothesis

The relationship between biodiversity, stability and productivity has been debated intensely by ecologists over the last few decades. Elton (1958) hypothesized that greater diversity within populations increases ecological stability and that diverse communities are less susceptible to invasion. Early empirical studies presented evidence for the idea that increased diversity and complexity lead to greater ecological stability (MacArthur 1955; Hutchinson 1959). However, controversy regarding the diversity-stability theory began when later studies reached the opposite conclusion that diversity decreased stability (Gardner and Ashby 1970; May 1974; MacDonald 1978; Pimm 1979).

There is no complete agreement on the terms used to describe ecological stability and its underlying causal mechanisms, but the most commonly used terms are those outlined by Pimm (1984). In his definition, species diversity is determined as a combination of species richness (the number of species) and the evenness of the species abundance distribution. When only a single species occurs, this is a 'species monoculture', while the term 'species mixture' is used to describe the co-occurrence of two or more species. The terms 'connectance' (calculated by dividing the actual number of interspecific interactions by the potential) and the term 'interaction strength' (the mean magnitude of interspecific interaction) are used to describe species diversity (Pimm 1984). Stability of a system refers to its ability to return to equilibrium following perturbation, an alteration of ecosystem function through a disturbance event (Pimm 1984). Resistance refers to the ability of the plant community to maintain productivity and resist change during perturbation (Tilman and Downing 1994). Recovery is defined as the system's ability to compensate for the perturbation-associated loss of productivity (Reusch *et al.* 2005). Resilience refers to the ability of the system to return to its pre-perturbation state after the perturbation event and is a combination of resistance and recovery (van Ruijven and Berendse 2010; Vogel *et al.* 2012). Ecological resistance, recovery and resilience may all have a role to play in enhancing system stability depending on the perturbation and the diversity present.

Until recently, few experiments have manipulated species diversity in the field to investigate the relationship between diversity and stability (Pimm 1984; Tilman *et al.* 2001; Pfisterer and Schmid 2002). Most studies used laboratory-based investigations or field observations and generally found that higher levels of diversity were associated with greater ecosystem stability (Tilman and Downing 1994; McNaughton 1995; Naeem and Li 1997; Tilman *et al.* 2001). Pioneering ecological research, in which species diversity was manipulated in a long-term grassland experiment, has contributed considerably to the diversity-stability debate (Tilman *et al.* 2001). The outputs from this experiment ranged from gaining a greater understanding of the effects of plant species diversity on temporal stability of ecosystems (Tilman *et al.* 2006), to studying the effects of drought stress on productivity and stability in plots varying in species diversity (Tilman and Downing 1994). Overall this research strongly supports the hypothesis that increased levels of plant diversity lead to an increase in ecological stability in these natural grasslands. This finding may be of great importance to

agriculturalists seeking to increase crop yield stability through application of appropriate plant diversity.

1.1.2 The diversity-productivity hypothesis

The theory that greater plant diversity can lead to an increase in plant productivity was originally suggested by Darwin (1872). Theoretical (Tilman *et al.* 1997b) and experimental studies (Harper 1977) have shown overyielding, in which mixtures yield higher than monocultures, to be associated with multispecies coexistence. An increase in productivity with diversity is thought to be the result of the increased likelihood of a productive species being present (sampling effect) that is also able to compensate for underyielding species (compensation), and from a greater chance of efficient exploitation of all available niches (complementation) (Tilman 1996; Hector *et al.* 1999; Hector *et al.* 2010). Together these effects increase utilization of limiting resources and enhance productivity through increased resource retention. The diversity-productivity hypothesis is therefore based on the prediction that functional complementarity can increase productivity through resource partitioning and/or positive interactions such as facilitation (Loreau 2000; Mulder *et al.* 2001; Tilman 2004). The key causal mechanisms suggested to cause increased productivity in diverse communities, i.e. compensation, complementation, and facilitation, are discussed in the following sections.

1.1.3 Mechanisms that increase stability and productivity: Compensation

The insurance hypothesis suggests that stability will increase with diversity as more diverse communities have a greater likelihood of containing a species able to increase performance and compensate for others in response to perturbation (Yachi and Loreau 1999; Hector and Bagchi 2007; Cardinale *et al.* 2011). Diverse communities can achieve greater stability if species vary in their response to perturbation and if some species are able to compensate for the decrease in productivity by poorly-adapted species. Compensation is observed when decreased productivity of poorly-adapted plants is counter-balanced by increased productivity by competitors through competitive release causing compensatory growth. Compensation is regularly shown to increase stability in terrestrial and aquatic plant ecosystems (McNaughton 1977; Leps *et al.* 1982; Tilman *et al.* 1996; Hughes and Stachowicz 2004; Bai *et al.* 2004). Tilman *et al.*

(1996) used results from a decade long grassland study to show that community productivity is often stabilised at the expense of population stability, which decreased in diverse communities, as a result of interspecific competition and compensation

The hypothesis of the sampling effect suggests that an increase in productivity with greater diversity is the result of the increased likelihood of a productive species being present and thus able to dominate the community. Many studies provide evidence for the sampling effect with often a single species increasing resistance and/or recovery from perturbation (Hector *et al.* 2002; Fargione and Tilman 2005; van Ruijven and Berendse 2010). However sampling effects and compensation restrict productivity to that of the most productive species and are therefore predicted to increase stability of productivity but not necessarily productivity itself (van Ruijven and Berendse 2010). Resolving the importance of these mechanisms in plant communities is therefore necessary if we want to exploit the relationship between diversity and productivity to develop higher yielding cropping systems and deploy biodiversity in agriculture.

1.1.4 Mechanisms that increase stability and productivity: Complementation

Complementarity is thought to increase productivity at higher levels of species diversity as interspecific differences in resource requirements and usage allow for increased utilization of limiting resources thereby increasing overall productivity (Naeem 1994; Tilman *et* al. 1997a; Lehman and Tilman 2000). Empirical support for this hypothesis comes from studies showing plant species diversity and niche complementarity to strongly affect ecosystem functioning by increasing productivity relative to species monocultures (Brassard *et al.* 2011; Tilman *et al.* 2001).

Plant biodiversity has been shown to increase aboveground biomass at the community and population level in high nutrient environments (Kirwan *et al.* 2007), and under heavy grazing by livestock (Isbell and Wilsey 2011), indicating potential applications to high input and intensively grazed agricultural systems. The effect of diversity on above-ground biomass production varies amongst functional groups e.g. forbs, herbs, legumes (Roscher *et al.* 2004; Roscher *et al.* 2011). This can be applied to intercropping systems in agriculture in which complementary species are grown together to benefit each other. Belowground complementarity has rarely been studied even though belowground

production can account for half of the total annual net productivity (Brassard *et al.* 2011). Soil space is more fully occupied by roots in species mixtures (e.g. trees and grass species) compared to monocultures (e.g. a single grass species), indicating niche differentiation and complementarity in root traits resulting in increased exploitation of soil resources (Brassard *et al.* 2011). However, despite the positive effect of diversity on biomass being shown in several studies (Tilman *et al.* 2001; van Ruijven and Berendse 2005; Marquard *et al.* 2009; Cardinale *et al.* 2011; Isbell and Wilsey 2011), some studies have shown species richness to have no effect on productivity as plant abundance, density or biomass have a greater impact upon the systems ability to respond to stress (Kahmen *et al.* 2005; Wang *et al.* 2007). These findings indicate the role of additional, often unknown, effects on the diversity-productivity relationship thus highlighting the need for rigorous experimental studies.

1.1.5 Mechanisms that increase stability and productivity: Facilitation

Protection from herbivores, provision of shade and accumulation of nutrients can increase fitness of neighbouring plants in a process called facilitation (Callaway 1995). Facilitation and resource partitioning reduce the intensity of interspecific competition relative to intraspecific competition, increasing resource capture in species mixtures. Compatible species increase productivity but not at the expense of others (Loreau 1998). Facilitation is thought to be greatest under extremely harsh environmental conditions (Bertness and Hacker 1994). However, competition is often the strongest interaction between plants when environmental conditions are less stressful (for reviews see Callaway 1995; Brooker and Callaway 2009), and increases as environmental conditions increase plant productivity (Bertness and Callaway 1994; Goldberg *et al.* 1999; Brooker and Callaway 2009). The effects of competition on ecological processes also increase as overall plant productivity increases whilst the inverse relationship is seen with facilitation (Brooker and Callaway 2009). Facilitation often requires greater levels of diversity than is typically present in species monocultures (even if multiple genotypes are present) as the positive interactions between plants often require a range of form and strategy often absent within species (Callaway 1995).

1.1.6 Negative or neutral effects of biodiversity on productivity and stability

Biodiversity does not always increase productivity in species mixtures (Hooper 1998; Loreau and Hector 2001). Antagonistic plant-plant interactions, whether physical or chemical, can lead to a reduction in biomass of mixtures compared to monocultures due to negative complementarity (Loreau and Hector 2001: Polley *et al.* 2003). When selection favours species with extreme phenotypes the result is often a decrease in mixture productivity relative to monocultures (Loreau 2000). Studies on the relationship between plant diversity and stability have also shown contradictory results when investigating the effects of species diversity on resistance to environmental stresses. Increased resistance to drought has been shown in diverse natural grasslands (Tilman and Downing 1994; Kahmen *et al.* 2005), yet some studies have shown a negative effect which may be due to random assemblage of species and disregard for competitive interactions taking place (Pfisterer and Schmid 2002; Van Peer *et al.* 2004; van Ruijven and Berendse 2010). Few studies have reported neutral effects of biodiversity on productivity and stability, likely because such findings are rarely published (A.C. Newton personal communication). The indication is therefore, that other factors than the diversity productivity relationship, as exemplified with the diversity biomass relationship, affect ecological resistance (Wang *et al.* 2007).

1.1.7 The effects of genotypic diversity on ecosystem stability

The effect of plant species diversity and plant functional group diversity on primary productivity (Hector *et al.* 1999; Tilman *et al.* 2001), ecosystem stability (Loreau 2000), nutrient cycling (Naeem *et al.* 1994; Tilman *et al.* 1996) and invasibility (Knops *et al.* 1999) has been well studied. Importantly, little attention has been paid to these relationships at the genotypic level. There is an indication that genetic diversity reduces the risk of invasion by alien species (Crutsinger *et al.* 2008), increases resistance to extreme climates (Reusch *et al.* 2005) and grazing in marine systems (Hughes and Stachowicz 2004), and reduces plant diseases such as rice blast through facilitation (Zhu *et al.* 2000), yet specific ecological processes acting at the level of genotypic diversity remain largely unknown.

Plant genotypes can vary in many ecologically important functional traits relating to competitive ability (Cahill *et al.* 2005), response to drought (van Ruijven and Benrendse

2010) and resistance to herbivores (Pan and Price 2001; Wise 2007; Kotowska *et al.* 2010). A study on the effect of genotypic selection on genotypic diversity in *Potentilla reptans* found that the strongest performing genotypes increase in abundance over time through possessing desirable traits such as high growth rates and the production of large seeds which interact positively with the local environment (Stuefer *et al.* 2009). Environmental conditions have been shown to alter the patterns and dynamics of genotypic diversity in computational and mathematical models (Nowak and Sigmund 2004; Violle *et al.* 2007), and in grassland systems (Reich *et al.* 2003; Silvertown 2004).

The importance of plant genotypic diversity on ecosystem stability and productivity has received little attention (but see Hughes *et al.* 2008; Kotowska *et al.* 2010). In natural populations genotypic diversity can vary from almost genotypic monoculture (Li *et al.* 2006) to extremely diverse genotypic mixtures (Stehlik and Holderegger 2000). Functional trait variation between different genotypes within a population will alter genotype frequency through time due to environmental interactions (Stearns 1989). Thus it should be possible to predict success of a particular genotype by examining its functional traits (Stuefer *et al.* 2009). For example competitive ability and persistence may be desirable traits in dense canopies thereby increasing selection for genotypes with high growth rates and large leaves that can contribute to community stability through compensation (Vermeulen *et al.* 2008). Ecosystems threatened by drought may favour plants with the ability to form dense root mats in the upper soil layer, a trait desirable for both the individual and the community as the growth of others is facilitated resulting in greater productivity and stability (van Ruijven and Berendse 2010). These studies highlight the importance of maintaining plant diversity over time, as the relative contribution of traits possessed by different genotypes for ecosystem functioning can change over relatively short time periods.

1.1.8 Relevance to agriculture of understanding such processes

Increasing plant species diversity often decreases stability of populations whilst increasing community stability and productivity (Tilman 1996). Complementary resource usage through niche partitioning in diverse communities increases nutrient storage, promoting stability by decreasing invasibility (van Ruijven *et al.* 2003). Greater

diversity decreases interspecific variation in traits among sites and decreases site-to-site variance in traits providing greater stability to the overall community (Tilman 1999). Application of these principles to agriculture can enhance crop production if the mechanisms contributing to increased stability and productivity in biologically diverse systems are properly understood such that they are effectively deployable in an agricultural setting. This thesis aims to further understanding in this research area through experimental investigation of the mechanisms responsible for increased yield and yield stability in diverse cropping systems.

1.2 Exploiting the benefits of plant diversity in agriculture

Current farming techniques rely heavily on the use of monoculture systems that are dependent upon high chemical inputs to buffer against environmental stresses and maintain optimum growing conditions. The fertilisers and pesticides required are energetically and economically costly to produce and are sometimes detrimental to the local environment sometimes leading to the creation of nitrate vulnerable zones in which restrictions to restrict watercourse pollution. Threats to modern farming are being compounded as environmental conditions become increasingly unpredictable, fuel prices escalate, the chemicals used to control pests are becoming more heavily regulated, and pests are evolving resistance to pesticides (Ruttan 1999; McDonald and Linde 2002; Morton 2007). Agroecosystem approaches to arable farming offer solutions to these issues through the application of ecological principles in an agricultural setting. Appropriate biological diversity can limit yield loss in low input and organic systems through use of varietal mixtures and through a range of ecological approaches discussed herein. Such approaches increase the system's potential to buffer against adverse environmental conditions, reduce fertiliser inputs, and control disease, among other benefits (Vandermeer 1989; Finckh and Wolfe 1998).

1.2.1 Deployment of diversity in agriculture

Plant diversity can be deployed at different levels in an agricultural setting: species, variety and gene. Monocultures and mixtures (also referred to as polycultures) can be deployed at each of these diversity levels. The term ‘monoculture’ often refers to the use of same species (species monoculture) for entire fields, but within a species there

may be many varieties, plants that are uniform for desired traits, each of which in themselves can be referred to as a 'monoculture' (variety monoculture). Varieties may differ in their genetic background but show uniformity for certain disease resistance genes, which can be referred to as 'gene monocultures' (Finckh and Wolfe 1998). This section begins by reviewing studies on agricultural diversity at the species level, and examines the use of species mixtures to benefit the main crop (cover-cropping), and produce a secondary crop yield (inter-cropping). The review will then discuss the ecological and economic advantages and disadvantages of growing varietal mixtures, focussing on the underlying mechanisms responsible for increased plant performance in genotypically mixed populations.

1.2.2 Application of species diversity in agriculture

Intercropping is the practice of simultaneously managing two or more crops in the same field (Willey 1979). Intercropping systems can provide substantial yield advantages, and the multiple crops provide insurance against total crop failure thereby safegarding small-holder livelihoods (Lithourgidis *et al.* 2011). For these reasons, intercropping methods continue to be popular with farmers from tropical and temperate regions (Li *et al.* 2006). Other benefits of intercropping relate to the enhancement of ecological function through processes such as compensation, complementation and facilitation resulting in higher land utilization efficiency and higher, more stable yields (Willey 1979).

Intercropping systems often rely on crop differentiation in resource requirements and usage, allowing for increased utilization of limiting resources and greater productivity through complementation (Vandermeer 1989). Intercropping of multiple species is primarily adopted to increase soil structure and fertility, commonly achieved by intercropping grasses with nitrogen-fixing legumes such as peas or beans which reduces the need for excessive nitrogen inputs (Stern 1993; Exner *et al.* 1999). Greater differentiation of resource requirements and usage within the crop reduces niche overlap between species which reduces competition intensity and results in increases in crop production through competitive release (Andersen *et al.* 2007).

Positive interactions between species can occur when one crop alters the growing environment of another crop leading to an increase in crop growth (Begon *et al.* 1996). Such facilitation can be achieved by increasing soil water retention through planting a combination of deep and shallow rooted crops (Morris and Garrity 1993). Examples include maize and faba bean intercropping systems common in Northern China (Li *et al.* 2006). Nutrient leaching and soil erosion can be minimized by plants with extensive root systems such as most trees species (Toledo 1985; Marten 1986). Insect pest outbreaks can also be reduced through intercropping by providing microhabitats for natural enemies to persist (Vandermeer 1989; Andow 1991), and increasing distance between host plants, reducing the spread and abundance of insect pests (Root 1973). Intercropping approaches to farming lead to an overall reduction in farm inputs (Kontturi *et al.* 2011).

Intercropping systems may require the crops to be fully mixed with no distinct row pattern, planted in rows narrow enough to permit interaction between crops, planted in strips wide enough to accommodate machinery and allow for separate harvesting, or planted in relay, where sowing dates of the crops are staggered (Finckh and Wolfe 1998). Mixing rates of intercrops are selected to achieve the required levels of interactions between crops to enhance crop function and achieve higher yields whilst considering the practicalities of farming multiple crops simultaneously in the same space. Interactions between intercrops occur both above and below-ground yet below-ground interactions have been shown to account for much of the increase in yield and nutrient uptake in intercropping systems such as wheat-maize mixtures (Li *et al.* 2006). Despite the advantages of growing crop species mixtures intercropping can sometimes reduce primary crop yields due to high levels of competition for resources (Akanvou *et al.* 2007). Maize-wheat intercropping systems have been shown to reduce maize yields through high levels of competition for below ground resources which highlights the important role of competition in diverse crop species mixtures (Li *et al.* 2001).

Cover crops are grown to benefit the primary crop which they do primarily by improving soil fertility and soil structure (Langdale *et al.* 1991). Cover crops are typically nitrogen-fixing legumes commonly grown prior to the main cash crop such that when they decompose, the nutrients return to the soil and become available for the cash crop (Shipley *et al.* 1992; Erenstein 2003). Legume cover crops have led to

increased yield stability without the addition of large amounts of fertiliser (Mundt 2002). This is achieved through the facilitation of primary crop growth by raising levels of available nutrients often achieved by planting grass-legume mixtures, such as winter wheat and white clover in the UK (Jones and Clements 1993). Non-legume cover crops, such as ryegrass or buckwheat, can also be used to recycle nutrients and reduce leaching of nitrogen into the groundwater (Clark *et al.* 1994; Ranells and Wagger 1997). Cover crops can also control weeds by out-competing weeds for resources prior to, and during the growth of the primary crop (Teasdale 1993). Despite the disadvantages of growing several crops simultaenously, such as managing many different harvest times, species mixtures offer ways to increase water-use efficiency, soil structure and fertility, pest control and provide many other benefits that subsistence farmers can afford (Gliessman 1995).

1.2.3 Application of within species diversity

Variety monoculture systems, in which a single high-yielding variety is grown throughout an entire field, dominate modern agricultural practices (Trewavas 2001). Monoculture systems are heavily dependent upon high levels of chemical inputs, such as pesticides and fertilisers, for soil improvement and pest control. An alternative to the monoculture system is the use of varietal mixtures, in which several genotypes are sown together at the same time to buffer against environmental stresses and improve yield stability, (Wolfe 1985; Lannou and Mundt 1996; Zhu *et al.* 2000). Mixtures are commonly deployed to control disease which they can do in several ways including the prevention of pathogen spread by increasing distance between susceptible host plants, or the use of resistant plants to form a barrier to prevent pathogen dispersal (Chin and Wolfe 1984; Zhu *et al.* 2000). The beneficial effect of mixtures on disease control has been observed in many crops, controlling major pathogens such as powdery mildew in barley (Wolfe 1992), stripe rust in wheat (Finckh and Mundt 1992), and blast in rice (Zhu *et al.* 2000). The use of varietal mixtures to buffer against the effects of abiotic stresses is also not an uncommon agricultural practice (Finckh and Wolfe 1998). For example, sowing a mixture comprised of high yielding varieties and winter hardy varieties effectively insures against excessive losses experienced in colder winters, particularly as survivors are able to overyield via compensation and competitive release (Finckh *et al.* 2000). Mixtures are commonly deployed to control a single stress, usually

a disease, yet mixtures can be used to control diverse and sometimes unpredicted stresses. Methods of using within-field genetic diversity to buffer against the effects of environmental fluctuations whilst maintaining productivity are particularly applicable to most cereal crops in which there is sufficient genetic diversity to allow such practices. However, this method may not be as effective if the available genetic diversity of a crop is too low, for instance in coffee (Hendre *et al.* 2008) and oil seed rape (Qian *et al.* 2009).

Yield is the most important factor for growers, yet yield stability is often hard to achieve in variety monocultures because they contain little variation for resistance to abiotic and biotic stresses. UK winter wheat yields have been particularly variable over the last few years. A wet harvest reduced yields in 2009 whereas dry conditions reduced yields in 2010. In 2011 there was a yield increase in all regions except the Eastern regions where yields were lower than in 2010 due to a very dry spring. 2012 saw wheat yields decline again with the greatest drop in yield (-31%) in the South West region (www.gov.uk). Due to such variation in crop yields, yield stability of varieties is becoming a significant breeding target. Ostergaard *et al.* (2005) found that varietal mixtures provided greater yield stability than monocultures. Variances associated with genotype by environment interactions are almost always lower for mixtures than their components in monoculture due to compensatory interactions occurring within the mixed population (Smithson and Lenne 1996; Cowger and Weisz 2008). When Polish barley trials were analysed to assess the yield stability of mixtures and pure stands, mixtures were found to have a much more stable yield than pure stands, but a lower yield on average (Eberhart and Russell 1966). The beneficial effect of mixtures on yield stability has been observed elsewhere in barley (Wolfe 1992) and also in wheat (Finckh and Mundt 1992). Within a mixture it is difficult to predict which variety will provide the highest yield, partly due to complexity of competitive interactions within the field and also variable field conditions, a fact that highlights the importance of maintaining genetic diversity as an essential practice for successful diverse cropping systems.

In the late 1970s, the use of varietal mixtures of wheat and barley became more popular with farmers, however, this transition was not as popular with the millers and maltsters due to issues regarding grain heterogeneity, grain verification and customer preference (Mundt 2002) as well as processing difficulties. There are also issues surrounding the

agronomy of growing mixtures, however, through the application of precision agricultural technologies available to farmers, these difficulties could be partially mitigated against, depending on the planting regimes adopted (Miller *et al.* 2001). Certain compromises must be made regarding the pesticide spray programmes and fertiliser application rates which must be adapted to the mean of the mixture components rather than tailored specifically to a single variety. Harvesting mixtures may also present problems to the farmer if there is variation in ripening dates between plants in the same field. Despite these issues varietal mixtures have potential for achieving high and stable yields whilst simultaneously requiring less chemical inputs, making their use appealing when attempting to meet future global food demands using sustainable approaches to farming.

1.2.4 Experimental approaches to investigate ecological processes occurring within varietal mixtures

There remains a significant gap in scientific knowledge about the relationship of environmental stress to yield in mixtures. Studies are often conducted under similar yet largely unreproducible environmental conditions, which reduces the ability to test hypotheses is adequately replicated experiments. Replicated trials across multiple soil types will indicate the consistency and any environmental dependency of such interactions taking place within the mixture. For disease studies, experimental plots are often artificially inoculated at higher concentrations than would be normal in nature, in an attempt to avoid stochastic effects in disease establishment (Mundt 2002). This may reduce the effectiveness of the mixture due to a reduction in the number of generations of pathogen increase that occur before the crops' carrying capacity is reached (Mundt 2002). Natural disease infection would allow the studies to be more accurately representative of the field environment. Non-diseased controls are often absent in field studies of mixtures, but are needed to allow for comparisons of the effects of disease on yields (Mundt 2002). Disease scoring of individual plants within both monoculture and mixture plots would aid understanding of the ability of mixed plant genotype populations to control disease and the alteration of population dynamics impacting upon population yield. Further, data from hand harvested plants and post-harvest varietal grain identification would provide insights into the population processes occurring within mixed variety populations under environmental stress.

1.3 **Arabidopsis** *as a model plant for varietal mixture studies*

Translational science from model systems into cropping systems can increase our understanding of the mechanisms contributing to high and stable crop production. Selection for traits that promote complementation, through facilitation and resource use efficiency, and compensatory interactions may enhance ecological resistance and resilience resulting in stability and productivity of the agricultural system. The following section describes the suitability of *Arabidopsis thaliana* as a model system to investigate the mechanisms leading to enhanced crop function in varietal mixtures. Traits contributing to the value of *Arabidopsis* as a model organism are discussed in detail. The use of *Arabidopsis* to investigate the effect of pathogens on plant fitness is then examined. The section finishes by focusing on the responses of *Arabidopsis* to abiotic stress and competition between plants, highlighting the suitability of this species for this project.

1.3.1 A model to understand varietal mixtures

Arabidopsis has been used extensively as a model organism to investigate the effects of a variety of stresses on plant fitness, including herbivory (Weinig *et al.* 2003; Arany *et al.* 2005), bacterial and viral pathogens (Kover and Schaal 2002; Pagan *et al.* 2008, 2009), drought (Bouchabke *et al.* 2008), and salinity (Zhu 2001). *Arabidopsis* has also aided our understanding of factors affecting the outcomes of competition between plants through manipulation experiments in which plants were grown in crowded, resource limited conditions and the vegetative and reproductive outputs were measured (Cahill *et al.* 2005; Masclaux *et al.* 2010). The proven track record of the model plant in these research areas prompted us to explore its potential for modelling crop varietal mixtures. Until now, the effects of stress on competition between closely related individuals in crop varietal mixtures have been studied on a large scale because of the high variance between experiments associated with the partly uncontrolled environment and genotype by environment interactions (Ceccarelli and Grando 1991). A suitable model system in which environmental conditions are more readily controlled would require fewer plants, making it feasible to manipulate and test the effects of specific interactions and to obtain insights into the mechanisms at work in crop mixture systems. Greater

understanding of the plant-plant interactions within varietal mixtures and the crop's interaction with the environment has the potential to inform rational choices of component varieties in mixtures.

1.3.2 The value of Arabidopsis *as a model organism*

Arabidopsis is a member of the mustard family (*Brassica*ceaae) with a wide distribution across the northern hemisphere (Meinke *et al.* 1998). It is a small dicotyledonous annual species that has been used extensively over the last 30 years as a model organism to increase understanding in many areas of plant biology such as biochemistry, physiology and genetics (Koornneef and Meinke 2010). *Arabidopsis* has many traits which make it ideal for use as a model organism. It has a rapid life cycle (as short as six weeks from germination to mature seed), high seed production (often >5000 seeds per plant) and can be easily cultivated in small spaces, reducing the resources required for large scale studies (Meinke *et al.* 1998). *Arabidopsis* was initially used to understand genetics and its small diploid genome (125Mb total) was the first plant genome to be to fully sequenced (*Arabidopsis* Genome Initiative 2000). The fully assembled genome and extensive genetic and physical maps of all five chromosomes made *Arabidopsis* an ideal model for genetic studies. Widespread use of this model has resulted in the production of a vast array of genetic resources available for experimental analysis including over 750 natural accessions and many mutant lines all catalogued at stock centres (ABRC, http://abrc.osu.edu/; NASC, http://*Arabidopsis*.info/). Examples of discoveries first made in *Arabidopsis* include the genetic regulation of flowering time (Koornneef *et al.* 1998) and much of the biology of floral development (Coen and Meyerowitz 1991). *Arabidopsis* was also used to identify plant receptors for phototropism (Huala *et al.*1997) and phytochrome action (Quail *et al.* 1995). Phytochrome mediated light perception (red:far red light ratio) is of particular importance to this study as it indicates the presence of neighbouring plants, and other studies have shown variation for developmental changes by *Arabidopsis* genotypes in response to this light ratio, which has particular relevance to ecological studies of competition (Dorn *et al.* 2000; Pigliucci *et al.* 1995a). *Arabidopsis* is closely related to economically important crops such as turnip, cabbage, broccoli and oil seed rape, making it a suitable model for *Brassica* crops. Genetic and molecular studies are simpler in the diploid *Arabidopsis* than in polyploid *Brassica* crops, thus providing a good springboard to understanding *Brassica*

genetics (Mitchell–Olds 2001). Negatives associated with the use of the *Arabidopsis* model system for understanding crop varietal mixtures include issues when attempting to extrapolate results from disease studies on *Arabidopsis* to crops. This is largely due to the fact that most coevolved pathogens are closely adapted to their specific host. Also, phenotypic diversity between genotypes is much greater for *Arabidopsis* than most crop species, which can create issues when attempting to translate research from model to crop.

1.3.3 The effects of pathogens on Arabidopsis

The variety of diseases that infect *Arabidopsis* and the variation in responses of different *Arabidopsis* genotypes to diverse pathogens have been exploited by many groups studying aspects of resistance and tolerance in plants. The range of pathogen types able to infect *Arabidopsis* includes bacteria (e.g. *Pseudomonas syringae*) (Boch *et al.* 2002), fungi (e.g. *Golovinomyces orontii*) (Adam and Somerville 1996), oomycetes (e.g. *Albugo candida*) (Cooper *et al.* 2008), viruses (e.g. *Cucumber mosiac virus*) (Pagan *et al.* 2008) and nematodes (e.g. *Meloidogyne incognita*) (Sijmons *et al.* 1991). *Arabidopsis* has been used to investigate natural variation for disease resistance and tolerance to naturally occurring pathogens such as *Hyaloperonospora arabidopsidis* (Salvaudon *et al.* 2007; Nemri *et al.* 2010), *Albugo* species and *Erysiphe* species (Cooper *et al.* 2008). It has also proven to be a useful model to study experimental pathogens of threat to crops related to *Arabidopsis*, such as *Turnip yellows virus* (TuYV) in *Brassicas* (Stevens *et al.* 2005) and *Cucumber mosaic virus* (CMV) in *Cucurbits* (Pagan *et al.* 2008, 2009). Susceptibility to a broad range of pathogens has further increased the popularity of *Arabidopsis* as a host in model pathosystems.

1.3.4 Naturally occurring pathogens of Arabidopsis

Hyaloperonospora arabidopsidis (*Hpa*) is an obligately biotrophic oomycete pathogen specific to *Arabidopsis*. Plant and parasite have a long coevolutionary history (Holub 2008). The *Arabidopsis*-*Hpa* model pathosystem has been used to study interactions between major resistance genes (*R* genes) and the oomycete effectors (*ATR* genes) identifying components of the interaction between host and pathogen, and exposing the complexity of such interactions that relate to plant immune systems and suppression of

immunity by pathogens (reviewed by Coates and Beynon 2010). Nemri *et al.* (2010) used association genetics of resistances to *Hpa* to increase genetic map resolution for different race-specific *R* genes in *Arabidopsis* indicating that combinations of association and linkage mapping could help discovery of novel resistance genes. Lapin *et al.* (2012) took the work on race-specific *R* genes further, identifying the source of broad-spectrum resistance of *Arabidopsis* accession C24 to *Hpa* as multiple combinations of isolate-specific loci. The quantitative resistance loci they identified can potentially assist the cloning of disease resistance-related genes, improving understanding of the complex molecular mechanism of disease immunity in plants. The potential for interactions between co-infecting species of pathogens complicates the plant-pathogen interaction further, as demonstrated in the study of interactions between *Arabidopsis* and two of its natural pathogens (Cooper *et al.* 2008). This study found suppression of host defence by the oomycete *Albugo candida* which allowed infection by avirulent strains of *Hpa* and powdery mildew (*Erysiphe* spp.). A similar suppression of resistance by a non-adapted pathogen isolate has been reported to be caused by pathogens such as rusts (Yarwood 1977). *Arabidopsis* is an extremely useful model for studying crop pathogens because of the similarities in its major pathogens epidemiology and dispersal mechanisms. Powdery mildews infect *Arabidopsis* (*Erysiphe* spp.), wheat and barley (*Blumeria* spp.), and share similar characteristics including aerial dispersal of spores and disease symptoms. Parallels between the model pathosystem and agronomically important pathosystems suggest that this model may be suitable for studying cropping systems under biotic stress.

Empirical studies have also provided insights into the evolutionary history of plant host and pathogen. Salvaudon *et al.* (2007) used the *Hpa-Arabidopsis* pathosystem to study trade-offs between host and parasite fitness. Host genotypic variation in compatibility with the pathogen had a significant effect on the relationship between host and plant fitness leading to differences in resource availability to both pathogen and host. Damgaard and Jensen (2002) studied the effect of disease on competitive ability in two genotypes of *Arabidopsis* and used the data to predict the outcome of long-term co-existence in the presence of the pathogen *Hpa* (then named *Peronospora parasitica*). They concluded that both *Arabidopsis* genotypes would co-exist in the absence of the pathogen, but if the pathogen was present the resistant genotype would outcompete the susceptible genotype and thereby reduce population genetic diversity. These studies

provide insight into ways in which plant population and community structure can be influenced by plant-pathogen interactions at the local scale.

The interaction between plant tolerance and resistance to viral and bacterial pathogens has also been investigated in *Arabidopsis*. Kover and Schaal (2002) measured plant and parasite fitness along with disease severity in search of selection for tolerance and resistance to the bacterial pathogen, *Pseudomonas syringae*. They concluded that *Arabidopsis* genotypes vary in their tolerance to *P. syringae* which reduces the strength of selection for resistance to the pathogen. Korves and Bergelson (2003) explored this idea further by investigating the developmental response of plants to the pathogens *P. syringae, Xanthomonas campestris* and *Hpa*. They found changes in flowering time, and branch architecture due to pathogen infection affect plant tolerance and disease resistance.

1.3.5 Experimental pathogens of Arabidopsis

Arabidopsis has been used extensively to study pathogens that do not normally naturally occur on this species. Pagan *et al.* (2008, 2009) investigated interactions between the parasite *Cucumber mosaic virus* (CMV) and plant density, monitoring differences in life history traits between *Arabidopsis* genotypes. Greater resource allocation to reproductive growth increased tolerance to the direct negative effect of the pathogen on plant fitness, while investment in vegetative growth increases tolerance to the indirect costs of infection, which reduces competitive ability through reduced plant fitness (Pagan *et al.* 2009). Westwood *et al.* (2013) found that CMV infection increased tolerance to drought in *Arabidopsis*. This effect was attributed to the induction of abscisic acid (ABA) regulated genes by the virus, which led to alteration of the root characteristics. The outcome of this interaction was beneficial to both plant and pathogen as increased plant tolerance to abiotic stress increases the likelihood of virus survival during periods of environmental stress.

Stevens *et al.* (2005) identified *Arabidopsis* as a host for *Beet mild yellowing virus* (BMYV) and *Turnip yellows virus* (TuYV) indicating the potential of this pathosystem for understanding the vector-virus-host interactions. Knowledge of these interactions is of particular relevance to important agronomic crops including oilseed rape (OSR) and

sugar beet that are threatened by both the aphid vector, *Myzus persicae*, and its symbiotic virus. *Arabidopsis* has also been used to study resistance to cabbage white butterflies (*Pieris* species) and leaf miners (*Scaptomyza* species.) (Hering 1957; Reymond *et al.* 2000). This genus of leaf miners is related to *Drosophila* indicating a potential combination of the genetic tools of *Arabidopsis* and *Drosophila* for a plant-insect-model system (Mitchell-Olds 2001).

1.3.6 The effects of abiotic stress on Arabidopsis

High levels of trait diversity among *Arabidopsis* accessions allow for rigorous testing of the response of plants to abiotic stress. The species has been used as a model to understand the effects of nutrient stress on plants, providing insight into mechanisms of nutrient uptake and accumulation (Raghothama 1999; Vert *et al.* 2002; Palmgren 2001) and also the interactions between nutrient status and other stresses such as salt stress (Wu *et al.* 1996; Zhu *et al.* 1998). *Arabidopsis* has proven to be a useful model in the study of the effect of salinity stress on plants, an area of particular agronomic importance as it affects one-fifth of cultivated land globally (Zhu 2001; Labidi *et al.* 2004). Bouchabke *et al.* (2008) found phenotypic variation in response to drought stress between accessions suggesting that *Arabidopsis* could potentially be used to identify important alleles for the complex traits of drought resistance in economically important plants. Advances in our understanding of tolerance to drought and salinity stress are critical for global crop production as they are responsible for yield losses of over 50% worldwide (Boyer 1982; Bray 2000; Wang *et al.* 2003).

Natural accessions of *Arabidopsis* can be widely variable in their morphology, development and physiology, opening up the potential for use of *Arabidopsis* in research areas such as evolutionary ecology (Mitchell-Olds 2001; Jorgensen 2012). High levels of self-pollination facilitate quantitative trait loci (QTL) mapping and testing of progeny of advanced lines, which can be problematic in outcrossing species (Karkkainen *et al.* 1999). The genomic sequence, QTL and association mapping allows for the study of natural selection on functional genes through the isolation of genes and identification of ecologically important polymorphisms (Johanson *et al.* 2000; Stahl *et al.* 1999). Findings from such studies can be tested experimentally through competition

experiments or reciprocal transplant experiments involving the use of near isogenic lines (NILs) to test the fitness of allelic/phenotypic variants.

1.3.7 Phenotypic plasticity and competition in Arabidopsis

The genetics and evolution of phenotypic plasticity has been extensively studied in *Arabidopsis*. Pigliucci *et al.* (1995a, b) found *Arabidopsis* to be an ideal model for investigation of genotype by environment interactions because of the wealth of information on genetics, physiology, development and high environmental sensitivity. They found genotypic variation for plasticity in response to light and nutrient treatments and also a correlation between the amount of phenotypic variation within a genotype and the range of variation in the environment. Later flowering genotypes have been associated with greater plasticity in many morphological and physiological traits indicating variation in life history strategy between early and late flowering genotypes (Zhang and Lechowicz 1994). Pigliucci and Kolodynska (2002) measured phenotypic plasticity in response to flooding in natural *Arabidopsis* accessions. Flooding conditions uncovered selection for different traits and trade-offs in allocation between roots and above-ground biomass, leaves and reproductive structures. Empirical studies on trade-offs in resource allocation and phenotypic plasticity in plants impact heavily upon our understanding of competitive interaction between plants across environments. Aarseen and Clauss (1992) used *Arabidopsis* to study r/K-selection theory which relates to a trade-off between the ability to reproduce quickly (r-selection) and compete successfully (K-selection) (MacArthur and Wilson 1967). There was great variation in fecundity allocation between plants of different sizes. However the authors did not take this work further by studying the outcomes of competition between r and K specialist genotypes. Cahill *et al.* (2005) investigated competition between *Arabidopsis* genotypes in monocultures and mixtures under different nutrient treatments. They found greater levels of competition under high nutrient conditions when resources were ample. Plant competitive ability was largely related to neighbour size (biomass) and shading ability, not genotypic identity. Masclaux *et al.* (2010) performed a similar study and found that the strength of the competitive ability of the neighbour, not its genetic identity, affected plant growth of focal plants. Willis *et al.* (2010) found a correlation between tolerance of competition and suppression of neighbours. Willis *et al.* (2010) found neighbour genetic identity to affect the fitness of focal plants, however, they may have confounded

genotype and size which could have been tested properly by using plants of different sizes or ages within a competing genotype.

The impact of resource availability on the outcome of competition remains a question of great scientific interest. The effect of carbon dioxide levels on competition between genotypes was explored by Andalo *et al.* (2001). At ambient CO_2 levels genotypic monocultures outperformed mixtures of competing genotypes and vice versa at elevated carbon dioxide levels in which increased plant growth increased competition for resources (Andalo *et al.* 2001). The interaction between plant traits and environmental conditions highlights the need for studies testing mixtures over a range of environments. The use of *Arabidopsis* as a model for such studies on competition can aid our understanding of the ecological processes that structure populations and communities and, in an agricultural setting, the effect of these processes on maximising and maintaining productivity. This makes *Arabidopsis* a suitable model for our research into the mechanisms underlying the increased performance of crop variety mixtures compared to their component monocultures.

1.4 Contents of this thesis

In Chapter 2, the ability of *Arabidopsis* genotypic diversity to buffer against abiotic stress, specifically nutrient and heat stress, was investigated using large-scale genotypic mixture experiments and pair-wise interaction experiments. Compensation was identified as the main ecological process conferring yield stability in these experiments.

Further investigation into the mechanisms contributing towards yield stability in a genotypically mixed population continues in Chapter 3. Support was provided for the role of compensatory interactions in the ability of *Arabidopsis* genotypic diversity to buffer against biotic stress, specifically the oomycete pathogen *Hyaloperonospora arabidopsidis* and viral pathogen *Turnip yellows virus*.

Findings from previous chapters regarding plant phenotypic traits involved in competition and compensation in *Arabidopsis* were translated into the crop winter barley in Chapter 4. The ecological resistance of winter barley varietal mixtures to abiotic and biotic stresses experienced under field growing conditions were

investigated. It was found that mixtures can stabilise yield despite unexpected stresses that were not anticipated when the experiment was designed.

Finally, in Chapter 5, I will discuss the implications and applications of this research, identifying key areas that require further investigation.

Chapter 2

Stabilisation of yield in plant genotype mixtures through compensation rather than complementation

This chapter has been accepted as a paper by Annals of Botany:

Creissen HE, Jorgensen TH, Brown JKM. 2013. Stabilisation of yield in plant genotype mixtures through compensation rather than complementation. *Annals of Botany*. **112**, 1439-1447. doi:10.1093/aob/mct209.

2.1 Introduction

Empirical studies have shown that higher levels of plant species diversity can result in greater above-ground productivity (Hector *et al.*1999; van Ruijven and Berendse 2005; Roscher *et al.* 2011) and ecosystem stability (Tilman *et al.* 2006). Previous studies on the relationships between plant diversity, stability and productivity of ecosystems have focused on diversity at the species level (Tilman 2001), yet these relationships are also observed at the functional group and genotype level (Hector *et al.* 1999; Hughes and Stachowicz 2011). The potential of plant diversity to increase or stabilise productivity is of great interest in crop systems (Zhu *et al.* 2000; Li *et al.* 2009). However, there is limited understanding of the actual mechanisms leading to correlations between plant diversity, productivity and stability which currently restricts the use of biologically diverse cropping systems in agriculture.

Ecological stability is commonly described using two main terms, resistance and resilience. Resistance refers to the ability of the system to resist change in response to perturbation, whereas resilience refers to the ability of the system to recover by returning to its pre-perturbation state (for reviews see Tilman 1996; Hooper *et al.* 2005). Resistance is the more relevant trait in annual plants, particularly when environmental stress occurs near or after the time of flowering. Note that resistance in the ecological sense used here, operating at the population or community level, is not the same as resistance of individual plants to stress or disease. Proposed mechanisms by which

stability is achieved by ecological resistance in diverse communities or populations include compensation, complementation and facilitation. Compensation occurs when a species displays resistance to perturbation and is able to compensate for more susceptible species. It requires variation between species or genotypes in response to stress and competition, allowing the stronger species or genotypes to compensate for weaker ones via competitive release (Tilman 1996). Such interactions increase stability in productivity at the community level but increase variability at the population and species level (Tilman 1996; Bai *et al.* 2004). Similar compensatory mechanisms may occur between genotypes in a diverse population of a single species (McLaren *et al.* 2011). Complementation, on the other hand, results from increased resource use efficiency in mixed communities or populations because individual plants often experience less niche overlap than in monoculture, which can lead to overyielding in species mixtures (Hector *et al.* 2002; Silvertown 2004). Finally, facilitation results from positive interactions between species or genotypes, which may increase productivity and stability by altering features of the local environment to the benefit of neighbouring plants, such as the accumulation of nutrients, provision of shade and protection from herbivores (Callaway 2002). Facilitation is indicated if plants perform significantly worse when a neighbour is removed and is common in stressful environments (Callaway 2002; Kikvidze *et al.* 2006).

Crop breeding programmes produce cultivars with increased yield potential which must be coupled with improved farming practices to achieve those yields (Calderini and Slafer 1998). In most situations, a single cultivar that is completely or almost completely genetically uniform is grown throughout a field (Trewavas 2001). Monocultures rely heavily on chemical inputs such as fungicides, pesticides and herbicides to maintain the specific environment required for successful cropping. However, selection for performance under high input conditions and low environmental variation can lead to a reduction in yield stability across environments (Calderini and Slafer 1999). The use of agro-chemicals may be heavily restricted in the future, forcing farmers to consider using alternative cropping systems that are adaptable to multiple environments (Hillocks 2012). If plant diversity within fields of agricultural crops contributes to achieve stable, high levels of production, it will promote food security, which is threatened by a ‘perfect storm’ of multiple interacting environmental and natural resource challenges (Beddington 2009).

Considering the current threat of global warming and the unpredictable ecological responses to climate change (Lavergne *et al.* 2010), the importance of increasing the adaptive power of crops is of great concern (Lobell 2008). Varietal mixtures, where several cultivars are grown together, are only used to a limited extent in modern, intensive farming owing to perceived disadvantages regarding heterogeneity of the end-product and variable agronomy (Newton *et al.* 2008b). Mixtures have the potential to increase yield stability and control pests and diseases whilst being less reliant on chemical inputs which generate a high demand for energy in their production and application (Wolfe 1985; Altieri 1999; Zhu *et al.* 2000).

Presently, evidence for the advantages and disadvantages of growing varietal mixtures comes from studies that are typically large in scale because of the high variances associated with the uncontrolled environment and genotype by environment interactions (Madden *et al.* 2007). A suitable model system in which environmental conditions are more readily controlled would require fewer plants, making it feasible to manipulate and test the effects of specific interactions and to obtain insights into the mechanisms at work in crop mixture systems. Greater understanding of the plant-plant interactions within varietal mixtures and the crops interaction with the environment has the potential to inform rational choices of component varieties in mixtures.

Arabidopsis thaliana (Brassicaceae) is a small annual weed that has been successfully used as a model for understanding plant biology (Mitchell-Olds 2001; Meldau *et al.* 2012; Jorgensen 2012). *Arabidopsis*, like most weedy species, is an *r*-strategist producing thousands of small seeds with little investment of resources per seed (MacArthur and Wilson 1967). It occurs naturally in highly disturbed environments with little competition but it can readily be used in competition studies because genotypes can differ greatly in biomass, seed production, resource requirements and competitive ability (Cahill *et al.* 2005; Masclaux *et al.* 2010). Phenotypic variation for traits relating to competitive ability observed within a genotype can be largely attributed to environmental variation (Clauss and Aarssen 1994) and several studies have found significant interactions between genotypes and environments (Pigluici *et al.* 1995a,b). The small size of *Arabidopsis* plants and short generation times under glasshouse conditions provide a model system in which the high levels of replication required for

competition experiments across environments can be reliably achieved. These attributes may make *Arabidopsis* a powerful tool for controlled ecological studies on competition between plants.

Here we examine *Arabidopsis* as a model system to study the effects of genotypic diversity on yield under glasshouse conditions. The roles of compensation and complementation in stabilising productivity in genotypic mixtures of *Arabidopsis* were determined for plants subjected to the types of abiotic stresses that may challenge present and future agricultural systems. We tested the hypotheses that: i) genotypic mixtures have greater yield stability than monocultures, particularly when under environmental stress, ii) the yield of individual genotypes is more variable within mixtures than monocultures but compensation by stronger competitors within the mixtures begets an increase in yield stability for the mixture as a whole, and iii) competitive ability can be predicted from plant phenotype.

2.2. Materials and methods

2.2.1 Four-way mixture experiments

Four genotypes of *Arabidopsis* were selected for study (Ler-1, Col-0, Gy-0, Ga-0) based largely on phenotypic variation for rosette size and seed production. Genotypes were selected to vary in flowering time by a few days at most so they would compete for resources at a similar time (Table 2.1). Four-way mixture experiments were conducted to investigate the effects of all the genotypes competing with each other. The experiment was conducted in large plastic trays (680 x 440 x 50 mm) in which inter-plant distance was 30mm for horizontally and laterally nearest neighbours, and 40mm between diagonally opposite neighbours, which generated intense competition between plants. In the absence of competitors under optimal growing conditions, genotypes ranged in rosette diameter from 30 to 110mm. Plants were cultivated as both monocultures and 4-way mixtures in which competition between genotypes was intensified by maximizing distance between plants of the same genotype (Fig. 2.1). Seeds were sown in small pots of peat-based compost (Levington F2 soil, Nitrogen 150: Phosphorous 200: Potassium 200 mg L^{-1}, pH 5.3 – 5.7) and were incubated at 4ºC for 4 days to break dormancy before being moved to the glasshouse at 21–23ºC on a 16 h

light/8 h dark cycle supplemented with 120 µmol m^{-2} s^{-1} fluorescent lighting for germination. After 7 days in the glasshouse, seedlings were transplanted into the experimental layout. Plants were grown under high or low nutrient conditions from the seedling stage until senescence. The high nutrient treatment consisted of eight parts compost (Levington F2 soil) to one part grit. Low nutrient conditions were created by diluting the high nutrient soil mixture with medium grade (2-5 mm) vermiculite (1:2 v/v).

A	B	C	D	A	B	C	D	A	B	C	D
C	D	A	B	C	D	A	B	C	D	A	B
B	A	C	D	B	A	C	D	B	A	C	D
C	D	B	A	C	D	B	A	C	D	B	A
B	A	D	C	B	A	D	C	B	A	D	C
D	C	B	A	D	C	B	A	D	C	B	A
A	B	D	C	A	B	D	C	A	B	D	C
D	C	A	B	D	C	A	B	D	C	A	B
C	A	B	D	C	A	B	D	C	A	B	D
B	D	C	A	B	D	C	A	B	D	C	A
A	B	D	C	A	B	D	C	A	B	D	C
D	C	A	B	D	C	A	B	D	C	A	B
A	B	D	C	A	B	D	C	A	B	D	C
C	A	B	D	C	A	B	D	C	A	B	D
B	D	C	A	B	D	C	A	B	D	C	A

Fig. 2.1: Planting design for four-way mixtures of *Arabidopsis* genotypes. Each small block represents an individual plant. Each letter represents a different genotype. Focal plants were sampled from inside the dark-bordered square to avoid edge effects. N=5 plants per genotype.

Each experimental repeat consisted of two replicates of each of the four monocultures under both nutrient conditions, and six replicates of four-way mixtures per nutrient condition, resulting in a total of 28 trays per experimental repeat. For each monoculture tray, ten focal plants were randomly selected for phenotypic trait analysis whereas for each mixture tray, ten focal plants of each genotype were sampled.

Two independent experiments were performed during autumn (beginning in October 2010) and winter (beginning in January 2011). Both experiments (autumn and winter)

had additional lighting for the duration of the experiment. Temperatures were fairly constant (mean temperature 19/20°C, daily maximum 26/27°C, standard deviation of mean temperature 1.3/1.8°C) during these replications. Another experiment ran during summer (beginning in June 2010) using the same design as the other two but it was exposed to additional heat stress, not replicated in other seasons. Temperature and light levels were substantially higher than in the other two experiments (mean temperature 21°C, daily maximum 31°C, standard deviation of mean temperature 2.6°C). No additional lighting was provided during the summer experiment.

Several measurements were taken for each focal plant to assess plant fitness, including days to first flower (phase 6, Boyes *et al.* 2001), height of longest inflorescence at the onset of silique maturation and total seed mass. Plants were bagged with individual clear, micro-perforated bags when the first siliques began to ripen to ensure all seeds were collected. Relative yield (yield in mixture/ yield in monoculture) (de Wit 1960) was calculated for each genotype to assess mixture performance.

2.2.2 Pair-wise interaction experiments

To test the hypothesis that competitive ability can be predicted from above-ground phenotypic traits, pair-wise interaction experiments were conducted to investigate the effect of a single competing genotype on the fitness of the focal genotype. Plants were treated as focal or competing, but not both, because it was not possible to bag adjacent plants for seed collection. The four genotypes (Ler-1, Col-0, Gy-0, and Ga-0) and a different set of four genotypes (Wei-0, Van-0, Ms-0, Ema-1) were assigned to a competitive group based on phenotypic traits relating to their predicted competitive ability such as seed production, rosette size and flowering time when grown as a single plant (Table 2.1). Genotypes received a ranking for each trait. These rankings were weighted to calculate predicted competitive ability based on preliminary study data; seed mass was given a weighting of 4, rosette size a weight of 2 and flowering time a weight of 1. Group 1 had the lowest predicted competitive ability due to its low yield, small rosette and early flowering whereas group 4 was predicted to be the most competitive.

Set	Competitive Group	Genotype	Days to flower	Rosette diameter at 4 weeks (mm)	Seed mass (g)
1	1	Ler-1	25.0 ± 0.0	28.5 ± 3.1	0.019 ± 0.008
1	2	Col-0	26.6 ± 1.7	57.4 ± 5.6	0.124 ± 0.013
1	3	Gy-0	31.0 ± 2.6	98.8 ± 21.4	0.177 ± 0.074
1	4	Ga-0	28.6 ± 2.7	84.4 ± 12.9	0.345 ± 0.079
2	1	Van-0	29.0 ± 0.0	34.6 ± 4.2	0.090 ± 0.013
2	2	Wei-0	25.8 ± 1.1	60.0 ± 11.9	0.098 ± 0.041
2	3	Ms-0	25.7 ± 1.2	50.4 ± 14.9	0.127 ± 0.074
2	4	Ema-1	34.0 ± 4.0	111.3 ± 10.7	0.516 ± 0.093

Table 2.1. Mean trait values (± SD) for eight *Arabidopsis* genotypes grown under high nutrient conditions in the absence of competitors. N=5 plants per genotype.

Growing conditions were the same as in the high nutrient treatment of the four-way experiment except that plants were grown in small pots (70mm x 70mm x 70mm), each of which contained four plants. Plants were spaced 30mm apart to achieve a similar intensity of competition as in the tray experiments. Below the soil surface, pots were either undivided or divided into four equal sections using plastic strips thus providing conditions in which below-ground competition was either allowed or prevented. Plants were grown either in monoculture (four plants of one genotype in the same pot) or a two way mixture containing two plants of each genotype with the same genotype at diagonally opposite corners of the pot. Plants were cultivated simultaneously in the same glasshouse from June-August 2011. Temperatures were quite variable (mean 20°C, max 35°C, standard deviation of mean temperature 3°C). Measurements taken were the same for plants in the four-way mixture experiment with the addition of a rosette diameter measurement at four weeks old, which was not possible to do in the large, crowded tray experiments.

2.2.3 Root growth assays

Seedling root growth assays were conducted to test if early root growth rates differed between genotypes. 30 seedlings of all eight genotypes were grown on plates containing ½ strength Murashige and Skoog (½MS) medium media in environmentally controlled cabinets (Snijders Economic Delux Dimmable containing Sylvania Britegro F36WT8/2084 bulbs). Cabinets were set to 16 hour photoperiod, 23/16 °C day/night temperature. Plants were grown as single plants. Total root length measurements were taken at six and ten days growth using the image processing package Fiji (Schindelin *et al.* 2012).

2.2.4. Statistical analysis: Four-way mixture experiments

Seed mass and flowering time were analysed in linear mixed models to evaluate differences between monocultures and mixtures. Fixed factors included growing season, nutrient level (high/low), cultivation type (monoculture/mixture), genotype and their interactions. Seed mass was log-transformed to normalise the distribution of residuals and to make them approximately independent of fitted values. Non-significant interaction terms were removed from the model. Random effects were the block (tray)

in which the plants were grown and the individual plants. Initially the model was run for the combined autumn and winter dataset. The summer dataset (including the additional heat stress) was analysed in a separate model. Finally a model was run for all three datasets combined to assess the effect of the additional heat stress in the summer season on plant fitness in mixtures and monocultures. Details of the models are given in the Results section. All unplanned two-way comparisons were tested by protected least significant difference (LSD).

2.2.5 Statistical analysis: Pair-wise interaction experiments

Initially a linear mixed model was run to test the strength of competition between genotypes within pots; fixed factors included competition (presence/absence of competitors) and the competitive group of the focal plant from which phenotypic measurements were taken. To evaluate differences between monocultures and mixtures in seed mass, rosette size and flowering time, a separate linear mixed model was then run on data from pots in which competitors were present. This included the main effect of each variable and the interactions between them. Fixed factors included genotype, competition type (above ground only, or above and below ground competition), cultivation type (mixture/monoculture) and their interactions. Seed mass was log transformed, as above. A separate linear mixed model included the effect of competition type and competitive group (of the focal and the competing genotypes) on seed mass, rosette size and flowering time. Seed mass was square-root transformed to normalise the distribution of residuals and to make them approximately independent of fitted values. All non-significant interactions terms were removed from the model. Random effects in this model were the pot in which the plants were grown and the individual plants. All other factors were treated as fixed. All statistical analysis was conducted using Genstat v.12 (Payne 2009).

2.3 Results

2.3.1 Four-way mixture experiment

Genotypic mixtures produced similar yields to those obtained in monocultures across all three experiments (Fig. 2.2; Table A2.1a, $F_{1,39}$=5.52, P=0.02). Gy-0 achieved the highest yields in monoculture in two of the three experiments (Fig. 2.3). The genotype Ga-0 consistently produced more seed mass in mixtures (mean relative yield = 1.5), whilst Ler-1 consistently produced less seed mass in mixtures (mean relative yield=0.6; Fig. 2.4; Table A2.1a, $F_{3,39}$=25.29, P<0.001). Despite differences in the yield of individual genotypes, the overall yield stability of mixtures (calculated by standard deviation) was approximately the same as that of the most stable genotype in monoculture (Fig. 2.5a).

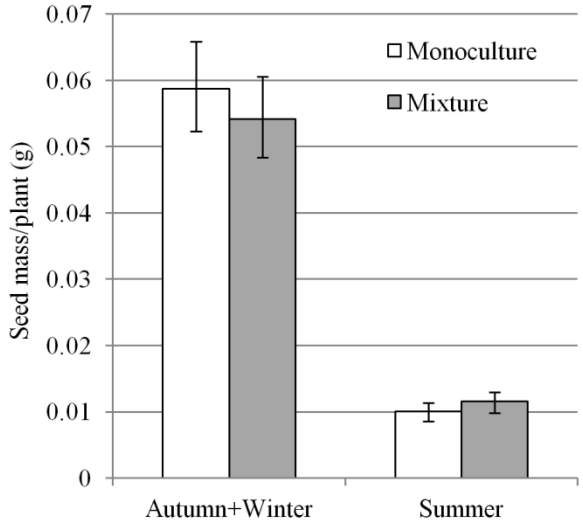

Fig. 2.2: Mean seed mass yields of *Arabidopsis* genotypes grown in mixture and in monoculture in the four-way mixture experiments during the autumn and winter seasons, and the summer season. N=1880. Error bars indicate 95% confidence interval of means.

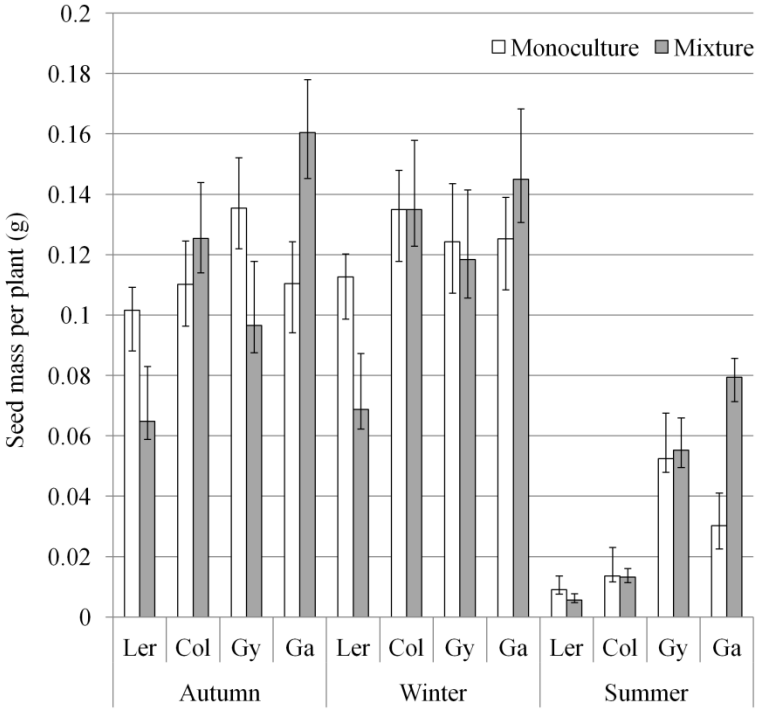

Fig. 2.3: Mean seed production per plant of each genotype in monoculture or mixture for each four-way mixture experiment. N=1880. Error bars show 95% confidence interval of means.

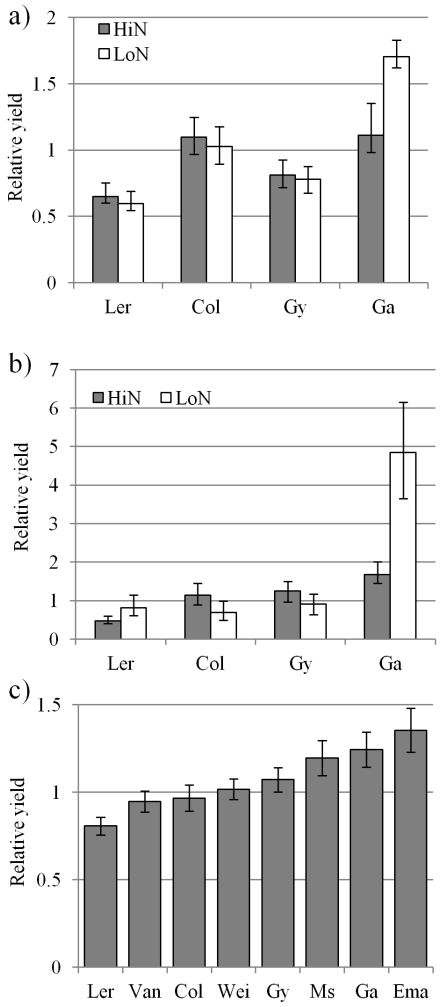

Fig. 2.4: Relative yield (mixture yield/monoculture yield) for each *Arabidopsis* genotype under high and low nutrient treatment in a four-way mixture experiment conducted during (a) the autumn and winter. N=1260, (b) the summer. N=620. (c) relative yields for eight genotypes in the pair-wise interaction experiment. Competitive groups of genotypes increase from left to right on the graph. N=639. Error bars show 95% confidence interval of means.

The highly significant interaction between growing season and genotype reflects differential responses of the four genotypes to different glasshouse environmental conditions across the three seasons, in particular the summer experiment in which the plants were subjected to additional heat stress (Fig. 2.3; Table A2.1a, $F_{6,39}$=56.17, P<0.001). To examine the effect of growing season, data from the summer experiment was separated from the autumn and winter experiments.

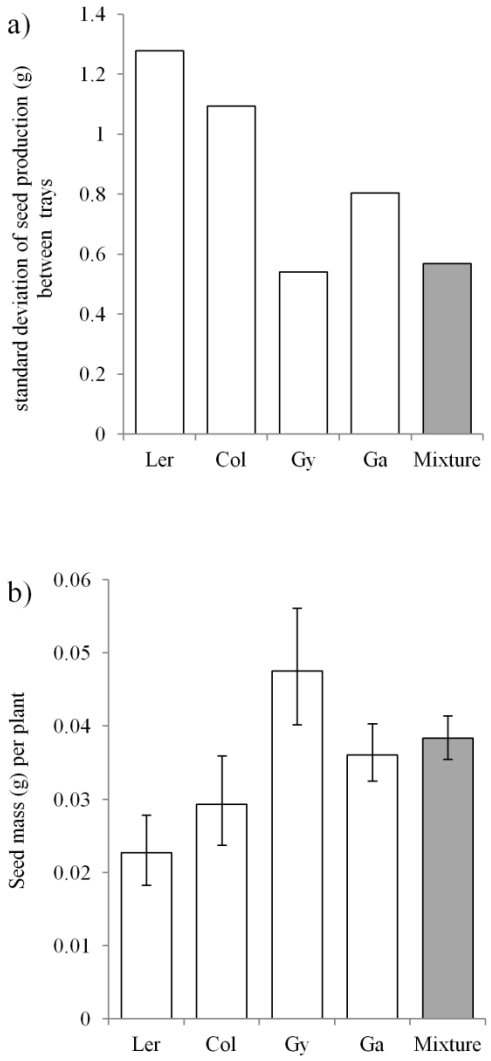

Fig. 2.5: (a) Standard deviation of the mean seed mass produced per tray (block) in a four-way mixture experiment. (b) Mean plant yield in genotypic monoculture and the four-way mixture averaged over entire experiment. N=1880. Error bars show 95% confidence interval of means.

Genotype had the largest effect on seed production in the autumn and winter experiments (Fig. 2.3; Table A2.1b, $F_{3,13}$=67.7, P<0.001) while the effect of growing season (autumn/winter) was comparatively small ($F_{1,13}$=2.12, P=0.02). The significant interaction between cultivation type (mixture/monoculture) and genotype in the autumn and winter experiments (Fig. 2.3; Table A2.1b, $F_{3,13}$=16.23, P<0.001) reflects differential responses of the four *Arabidopsis* genotypes to the two cultivation types in which they were grown. As expected, plants produced more seed under high nutrient conditions (Fig. 2.6; Table A2.1b, $F_{1,13}$=12.53, P<0.001).

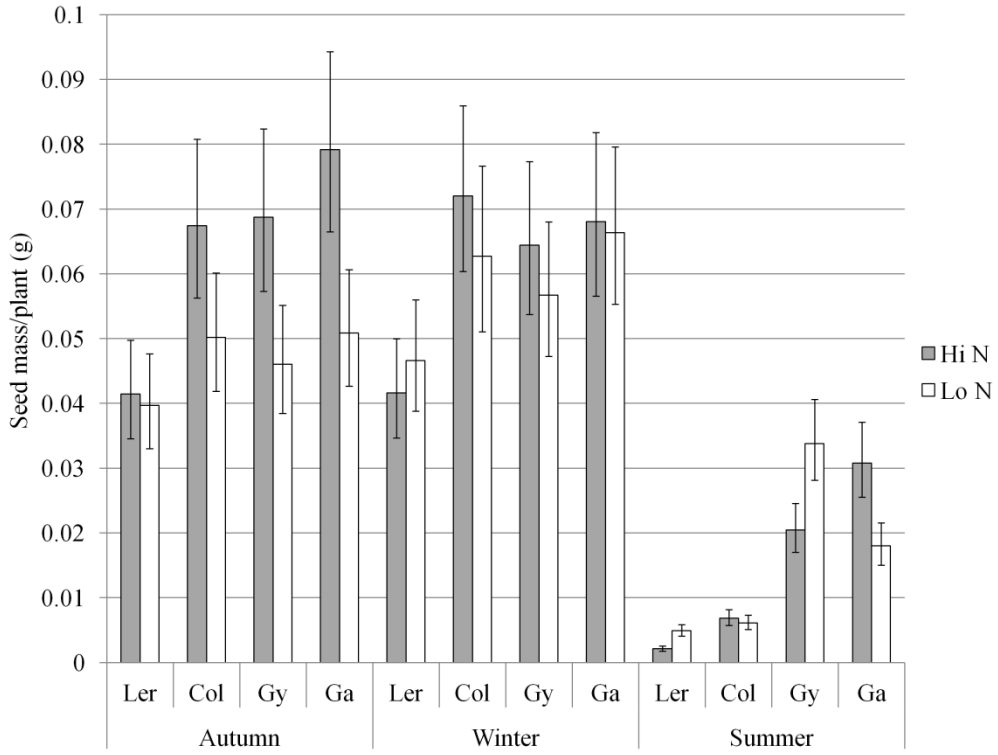

Fig. 2.6: Mean seed production per plant of each genotype under high or low nutrient treatment for each experiment. N=1880. Error bars show 95% confidence interval of means.

The additional heat stress substantially reduced growth of Col-0 and Ler-1 in the summer experiment. In the other two seasons, Ler-1 and Col-0 were 82% and 60% taller respectively (Fig. 2.7). Genotype had the largest effect on seed production in summer (Fig. 2.3; Table A2.1c, $F_{3,15}$=156.92, P<0.001). Despite the additional heat stress in summer, genotype performance was qualitatively similar across the entire experiment; in particular Ga-0 consistently overyielded in mixtures (Fig. 2.4, relative yield>1). However, there were substantial quantitative differences between the summer experiment and the other two experiments. In the summer, plants produced much less seed (Fig. 2.2, 72% overall reduction in seed mass). There was a larger effect of cultivation method on seed production in summer (Table A2.1c; $F_{4,15}$=39.17, P<0.001), largely because Ga-0 individuals receiving the low nutrient treatment produced significantly less seed in monoculture than they did in mixtures (Fig. 2.4; P<0.01 for difference from 1, LSD).

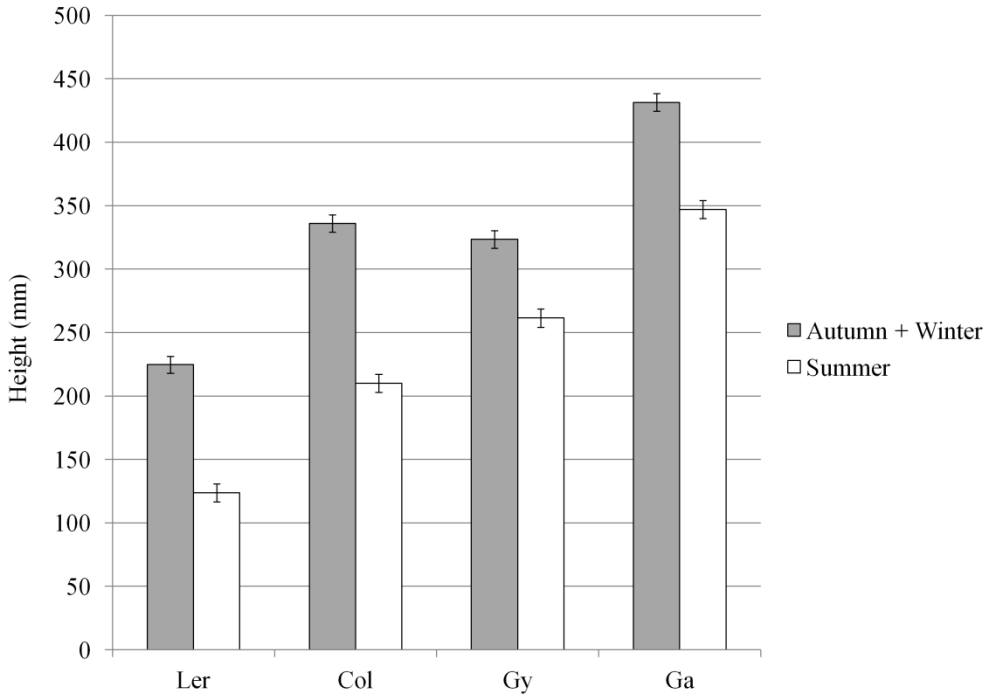

Fig. 2.7: Mean plant height for each genotype grown in each experiment. N=1880. Error bars show 95% confidence interval of means.

The number of days taken to flower differed between genotypes (Fig. 2.8; Table A2.2; $F_{3,47}$=876.13, P<0.001) and between seasons ($F_{2,47}$=284.45, *P*<0.001) with some interaction between the two factors ($F_{6,47}$=45.47, *P*<0.001). There was an overall reduction in days taken to flower in the summer season (Fig. 2.8). There was a small but significant interaction between genotype and cultivation method (Table A2.2, $F_{3,47}$=10.30 P<0.001), attributable to slightly delayed flowering of Ga-0 in mixtures (Fig. 2.8, *P*<0.01, LSD). Gy-0 showed a delay in flowering when under low nutrient conditions in the summer which led to an unexpected increase in seed mass (Fig. 2.6, Fig. 2.8).

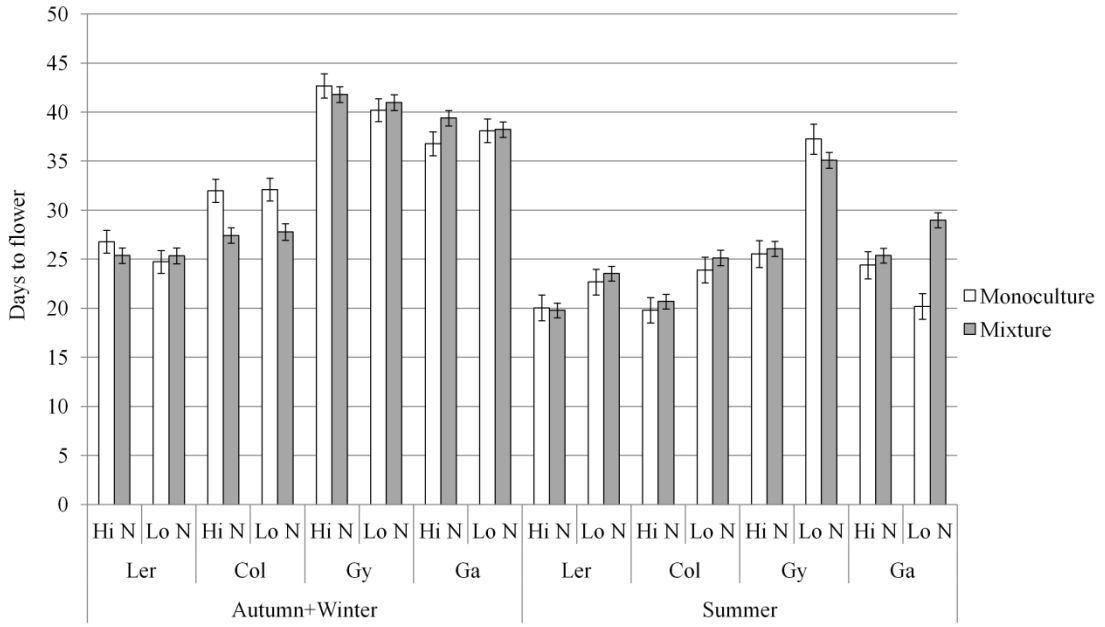

Fig. 2.8: Mean days taken to flower for four *Arabidopsis* genotypes grown in monoculture and mixture in a four-way mixture experiment. N=1880. Error bars indicate 95% confidence interval of means.

2.3.2 Pair-wise interaction experiment

Competition was studied in the absence and presence of below-ground competition to test whether above-ground traits or below-ground traits had the greatest effect on competitive ability. Genotype had the greatest effect on seed production (Table A2.3, $F_{7,24}$=137.76, P<0.001). There was a small overall effect of competition type (either above ground competition only or both above and below ground competition) on seed production, largely due to an interaction between competition type and genotype (Table A2.3, $F_{14,24}$=7.91, P<0.001). Mixtures achieved slightly greater yields than monocultures (Fig. 2.9; Table A2.3, $F_{1,24}$=9.87, P<0.001). Mixture performance of genotypes increased with competitive group (Fig. 2.4c).The factor affecting seed production most strongly was the phenotype of the focal plant, as large rosette size (x) was consistently associated with increased seed production (y=301.41x + 30.72, R^2 = 0.55). The competitive group of both the focal plant (Table A2.4, $F_{3,13}$=143.6, P<0.001) and competing plant (Table A2.4, $F_{3,13}$=6.16, P<0.001) significantly affected seed

production. More competitive groups showed a larger reduction in seed production in the presence of competition (Fig. 2.10; Table A2.5, $F_{3,7}$=16.1, *P*=0.001) indicating that these highly competitive genotypes have the greatest yield potential and the ability to utilize limited resources allows them to overyield in mixture, but they may not perform so well in monoculture. Yield of the focal plant decreased when the competitive ability of the neighbour increased but only when competition was unrestricted (Fig. 2.11; Table A2.4, $F_{3,13}$=3.77, *P*=0.01).

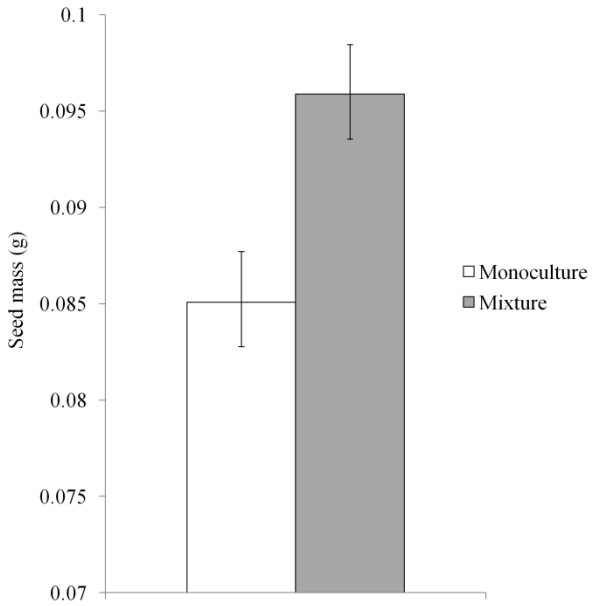

Fig. 2.9: Mean seed production in mixtures and monocultures in the pair-wise interaction experiments. N=639. Error bars indicate 95% confidence interval of means.

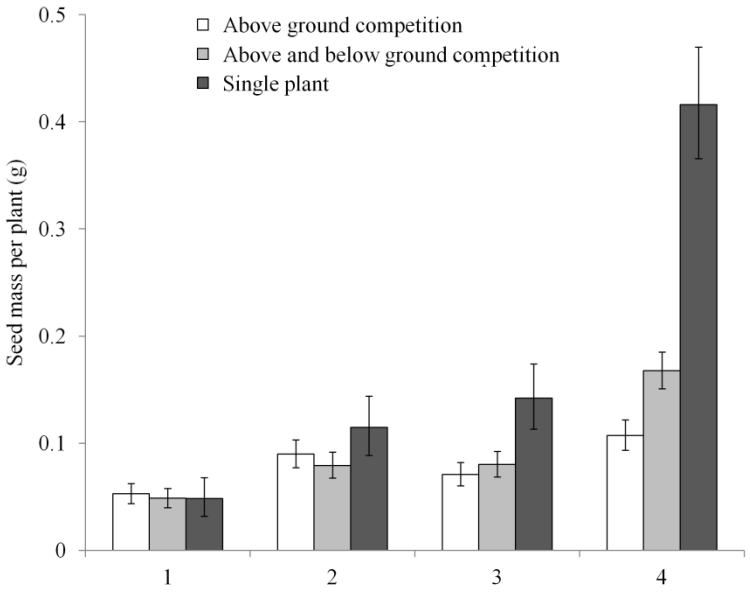

Fig. 2.10: Mean seed production of focal *Arabidopsis* plants from four competitive groups (1= least competitive, 4= most competitive) under three competition treatments (above ground competition only, above and below ground competition, single plant) in a pair-wise interaction experiment. N=639. Error bars indicate 95% confidence interval of means.

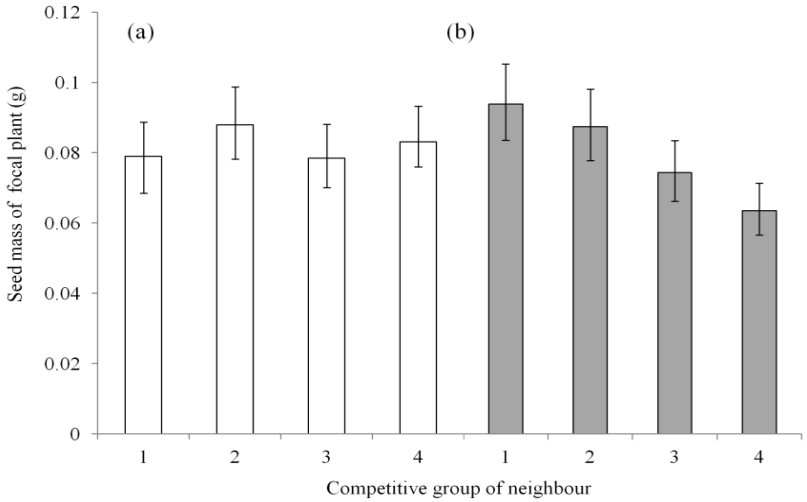

Fig. 2.11: Mean seed production of focal *Arabidopsis* plants grown with plants of four competitive groups (1= least competitive, 4= most competitive) in a pair-wise interaction experiment, (a) when competition was restricted to above ground only, (b) when competition occurred both above and below ground. For competitive groups see Table 2.1. N=639. Error bars indicate 95% confidence interval of means.

2.3.3 Root growth assays

Seedling root growth assays showed no significant effect of genotype on initial root growth rates (Fig. 2.12) although the conditions in which root growth was measured were inevitably not the same as those used in the glasshouse, where the plants were grown in soil.

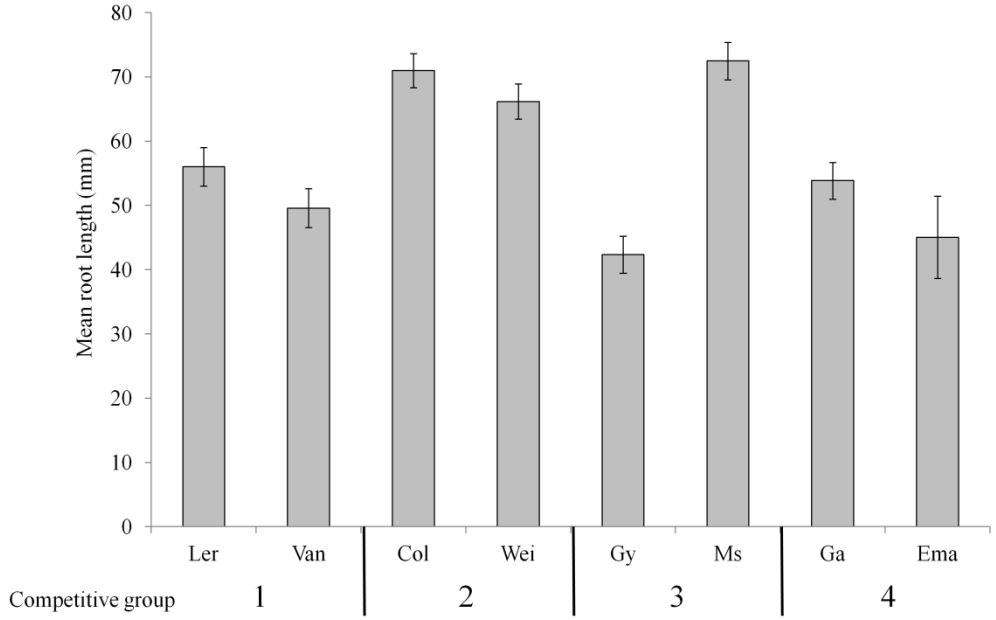

Fig. 2.12: Mean seedling root length after ten days growth on ½ MS plates. N=128. Error bars indicate 95% confidence interval of the means.

2.4 Discussion

We investigated the suitability of *Arabidopsis thaliana* as an ecological model for studying intra-specific competition between plants at different levels of genetic and phenotypic diversity. In this study, *Arabidopsis* genotypic diversity enhanced ecological resistance of the population to nutrient stress and the combination of nutrient and heat stress shown by an increase in yield and yield stability compared to the average monoculture. Mixtures produced yields that were as stable and almost as high as the best performing monoculture (Gy-0) over the entire experiment, supporting the hypothesis that biodiversity increases ecological stability (Yachi and Loreau 1999; Hooper *et al.* 2005; Tilman *et al.* 2006). Yield stability was achieved through compensation in which the fittest, most plastic genotype with high yield potential (e.g. Ga-0) overyielded in genetic mixtures, compensating for the lower yield of less fit genotypes (e.g. Ler-1). This effect was greatest in the summer experiment when plants were under the highest levels of abiotic stress. Compensation was seen throughout the study despite genotypic variation in responses to environmental conditions. There was no transgressive overyielding, which would have been an indication of complementary resource usage, and plants always performed better in the absence of others indicating that facilitation did not occur (Callaway 2007; Brooker 2008). As complementation was not detectable in this experiment, we conclude that compensation was responsible for the increased ecological resistance of *Arabidopsis* mixtures to nutrient stress and also the combination of nutrient and heat stress.

The role of root competition in plant genetic mixtures is intriguing and appears to have been important in this experiment. Although competition for space above-ground is obvious, the results of the pair-wise interaction experiments indicate that, in fact, competition between *Arabidopsis* plants depends more on below-ground interactions. The most competitive genotypes decreased the yield of focal plants only when below-ground competition was permitted indicating that below-ground competition may be more important than above-ground competition in *Arabidopsis* when securing resources for seed production. The growth habit of the *Arabidopsis* rosette prevented the partitioning of the aerial space in a similar way to that done for the soil space, a common method for separating above and below-ground competition (Semere and Froud-Williams 2001; Cahill, 2002). Restricting competition with partitions can also

create artificial effects including alteration of the root system architecture (McPhee and Aarssen 2001). No significant interaction was identified between competitive ability and seedling root growth indicating that some property of adult plant roots allows certain genotypes to outcompete others for below ground resources. Below-ground competition for nutrients, water and space often affects plant growth more than above-ground competition yet it remains overlooked in many studies of competition between plants (Casper and Jackson 1997). This study implies that it is crucial to understand below-ground interactions between adult plants in order to predict accurately the outcome of competition between cultivars and design sustainable cropping systems.

Nevertheless, the competitive ability of genotypes was predictable from above-ground phenotype. The most competitive genotypes had larger rosettes, took longer to flower, were more plastic in their flowering time, and produced more seed, confirming predictions from the four-way mixture experiment. These results suggest that competitive ability can be predicted in crops prior to competition experiments. Such data can be used to estimate the mixing ability of genotypes and increase the efficiency of mixture selection (Knott and Mundt 1990). Certain genotypes may contribute more yield in mixtures than in monoculture; for example, in the four-way mixture experiment, Gy-0 monocultures produced more seed than Ga-0 monocultures yet Ga-0 was the highest yielding genotype in mixtures. This implies that high levels of intra-genotypic competition decreased the yield of individual Ga-0 plants in monoculture, indicating that while Ga-0 is a strong competitor with other genotypes, it is not well-adapted to intra-genotypic competition. This effect was amplified under low nutrient conditions where Ga-0 showed a significant reduction in yield when grown in monoculture compared to the mixture. The phenotypic plasticity of Ga-0 (e.g. a delay in flowering time in mixtures) allowed the genotype to respond to different growing conditions in a way that the more static Gy-0 did not. Under less predictable environmental conditions (seen in the summer experiment) phenotypic plasticity and high yield potential enables genotypes such as Ga-0 to compensate for less fit genotypes, thereby increasing yield stability through enhanced resistance.

The pair-wise interaction experiments suggested little advantage of being in the lowest competitive groups (group 1 and 2), but their reduced time taken to flower may be advantageous in very unpredictable environments in which setting seed quickly

provides escape from potentially fatal environmental conditions. We speculate that if an additional drought stress was included in the summer experiment then Ga-0 and Gy-0 individuals would have died before setting seed. These experiments showed the seed production of group 4 genotypes to be most restricted by the presence of competition, a trait that increases the potential for compensation in mixtures via competitive release. This finding highlights the importance of mixture selection because successful mixtures must contain components that are not only good performers but also good neighbours (Mundt *et al.* 1995).

To date, the majority of studies of genetic mixtures in agriculture have been conducted under field conditions and have focussed on the ability of mixtures to control disease (Finckh *et al.* 2000; Zhu *et al.* 2000; Mundt 2002). Varietal mixture studies often report general trends in yield and disease severity for the population (Mundt 2002; Philips *et al.* 2005; Newton and Guy 2009) but few studies have focussed on the plant-plant interactions taking place within mixtures (Allard and Adams 1969; Finckh and Mundt 1992). Empirical studies that attempt to separate the effects of abiotic and biotic stress on mixtures are impractical because of the unique environmental conditions of the field (Finckh *et al.* 2000). *Arabidopsis* provides a model system in which individual stresses can be applied separately and in combination, and in which genotype by environment interactions can be closely studied under environmentally controlled conditions in an efficient and repeatable way. In this study, *Arabidopsis* provided insight into the mechanisms of plant competition within genetic mixtures and demonstrated its potential in ecological research.

Agricultural weed ecology may benefit from the use of *Arabidopsis* as a model. Studies investigating competitive ability of varieties above and below ground will become increasingly important as the use of herbicides becomes restricted by legislation or by insensitivity of target weeds. At present, there is no intentional selection for increased competitive ability, because competition from weeds is minimised by the use of herbicides. Increased competitive ability in crops may be associated with lower yield (Lemerle *et al.* 2006) but greater weed suppression (Jordan 1993; Song 2010). Different varieties and mixtures may be selected depending on the cropping system (conventional, low-input or organic) and there may be a trade-off between reducing competition between crop plants and increasing competition against weeds.

The *Arabidopsis* model system has the potential to be used to study pest and pathogen dynamics in crops. The use of genetic diversity to control pests and pathogens will become increasingly important as the chemicals used to control them become more heavily regulated. Cropping systems will need to be less reliant on chemical input, less expensive to manage and show greater adaptability to the changing environment if future food security is to be achieved (FAO, WFP, IFAD 2012; Hillocks 2012). Genetically diverse crops, able to adapt to a wider range of environments, will contribute to stable, high productivity by buffering against diverse and sometimes unpredictable stresses.

Chapter 3:
Yield of plant genotype mixtures under disease pressure

3.1 Introduction

The relationship between plant diversity, ecological stability and ecosystem productivity is of great importance to natural systems. Plant pathogens alter such relationships by affecting plant fitness, reducing the growth and competitive ability of diseased plants which can impact heavily upon plant population and community structure (Burdon *et al.* 2006; Bradley *et al.* 2008; Maron *et al.* 2011; Latz *et al.* 2012). Pathogens can promote host plant biodiversity by preventing competitive exclusion if they have a greater negative impact upon the dominant species in a community, such that a trade-off exists between plant competitive ability and susceptibility to pathogens (Alexander and Holt 1998; Bradley *et al.* 2008; Allan *et al.* 2010). However, if pathogens have a greater detrimental impact upon uncommon and less competitive species then biodiversity will be reduced (Peters and Shaw 1996). Studies investigating the effect of biodiversity on the system's ability to buffer against disease have been largely observational; for example, increased species richness has been shown to reduce disease caused by foliar and soil borne pathogens and increase productivity in grassland communities (Allan *et al.* 2010; Maron *et al.* 2011). Despite the pertinence of understanding the impact of pathogens on plant diversity at all complexity levels, studies investigating the ability of pathogens to promote plant genotypic diversity in natural systems are rare, and experimental tests of the mechanisms of pathogen-induced changes to diversity are even rarer. Theory predicts that increased stability can be achieved through resistance to change or recovery after perturbation (Pimm 1984). Knowledge of plant-plant interactions contributing to such mechanisms can facilitate appropriate exploitation of plant genotypic diversity, stabilising productivity in natural and agricultural systems. Manipulation experiments, in which diversity and environmental stresses are regulated, can improve understanding of the mechanisms contributing to increased productivity and ecological stability in diseased populations.

Host fitness and competitive ability can be reduced by susceptibility to the pest or pathogen, or through costs of mounting a defence response (Brown 2002; Damgaard and Jensen 2002; Tian *et al.* 2003; Bedhomme *et al.* 2005). When costs of resistance is

associated with reduced plant growth or reproduction these may be traded-off against competitive ability since the latter is expected to be associated with an increased allocation to growth (Chase *et al.* 2002; Viola *et al.* 2010). Empirical support for a trade-off between competitive ability and defence is inconclusive. In *Arabidopsis*, greater resource allocation to reproductive growth can increase tolerance to the direct negative effect of the viral pathogen, *Cucumber mosaic virus*, on plant fitness (Pagan *et al.* 2008, 2009). Investment in vegetative growth can increase tolerance to the indirect costs of infection, reducing competitive ability through reduced plant fitness (Pagan *et al.* 2009). However, defence costs are reported to decrease or even disappear in the presence of plant competition as was observed in *Arabis perennans* infected by *Plutella xylostella,* which indicates that some defence mechanisms may be advantageous in competitive environments (Siemens *et al.* 2003). These contradictory findings raise questions regarding the overall importance of defence costs in plant ecology and evolution. Studies testing the effect of various biotic stresses on plants grown under different competition scenarios could provide insight into the relative costs and benefits of plant defence.

Plant pathogens are a major threat to food security reducing global crop yields by 20-40% (FAO 2013), such that agricultural systems must combat disease pressures. Modern arable farmers routinely grow a single high yielding variety throughout an entire field to maximise yield potential (Trewavas 2001). Such monocultures exert a strong selection pressure on pathogen races to overcome resistance, increasing reliance on fungicides that are becoming heavily restricted (Bai and Shaner 1994; Brown and Hovmoller 2002; Hillocks 2012). Varietal mixtures offer a potential solution to these problems through the use of several partially- and fully-resistant genotypes sown together at the same time to control disease (Wolfe 1985; Lannou and Mundt 1996; Zhu *et al.* 2000). Several studies have investigated the competitive interactions between plants in mixtures contributing to yield stability under disease pressure e.g. Burdon *et al.* 1984; Finckh and Mundt 1992; Cowger and Mundt 2002. However, results are often highly variable due to large genotype by environment interactions and it is therefore difficult to draw firm conclusions from these experiments. A model system under controlled environmental conditions would provide the level of experimental control required to study the ecological mechanisms behind resistance of varietal mixtures to pathogens. *Arabidopsis thaliana* (Brassicaceae) has traits that make it particularly

suitable to such studies, including small size and short generation time, and therefore provides an excellent model system to study competition under glasshouse conditions in which environmental conditions are readily controlled.

This study examined the effect of plant genotypic diversity in stabilising plant productivity in populations under pathogen attack. We tested the hypothesis that compensation by better-adapted plants increases yield stability in phenotypically diverse mixtures, and that this effect is greatest when susceptible and resistant genotypes are combined in the presence of the pathogen. We predicted that biologically dissimilar pathogens have different effects on the relationship between plant diversity, stability and productivity because of differences in disease transmission, progression and impact on plant fitness.

3.2. Materials and methods

3.2.1 The model system

Arabidopsis thaliana is an excellent model for studying the effect of competition on productivity because seed mass correlates positively with vegetative biomass and overall fitness (Aarssen and Clauss 1992; Clauss and Aarssen 1994). Its small size, rapid life cycle and limited requirement for space are convenient for competition studies that require high levels of replication. To study the ability of *Arabidopsis* genotype mixtures to buffer against disease and stabilize yield in different environments two pathogens were selected that differ greatly in their taxonomy, transmission method and impact on plant fitness.

Hyaloperonospora arabidopsidis (*Hpa*) is an obligate oomycete pathogen causing downy mildew in natural populations of *Arabidopsis* (Holub *et al.* 1994; Koch and Slusarenko 1990). *Arabidopsis* genotypes show high levels of variation in their interactions with different *Hpa* isolates both in terms of resistance (Nemri *et al.* 2010) and tolerance (Salvaudon *et al.* 2008). There is also good indication that plant competitive ability can be altered by the presence of the pathogen (Damgaard and Jensen 2002). This pathosystem therefore offers a tool to investigate mechanisms leading to increased yield and yield stability in plant genotypic mixtures.

The Polerovirus *Turnip yellows virus* (TuYV) is a major viral disease of oilseed rape (*Brassica napus*) with potential to decrease yield by 26% in the UK (Jay *et al.* 1999). Typical symptoms include reddening and purpling of leaf margins and inter-veinal yellowing and reddening (Stevens *et al.* 2008). TuYV is insect-borne with the main vector being the peach-potato aphid (*Myzus persicae*). There are currently no known *Arabidopsis* genotypes that are fully resistant to TuYV, though variation in tolerance to the virus is known (Stevens *et al*. 2005). This virus was used as it represents a group of agriculturally important pathogens that have natural representatives infecting *Arabidopsis*. Viruses are biologically very different from oomycete pathogens, using host resources in different ways. Experiments with TuYV can indicate how universal the consequences of pathogen infection for host plant fitness and competitive ability are.

3.2.2 Experimental design

Four *Arabidopsis* genotypes were selected for the *Hpa* experiment and two for the TuYV experiment (see below). Plants were sown in pots (70mm x 70mm x 70mm) with four plants per pot 30mm apart, generating intense competition (Creissen *et al.* 2013). Under optimal growing conditions, rosette diameter at five weeks varied between genotypes by 50 to 110mm. Phenotypic measurements were taken for one focal plant in each pot, with other plants acting as competing neighbours only. Plants were cultivated as monocultures and as mixtures of two or four genotypes to assess the effect of competition between different genotypes. This design also enabled examination of the effect of diversity on competitive ability of focal plants, measured by relative yield (RY = yield in mixture/yield in monoculture, de Wit 1960).

3.2.3 Plant growth conditions

Seeds were sown into media consisting of eight parts peat-based compost (Levington F2 soil, Nitrogen 150: Phosphorous 200: Potassium 200 mg L^{-1}, pH 5.3 – 5.7) to one part grit and incubated at 4°C for four days in a controlled environment room to break dormancy. Once dormancy had been broken, seedlings were moved to a glasshouse for germination at 18°C during the day/12°C at night on an 8h dark/16h light cycle supplemented with high pressure sodium lighting (240 μmol m^{-2} s^{-1}). After ten days in

the glasshouse seedlings were transplanted into the experimental design. When plants began to flower (phase 6, Boyes *et al.* 2001) glasshouse temperatures were increased to 23°C during the day/16°C at night to hasten seed production.

3.2.4 Experiments with Hyaloperonospora arabidopsidis

Four genotypes of *Arabidopsis* (Van-0, Ga-0, NFA-10, NFA-8) were selected from an initial screen of 15 genotypes, based on phenotypic variation for traits related to plant fitness (including rosette size and seed production) and compatibility with *Hpa* in the absence of competition (Table 3.1). Variation for flowering time was restricted to a window of one week so the peak requirement for resources would occur at a similar time. *Hpa* isolate Emoy2 was maintained on a susceptible host genotype, NFA-8, and inoculated by spraying a suspension of 5 x 10^4 conidiospores mL^{-1} in distilled water onto 18 day old plants as previously described (Reignault *et al.* 1996). After inoculation, plants were covered with a transparent lid to maintain high humidity (90-100%). Control plants were sprayed with water and subjected to the same growing conditions as inoculated plants. Control and infected plants were grown in adjacent rooms of the same glasshouse to prevent *Hpa* infection of control plants while making growth conditions as similar as possible. Marginal differences in temperature and humidity were observed between rooms in the same experimental repeat (data not shown) therefore rooms were swapped between replicates as part of the split-plot crossover design. This experimental design was chosen over the alternative of conducting the experiment in the same room and spraying control plants with fungicide because of the range of effects such chemicals can have on plant physiology. Each experiment included 22 treatments, namely the four genotypic monocultures, all six possible two-genotype combinations (2-way mixtures) and a mixture of all four genotypes (4-way mixture), all in the presence and absence of the pathogen. There were 20 replicates of each of the 11 monocultures and mixtures within each pathogen treatment in each experiment, and the 220 pots were completely randomised. Two independent experiments were carried out beginning in October 2011 and March 2012. During the second experiment, internal glasshouse temperatures were more variable (standard deviation of mean temperature in 2011 was 5.4°C compared to 6.2°C in 2012) and the maximum temperature was higher (2011: 32°C, 2012: 38°C) as was humidity

(2011: 56%, 2012: 63%). This was the result of a few days of very high external temperatures during March and April 2012.

Measurements were taken for each focal plant to assess plant fitness. These included days to first flower (phase 6, Boyes *et al.* 2001), rosette diameter after five weeks growth and total seed mass. Disease severity was assessed twice. At six days post inoculation (dpi) the number of leaves with conidiospores present was scored and the proportion of leaves infected recorded. At ten dpi plants were scored using the following 0-4 scale to describe disease development:

0 = plants showing no signs of sporulation.
1 = a few sporulating conidiospores detectable on leaves using a hand lens (4x magnification).
2 = plants with approximately 25% leaf area covered in conidiospores.
3 = plants with 50% leaf area covered by conidiospores.
4 = plants with 75-100% leaf area covered in conidiospores.

Plants were bagged with individual clear, micro-perforated bags when the first siliques began to ripen to ensure all seeds were collected. RY were calculated for each genotype to assess mixture performance and competitive ability in the presence and absence of the pathogen.

Genotype	**Disease score (0-4)**	**Days taken to flower**		**Rosette diameter at 5 weeks (mm)**		**Seed mass (g)**	
Hpa	present	absent	present	absent	present	absent	present
Van-0	0±0.2	49±9	52±9	72±19	68±14	0.80±0.29	0.78±0.2
Ga-0	0.2±0.5	53±6	57±9	69±17	66±13	0.87±0.31	0.88±0.2
NFA-10	1.8±0.6	57±9	56±7	71±20	61±11	0.93±0.32	1.10±0.3
NFA-8	2.7±0.7	55±9	55±7	88±22	63±7	0.90±0.22	0.78±0.2

Table 3.1: Mean trait values (± SD) for four *Arabidopsis* genotypes grown in the absence of competition and the presence/absence of *Hpa*. N=40 plants/genotype.

3.2.5 Experiments with Turnip yellows virus

Two *Arabidopsis* genotypes (Col-0, Ler-1) were selected from a preliminary screen of 12 genotypes using the same criteria for phenotypic variation as in the experiments with *Hpa* (data not shown). As there are no known *Arabidopsis* genotypes that are immune to TuYV (Stevens *et al.* 2005), so genotypes were selected for variation in tolerance to the pathogen. Tolerance occurs when the host plant compensates for damage caused by the pathogen (Brown and Handley 2005), and was characterized in this study by smaller reductions in overall plant fitness (seed production, rosette size) in the presence of high levels of viral antigen within infected leaf tissue, assessed by enzyme-linked immunosorbent assay (ELISA) (Clark and Adams 1977) four weeks after inoculation. Col-0 suffered greater yield loss than Ler-1 despite similar levels of viral titre within leaf tissue four weeks after infection with TuYV in a preliminary experiment (data not shown). Ler-1 is therefore judged to be more tolerant to the virus than Col-0. No significant effect of non-viruliferous aphids on plant fitness was found (data not shown).

After 14 days in the glasshouse, plants were inoculated with TuYV isolate BrYV-GB by placing three viruliferous *M. persicae* (RRes genotype 0; Bos *et al.* 2010) aphids onto each individual plant using a paint brush. All trays of inoculated plants were covered with clear plastic lids to prevent the spread of aphids onto uninoculated plants. The experiment, beginning in October 2012, included four treatments, namely the two genotypic monocultures, and a mixture of both genotypes, both in the presence and absence of the pathogen. There were 25 replicates of each of the monocultures and the 50 of the mixture within each pathogen treatment in the experiment, and the 200 pots were completely randomised. The planting design was the same as in the *Hpa* experiment except for the absence of a 4-way mixture. All pots received a compost drench treatment with the insecticide Intercept™ 70 WG (Scotts UK, active ingredient imidacloprid, 0.2g/L water) one week after inoculation to kill aphids and thus prevent further virus transmission. After seven days, once all aphids were dead, the plastic lids were removed. Virus-inoculated and control plants were grown in the same glasshouse compartment at 20°C during the day/18°C at night on a 16h light / 8h dark cycle, as above. Forty plants of each genotype per treatment (aphids/no aphids) grown amongst the focal plants in separate pots were tested by ELISA to confirm the presence of the

TuYV in the inoculated plants and the absence of the virus in the controls without damaging focal plants. Days to first flower, rosette diameter and total seed mass were measured as in the *Hpa* experiment.

3.2.6. Statistical analysis

Linear mixed modelling was used to evaluate differences in seed mass, rosette size, flowering time and disease scores between monocultures and mixtures of *Arabidopsis* genotypes, for each disease. The model included the main effect of each factor and all interactions between them. Fixed factors included genotype, presence/absence of the pathogen and cultivation (monoculture, 2-way or 4-way mixture). A separate linear mixed model analysed the effect of genotype and cultivation on each of the following variables: seed mass, rosette size, flowering time and disease score. All non-significant (P>0.05, F-test) interactions between the main terms were removed from the analysis. The random effect for each model was the pot in which the plants were grown. All statistical analysis was conducted using Genstat v.14 (VSN International 2011).

3.3 Results

3.3.1 Hyaloperonospora arabidopsidis

The relative yields of genotypes in 2-way mixtures were altered by the presence of the pathogen, as shown by a significant interaction between genotype, cultivation (2-way mixture or monoculture) and the presence or absence of *Hpa* (Fig. 3.1; Table A3.1, $F_{4,23}$=3.54, P=0.007). The outcome of specific competitive interactions in the 2-way mixtures was heavily dependent on the pathogen. In 2-way mixtures, *Hpa* reduced seed production in the most susceptible genotypes, NFA-8 and NFA-10 (Fig. 3.2a), which was associated with a reduction in rosette diameter (Fig. 3.2b) and a significant reduction in competitive ability, assessed by relative yield, for the most susceptible genotype NFA-8 (Fig. 3.1). By reducing fitness of susceptible genotypes, *Hpa* indirectly increased the competitive ability in mixtures of the more resistant genotypes, Ga-0 and Van-0, which had higher relative yield in 2-way mixtures when they were attacked by the pathogen (Fig. 3.1).

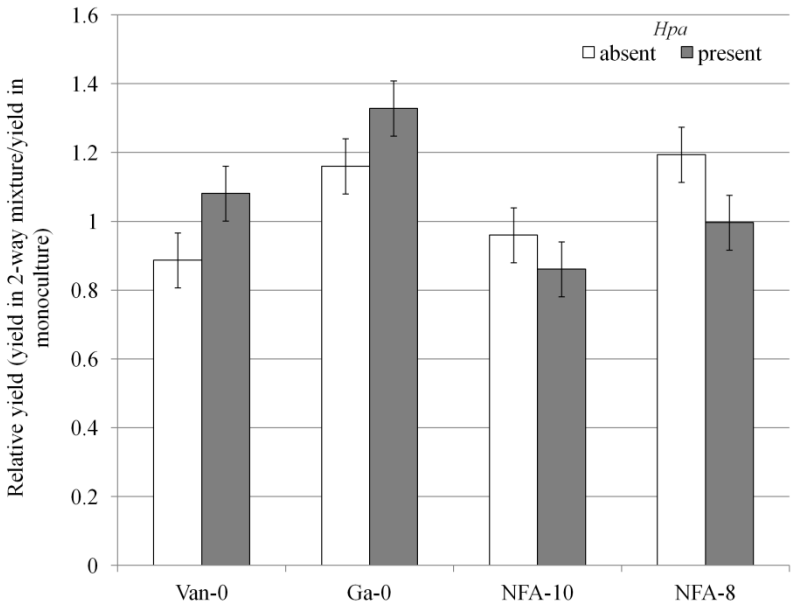

Fig. 3.1: Relative seed mass yields (yield in 2-way mixture/yield in monoculture) of four *Arabidopsis* genotypes grown in the presence and absence of *Hpa* in both experiments. N=1600. Error bars show 95% confidence interval of means.

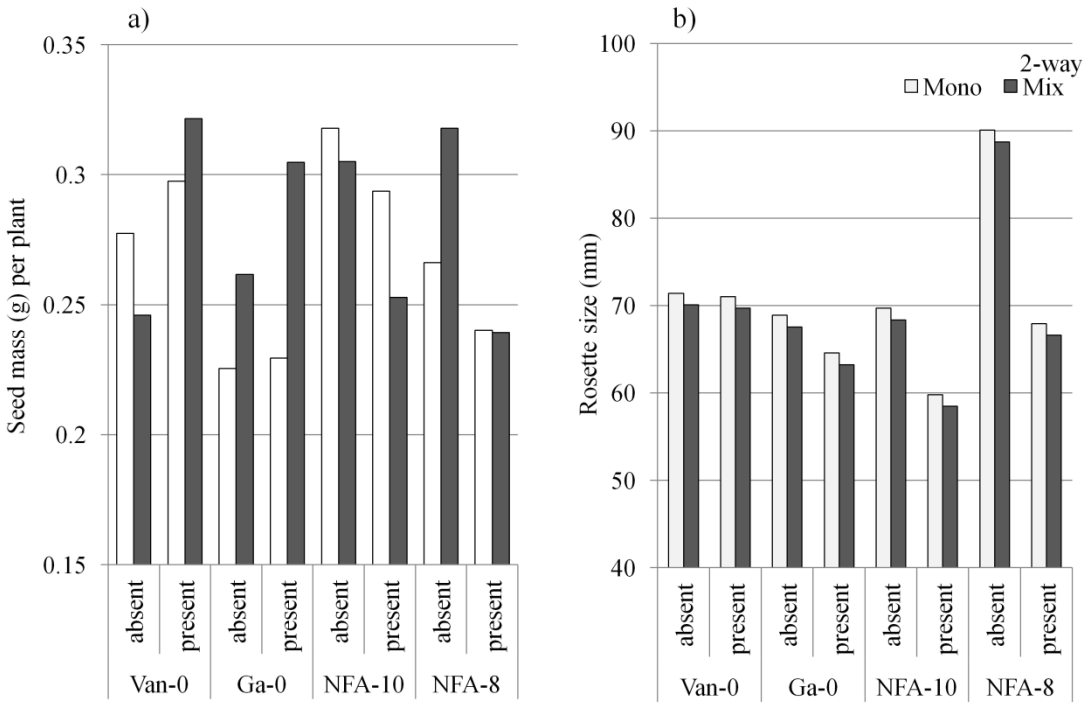

Fig. 3.2: Phenotypic fitness measurements taken for four *Arabidopsis* genotypes grown in monoculture and 2-way genotype mixtures and in the presence and absence of *Hpa*. a) Mean seed mass produced per plant. 95% confidence interval of mean = 0.015 (mono), 0.007 (mix). b) Mean rosette diameter. 95% confidence interval of mean = 1.1 (mono), 1.0 (mix). N=1600.

Certain combinations of two genotypes produced significantly more seed than monocultures of either of the component genotypes in both the presence and absence of *Hpa* (Fig. 3.3; Table A3.1, $F_{4,23}$=3.54, *P*=0.007). Genotypes that performed better in 2-way mixtures than monoculture were identified by high relative yield (RY>1, Fig. 3.1), and classed as highly competitive. The partially resistant Ga-0 and the highly susceptible NFA-8 produced the least seed in monoculture and when combined together in 2-way mixture (Fig. 3.3). Treatments containing only these highly competitive genotypes were the lowest yielding overall, whereas pots containing less competitive genotypes, the moderately susceptible NFA-10 and the fully resistant Van-0, were the highest yielding overall, both in monoculture and in the respective 2-way mixture (Fig. 3.3).

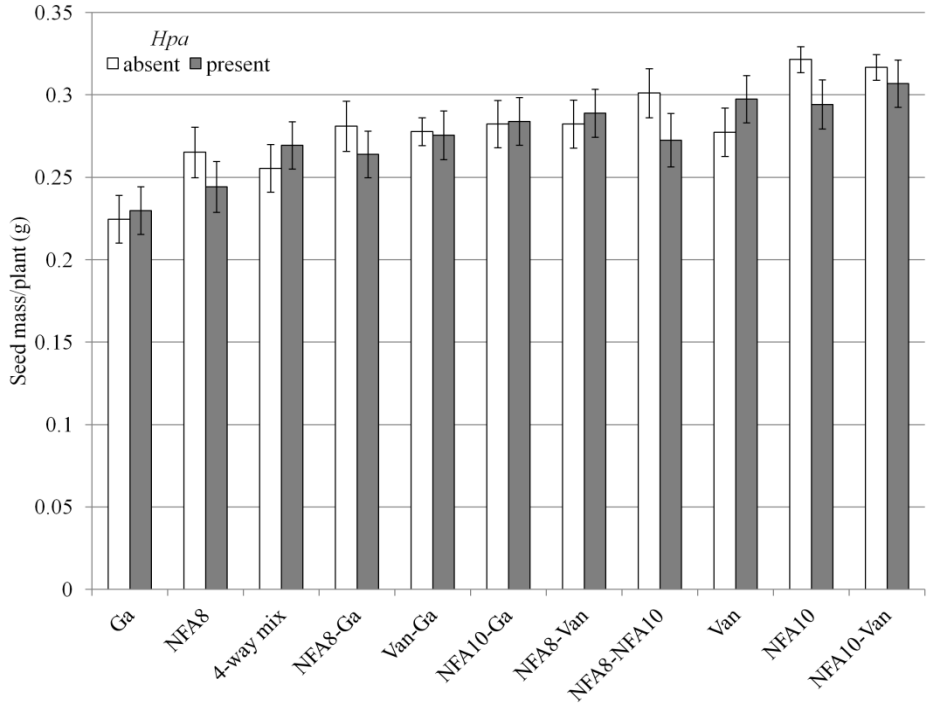

Fig. 3.3: Mean seed production for an individual plant grown in competition with three other plants. The competing plants may be of the same genotype (single genotype name on chart). Two genotype names indicate the presence of two genotypes. '4-way mix' have all four genotypes competing in the same pot. Genotype combination treatments are ordered by increasing mean seed production per plant summed across both pathogen treatments and displayed from left to right on the x-axis. N=1600. Error bars show 95% confidence interval of means.

Rosette size, flowering time, disease score, seed production and consequently competitive ability varied between experiments (Fig. 3.4, Fig. 3.5). The first experiment was conducted in a glasshouse over winter and the second experiment over spring when the external light levels were higher for longer. This change in light intensity and day length had an impact on disease progression and plant development (Fig. 3.4, Fig. 3.5a, b). Disease scores six days after inoculation (dai) were significantly higher for NFA-8 and NFA-10 in the second experiment than in the first, although they remained the most susceptible genotypes (Fig. 3.4a; Table A3.2a, $F_{3,8}$=35.43, P<0.001). By contrast, no overall significant difference between experimental repeats was seen for disease scores at ten dai indicating that by this stage the pathogen had achieved maximum disease levels (Fig. 3.4b; Table A3.2b, $F_{1,8}$=0.85, P=0.4). However Ga-0 was more resistant in the second experiment at both six and ten dai (Fig. 3.4). In the second experiment rosette diameter was significantly greater after five weeks growth (Fig. 3.5a; Table A3.3, $F_{1,16}$=2145.41, P<0.001), and the number of days to flower significantly fewer (Fig. 3.5b; Table A3.4, $F_{1,30}$=3265.15, P<0.001), which ultimately led to increased seed production (Fig. 3.5c; Table A3.1, $F_{1,23}$=209.68, P<0.001). Despite varying genotypic responses to the environmental conditions experienced in each replicate, the overall effects on plant competitive ability (relative yield) and the outcomes of competition under each treatment were consistent between replicates (Fig. 3.5d; Table A3.1, $F_{4,23}$=3.54, P=0.007).

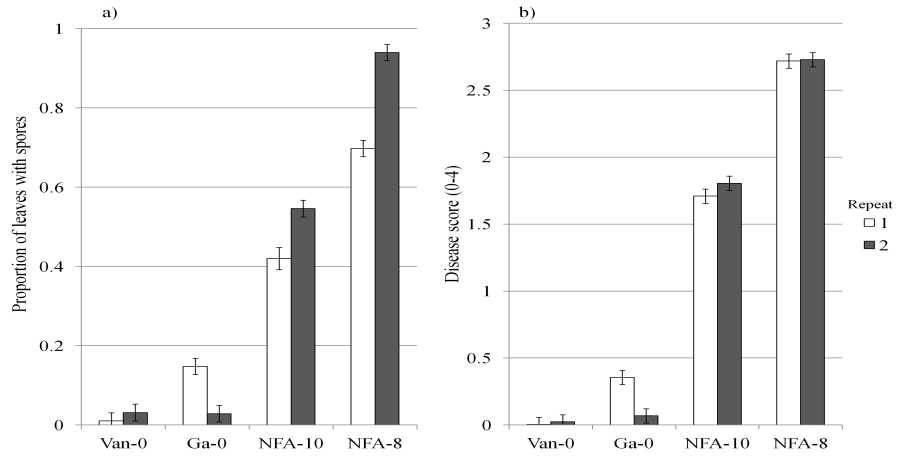

Fig. 3.4: Mean disease scores for individual plants infected with *Hpa* in experimental repeat 1 and 2. a) Proportion of leaves showing signs of sporulation six days post-inoculation. b) Disease score (0=no disease, 4=over 75% leaf area covered in spores) ten days post infection. N=1600. Error bars show 95% confidence interval of means.

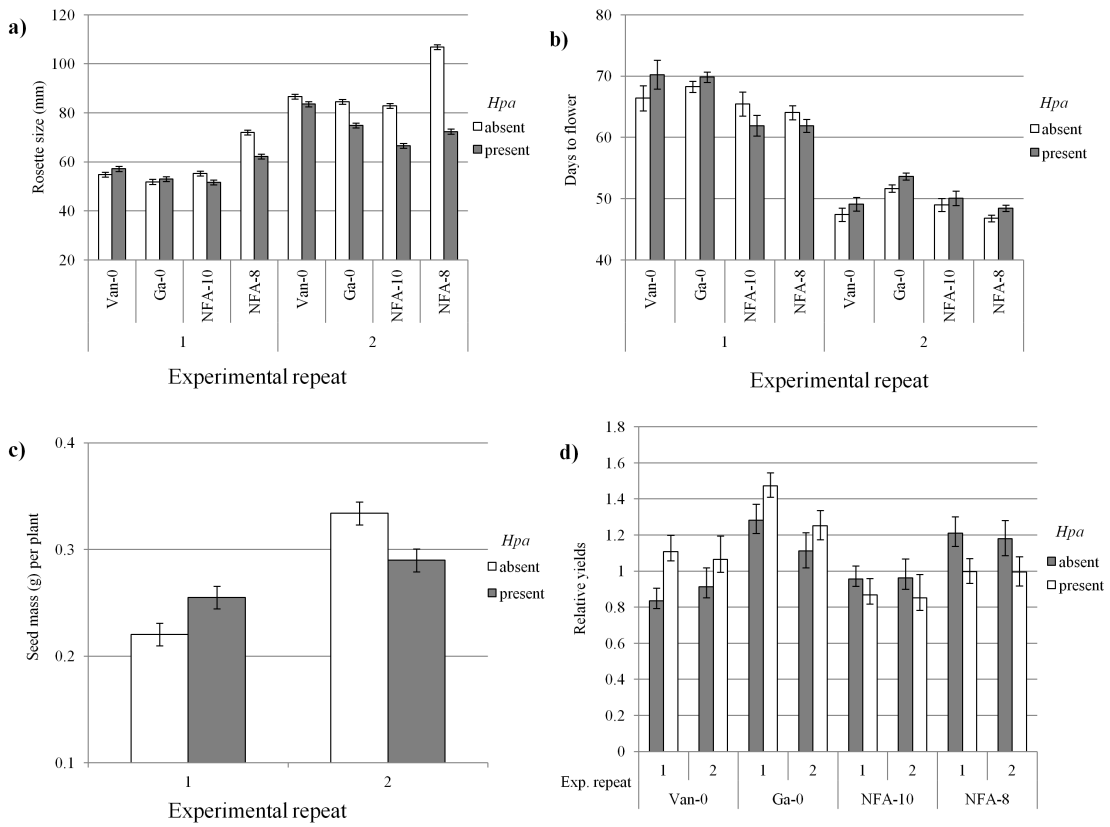

Fig. 3.5: Phenotypic fitness measurements taken for four *Arabidopsis* genotypes grown in experimental repeat 1 and 2, in the presence and absence of *Hpa*. a) Mean rosette diameter after five weeks growth. b) Mean number of days taken to flower. c) Mean seed mass produced per plant. d) Relative yield (yield in mixture/yield in monoculture), an indicator of competitive ability. Error bars show 95% confidence interval of means.

On average across treatments and genotypes, 2-way mixtures achieved greater yields than monocultures and 4-way mixtures (Fig. 3.6; Table A3.5, $F_{2,31}$=6.76, P=0.001). 4-way mixtures produced the lowest yields in the absence of the pathogen, and yields similar to the sum of the monoculture yields in the presence of *Hpa.* A likely cause is the high levels of inter-plant competition resulting from the presence of NFA-8 and Ga-0. However yields were variable in 2-way mixtures due to high levels of variation in competition intensity in pots containing different competing genotypes (Fig. 3.7). Seed production decreased as genotypic diversity increased for the less competitive genotypes Van-0 (fully resistant) in the absence of *Hpa*, and NFA-10 (moderately susceptible) in the presence of *Hpa* (Fig. 3.8; Table A3.5, $F_{8,31}$=2.44, P=0.01). Ga-0 was

the only genotype to significantly overyield in the 4-way mixture compared to monoculture, further illustrating its stronger competitive ability (Fig. 3.8, $P<0.01$ for difference from 1, LSD). Genotypic diversity and composition both contributed towards competitive intensity between plants, ultimately affecting yield and yield stability in this pathosystem.

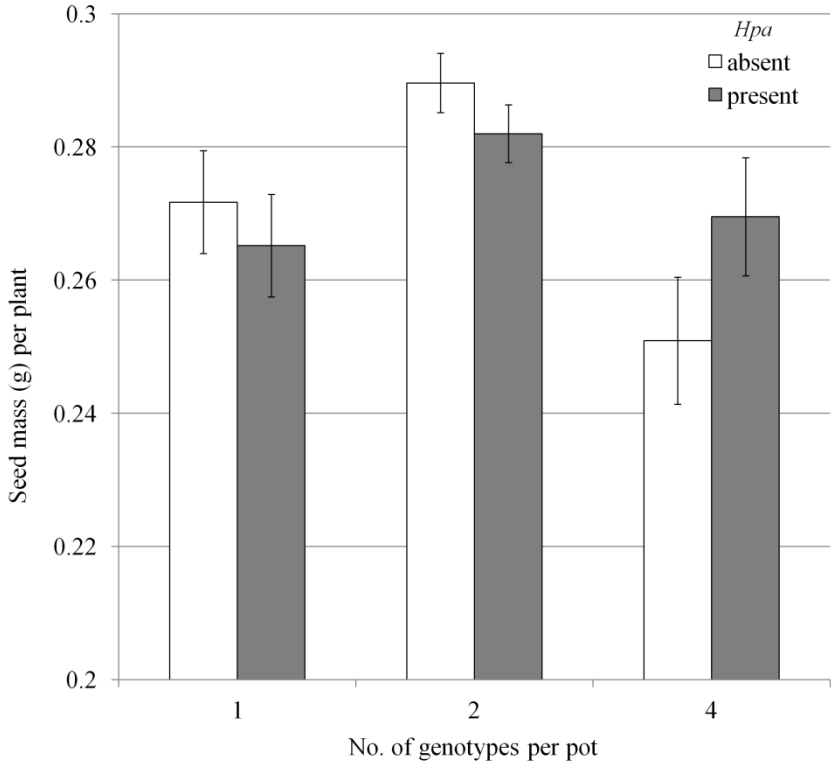

Fig. 3.6: Mean seed mass yields for *Arabidopsis* plants grown as 1, 2 or 4 genotypes per pot in the presence or absence of *Hpa*. N=1600. Error bars show 95% confidence interval of means.

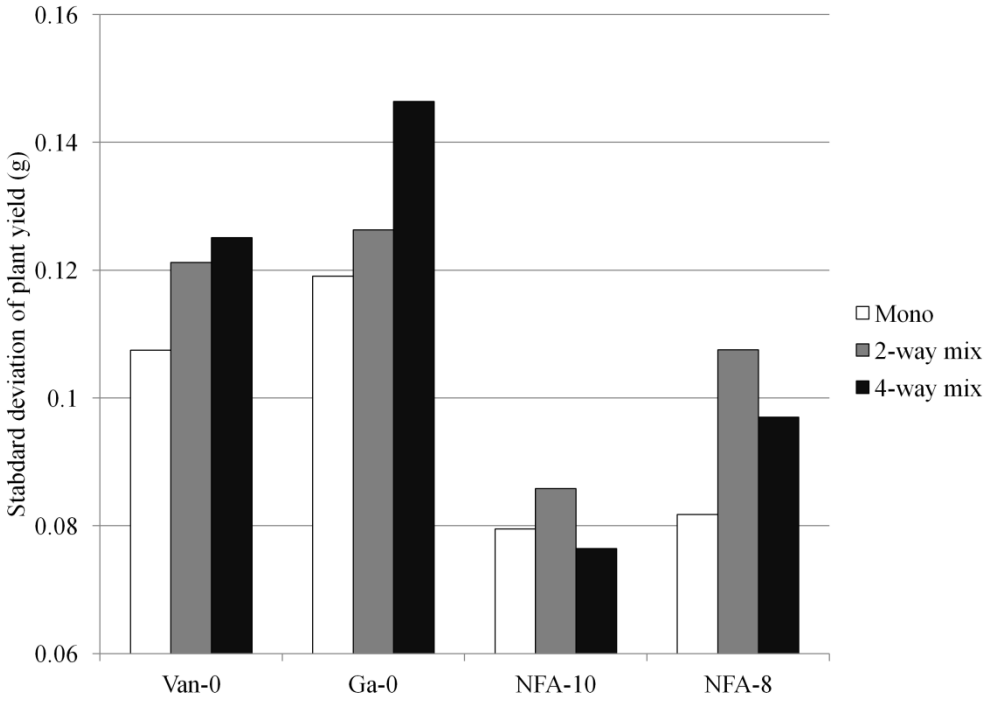

Fig. 3.7: Standard deviation of the mean seed mass produced per plant for *Arabidopsis* genotypes grown in monoculture, 2-way mixture and 4-way mixture.

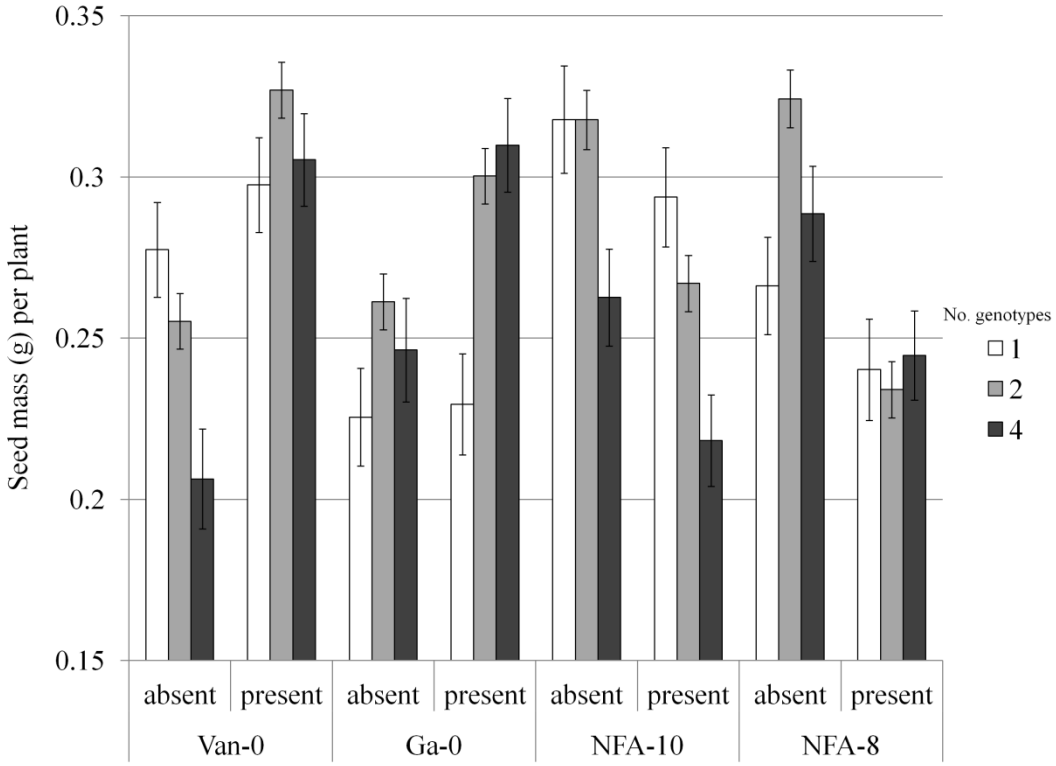

Fig. 3.8: Mean seed mass produced per plant for each genotype in pots containing 1, 2 or 4 genotypes in the presence and absence of *Hpa*. N=1600. Error bars show 95% confidence interval of means.

3.3.2 Turnip yellows virus

Col-0 and Ler-1 were susceptible to TuYV, allowing viral titre to reach similar levels in both genotypes (Fig. 3.9) and observed visually by a purpling of the leaves (Fig. 3.10). Both genotypes showed a delay in flowering time in mixtures compared to monocultures in the presence of TuYV but the delay was greatest for Col-0 when grown in mixture (Fig. 3.11a; Table A3.6, $F_{2,7}$=4.12, *P*=0.02). Ler-1 produced a larger rosette after five weeks growth when grown in monoculture compared to mixture, possibly due to higher levels of inter-plant competition in the mixture (Fig. 3.11b; Table A3.7, $F_{1,4}$=3.96, *P*=0.05). The more competitive genotype, Col-0, overyielded in uninfected mixtures at the expense of Ler-1, which produced less seed (Fig. 3.11c; Table A3.8, $F_{2,7}$=6.58, *P*=0.002). However when the virus was present both genotypes performed as well in mixture as they did in monoculture, due to a large reduction in the competitive ability of Col-0 (Fig. 3.12). Despite changes in competitive ability due to pathogen infection the average yield in mixtures and the average of the monocultures was stable whether the pathogen was present or absent, and there was no overall yield penalty as a result of growing mixtures (Fig. 3.13).

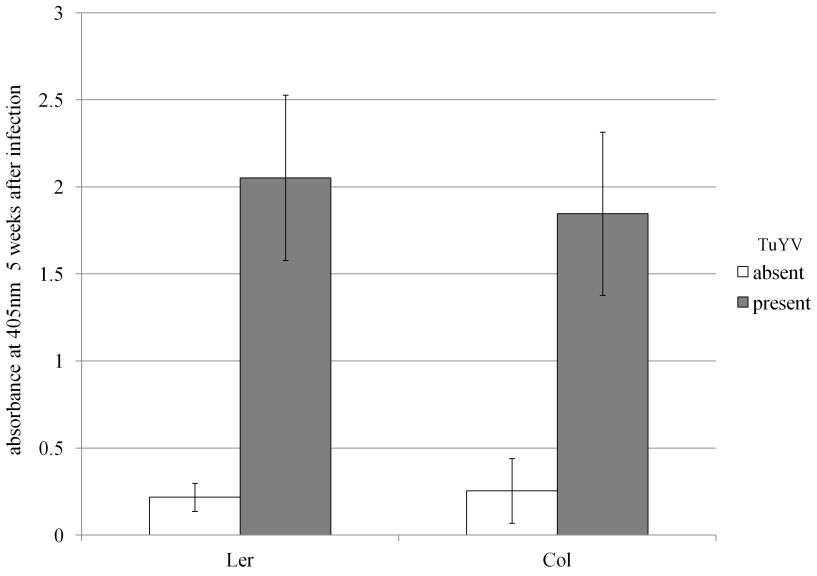

Fig. 3.9: Enzyme-linked immunosorbent assay (ELISA) detection of TuYV for two *Arabidopsis* genotypes grown in the absence and presence of TuYV. Readings show absorbance at 405 nm five weeks post inoculation. N=400. Error bars show 95% confidence interval of means.

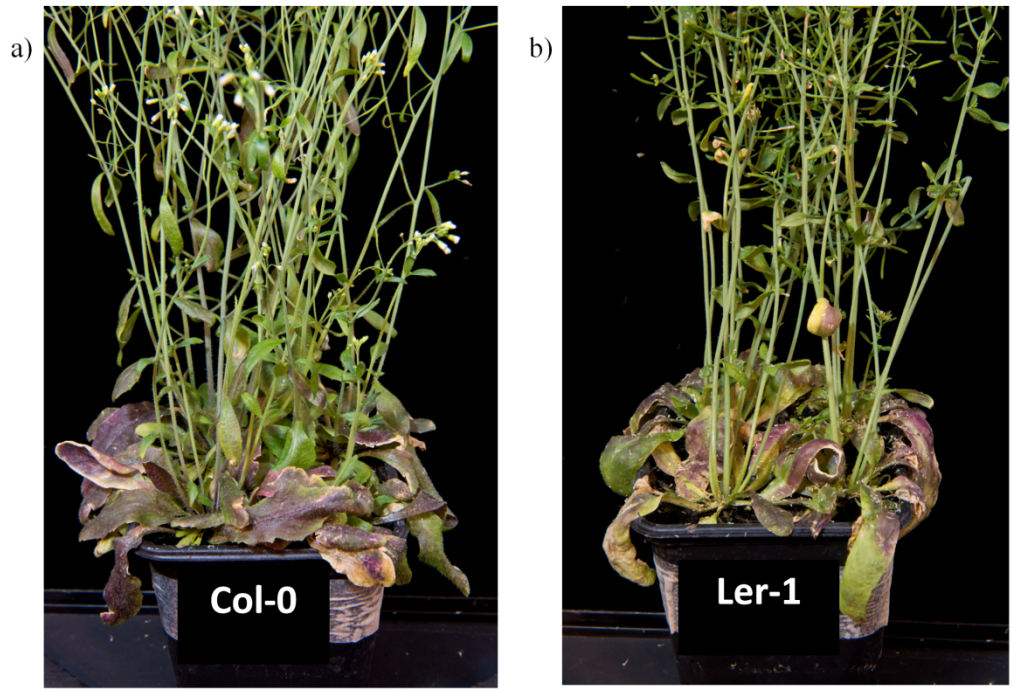

Fig. 3.10: Photographs of TuYV infected *Arabidopsis* after ten weeks growth. a) Monoculture of four Col-0 plants. b) Monoculture of four Ler-1 plants.

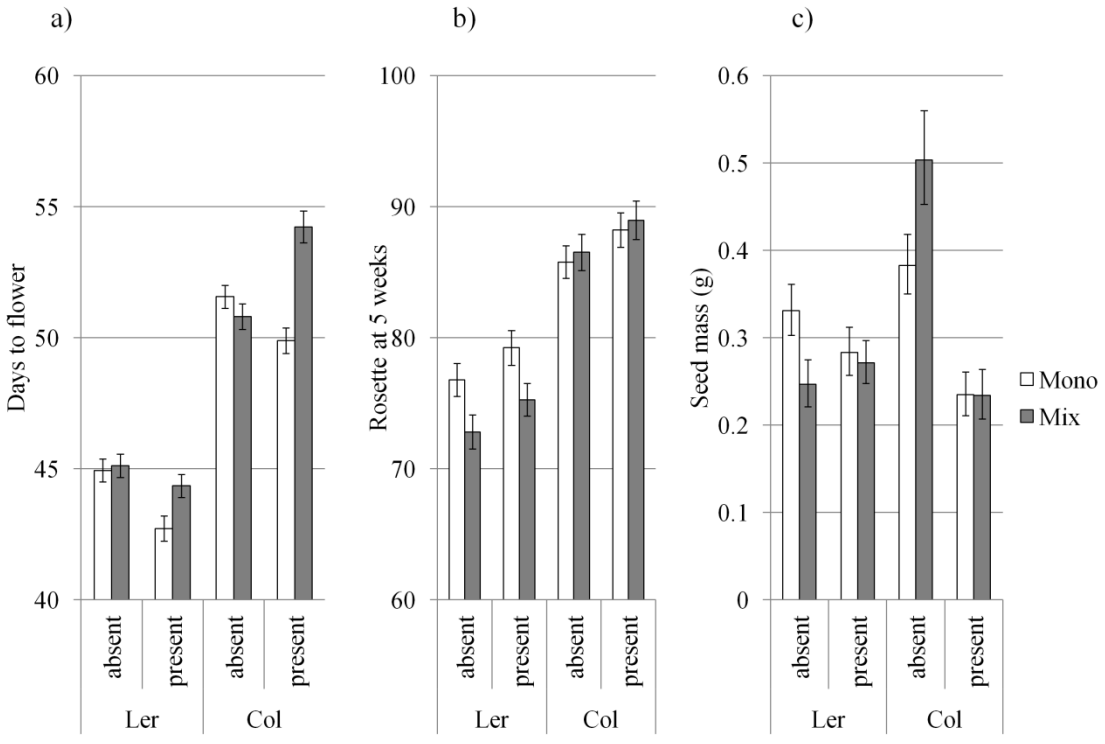

Fig. 3.11: Phenotypic fitness measurements taken for two *Arabidopsis* genotypes grown in the presence and absence of TuYV. a) Mean number of days taken to flower. b) Mean rosette diameter after five weeks growth. c) Mean seed mass produced. N=400. Error bars show 95% confidence interval of means.

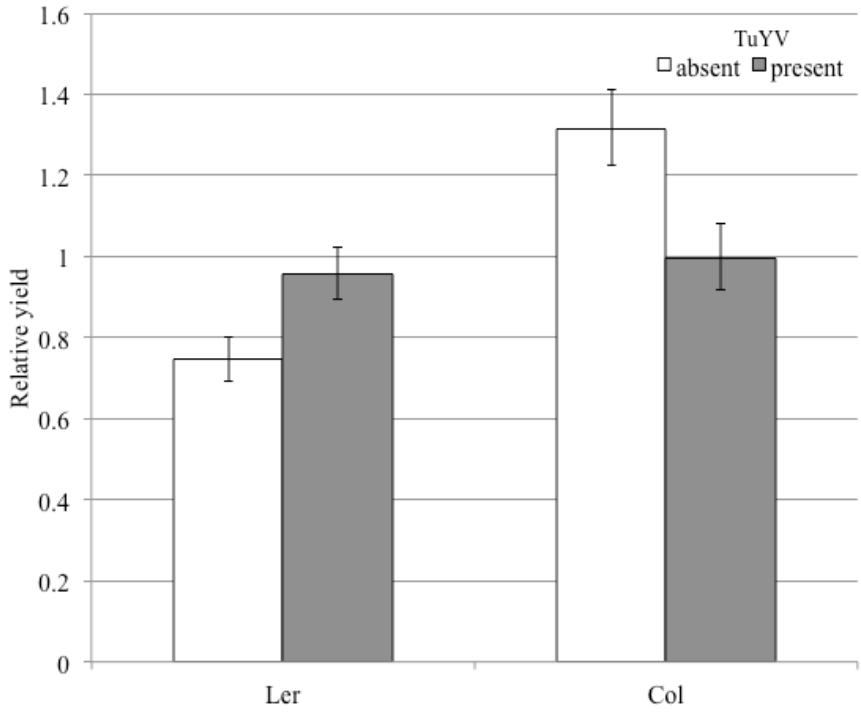

Fig. 3.12: Relative seed mass yields (yield in mixture/yield in monoculture) of two *Arabidopsis* genotypes grown in the presence and absence of TuYV. N=400. Error bars show 95% confidence interval of means.

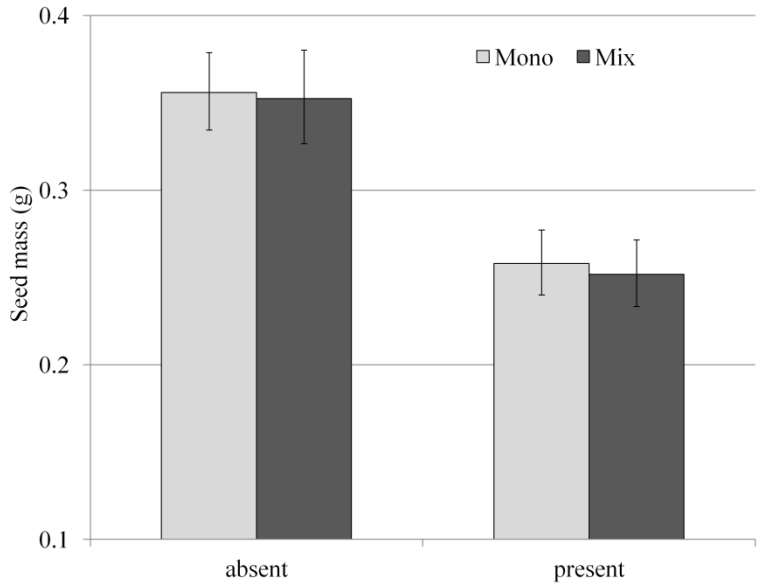

Fig. 3.13: Mean yields of *Arabidopsis* genotypes grown in monoculture and mixture and in the presence and absence of TuYV. N=400. Error bars show 95% confidence interval of means.

3.4 Discussion

This study investigated the suitability of *Arabidopsis thaliana* as a model for understanding the effects of disease on genotypically and phenotypically diverse plant populations. *Arabidopsis* genotypes were selected to vary in competitive ability and compatibility with two different types of plant pathogen, an oomycete and a virus. Genotypic diversity enhanced ecological resistance of the plant population to attack by *Hyaloperonospora arabidopsidis* as shown by an increase in yield in 2-way mixtures compared to the average of the component monocultures or 4-way mixtures. Ecological resistance refers to the ability of the system to resist change following perturbation (Pimm 1984) and is not the same as resistance of individual plants to stress or disease. The evidence produced from this investigation supports the diversity-productivity hypothesis which states that greater plant diversity can lead to an increase in plant productivity, a theory originally proposed by Darwin (1872).

Susceptible genotypes suffered greatly in the presence of both pathogens due to an overall reduction in plant fitness and competitive ability which led to reduced yield of seed production in susceptible monocultures. In mixtures containing genotypic variation for disease susceptibility, a reduction in competitive ability of susceptible genotypes consequently increased competitive ability of more resistant or tolerant genotypes via competitive release. In certain mixtures, fitter genotypes compensated for less fit genotypes in both the absence and presence of the pathogen, leading to increased yield in 2-way mixtures. Competitive interactions are thought to play a significant role in driving the diversity-productivity relationship in natural systems (Schmid 1994; Tilman *et al.* 1996; Hector *et al.* 1999). Increases in productivity with greater diversity are thought to be the result of the increased likelihood of a productive species being present (sampling effect and compensation) and from a greater chance of efficient exploitation of all available niches (complementation) (Tilman 1996; Hector *et al.* 1999). Evidence for plant diversity enhancing yield and yield stability under disease pressure is supported by this study and by several studies of natural systems in which plant pathogens promoted biodiversity by preventing competitive exclusion (Bradley *et al.* 2008; Maron *et al.* 2011).

Mixtures consisting of two genotypes were identified as containing the optimum level of genotypic diversity required to maintain high yields in both the presence and absence of *Hpa*. 4-way mixtures produced the lowest yields in the absence of *Hpa*, apparently because of the presence of two highly competitive genotypes (Ga-0 and NFA-8) that outcompeted neighbours for resources and overyielded in mixture, yet produced less seed than weaker competing genotypes. Mixture composition rather than genotypic diversity had a greater effect on productivity in this study. This finding contrasts with several agricultural studies that show a trend towards greater yields from increased number of varieties in the mixture, largely because of superior disease control (Newton *et al.* 1997; Newton *et al.* 2008a). The highest yielding 2-way mixtures consisted of genotypes with relatively low competitive abilities and greater investment in reproductive effort (Van-0 and NFA-10), providing evidence for a fundamental tenet of life history theory that reproduction is costly and results in trade-offs with other fitness components, regularly observed in crop plants (Lemerle *et al.* 2006; Song *et al.* 2010).

Compensation occurring through competitive release buffered against pathogen-induced alterations to host competitive ability and resulted in yield stability of the mixed genotype population, supporting work conducted in both agricultural (Finckh *et al*. 2000) and natural systems (Tilman 1996). Mixtures had a yield advantage when plants were inoculated with *Hpa*, but not when inoculated with TuYV, yet yield stability was still achieved in both experiments. In the absence of the TuYV, the high yielding Col-0 genotype maintained high yields in monoculture despite being under higher competition levels than Ler-1 in monoculture. This result contrasts findings from the *Hpa* experiment in which the highly competitive genotypes produced significantly less seed in non-diseased monocultures. A possible explanation for this finding is that the most competitive genotype in the TuYV experiment was less competitive than the most competitive genotypes in the *Hpa* experiment and therefore was under less competition in monoculture.

Within-plant compensation was observed in the TuYV experiments as Ler-1 was able to maintain seed production in mixture despite a reduction in rosette size through alteration of resource allocation. No signs of within-plant compensation were observed in the *Hpa* experiment possibly due to the pathogen isolate or plant genotypes used. This highlights the fact the different pathogens interact with hosts in different ways

(Jones and Dangl 2006) and that successful plant genotypic mixtures must confer resistance to multiple pathogens. Further, TuYV infection resulted in a delay in flowering time of both genotypes in mixtures indicating an alteration of plant development strategy in response to pathogen. The finding that plants can have a developmental response to stress is supported by work on plants subjected to many stresses including nutrient stress (Martinez-Zapater *et al.* 1994), shade (Halliday *et al*. 1994) and pathogen infection (Korves and Bergelson 2003). Plants may delay or accelerate the transition into reproductive growth in response to disease (Korves and Bergelson 2003). Most life history evolution studies predict that organisms at risk of severe disease will evolve fast reproduction strategies to reduce damage from disease (Forbes 1993; Angew *et al*. 2000). Delaying the transition into reproductive growth allows for greater investment in vegetative growth prior to flowering, extending the plants lifespan and increasing seed production (Bazzaz 1987). Pathogens can also benefit from an extension of the host's life cycle as increasing the contact time between the host and the vector enhances the pathogens dispersal ability (Brown and Tellier 2011). No firm conclusions regarding the effect of *Hpa* infection on flowering time can be drawn due to inconsistencies between experimental replications. Although no direct effect of *Hpa* infection on flowering could be discerned, an indirect effect caused by pathogen-induced alterations to plant competitive ability may have resulted in delayed flowering in resistant genotypes. This study indicates that host plant responses to the pathogens can vary greatly depending on their interaction, which must be considered when assessing and predicting plant population responses to multiple pathogens. In this study genotypic mixtures ultimately lead to yield stability in infected and uninfected populations through a combination of altered plant resource allocation and compensation by fitter genotypes.

Understanding the mechanisms of plant competition increases the predictability of the outcome of competition for different resources. Light competition favours taller plants with flatter canopies than are optimal in the absence of competition (Spehn *et al.* 2000; Craine and Dybzinski 2013), competition for low levels of nutrients favours plants with roots longer than is optimal in nutrient rich soil (Craine 2006; Craine and Dybzinski 2013). Understanding the mechanisms of plant competition responsible for enhanced yield stability in variety mixtures could greatly improve the efficient deployment of mixtures in agriculture (Knott and Mundt 1990). Mixtures containing diversity for

important functional traits relating to competitive ability (Cahill *et al.* 2005; Creissen *et al.* 2013) and response to environmental stresses such as drought (van Ruijven and Berendse 2010), herbivory (Kotowska *et al.* 2010) and disease (Mundt 2002) are predicted to have greater ecological resistance and achieve greater yield and yield stability in variable environments through ecological processes such as compensation, complementation and facilitation. Indeed this study has provided insight into mechanisms by which diverse plant populations buffer against disease, identifying favourable traits for mixture components, such as disease resistance and high reproductive allocation, that contribute towards high, stable yields and ecological resistance of the plant population to pathogen attack.

In agriculture, variety mixtures can provide similar productivity to high yielding monocultures but with a lower risk of excessive yield loss. However, studies investigating plant traits and mechanisms responsible for the enhanced function of mixtures are rare (Wolfe 1985; Mundt 2002; Newton *et al.* 2008b). *Arabidopsis* can be used as a model system to identify traits that affect competitive ability and mixture performance such as vegetative growth capacity, establishment capability, flowering time plasticity and alteration of resource allocation. Information regarding the competitive ability of varieties is important when attempting to minimize yield losses associated with competition between crop plants and competition from weeds (Jordan 1993; Lemerle *et al.* 2006; Song *et al.* 2010). Determining the suitability of a variety for mixture usage prior to competition studies will increase the efficiency of varietal mixture selection. This may support future commercial cropping systems which will need to be less reliant on chemical inputs, less expensive to manage and show greater adaptability to the changing environment if future food security is to be achieved (FAO, WFP, IFAD 2012; Hillocks 2012).

Chapter 4:
Increased yield stability of field-grown winter barley (*Hordeum vulgare* L.) varietal mixtures compared to monocultures

4.1 Introduction

Advances in modern agriculture, including plant breeding techniques and the development of inorganic fertilizers and pesticides, have led to modern arable farmers routinely growing a single high-yielding variety throughout an entire field in order to maximise yield potential, a practice termed monoculture (Trewavas 2001). Ecosystem services normally provided by crop diversity, such as soil improvement and pest control, have been replaced by chemical inputs, which can be detrimental to the environment (Tilman *et al.* 2002). Despite attempts to minimise the problems associated with reduced crop diversity, variety monocultures remain susceptible to severe disease epidemics and the associated drastic reductions in yield (Newton *et al.* 2008b).

An alternative to the variety monoculture system is the use of varietal mixtures in which several genotypes are sown together at the same time to buffer against environmental stresses, including disease, and improve yield stability (Wolfe 1985; Lannou and Mundt 1996; Zhu *et al.* 2000). To date, varietal mixtures have primarily been deployed against crop diseases, controlling major pathogens such as powdery mildew of barley (Wolfe and Barrett 1980), Rhynchosporium scald of barley (Newton *et al.* 1997), wheat yellow rust (Sapoukhina *et al.* 2013), and rice blast (Zhu *et al.* 2000). Mixtures can reduce disease severity by reducing pathogen spread, either by increasing the distance between susceptible host plants, or by resistant plants forming a barrier to prevent pathogen dispersal (Chin and Wolfe 1984; Zhu *et al.* 2000).

The theory underpinning the use of mixtures is largely based on the hypothesis that biodiversity increases ecological stability (Yachi and Loreau 1999). This approach relies on beneficial ecological processes to increase the system's potential to buffer against adverse environmental conditions, reduce fertiliser inputs and control disease (Finckh and Wolfe 1998). Variation between mixture components in response to common pathogens allows ecologically beneficial processes

such as compensation, complementation and facilitation to occur (Wolfe 1985). Complementation between crop plants can increase productivity in mixtures through niche differentiation and resource partitioning (Loreau 2000; Mulder *et al.* 2001; Tilman 2004). Facilitation can occur within mixed populations if the fitness of neighbouring plants is increased through inter-plant interactions such as provision of shade and deterrence of pests (Callaway 1995). When weaker individuals are harmed by environmental stress, stronger plants can increase their yields through compensation via competitive release (Tilman 1996). Compensation is thought to be the major ecological process contributing to yield stability in diverse mixtures (Eberhart and Russell 1966; Wolfe 1985; Smithson and Lenne 1996; Mundt 2002; Ostergaard *et al.* 2005; Cowger and Weisz 2008) but other beneficial processes may also be involved (Finckh *et al.* 2000). For example, a mixture consisting of high yielding varieties and winter hardy varieties insures against excessive losses experienced in colder winters, particularly as stress tolerant plants are able to overyield through competitive release (Finckh *et al.* 2000). The potential to exploit beneficial plant-plant interactions therefore depends on the presence of suitable mixture components. Field trials are necessary for accurate mixture assessment as it is often difficult to predict the performance of a variety in mixture from its monoculture yield due to the complexity of competitive interactions taking place within the crop and variation in field environments (Lopez and Mundt 2000; Mille *et al.* 2006).

Varietal mixture studies are often conducted under similar yet largely unreproducible environmental conditions, which reduces the strength of any conclusions drawn (Mundt 2002). In contrast, replicated trials across multiple sites would indicate the consistency and any environmental dependency of such interactions taking place within the mixture. For disease studies, experimental plots are often either artificially inoculated at higher concentrations than would be present in nature to ensure disease establishment, or repeatedly infected with 'spreader plants' in a way unrepresentative of field infection (Finckh *et al.* 2000). Yield and disease data from both the level of individual plants and that of populations within field trial plots, can provide insight into the population processes occurring within mixed variety populations that lead to yield stability under environmental stress. In turn, this can contribute to understanding of the ecological mechanisms by which mixed plant genotype populations control disease in mixtures, and thus improve the predictability of the performance of variety mixtures.

This study examines the effect of varietal mixtures in stabilising yields in populations under natural levels of environmental stress. We test the hypothesis that compensation by better-adapted plants increases yield stability in phenotypically diverse mixtures, and that this effect is greatest when susceptible and resistant varieties are combined in the presence of the pathogen. We predict that genotype by environment interactions will alter the competitive ability and fitness of individual varieties, yet the overall mixture yield will be maintained through beneficial ecological processes such as compensation, complementation and facilitation.

4.2. Materials and methods

4.2.1 Mixture design

To assess the ability of winter barley variety mixtures to buffer against environmental stress and stabilise yield, UK commercial varieties were selected based on phenotypic information contained within the HGCA (Home Grown Cereals Authority) Recommended List for 2011/2012 (http://www.hgca.com). Two mixtures were designed to contain three varieties varying in disease resistance, competitive ability and classification group which is based on morphological differences in the ear (2-row or 6-row) and the crops end-usage (malting or feed). Each variety had a set of unique phenotypic traits for easy identification in the field. Each 3-way mixture contained the hybrid 6-row Element (Syngenta, Fulbourn, Cambridge, CB21 5XE), the red-awned 2-row Winsome (Syngenta), and one other white grain 2-row variety, either Cassata (Limagrain UK Ltd, Rothwell, Lincolnshire, LN7 6DT) or Saffron (KWS, Thriplow, Hertfordshire, SG8 7RE). Information from previous trials conducted at the John Innes Centre (JIC) and observations made on commercial farms around the site area in Norfolk, indicated major biotic threats to crop yield to be the fungal diseases Rhynchosporium scald (caused by *Rhynchosporium commune*) and brown rust (caused by *Puccinia hordei*). For the present study, each mixture therefore contained one variety susceptible to common isolates of either Rhynchosporium or brown rust. Saffron was predicted to be the most susceptible variety to Rhynchosporium infection based on HGCA resistance ratings (Table 4.1), and was included in the mixture designed to investigate mixture responses to this disease (Mixture A). Element was present in both mixtures but was the most susceptible variety to brown rust in Mixture B (Table 4.1)

because other varieties in the mixture possessed good resistance to brown rust and Rhynchosporium.

Table 4.1: Mixture composition (A and B) and information from HGCA recommended list 2011/2012. Ratings for the winter barley varieties used in this study. 1=poor resistance, 9=high resistance.

Variety	Mix	Rhyncho-sporium	Brown Rust	Net blotch	Mildew	Lodging resistance	Straw height (cm)	Yield with fungicide (t/ha)	Yield no fungicide (t/ha)
Element	A+B	7	4	7	6	6	103	9.3	7.7
Winsome	A+B	8	6	8	7	6	93	8.6	7.0
Saffron	A	4	7	4	3	8	87	9	7.1
Cassata	B	8	7	8	4	8	87	8.5	7.0

Target plant populations were set according to plant breeding companies' recommendations of 300 plants/m^2 for the 2-row varieties, and 200 plants/m^2 for the 6-row variety. The amount of seed required per m^2 was calculated using the following equation: thousand grain weight (TGW) x target plant population/ 95% establishment = seed/ m^2. All plots were 6 m^2 (1.5m x 4m), so for each monoculture plot Winsome and Cassata (TGW 54g) required 128g seed, Saffron (TGW 64g) 150g and Element (TGW 49g) 75g. For each mixture plot, one third of the seed mass required for each variety's monoculture plot was thoroughly mixed by hand, prior to sowing in the field with a Hege 80 drill (Wintersteiger, Austria).

4.2.2 Trial sites and experimental design

Trials were sown on 30th September 2011 at three different sites ranging in soil type from a very light sandy clay loam (JIC; OS 52.62250, 1.2184417), to a light sandy clay loam (light land on a well-drained area of Church Farm, Bawburgh; OS 52.625092, 1.1745071), and a heavy sandy clay loam (heavy land prone to water logging at Church farm, Bawburgh; OS 52.628713, 1.1786270). All plots received 40 Nitrogen kg/ha on 2nd March 2012 and 100 Nitrogen kg/ha on 3rd April 2012. The experiments at each site consisted of four plots of each monoculture, mixture A and mixture B for both the fungicide and no fungicide treatments, giving 48 plots per site and 144 plots in total.

4.2.3. Chemical treatments

Chemical treatments were based on recommendations from a local agronomist and applied manually using a knapsack sprayer (Cooper Pegler CP3 with 1.2 metre spray boom). All plots received the plant growth regulator Chormequat (BASF), and the herbicides Ally max (DuPont) and Oxytril (Bayer). Non-disease control plots received a full fungicide treatment programme consisting of full rate applications of Bravo (Syngeta), Opus (BASF), Cyflamid (Certis), Proline (Bayer) and Comet 200 (BASF).

4.2.4. Disease scoring

Disease levels were assessed twice, on 31st October 2011 and 25th June 2012, 31 and 269 days after sowing, respectively. Disease was measured as percentage green leaf area covered in symptoms on the flag and first leaf (Peterson *et al.* 1948; James *et al.* 1968; James 1971). Ten plants of each variety per plot were scored. Diseases scored included brown rust, powdery mildew (caused by *Blumeria graminis* f. sp. *hordei*), Rhynchosporium and net blotch (caused by *Pyrenophora teres*).

4.2.5. Plant height measurement

Maximum plant height measurements were taken on 30th April 2012, 213 days after sowing, when height differences were greatest between sites and plots. Height measurements were taken from three randomly selected individual plants from within each plot, as it was impossible to identify individual varieties within mixture plots at this early stage. Plants on the outer rows were not measured to avoid edge effects.

4.2.6. Yield

Plots were harvested at JIC on 25th July (299 days after sowing), at the Light land site on 1st August (306 days after sowing) and at the heavy land site on 5th August 2012 (310 days after sowing). Total plot yield (g) and mean grain humidity (%) data were recorded using a Zurn 150 plot combine (Zurn GmbH & Co., Germany). Yield

component measurements were also taken for each variety in mixture and monoculture. Thirty ears of each variety were hand-harvested per plot and threshed using a single ear thresher. Measurements including mass, number of seeds and average seed mass were taken for each ear using the Marvin Universal seed counter (GTA Sensorik GmbH, Germany). For each mixture plot 100 grains were randomly selected for varietal identification through analysis of visually assessable phenotypic characteristics by the National Institute of Agricultural Botany (NIAB, http://www.niab.com/pages/id/21/Seed_Certification; Table A4.1). These data were used to estimate the relative proportions (%) contributed by each variety to the total mixture yield (g). Mean relative yields (RY=yield in mixture/monoculture) were calculated for each variety at each site under both fungicide/non-fungicide treatments as follows: the estimated proportions contributed by each variety to the overall mixture plot yield (%) were multiplied by the total mixture plot yield (g), and this value was then divided by the average plot yield of the variety in monoculture.

4.2.7. Statistical analysis

Linear mixed modelling was used to evaluate differences in yield and disease between monocultures and mixtures of winter barley varieties. Plot yield, disease scores, ear mass, mean seed mass per ear, mean number of seed per ear were analysed in separate models all including the main effects of variety, site and cultivation (monoculture/mixture A/mixture B) as fixed factors and all interactions between them. The plot in which the plants were grown was included as a random effect. All non-significant (P>0.05, F-test) interactions between the main terms were removed from the analysis. Statistical analysis was conducted using Genstat v.14 (VSN International 2011).

4.3 Results

Mean yields of mixtures and monocultures were similar across the entire experiment (Table A4.2, $F_{3,10}$=2.23, P=0.141) but mixture yields were more stable than monoculture yields as shown by lower coefficient of variation in the mixtures compared to the sum of their component monocultures (Fig. 4.1). Mixture yields were stable, despite the presence of a variety with highly variable yields, Winsome, in both mixtures

(Fig. 4.2). Mixture performance of each variety was altered by the site and fungicide treatment, shown by a change in relative yield (Fig. 4.3). Element generally performed better in mixture indicating that inter-plant competition was greater within monocultures (Fig. 4.3). The success of Element in mixtures is partly due to plasticity in certain yield components, which allowed the variety to increase mean ear mass in mixture (Fig. 4.4; Table A4.3a, $F_{3,9}$=3.6, *P*=0.013). Plasticity in mean ear mass data was dependent on cultivation (mixture/monoculture) and not site (Table A4.3a, $F_{1,9}$=0.42, *P*=0.516). Cassata's low competitive ability in mixtures led to a reduction in yield and yield components including mean mass per ear (Fig. 4.3, Fig. 4.4; Table A4.3a, $F_{3,9}$=3.6, *P*=0.013). Element overyielded in mixture to compensate for under-yielding varieties, resulting in high and stable mixture yields across the entire experiment (Fig. 4.2).

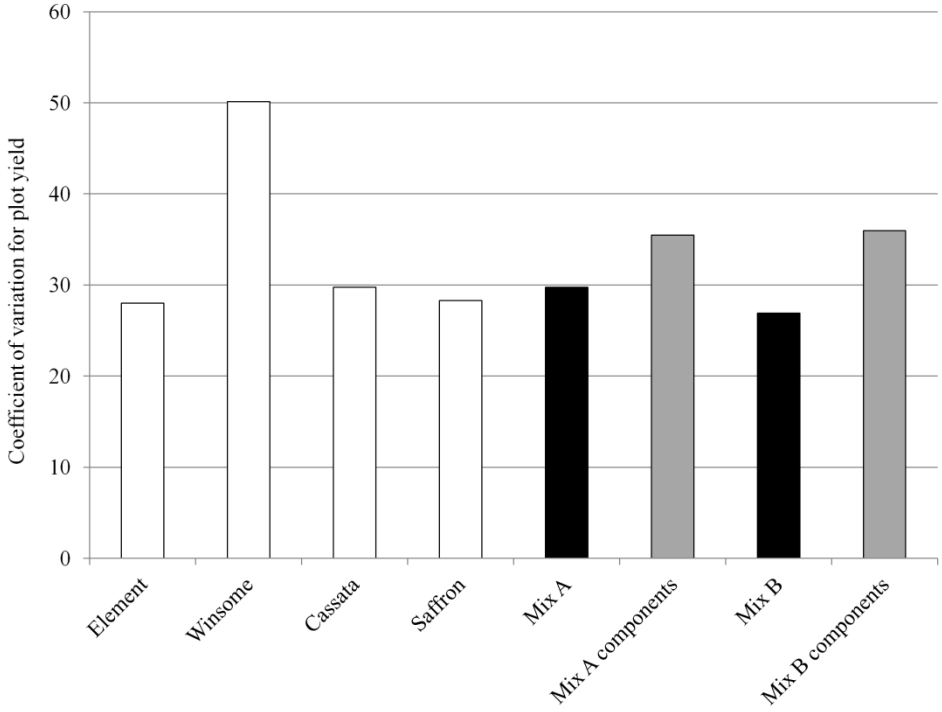

Fig. 4.1: Coefficient of variation (a measure of yield stability) for 6m^2 plot yields of winter barley monocultures and mixtures in a field trial experiment conducted over three different sites. White bars indicate variety monocultures. Black bars represent mixtures. Grey bars show the mean coefficient of variation for the mixture component varieties when grown in monoculture. N=1600.

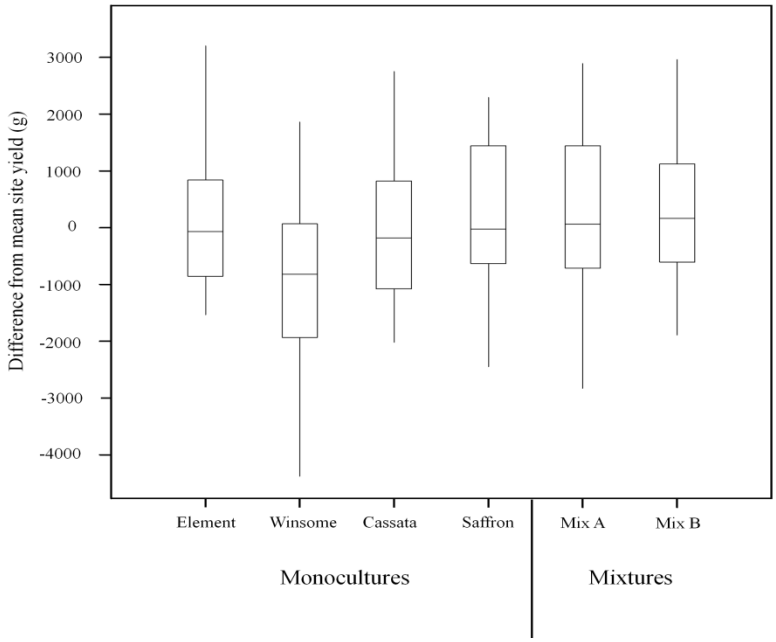

Fig. 4.2: Difference from mean site yield (g) for each monoculture and mixture in a winter barley field trial experiment conducted over three different sites. Mix A includes varieties Element, Winsome and Saffron. Mix B includes varieties Element, Winsome and Cassata. The bottom and top of the boxes represent the first and third quartiles. Lines within the box represent the median. Lines outside the box display the range. N=1600.

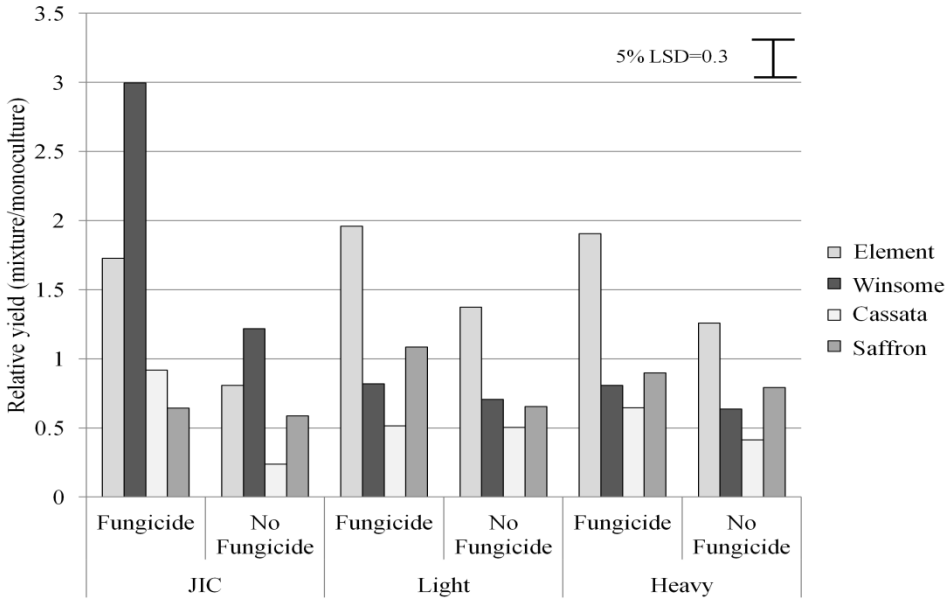

Fig. 4.3: Relative yields (yield in 3-way mixture/yield in monoculture) of four winter barley varieties grown in fungicide treated or untreated plots in a field trial conducted over three different sites. N=1600.

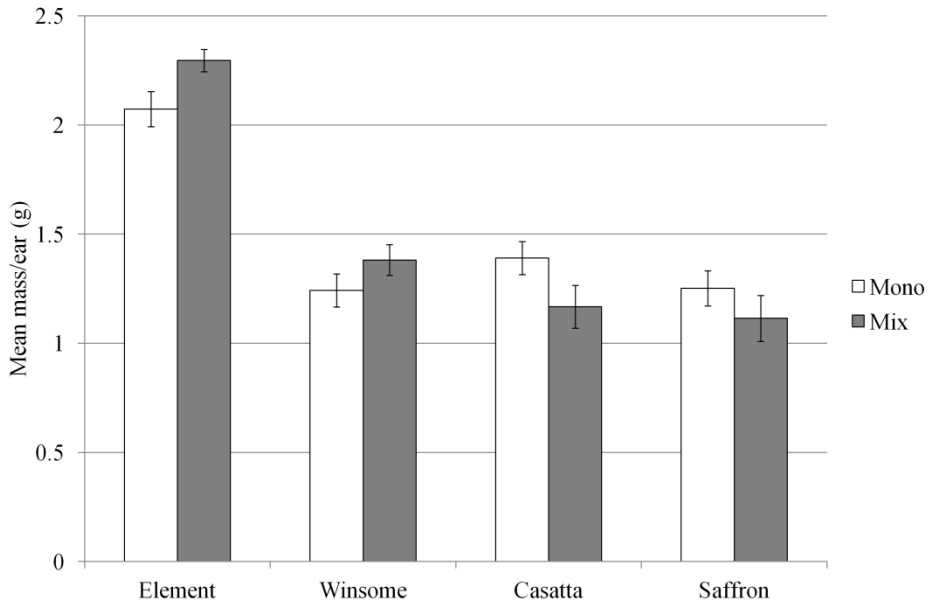

Fig. 4.4: Mean mass per ear (g) for four winter barley varieties grown in monoculture or 3-way mixture in fungicide treated and untreated plots in a field experiment. N=1600. Error bars show 95% confidence interval of means.

Cassata and Saffron were heavily infected with powdery mildew by 31st October 2012, just four weeks after drilling whereas Winsome and Element showed no signs of infection. Average leaf area covered in mildew colonies was 55±15% (BAPB score=7, Newton and Hackett 1994) for Cassata, and 25±10% (BAPB score=6) for Saffron. Low temperatures in January and February 2012 arrested mildew development allowing the plants to begin recovery from the infection. Plant development remained slower for Cassata and Saffron, such that in the first week of May 2012 at the light land site, Cassata was at GS32 (Zadoks Growth Stage, Zadok *et al.* 1974), whereas the other varieties were at GS35. At the heavy land site, Cassata was at GS41 and other varieties at GS49. At JIC, the most heavily diseased site and the only site showing mildew symptoms on 5th May 2012, Element was at ear emergence about to enter flowering (GS59) and Winsome had nearly finished booting (GS49), whereas susceptible varieties Cassata and Saffron were just entering the booting phase (GS41) with no awns yet visible.

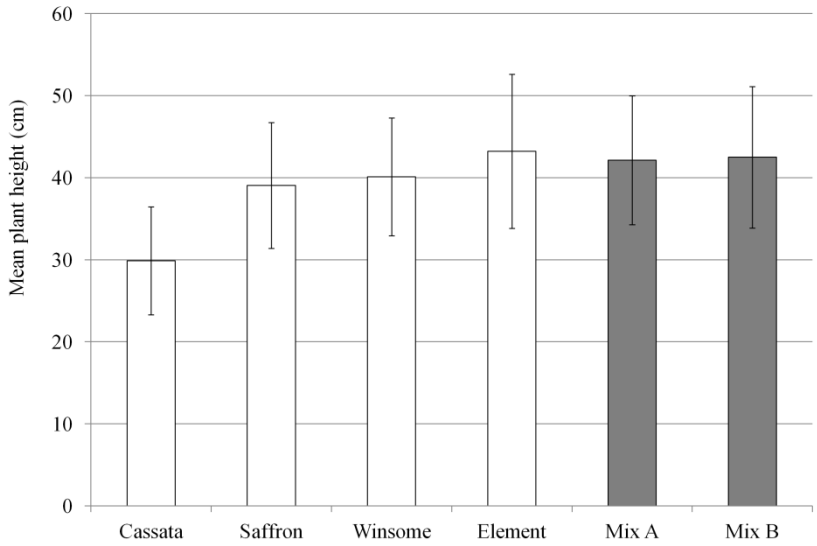

Fig. 4.5: Mean height measurements (cm) taken for four winter barley variety monocultures and two 3-way mixtures in a field trial . N=1600. Error bars show 95% confidence interval of means.

HGCA recommended list 2011-12 disease resistance ratings were reflected in the disease levels observed on the varieties used in this study, however, Winsome (brown rust rating=6) had significantly more brown rust than Element at JIC and on the light land trial (brown rust rating=4). Brown rust was by far the most prevalent disease, assessed by total green area covered in disease on the flag and first leaves at the end of the growing season, followed by powdery mildew and net blotch. Unexpectedly, the least prevalent disease was Rhynchosporium scald with only six plants showing signs of infection (data not shown).

Brown rust scores, recorded as the percentage leaf area infected with the fungus, were heavily dependent upon interactions between variety and cultivation (mixture/monoculture) (Fig. 4.6; Table A4.4, $F_{3,15}$=4.31, P=0.006). Disease levels were consistently reduced for Winsome when grown in mixture compared to monoculture, indicating a positive effect of mixtures in reducing disease severity for susceptible varieties (Fig. 4.6; Table A4.4, $F_{3,15}$=4.31, P=0.006). There was no significant difference in disease levels between the different mixtures, as the most diseased varieties, Winsome and Element, were present in both mixtures (data not shown).

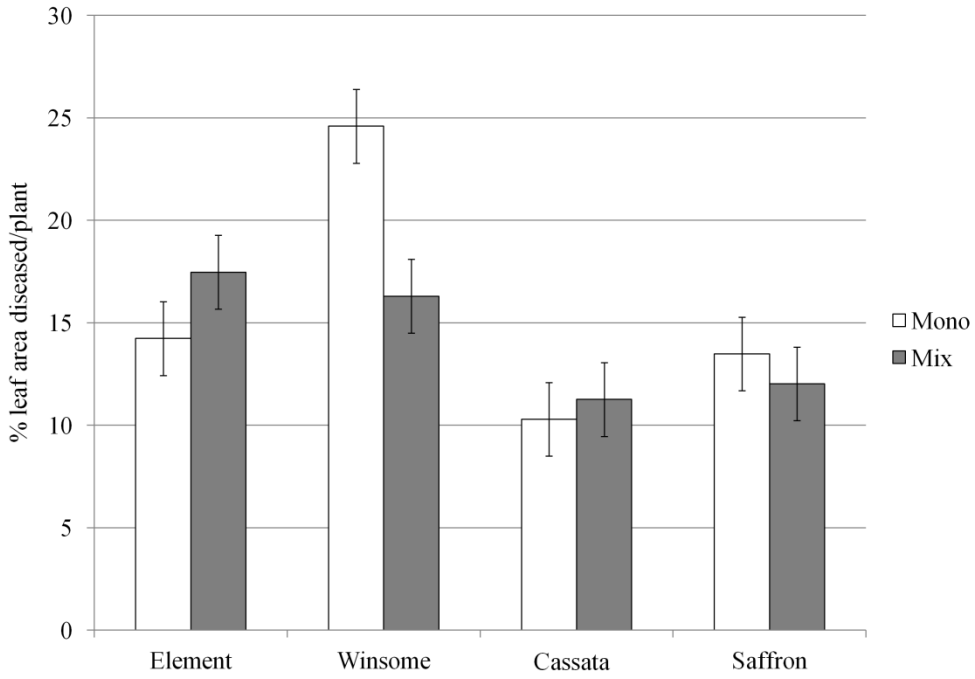

Fig. 4.6: Mean percentage green leaf area on the flag and first leaves showing signs of brown rust infection. Disease scores are for individual plants grown in monoculture or 3-way mixture and were naturally infected under field conditions. Specific data for individual sites is not shown due to a non-significant interaction between site and cultivation (mixture/monoculture). N=1600. Error bars show 95% confidence interval of means.

As expected, mean seed mass was greater in fungicide treated plots (Fig. 4.7; Table A4.3b, $F_{3,15}$=4.31, P=0.006)) which led to higher yield in these plots (Fig. 4.8). Yields from untreated plots were greatest at the light land site, which had the lowest disease levels (Fig. 4.8, Fig. 4.9). The JIC site had significantly more disease than the light land and heavy land sites (Fig. 4.9; Table A4.4, $F_{2,15}$=19.37, P<0.001) and consequently suffered the most from a lack of fungicide application, yielding over 50% less in untreated plots (Fig. 4.8).

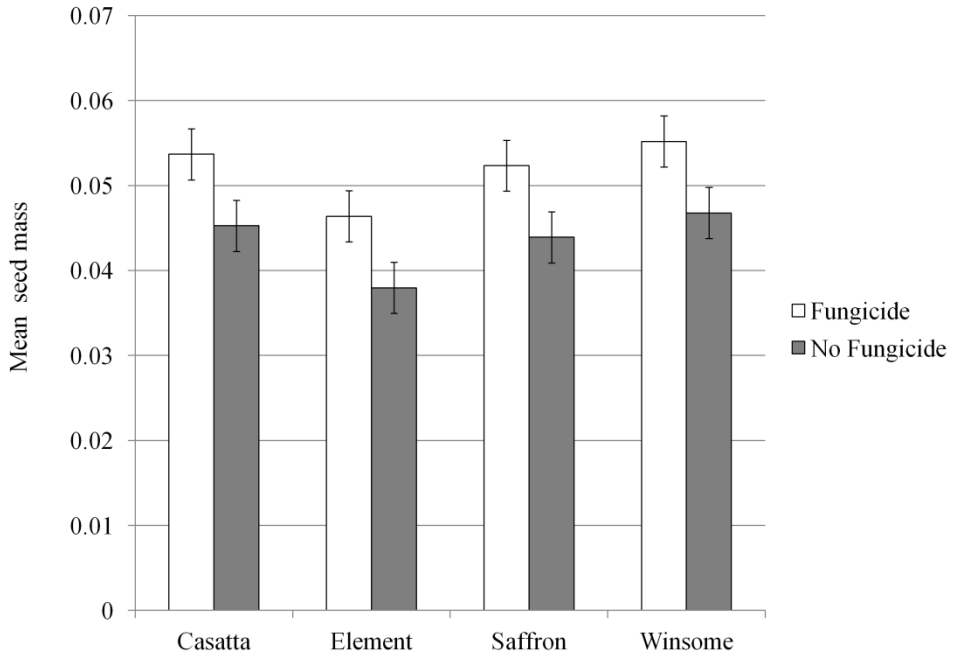

Fig. 4.7: Mean seed mass for four winter barley varieties grown in monoculture and 3-way mixture in fungicide treated or untreated plots in a field trial. N=1600. Error bars show 95% confidence interval of means.

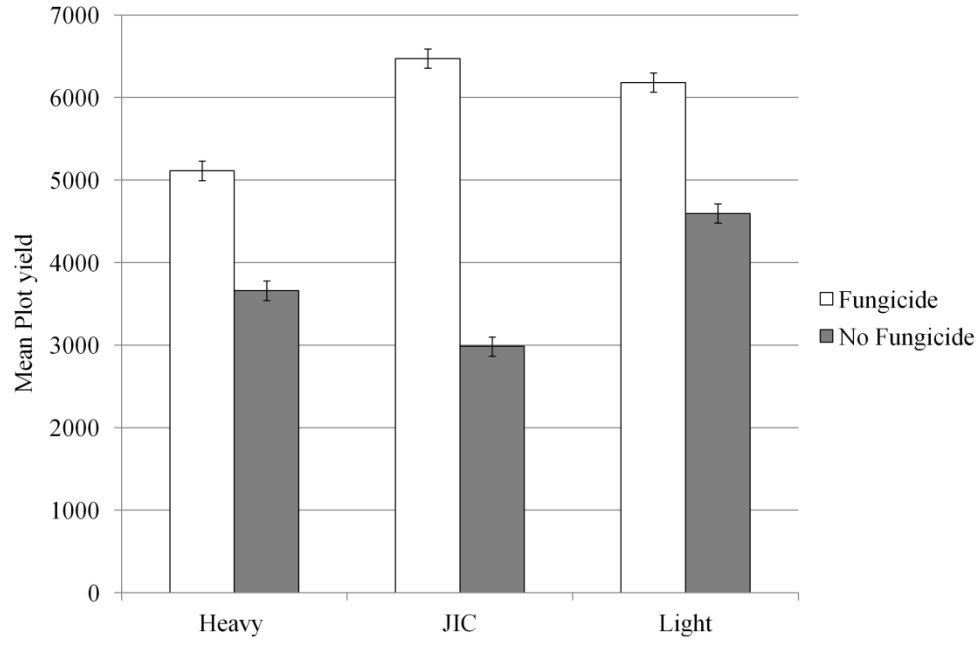

Fig. 4.8: Mean plot yields (per $6m^2$) for winter barley variety monocultures and two 3-way varietal mixtures in fungicide treated or untreated plots in a field trial at three different sites. N=1600. Error bars show 95% confidence interval of means.

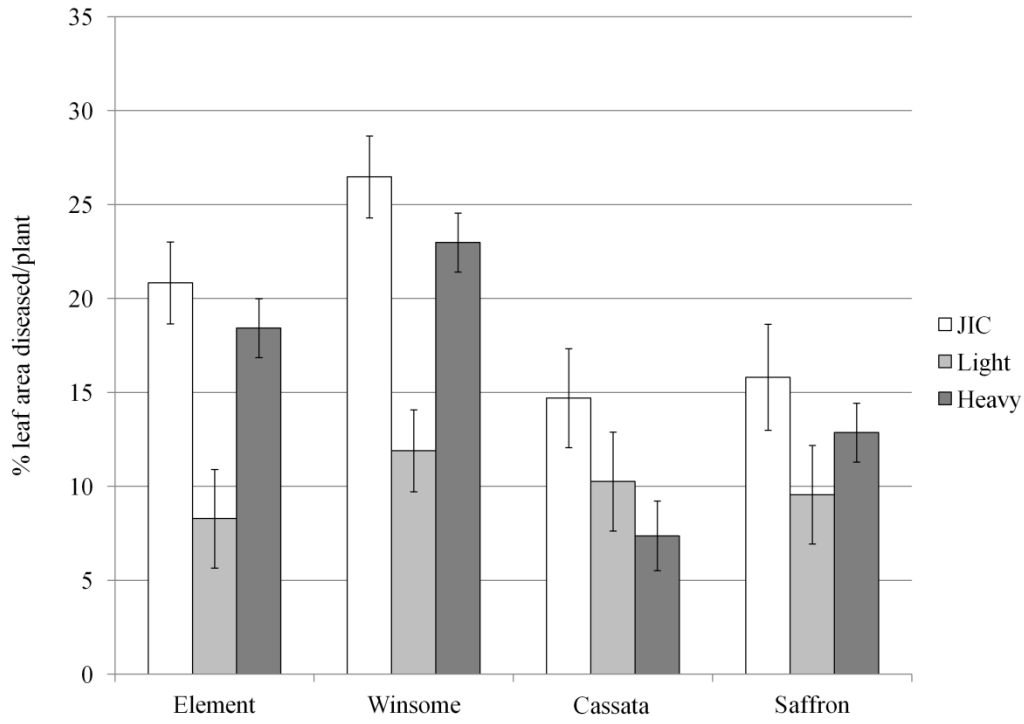

Fig. 4.9: Mean percentage green leaf area on the flag and first leaves showing signs of brown rust infection. Disease scores for individual plants grown in mixture and monoculture and naturally infected under field conditions conducted at three sites located in Norfolk. JIC=John Innes Centre site. Light= light land trial site at Bawburgh. Heavy= heavy land trial site at Bawburgh. N=1600. Error bars show 95% confidence interval of means.

Winsome was very prone to lodging especially on the light sandy soil of JIC, indicating that the growth regulators had a poor effect on reducing lodging at this site. (Fig. 4.10; Table A4.5). Severe lodging of Winsome in monoculture resulted in a very high relative yield of mixtures in fungicide treated plots at JIC (Fig. 4.3). Winsome did not lodge on the heavy land site due to reduced plant height and fewer pigeons, which contributed to the severe lodging at JIC by flattening the crop to feed on grain close to the ground (personal observation) (Fig. 4.10). Mixtures were more resistant to lodging through facilitation by Element. This variety has strong straw, and therefore reduced lodging in neighbouring Winsome plants, which contributed to increased yield stability in mixtures (Fig. 4.10).

a) b)

Fig. 4.10: Lodging of monoculture plots of winter barley variety Winsome at the John Innes Centre field trial site. a) Photo taken on 1st June 2012. b) Photo taken on 22nd June 2012.

4.4 Discussion

This study investigated the ability of winter barley varietal mixtures to buffer against environmental stresses, mainly disease but also unexpected abiotic stresses, and stabilise yield across multiple natural environments. Mixture yields were as high as the best performing monocultures, indicating no yield penalty of growing mixtures. Varietal mixtures enhanced yield stability compared to component monocultures, providing further support for the hypothesis that biodiversity increases ecological stability (Yachi and Loreau 1999). Yield stability was largely achieved through the ecological processes of compensation and facilitation. In mixtures, the most competitive variety (the hybrid 6-row variety, Element) compensated for yield losses associated with less competitive varieties (e.g. Cassata) through competitive release. Element also reduced lodging in mixtures, increasing fitness of neighbouring plants by facilitation. Previous work with the model plant *Arabidopsis thaliana* demonstrated that genotypes with the highest yield potential are often the most competitive, allowing them to over-yield in mixture

through a reduction in competition intensity compared to monoculture (Creissen *et al.* 2013). Indeed in this study, the 6-row cultivar, which had the highest yield potential, was responsible for compensation observed within the mixtures.

Ecological processes that contribute towards increased yield stability in mixtures can be identified under laboratory or glasshouse conditions (Creissen *et al.* 2013), yet experimentation under field conditions may be specific to the variety and particular combination of stresses present. Crops are grown in environments in which multiple stresses are present that prevent them from achieving their yield potential (Bray *et al.* 2000). Abiotic stresses alone can reduce average yields by more than 50% (Bray *et al.* 2000), yet plants must cope with stresses such as cold, drought and salinity whilst simultaneously defending themselves from pests and pathogens ranging from fungi and bacteria, to nematodes and insects (Hammond-Kosack and Jones 2000). Examining stress tolerance by exposing the plant to individual stresses may lead to inaccurate predications, even if care is taken to relate experimental conditions to natural or field conditions (Mittler and Blumwald 2010). Interactions between biotic and abiotic stresses experienced by plants grown under field conditions can result in varieties responding unpredictably (Mittler 2006; Atkinson and Urwin 2012). Abiotic stresses can have positive or negative effects on disease susceptibility in ways difficult to replicate under laboratory or glasshouse conditions. Indeed this study showed high levels of variation in disease severity between geographically similar sites. The most heavily diseased site, JIC, was slightly sheltered by nearby buildings leading to higher humidity and temperature, providing a more conducive environment for disease than at the other two sites. Studies investigating the effect of multiple stresses on plant productivity and stability under field conditions are vital as they more accurately represent the unpredictable environmental conditions experienced by crop plants in agricultural systems.

The majority of empirical studies on variety mixtures have focussed on disease control (Finckh *et al.* 2000; Zhu *et al.* 2000; Mundt 2002), reporting trends in yield and disease severity for the population (Mundt 2002; Philips *et al.* 2005; Newton and Guy 2009), yet varietal mixtures also offer protection against unexpected stresses related to unpredictable environmental conditions. Few studies have focussed on the plant-plant interactions and ecological processes taking place within mixtures (but see Allard and

Adams 1969; Finckh and Mundt 1992; Revilla-Molina *et al.* 2009). Despite the prediction from HGCA recommended lists (http://www.hgca.com) that Element would be the most susceptible to brown rust infection, Winsome was the most susceptible in the trials reported here. Despite high levels of disease in Winsome monocultures, brown rust infection was reduced on Winsome in mixtures. This reduction in disease severity may act through a combination of increased distance between susceptible hosts, and barriers of resistant plants preventing pathogen spread (Chin and Wolfe 1984; Zhu *et al.* 2000). Despite high levels of disease in mixtures, compensation by resistant plants increased yield stability through competitive release.

Compensation did indeed play a role in enhancing yield stability in varietal mixtures in this study, however yield stability was also achieved by unexpected methods in response to unpredictable stresses such as the combination of lodging and herbivory by pigeons. Lodging poses a significant global threat to farming and can reduce crop yields by up to 60% (Rajkumara 2008). In this study Winsome proved to be highly prone to lodging (especially at JIC) due to a combination of weak straw, irrigation (only at JIC site), early ripening (especially on fungicide-untreated plots), and large numbers of pigeons that further flattened the plants to feed on the grain lying on the ground. The lodging-resistant variety Element reduced lodging of the entire plant population through facilitation. This finding was not totally unexpected, as Element is recommended by the HGCA for use in the North of the UK, where crops are more likely to experience environmental stresses such as rain and high winds. Such stresses increase lodging so a variety with stronger straw, providing increased lodging resistance, is generally favoured. Barley plants in this study experienced conditions more typical of a northern environment, with heavy wind and rain in the weeks prior to harvest that were unusual for East Anglia. The beneficial effect of mixtures on reducing lodging has been observed previously in several crops, including winter barley (Stutzel and Aufhammer 1989) and rice (Revilla-Molina *et al.* 2009). Unforeseen environmental stresses due to climate observed in our study highlight the importance of careful mixture selection and experimentation under field conditions for accurate assessment of mixture performance.

Despite the many advantages of growing barley varietal mixtures, such as increased disease resistance, increased tolerance to abiotic stresses, increased yield and yield stability, adoption of this practice remains restricted (Newton *et al.* 1997; Finckh *et al.*

2000; Mundt 2002; Newton and Guy 2009). Mixtures have historically been unacceptable to maltsters and millers who have issues regarding grain heterogeneity, grain verification and customer preference, as well as processing difficulties. However, grain consistency and grain quality has been shown to be equal to and even better than the sum of the mixture components (Newton *et al.* 2008a).Approximately 50% of the barley produced in the UK is used for animal feed for which grain consistency is less of a concern than it is for brewers (Newton *et al.* 2011). A major problem of growing mixtures is the uncertainty about the agronomy in which the requirements of multiple varieties must be considered. Variation in heading date between varieties in varietal mixtures may also create problems at harvest. Despite these issues the benefits of growing mixtures include reduced cost of chemical inputs, in turn reducing the cost of crop production, which makes the lack of uptake across the UK surprising. Future cropping systems will need to be less reliant on chemical input, less expensive to manage and show greater adaptability to the changing environment if future food security is to be achieved (FAO, WFP, IFAD 2012; Hillocks 2012). Varietal mixtures designed to exploit beneficial ecological processes such as compensation and facilitation will be more adaptable to a wider range of environments enabling them to achieve high and stable yields by buffering against diverse and sometimes unpredictable stresses.

Chapter 5
General Discussion

Variety monocultures able to achieve high yields dominate commercial production (Soliman and Allard 1991; Trewavas 2001). However such systems are only high yielding under specific environmental conditions and agronomic practices, which makes them susceptible to high yield losses associated with environmental stress (Calderini and Slafer 1999). Varietal mixtures have the potential to increase yield stability and buffer against environmental stresses whilst being less reliant on chemical inputs (Wolfe 1985; Altieri 1999; Zhu *et al.* 2000). Previous studies investigating the advantages and disadvantages of growing varietal mixtures have typically been large in scale because of the high levels of variation associated with uncontrolled environments and genotype by environment interactions (Ceccarelli and Grando 1991: Madden *et al.* 2007). This project used a model system in which experiments could be conducted under more readily controlled environmental conditions, to allow for detailed investigation of the mechanisms and ecological processes contributing to the success of mixtures in plant ecosystems. Work conducted in the model system was translated into the crop winter barley to facilitate the exploitation of varietal mixtures in agriculture.

Chapters 2 and 3 of this thesis tested the suitability of *Arabidopsis thaliana* (herein referred to as *Arabidopsis*) as a model for studying the mechanisms underlying the enhanced stability of genotypic mixtures across environments. The ability of *Arabidopsis* to grow well under glasshouse conditions means that fewer plants and smaller growing spaces are required to achieve consistent results across experiments. However, in practice this is not always the case as was observed in Chapter 2 in which inconsistencies in glasshouse conditions between experimental repeats affected plants growth and seed production. Despite these inconsistencies *Arabidopsis* genotypic diversity enhanced ecological resistance of the population to nutrient stress, and the combination of nutrient and heat stress, shown by an increase in yield and yield stability compared to the average monoculture. Yield stability was achieved through compensation in which the fittest, most competitive genotype with high yield potential overyielded in genotypic mixtures, compensating for the sub-optimal yield of others. The role of compensation in buffering against stress is supported by findings from

previous studies in agricultural (Finckh *et al.* 2000), and grassland systems (Tilman 1996). The outcome of competition was predictable from above-ground traits such as flowering time, rosette size and seed production, yet below ground competition was identified as being more important than above ground competition in *Arabidopsis.* Findings from chapter 2 highlighted the importance of studying below ground interactions between adult plants in order to predict accurately the outcome of competition between genotypes, an area that has previously been overlooked in many competition studies (Casper and Jackson 1997). The accurate estimation of genotype mixing ability may increase the efficiency of mixture selection substantially in the future (Knott and Mundt 1990).

The role of intra- and inter-genotypic competition in ecological processes operating at the population scale in diseased *Arabidopsis* populations was investigated experimentally in chapter 3. In these large-scale experiments I took advantage of results from chapter 2 that identified traits affecting competitive ability of genotypes and suitable experimental procedures. Phenotypically dissimilar genotypes varying in pathogen compatibility with the oomycete *Hyaloperonospora arabidopsidis* or the *Turnip yellows virus* were grown in a competitive environment to examine the effects of competition on seed production for both individuals and populations. Host fitness and competitive ability were predicted to be reduced by either susceptibility to the pathogen or through costs of resistance (Brown 2002; Damgaard and Jensen 2002; Tian *et al.* 2003; Bedhomme *et al.* 2005; Pagan *et al.* 2009). A pathogen-induced reduction in competitive ability for susceptible genotypes led to an increase in competitive ability for resistant genotypes. Compensation occurring within diseased and non-diseased populations led to high and stable yields in mixtures of two genotypes supporting findings from previous mixture studies (Stutzel and Aufhammer 1989; Kiaer *et al.* 2012). The most genotypically diverse mixtures (4-way mixtures) achieved lower and less stable yields than pots containing fewer genotypes. This indicates an optimum level of diversity, which contrasts with the majority of crop varietal mixture studies that show a trend towards greater yields from increased number of varieties (Mundt *et al.* 1994; Newton *et al.* 1997; Newton *et al.* 2008a). The highest yields were produced by mixtures and monocultures of weakly competing, relatively fecund genotypes. Mixtures and monocultures of highly competitive genotypes achieved the lowest yields due to high levels of competition forcing the plants to invest excessively in vegetative growth.

This reduced reproductive output and the results therefore support the findings from previous studies (Khalifa and Qualset 1974; Creissen *et al.* 2013). Results of chapter 3 indicate that suitable mixture components are moderately high yielding but not highly competitive. This is valuable knowledge to those designing mixtures since high levels of competition can dramatically reduce plant yield.

The possibilities for experimental research into ecological processes using the *Arabidopsis* model system cover a wide range of scenarios relevant to arable crops. Research presented in chapters 2 and 3 indicate that *Arabidopsis* has great potential to provide further insights into the mechanisms occurring within genotypic mixtures, but it also has potential to be exploited as a model in other areas of crop research such as intercropping. Experiments in which *Arabidopsis* and other species, such as nitrogen-fixing clovers (*Trifolium*), compete for resources would for example allow for investigations of complementation and facilitation between intercrops. Knowledge of these mechanisms gained from such model systems can be translated into the crops and increase efficiency of intercrop selection.

Arabidopsis genotypes (often referred to as accessions) are highly variable for many physiological traits (e.g. flowering time, pest and disease resistance) and developmental traits (e.g. leaf shape, size and number). *Arabidopsis* genotypes are often referred to as ecotypes as they show signs of local adaptation to specific environments which contribute to the high levels of trait diversity within the species. Conversely, domestication of crop plants has heavily shaped their evolution over the last 11,000 years through selective breeding by man (Ceccarelli 2009). Presently, crop plants are bred largely for high yields and uniformity in important agronomic traits such as height and maturity, for ease of harvest. Widely grown varieties of heavily bred crops such as rice, wheat and maize are very closely related and genetically uniform. However *Arabidopsis* genotypes are considerably more phenotypically diverse due to the broad range of environments in which the weed occurs, which raises issues regarding its suitability as a model for heavily bred cereal crops. As a result of high trait diversity the effect of genotypic diversity on interplant competition and population processes is likely to be amplified in the model species relative to the crop, which may create issues when attempting to extrapolate findings from the model to the crop.

Arabidopsis has, in the work presented here, provided insight into the ecological processes occurring within genotypic mixtures (Chapter 2, 3). Issues can, of course, be raised regarding the suitability of this brassica-relative as a model for cereal varietal mixtures. Brassicas are cruciferous vegetables including cabbages, broccoli and mustards that are dramatically different from cereals in both physiology and form. An important difference between cereals and brassicas is that cereals, unlike most Brassicaceae, form mycorrhizae which can greatly affect plant growth (Khan 1975). Direct comparisons of traits relating to competitive ability and yield can be difficult between brassicas and cereals, which can restrict translational science from the *Arabidopsis* model to cereal crops. There is an argument to be made for the use of the wild grass *Brachypodium distachyon* (herein referred to as *Brachypodium*) as a model for cereal cropping systems. *Brachypodium* is related to small grain cereals and possesses model organism traits such as a short life cycle and a small genome (Opanowicz *et al.* 2008). Many important diseases of cereal crops infect *Brachypodium* allowing the model to contribute towards an understanding of the mechanisms of plant defence in response to fungal diseases such as fusarium head blight, eyespot and ramularia leaf spot (Peraldi *et al.* 2011: Peraldi *et al.* 2013). However, the use of a model organism to study specific crop diseases is often limited due to highly specific host-pathogen interactions. Despite not being useful for studying the effects of a specific disease of cereals, *Arabidopsis* may be of value for studying the effects of disease in general or even of a particular class of disease. *Arabidopsis* is ideally suited to ecological experiments as seeds can be sown into experimental layout quickly with minimal preparation time. Preparation for ecological studies in *Brachypodium* is more laborious as seed cases must be removed for synchronous germination (A. Peraldi personal communication), which is necessary as asynchrony of germination can dramatically affect the outcome of competition (Bengtsson *et al.* 1994). Genetic resources readily available to scientists are substantially less for *Brachypodium* than for *Arabidopsis* which has over 750 natural accessions and many mutant lines, catalogued at stock centres (ABRC, http://abrc.osu.edu/; NASC, http://*Arabidopsis*.info/). However, as the studies of *Brachypodium* as a model plant increase, the number of genetic resources available for research purposes is likely to increase (Mur *et al.* 2011). Presently, genotypic variation is less documented for *Brachypodium* than for *Arabidopsis,* which means that preliminary phenotypic assessments required for competition studies must be more extensive.

Key plant traits relating to competitive ability (vegetative growth allocation, disease resistance etc.), and key processes (compensation), were identified in the *Arabidopsis* studies of Chapters 2 and 3. These findings were translated into a study investigating the ability of winter barley varietal mixtures to buffer against environmental stresses, namely disease, and stabilise yield over multiple environments (Chapter 4). Predictions on the outcomes of competition and compensatory interactions occurring within the mixtures were made based on traits such as height, disease resistance, classification (2- or 6-row, malting or feed), and straw strength. Varietal mixtures achieved greater yield stability across sites and treatments and produced yields equal to the best performing monoculture indicating no yield penalty of growing mixtures. Compensation played a role in the yield stability of mixtures, which was predicted from the work conducted in *Arabidopsis* (chapters 2 and 3) and previous work in mixtures (Finckh *et al.* 2000). Unexpectedly facilitation played a major role in the ecological resistance of barley varietal mixtures to environmental stress. Facilitation occurred when the 6-row variety reduced lodging, and the subsequent damage by feeding pigeons, in mixtures that contained a 2-row variety prone to lodging. The role of facilitation in varietal mixtures is often overlooked though studies have observed its role in reducing lodging (Stutzel and Aufhammer 1989; Revilla-Molina *et al.* 2009). Facilitation may also be responsible for the reduction in brown rust infection in mixtures. By increasing distance between host plants disease was reduced for the most susceptible variety. This contrasts with many mixture studies that have shown an 'averaging effect' of mixtures in decreasing disease on the most susceptible varieties but increasing disease on more resistant varieties (Wolfe 1985). Environmental stresses experienced by crops grown under field conditions can result in unpredictable responses and in this case unexpected ecological processes (Mittler 2006; Atkinson and Urwin 2012). This finding makes the point that conclusions from lab based experiments on a model system, though often insightful, are inevitably limited. In this study, interactions between genotype, disease severity and soil types confirmed the need for experimentation under field conditions in which plants are exposed to multiple, interacting stresses (Mittler and Blumwald 2010). Results of Chapter 4 suggest that predicting the response of both monocultures and mixtures to local environmental conditions can be difficult. Higher levels of trait diversity increase the adaptive capability of the crop, allowing the population to respond favourably to environmental change. Maintenance of crop trait diversity and identification of suitable

mixture components designed to promote beneficial ecological processes, are necessary to ensure the successful exploitation of mixtures in agriculture.

Alternative methods of increasing within-field genetic diversity include the use of composite cross populations (CCP) in which diverse genotypes are re-hybridized and the progeny bulked prior to the subjection to natural selection (Suneson 1956). The success of a CCP depends upon recombination and segregation over many generations and the relationship between survival and agronomic value (Allard and Hansche 1964). Potential advantages of CCP are similar to those of varietal mixtures such as increased buffering capacity against environmental stresses, and increased yield and yield stability compared to monocultures through compensation and complementation (Allard 1961; Rasmusson *et al.* 1967; Hockett *et al.* 1983; Danquah and Barrett 2002). However, despite the potential advantages, inter-plant competition occurring within CCP can often lead to natural selection for taller plants that are more prone to lodging, and later maturity dates which create problems at harvest (Patel *et al.* 1987). *Arabidopsis* could be used as a model to study competition and adaptation in CCP through the use of Multiparent Advanced Generation Inter-Cross (MAGIC) lines, a set of recombinant inbred lines descended from 19 intermated accessions (Kover *et al.* 2009). Experiments in which the MAGIC lines are grown in competition under glasshouse conditions would allow for testing of the effects of specific single and multiple stress combinations on population dynamics in diverse populations. As with varietal mixture, issues remain regarding quality consistency of end-products produced by CCP, yet evidence suggests that quality can be more stable in variety mixtures and CCP than monocultures (Sarandon and Sarandon 1995; Newton *et al.* 1998). Despite findings from such studies, many grain processers, maltsters in particular, will only accept grain from single variety monocultures for ease of processing (Newton *et al.* 2008b). The cultivation of CCP and varietal mixtures may be a particularly viable strategy for low-input and subsistence farming in developing countries in which yield stability remains the highest priority (Ceccarelli 1996; Danquah and Barrett 2002). Future commercial cropping systems will need to be less reliant on chemical inputs, less expensive to manage and show greater adaptability to the changing environment if future food security is to be achieved (FAO, WFP, IFAD 2012; Hillocks 2012). CCP and varietal mixture approaches to farming offer a potential solution to such important food production issues.

Subsistence farming, in which the main output is directly consumed, is common in the poorest areas of the world such as Africa and Asia (Nagayets 2005; von Braun 2005). It is estimated that approximately 50% of the world's population are small-scale subsistence farmers (Jazairy *et al.* 1992). The high prevalence of subsistence farmers in poverty stricken areas prone to hunger and malnutrition highlights the need for improvement of agricultural practices. Yield stability is the main priority because crop failure can have hugely detrimental effects on the livelihoods of the smallholder. In contrast to commercial farming, subsistence farming approaches consist of very low levels of technology and high labour costs. The crops grown also differ substantially between commercial and subsistence farming systems. Commercial farmers grow high yielding improved varieties whereas subsistence farmers favour locally adapted, genetically diverse crop landraces (Smithson and Lenne 1996). Varietal mixtures are regularly deployed in subsistence farming, and often involve landraces, improved varieties and populations (Smithson and Lenne 1996). Diverse mixtures containing multiple species and varieties are common. The mixture *hanfets* is an example, containing multiple varieties of both wheat and barley cultivated in North Africa. Woldeamlak *et al.* (2008) showed that *hanfets* can achieve 50% greater yields than the pure crops, thought to be largely due to complementation in root architecture which increases water-use efficiency in drought stricken areas. The lack of research conducted in such unusual cropping systems restricts the potential for agronomic improvement, because the plant traits and ecological processes responsible for increased yield and yield stability remain poorly understood.

Participatory plant breeding, in which farmers collect, propagate and select germplasm under local agro-ecological conditions leading to the creation of locally adapted varieties, also plays a significant role in subsistence farming. Participatory rice breeding in particular has led to a huge amount of varieties adapted to a wide range of environmental conditions such as dryland or paddy, upland or lowland (Medina 2012). Unfortunately a lot of this diversity has been lost though the development of high yielding varieties during the green revolution. Such varieties are dependent upon high chemical inputs, which have led to a reduction in sustainability due to depletion of soil organic matter, mineral deficiencies, toxicities etc. In Indonesia, for example, approximately 1500 rice varieties have were lost between 1975 and 1990 (Ryan 1992). Green revolution technologies resulted in rice farmers becoming disconnected with their

crop. Participatory breeding programmes provide training for farmers at all stages of the breeding process which increases farmer confidence in their ability to breed crops.

Research into subsistence farming systems remains minimal due to the many issues regarding agricultural research in poor, often politically unstable areas of the world where subsistence farming is the dominant source of food production. Subsistence farming systems are highly vulnerable to climate change, as they are largely located in the tropics where even small changes in temperature (1-2ºC) could dramatically reduce productivity of the major crops such as rice and maize (Morton 2007). Various demographic and socioeconomic trends also restrict adaptation to change in such places. The future problems facing subsistence farmers accentuate the need for increased research efforts to improve subsistence farming practices (Morton 2007). Detailed knowledge of the roles of ecological processes in such systems may facilitate the exploitation of plant diversity in subsistence agroecosystems and contribute to increased food production and financial security of the world's poorest people. This project has provided valuable insight into the use of model systems to investigate plant traits responsible for determining the outcome of competitive interactions and the influence of such interactions on the relative roles of specific ecological processes (compensation and facilitation) in achieving yield stability in genotypic mixtures. Findings from these empirical studies should be considered by plant breeders, agronomists and agroecologists when selecting suitable, functionally complementary mixture components. Efficient mixture choice will ensure crop success in the future in which changing environmental conditions will expose plants to multiple, unpredictable, interacting stresses.

Refrence:

Aarssen LW, Clauss MJ. 1992. Genotypic variation in fecundity allocation in *Arabidopsis thaliana. Journal of Ecology* **80**, 109-114.

Adam L, Somerville SC. 1996. Genetic characterization of five powdery mildew disease resistance loci in *Arabidopsis thaliana*. *Plant Journal* **9**, 341-356.

Agnew P, Koella JC, Michalakis Y. 2000. Host life history responses to parasitism. *Microbes and Infection* **2**, 891-896.

Akanvou RK, Becker M, Bastiaans L, Kropff MJ. 2007. Morpho-physiological characteristics of Cover crops for analysis of upland rice production in relay intercropping systems. *Sciences and Nature* **4,** 205-216.

Alexander HM, Holt RD. 1998. The interaction between plant competition and disease. *Perspectives in Plant Ecology, Evolution and Systematics* **1**, 206-220.

Allan E, van Ruijven J, Crawley MJ. 2010. Foliar fungal pathogens and grassland biodiversity. *Ecology* **91**, 2572-2582.

Allan E, Weisser W, Weigelt A, Roscher C, Fischer M, Hillebrand H. 2011. More diverse plant communities have higher functioning over time due to turnover in complementary dominant species. *Proceedings of the National Academy of Sciences* **108**, 17034-17039.

Allard RW, Adams J. 1969. Population studies in predominantly self-pollinating species. 13. Intergenotypic competition and population structure in barley and wheat. *American Naturalist* **103**: 621-645.

Allard RW, Hansche PE. 1964. Some parameters of population variability and their implications in plant breeding. *Advances in Agronomy* **16**, 281–325.

Allard RW. 1961. Relationship between genetic diversity and consistency of performance in different environments. *Crop Science* **1**, 127–133.

Altieri MA. 1999. The ecological role of biodiversity in agroecosystems. *Agriculture Ecosystems & Environment* **74**, 19-31.

Andalo C, Goldringer I, Godelle B. 2001. Inter- and intragenotypic competition under elevated carbon dioxide in *Arabidopsis thaliana. Ecology* **82**, 157-164.

Andersen MK, Hauggaard-Nielsen H, Weiner J, Jensen ES. 2007. Competitive dynamics in two- and three-component intercrops. *Journal of Applied Ecology,* **44**, 545-551.

Andow DA. 1991. Vegetational diversity and arthropod population responses. *Annual review of Entomology,* **36**, 561-586.

Antonelli A, Humphreys AM, Lee WG, Linder HP. 2011. Absence of mammals and the evolution of New Zealand grasses, *Proceedings of the Royal Society B-Biological Sciences* **278**, 695-701,

Arany AM, de Jong TJ, van der Meijden E. 2005. Herbivory and abiotic factors affect population dynamics of *Arabidopsis thaliana* in a sand dune area. *Plant Biology* **7**, 549-555.

Atkinson NJ, Urwin PE. 2012. The interaction of plant biotic and abiotic stresses: from genes to the field. *Journal of Experimental Botany* **63**, 3523-3543.

Bai GH, Shaner G. 1994. Scab of wheat - prospects for control. *Plant Disease* **78**, 760-766.

Bai YF, Han XG, Wu JG, Chen ZZ, Li LH. 2004. Ecosystem stability and compensatory effects in the Inner Mongolia grassland. *Nature* **431**, 181-184.

Bazzaz FA, Chiariello NR, Coley PD, Pitelka LF. 1987. Allocating resources to reproduction and defense. *Bioscience* **37**, 58-67.

Bazzaz FA, McConnaughay KDM. 1992. Plant plant interactions in elevated CO2 environments. *Australian Journal of Botany* **40**, 547-563.

Beddington J. 2009. *Food, Energy, Water and the Climate: a Perfect Storm of Global Events? Lecture to Sustainable Development UK 09 Conference*. [WWW document] URL http://www.bis.gov.uk/assets/goscience/docs/p/perfect-storm-paper.pdf [accessed 18 February 2013].

Bedhomme S, Agnew P, Vital Y, Sidobre, C, Michalakis Y. 2005. Prevalence-dependent costs of parasite virulence. *PLoS Biology* **3**, 1403-1408.

Begon M, Harper JL, Townsend CR. 1996. *Ecology: individuals, populations and communities.* 3rd edn. Blackwell Science London.

Bengtsson J, Fagerstrom T, Rydin H. 1994. Competition and coexistence in plant communities. *Trends in Ecology & Evolution* **9**, 246-250.

Bertness MD, Callaway R. 1994. Positive forces in natural communities. *Trends in Ecology and Evolution* **9**, 191-193.

Bertness MD, Hacker SD. 1994. Physical stress and positive associations among march plants. *American Naturalist* **144**, 363-372.

Boch J, Joardar V, Gao L, Robertson TL, Lim M, Kunkel BN. 2002. Identification of *Pseudomonas syringae* pv tomato genes induced during infection of *Arabidopsis thaliana. Molecular Microbiology* **44**, 73-88.

Bonser SP, Ladd B. 2011. The evolution of competitive strategies in annual plants. *Plant Ecology* **212,** 1441-1449.

Bos JIB, Prince D, Pitino M, Maffei ME, Win J, Hogenhout SA. 2010. A functional genomics approach identifies candidate effectors from the aphid species *Myzus persicae* (Green Peach Aphid). *Plos Genetics* **6**.

Bouchabke O, Chang F, Simon M, Voisin R, Pelletier G, Durand-Tardif M. 2008. natural variation in *Arabidopsis thaliana* as a tool for highlighting differential drought responses. *Plos One* **3**.

Boyer JS. 1982. Plant Productivity and Environment. *Science* **218**, 443-448.

Boyes DC, Zayed AM, Ascenzi R *et al.* 2001. Growth stage-based phenotypic analysis of *Arabidopsis*: A model for high throughput functional genomics in plants. *Plant Cell* **13**, 1499-1510.

Bradley DJ, Gilbert GS, Martiny JBH. 2008. Pathogens promote plant diversity through a compensatory response. *Ecology Letters* **11**, 461-469.

Brassard BW, Chen HYH, Bergeron Y, Pare D. 2011. Differences in fine root productivity between mixed- and single-species stands. *Functional Ecology* **25**, 238-246.

Bray EA, Bailey-Serres J, Weretilnyk E. 2000. Responses to abiotic stresses. *In* W Gruissem, B Buchannan, R Jones, eds, Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologists, Rockville, MD, pp 1158–1249.

Brooker RW, Callaway RM. 2009. Facilitation in the conceptual melting pot. *Journal of Ecology* **97**, 1117-1120.

Brooker RW, Maestre FT, Callaway RM e*t al.* 2008. Facilitation in plant communities: the past, the present, and the future. *Journal of Ecology* **96**, 18-34.

Brown JKM, Handley RJ. 2005. Evolutionary genetics: Fight or flinch? *Heredity*, **96**, 3-4.

Brown JKM. 2002. Yield penalties of disease resistance in crops. *Current Opinion in Plant Biology* **5:** 339-344.

Brown JKM, Hovmoller MS. 2002. Epidemiology - Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science* **297,** 537-541.

Brown JKM, Tellier A. 2011. Plant-Parasite Coevolution: Bridging the Gap between Genetics and Ecology. *In*: VanAlfen NK, Bruening G, Leach JE eds. *Annual Review of Phytopathology* **49**, 345-367.

Burdon JJ, Groves RH, Kaye PE, Speer SS. 1984. Competition in mixtures of susceptible and resistant genotypes of *Chondrilla juncea* differentially infected with rust. *Oecologia* **64**, 199-203.

Burdon JJ, Thrall PH, Ericson L. 2006. The current and future dynamics of disease in plant communities. *Annual Review of Phytopathology*. **44**, 19-39.

Cahill JF, Kembel SW, Gustafson DJ. 2005. Differential genetic influences on competitive effect and response in *Arabidopsis thaliana*. *Journal of Ecology* **93**, 958-967.

Cahill JF. 2002. Interactions between root and shoot competition vary among species. *Oikos* **99**, 101-112.

Calderini DF, Slafer GA. 1998. Changes in yield and yield stability in wheat during the 20th century. *Field Crops Research* **57,** 335-347.

Calderini DF, Slafer GA. 1999. Has yield stability changed with genetic improvement of wheat yield? *Euphytica* **107**, 51-59.

Callaway RM. 1995. Positive interactions among plants. *Botanical Review* 61, 306-349.

Callaway RM. 2007. *Positive Interactions and Interdependence in Plant Communities, XI*. New York, USA: Springer.

Callaway RM, Brooker RW, Choler P *et al.* 2002. Positive interactions among alpine plants increase with stress. *Nature* **417**, 844-848.

Callaway RM, Walker LR. 1997. Competition and facilitation: a synthetic approach to interactions in plant communities. *Ecology* **78**, 1958-1965.

Cardinale BJ, Matulich KL, Hooper DU *et al.* 2011 The functional role of producer diversity in ecosystems. *American Journal of Botany* **98**, 572-592.

Casper BB, Jackson RB. 1997. Plant competition underground. *Annual Review of Ecology and Systematics* **28**, 545-570.

Ceccarelli S. 1996. Adaptation to low high input cultivation. *Euphytica* **92**, 203-214.

Ceccarelli S. 2009. Evolution, plant breeding and biodiversity. *Journal of Agriculture and Environment of International Development* **103**, 131-145.

Ceccarelli S, Grando S. 1991. Selection environment and environmental sensitivity in barley. *Euphytica* **57**, 157-167.

Chang C, Kwok SF, Bleecker AB, Meyerowitz EM. 1993. Arabidopsis ethylene-response gene ETR1 – similarity of product to 2-component regulators. *Science* **262**, 539-544.

Chase JM, Abrams PA, Grover JP *et al.* 2002. The interaction between predation and competition: a review and synthesis. *Ecology Letters* **5**, 302-315.

Chin KM, Wolfe MS. 1984. The spread of *Erysiphe-graminis* f.sp. *hordei* in mixtures of barley varieties. *Plant Pathology* **33**, 89-100.

Cipollini DF. 2002. Does competition magnify the fitness costs of induced responses in *Arabidopsis thaliana*? A manipulative approach. *Oecologia* **131**, 514-520.

Clark AJ, Decker AM, Meisinger JJ. 1994. Seeding rate and kill date effects on hairy vetch-cereal rye cover crop mixtures for corn production. *Agronomy Journal* **86**, 1065-1070.

Clauss MJ, Aarssen LW. 1994. Phenotypic plasticity of size-fecundity relationships in *Arabidopsis* thaliana. *Journal of Ecology* **82**, 447-455.

Clark MF, Adams AN. 1977. Characteristics of microplate method of enzyme-linked immunosorbent assay for detection of plant viruses. *Journal of General Virology* **34**, 475-483.

Coates ME, Beynon JL. 2010. *Hyaloperonospora arabidopsidis* as a Pathogen Model. *Annual Review of Phytopathology* **48**, 329-345.

Coen ES, Meyerowitz EM. 1991. The war of the whorls - genetic interactions controlling flower development. *Nature* **353**, 31-37.

Coley PD, Bryant JP, Chapin FS III. 1985. Resource availability and plant anti-herbivore defence. *Science* **230**, 895-899.

Cooper AJ, Latunde-Dada AO, Woods-Tor A *et al.* 2008. Basic compatibility of *Albugo candida* in *Arabidopsis thaliana* and *Brassica juncea* causes broad-spectrum suppression of innate immunity. *Molecular Plant-Microbe Interactions* **21**, 745-756.

Cowger C, Mundt CC. 2002. Effects of wheat cultivar mixtures on epidemic progression of Septoria tritici blotch and pathogenicity of *Mycosphaerella graminicola*. *Phytopathology* **92**, 617-623.

Cowger C, Weisz R. 2008. Winter wheat blends (mixtures) produce a yield advantage in north Carolina. *Agronomy Journal* **100**, 169-177.

Craine JM. 2006. Competition for nutrients and optimal root allocation. *Plant Soil* **285**, 171-185.

Craine JM, Dybzinski R. 2013. Mechanisms of plant competition for nutrients, water and light. *Functional Ecology* **27**, 833-840.

Creissen HE, Jorgensen, TH, Brown JKM. 2013. Stabilisation of yield in plant genotype mixtures through compensation rather than complementation. *Annals of Botany* **112**, 1439-1447.

Crutsinger GM, Souza L, Sanders NJ. 2008. Intraspecific diversity and dominant genotypes resist plant invasions. *Ecology Letters* **11**, 16-23.

Damgaard C, Jensen BD. 2002. Disease resistance in *Arabidopsis thaliana* increases the competitive ability and the predicted probability of long-term ecological success under disease pressure. *Oikos* **98** 459-466.

Danquah EY, Barrett JA. 2002. Grain yield in Composite Cross Five of barley: effects of natural selection. *Journal of Agricultural Science* **138**, 171-176.

Darwin C. 1872. *The expression of the emotions in man and animals*. London: John Murray.

De Boeck HJ, Lemmens CMHM, Zavalloni C *et al.* 2008. Biomass production in experimental grasslands of different species richness during three years of climate warming. *Biogeosciences* **5**, 585-594.

de Wit CT. 1960. On competition. *Verslag Landbouwk Onderzoek* **66**, 1–82.

Dobson A, Crawley W. 1994. Pathogens and the structure of plant communities. *Trends in Ecology and Evolution* **9**, 393-398.

Dorn LA, Pyle EH, Schmitt J. 2000. Plasticity to light cues and resources in *Arabidopsis thaliana*: Testing for adaptive value and costs. *Evolution* **54,** 1982-1994.

Duffy JE. 2009. Why biodiversity is important to the functioning of real-world ecosystems. *Frontiers in Ecology and the Environment* **7**, 437-444.

Duffy JE, Macdonald KS, Rhode JM, Parker JD. 2001. Grazer diversity functional redundancy and productivity in seagrass beds: An experimental test. *Ecology* **82**, 2417-2434.

Eberhart SA, Russell WA. 1966. Stability parameters for comparing yield. *Crop Science* **6**: 36-40.

Elton CS. 1958. *The ecology of invasions by animals and plants*. The University of Chicago press, Chicago, USA.

Erenstein O. 2003. Smallholder conservation farming in the tropics and sub-tropics: a guide to the development and dissemination of mulching with crop residues and

cover crops. *Agriculture Ecosystems and Environment* **100**, 17–37.

Exner DN, Davidson DG, Ghaffarzadeh M, Cruse RM. 1999. Yields and returns from strip intercropping on six Iowa farms. *American Journal of Alternative Agriculture* **14**, 69-77.

FAO, WFP, IFAD. 2012. *The State of Food Insecurity in the World 2012. Economic growth is necessary but not sufficient to accelerate reduction of hunger.* Rome, Italy: FAO.

FAO. 2013. http://www.fao.org/agriculture/crops/core-themes/theme/compendium/tools-guidelines/what-is-agricultural-biodiversity/en/

Fargione JE, Tilman D. 2005. Diversity decreases invasion via both sampling and complementarity effects. *Ecology Letters* **8**, 604-611.

Finckh MR, Mundt CC. 1992. Plant competition and disease in genetically diverse wheat populations. *Oecologia* **91**, 82-92.

Finckh MR, Wolfe MS. 1998. Diversification strategies. *In*: Cooke BM, Jones GD, Kaye B, eds. *The Epidemiology of Plant Diseases*. Chapman and Hall.

Finckh MR, Gacek ES, Goyeau H *et al.* 2000. Cereal variety and species mixtures in practice, with emphasis on disease resistance. *Agronomie* **20**, 813-837.

Forbes MRL. 1993. Parasitism and host reproductive effort. *Oikos* **67**, 444-450.

Fridley JD. 2002. Resource availability dominates and alters the relationship between species diversity and ecosystem productivity in experimental plant communities. *Oecologia* **132**, 271-277.

Gamfeldt L, Hillebrand H. 2008. Biodiversity effects on aquatic ecosystem functioning - maturation of a new paradigm. *International Review of Hydrobiology* **93**, 550-564.

Gardner MR, Ashby WR. 1970. Connectance of large dynamic (cybernetic) systems: critical values for stability. *Nature* **228**, 784.

Gliessman SR. 1995. Sustainable agriculture: an agroecological perspective. *Advances in Plant Pathology* **11**, 45-57.

Goldberg DE, Rajaniemi T, Gurevitch J, Stewart-Oaten A. 1999. Empirical approaches to quantifying interaction intensity: Competition and facilitation along productivity gradients. *Ecology* **80**, 1118-1131.

Griffin JN, Jenkins SR, Gamfeldt L, Jones D, Hawkins SJ, Thompson RC. 2009. Spatial heterogeneity increases the importance of species richness for an ecosystem process. *Oikos* **118**, 1335-1342.

Halliday KJ, Koornneef M, Whitelam GC. 1994. Phytochrome B and at least one other phytochrome mediate the accelerated flowering response of *Arabidopsis thaliana* L. to low red/far red ratio. *Plant Physiology* **104**, 1311-1315.

Hammond-Kosack KE, Jones JDG. 2000. Response to plant pathogens. *In*: Buchannan B, Gruissem W, Jones R, eds. *Biochemistry and molecular biology of plants*. Rockville, MD: American Society of Plant Physiologists, 1102–1157.

Harper J. 1977. Population biology of Plants. Academic press; New York-London-San Francisco.

Harpole WS, Tilman D. 2007. Grassland species loss resulting from reduced niche dimension. *Nature* **446**, 791-793.

Hector A, Bagchi R. 2007. Biodiversity and ecosystem multifunctionality. *Nature* **448**, 188-186.

Hector A, Bazeley-White E, Loreau M, Otway S, Schmid B. 2002. Overyielding in grassland communities: testing the sampling effect hypothesis with seasond biodiversity experiments. *Ecology Letters* **5**, 502-511.

Hector A, Hautier Y, Saner P, Wacker L *et al.* 2010. General stabilising effects of plant diversity on grassland productivity through population asynchrony and overyielding. *Ecology* **91**, 2213-2220.

Hector A, Schmid B, Beierkuhnlein C *et al.* 1999. Plant diversity and productivity experiments in European grasslands. *Science* **286**, 1123-1127.

Hendre PS, Regur P, Annapurna V, Lalremruata A, Aggarwal RK. 2008. Development of new genomic microsatellite markers from robusta coffee (*Coffea canephora* Pierre ex A Froehner) showing broad cross-species transferability and utility in genetic studies. *BMC Plant Biology* **8**.

Herring EM. 1957. *Bestimmungstabellen Der Blattminen Von Europa*: Einschlie Lich DES Mittelmeerbeckens Und Der Kanarischen Inseln Kluwer Academic Publishers.

HGCA recommended list 2011/2012 - Barley. HGCA, Stoneleigh Park, Warwickshire.

Hillocks RJ. 2012. Farming with fewer pesticides: EU pesticide review and resulting challenges for UK agriculture. *Crop Protection* **31**, 85-93.

Hockett EA, Eslick RF, Qualset CO, Dubbs AL, Stewart VR. 1983. Effects of natural selection in advanced generations of barely composite cross II. *Crop Science* **23**, 752-756.

Holub EB. 2008. Natural history of *Arabidopsis thaliana* and oomycete symbioses. *European Journal of Plant Pathology* **122**, 91-109.

Holub EB, Beynon LJ, Crute IR. 1994. Phenotypic and genotypic characterization of interactions between isolates of *Peronospora parasitica* and accessions of *Arabidopsis thaliana*. *Molecular Plant-Microbe Interactions* **7**, 223-239.

Hooper DU. 1998. The role of complementarity and competition in ecosystem responses to variation in plant diversity. *Ecology* **79**, 704-719.

Hooper DU, Chapin FS, Ewel JJ *et al.* 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecological Monographs* **75**, 3-35.

Huala E, Oeller PW, Liscum E, Han IS, Larsen E, Briggs WR. 1997. Arabidopsis NPH1: A protein kinase with a putative redox-sensing domain. *Science* **278**, 2120-2123.

Hughes AR, Inouye BD, Johnson MTJ, Underwood N, Vellend M. 2008. Ecological consequences of genetic diversity. *Ecology Letters* **11**, 609-623.

Hughes AR, Stachowicz JJ. 2004. Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *Proceedings of the National Academy of Sciences* **101**, 8998-9002.

Hughes AR, Stachowicz JJ. 2011. Seagrass genotypic diversity increases disturbance response via complementarity and dominance. *Journal of Ecology* **99**, 445-453.

Hutchinson GE. 1959. Homage to Santa Rosalia or Why are there so many kinds of animals *American Naturalist* **93**, 145-159.

Isbell F, Calcagno V, Hector A. 2011. High plant diversity is needed to maintain ecosystem services. *Nature* **477**, 199-196.

Isbell FI, Wilsey BJ. 2011. Increasing native but not exotic biodiversity increases aboveground productivity in ungrazed and intensely grazed grasslands. *Oecologia* **165**, 771-781.

James WC. 1971. An illustrated series of assessment keys for plant diseases their preparation and usage. *Canadian Plant Disease Survey* **51**, 39-65.

James WC, Jenkins JEE, Lemmett JL. 1968. The relationship between leaf blotch caused by *Rhynchosporium secalis* and losses in grain yield of spring barley. *Annals of Applied Biology* **62**. 273-288.

Jay CN, Rossall S, Smith HG. 1999. Effects of beet western yellows virus on growth and yield of oilseed rape (*Brassica napus*). *Journal of Agricultural Science* **133**, 131-139.

Jazairy I, Alamgir M, Panuccio T. 1992. *The state of world rural poverty: an inquiry into its causes and consequences*. New York University Press for IFAD, New York.

Johanson U, West J Lister C, Michaels S, Amasino R, Dean C. 2000. Molecular analysis of FRIGIDA a major determinant of natural variation in Arabidopsis flowering time *Science* **290**, 344-347.

Jones L, Clements RO. 1993. Development of a low-input system for growing wheat (*Triticum vulgare*) in a permanent understorey of white clover (*Trifolium repens*). *Annals of Applied Biology* **123**, 109-119.

Jones JDG, Dangl JL. 2006. The plant immune system. *Nature* **444**, 323-329.

Jordan N. 1993. Prospects for weed control through crop interference. *Ecological Applications* **3**, 84-91.

Jorgensen TH. 2012. The effect of environmental heterogeneity on RPW8-mediated resistance to powdery mildews in *Arabidopsis thaliana*. *Annals of Botany* **109**, 833-842.

Jousset A, Schmid B, Scheu S, Eisenhauer N. 2011. Genotypic richness and dissimilarity opposingly affect ecosystem functioning. *Ecology Letters* **14**, 537-545.

Juska A, Busch L, Tanaka K. 1997. The blackleg epidemic in Canadian rapeseed as a"normal agricultural accident". *Ecological applications* **7**, 1350-1356.

Kahmen A, Perner J, Buchmann N. 2005. Diversity-dependent productivity in semi-natural grasslands following climate perturbations. *Functional Ecology* **19**. 594-601.

Karkkainen K, Kuittinen H, van Treuren R, Vogl C, Oikarinen S, Savolainen O. 1999. Genetic basis of inbreeding depression in *Arabis petraea*. *Evolution* **53**, 1354-1365.

Kembel SW, Cahill JF. 2005. Plant phenotypic plasticity belowground: A phylogenetic perspective on root foraging trade-offs. *American Naturalist* **166**, 216-230.

Khalifa MA, Qualset CO. 1974. Intergenotypic competition between tall and dwarf wheats.1. in mechanical mixtures. *Crop Science* **1**, 795-799.

Khan AG. 1975. Effect of vesicular arbuscular mycorrhizal associations on growth of cereals .2. Effect on wheat growth. *Annals of Applied Biology* **80**, 27-36.

Kiaer LP, Skovgaard IM, Ostergard H. 2012. Effects of inter-varietal diversity, biotic stresses and environmental productivity on grain yield of spring barley variety mixtures. *Euphytica* **185**, 123-138.

Kikvidze Z, Armas C, Pugnaire FI. 2006. The effect of initial biomass in manipulative experiments on plants. *Functional Ecology* **20**, 1-3.

Kirwan L, Luescher A, Sebastia MT *et al.* 2007. Evenness drives consistent diversity effects in intensive grassland systems across 28 European sites. *Journal of Ecology* **95**, 530-539.

Knops JMH, Tilman D, Haddad NM *et al.* 1999. Effects of plant species richness on invasion dynamics disease outbreaks insect abundances and diversity. *Ecology Letters* **2**, 286-293.

Knott EA, Mundt CC. 1990. Mixing ability analysis of wheat cultivar mixtures under diseased and non-diseased conditions. *Theoretical and Applied Genetics* **80**, 313-320.

Koch E, Slusarenko A. 1990. *Arabidopsis* is susceptible to infection by a downy mildew fungus. *Plant Cell* **2**, 437-445.

Kontturi M, Laine A, Niskanen M, Hurme T, Hyovela M, Peltonen-Sainio P. 2011. Pea-oat intercrops to sustain lodging resistance and yield formation in northern European conditions. *Acta Agriculturae Scandinavica B-Soil and Plant* **61**, 612-621.

Koornneef M, Alonso-Blanco C, Peeters AJM, Soppe W. 1998. Genetic control of flowering time in Arabidopsis. *Annual Review of Plant Physiology and Plant Molecular Biology* **49**, 345-370.

Koornneef M, Meinke D. 2010. The development of *Arabidopsis* as a model plant. *The Plant Journal* **61**, 909-921.

Korves TM, Bergelson J. 2003. A developmental response to pathogen infection in Arabidopsis. *Plant Physiology* **133**, 339-347.

Kotowska AM, Cahill JF, Keddie BA. 2010. Plant genetic diversity yields increased plant productivity and herbivore performance. *Journal of Ecology* **98**, 237-245.

Korves TM, Bergelson J. 2003. A developmental response to pathogen infection in Arabidopsis. *Plant Physiology* **133**, 339-347.

Kover PX, Schaal BA. 2002. Genetic variation for disease resistance and tolerance among *Arabidopsis thaliana* accessions. *Proceedings of the national Academy of Sciences* **99**, 11270-11274.

Kover PX, Valdar W, Trakalo J *et al.* 2009. A Multiparent Advanced Generation Inter-Cross to Fine-Map Quantitative Traits in *Arabidopsis thaliana*. *Plos Genetics* **5**.

Labidi N, Lachaal M, Soltani A, Grignon C, Hajji M. 2004. Variability of the effects of salinity on reproductive capacity of *Arabidopsis thaliana. Journal of Plant Nutrition* **27**, 1561-1573.

Langdale GW, Blevins RL, Karlen DL *et al.* 1991. Cover crop effects on soil erosion by wind and water, 15-22. *In*: WL Hargrove, ed. *Cover Crops for Clean Water*. Soil and Water Conservation Society, Ankeny.

Lannou C, Mundt CC. 1996. Evolution of a pathogen population in host mixtures: Simple race-complex race competition. *Plant Pathology* **45**, 440-453.

Lapin D, Meyer RC, Takahashi H, Bechtold U, Van den Ackerveken G. 2012. Broad-spectrum resistance of Arabidopsis C24 to downy mildew is mediated by different combinations of isolate-specific loci. *New Phytologist* **196**, 1171-1181.

Latz E, Eisenhauer N, Rall BC *et al.* 2012. Plant diversity improves protection against soil-borne pathogens by fostering antagonistic bacterial communities. *Journal of Ecology* **100**, 597-604.

Lavergne S, Mouquet N, Thuiller W, Ronce O. 2010. Biodiversity and Climate Change: Integrating Evolutionary and Ecological Responses of Species and Communities, *In*: Futuyma DJ, Shafer HB, Simberloff D, eds. *Annual Review of Ecology, Evolution, and Systematics*, **41**. 321-350.

Lawton JH, Brown VK. 1993. Redunduancy in ecosystems 255-270. *In*: Schultze ED and Mooney HA, eds. *Biodiversity and ecosystem function.* Springer-Verlag Berlin Germany.

Lehman CL, Tilman D. 2000. Biodiversity stability and productivity in competitive communities. *American Naturalist* **156**, 534-552.

Lemerle D, Smith A, Verbeek B, Koetz E, Lockley P, Martin P. 2006. Incremental crop tolerance to weeds: A measure for selecting competitive ability in Australian wheats. *Euphytica* **149**, 85-95.

Leps J, Osbornovakosinova J, Rejmanek M. 1982. Community stability complexity and species life-history strategies. *Vegetation* **50**, 53-63.

Li C, He X, Zhu S *et al.* 2009. Crop diversity for yield increase. *PLoS ONE* **4.**

Li L, Sun JH, Zhang FS, Li XL, Rengel Z, Yang SC. 2001. Wheat/maize or soybean strip intercropping II Recovery or compensation of maize and soybean after wheat harvesting. *Field Crops Research* **71**, 173–181.

Li L, Sun JH, Zhang FS *et al.* 2006. Root distribution and interactions between intercropped species. *Oecologia* **147**, 280-290.

Lithourgidis AS, Dordas CA, Damalas CA, Vlachostergios DN. 2011. Annual intercrops: an alternative pathway for sustainable agriculture. *Australian journal of crop science* **5**, 396-410.

Lobell DB, Burke MB, Tebaldi C, Mastrandrea MD, Falcon WP, Naylor RL. 2008. Prioritizing climate change adaptation needs for food security in 2030. *Science* **319**, 607-610.

Lopez CG, Mundt CC. 2000. Using mixing ability analysis from two-way cultivar mixtures to predict the performance of cultivars in complex mixtures. *Field Crops Research* **68,** 121-132.

Loreau M, Hector A. 2001. Partitioning selection and complementarity in biodiversity experiments. *Nature* **412**, 72-76.

Loreau M, Mouquet N, Gonzalez A. 2003. Biodiversity as spatial insurance in heterogeneous landscapes. *Proceedings of the National Academy of Sciences* **100**, 12765-12770.

Loreau M, Naeem S, Inchausti P. 2001. Ecology - Biodiversity and ecosystem functioning: Current knowledge and future challenges. *Science* **294**, 804-808.

Loreau M. 1998. Biodiversity and ecosystem functioning: A mechanistic model. *Proceedings of the National Academy of Sciences* **95**, 5632-5636.

Loreau M. 2000. Biodiversity and ecosystem functioning: recent theoretical advances. *Oikos* **91**, 3-17.

MacArthur RH. 1955. Fluctuations of animal populations and a measure of community stability. *Ecology **36***, 533-536.

MacArthur RH, Wilson ED. 1967. *The theory of island biogeography*. Princeton, NJ: Princeton University Press.

MacDonald N 1978. Complexity and stability. *Nature* **275**, 117-118.

Madden LV, Paul PA, Lipps PE. 2007. Consideration of nonparametric approaches for assessing genotype-by-environment (G x E) interaction with disease severity data. *Plant Disease* **91**, 891-900.

Maron JL, Marler M, Klironomos JN, Cleveland CC. 2011. Soil fungal pathogens and the relationship between plant diversity and productivity. *Ecology Letters* **14**, 36-41.

Martinez-Zapater JM, Coupland G, Dean C, Koornneef M 1994. The Transition to Flowering in Arabidopsis. *In*: Meyerowitz EM, Somerville CR eds. *Cold Spring Harbor Monograph Series; Arabidopsis*, 403-433.

Marquard E, Weigelt A, Temperton VM *et al.* 2009. Plant species richness and functional composition drive overyielding in a six-year grassland experiment. *Ecology* **90**, 3290-3302.

Marten GG. 1986. *Traditional Agriculture in Southeast Asia: a Human Ecology Perspective*. Westview Press. Boulder, Colorado.

Masclaux F, Hammond RL, Meunier J, Gouhier-Darimont C, Keller L, Reymond P. 2010. Competitive ability not kinship affects growth of *Arabidopsis thaliana* accessions. *New Phytologist* **185**, 322-331.

May RM. 1973. Stability and complexity in model ecosystems. Princeton University Press, New Jersey, USA.

McDonald BA, Linde C. 2002. Pathogen population genetics evolutionary potential and durable resistance. *Annual Review of Phytopathology* **40**, 349-79.

McLaren JR, Turkington R. 2011. Biomass compensation and plant responses to 7 years of plant functional group removals. *Journal of Vegetation Science* **22,** 503-515.

McNaughton SJ. 1995. *Biodiversity and function of grazing ecosystems*. Symposium on biodiversity and ecosystem function, Germany **99**, 361-383.

McNaughton SJ. 1977. Diversity and stability of ecological communities - comment on role of empericism in ecology. *American Naturalist* **111**, 515-525.

McPhee CS, Aarssen LW. 2001. The separation of above-and below-ground competition in plants: A review and critique of methodology. *Plant Ecology* **152**, 119-136.

Medina CP. 2012. Rice: Crop breeding using farmer-led participatory plant breeding. *In:* Lammerts van Bueren ET, Myers JM, eds. *Organic Crop Breeding*. Wiley-Blackwell, UK.

Meinke DW, Cherry JM, Dean C, Rounsley SD, Koornneef M. 1998. *Arabidopsis thaliana*: A model plant for genome analysis. *Science* **282**, 662-682.

Meldau S, Erb M, Baldwin IT. 2012. Defence on demand: mechanisms behind optimal defence patterns. *Annals of Botany* **110**, 1503-1514.

Mille B, Fraj MB, Monod H, de Vallavieille-Pope C. 2006. Assessing four-way mixtures of winter wheat cultivars from the performances of their two-way and individual components. *European Journal of Plant Pathology* **114**, 163-173.

Miller P, Lane A, Wheeler H. 2001. Matching spray applications to canopy characteristics in cereal crops. *Pesticide Outlook* **12**, 100-102.

Mitchell-Olds T. 2001. *Arabidopsis thaliana* and its wild relatives: a model system for ecology and evolution. *Trends in Ecology and Evolution* **16**, 693-700.

Mittler R, Blumwald E. 2010. Genetic Engineering for Modern Agriculture: Challenges and Perspectives. *In*: Merchant S, Briggs WR, Ort D eds. *Annual Review of Plant Biology* **61**, 443-462.

Mittler R. 2006. Abiotic stress, the field environment and stress combination. *Trends in Plant Science* **11**, 15-19.

Moreno JE, Tao Y, Chory J, Ballare CL. 2009. Ecological modulation of plant defense via phytochrome control of jasmonate sensitivity. *Proceedings of the National Academy of Sciences* **106**, 4935-4940.

Morris RA, Garrity DP. 1993. Resource capture and utilization in intercropping-non-nitrogen nutrients. *Field Crops Research* **34**, 319–334.

Morton JF. 2007. The impact of climate change on smallholder and subsistence agriculture. *Proceedings of the National Academy of Sciences* **104**, 19680-19685.

Mulder CPH, Uliassi DD, Doak DF. 2001. Physical stress and diversity-productivity relationships: The role of positive interactions. *Proceedings of the National Academy of Sciences* **98**, 6704-6708.

Mundt CC, Brophy LS, Schmitt MS. 1995. Choosing crop cultivars and cultivar mixtures under low versus high disease pressure: A case-study with wheat. *Crop Protection* **14**, 509-515.

Mundt CC, Hayes PM, Schon CC. 1994. Influence of barley mixtures on severity of scald and net blotch and on yield. *Plant Pathology* **43**, 356-361.

Mundt CC. 2002. Use of multiline cultivars and cultivar mixtures for disease management. *Annual Review of Phytopathology* **40**, 381-410.

Mur LAJ, Allainguillaume J, Catalan P *et al.* 2011. Exploiting the Brachypodium Tool Box in cereal and grass research. *New Phytologist* **191**, 334-347.

Naeem S, Thompson LJ, Lawler SP, Lawton JH, Woodfin RM. 1994. Declining biodiversity can alter the performance of ecosystems. *Nature* **368**, 734-737.

Naeem S. 1998. Species redundancy and ecosystem reliability. *Conservation Biology* **12**, 39-45.

Naeem S, Li S. 1997. Biodiversity enhances ecosystem reliability. *Nature* **390**, 507-509.

Nagayets O. 2005. Small farms: current status and key trends. Information brief prepared for the future of small farms research workshop Wye College, June 26–29, 2005.

Nemri A, Atwell S, Tarone AM *et al.* 2010. Genome-wide survey of Arabidopsis natural variation in downy mildew resistance using combined association and linkage mapping. *Proceedings of the National Academy of Sciences* **107**, 10302-10307.

Newton AC, Hackett CA. 1994. Subjective components of mildew assessment on spring barley. *European Journal of Plant Pathology* **100**, 395-412.

Newton AC, Ellis RP, Hackett CA, Guy DC. 1997. The effect of component number on *Rhynchosporium commune* infection and yield in mixtures of winter barley cultivars. *Plant Pathology* **46**, 930-938.

Newton AC, Hackett CA, Swanston JS. 2008a. Analysing the contribution of component cultivars and cultivar combinations to malting quality, yield and disease in complex mixtures. *Journal of the Science of Food and Agriculture,* **88**, 2142-2152.

Newton AC, Begg GS, Swanston JS. 2008b. Deployment of diversity for enhanced crop function. *Annals of Applied Biology* **154**, 309-322.

Newton AC, Guy DC. 2009. The effects of uneven, patchy cultiver mixtures on disease control and yield in winter barley. *Field Crops Research* **110**, 225-228.

Newton AC, Flavell AJ, George TS *et al.* 2011. Crops that feed the world 4. Barley: a resilient crop? Strengths and weaknesses in the context of food security. *Food security* **3**, 141-178.

Newton AC, Swanston JS, Guy DC, Ellis RP. 1998. The effect of cultivar mixtures on malting quality in winter barley. *Journal of the Institute of Brewing* **104**, 41-45.

Nowak MA, Sigmund K. 2004. Evolutionary dynamics of biological games. *Science* **303**, 793-799.

Opanowicz M, Vain P, Draper J, Parker D, Doonan JH. 2008. *Brachypodium distachyon*: making hay with a wild grass. *Trends in Plant Science* **13**, 172-177.

Ostergaard H, Kristensen K, Jensen JW. 2005. Stability of variety mixtures of spring barley. *In*: Proceedings of the COST SUSVAR/ECO-PB. Workshop on Organic Plant Breeding Strategies and the Use of Molecular markers Driebergen. The Netherlands.

Pagan I, Alonso-Blanco C, Garcia-Arenal F. 2008. Host responses in life-history traits and tolerance to virus infection in *Arabidopsis thaliana. PLoS Pathogens* **4**.

Pagan I, Alonso-Blanco C, Garcia-Arenal F. 2009. Differential Tolerance to Direct and Indirect Density-Dependent Costs of Viral Infection in *Arabidopsis thaliana*. *PLoS Pathogens* **5**.

Palmgren MG. 2001. Plant plasma membrane H+-ATPases: Powerhouses for nutrient uptake. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**, 817-845.

Pan JJ, Price JS. 2001. Fitness and evolution in clonal plants: the impact of clonal growth. *Evolutionary Ecology* **15**, 583-600.

Patel JD, Reinbergs E, Mather DE, Choo TM, Sterling JDE. 1987. Natural selection in a double-haploid mixture and a composite cross of barley. *Crop Science* **27**, 474-479.

Payne RW. 2009. GenStat. *Wiley Interdisciplinary Reviews: Computational Statistics* **1**, 255–258.

Peraldi A, Beccari G, Steed A, Nicholson P. 2011. *Brachypodium distachyon*: a new pathosystem to study Fusarium head blight and other Fusarium diseases of wheat. *BMC Plant Biology* **11**.

Peraldi A, Griffe LL, Burt C, McGrann, Nicholson P. 2013. *Brachypodium distachyon* exhibits compatible interactions with *Oculimacula* spp. and *Ramularia collo-cygni*, providing the first pathosystem model to study eyespot and ramularia leaf spot diseases. *Plant pathology*. doi 10.1111/ppa/12114.

Peters JC, Shaw MW. 1996. Effect of artificial exclusion and augmentation of fungal plant pathogens on a regenerating grassland. *New Phytologist* **134**, 295-307.

Peterson RF, Campbell AB, Hannah AE. 1948. A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. *Canadian Journal of Research Section C-Botanical Sciences* **26**, 496-400.

Pfisterer AB, Schmid B. 2002. Diversity-dependent production can decrease the stability of ecosystem functioning. *Nature* **416**, 84-86.

Phillips SL, Shaw MW, Wolfe MS. 2005. The effect of potato variety mixtures on epidemics of late blight in relation to plot size and level of resistance. *Annals of Applied Biology* **147**, 245-252.

Pigliucci M, Kolodynska A. 2002. Phenotypic plasticity and integration in response to flooded conditions in natural accessions of *Arabidopsis thaliana* (L) Heynh (Brassicaceae). *Annals of Botany* **90**, 199-207.

Pigliucci M, Whitton J, Schlichting CD. 1995a. Reaction norms of *Arabidopsis*.1. Plasticity of characters and correlations across water, nutrient and light gradients. *Journal of Evolutionary Biology* **8**, 421-438.

Pigliucci M, Schlichting CD, Whitton J. 1995b. Reaction norms of *Arabidopsis*. 2. Response to stress and unordered environental variation. *Functional Ecology* **9**, 537-547.

Pimm SL. 1979. The structure of food webs. *Theoretical Population Biology* **16**, 144−158.

Pimm SL. 1984. The complexity and stability of ecosystems. *Nature* **307**, 321-326.

Polley WH, Wilsey BJ, Derner JD. 2003. Do species evenness and plant density influence the magnitude of selection and complementarity effects in annual plant species mixtures. *Ecology Letters* **6**, 248-256.

Qian W, Li Q, Noack J *et al.* 2009. Heterotic patterns in rapeseed (Brassica napus L): II Crosses between European winter and Chinese semi-winter lines. *Plant Breeding* **128**, 466-470.

Quail PH, Boylan MT, Parks BM, Short TW, Xu Y, Wagner D. 1995. Phytochromes – photosensory perception and signal-transduction. *Science* **268**, 675-680.

Raghothama KG. 1999. Phosphate acquisition. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 665-693.

Rajkumara S. 2008. Lodging in cereals - a review. *Agricultural Reviews* **29**, 55-60.

Ranells NN, Wagger MG. 1997. Winter grass-legume bicultures for efficient nitrogen management in no-till corn. *Agriculture Ecosystems and Environment* **65**, 23-32.

Rasmusson DC, Beard BH, Johnson FK. 1967. Effect of natural selection on performace of a barley population. *Crop Science* **7**, 543.

Reich PB, Knops J, Tilman D *et al.* 2001. Plant diversity enhances ecosystem responses to elevated CO2 and nitrogen deposition. *Nature* **410**, 809-812.

Reich PB, Wright IJ, Cavender-Bares J *et al.* 2003. The evolution of plant functional variation: Traits spectra and strategies. *International Journal of Plant Sciences* **164**, S143-S164.

Reignault P, Frost LN, Richardson H, Daniels MJ, Jones JDG, Parker JE. 1996. Four *Arabidopsis* RPP loci controlling resistance to the Noco2 isolate of *Peronospora parasitica* map to regions known to contain other RPP recognition specificities. *Molecular Plant-Microbe Interactions* **9**, 464-473.

Reusch TBH, Ehlers A, Hammerli A, Worm B. 2005. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proceedings of the National Academy of Sciences* **102**, 2826-2831.

Revilla-Molina IM, Bastiaans L, Van Keulen H *et al.* 2009. Does resource complementarity or prevention of lodging contribute to the increased productivity of rice varietal mixtures in Yunnan, China? *Field Crops Research* **111**, 303-307.

Reymond P, Weber H, Damond M, Farmer EE. 2000. Differential gene expression in response to mechanical wounding and insect feeding in Arabidopsis. *Plant Cell* **12,** 707-719.

Ridder B. 2008. Questioning the ecosystem services argument for biodiversity conservation. *Biodiversity and Conservation* **17**, 781-790.

Root RB. 1973. Organization of a plant-arthropod association in simple and diverse habitats; the fauna of collards (*Brassica oleraceae*). *Ecological monographs* **43**, 95-124.

Roscher C Schumacher J Baade J *et al.* 2004 The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. *Basic and Applied Ecology* **5**, 107-121.

Roscher C, Scherer-Lorenzen M, Schumacher J, Temperton, VM, Buchmann N, Schulze ED. 2011. Plant resource-use characteristics as predictors for species contribution to community biomass in experimental grasslands. *Perspectives in Plant Ecology Evolution and Systematics* **13**, 1-13.

Roscher C, Weigelt A, Proulx R, Marquard *et al.* 2011. Identifying population- and community-level mechanisms of diversity-stability relationships in experimental grasslands. *Journal of Ecology* **99**, 1460-1469.

Rosenfeld JS. 2002. Functional redundancy in ecology and conservation. *Oikos* **98**, 156-162.

Ruttan VW. 1999. The transition to agricultural sustainability. *Proceedings of the National Acedemy of Sciences* **96**, 5960-5967.

Ryan JC. 1992. Conserving biological diversity. *In:* Brown L, Lester R *et al.* eds. *State of the world 1992*. Norton WW, New York.

Salvaudon L, Heraudet V, Shykoff JA. 2007. Genotype-specific interactions and the trade-off between host and parasite fitness. *BMC Evolutionary Biology* **7**.

Salvaudon L, Heraudet V, Shykoff JA. 2008. *Arabidopsis thaliana* and the Robin Hood parasite: a chivalrous oomycete that steals fitness from fecund hosts and benefits the poorest one? *Biology Letters* **4**, 526-529.

Sapoukhina N, Paillard S, Dedryver F, de Vallavieille-Pope C. 2013. Quantitative plant resistance in cultivar mixtures: wheat yellow rust as a modeling case study. *New Phytologist*. doi: 10.1111/nph.12413.

Sarandon SJ, Sarandon R. 1995. Mixture of cultivars - pilot field trial of an ecological alternative to improve production or qualtity of wheat (*Triticum aestivum*). *Journal of Applied Ecology* **32**, 288-294.

Schenk PM, Kazan K, Wilson I *et al.* 2000. Coordinated plant defense responses in Arabidopsis revealed by microarray analysis. *Proceedings of the National Academy of Sciences* **97**, 11655-11660.

Schindelin J, Arganda-Carreras I, Frise E *et al.* 2012. Fiji: an open-source platform for biological-image analysis. *Nature Methods* **9**, 676–682.

Schmid B. 1994. Effects of genetic diversity in experimental stands of *Solidago altissima* - Evidence for the potential role of pathogens as selective agents in plant populations. *Journal of Ecology* **82**, 165-175.

Semere T, Froud-Williams RJ. 2001. The effect of pea cultivar and water stress on root and shoot competition between vegetative plants of maize and pea. *Journal of Applied Ecology* **38,** 137-145.

Shipley PR, Meisinger JJ, Decker AM. 1992. Conserving residual corn fertiliser nitrogen with winter cover crops. *Agronomy Journal* **84**, 869-876.

Siemens DH, Lischke H, Maggiulli N, Schurch S, Roy BA. 2003. Cost of resistance and tolerance under competition: the defense-stress benefit hypothesis. *Evolutionary Ecology* **17**, 247-263.

Sijmons PC, Grundler FMW, Vonmende N, Burrows PR, Wyss U. 1991. *Arabidopsis thaliana* as a new model for host plant-parasitic nematodes. *Plant Journal* **1**, 245-254.

Silvertown J. 2004. Plant coexistence and the niche. *Trends in Ecology and Evolution* **19**, 605-611.

Smithson JB, Lenne JM. 1996. Varietal mixtures: A viable strategy for sustainable productivity in subsistence agriculture. *Annals of Applied Biology* **128**, 127-158.

Soliman KM, Allard RW. 1991. Grain-yield of composite cross populations of barley-effect of natural selection. *Crop Science* **31**, 705-708.

Song L, Zhang DW, Li FM, Fan XW, Ma Q, Turner NC. 2010. Soil water availability alters the inter- and intra-cultivar competition of three spring wheat cultivars bred in different eras. *Journal of Agronomy and Crop Science* **196**, 323-335.

Spehn EM, Joshi J, Schmid B, Diemer M, Korner C. 2000. Above-ground resource use increases with plant species richness in experimental grassland ecosystems. *Functional Ecology* **14**, 326–337.

Stahl EA, Dwyer G, Mauricio R, Kreitman M, Bergelson J. 1999. Dynamics of disease resistance polymorphism at the Rpm1 locus of Arabidopsis. *Nature* **400**, 667-671.

Stearns SC. 1989. Trade –offs in life-history evolution. *Functional Ecology* **3** 259-268.

Stehlik I, Holderegger R. 2000. Spatial genetic structure and clonal diversity of Anemone nemorosa in late successional deciduous woodlands of Central Europe. *Journal of Ecology* **88**, 424-435.

Stern WR. 1993. Nitrogen fixation and transfer in intercrop systems. *Field Crops Research* **34**, 335–356.

Stevens M, Freeman B, Liu HY, Herrbach E, Lemaire O. 2005. Beet poleroviruses: close friends or distant relatives? *Molecular Plant Pathology* **6**, 1-9.

Stevens M, McGrann G, Clark B. 2008. *Turnip yellows virus* (syn. *Beet western yellows virus*): an emerging threat to European oilseed rape production? *HGCA Research Review*, 36 pp.

Stotz HU, Pittendrigh BR, Kroymann J *et al.* 2000. Induced plant defense responses against chewing insects Ethylene signaling reduces resistance of Arabidopsis against Egyptian cotton worm but not diamondback moth. *Plant Physiology* **124**, 1007-1017.

Stuefer JF, Anten NPR, De Kroon H *et al.* 2009. Genotypic selection shapes patterns of within-species diversity in experimental plant populations. *Journal of Ecology* **97**, 1020-1027.

Stutzel H, Aufhammer W. 1989. Effects of winter barley cultivars on lodging. *Journal of Agricultural Science* **112**, 47-55.

Suneson CA. 1956. An evolutionary plant breeding method. *Agronomy Journal* **48**, 188–191.

Szekeres M, Nemeth K, KonczKalman Z *et al.* 1996. Brassinosteroids rescue the deficiency of CYP90 a cytochrome P450 controlling cell elongation and de-etiolation in Arabidopsis. *Cell* **85**, 171-182.

Teasdale JR 1993. Interaction of light soil moisture and temperature with weed suppression by hairy vetch residue. *Weed science* **41**, 46-51.

Tian D, Traw MB, Chen JQ, Kreitman M, Bergelson J. 2003. Fitness costs of R-gene-mediated resistance in Arabidopsis thaliana. *Nature* **423**, 74-77.

Tilman D.1996. Biodiversity: Population versus ecosystem stability. *Ecology* **77**, 350-363.

Tilman D. 1997. Community invasibility recruitment limitation and grassland biodiversity. *Ecology* **78**, 81-92.

Tilman D. 1999. The ecological consequences of changes in biodiversity: A search for general principles. *Ecology* **80**, 1455-1474.

Tilman D. 2004. Niche tradeoffs, neutrality, and community structure: A stochastic theory of resource competition, invasion, and community assembly. *Proceedings of the National Academy of Sciences* **101**, 10854-10861.

Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S. 2002. Agricultural sustainability and intensive production practices. *Nature* **418**, 671-677.

Tilman D, Downing JA. 1994. Biodiversity and stability in grasslands. *Nature* **367**, 363-365.

Tilman D, Knops J, Wedin D, Reich P, Ritchie M, Siemann E. 1997a. The influence of functional diversity and composition on ecosystem processes. *Science* **277**, 1300-1302.

Tilman D, Lehman CL, Thomson KT. 1997b. Plant diversity and ecosystem productivity: Theoretical considerations. *Proceedings of the National Academy of Sciences* **94** 1857-1861.

Tilman D, Reich PB, Knops J. 2006. Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature* **441**, 629-632.

Tilman D, Reich PB, Knops J, Wedin D, Mielke T, Lehman C. 2001. Diversity and productivity in a long-term grassland experiment. *Science* **294**, 843-845.

Tilman D, Wedin D, Knops J. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature,* **379**, 718-720.

Toledo UM. 1985. Ecologia y Autosuficiencia Alimentaria Siglo XXI Editors, Mexico City 118 pp.

Trewavas A. 2001. Urban myths of organic farming. *Nature* **410**, 409-410.

Van Peer L, Nijs I, Reheul D, De Cauwer B. 2004. Species richness and susceptibility to heat and drought extremes in synthesized grassland ecosystems: compositional vs physiological effects. *Functional Ecology* **18**, 769-778.

van Ruijven J, Berendse F. 2005. Diversity-productivity relationships: Initial effects long-term patterns and underlying mechanisms. *Proceedings of the National Academy of Sciences* **102**, 695-700.

van Ruijven J, Berendse F. 2010. Diversity enhances community recovery but not resistance after drought. *Journal of Ecology* **98**, 81-86.

van Ruijven J, De Deyn GB, Berendse F. 2003. Diversity reduces invasibility in experimental plant communities: the role of plant species. *Ecology Letters* **6**, 910-918.

Vandermeer JH. 1989. *The ecology of intercropping.* Cambridge UK, Cambridge University Press.

Vermeulen PJ, Anten NPR, Schieving F, Werger MJA, During HJ. 2008. Height convergence in response to neighbour growth: genotypic differences in the stoloniferous plant *Potentilla reptans*. *New Phytologist* **177**, 688-697.

Vert G, Grotz N, Dedaldechamp F *et al.* 2002. IRT1 an Arabidopsis transporter essential for iron uptake from the soil and for plant growth. *Plant Cell* **14**, 1223-1233.

Viola DV, Mordecai EA, Jaramillo *et al.* 2010. Competition-defense tradeoffs and the maintenance of plant diversity. *Proceedings of the National Academy of Sciences* **107**, 17217-17222.

Violle C, Navas M-L, Vile D *et al.* 2007. Let the concept of trait be functional! *Oikos* **116**, 882-892.

Vogel A, Scherer-Lorenzen M, Weigelt A. 2012. Grassland resistance and resilience after drought depends on management intensity and species richness. *Plos One* **7**.

von Braun J. 2005. Small scale farmers in a liberalized trade environment. *In Small-scale farmers in liberalised trade environment*, T. Huvio, J. Kola, and T. Lundström, eds. Proceedings of the seminar, October 18–19, 2004, Haikko, Finland. Department of Economics and Management Publications No. 38. Agricultural Policy. Helsinki: University of Helsinki. <http://honeybee.helsinki.fi/mmtal/abs/Pub38.pdf> Accessed June 2005.

VSN International. 2011. GenStat *for Windows* 14th Edition. VSN International, Hemel Hempstead, UK. Web page: GenStat.co.uk

Walther GR., Post E, Convey P *et al.* 2002. Ecological responses to recent climate change. *Nature* **416,** 389-395.

Wang WX, Vinocur B, Altman A. 2003. Plant responses to drought salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta* **218**, 1-14.

Wang Y, Yu S, Wang J. 2007. Biomass-dependent susceptibility to drought in experimental grassland communities. *Ecology Letters* **10**, 401-410.

Weinig C, Stinchcombe JR, Schmitt J. 2003. Evolutionary genetics of resistance and tolerance to natural herbivory in *Arabidopsis thaliana*. *Evolution* **57**, 1270-1280.

Westwood JH, McCann L, Naish M *et al.* 2013. A viral RNA silencing suppressor interferes with abscisic acid-mediated signalling and induces drought tolerance in *Arabidopsis thaliana*. *Molecular Plant Pathology* **14**, 158-170.

Willey RW. 1979. Intercropping - its importance and research needs. Part 1: Competition and yield advantages. *Field Crops Abstracts* **32**,1-10.

Willis CG, Brock MT, Weinig C. 2010. Genetic variation in tolerance of competition and neighbour suppression in *Arabidopsis thaliana*. *Journal of Evolutionary Biology* **23**, 1412-1424.

Wise MJ. 2007. Evolutionary ecology of resistance to herbivory: an investigation of potential genetic constraints in the multiple-herbivore community of *Solanum carolinense*. *New Phytologist* **175**, 773-784.

Woldeamlak A, Grando S, Maatougui M, Ceccarelli S. 2008. Hanfets, a barley and wheat mixture in Eritrea: Yield, stability and farmer preferences. *Field Crops Research* **109**, 50-56.

Wolfe MS. 1985. The current status and prospects of multiline cultivars and variety mixtures for disease resistance. *Annual Review of Phytopathology* **23,** 251-273.

Wolfe MS. 1992. Barley diseases: maintaining the value of our varieties. *In* Munk L, eds. *Barley genetics VI*. Munksgaard International publishers, Copenhagen, 1055-1067.

Wolfe MS, Barrett JA. 1980. Can we lead the pathogen astray? *Plant Disease* **64**, 148-155.

Wu SJ, Ding L, Zhu JK. 1996. SOS1 a genetic locus essential for salt tolerance and potassium acquisition. *Plant Cell* **8**, 617-627.

Yachi S, Loreau M. 1999. Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. *Proceedings of the National Academy of Sciences* **96**, 1463-1468.

Yarwood CE. 1977. *Pseudoperonospora cubensís* in *Uromyces phaseoli* rust-infected bean. *Phytopathology* **67**, 1021-1022.

Zadoks JC, Chang TT, Konzak CF. 1974. A decimal code for the growth stages of cereals. *Weed Research* **14**, 415-421.

Zavaleta ES, Pasari JR, Hulvey KB, Tilman GD. 2010. Sustaining multiple ecosystem functions in grassland communities requires higher biodiversity. *Proceedings of the National Academy of Sciences* **107**, 1443-1446.

Zhang JH, Lechowicz MJ. 1994. Correlation between time of flowering and phenotypic plasticity in *Arabidopsis thaliana* (Brassicaceae). *American Journal of Botany* **81** 1336-1342.

Zhu JK, Liu JP, Xiong LM. 1998. Genetic analysis of salt tolerance in Arabidopsis: Evidence for a critical role of potassium nutrition. *Plant Cell* **10**, 1181-1191.

Zhu JK. 2001. Plant salt tolerance. *Trends in Plant Science* **6**, 66-71.

Zhu YY, Chen HR, Fan JH *et al.* 2000. Genetic diversity and disease control in rice. *Nature* **406**, 718-722.

Appendix 1:

Fixed effect	F	n.d.f.	d.d.f.	P
season	305.66	2	63.5	<0.001
nutrient level	0.92	1	60.3	0.3
cultivation	5.52	1	61.7	0.02
genotype	153.45	3	940.5	<0.001
season x nutrient	11.99	2	65.3	<0.001
season x cultivation	13.39	2	66.9	<0.001
genotype x season	56.17	6	888.6	<0.001
genotype x nutrient level	5.26	3	921.1	0.001
genotype x cultivation	25.29	3	135.4	<0.001
genotype x season x nutrient level	4.48	6	834.9	<0.001
genotype x season x cultivation	2.48	6	157.4	0.03
genotype x nutrient level x cultivation	3.68	4	108.4	0.007

Table A2.1: The effect of *Arabidopsis* genetic diversity and different growing conditions on seed productivity in the four-way mixture experiments. In each case, a linear mixed model was used to analyse each factor and the interactions between them. Fixed effects included growing season (autumn, winter, summer), nutrient level (high/low) and cultivation monoculture/mixture) and genotype. Non-significant interaction terms were removed from each model. *F* and *P* values refer to ANOVA tests of each factor separately and the interactions between them.

Table A2.1a: Analysis of combined data from all three experiments. N=1880.

Fixed term	F	n.d.f.	d.d.f.	P
season	2.12	1	47.5	0.02
nutrient level	12.53	1	46.5	<0.001
cultivation	2.39	1	47.3	0.1
genotype	67.7	3	750.4	<0.001
season x nutrient level	5.43	1	47.1	0.02
genotype x nutrient level	3.14	3	751.4	0.03
genotype x cultivation	16.23	3	105.4	<0.001

Table A2.1b: Analysis of data from the autumn and winter seasons of the four-way mixture experiment. N=1260.

Fixed term	F	n.d.f.	d.d.f.	P
nutrient level	10.66	1	21.0	0.004
cultivation	39.17	1	23.0	<0.001
genotype	156.92	3	184.8	<0.001
genotype x nutrient level	7.43	3	159.1	<0.001
genotype x cultivation	11.82	3	62.0	<0.001
genotype x nutrient level x cultivation	3.22	4	49.9	0.02

Table A2.1c: Analysis of data from the summer season of the four-way mixture experiment. N= 620.

Fixed effect	F	n.d.f.	d.d.f.	P
season	284.45	2	55.5	<0.001
nutrient level	9.60	1	52.9	0.003
cultivation	0.01	1	56.9	0.9
genotype	876.13	3	1125.3	<0.001
season x nutrient level	20.58	2	53.8	<0.001
season x genotype	45.47	6	1204.2	<0.001
nutrient x genotype	3.80	3	1124.0	0.01
cultivation x genotype	10.30	3	124.6	<0.001
season x nutrient level x genotype	7.46	6	1097.0	<0.001
season x cultivation x genotype	13.17	8	105.0	<0.001
season x nutrient level cultivation x genotype	2.01	12	102.5	0.03

Table A2.2: The effect of *Arabidopsis* genotypic diversity and different growing conditions on days to flowering in all three growing seasons of a four-way mixture experiment. A linear mixed model analysed each factor individually and the interactions between them. Fixed factors include experimental season, nutrient level (high/low) and cultivation (monoculture/mixture) and genotype. N=1880. Non-significant interaction terms were removed from each model. *F* and *P* values refer to ANOVA tests of each factor separately and the interactions between them.

Fixed term	F	n.d.f.	d.d.f.	P
genotype	137.76	7	275.3	<0.001
competition type	23.47	2	173.2	<0.001
cultivation	9.87	1	287.2	<0.001
genotype x competition type	7.91	14	176.7	<0.001

Table A2.3: The effect of *Arabidopsis* genetic diversity and different growing conditions on seed productivity in a pair-wise interaction experiment. A linear mixed model was used to analyse each factor and all interactions between them. Fixed effects included genotype, competition type (above ground only/above and below ground) and cultivation (mixture/monoculture). Non-significant terms were eliminated from the model. *F* and *P* values refer to ANOVA tests of each factor separately and the interactions between them. N=639.

Fixed term	F	n.d.f.	d.d.f.	P
competition type	4.58	1	295.5	0.03
competitive group of focal plant	143.6	3	295.6	<0.001
competitive group of competing plant	6.16	3	295.5	<0.001
competition type x competitive group of focal plant	19.92	3	295.7	<0.001
competition type x competitive group of competing plant	3.77	3	295.5	0.01

Table A2.4: The effect of competitive group of the focal and competing plant, competition type (above ground only/above and below ground) on seed productivity of *Arabidopsis* plants in the pair-wise interaction experiments. A linear mixed model was used to analyse each factor and the interactions between them. Fixed effects included growing season, competition type (above/below-ground), competitive group of focal plant competitive group of competing plant. Non-significant interaction terms were removed from each model. *F* and *P* values refer to ANOVA tests of each factor separately and the interactions between them. N=639.

Fixed term	F	n.d.f.	d.d.f.	P
competition (presence/absence)	18.39	1	212.0	<0.001
competitive group of focal plant	347.7	3	308.5	<0.001
competition (presence/absence) x competitive group of focal plant	16.1	3	212.1	0.001

Table A2.5: The effect of competition (presence/absence of competitors) and competitive group of the focal plant on seed productivity of *Arabidopsis* plants in a pair-wise interaction experiment. A linear mixed model was used to analyse each factor and all interactions between them. Fixed effects included growing season, competition type (above/below-ground), competitive group of focal plant competitive group of competing plant. Non-significant interaction terms were removed from each model. *F* and *P* values refer to ANOVA tests of each factor separately and the interactions between them. N=639.

Appendix 2:

Fixed term	F	n.d.f.	d.d.f.	P
Experiment	209.68	1	587.1	<0.001
Genotype	5.69	3	586.7	<0.001
Cultivation method	7.34	1	858.6	0.007
Hpa	0.24	1	586.9	0.621
Experiment. Genotype	86.57	3	587.1	<0.001
Genotype. Cultivation	5.30	3	586.0	<0.001
Experiment. *Hpa*	58.50	1	587.1	<0.001
Genotype. *Hpa*	32.63	3	587.0	<0.001
Genotype. *Hpa.* Experiment	6.00	3	587.2	<0.001
Genotype. *Hpa.* Cultivation	3.54	4	585.5	0.007

Table A3.1: The effect of *Arabidopsis* genotypic diversity and *Hpa* on seed mass produced per plant in a pair-wise interaction experiment. A linear mixed model was used to analyse each factor and all interactions between them. Fixed effects included experimental repeat, genotype, cultivation (2-way mixture/monoculture) and *Hpa* (presence/absence). Non-significant terms were eliminated from the model. *F* and *P* values refer to ANOVA tests of each factor separately and the interactions between them. N=1600.

Fixed term	F	n.d.f.	d.d.f.	P
Experiment	38.41	1	334.8	<0.001
Cultivation	1.79	1	362.5	0.2
Genotype	664.52	3	634.3	<0.001
Experimental. Genotype	35.43	3	634.0	<0.001

Table A3.2a: The effect of *Arabidopsis* genotypic diversity and *Hpa* on the initial disease score at six days after infection in a pair-wise interaction experiment. Disease was scored as the proportion of leaves showing sporulation. A linear mixed model was used to analyse each factor and all interactions between them. Fixed effects included experimental repeat, genotype and cultivation (2-way mixture/monoculture). Non-significant terms were eliminated from the model. *F* and *P* values refer to ANOVA tests of each factor separately and the interactions between them. N=1600.

Fixed term	F	n.d.f.	d.d.f.	P
Experiment	0.85	1	349.2	0.4
Cultivation	0.15	1	394.1	0.7
Genotype	1155.94	3	631.2	<0.001
Experimental. Genotype	4.94	3	631.1	0.002

Table A3.2b: The effect of *Arabidopsis* genotypic diversity and *Hpa* on the second disease score at ten days after infection in a pair-wise interaction experiment. Disease was scored on a scale of 0-4, with 0=no disease and 4=over 75% of leaves covered in spores. A linear mixed model was used to analyse each factor and all interactions between them. Fixed effects included experimental repeat, genotype and cultivation (2-way mixture/monoculture). Non-significant terms were eliminated from the model. *F* and *P* values refer to ANOVA tests of each factor separately and the interactions between them. N=1600.

Fixed term	F	n.d.f.	d.d.f.	P
Experiment	2145.41	1	584.9	<0.001
Genotype	174.29	3	584.9	<0.001
Cultivation	0.08	1	584.9	0.771
Hpa	334.82	1	584.9	<0.001
Experiment. Genotype	12.57	3	584.9	<0.001
Experiment. *Hpa*	146.6	1	584.9	<0.001
Genotype. *Hpa*	100.35	3	584.9	<0.001
Experiment. Genotype. *Hpa*	13.73	3	584.9	<0.001

Table A3.3: The effect of *Arabidopsis* genotypic diversity and *Hpa* on rosette diameter in a pair-wise interaction experiment. A linear mixed model was used to analyse each factor and all interactions between them. Fixed effects included experimental repeat, genotype, cultivation (2-way mixture/monoculture) and *Hpa* (presence/absence). Non-significant terms were eliminated from the model. *F* and *P* values refer to ANOVA tests of each factor separately and the interactions between them. N=1600.

Fixed term	F	n.d.f.	d.d.f.	P
Experiment	3265.15	1	446.3	<0.001
Genotype	57.10	3	434.7	<0.001
Cultivation	0.008	1	429.3	0.771
Hpa	2.49	1	426.0	0.115
Experiment. Genotype	11.89	3	544.4	<0.001
Experiment. *Hpa*	39.83	1	443.5	<0.001
Genotype. *Hpa*	8.83	3	433.7	<0.001
Experiment. Genotype. Cultivation	3.61	7	479.6	<0.001
Experiment. Genotype. *Hpa*	11.72	3	551.8	<0.001
Experiment. Genotype. Cultivation. *Hpa*	4.94	7	449.2	<0.001

Table A3.4: The effect of *Arabidopsis* genotypic diversity and *Hpa* on days to flower in a pair-wise interaction experiment. A linear mixed model was used to analyse each factor and all interactions between them. Fixed effects included experimental repeat, genotype, cultivation (2-way mixture/monoculture) and *Hpa* (presence/absence). Non-significant terms were eliminated from the model. *F* and *P* values refer to ANOVA tests of each factor separately and the interactions between them. N=1600.

Fixed term	F	n.d.f.	d.d.f.	P
Experiment	236.07	1	636.1	<0.001
Genotype	1.65	3	1116.6	0.2
Cultivation	6.76	2	484.5	0.001
Hpa	0	1	635.0	0.98
Experiment. Genotype	130.56	3	1111.8	<0.001
Genotype. Cultivation	6.44	6	1179.4	<0.001
Experiment. *Hpa*	60.04	1	636.6	<0.001
Genotype. *Hpa*	48.26	3	1112.9	<0.001
Experiment. Genotype. *Hpa*	10.09	3	1110.4	<0.001
Genotype. Cultivation. *Hpa*	2.44	8	990.4	0.01

Table A3.5: The effect of *Arabidopsis* genotypic diversity and *Hpa* on seed productivity in a pair-wise interaction experiment. A linear mixed model was used to analyse each factor and all interactions between them. Fixed effects included experimental repeat, genotype, cultivation (monoculture,/2-way mixture,/4-way mixture), *Hpa* (presence/absence). Non-significant terms were eliminated from the model. *F* and *P* values refer to ANOVA tests of each factor separately and the interactions between them. N=1600.

Fixed term	F	n.d.f.	d.d.f.	P
Genotype	442.18	1	165.7	<0.001
TuYV	3.55	1	164.4	0.06
Cultivation	9.52	1	164.6	0.002
Genotype. TuYV	7.80	1	166.3	0.006
TuYV. Cultivation	19.73	1	164.9	<0.001
Genotype. TuYV. Cultivation	4.12	2	166.5	0.02

Table A3.6: The effect of *Arabidopsis* genotypic diversity and TuYV on days to flower in a pair-wise interaction experiment. Fixed effects included genotype, TuYV (presence/absence) and cultivation (mixture/monoculture). Non-significant terms were eliminated from the model. *F* and *P* values refer to ANOVA tests of each factor separately and the interactions between them. N=400.

Fixed term	F	n.d.f.	d.d.f.	P
Genotype	90.99	1	165.0	<0.001
TuYV	3.5	1	165.0	0.06
Cultivation	2.24	1	165.0	0.1
Genotype. Cultivation	3.96	1	165.0	0.05

Table A3.7: The effect of *Arabidopsis* genotypic diversity and TuYV on rosette diameter in a pair-wise interaction experiment. Fixed effects included genotype, TuYV (presence/absence) and cultivation (mixture/monoculture). Non-significant terms were eliminated from the model. *F* and *P* values refer to ANOVA tests of each factor separately and the interactions between them. N=400.

Fixed term	F	n.d.f.	d.d.f.	P
Genotype	18.22	1	137.6	<0.001
TuYV	72.51	1	138.0	<0.001
Cultivation	0	1	137.8	0.9
Genotype. TuYV	56.56	1	137.7	<0.001
Genotype. Cultivation	19.22	1	137.6	<0.001
Genotype. TuYV. Cultivation	6.58	2	138.2	0.002

Table A3.8: The effect of *Arabidopsis* genotypic diversity and TuYV on seed productivity in a pair-wise interaction experiment. A linear mixed model was used to analyse each factor and all interactions between them. Fixed effects included genotype, TuYV (presence/absence) and cultivation (mixture/monoculture). Non-significant terms were eliminated from the model. *F* and *P* values refer to ANOVA tests of each factor separately and the interactions between them. N=400.

Appendix 3:

Character	Winsome	Cassata	Saffron	Element
Rachilla hair type	short	long	long	long
Ventral furrow hair	absent	absent	present	present
Aleurone colour	white	white	blue	blue but weak
Pigment	strong	absent/weak	absent	medium
Spicules ILN	absent/weak	strong	medium	very strong

Table A4.1: The morphological grain characters used by NIAB to identify the amount of grain contributed by each winter barley variety to the mixture plot yield.

Fixed term	F	n.d.f.	d.d.f.	P
Site	15.34	2	94.9	<0.001
Fungicide	156.36	1	50.5	<0.001
Gcomp	2.23	5	53.5	0.141
Site.Fungicide	18.43	2	95.5	<0.001

Table A4.2: The effect of winter barley varietal diversity on plot yield (per $6m^2$) in a field trial conducted over three different sites. A linear mixed model was used to analyse each factor and all interactions between them. Fixed effects included trial site, fungicide treatment (treated Vs untreated) and Gcomp (Genotypic composition, 6 levels including each monoculture and both mixtures). Non-significant terms were eliminated from the model. *F* and *P* values refer to ANOVA tests of each factor separately and the interactions between them. N=1600.

a)

Fixed term	F	n.d.f	d.d.f	P
Site	0.42	1	3587	0.516
Fungicide	6.86	1	3581.9	0.009
Cultivation	30.79	1	3583.1	<0.001
Variety	90.85	3	3580.7	<0.001
Cultivation.Variety	3.6	3	3581.3	0.013

b)

Fixed term	F	n.d.f.	d.d.f	P
Site	0.42	1	3567	0.516
Fungicide	6.88	1	3562	0.0009
Mixmono	30.87	3	3563	<0.001
Variety	91.07	3	3560	<0.001

Table A4.3: The effect of four winter barley varietal diversity on a) mean mass per ear and b) mean mass per grain, in a field trial conducted over three different sites. A linear mixed model was used to analyse each factor and all interactions between them. Fixed effects included trial site, fungicide treatment (treated Vs untreated) and cultivation (monoculture/mixture). Non-significant terms were eliminated from the model. *F* and *P* values refer to ANOVA tests of each factor separately and the interactions between them. N=1600.

Fixed term	F	n.d.f.	d.d.f	P
Site	19.37	2	144	<0.001
Variety	13.39	3	144	<0.001
Cultivation	0.2	1	144	0.652
Site.Variety	2.37	6	144	0.033
Variety.Cultivation	4.31	3	144	0.006

Table A4.4: The effect of winter barley varietal diversity on brown rust disease severity (measured as % green leaf area covered in disease on the flag and first leaf) in a field experiment conducted over three different sites. A linear mixed model was used to analyse each factor and all interactions between them. Fixed effects included trial site, cultivation and variety (mixture/monoculture). Non-significant terms were eliminated from the model. *F* and *P* values refer to ANOVA tests of each factor separately and the interactions between them. N=1600.

Date	Site	Mix Mono	Plot	Fungicide	% Lodged
01/06/2012	JIC	Mono	Winsome	no fungicide	100
01/06/2012	JIC	Mono	Winsome	fungicide	50
25/06/2012	JIC	Mono	Winsome	no fungicide	100
25/06/2012	JIC	Mono	Winsome	fungicide	100
25/06/2012	JIC	Mix	A	no fungicide	50
25/06/2012	JIC	Mix	A	fungicide	25
25/06/2012	JIC	Mix	B	no fungicide	25
25/06/2012	JIC	Mix	B	fungicide	25
25/06/2012	Light	Mono	Winsome	no fungicide	25
25/06/2012	Light	Mono	Winsome	fungicide	10

Table A4.5: Percentage of plots lodged in a field trial of mixtures and monocultures of winter barley. Plots not represented showed no signs of lodging. All plots contain the barley variety Winsome. Monocultures N=24. Mixtures N=48.

Appendix 4:

Creissen, H.E., Jorgensen, T.H., Brown, J.K.M. 'Diversity Awareness: using Arabidopsis as a model for crop varietal mixtures'. Ecological Society of America annual conference in Portland, Oregon, 2012.

Creissen, H.E., Jorgensen, T.H., Brown, J.K.M. 'Diversity Awareness: using genetic mixtures to improve yield stability'. European Plant Sciences Retreat for PhD students 2012 (prize for best Plant Pathology talk).

Creissen, H.E., Jorgensen, T.H., Brown, J.K.M. 'Diversity Awareness: using genetic mixtures to improve yield stability'. British Ecological Society annual conference, Birmingham, UK, 2012.

Creissen, H.E., Jorgensen, T.H., Brown, J.K.M. 'Downy but not out: using genetic diversity to improve yield stability under disease pressure'. Ecological Genetics Group annual meeting, Belfast, UK, 2013.

Creissen, H.E., Jorgensen, T.H., Brown, J.K.M. 'Downy but not out: using genetic diversity to improve yield stability under disease pressure'. INTECOL, London, UK, 2013.